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Management Practices for Improving Sustainable Crop Production in Tropical Acid Soils

Results of a Coordinated Research Project organized by the Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture

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MANAGEMENT PRACTICES FOR IMPROVING SUSTAINABLE CROP PRODUCTION IN TROPICAL ACID SOILS

PROCEEDINGS SERIES

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OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

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FOREWORD

As a result of the burgeoning world population, there is an urgent need to increase food crop production. This can be achieved through intensification, diversification and specialization of agricultural production systems in existing cultivated land or by expansion of the land under cultivation. According to FAO estimates, only 11% of the earth's surface is currently cultivated (1406 Mha) and about 24% (3900 Mha) is potentially arable, most of which, 2500 Mha, is composed of acid soils with 1700 Mha located in the humid tropics. Thus, the greatest potential for expanding agricultural land lies in the tropical forest and savannah regions dominated by highly weathered, acid, infertile soils, mostly Oxisols and Ultisols. Soil acidification problems are also likely to increase, with rising CO_2 levels in the atmosphere, continuous use of ammonium-based nitrogenous fertilizers, removal of nutrients in farm products without replenishment and nitrate leaching. The savannahs are mainly located in humid and subhumid tropical areas and suitable for rainfed cropping conditions. They comprise a sizeable amount of the agricultural land in many countries of Africa and Latin America and include also the largely anthropic savannahs of tropical Asia. The acid savannah soils are mostly considered marginal because of their inherent low fertility and high susceptibility to rapid degradation. The cultivation of these soils without proper soil management and conservation practices has resulted in an accelerated rate of degradation of the natural resource base. Therefore, management practices must be developed/improved to avoid further degradation of the resource base and to sustain cop productivity in tropical acid soils. This Coordinated Research Project (CRP) was conceived as a follow-up of the CRP on "The use of nuclear and related techniques to evaluate the agronomic effectiveness of phosphatic fertilizers in particular rock phosphates" (1993–1998), where P fertilizer management practices were developed for P-deficient tropical acid soils. A Consultants Meeting (CM) was organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Vienna from 1 to 3 March 1999 to review advances in strategies and approaches and the role of nuclear techniques to develop management practices for sustainable crop production in the tropical acid soils of the savannah areas of Africa and Latin America. The proceedings of the meeting were published in IAEA TECDOC-1159 "Management and Conservation of Tropical Acid Soils for Sustainable Crop Production". Based on the recommendations of this CM, a Coordinated Research Project on "The Development of Management Practices for Sustainable Crop Production Systems in Tropical Acid Soils Through the Use of Nuclear and Related Techniques" was approved and implemented between 1999 and 2004. The overall objective of the CRP was to develop integrated soil, water, and nutrient management practices to increase and sustain productivity of tropical acid soils.

This publication contains the manuscripts prepared by the participants, and was edited by F. Zapata, former Project Officer. The IAEA officer responsible for this publication is L.-M. Nguyen, of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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SUMMARY

This Coordinated Research Project (CRP) established a research network and supported the efforts of teams of scientists in ten Member States with the overall objective of developing integrated soil, water and nutrient management practices to increase and sustain crop productivity in the moist savannahs of Africa and Latin America. The specific objective of the project was to improve agricultural production of tropical acid soils through (a) the use of adapted plants; (b) the amelioration of soil acidity and infertility; and (c) improved soil, water, nutrient and crop management.

Thus, the research teams adopted an integrated approach to crop, soil, water and nutrient management in predominant cropping systems during project implementation. Studies were conducted along three main areas of investigation to: (1) Identify and utilise acid-tolerant and P- and N-efficient genotypes; (2) Address issues of soil acidity and infertility in particular N and P deficiencies; and (3) Develop improved soil and water management and conservation practices for acid savannah soils. Nuclear, isotopic and related conventional techniques were employed to obtain quantitative information on the dynamics of water and nutrients in predominant cropping systems and to better understand the processes and factors influencing their productivity, and to evaluate and pilot-test improved management practices designed to alleviate soil constraints to agricultural production while conserving the natural resource base. For instance ^{15}N isotopic techniques were employed for measuring various N cycling processes (N recovery from organic residues and chemical fertilizers, biological nitrogen fixation in legumes, N losses from applied fertilizers, etc.) and establishing a N balance in crop rotations and tillage systems; ^{32}P radioisotopic techniques for identifying P-efficient crop genotypes, evaluating the effect of management practices on soil P dynamics and the agronomic effectiveness of P fertilizers, in particular phosphate rock-derived products; ^{13}C isotopic techniques for the identification of sources of organic carbon in soil C stocks and evaluating long-term soil organic matter dynamics in cropping systems.

The agricultural scenario of the humid savannahs of Africa and Latin America was very contrasting with regard to main soil types, climatic conditions and scale of farming (including the level of inputs utilized), thus determining the specific approaches and technologies to be developed and tested by the individual research teams. While, small scale farming and weakly acidic soils of very low P and N (in that order) fertility status and low buffer capacity, but prone to acidification (mostly Alfisols or Lixisols) with cultivation were predominant features in Africa, medium to large scale farming and strongly weathered and acidic soils, of inherent low fertility (Ultisols and Oxisols or Acrisols and Ferralsols) were found in Latin America. Therefore, the field experiments of this CRP covered a wide range of representative moist savannah environments and cropping systems such as maize-soybean and wheat-soybean in Brazil; sorghum-soybean in Venezuela; maize/sorghum-common bean in Mexico and Cuba; sorghum-cowpea/groundnut in Burkina Faso; maize-soybean in Benin and Nigeria. Most of the study sites were characterized by high annual rainfall (more than 1000 mm) but variable and with occasional dry spells during the growing season, isohypertermic temperature regimes and the soils were slightly to strongly acidic, low in organic matter and plant nutrients, in particular N and P but also Ca, Mg, K and micronutrients. Moreover, controlled studies such as the characterization of soil P status using the ^{32}P isotopic exchange kinetics technique; evaluation of nutrient use efficiency of a large number of plant genotypes including transgenic plants; isolation and characterization of rhizobial strains from tropical legumes and testing of various microbial inoculants were conducted under greenhouse and laboratory conditions. The main research results and recommendations arising from this CRP are summarized according to the objectives of the project.

1. IDENTIFICATION OF CROP GENOTYPES ADAPTED TO TROPICAL ACID SOILS

One of the key elements of sustainable cropping systems on tropical acid soils of the savannah agro-ecosystems of Africa and Latin America is the integration of crops and/or crop cultivars with high Al resistance, and enhanced P and N use efficiencies. Studies were undertaken carried out to identify crop genotypes with these attributes using isotopic and conventional techniques and to understand the mechanisms responsible for these attributes.

With regard to Al resistance (and soil acidity tolerance), a simplified screening technique based on Al-induced callose formation in root apices of maize after short-term Al treatment has been developed and validated in field conditions and hydroponics. This screening technique proved to be a powerful tool in the identification of maize germplasm with high combining ability for adaptation to acid Al-toxic soils. As the application of this technique failed with common bean, the Al-induced inhibition of root elongation in hydroponics was employed for the screening of bean genotypes. The prospects and limitations of the Al-induced callose technique should be further evaluated for other crops. Related physiological studies provided experimental evidence of the root apoplast involvement in Al toxicity and resistance in addition to the release of organic acid anions. Also, the possible involvement of plasma-membrane properties in Al sensitivity and resistance was investigated. Further studies are needed to get a more in-depth understanding of Al resistance and Al toxicity to facilitate and accelerate the breeding of Al-resistant crops (Horst *et al.*, p. 113).

Results from greenhouse and field experiments in Cuba and Mexico (common bean, Garcia *et al.* p. 151, and Peña Cabriales *et al.*, p. 277), Venezuela (sorghum and rice, Lopez *et al.*, p. 165), Brazil (corn and soybean, Muraoka *et al.*, p. 139) and West Africa (soybean and cowpea, Bado *et al.*, p. 293 and Diels *et al.*, p. 65) revealed important genotypic differences in P use efficiency (PUE). ^{32}P isotopic techniques were used for estimation of available P in the laboratory and greenhouse. The L value (labile P or plant-available P) technique based on ^{32}P dilution principles was improved for its use in tropical acid (low P and strongly P-fixing) soils and it was applied in Nigeria to assess cowpea genotypic differences in the mobilization of sparingly soluble soil-P fractions (Diels *et al.*, p. 65). Greenhouse experiments conducted in Mexico with three sorghum transgenic lines expressing a bacterial citrate synthase and thus an enhanced excretion of citrate from roots did not show enhanced PUE (Peña Cabriales *et al*., p. 259). Field experiments carried out in the Northern Guinea savannah of Nigeria and accompanying pot-experiments revealed a positive rotational effect of several P-efficient legume cover crops with enhanced P mobilisation capacity on subsequent maize growth and grain yields, particularly with return of crop residues (Horst *et al.*, p. 113). A relationship between P mobilisation capacity and exudation of organic acid anions and P use efficiency under field conditions could not be established yet. In acid soils of the Cerrado region of Brazil marked differences in specific activities were found between 22 plant species commonly cultivated, using ^{32}P isotopic techniquesThe most P-efficient species were pigeon pea, cotton, eucalyptus, rice and white lupin, whereas cowpea, maize and soybean belonged to the less P-efficient species. In another experiment genotypic differences in PUE were also found amongst 30 maize hybrids, which were developed for acid environments of Brazil. Approximately 20% of the studied genotypes were ranked as P-efficient; another 20% were found inefficient and the remaining 60% were considered of intermediate efficiency. (Muraoka *et al.*, p. 139).

The results from the CRP demonstrated that biological N_2 fixation (BNF) capacity of the grain legumes such as cowpea, common bean and soybean commonly grown in the acid savannah soils of West Africa is much lower (less than 50% Ndfa) than that for soybean in the savannah soils of Brazil (over 80% Ndfa). Therefore, there is much scope to enhance the N derived from N_2 fixation in these legumes under such conditions. The BNF capacity of selected grain legume genotypes was evaluated using the ^{15}N isotopic dilution technique. For instance the BNF in seven common bean cultivars in Cuba (Garcia *et al.*, p. 151), 28 common bean cultivars in Mexico (Peña Cabriales *et al.*, p. 277), 24 cowpea cultivars in Burkina Faso (Bado *et al.*, p. 293) and 20 soybean cultivars in Benin (Houngnandan *et al.*, p. 329) was compared in both pot and field experiments. Important genotypic differences in BNF capacity were found. Participants assessed the fertilizer N use-efficiency for 5 sorghum (5–18% FNUE) and maize (7–18% FNUE) cultivars, each, in Mexico indicating genotypic differences in N use efficiency among these cereals (Peña Cabriales *et al.*, p. 259).

The incorporation of Al-resistant, N and P-efficient crop species and cultivars into cropping systems on acid soils should be recommended and adopted particularly by small farmers to reduce the necessary inputs such as lime, N and P fertilizers and thus production costs. Efforts should be made by plant and soil scientists to integrate the 3 following plant characteristics: Al resistance, P use and N use efficiencies. This can be done under a new CRP on selection of crop genotypes tolerant to nutritional stress in the African and Latin American savannas. This is a pre-requisite for the adoption of such genotypes by small farmers into cropping systems on tropical acid soils of the savannah areas. Close collaboration between soil scientists, plant physiologists, plant molecular biologists and plant breeders is necessary to achieve the required progress in this rather unexploited area of breeding crops tolerant to nutritional constraints.

2. IMPROVED SOIL/WATER MANAGEMENT OF TROPICAL ACID SOILS

These investigations focused mainly on the impact of zero tillage, improved cropping systems and other selected management practices on the soil fertility status, in particular nutrient cycling and crop production in tropical acid soils. ^{15}N and ^{13}C techniques were proven essential tools in collecting quantitative information related to N cycling and C dynamics in improved cropping systems grown in the humid savannah areas. Most participants assumed that water was not a limiting factor in these areas, though there were major variations in total rainfall and its distribution during the growth season affecting crop yields, as reported in the long term experiments of Burkina Faso (Bado *et al.*, p. 47).

Integration of grain legumes and/or herbaceous legumes (cover crops/green manure) and their nitrogen inputs through BNF in cropping systems enhances their overall productivity with positive impacts on soil fertility status and recovery of applied N fertilizer (Urquiaga *et al.,* p. 13, Peña Cabriales *et al.*, p. 277 and Silva *et al.*, p. 311).

Inclusion of legumes in cropping systems may have either positive (e.g. earthworms) or negative (e.g. nematodes) impact on the diversity and function of soil biota (Peña Cabriales *et al.* p. 259 and Bado *et al.*, p. 293 respectively).

Certain tree species like Senna in agroforestry systems in the West African moist savannah zone are able to capture nutrients from the deeper soil layers if the subsoil contains adequate nutrient stocks with consequent positive recycling effects on the topsoil pH and other soil properties (Vanlauwe *et al.*, p. 85).

Zero or no tillage (ZT or NT) in the savannahs (Cerrado) of Brazil does maintain crop yield, enhance the N balance (less N losses, higher BNF, higher fertilizer N recoveries), and improve soil C stocks with time. Losses of N as ammonia volatilisation (maximum 15 kg N-

NH_3 ha^{-1} yr^{-1}) and N_2O emissions (less than 900 g N-N_2O ha^{-1} yr^{-1}) are relatively low and have little impact on the overall N balance of the system (Urquiaga *et al.*, p. 13).

In long term experiments, studies using ^{13}C natural abundance provided useful information on the SOM dynamics and the relative contribution of C_3 and C_4 plants to soil organic carbon when grown as intercropped/rotated in a complex pattern of cropping systems in tropical humid savannahs of Africa (Diels *et al.*, p. 65 in Nigeria) and Latin America (Urquiaga *et al.*, p. 13 in Brazil).

3. CARBON/NITROGEN CYCLING STUDIES IN CROP ROTATIONS AND TILLAGE SYSTEMS

Zero tillage (ZT) was compared to conventional tillage (CT) in field investigations carried out in several locations of the Cerrado of Brazil on Ferralsols/Acrisols under a series of legume-cereal rotations (Soybean – wheat or soybean/maize rotations with inclusion of green manure legumes). There were no marked differences between tillage systems (ZT and CT) in grain yields or N accumulation by the crops, but BNF was higher in the soybean crop under ZT. The BNF system in soybean (above 80%) is so efficient that attempts to increase grain yields by addition of N fertilizer are hardly ever successful if the plants have been effectively inoculated with the recommended *Bradyrhizobium* strains. Since N balance estimates (N inputs from BNF in the whole plant minus offtake as total N in grain) of the new soybean varieties are almost nil or negative, it may be deduced that the rapid SOM mineralization induced by the soybean residues favoured the grain yield of succeeding cereal crops by increasing soil N availability. This stimulation effect of soybean residues on soil N supply could gradually reduce SOM and soil N stocks under the traditional soybean/wheat crop sequence. N fertilizer use efficiency varied from 40 to 60% for a maize crop fertilised with 80 kg N ha^{-1}, resulting in a grain yield of about 7 Mg ha^{-1}. Major N losses via ammonia volatilization (a maximum of 10% of the 40 kg N ha^{-1} applied to maize) occurred when the urea was spread in the soil surface under ZT. When the N-fertilizer was buried in the soil surface layer the N losses were insignificant. It was estimated that less than 900 g N-N_2O ha^{-1} yr^{-1} was lost from the soil surface, where the highest values were found under CT (Urquiaga *et al.*, p. 13).

The large contribution of BNF (about 100–200 kg N ha^{-1}) to the lupin and vetch crops used as green manures is sufficient to balance, or even to increase significantly the soil N content, with good potential to reduce the N-fertiliser inputs without yield reduction in subsequent cereal crops. At the end of 13 years under a continuous sequence of wheat/soybean there was no significant difference in soil C stocks between ZT and CT. However, in the rotations where the N_2-fixing legume vetch was included as a winter green manure crop, soil C stocks in the topsoil (0–30 cm depth) and in the soil profile (to 100 cm depth) under ZT were increased by approximately 10 Mg ha^{-1} and 17 Mg ha^{-1} respectively. Where soil C stocks under ZT were higher than CT, much of the N gain was at depths below the plough layer, suggesting that most of the accumulated soil C is derived from root residues. The ^{13}C natural abundance data suggested that in comparison to CT, the ZT system promoted the preservation the native soil organic C (Urquiaga *et al.*, p. 13).

Field fertilization experiments were conducted on Ferralsols of Brazil with crotalaria or millet – maize rotation under zero tillage on Ferralsols, the highest maize yield was obtained in the crotalaria-maize rotation, followed by fallow-maize and millet-maize. This was also true for the residual effects during the second year. Grain yield response to fertilizer N application improved slightly in the crotalaria-maize system compared with the other two systems. The quantity of N derived from fertilizer increased with increasing N application rate. Best values were obtained in the crotalaria-maize rotation. The maximum fertilizer N use efficiency was obtained with 100 kg N ha^{-1} (Silva *et al.*, p. 311).

In Mexico, from investigations conducted with legume green manures (mucuna, canavalia, cajanus) – maize associations under conventional tillage on Acrisols, the application of earthworms and mucuna as green manure was found a promising agricultural practice to increase maize biomass yield by 58%. *C. ensiformis* and *C. cajan* with 260–370 and 133–170 kg fixed N kg ha^{-1} respectively, have very good potential for use as green manures in the Ultisols of the savannah of Tabasco, specially when they are P-fertilized. For the plant population density used in the study, the association of recommended maize genotypes and legumes green manures resulted in a higher nitrogen fixation by legumes (70–90 % Ndfa in association versus 40–80% in monoculture) and a relatively lower maize yield (1.71–3.79 in association versus 2.15–4.20 in monoculture) and fertilizer N use efficiency (6–20% FNUE in association versus 15–38% FNUE in monoculture) (Peña-Cabriales *et al.*, p. 277).

In Burkina Faso, field experiments were carried out with grain legumes (groundnut, cowpea) – sorghum rotations under conventional tillage on Lixisols. Compared to continuous cultivation of sorghum, the cowpea and groundnut grwon in rotation increased succeeding sorghum yields (2.9–3.1 times more grain yields). The nitrogen fertilizer equivalency (NFE) of groundnut (35 kg N ha^{-1}) was higher than that of cowpea (25 kg N ha^{-1}). A better fertilizer N use efficiency (FNUE) was observed in legume-sorghum rotations. In continuous sorghum, FNUE was 20%, compared to 28 and 37% in cowpea-sorghum and groundnut-sorghum rotations, respectively. Based on grain yield potential estimates, fertilizer N recommendation was 40 kg N $ha^{-1}$for succeeding sorghum in mono-cropping and 51 and 58 kg N ha^{-1} when succeeding sorghum was rotated with groundnut or cowpea respectively. Legume-sorghum rotations increased soil mineral N by 40–50%, thus N supply to succeeding sorghum was higher in these soils compared to monocropping of sorghum. In cowpea-sorghum rotation soil and succeeding sorghum roots were mostly infected by nematodes while groundnut decreased nematode infections (Bado *et al,.* p. 293). Soil organic carbon (SOC) was the lowest (0.36%) in continuous sorghum and increased to 0.39% and 0.54% in cotton-groundnut-sorghum and fallow-sorghum rotations, respectively. Only fallow-sorghum rotation maintained SOC, exchangeable acidity and base saturation at same levels like those of original soil. Highest available soil P status (7.9 ppm P-Bray I) was observed in soils of monocropping of sorghum and lowest (4.1 ppm P) was found in fallow-sorghum rotation. Manure applications increased soil organic carbon, total N and available P. Except for fallow-sorghum rotation, all rotations increased aluminium saturation and decreased soil pH compared to the original soil. Manure or dolomite applications decreased exchangeable acidity and maintained soil pH and base saturation at same levels as the original soil (Bado *et al.*, p. 47).

In Nigeria, studies were made with maize intercropped with trees (*Leucaena*, *Senna*, *Gliricidia*) under minimum tillage on Lixisols. In a long term trial with inputs of tree-derived organic resources (*Leucaena leucocephala*, *Senna siamea*), and fertilizer, alone or in combination, the *Senna*-based system with fertilizers was the more resilient one, both in terms of maize crop yields and soil fertility status. Fertilizer N use efficiency was usually higher (47% FNUE) in the *Senna* treatment compared to the control (28% FNUE) or the *Leucaena* (30% FNUE) treatments. Interactions between fertilizer and organic matter additions were negative for the *Leucaena* treatments in the first three years, and positive for the *Senna* treatment in the last 6 years. It was also found that trees had a positive impact on the maintenance of exchangeable base cations in the topsoil. Exchangeable Ca, Mg, and K – and hence ECEC – were only slightly reduced after 16 years of cropping in the tree-based systems, and even increased in the *Senna* treatment (Vanlauwe *et al.*, p. 85).

In Nigeria, the potential of deep-rooting hedgerow trees to recycle basic cations from the subsoil and increase the topsoil pH was evaluated in a set of medium to long term alley cropping trials. Topsoil Ca content, ECEC, and pH were substantially higher under *Senna* than under *Leucaena* or *Gliricidia* or the no-tree control plots at sites with a Bt horizon, rich in exchangeable Ca. This effect was largely related to the recovery of Ca from the subsoil under Senna trees (Vanlauwe *et al.*, p. 85).

Results from a 16-year continuous-cropping field experiment in Ibadan, Nigeria, provided information on long-term changes in soil organic carbon (SOC) contents in savannah soils with sandy topsoil. In the control treatments with continuous maize and cowpea cropping without trees, SOC levels dropped from the initial 15.4 Mg C ha^{-1} to 7.3–8.0 Mg C ha^{-1} in 16 years (SOC content in 1700 Mg ha^{-1} equivalent soil mass). In the two continuously cropped alley cropping (AC) systems (*Leucaena* and *Senna*), the SOC levels dropped to between 10.7 and 13.2 Mg C ha^{-1}. The ^{13}C natural abundance technique yielded useful information to test the ROTHC-26.3 SOC model in sub-humid tropical conditions under a complex pattern of cropping systems in which both C_3 and C_4 plants were intercropped/rotated. Contrasting $\delta^{13}C$ values of the SOC were found because the relative OM contribution of the C_3 and C_4 components differed significantly between the treatments compared. SOC decomposition rates werevery fast, because all decomposition rate constants utilised in the model had to be doubled in order to better simulate the measured SOC contents and $\delta\ ^{13}C$ of the AC treatments and the no-tree controls (Diels *et al.*, p. 65).

In Benin, experiments were done in farmer's fields with soybean-maize rotation under minimum tillage on degraded Acrisols. Residual effects of previous soybean, amended with manure or N fertilized resulted in significant improvements in maize grain yield although the values (less than 1 t ha^{-1}) remained low (Houngnandan, p. 329).

An economic evaluation of zero tillage versus conventional tillage should be conducted, with special reference to labour, energy and environmental issues, including above and below-ground biodiversity effects as well. Efforts should be directed towards a better understanding of importance of root (below-ground) biomass and its nutrient turnover as the driving forces behind C accumulation/sequestration under zero tillage systems. Also, investigations should be made to assess the effects of zero or minimum tillage on soil water storage and crop biomass, including C accumulation/sequestration. Future field work should include multi-locational on-farm approaches in order to enhance the validity and applicability of the recommendations with a farmer participatory approach. Socio-economic studies should also be conducted to enable policy and decision-makers to formulate appropriate policies to promote the adoption of the best management practices in their countries. This can be best achieved under IAEA technical co-operation and other projects. Efforts should be made to initiate new long-term trials in the African and Latin American savannas to properly study the impact of new soil management practices (e.g. minimum tillage in Africa) in the long run under the "Conservation Agriculture" initiative.

4. AMELIORATING SOIL ACIDITY AND NITROGEN INFERTILITY OF TROPICAL ACID SOILS

In the course of this CRP, ^{15}N methodologies and related techniques provided reliable information to address limitations of soil acidity and nitrogen infertility in a range of cropping systems on acid tropical soils in Latin America and Africa.

In fertilizer N studies, observed fertilizer N recoveries (FNR) by cereals varied widely across the different sites reflecting the influence of local prevailing environmental factors and management practices. Both the inclusion of legumes in the cropping system and the shift from conventional to minimum tillage increased the FNR but this improvement was site-specific (soil, climate and farming practices) and related to the fertilizer N application rate. In general FNR seemed to decrease with increased fertilizer N application rates.

Participants conducted studies on biological nitrogen fixation (BNF) assessment in grain legumes such as soybean in Brazil (Urquiaga *et al.*, p. 13), Benin (Houngnandan, p. 329), and Venezuela (España *et al.*, p. 343); common bean in Mexico (Peña-Cabriales *et al.*, p. 277) and Cuba (Garcia *et al.*, p. 205), cowpea and groundnut in Burkina Faso (Bado *et al.*, p. 293), and pigeon pea in Mexico and Venezuela (Peña-Cabriales *et al.*, p. 277 and España *et al.*, p. 343 respectively) using the ^{15}N methodology. A wide range of nitrogen fixation capabilities (proportion and amounts) within species and across environments was found indicating that there is a great scope for increasing BNF, especially for common bean and to some extent for cowpea and groundnut. This can be achieved through a better agronomic management, rhizobial strain selection, and further plant genetic improvement. In Burkina Faso, satisfactory agreement was observed between the ^{15}N isotopic dilution and the total N difference method for BNF assessment in cowpea (Bado *et al.*, p. 293).

Liming with $CaCO_3$ increased % Ndfa of common bean from 28 to 38% on an Acrisol (initial pH-KCl of 3.8) in Cuba (Garcia *et al.*, p. 205). Application of dolomite to cowpea doubled the amount of Ndfa on a sandy Ultisol (pH-H_20 6.5) in Burkina Faso (Bado *et al.*, p. 293).

Studies in Brazil and Mexico showed that the inclusion of multipurpose legume species such as lupins, vetch, cajanus, centrosema, crotalaria, mucuna, etc; as cover crops/green manures in the cropping system was sufficient to balance, or even to increase significantly the soil N content, with good potential to reduce the N-fertiliser inputs without yield reduction in subsequent cereal crop (Urquiaga *et al.*, p. 13; Silva *et al.*, p. 311; Peña-Cabriales *et al.*, p. 259 and 277).

There is a need to identify and elucidate the key mechanisms responsible for the observed ‘rotational’ effects (i.e. effects other than N effects) of legumes in legume-cereal systems over long periods of time. Better understanding of these is required to optimise cropping systems in tropical acid soils. Also, it is necessary to improve the BNF potential of legumes in the African savannahs. As indicated above, there is a need to confirm the results from these studies in multi-location experiments, preferably in on-farm conditions.

5. AMELIORATING LOW P STATUS OF TROPICAL ACID SOILS

Valuable information to ameliorate low P status on acid tropical soils and to supply adequate P requirements to a range of cropping systems was obtained utilising ^{32}P isotopic and related techniques in tropical acid soils.

The application of P fertilizer resulted in excellent crop responses in P-deficient soils as it was found in Brazil (Muraoka *et al.*, p. 243), Burkina Faso (Bado *et al.*, p. 293), Cuba (Garcia *et al.*, p. 205) and Mexico (Peña-Cabriales *et al.*, p. 259). Specific crop genotype responses were related to P rates and P sources, including phosphate rock (PR) products. For instance in Cuba there was significant grain yield response of the local common bean genotypes to the application of appropriate PR products (Garcia *et al.*, p. 151) whereas in Mexico, no response from sorghum transgenic lines was obtained to the application of the local Baja California PR (Peña-Cabriales *et al.*, p. 259). Results from Mexico, Cuba, Brazil and Burkina Faso confirmed the need of P application for adequate nitrogen fixation in legumes.

^{32}P isotopic techniques were improved for their application in tropical acid soils exhibiting very low P and high P sorption (fixation) (Diels *et al.*, p. 65). Furthermore they were used in the laboratory and greenhouse for accurately estimating plant-available P (Diels *et al.*, p. 65) and for the identification of P-efficient genotypes (Muraoka *et al.*, p. 139).

Studies on the interaction of lime and PR products for crop production in strongly acid soils were conducted in Cuba and the USA. The results from Cuba confirmed the need for liming a strongly acid (pH-KCl 3.8) Acrisol soil to reduce Al toxicity and enhance soil P availability but only when the PR product (C40) is applied at normal maintenance P rates (40 and 80 kg P_2O_5 ha^{-1}). Liming reduced P availability of C40 applied at the highest P rate (120 kg P_2O_5 ha^{-1}) (Garcia *et al.*, p. 205). The results from the greenhouse study in the USA indicated that the reactive Sechura PR will not only provide available P nutrient, but also reduce potential Al toxicity for upland rice and soybean contributing to sustainable crop production and BNF in the legume crop, especially for its good residual P effect, in an acid Ultisol. The possible use of the more cost-effective Huila PAPR can be also an alternative P source to TSP in this soil due to its good agronomic performance. However, the liming requirements should be carefully determined based on the possible soil-plant-fertilizer interactions and their effect on the soil properties continuously monitored because liming upon reaction with soils can significantly reduce the agronomic effectiveness of PR (Chien, p. 185). Liming increased grain yield by about 2-fold and nitrogen fixation (from 27 to 38% Ndfa and from 6 to 19 kg N ha^{-1} in unlimed and limed soil respectively) of seven common bean genotypes in Cuba. (Garcia *et al.,* p. 205).

The effects of soil amendments such as lime (combined effects of pH and Ca) and gypsum (Ca effect) applications, e.g., in Brazil, on the soil P and micronutrient status and in particular their effects on the P-PR availability, need to be further assessed in long-term field experiments.

Indigenous PR were utilised as P sources in Cuba and Brazil. Under unfavourable conditions for PR use, e.g. low reactivity, high soil pH, etc., modifications of the phosphate rock by (i) partial acidulation of PR, (ii) dry compaction of PR with WSP, or (iii) wet granulation of PR with WSP are recommended to improve their agronomic and economic feasibility. This was done in the field and greenhouse experiments in Cuba to improve the reactivity and agronomic effectiveness of the local Trinidad de Guedes PR for its application as P source for annual crops (Garcia *et al.*, p. 205). Powdered mixtures of PR and WSP without compaction or granulation proved to be ineffective for increasing PR agronomic effectiveness of the low-reactive Patos PR as shown by the results from Brazil utilizing a high P-fixing Oxisol soil (Muraoka *et al.*, p. 243).

A PR database based on standardized PR solubility measurements including entries from geological and agronomic key references has been created and a decision support system for direct application of phosphate rock sources (PR DSS) was developed under a joint FAO/IAEA and IFDC collaborative project. The current PRDSS predicts the relative agronomic effectiveness (RAE) of PR with respect to water-soluble P fertilizer (WSP) for the initial PR application. The RAE as predicted by the PRDSS depends on: (1) PR sources as quantified by PR solubility determined using the second extraction with neutral ammonium citrate (NAC_2); (2) soil pH; (3) crops as they influence the rhizosphere, root quantity and distribution, uptake of Ca, crop duration, Al toxicity/tolerance, etc.; (4) soil P-fixation capacity; (5) soil texture; (6) soil organic matter content; (7) Al saturation; (8) moisture and rainfall regime; and (9) free calcium carbonate content of the PR source. The current version as presented here is ready for future web application. Additional features sich as the cumulative effect of annual PR application or the residual effect of PR will be included in the next version of the PRDSS (Singh *et al.*, p. 223).

Field experiments in Burkina Faso, Tanzania, Malaysia, Vietnam, Argentina, Brazil, Togo and additional sites in Mali and Thailand through the University of Hawaii are underway to validate the PRDSS and also evaluate effect of one-time versus annual application over a two-year period to capture immediate and residual effects and potential effect of PR as liming agent.

All these data will be compiled and a web-based PRDSS version will be posted in the DAPR (Direct Application of Phosphate Rock) website created jointly by FAO/IAEA and IFDC The web-based PRDSS will result in greater accessibility of information and decision choices to a large audience comprising of researchers, extension workers, farm managers, progressive farmers, policy makers, and fertilizer companies. Based on the RAE of the PR in study and its economic feasibility provided by the PRDSS, the users will be able to make better informed decisions for direct application of PR as compared with WSP. This will avoid trial and error use of PR and thus save time and money. Alternatively, if direct application of a particular PR is not suitable, possible modifications/suggestions may be provided. At a final stage, it is planned to establish linkages to DSSAT P modeling to predict crop yield to be obtained with WSP and PR sources and made an economic assessment (cost/benefit ratio) to determine whether use of PR or WSP versus no P application would be most profitable to the farmers in the tropical regions of acid soils.

Integrated Management of Tropical Acid Soils

NITROGEN DYNAMICS IN SOYBEAN-BASED CROP ROTATIONS UNDER CONVENTIONAL AND ZERO TILLAGE IN BRAZIL

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Abstract

Approximately 70% of Brazilian agriculture is based on the cultivation of highly weathered soils located in tropical and subtropical savannah areas, where the zero or no tillage (ZT) system is now considered as the best alternative to the traditional conventional tillage (CT). During the period 1999-2004 the Agrobiology Centre of Embrapa implemented a research program using nuclear and related techniques to study the main processes involved in the dynamics and cycling of N for predominant crop rotations grown at five locations under ZT in comparison to CT, on acidic savannah soils of both southern and Cerrado regions of Brazil. The most relevant results of these studies were: (1) The soybean crop derived a high proportion of its N (over 80%) from biological nitrogen fixation (BNF). The BNF system is so efficient that attempts to increase grain yields by addition of N fertilizer are hardly ever successful as long as the plants have been effectively inoculated with the recommended *Bradyrhizobium* strains. (2) The N fertilizer use efficiency by the maize crop fertilized with 80 to 90 kg N ha^{-1} varied from 40 to 60%. (3). A maximum 10% of N-urea applied broadcast over the soil surface (40 kg N ha^{-1}) in a maize crop was lost via NH_3 volatilization, with the highest values under ZT. (4). The inclusion of green manure legumes (GMLs) such as lupin and vetch, which are highly efficient in BNF was essential to promote a positive soil N balance in crop rotations and to increase the stock of soil organic C. (5) Less than 900 g N-N_2O ha^{-1} yr^{-1} were lost from the soil surface, and the highest values were found under CT. (6) Even thought the contribution of BNF to the Brazilian soybean crop is high (over 80%), the accurate assessment of this input to the soil N balance still remain unclear due to methodological problems using the leaf labeling technique to estimate residual below-ground N. Results obtained so far suggest that these methodological issues need to be overcome before these estimates can be considered as reliable. These results demonstrate the enormous potential impact of BNF in legumes such as soybean and green manures and their management under ZT and CT on the N cycling processes and overall C and N stocks in tropical acid savannah soils, thus contributing to the productivity and sustainability of the agricultural production systems.

1. INTRODUCTION

Brazil is the largest country in South America with a population of some 180 million inhabitants. Agricultural production is based on the cultivation of around 53 Mha of mainly Oxisols and Ultisols located in tropical and subtropical savannah areas. In this country, although great advances have been achieved in the management of these highly weathered acid and infertile soils, serious problems still persist, principally those related to soil conservation under intensive agricultural production systems. However, in recent years an ever-increasing area of land under mechanized grain production is being converted from conventional tillage (CT) to the zero tillage (ZT) system.

By preserving crop residues on the soil surface, the ZT system contributes to control soil erosion and it is now recommended not only as a means to contribute to reduce soil erosion, but also to mitigate greenhouse emissions to the atmosphere. Besides using the ZT system, the diversification of species in crop rotations is a practice that is being increasingly adopted. In addition to the traditional annual crops such as soybean, maize, wheat, *Phaseolus* beans and rice, others crops like cotton, sunflower and green manure/cover crop legumes are being included. Thus, as the low levels of available soil N is one of the most limiting soil fertility factors for the intensification and diversification of the Brazilian agriculture, it is important to generate better knowledge of the N dynamics and the main processes occurring in the soil/plant system in order to devise best management practices that contribute to the productivity and sustainability of these agricultural production systems.

Efficient N fertilizer use by the crops and a positive N balance for the soil/plant system are two prerequisites to guarantee sustainable crop production under these conditions. The tillage system affects grain yield and fertilization response through its effects on nutrient and water availability. Biological N_2 fixation (BNF) plays a critical role in guaranteeing a major part of the N supply for crop production in these rotations as they invariably include soybean and often winter green manure legumes. It is common practice by farmers to apply the same quantities of N fertilizer recommended for each of the cereal grain crops (principally maize and wheat) without regard to the export of N in grain compared to the total N inputs from fertilizer and BNF.

In 1999 the National Centre for Agrobiology Research of Embrapa, started a research program at selected sites on acid savannah soils of both the southern and the Cerrado regions of Brazil to study the biological processes involved in the dynamics of N in the soil/plant system under different crop rotations under ZT, in comparison with CT. This project has received important assistance from the IAEA through the research contract Nº 10953 that was implemented during the period 2000–2004 as part of the Coordinated Research Project (CRP) on “The development of management practices for sustainable crop production systems on Tropical Acid Soils through the use of nuclear and related techniques”.

The main objectives of this research project were: (a) To quantify the contribution of biological nitrogen fixation (BNF) to soybean and green manure legumes and the benefit of the residual N to the subsequent cereal crops under both ZT and CT, (b) To evaluate the influence of crop rotation including legumes crops and tillage systems on the soil N availability, (c) To estimate the nitrogen fertilizer use efficiency (NFUE) and the total balance of fertilizer N with a view to evaluating potential N losses under the two different tillage systems, (d) To evaluate the influence of BNF and crop residues of different crop rotations (including green manure legumes) under both ZT and CT systems as a source of organic C and N to the stock of these elements cumulated in the soil profile, based on long-term experiments, and (e) To assess the emission fluxes of the greenhouse gases N_2O and CH_4 from the soil surface with different crop rotations under ZT and CT, in comparison with the soil under native vegetation. A series of studies were carried out at five locations on acid savannah soils in both southern and Cerrado region of Brazil using nuclear and related techniques.

2. EXPERIMENTAL SITES

This study has been developed by a multidisciplinary group of researchers from the following institutions: Embrapa-Agrobiologia (Seropédica, Rio de Janeiro, RJ), Embrapa-Cerrados (Planaltina, Federal District, DF), Embrapa-Soybean Research Centre (Londrina, Paraná, PR), Embrapa-Wheat Research Centre (Passo Fundo, Rio Grande do Sul, RS) and the Fazenda Foresta do Lobo (Uberlândia, Minas Gerais, MG).

2.1. Description of the experimental sites

2.1.1. Embrapa Soybean Research Centre (Experimental Station), Londrina, Paraná State

This site is in the southern region of Brazil where zero tillage was introduced 25 years ago. In the experimental area the ZT started in 1995.

- Soil type: Dusky red Latosol. Rhodic Haplustox (US Soil Taxonomy Classification).
- Soil texture: 7% sand, 19.6 silt, 73.4% clay.
- Soil fertility: pH (H_2O)-5.6, Ca-6.2 $cmol_c\ dm^{-3}$, Mg-2.8 $cmol_c\ dm^{-3}$ P- 9 mg dm^{-3}, K-338 mg dm^{-3}. (Determined using standard methods according to the Embrapa soil analysis manual [1]).
- Localization: 23° 23´S and 51° 11´W.
- Altitude: 566 m above sea level.
- Rainfall (1700 mm yr^{-1}) is reasonably evenly distributed during the year, with hot summers and cool winters (5–10 days of frost).
- Average temperature: Monthly temperatures oscillate from 28^{o}C in the wetter period (October to April) to around 12^{o}C during autumn and winter months (May to August).

NB: Climatic data from the meteorological station at the Embrapa Soybean Research Centre

2.1.2. Fazenda Floresta do Lobo (Private farm), Uberlandia, Minas Gerais State

- This site is in the Cerrado region, the tropical savanna. Zero tillage is fast being introduced into this region. In the experimental area ZT started in 1996.
- Soil type: Dark red Latosol. Typic Haplortox – US Soil Taxonomy Classification.
- Soil texture: 25.3% sand, 11.1% silt, 63.6% clay.
- Soil fertility: pH (H_2O)-5.3, Al-0.2 $cmol_c\ dm^{-3}$, Ca-1.7 $cmol_c\ dm^{-3}$, Mg-0.5 $cmol_c\ dm^{-3}$ P-14 mg dm^{-3}, K-113 mg dm^{-3}.[1].
- Localization: 18^o 55´S and 48^o 17´W.
- Altitude: 860 m above sea level.
- Rainfall (1300 mm yr^{-1}) is concentrated almost exclusively in the months of October to April.
- Average temperature: Monthly temperatures oscillate from 18.8–24.7^{o}C.

NB: Climatic data from the meteorological station at the Federal University of Uberlândia.

2.1.3. *Embrapa Cerrados (Experimental Station), Planaltina, near Brasilia, D.F.*

- This site is in the Cerrado region, a typical tropical savanna. Zero tillage is also fast being introduced in this region. In this experiment ZT started over 20 years ago.
- Soil type: Typic Haplustox (US Soil Taxonomy Classification).
- Soil texture: 49.1% sand, 9.9% silt, 41.1% clay.
- Soil fertility: pH(H_2O)-5.7, Al-3.8 $cmol_c\ dm^{-3}$, Ca-1.4 $cmol_c\ dm^{-3}$, Mg-0.1 $cmol_c\ dm^{-3}$ P-11 $mg\ dm^{-3}$, K-167 $mg\ dm^{-3}$. [1].
- Localization: 15° 35´S and 47° 42´W.
- Altitude: 1007 m above sea level.
- Rainfall: 1300 mm.
- Average temperature: Monthly temperatures oscillate from 19.2–22°C.

NB: Climatic data from the meteorological station at the Embrapa Cerrados Centre.

2.1.4. *Embrapa Wheat Research Centre (Experimental Station), Passo Fundo, Rio Grande do Sul State*

- This site is in the southern region of Brazil, where zero tillage was introduced 28 years ago.
- Soil type: Hapludox (US Soil Taxonomy Classification).
- Soil texture: 24% sand, 13% silt, 63% clay.
- Soil fertility: pH (H_2O)-5.0, Al-0.6 $cmol_c\ dm^{-3}$, Ca-4.0 $cmol_c\ dm^{-3}$, Mg-1.8 $cmol_c\ dm^{-3}$, P-18 $mg\ dm^{-3}$, K-163 $mg\ dm^{-3}$. [1].
- Localization: 28° 15’S and 52° 24´W.
- Altitude: 684 m above sea level.
- Rainfall: 1800 mm.
- Average temperature: Monthly temperatures fluctuate between 13.2–23.6°C.

NB: Climatic data from the meteorological station at the Embrapa Wheat Research Centre.

3. RESPONSE OF SOYBEAN TO N FERTILIZER APPLICATION

Soybean is the most important export crop of Brazil. During the last few years the cropped area of this grain legume has grown significantly. Thus, from the season 2000/2001 to 2003/2004 the soybean area increased from 12.5 to 21.3 Mha, with an average grain yield of approximately 2.4 Mg ha^{-1}. This crop normally accumulates as much as 250 kg N ha^{-1} for a grain yield of 3.5 Mg ha^{-1}, which is frequently attained in many locations. This amount of

N accumulated in the crop biomass is very high considering that the crop is only inoculated with *Bradyrhizobium spp.*and without application of N-fertilizer. For this reason many farmers are asking if it is possible to further increase the grain yield through the application of N-fertilizer at flowering stage when the N demand of this crop is normally the highest. This question is further stimulated by the marketing strategies of N-fertilizer-producing industries. To answer this question, this study was carried out to evaluate the response of the soybean crop to the application of two rates of N-fertilizer, 30 and 60 kg N ha^{-1} at flowering stage using ammonium sulphate labeled with 2.0 atom % ^{15}N excess. The treatment without N-fertilizer was inoculated with *Bradyrhizobium spp.* This study was conducted from November 2000 to April 2001 at the “Fazenda Floresta do Lobo” on an Oxisol (Dark Red Latosol) of the Cerrado of Uberlândia in the State of Minas Gerais. The soybean variety used was Conquista, which is widely grown in the Brazilian Cerrado region. The labeled fertilizer was applied to micro-plots of 4.5 m^2. The basal fertilization for the main plots included 160 kg $ha^{-1}$of a compound NPK fertilizer 0-20-6.

The results showed that this crop inoculated with *Bradyrhizobium spp.* can produce over 3.4 Mg ha^{-1} of grain, 1 Mg more than the current Brazilian average grain yield, without any significant response to the application of N-fertilizer (30 and 60 kg N ha^{-1}) at flowering stage (Table I). From these results it may be inferred that BNF is very efficient and enough to cover most of the whole N demand of this crop under the conditions of this study.

TABLE I. RESPONSE OF SOYBEAN, CULTIVAR ‘CONQUISTA’ TO THE APPLICATION OF N-FERTILIZER AT THE FLOWERING STAGE IN AN OXISOL OF UBERLÂNDIA, MG. MEANS OF FOUR REPLICATES.

Tillage system	N-fertilizer kg ha^{-1}	Grain		Whole plant		
		Yield kg ha^{-1}	Total N kg ha^{-1}	Total N kg ha^{-1}	N-harvest index (%)	N-FUE (%)
ZT	0	3352a	186a	210a	89a	-
	30	3307a	189a	214a	88a	69a
	60	3663a	210a	236a	89a	47b
	Mean	**3441**	**195**	**220**	**89**	**54**
CT	0	3414a	189a	210a	90a	-
	30	3403a	190a	213a	89a	47b
	60	3196a	181a	199a	91a	56a
	Mean	**3338**	**187**	**207**	**90**	**53**

N-fertilizer: Ammonium sulphate (2.0 at. %^{15}N exc.) applied at the flowering stage of the soybean crop on the soil surface. In each column, values with the same letter are not significant at $P<0.05$ (Tukey’s HSD test).

It was also observed that this crop had high N harvest indices (about 90%), which resulted in the export from the soil/plant system of some 190 kg N ha^{-1}. The efficient contribution of BNF that is normally approximately 85% under these conditions, plus the soil N supply can guarantee the total demand of N to this crop. In this study the soybean crop recovered on the average 54% of the N-fertilizer applied (N-FUE) at flowering stage. Though this value was high, it did not contribute to further increase the grain yields. Thus, under these conditions, high grain yields of soybean can be obtained either with the application of N fertilization or through the use of an efficient *Bradyrhizobium* inoculant. However it would be uneconomical and environmentally inappropriate to produce soybean based on the use of N fertilization. On the average no major differences between total biomass and grain yield, total and grain N yield, nitrogen harvest indices and fertilizer N recoveries were found between both tillage systems studied.

4. QUANTIFICATION OF THE BIOLOGICAL NITROGEN FIXATION (BNF) CONTRIBUTION TO SOYBEAN

4.1. Soybean BNF in long-term crop rotation experiments

In order to construct a simple N balance for the different crop sequences in the rotations at the experimental sites the first step was to estimate the contribution of BNF to soybean in the long-term experiments at the Embrapa Soybean Centre (Londrina, PR) and at the Fazenda Floresta do Lobo (Uberlândia, MG). At both sites the total dry matter and total N accumulation of the soybean crops were measured for 10 plants per plot (4 replicate plots per experiment) at all three sites taken at mid-pod-fill stage when N accumulation is maximum. The proportion of N derived from BNF (% Ndfa) was estimated by comparing the ^{15}N natural abundance of the soybean samples (whole shoot tissue) with that of weeds in the same plots, as reference non N-fixing species as described by Ramos *et al.* [2]. The ^{15}N natural abundance of the N derived from BNF by the soybean (the *B* value) was estimated to be -1.30 ‰, a value taken from a survey of the literature conducted by Boddey *et al.* [3].

At Londrina the soybean was preceded by a winter crop of black oats, which in turn was preceded by maize in the previous summer season and both crop sequences were managed under either ZT or CT. The soybean crop was only inoculated with *Bradyrhizobium spp.* and with no application of N-fertilizer. The results showed that there was no significant difference in grain yield, total dry matter or total N accumulation of the soybean between the tillage systems (Table II). However, the proportion of N derived from BNF under ZT (82%) was significantly higher than under CT (72%), which can be attributed to the greater mineralization of soil N by the tillage operations under CT, than when the soil was virtually undisturbed under ZT. The total N exported in grain was marginally (not significant at $P<0.05$) higher under CT than under ZT (182 versus 175 kg N ha^{-1}), and as the % Ndfa for the soybean under CT was lower than under ZT, the overall N balance (input of N from BNF – N exported in grain) was almost 20 kg N ha^{-1} lower under CT than under ZT. In addition, N inputs from BNF were lower than N exported in grains under CT and ZT, the N balance in both systems was negative.

TABLE II. GRAIN YIELD, RESIDUE DRY MATTER, N ACCUMULATION AND BNF CONTRIBUTION TO THE SOYBEAN CROP UNDER ZERO TILLAGE (ZT) AND CONVENTIONAL TILLAGE (CT), IN LONDRINA, PR, SUMMER 2000. MEANS OF FOUR REPLICATES

Tillage system	Grain yield	Residues[1]	Grain N	Residue N	Plant total N	BNF	N balance[2]
	Mg ha^{-1}		kg ha^{-1}				
CT	3.03	3.93	181.8	38.1	219.5	158.4	- 23.4
ZT	2.91	3.92	174.6	31.4	206.0	168.9	- 5.7
t test	ns	ns	ns	ns	ns	*	*
CV(%)	35.5	20.5	32.7	14.8	30.1	-	-

[1] Except grain, all plant dry matter including recoverable roots.
[2] N derived from BNF (whole plant) minus total N in grain.
ns and *, means not significantly different or significantly different at P<0.05, respectively (t test).

Considering that no N losses were quantified in this part of the study, it is evident that the actual N balances for soybean in this experiment were even more negative.

At the site near Uberlândia (Fazenda Floresta do Lobo) in the Cerrado region, the cropping season is from October to May. From May to September there is virtually no rainfall at all. The cropping sequence here was soybean planted in October and harvested in February followed by maize harvested in May. At this site soybean grain yields were approximately 4 Mg ha^{-1}, somewhat higher under CT than under ZT (Table III). Again the % Ndfa under CT (67%) was significantly lower than under ZT (76%) and this led to the N balance for soybean at this site being almost 30 kg N ha^{-1} lower under CT than under ZT.

TABLE III– GRAIN YIELD, RESIDUE DRY MATTER, N ACCUMULATION AND BNF CONTRIBUTION TO THE SOYBEAN CROP UNDER ZERO TILLAGE (ZT) AND CONVENTIONAL TILLAGE (CT), IN UBERLÂNDIA, MG, SUMMER 2000. MEANS OF FOUR REPLICATES

Tillage system	Grain yield	Residues[1]	Grain N	Residue N	Plant total N	BNF	N balance[2]
	--------Mg ha^{-1}-------		---------------------------kg ha^{-1}-------------------------				
CT	4.06	5.60	217.5	67.8	285.3	191.7	-25.8
ZT	3.74	5.37	199.9	65.8	265.7	202.8	2.9
t test	ns	ns	ns	ns	ns	*	**
CV(%)	35.5	20.5	32.7	14.8	30.1	-	-

[1] Except grain, all plant dry matter including recoverable roots.
[2] N derived from BNF (whole plant) minus Grain N (total N in grain).
ns, *, ** means not significantly different or significantly different at P<0.05 or P<0.01, respectively (t test).

4.2. On farm assessment of soybean BNF in the north western region of Paraná

A survey was conducted in the 2000/2001 growing season on 21 farms in the northwest of the state of Paraná, near to the cities of Londrina and Campo Mourão. Nearly all soybean crop in this area is planted under ZT system, and this was true for all the crops sampled. A questionnaire was distributed to the owners of all 21 farms with a series of questions concerning management practices of the soybean crop. Not all questions were answered but information from all farms was obtained on the number of years since the adoption of ZT, planting date of the soybean and applied rate of *Bradyrhizobium* inoculant.

For soil and plant sampling areas of approximately 1 ha, which visually presented uniform soybean stands, and had been managed in a similar manner was chosen at each farm. For plant sampling, 50 soybean plants (all shoot tissue) were sampled at the mature grain stage while walking through the rows across the whole of each 1 ha area. Samples of neighbouring non-N_2-fixing weed species were taken at the same time. These samples were oven-dried and transported to Embrapa Agrobiologia for analysis of ^{15}N natural abundance by mass spectrometry.

At all sites except one (site R) it was found possible to sample two or more different non-leguminous, non-N_2-fixing, weed species (reference plants) for stable nitrogen isotopic analysis to assess the ^{15}N abundance of the plant-available soil N (Table IV). At three sites (C, G, N) the difference between the $\delta\ ^{15}N$ values of the weed species was over 2‰, at 7 sites between 1 and 2‰ and in the remaining 10 sites less than 1‰. With the exception of three sites (H, L and U) where the mean $\delta\ ^{15}N$ values of the weed species were, respectively, +6.22, +5.21 and +4.26‰, the ^{15}N abundance of the weed species ranged from 2 to 4 ‰. In natural ecosystems where a wide range of $\delta^{15}N$ values is normally found amongst non-leguminous and non-nodulating legume plants (e.g. Pate *et al.* [4]; Gehring and Vlek [5]) it has been recommended that the ^{15}N abundance of such non-N_2-fixing reference species should be at least 5‰ greater than that of the target legume N_2-fixing species to quantify BNF inputs [6]. However, in agricultural ecosystems it has been also found that variations in δ ^{15}N between different reference plants are considerably lower [3, 7] suggesting that a difference of 2‰ between the reference and legume fixing plants was sufficient to obtain useful estimates of BNF contributions.

The criterion of a difference of at least 2‰ between the δ ^{15}N value of the N_2-fixing soybean and the mean of the non-N_2-fixing reference plants was met for all 21 sites and for 17 of the 21 sites differences between the means for the reference plants was less than 2‰. It was, therefore, considered that these data could be used to estimate BNF contributions.

At 18 of the 21 sites, the ^{15}N abundance of the soybean grain was negative indicating that the proportion of N derived from BNF (% Ndfa or % N-BNF) was high. The values estimated using a '*B*' value (the ^{15}N natural abundance of the N derived from BNF by the soybean) of -1.3‰ ranged from 60 to 99.8%. The highest estimated values, those above 90 or 95% seem unrealistically high, as there is no explanation why the soils at these sites should exhibit extreme N deficiency. This probably means that the '*B*' values used in the calculations were less negative than those experienced by the soybean in the field. This hypothesis is supported by recent data from Okito *et al.* [8], who found that the '*B*' value of the aerial tissue of soybean (variety Celeste) could range from -2.4 to -4.5‰. However, as the aim of this work was to assess the effect of different management practices and soil fertility parameters on the contribution of BNF to the soybean, the actual % N-BNF values were not essential.

Applying a multivariate statistical design to explain variability in the BNF values observed in the soybean crop in the Paraná State, it was found that the most limiting factors are related to (a) the non-inoculation or poor quantity of inoculant of *Bradyrhizobium spp.* used, (b) sowing in periods of low rainfall, and (c) sowing in the older areas under ZT. A possible explanation for ZT may be that when it is first introduced available soil N is strongly immobilized in the build-up of soil organic matter. As SOM increases with time, a new N mineralisation-immobilization equilibrium level is attained such that less N is immobilized. The observed decline in the BNF inputs may reflect an increased availability of soil N to the soybean plants.

TABLE IV. δ ^{15}N VALUES OF SOYBEAN FIXING CROP AND WEED (REFERENCE) PLANTS AND CONTRIBUTION OF BNF (%) TO THE SOYBEAN CROP GROWN IN 21 REPRESENTATIVE COMMERCIAL FARMING AREAS OF THE STATE OF PARANÁ

Site	Soybean and reference plants[1]	δ ^{15}N (‰)	N-BNF (%)
A	Soybean	-1.22	97.7
	Sida rhombifolia L.	+2.71	
	Euphorbia heterophylla L.	+1.79	
	Mean of weed plants	+2.25	
B	Soybean	+0.48	60.0
	Euphorbia heterophylla L.	+3.81	
	Commelina sp.	+2.48	
	Mean of weed plants	+3.15	
C	Soybean	-0.82	90.7
	Ipomoea sp.	+5.58	
	Commelina sp.	+2.18	
	Mean of weed plants	+3.88	
D	Soybean	-0.69	86.7
	Ipomoea sp.	+3.45	
	Bidens pilosa L.	+3.12	
	Mean of weed plants	+3.29	
E	Soybean	-1.03	92.4
	Sida rhombifolia L.	+2.72	
	Phyllanthus tenelus L.	+1.80	
	Mean of weed plants	+2.26	
F	Soybean	-1.22	97.7
	Sida rhombifolia L.	+2.42	
	Bidens pilosa L.	+2.04	
	Mean of weed plants	+2.23	
G	Soybean	-1.09	95.6
	Sida rhombifolia L.	+2.52	
	Euphorbia heterophylla L.	+2.86	
	Solanum americanum Mill	+4.97	
	Mean of weed plants	+3.45	
H	Soybean	+0.62	74.5
	Sida rhombifolia L.	+6.05	
	Solanum americanum Mill	+6.39	
	Mean of weed plants	+6.22	
I	Soybean	-1.22	97.9
	Triumfetta bartramia L.	+3.29	
	Euphorbia heterophylla L.	+2.89	
	Solanum americanum Mill	+2.26	
	Commelina sp.	+1.56	
	Mean of weed plants	+2.50	
J	Soybean	-0.91	92.0
	Ipomoea sp.	+3.39	
	Leonotis nepetifolia L.	+4.45	
	Euphorbia heterophylla L.	+2.83	
	Mean of weed plants	+3.56	

Site	Soybean and reference plants[1]	$\delta\ ^{15}N$ (‰)	N-BNF (%)
K	Soybean	-1.29	99.8
	Sida rhombifolia L.	+2.57	
	Bidens pilosa L.	+2.93	
	Mean of weed plants	+2.75	
L	Soybean	-0.80	92.3
	Senecio brasiliensis Less.	+5.61	
	Solanum americanum Mill	+4.80	
	Mean of weed plants	+5.21	
M	Soybean	-1.07	94.5
	Ipomoea sp.	+3.05	
	Euphorbia heterophylla L.	+2.66	
	Mean of weed plants	+2.86	
N	Soybean	+0.11	71.8
	Euphorbia heterophylla L.	+4.77	
	Commelina sp.	+2.62	
	Mean of weed plants	+3.70	
O	Soybean	-0.91	90.0
	Amaranthus sp.	+3.24	
	Ipomoea sp.	+2.05	
	Sida rhombifolia L.	+2.93	
	Commelina sp.	+2.16	
	Mean of weed plants	+2.60	
P	Soybean	-0.77	88.8
	Ipomoea sp.	+4.17	
	Euphorbia heterophylla L.	+2.67	
	Mean of weed plants	+3.42	
Q	Soybean	-1.12	96.1
	Leonotis nepetifolia L.	+4.16	
	Euphorbia heterophylla L.	+2.42	
	Mean of weed plants	+3.29	
R	Soybean	-0.98	90.6
	Euphorbia heterophylla L.	+2.09	
	Mean of weed plants	+2.09	
S	Soybean	-0.59	79.2
	Euphorbia heterophylla L.	+2.96	
	Commelina sp.	+1.26	
	Mean of weed plants	+2.11	
T	Soybean	-0.78	88.5
	Sida rhombifolia L.	+3.05	
	Euphorbia heterophylla L.	+3.37	
	Mean of weed plants	+3.21	
U	Soybean	-0.65	88.3
	Ipomoea sp.	+4.17	
	Commelina sp.	+4.34	
	Mean of weed plants	+4.26	

[1] Plant samples: grain for soybean and the aerial part of weed plants used as reference crop.

5. QUANTIFICATION OF BIOLOGICAL NITROGEN FIXATION (BNF) IN LEGUME GREEN-MANURES

Lupins (*Lupinus albus*) are grown as winter crop in crop rotations in the southern region of Brazil. They are usually sown in April and cut in August or September, to provide N to a following summer maize crop. As this crop is only grown as green manure, no fraction of the resulting biomass is removed from the field, so that all N in the biomass derived from BNF can be regarded as an N input to the system.

In an experiment at the Embrapa Soybean Centre (Londrina) the contribution of BNF to this legume following soybean was estimated using essentially the ^{15}N natural abundance methodology applied for soybean (Section 3 above). In this case samples of the whole shoot tissues of the lupins were taken just before cutting (at full flowering stage) and those of a neighboring oat crop were used as the non-N_2-fixing reference crop. This material was oven-dried and ground and analyzed for ^{15}N abundance by mass spectrometry. The contribution of BNF (% N-BNF) to the lupins was calculated as described above, but a '*B*' value of the shoot tissue was of -0.55‰ was employed, being the mean of values published in the literature [3].

No significant differences in the parameters shown in Table V were found for both tillage systems studied. The total dry matter yield of the lupins (mean of 10 Mg ha^{-1} of plant biomass) showed a tendency to be higher under CT than under ZT and the total N accumulation was 315 and 272 kg N ha^{-1} respectively (Table V). There was also a tendency for the proportion of N derived from BNF to be lower under CT than under ZT as it was observed for soybean, and the explanation is likely to be the same: the CT stimulated more mineralization of N from crop residues and the soil, hence causing a reduction in the BNF input. The total N in the lupin plants derived from soil N including crop residues was estimated to be 98 kg N ha^{-1} under CT, but only 69 kg N ha^{-1} under ZT.

TABLE V. DRY MATTER AND NITROGEN ACCUMULATION AND BNF ESTIMATES IN LUPIN CROPS, UNDER ZERO TILLAGE (ZT) AND CONVENTIONAL TILLAGE (CT), IN LONDRINA, PR, WINTER 2000. MEANS OF FOUR REPLICATIONS

Tillage system	D.M.[a]. Mg ha^{-1}	N-accumulated[a] kg ha^{-1}	N-BNF[b] %	N-BNF[b] kg ha^{-1}
CT	11.20 (0.95)[c]	314.8 (11.6)	68.8 (0.8)	216.5 (12.1)
ZT	9.44 (0.42)	271.5 (4.4)	74.4 (2.3)	202.5 (16.0)
t test	ns[d]	ns	ns	ns
C.V.(%)	16.4	13.6	6.1	13.0

[a] Dry matter and N accumulation, included stems, leaves and recoverable roots.
[b] BNF evaluated by δ ^{15}N technique, using oats as the reference crop and a value of 'B' of –0,55 ‰ for the lupin.
[c] Values in parentheses indicate standard errors of the means.
[d] ns – means not significantly different at $P<0.05$ (t test).

Vetch (*Vicia villosa*) is also used as a legume green manure in the soybean-based crop rotations in the southern region of Brazil, especially in the southernmost areas as it is more resistant to cold winters than lupins. In the long-term experiment at the Embrapa Wheat Centre (Passo Fundo, RS) – for full details see Sisti *et al.* [9], hairy vetch (*Vicia villosa*) was used as a winter crop in two of the three rotations following soybean and preceding maize. Whole shoot tissue of the vetch was sampled along with 5 species of non-N_2-fixing weeds and the samples of these materials were oven-dried and ground and analyzed for ^{15}N abundance. The contribution of BNF (% Ndfa) to the vetch was calculated as described above for soybean and lupin, but the value used for '*B*' of the shoot tissue was -0.76‰, the value published for *Vicia sativa* by Yoneyama *et al.* [10].

TABLE VI. $\delta^{15}N$ VALUES OF VETCH (*Vicia villosa*) AND SPONTANEOUS WEEDS IN THE LONG TERM EXPERIMENT AT PASSO FUNDO, RS (SISTI *et al.*, 2004) AND BNF ESTIMATES (%NDFA) IN THIS GREEN MANURE CROP

Crop rotation	Vetch and *reference plants*	^{15}N abundance $\delta^{15}N$ (‰)	Ndfa estimates* (%)
[a]R2	Vetch – ZT	+0.04	76a
R2	Vetch – CT	-0.25	78a
[b]R3	Vetch – ZT	-0.05	87a
R3	Vetch – CT	-0.07	78a
	Lolium multifolium L.	+2.13	
	Stachys arvensis L.	+2.05	
	Sonchus oleraceis L.	+2.38	
	Erigeron bonariensis L.	+2.32	
	Gamochaeta spicata	+2.12	
	Mean of *reference plants*	+2.20	

[a] R2– Crop rotation 2: wheat/soybean /vetch/maize.
[b] R3 – Crop rotation 3: wheat/soybean/oats/soybean/vetch/maize.
*In this column, values with the same letter are not significant at P<0.05 (Tukey's HSD test).

.The ^{15}N natural abundance values of the non-N_2-fixing reference plants were low (mean of +2.2 ‰) and all within a narrow range (Table VI). This suggests that $\delta^{15}N$ of available soil N within and between plots was reasonably uniform in time and space. Under these conditions, the % Ndfa estimates can be considered to be more reliable than those obtained when reference crops show a wide range of ^{15}N abundance [3]. In rotation 2, there were no differences in the % Ndfa of vetch between CT and ZT, but in rotation 3, it appeared that the proportion of N derived from BNF was somewhat higher under ZT than CT though not statistically different.

6. SIMPLE N BALANCE OF CROP SEQUENCES

An experiment including a 3-year crop rotation soybean/wheat - soybean/lupines - maize/oats was laid down at Londrina, PR in the summer 97/98, in plots under conventional (CT) and no-tillage (ZT) arranged in a randomized block design with 4 replicates. A simple N balance for the 3-year crop rotation under CT and ZT systems was established. As the experiment had three treatments where the rotation started in three different years, it was possible in the period of 18 months to study all of the 3 rotations simultaneously.

The mean grain yield of soybean was about 4.4 Mg ha^{-1} for the season 1998/99. The yield data obtained under both tillage systems (CT and ZT) were very similar (Table VII). This soybean grain yield is 85% higher than the Brazilian average yield for this crop and probably these data are an artifact of extrapolation from small plots. The total N accumulation by this crop was over 340 kg N ha^{-1}, most of which (>80%) was exported in the grain (Table VII). The BNF contributed 82% of the entire soybean plant N under ZT and 71% under CT. The N balance for soybean (the difference between the BNF input and the N exported in grain) was estimated as being +1 and -34 kg N ha^{-1} for ZT and CT, respectively. The higher value of BNF under ZT is attributed to the relatively high rate of N immobilization in the upper layer of the soil by the decomposition of residues of high C/N ratio of the previous oat crop [11].

TABLE VII. SOYBEAN GRAIN YIELD, TOTAL PLANT N, BNF CONTRIBUTION AND N BALANCE ESTIMATED FOR THE HARVEST 1998/1999 UNDER CONVENTIONAL AND NO TILLAGE CROPPING SYSTEMS

Cropping system	Grain yield Mg ha^{-1}		Grain N kg ha^{-1}		Plant total N[1] kg ha^{-1}		BNF %		N balance[2] kg ha^{-1}	
CT	4.38	(0.46)[3]	278.3	(26.6)	344.1	(30.0)	70.9	(4.5)	-34.3	(8.6)
ZT	4.51	(0.42)	288.6	(24.5)	351.5	(28.1)	82.4	(2.0)	1.1	(4.0)
Student LSD test	ns[4]		ns		ns		*		*	

[1] Including stems, senescent leaves and roots.
[2] Difference between grain N and total N derived from BNF.
[3] Standard error of the mean (values between brackets).
[4] ns and *, means not significantly different or significantly different at $P<0.05$, respectively (Student LSD test).

The tillage system did not significantly affect the total grain yield of soybean and wheat, except that wheat and maize yields were highly influenced by the inclusion of soybean or lupins as the preceding crop (Tables VIII and IX).

TABLE VIII. WHEAT GRAIN YIELD, GRAIN N AND N BALANCE ESTIMATED FOR THE GROWING SEASON 1998/1999 AFTER SOYBEAN OR FALLOW (OATS RESIDUES) AND N FERTILIZER USE EFFICIENCY (NFUE) UNDER CONVENTIONAL AND NO TILLAGE CROPPING SYSTEMS

Cropping system	Grain yield Mg ha^{-1}	Grain N kg ha^{-1}	N balance[1] kg ha^{-1}	NFUE[2] %
CT after soybean	2.3 a[3]	55.4 a	-15.4	49.8
ZT after soybean	2.5 a	56.7 a	-16.7	55.0
ZT after fallow	1.8 b	41.0 b	-1.0	---

[1] Difference between grain total N and N added as fertilizer (40 kg N ha^{-1}).
[2] N fertilizer use efficiency based on recovery of ^{15}N-labelled ammonium sulphate.
[3] Values with the same letter do not differ statistically at $P<0.05$ (Student LSD test).

In this study and the data presented in Tables I and II it can be observed that N outputs in grain exceeded N inputs from BNF. This suggests that substantial N outputs in grain must be derived from the mining of soil N. This nitrogen mining associated with the decrease in soil organic matter and N availability may be one of the main causes of soil degradation in many areas of the Cerrado region with soybean monoculture , especially under CT[12].

In the treatment where wheat followed soybean, its grain yield reached 2.4 Mg ha^{-1} under both ZT and CT, which was approximately 30% greater than when wheat followed a summer fallow (Table VIII). Since the N balance of soybean was almost null or negative, it could be deduced that the rapid mineralization of the soybean residues favoured wheat growth and grain yield by increasing soil N availability [13]. This stimulation of mineralization of SOM induced by the soybean crop residues could gradually decrease soil N stocks (see negative balance for the wheat crop in Table VIII) and contribute to soil degradation.

In Londrina, as in many areas of southern Brazil, the use of green manures is not as uncommon as might be supposed for a crop with no immediate economic return. The results displayed in Table V indicate that lupin produced an input of N-BNF equivalent to 216 and 202 kg N ha^{-1} for CT and ZT, respectively. These large quantities of N are enough to significantly increase the soil N content, offering good perspectives to reduce the N-fertilizer inputs without yield decline of subsequent cereal crops. However, they also represent a high risk for surface and groundwater and atmosphere contamination due to their great potential for leaching through the soil profile and gaseous losses.

During the cropping period 1998/99 the N-fertilizer use efficiency (NFUE) was estimated based on the recovery of 40 and 80 kg N ha^{-1} applied as ^{15}N labeled ammonium sulphate (2 atom $\%^{15}N$ excess) to the wheat and maize crops respectively in Londrina (Tables VIII and IX). The NFUE values of wheat (Table VIII) were very similar to those of maize (Table IX), and in both cases higher values were recorded under ZT. The finding that NFUE was higher under no-tillage may confirm the hypothesis that CT stimulated SOM mineralization, resulting also in higher grain yields and grain N. The maize crop was grown following a lupin used as green manure under CT and ZT systems. The maize grain yield under ZT of 9.3 Mg ha^{-1} was significantly higher than under CT (7.8 Mg ha^{-1}) (Table IX). These high yields reflect the positive influence of the previous green manure crop in improving the soil N status. Under these conditions even though the green manure contributed with an input of over 200 kg N ha^{-1} (Table V) to the soil/plant system, the maize crop still recovered a large proportion (49 to 58 %) of the applied N-fertilizer (80 kg N ha^{-1}). The NFUE of maize under the two tillage systems was not statistically different, but the results clearly indicate that the ZT system presented a strong tendency to improve the N-fertilizer recovery of this crop.

TABLE IX. MAIZE GRAIN YIELD, GRAIN N, N BALANCE AND N FERTILIZER USE EFFICIENCY (NFUE) ESTIMATED FOR THE HARVEST OF 1998/1999 WITH LUPINS AS A PRECEDING CROP UNDER CONVENTIONAL AND NO TILLAGE CROPPING SYSTEMS

Cropping system	Grain yield Mg ha^{-1}		Grain N kg ha^{-1}		N balance[1] kg ha^{-1}	NFUE[2] %
CT	7.8	(0.2)[3]	108.7	(3.2)	28.7	49.3
ZT	9.3	(0.3)	126.3	(6.4)	46.3	58.1
Student LSD test[4]	**		*		---	-----

[1] Difference between grain total N and N added as fertilizer (80 kg N ha^{-1})
[2] N fertilizer use efficiency based on recovery of ^{15}N-labelled ammonium sulphate
[3] Values in parentheses are standards errors of the means
[4] *, ** means statistically different at $P<0.05$ or $P<0.01$ respectively (Student LSD test)

In the 2000/2001 season maize was grown in the plots containing the residues of oats and lupins cropped as preceding green manure during the winter of 2000. Maize grain yield under ZT and CT reached 7.1 and 7.9 Mg ha^{-1}, respectively, when maize was planted after lupins with no N fertilizer addition, and 5.9 and 7.5 Mg ha^{-1} after oats where maize received 80 kg N ha^{-1} (Table X).

TABLE X. MAIZE YIELD OF THE HARVEST 2000/2001 UNDER CONVENTIONAL AND NO TILLAGE CROPPING SYSTEMS WITH OATS OR LUPINS AS A PRECEDING GREEN MANURE

Cropping system	Grain yield Mg ha^{-1}
Maize CT after oats[1]	7.5 ab[2]
Maize ZT after oats[1]	5.9 c
Maize CT after lupins [3]	7.9 a
Maize ZT after lupins[3]	7.1 b

[1] Oats residues (106 kg N ha^{-1}) plus 80 kg N ha^{-1} as N fertilizer equals 186 kg N ha^{-1}
[2] Means followed by the same letter do not differ according Student LSD test ($P<0.05$)
[3] Lupin residues only equal to 293 kg N ha^{-1}

Under ZT maize after oats, soil and fertilizer N were immobilized with the oat residues [11], thus resulting in a lower maize yield. However, this yield depression was apparently eliminated by stimulation of soil N mineralization under CT, where maize yield was the highest. Maize yield after lupins only (without N fertilizer) under CT equaled that obtained in maize under CT after oats plus 80 kg N ha^{-1}, being a little lower under ZT after lupins. These data on the N gains that can be obtained using lupins suggested that this crop has good potential for inclusion in crop rotations, especially if the N fertilizer application can be reduced or eliminated, for the summer crop.

The results of the nitrogen balance (kg N ha^{-1}) in three crop sequences (soybean-wheat-soybean; soybean-lupin-maize; maize-oats-soybean) established under conventional or no tillage systems are shown in Fig. 1. These data clearly show that the introduction of a legume cover/green manure crop will guarantee the maintenance of soil N reserves and the sustainability of crop production. Under CT, although the contribution of BNF to soybean was high (190 to 315 kg N ha^{-1}), more N was also removed in the grain resulting in a negative balance. Under ZT the N balance was occasionally slightly positive. In the case of lupin, as no N was removed from the system, there was a large N contribution to the subsequent maize crop, increasing the maize grain yield to almost 10 Mg ha^{-1} (containing 150 kg N ha^{-1}), although only 80 kg N ha^{-1} of N fertilizer was applied. The ZT system favoured the immobilization of soil N and a higher BNF contribution to the legumes.

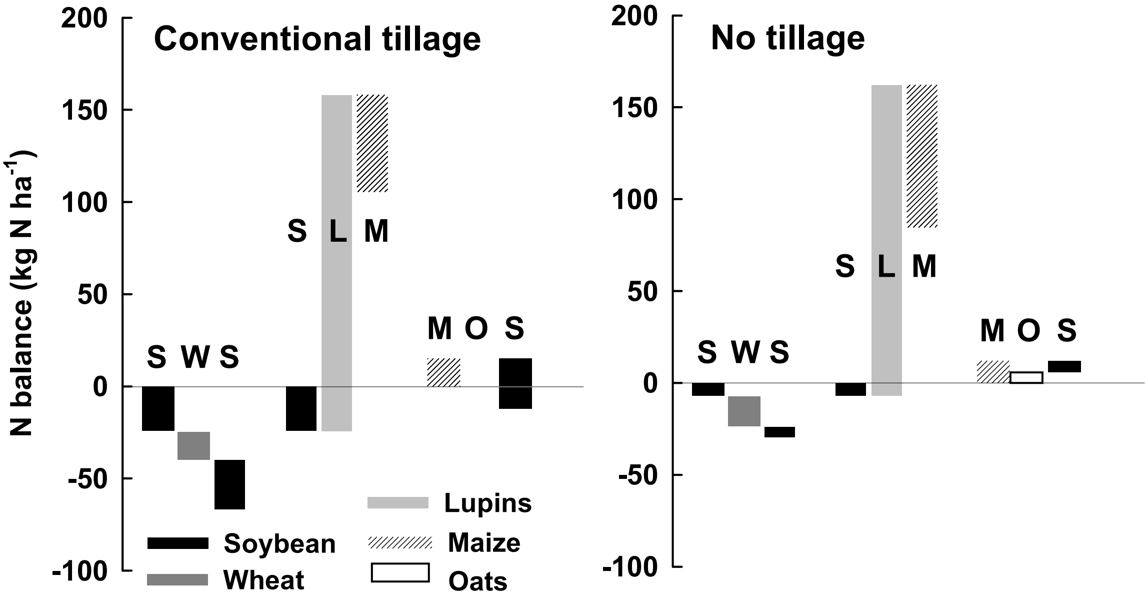

FIG. 1. Nitrogen balance (kg N ha^{-1}) in three crop sequences (soybean-wheat-soybean; soybean-lupin-maize; maize-oats-soybean) established under conventional or no tillage systems.

7. QUANTIFICATION OF RESIDUAL BELOW-GROUND N OF SOYBEAN

Over the last few years, Australian research workers have developed a ^{15}N isotopic technique to estimate the quantity of N, which remains in the soil after the harvest of a legume [14, 15]. It is evident that in many soils, especially those of high clay content and with strongly bound aggregates, that recovery of 100% of the root systems by washing and sieving the soil is not feasible. Furthermore, it has been suggested that considerable N may be exuded into the soil during crop growth and that the N in senescent roots and nodules may also be a significant source of N left in the soil after the grain harvest [15, 16]. In order to estimate this non-recoverable below-ground N (NRBGN), the aerial tissue of the legume plant (stem or leaf) is fed with an enriched ^{15}N-labelled N source (in our study, as in most others, ^{15}N-labelled urea) so that the whole plant becomes labeled with ^{15}N. After some weeks the plants are uprooted and both the roots and soil are analyzed for total N and ^{15}N enrichment. It is assumed that the N deposited in the soil has the same ^{15}N enrichment as the N in the roots, and hence knowing the ^{15}N enrichment and the total N of the soil it is possible to calculate the total quantity of N in the soil derived from the legume roots. A series of experiments was carried out to evaluate the below-ground N input of soybean under field conditions in several locations.

The first study was conducted at the field station of Embrapa Agrobiologia, RJ. Soybean plants (variety Celeste) were grown in open plastic cylinders (2 plants per cylinder, 2 replicate cylinders in each of 4 blocks) of 25 cm diameter and 60 cm length sunk into the soil so that the cylinder top was flush with the soil surface. The central vein of one leaf of each plant was isolated by cutting from the surrounding leaf tissue and the tip immersed in a 1.5 ml plastic Eppendorf tube as described McNeill *et al.* [15] containing a solution of ^{15}N-labelled urea (5 g L^{-1}, 71.3 atom % ^{15}N). The tube was sealed with Parafilm® and left for 3 days.

The harvest for below ground N determination was made at 120 days after emergence (DAE). From one cylinder in each block, all plant aerial tissue was harvested, oven-dried and ground for the analysis of total N and ^{15}N enrichment. The cylinders were removed from the soil cut into three lengths of 20 cm, and the soil carefully removed, washed through a 2 mm sieve to recover as many roots as possible (denominated recoverable roots). All roots were dried and ground for subsequent analysis of total N and ^{15}N enrichment. The bulk soil in each part of the cylinder was thoroughly mixed; sub-samples were dried, finely ground and also analyzed for total N and ^{15}N enrichment.

With the traditional method of manual separation of roots it was found that 51 mg N was present in the recoverable roots, which represented 3.6% of all plant N (Table XI). The estimate of the quantity of N present in the soil derived from the plants (NRBGN) was 15.5% of the total N accumulated in the whole plant resulting that 19.1% of whole plant N was to be found in the soil.

TABLE XI. ^{15}N ENRICHMENT AND RECOVERY OF N IN THE SOIL DERIVED FROM THE LABELLED SOYBEAN PLANTS HARVESTED AT THE LATE GRAIN FILLING STAGE (120 DAE). EXPERIMENT PERFORMED AT EMBRAPA-AGROBIOLOGIA, RJ

Plant tissue	^{15}N enrichment (atom % ^{15}N excess)[a]	Plant N	
		mg/2 plants	%
Grain	0.28 ± 0.02	926.9	65.6
Stems	0.41 ± 0.01	225.0	15.9
Leaves	0.54 ± 0.08		
Roots (depth – cm)			
0 – 20	0.59 ± 0.07	51.0	3.6
20 – 40	0.59 ± 0.08		
40 – 60	0.61 ± 0.10		
Soil (depth – cm)			
0 – 20	18.83 + 3.9	220.7	15.6
20 – 40	15.7 ± 1.9		
40 – 60	7.28 ± 1.3		
Total		1413.5	100.0

[a] Values are means of four replicates ± standard errors of the mean.

A further study was conducted in the field at the Embrapa Soybean Centre (Londrina, PR) using exactly the same methodology with soybean variety BR 43 but two harvests were made to evaluate NRBGN at 80 and 120 days after emergence (DAE) (Table XII). With the traditional method of manual root separation it was found that the quantity of N in the root system plus the recoverable nodules at 80 DAE, represented 8% of the total N accumulated in the whole plant. This proportion decreased at the final harvest (120 DAE), where only 2% of the total N accumulated by the plant was found in the roots. This could be explained by the large remobilization within plant parts, in particular increase of the total N accumulated in the grain and senescence and turnover of roots and nodules when approaching maturity.

TABLE XII. ESTIMATION OF THE BELOW-GROUND N OF SOYBEAN PLANTS BY THE TRADITIONAL MANUAL SEPARATION OF ROOTS AND THE ^{15}N LEAF LABELING TECHNIQUES, AT 80 DAYS AFTER EMERGENCE (DAE) AND AT HARVESTING STAGE (120 DAE), UNDER FIELD CONDITIONS, AT EMBRAPA-LONDRINA, PR

Part of the plant	80 DAE			120 DAE		
	Dry matter	Total N	N distribution	Dry matter	Total N	N distribution
	mg/plant		%	mg/plant		%
A. Traditional method						
Shoots	20	372	41	13	255	23
Pods	13	456	51	16	852	75
Aerial part	33	828	92	29	1107	98
Roots	5	34	4	2.3	17	1.5
Nodules	2	40	4	0.2	8	0.7
Roots + nodules	7	74	8	2.5	25	2
Whole plant		902	100		1132	100
B. Isotopic method						
Aerial part	32.9	828	53	29.3	1107	77
Roots + nodules	7.3	74	5	2.5	25	2
Unrecovered roots	-	647	42		313	21
Below-ground N	-	721	47		338	23
Whole plant		1549	100		1445	100

From the soil ^{15}N enrichment data, it was estimated that there was 647 mg N/cylinder derived from the soybean plants at 80 DAE but this decreased to 313 mg N at 120 DAE. This would suggest that there is a serious problem with this technique, at least in this study in that there was a large loading of plant-derived ^{15}N-enriched N soon after leaf labeling and with time much of this enriched N was lost from the system. This is not the expected scenario; because the plant derived N would have been gradually released into the soil with time via root exudation/senescence and at the final harvest there would be more non-recoverable roots than at earlier growth stages. The estimate of the proportion of the N in residual below-ground N at 120 DAE was similar to that of the previous study at Embrapa-Agrobiologia (23% compared to 19%). This inexplicable behavior on the accumulation of plant derived ^{15}N-labelled N in the soil with time deserved further investigation and hence another experiment was performed under greenhouse conditions at Embrapa- Agrobiologia

For this study, soybean plants (variety Celeste) were grown in pots containing 4 kg of soil (1 plant per pot). The experiment consisted of three treatments (harvests after 13, 44 and 74 days after leaf labeling) and each treatment had four replicates arranged in a complete randomized block design. Leaf labeling with a solution of ^{15}N-labelled urea (5 g L^{-1}, 71.3 atom % ^{15}N) was conducted exactly as described above at 27 days after emergence (DAE). At each harvest the whole shoot tissue was harvested and the soil was sieved through a 2 mm sieve to extract all visible roots. The roots were divided into primary, secondary and fine roots. The soil was thoroughly mixed, divided into two equal portions, one of which was used to determine dry weight, and the other washed sequentially through sieves of 1.0, 0.5 and 0.125 mm to further separate remaining fine roots. All plant and soil samples were dried, weighed, ground and analyzed for total N and ^{15}N enrichment.

At the first harvest (40 DAE, 13 days after labeling – DAL), a small sample of fine roots was taken from all plants in the experiment to investigate the uniformity of the leaf labeling process between replicate plants. The mean ^{15}N enrichment was 0.5650% with a standard error of 0.0750% indicating that the labeling process was very reproducible between plants.

The ^{15}N enrichment of the shoot tissue declined from 1.23 to 0.23 atom % ^{15}N excess in the period from 13 to 74 days after leaf labeling. As the quantity of excess ^{15}N added was fixed but the plant was continuing to accumulate N from the unlabelled soil and possibly from the atmosphere (via fixation), this result was expected (Table XIII). The same pattern was observed for the roots, but consistently the ^{15}N enrichment decreased in the order: primary, secondary, fine roots and these differences were statistically significant ($P<0.05$) at 44 and 74 DAL. Thus not only the ^{15}N enrichment of the roots varies with root size but also with time. This would mean that choosing a single ^{15}N enrichment to represent the ^{15}N enriched N, which was deposited in the soil for all times, becomes a virtually impossible task.

TABLE XIII. ^{15}N ENRICHMENT OF SHOOT TISSUE; PRIMARY, SECONDARY AND FINE ROOTS AND SOIL (atom % ^{15}N excess) TAKEN FROM SOYBEAN PLANTS LABELLED WITH ^{15}N ENRICHED UREA AT 13, 44 AND 74 DAYS AFTER LABELLING (DAL)

	Days after leaf labelling		
	13 DAL (V_6)	44 DAL (R_4)	74 DAL (R_6)
Shoot tissue	1.2306	0.4416	0.2281
Primary roots	0.7514 ns	0.4654 a	0.3117 a
Secondary roots	0.5879	0.3534 ab	0.2184 ab
Fine roots	0.4697	0.2784 b	0.1764 b
Coef. of variation (%)	17	7	10
Soil	0.0050	0.0040	0.0033

Differences between ^{15}N enrichment of different root classes.
ns = not significant different at P< 0.05 (Tukey's HSD test).
Means of 15N enrichment of different root classes followed by the same letter in the same column are not significant different at P< 0.05 (Tukey's HSD test).

Khan *et al.* [16] discussed the differences in ^{15}N enrichment between different root size classes, but these authors generally found lower ^{15}N enrichment in primary roots where the majority of the N_2-fixing nodules are found. In this study, however, for reasons, which we do not understand the soybean plants did not nodulate so the differences in ^{15}N enrichment across root size classes were not related to inputs of unlabelled N derived from BNF. The ^{15}N enrichments found in the soil were rather low. For the calculation of non-recoverable below-ground N (NRBGN) at each harvest, the weighted mean ^{15}N enrichment of all roots collected at that harvest was used.

The N accumulated in the (recoverable) primary and secondary roots increased considerably from 13 to 44 DAL and then almost stabilized until 74 DAL (Table XIV). However, the recoverable fine roots increased by more than 7-fold between 13 and 44 DAL, but then decreased by 31% (almost 8 mg/plant) until 74 DAL. Hence if these roots senesced or were subsequently more difficult to remove from the soil, this N should be recovered in the non-recoverable root N. However, the NRBGN was approximately stable at between 14 and 15 mg N/plant, which shows that the technique did not detect this N.

TABLE XIV. NITROGEN ACCUMULATED (mg/plant) BY SOYBEAN SHOOT AND ROOTS AND ESTIMATES OF THE NRBGN COMPUTED BASED ON THE WEIGHTED MEAN ^{15}N ENRICHMENT OF THE THREE DIFFERENT ROOT SIZE CLASSES

	Days after leaf labelling		
	13 DAL (V_6)	44 DAL (R_4)	74 DAL (R_6)
Shoot tissue	85.67 (7.43)	164.80 (5.46)	170.60 (12.1)
Primary roots	2.20 (0.27)	3.18 (0.31)	3.28 (0.13)
Secondary roots	4.29 (0.44)	6.30 (0.60)	6.98 (1.31)
Fine roots	3.43 (0.41)	25.05 (2.56)	17.11 (2.94)
Soil	15.04 (3.5)	14.05 (3.41)	14.15 (3.18)

Values are means of four replicates.
Values in parentheses indicate standard errors of the means.

It can be inferred from this study, as well as the field experiment conducted at the Embrapa Soybean Centre (Londrina, PR), that more investigations are necessary before this technique can be recommended to estimate the residual N left in the soil by soybean or other legumes. From these preliminary studies it may be concluded that the traditional physical separation of roots underestimates the underground N of this crop, while it is increasingly apparent that the isotopic method may be overestimating NRBGN. However, it is still too early to conclude, as Khan *et al.* [16] did, that we must radically revise our existing N budgets to allow for large quantities of residual below-ground N.

8. INFLUENCE OF N INPUTS FROM A LEGUMINOUS GREEN-MANURE CROP ON SOIL CARBON STOCKS UNDER A LONG-TERM CROP ROTATION EXPERIMENT MANAGED UNDER ZERO OR CONVENTIONAL TILLAGE

This long-term study was installed at the Embrapa Wheat Centre (Passo Fundo, RS) in 1986. In November of 1985 the soil was ploughed and limed with 7 Mg ha^{-1} of dolomitic lime and during this study no further lime was added. The experiment had 4 tillage treatments: (1) No tillage (ZT)), (2) Minimum cultivation, (3) Conventional tillage (CT)- disc plough and (4) Conventional tillage (CT)-mouldboard plough, and three crop rotations of 1, 2 and 3 years duration respectively. These rotations were R1) wheat/soybean (W/S), R2) wheat/soybean – vetch (*Vicia villosa*)/maize (W/S-V/M), and R3) wheat/soybean – white oats (*Avena sativa*)/soybean – vetch/maize (W/S-O/S-V/M). The wheat, vetch and oats were planted in the winter and the soybean and maize were summer crops. In the rotations containing maize (R2 and R3), for just one year (1996 for R2 and 1995 for R3), maize was substituted by sorghum owing to theft of the previous year's maize cobs. The experiment was arranged in a randomized complete block design with three replicates, tillage treatments were in main plots and rotations in subplots of 10 × 4 m.

From 1987 onwards all summer crops of this experiment were direct drilled into the shoot residues remaining from the preceding winter crop, and ploughing before planting was only practiced for the winter crops in the CT treatments. Soil samples were taken from all three replicates of all three rotations under ZT, and from CT with a disc plough. The soil was sampled at the end of 1999 by composite samples consisting of 6 sub-samples that were taken at depths of 0–5, 5–10, 10–15, 15–20, 20–30, 30–40, 40–55, 55–70, 70–85 and 85–100 cm in each sub-plot and from three areas (6 sub-samples per area) separated by approximately 30 m in a neighbouring area of native forest vegetation (Araucaria woodland). Bulk density measurements of the soil were made in each sub-plot at each depth interval sampled using a beveled ring of known volume (145 cm^3), and the soil within was removed and weighed after drying at 110°C for 72h.

Soil samples were air dried, passed through a 2 mm sieve, thoroughly homogenized, and sub-samples taken for analyses of total N and C, and the stable ^{13}C isotopic abundance. Further sub-samples were ground to a fine powder (<0.15 mm) using a roller mill similar to that described by Smith and Myung [17]. Total N content was determined using semi-micro Kjeldahl digestion as described by Urquiaga *et al.* [18]. Total C analysis was performed using a total C analyser (LECO model CHN 600). The ^{13}C isotopic abundance of the soil samples was determined using a continuous-flow isotope-ratio Finnigan DeltaPlus mass spectrometer coupled to a Carlo Erba EA 1108 total C and N analyser.

Stocks of organic C in the soil under the three rotations of both tillage systems (ZT and CT) to depths of 30 cm and 100 cm are displayed in Fig. 2. It was observed that significant quantities of organic C accumulated in the soil profile with great differences among crop rotations and tillage systems. It is clear that under ZT the soil below 30 cm depth accumulated higher quantities of organic C than under CT. These results were confirmed by the data (not shown) on total N content. This greater accumulation of soil organic matter (SOM) at depth under ZT means that if sampling is restricted only to the 20 or 30 cm depth (the maximum depth affected by ploughing under CT), the gain in C and N stocks by the adoption of ZT compared to CT would be grossly underestimated. When C and N stocks were calculated to a depth of 30 cm, it was apparent that there was no significant difference in the quantity of SOM under ZT and CT in R1 (continuous wheat/soybean – Fig. 2A), but there were significantly greater C and N stocks in the soil under ZT compared to CT under the other two rotations (R2 and R3), amounting to differences of 5.3 and 9.1 Mg C ha^{-1} and 0.31 and 1.38 Mg N ha^{-1}, for R2 and R3, respectively. Because of the significantly higher C and N contents under ZT of the soil at depths greater than 30 cm, the differences in C and N stocks between ZT and CT calculated to 100 cm depth were even greater, amounting to 17 and 16 Mg C ha^{-1} and 1.1 and 2.0 Mg N ha^{-1}, for R2 and R3, respectively (Fig. 2B).

Recently, samples of soybean and vetch were taken from these plots in order to quantify the contribution of BNF from these legumes. The preliminary data confirm the results of the studies performed at the Embrapa Soybean Centre (Londrina, PR – Table II) and at the Fazenda Floresta do Lobo (Uberlândia, MG – Table III) that the quantity of N exported from the field in soybean grain was equal to or greater than the BNF input from the soybean crop. This indicates that in the continuous wheat/soybean (Rotation 1) the N balance for the system was either zero or somewhat negative. However, in the Rotation 2 and 3 where hairy vetch was included every two or three years, respectively, the N balance for the system would have been positive, the estimates of the BNF input being approximately 127 kg N ha^{-1} for each vetch crop. The estimated proportion of N derived from BNF by the vetch at this site are displayed in Table VI above, and the total N accumulation of this crop (160 kg N ha^{-1} per crop) was taken from the estimates made for previous years by Sisti *et al.*, 2004 [9].

The above results suggest that SOM accumulation (C sequestration) requires not only C but also N inputs. This would explain why there were no significant differences in C stocks under the soybean/wheat rotation (N fertilizer inputs to wheat were only ~40 kg N ha^{-1} yr^{-1} which is approximately equal to the N exported in grain) under either tillage system. The main difference between R1 and the other 2 rotations was that in the latter vetch, a leguminous green manure (all residues left in the field) was included. Thus it may be concluded that the inclusion of the vetch as green manure under ZT was a key factor for the large increase in soil C compared to either the soil under R1, or to R2 and R3 under CT. It would appear that under CT, N_2 fixation inputs were lower and/or most of the fixed N was lost as mineral N by leaching (NO_3^-) or in a gaseous form (NH_3, N_2O, N_2) when the ploughing stimulated N mineralization.

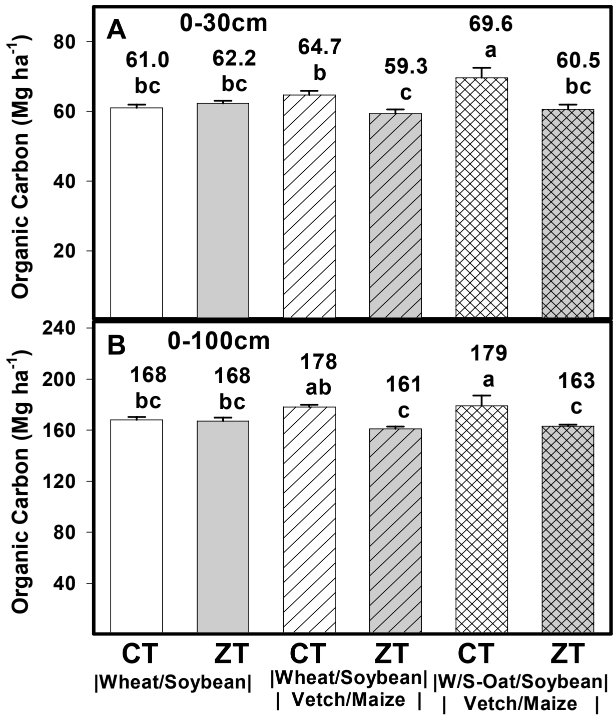

FIG.2. Stock of organic C in soil under the three rotations of both tillage systems (ZT and CT) A to a depth of 30 cm and B to a depth of 100 cm. Soil C stocks were corrected for differences in soil bulk density. Values are means for 3 replicates. Values above columns are total C contents in Mg C ha-1 and the same letter indicates that the C content was not significantly different at P<0.05 (Student LSD test).

9. ESTIMATES OF LOSSES OF N FROM SOYBEAN-BASED CROP ROTATIONS MANAGED UNDER ZERO OR CONVENTIONAL TILLAGE

9.1. Ammonia volatilization from a maize crop under zero or conventional tillage

This study was conducted in the plots of the long-term crop rotation experiment established at the Embrapa Soybean Centre (Londrina, PR). The treatments chosen were maize preceded by either lupins or oats under both zero and conventional tillage (ZT and CT). In the latter case the maize crop was fertilized with 80 kg N ha^{-1} as urea, in a two-split of 40 kg at planting and 40 kg at full leaf cover. The second split was added at full leaf cover as ^{15}N labeled urea (3.0 atom % ^{15}N excess) and broadcast over the soil surface (or trash layer in the case of ZT) or incorporated at a depth of 5 cm between the rows.

Ammonia volatilization was evaluated using open tubes made from 2 litre plastic beverage bottles equipped with filter paper moistened with sulphuric acid (0.05%) to trap the ammonia as described by Marsola *et al.* [19]. The filter papers were changed every day and the ammonia volatilization was monitored for 14 days after the application of the ^{15}N-labelled urea. Ammonia was recovered from the filter paper strips by direct steam distillation and after titration the sample was acidified and dried for analysis of ^{15}N enrichment.

The maize yields after the lupin crop were somewhat higher than those of maize after oats and fertilized with 80 kg N ha^{-1} as urea, and this difference was significant under zero tillage (Fig. 3). The CT tillage system did significantly increase the grain yield of maize, especially after the oat crop, probably due to enhanced soil N mineralization.

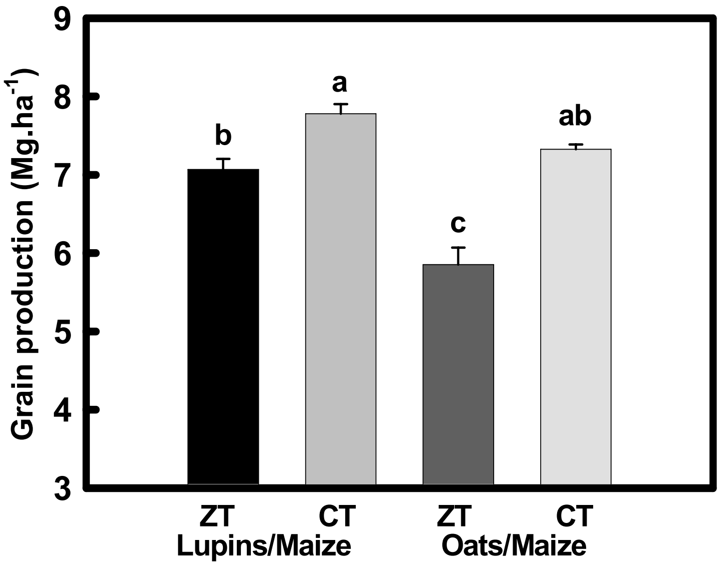

FIG.3. Grain dry matter (Mg ha^{-1}) of maize planted under conventional and zero-tillage after oats and lupines (different letters above the data bars means differences at $P < 0.01$ using t test). Maize after oats was fertilized with 80 kg N ha^{-1} as urea enriched with 3 atom % ^{15}N excess.

Ammonia volatilization was found to occur when the ^{15}N-labeled urea was broadcast over the surface of the soil and especially when applied over the residues under ZT (Fig. 4). Integrating the data of total ammonia volatilization rate over a 14 day periodafter urea application it was found that a maximum of 15 kg N-NH_3 ha^{-1} was lost in this period under ZT, with a maximum of 4 kg N ha^{-1} derived from the surface applied urea. This loss represented less than 10% of the N-fertilization rate applied to the maize crop (Fig. 5).

Results of this study confirm data reported in the literature that the greatest N losses via ammonia volatilization occur when N fertilizer was applied broadcast on the soil surface. When the N-fertilizer was buried or incorporated in the surface 5 cm layer of the soil, the N losses were insignificant. Significant quantities of N derived from the soil (7 to 12 kg N ha^{-1}) were lost from the system as N-NH_3, apparently promoted by the application of urea especially under ZT. This phenomenon needs more investigation.

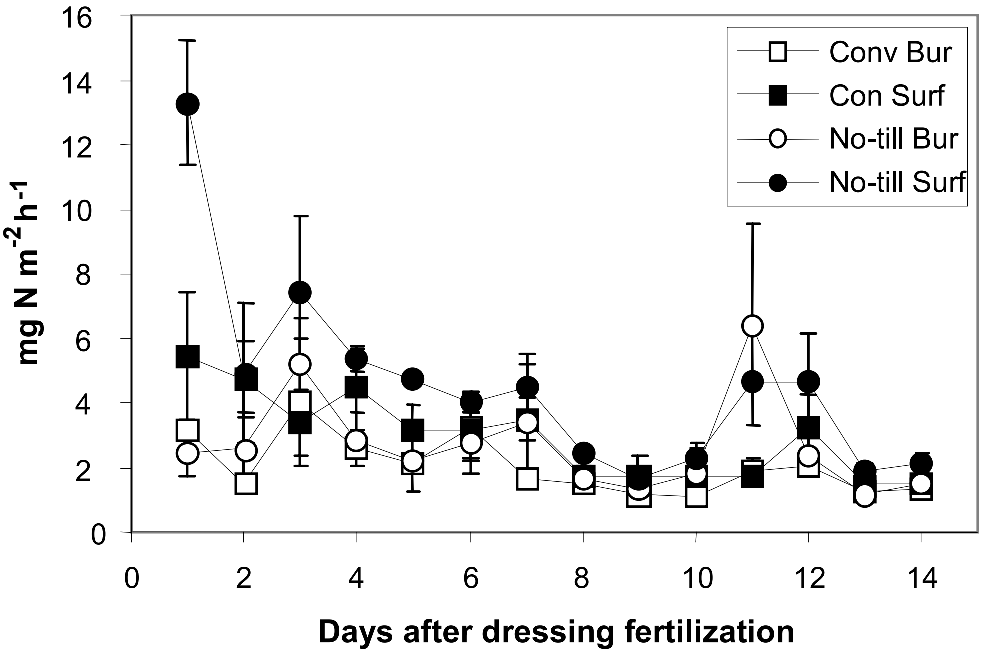

FIG.4. [15]N-Ammonia volatilization rate (mg N m^{-2} h^{-1}) from the soil surface during 14 days after fertilization of 40 kg N ha^{-1} as urea labeled with 3 atom % ^{15}N excess applied to the soil surface (Surf) or buried (Bur) to a maize crop under ZT (No till) and CT (Conv.).

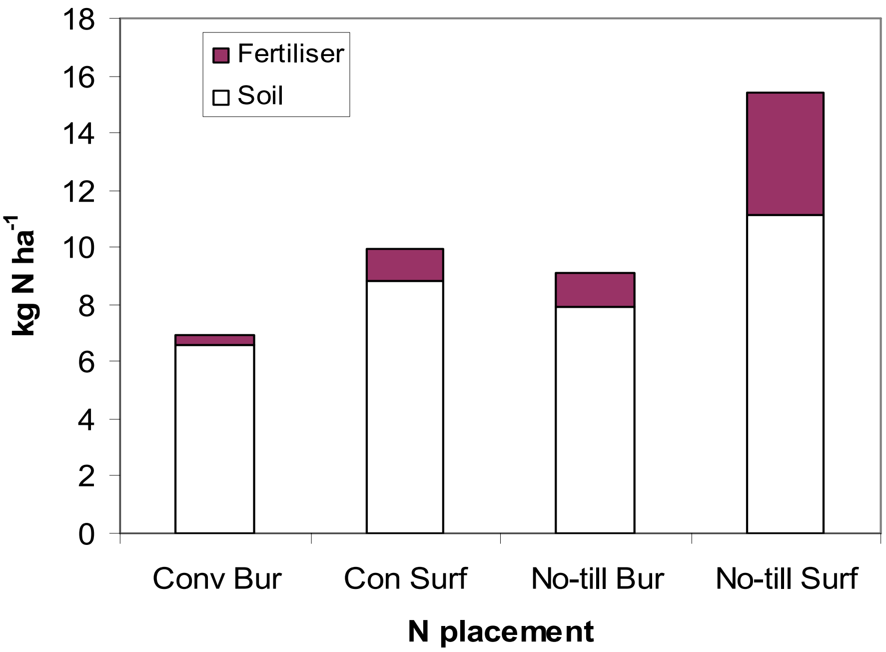

FIG.5. Total N lost via ammonia volatilization derived from the soil and fertilizer,over a 14- day period after fertilization of 40 kg N ha^{-1} as urea labeled with 3 atom % ^{15}N excess applied to the soil surface (Surf) or buried (Bur) to a maize crop under ZT (no till) and CT (Conv.).

9.2. N_2O and CH_4 emissions

Studies to quantify the emission of the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) from the soil were carried out at the experimental area of the Embrapa Wheat

Research Centre (Passo Fundo, RS) from November 2002 until September 2003. On a long term experiment, which had been running for 16 years (Section 8 of this manuscript), this study was set up to evaluate the influence of the tillage system ZT (used at planting for both the winter and summer crops) and CT (used only for the winter crop) and three crop rotations (R1-Wheat/soybean; R2A-Wheat/Soybean/Vetch/Maize; R2B-Vetch/Maize/Wheat/Soybean) were evaluated. The rotations R2A and R2B are in practice the same but the cropping system began with a different crop in the sequence.

The fluxes of N_2O and CH_4 from the soil surface were evaluated through the use of static chambers consisting of two parts; a base of 25 cm diameter and 6 cm high was inserted into the soil to a depth of 5 cm and remained installed during the whole period. Two chambers in each experimental plot and six in the native forest (control treatment) near the experimental area were laid down. The chambers were set up in the field at the end of November 2002, when this study started, immediately after seeding the summer crops. In this case there was no application of N-fertilizer. During the first week the chambers were sampled daily and weekly thereafter.

Air samples were always taken in the morning period. The soil water content and the air temperature were evaluated daily. The soil porosity occupied by water (water-filled pore space, WFPS) was also calculated. Air samples (12 ml) from each chamber were taken through the rubber septum Suba-Seal® using a syringe of 20 ml. The air samples were stored in pre-evacuated flasks. The air samples from all chambers were taken at zero and 60 min after placing the superior part of each chamber in its respective base. The air samples were analyzed for N_2O and CH_4 using two gas chromatographs (Perkin Elmer-Autosystem XL), each equipped with an electron capture detector (ECD) and a flame ionization detector (FID) respectively.

The most relevant results of this study were: The highest values of N_2O emission occurred during the first 10 days after sowing reaching a maximum of 154 μg N m^{-2} h^{-1} in the treatment R1 under CT (CTR1) (Figs 6 and 7). In the treatments under ZT, crop rotations R1 (ZTR1) and R2A (ZTR2A) showed similar N_2O emissions during the whole evaluation period, being that after the sowing time (Fig. 6) the fluxes of November/December 2002 were very variable with means of approximately of 60 μg N m^{-2} h^{-1}, which is equivalent to a total of 144 g N-N_2O ha^{-1} in 10 days. During this period, under ZT the lower values were observed under the crop rotation R2B varying around 20 μg N m^{-2} h^{-1} similar to the reference treatment (native vegetation). During the following period, December 2002/January 2003 (Fig. 7) the N_2O emission values were significantly lower, being approximately 8 μg N m^{-2} h^{-1}, similar to those recorded from the soil under native vegetation.

Under the CT system (tillage only in the winter), the treatments of crop rotation R1 (CTR1) and rotation R2B (CTR2B), were those that emitted more N_2O after sowing. Although their fluxes were very variable, the average values of both treatments were similar, varying closely around 80 μg N m^{-2} h^{-1}, which was equivalent to a total of 192 g N-N_2O ha^{-1} in 10 days, or 48 g N-N_2O ha^{-1} higher than the treatments under permanent ZT. During this period it was also observed that the lower values of N_2O emission corresponded to the crop rotation R2A (CTR2A) showing values similar to that of the control soil with values varying around 10 μg N m^{-2} h^{-1}, equivalent to 24 g N-N_2O ha^{-1} in 10 days. During the following

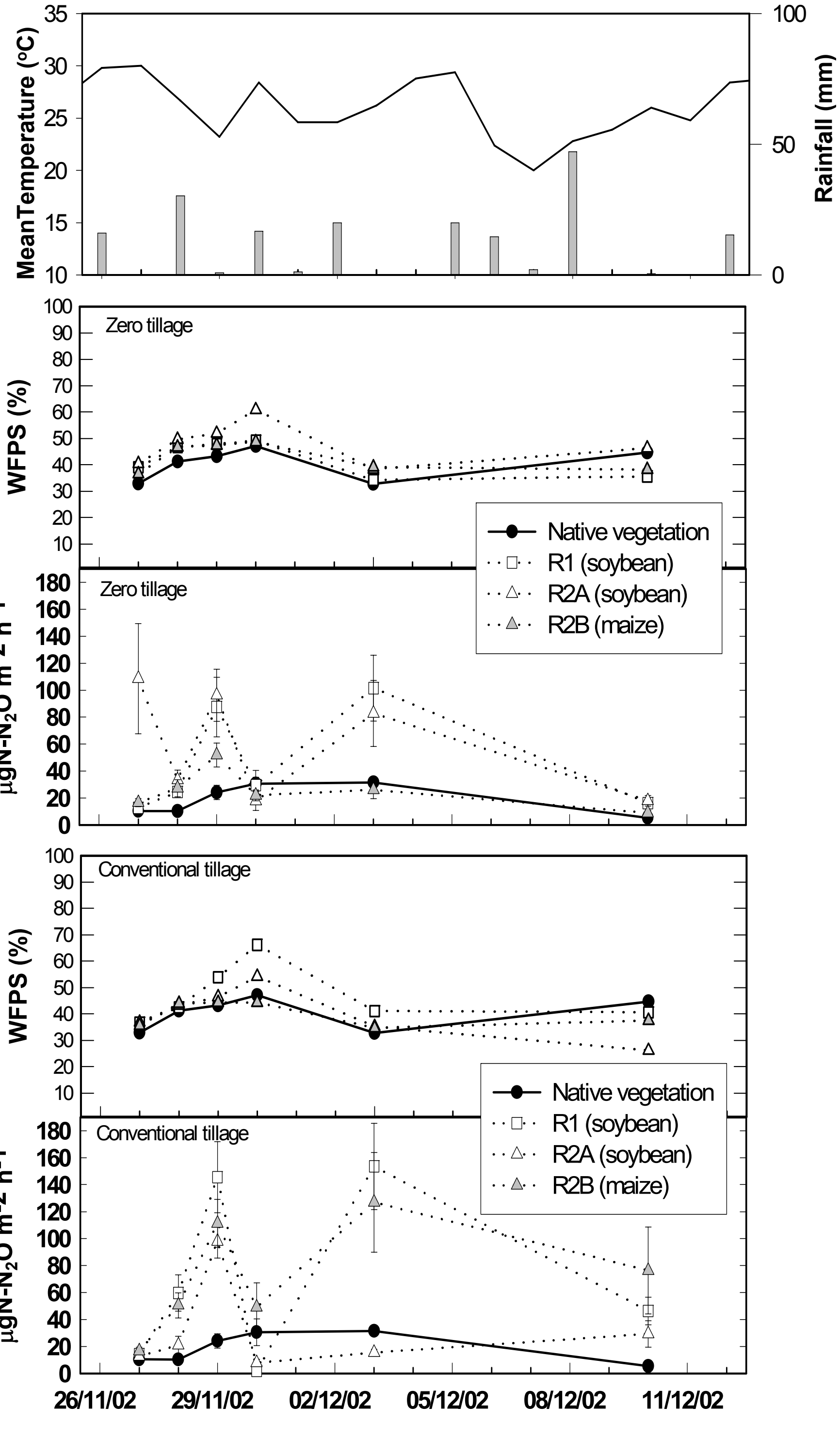

FIG.6. Emissions of N_2O from the soil surface of an Oxisol in a long term experiment under different crop rotations and tillage systems at Passo Fundo, RS. Period A-November to December 2002.

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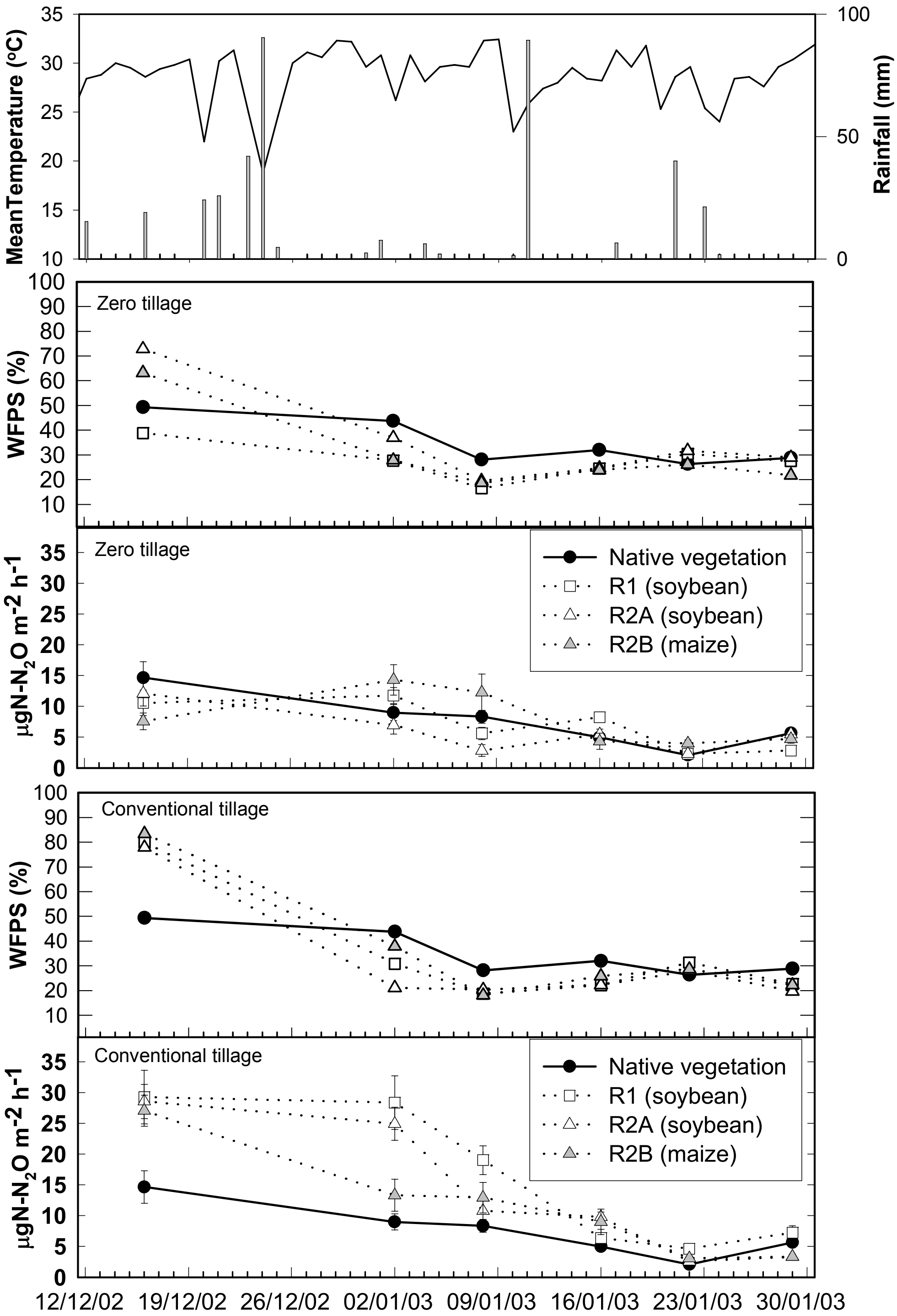

FIG.7. Emissions of N_2O from the soil surface of an Oxisol in a long term experiment under different crop rotations and tillage systems at Passo Fundo, RS.Period B-December 2002 to January 2003.

period of December 2002 to January 2003, under both ZT and CT the emission of N_2O from all treatments decreased significantly until the level was similar to that from the reference soil under native vegetation (around 8 μg N m^{-2} h^{-1}).

Emissions during the winter period of 2003 (May to September, 2003) were low being only marginally higher than from the native vegetation and with little differences between crops (Fig. 8).

Integrating all the N_2O emission data of the summer and winter periods for the agricultural year 2002 to 2003, it was apparent that emissions were higher in the summer than in winter but they did not exceed a total of 900 g N ha^{-1} $year^{-1}$ under any of the crops (Fig. 9). The spatial variability of the measurements was high, as was expected, but there was a strong tendency for N_2O emissions to be higher under wheat in the continuous soybean/wheat sequence (R1) and under the vetch in rotation R2B in the CT treatments than for these crops managed with the ZT system.

Regarding the CH_4 emissions from the soil surface the values were very low and similar to the control treatment (native forest) (Fig. 10). The average maximum values varied around 20 mg CH_4 m^{-2} h^{-1}, equivalent to 4.8 kg CH_4 ha^{-1} day^{-1}, but significantly higher than the N_2O emissions mentioned before. It is important to note that in general 10 days after sowing all treatments showed a significant reduction in the emission of CH_4, and in some cases there were days in which this gas was consumed. After the second week of January there was a tendency for the emission of this gas to increase, which probably was associated with the fall of the first senescent leaves of the crops, especially soybean.

It is important to note that even under the apparent free-drainage conditions of the soil where this study was developed, some peaks of methane were registered. This could be associated with the higher moisture content of the soil surface. It is likely that even low rainfall events can create saturated conditions of the soil surface in some microsites and/or locations of concave relief in the landscape to produce methane. More studies are necessary for a better understanding of this phenomenon.

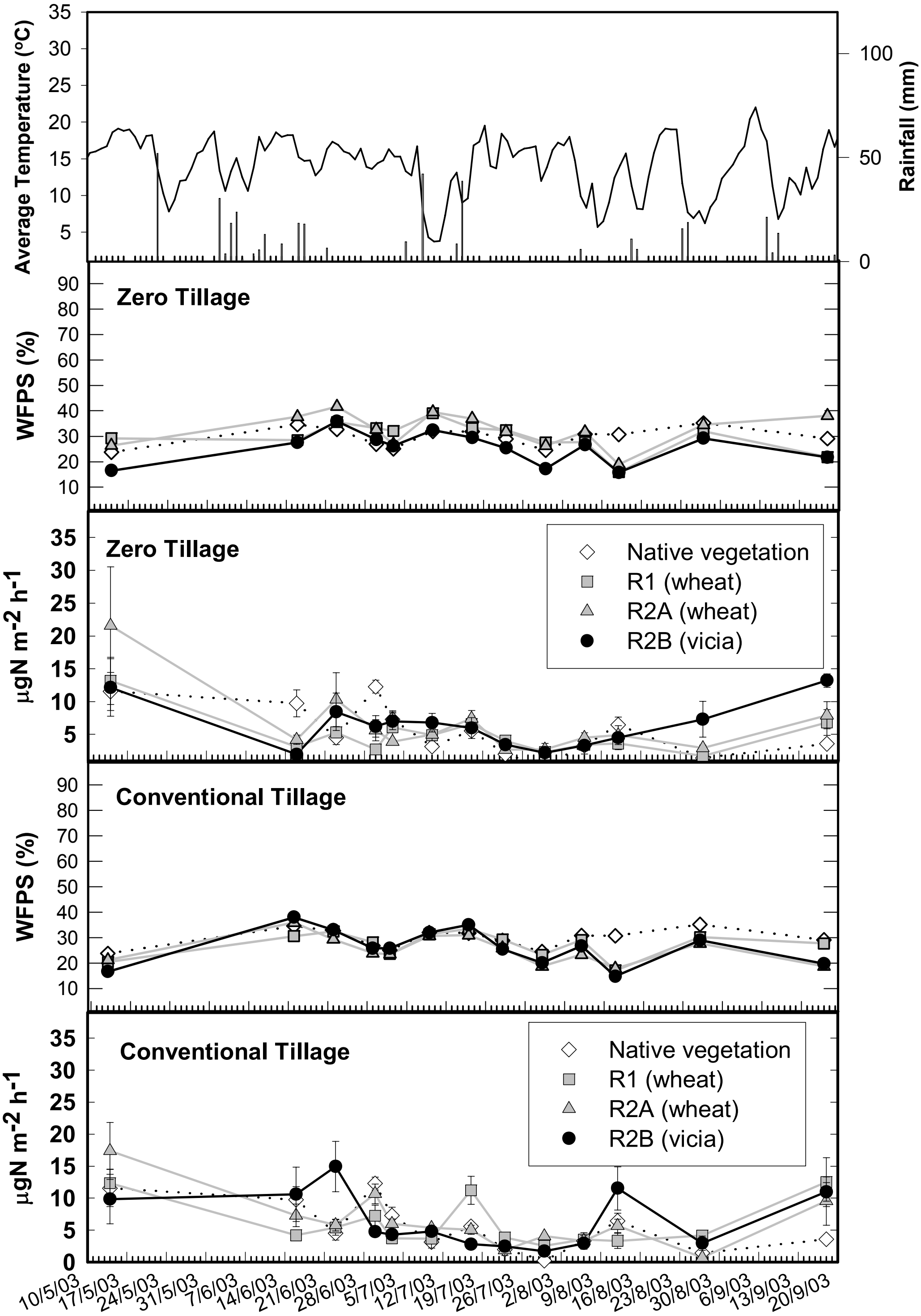

FIG.8. Emissions of N_2O from the soil surface of an Oxisol in a long term experiment under different crop rotations and tillage systems at Passo Fundo, RS. Period C- May 2003 to September 2003.

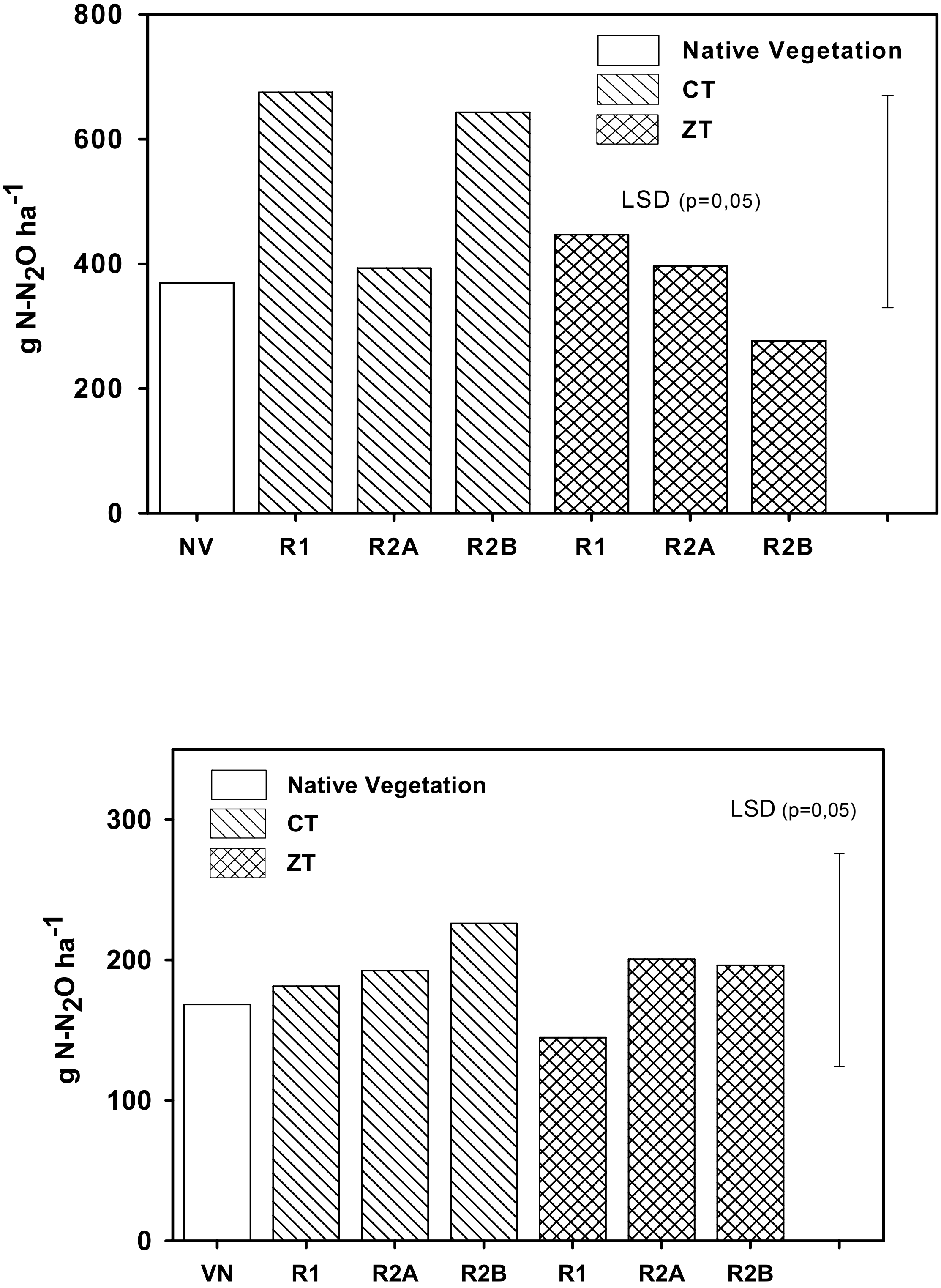

FIG.9. Emissions of N_2O during the summer (top graph) and winter (bottom graph) seasons in a long-term experiment under the different crop rotations and tillage systems and from a neighboring area of native vegetation at the Embrapa Wheat Centre (Passo Fundo, RS).

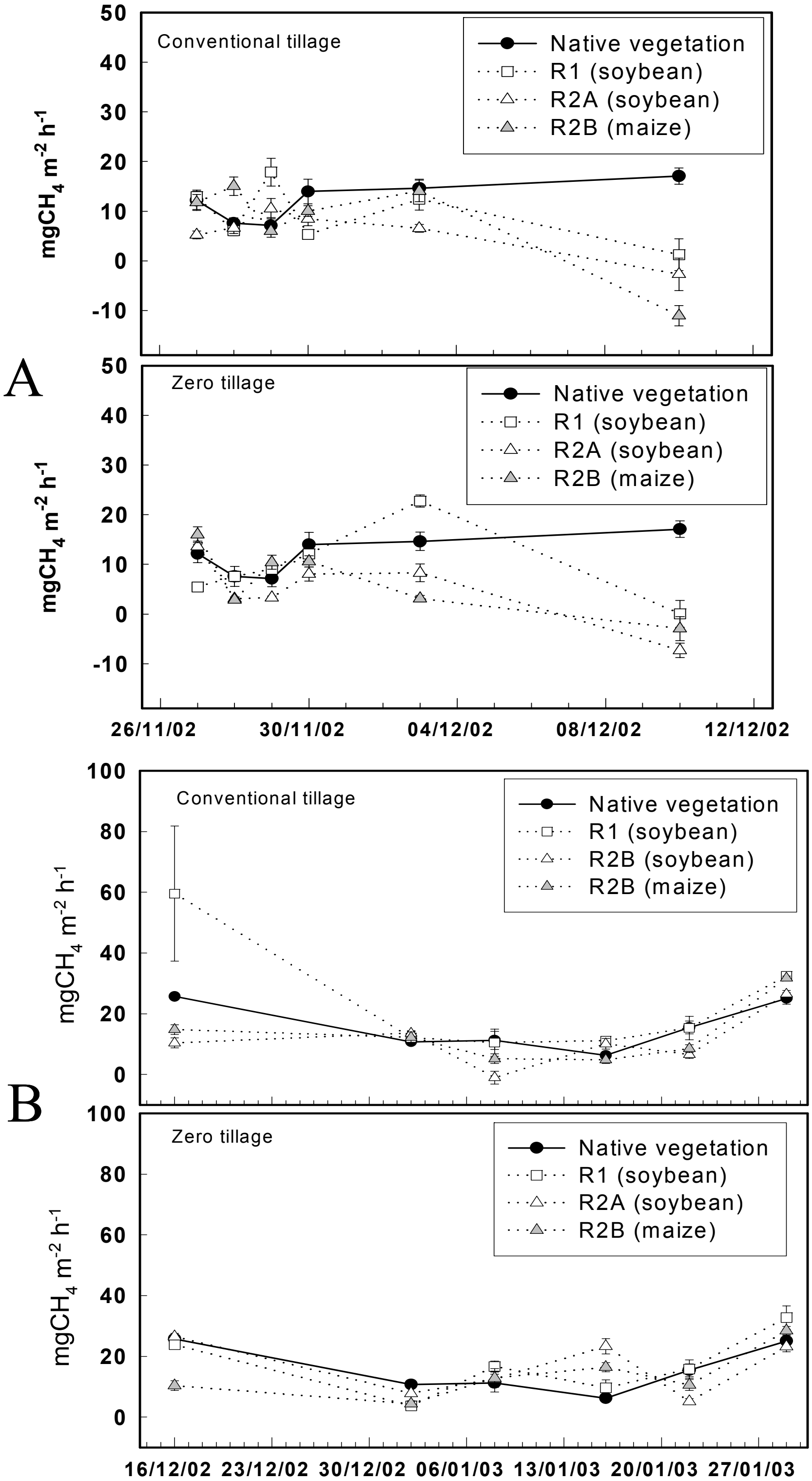

FIG.10. Emissions of CH_4 from the soil surface of an Oxisol in a long term experiment under different crop rotations and tillage systems at Passo Fundo, Rio Grande do Sul. A - November to December 2002. B - December 2002 to January 2003.

10. CONCLUSIONS

The main conclusions of these studies were:

The BNF process in the soybean crop grown under ZT and CT, is efficient atmeeting the grain N demand of the crop. Attempts to further increase grain yields by addition of N fertilizer are hardly ever successful if the plants have been effectively inoculated and nodulated with the recommended *Bradyrhizobium* strains.

The contribution of BNF to the lupin green manure crop in both tillage systems is sufficient to significantly increase the soil N status, with good potential to reduce the N-fertilizer needs of the subsequent cereal in the cropping system without grain yield reduction.

Although for both ZT and CT systems the estimated contribution of BNF to soybean was over 170 kg N ha^{-1}, the benefit to the subsequent crop was mainly due to the fast N release from the good quality soybean residues rather than a net gain of N derived from BNF.

The ZT system improved the BNF contribution to legume crops and increased the N-fertilizer use efficiency by cereals.

The aerial part of the lupin crop accumulated large amounts of N (over 200 up to 300 kg N ha^{-1}) that can be used as a source of N for subsequent cereal crops. Under CT maize grain yields (of 7.5 Mg ha^{-1}) after lupins without N fertilizer were comparable to those obtained in maize after oats plus 80 kg N ha^{-1}. Soybean crop resulted in a a negative N balance due to its high grain yield and high N harvest index.. The introduction of the lupin crop as a green manure in soybean-based crop rotations contributed to the maintenance of the soil N reserves and the sustainability of crop production.

Under the conditions of this study a maximum of 15 kg N-NH_3 ha^{-1} was lost under ZT by ammonia volatilization with a maximum of 4 kg N ha^{-1} derived form the surface broadcast application of urea and the remaining 11 kg N ha^{-1} originated from the soil. The loss from urea represented less than 10% of the N-fertilization rate applied to the maize crop.

A soybean crop inoculated with *Bradyrhizobium* spp grown in an Oxisol produced over 3.4 Mg ha^{-1} of grain yield independent of the tillage system in this study (ZT and CT), and there was no significant response in grain yield to the application of 60 kg N ha^{-1} at the flowering stage, even though the N fertilizer recovery was high (54% N-FUE).

At the end of 13 years under a continuous sequence of wheat/soybean there was no significant difference in soil C stocks between ZT and CT. However, in the rotations (R2-Wheat/soybean/vetch/maize and R3-Wheat/soybean/vetch/soybean/oats/maize) where the N_2-fixing legume vetch was included as a winter green manure crop, soil C stocks in the topsoil (0–30 cm depth) under ZT were increased by approximately 10 Mg ha^{-1}. Furthermore, the soil C stocks in the soil profile (to 100 cm depth) in R2 and R3 rotations were 17 Mg ha^{-1} higher under ZT than under CT.

The methane emissions, irrespective of the treatments studied, were always similar to the control soil. The values varied around 10 mg CH_4 m^{-2} day^{-1} from November to December 2002 and doubled from December 2002 to January 2003. Only during the first period of this study there were days on which this gas was consumed, instead of being released.

By extrapolation of results of N-N_2O losses, it was estimated that less than 900 g N-N_2O ha^{-1} yr^{-1} were lost from the soil surface, where the highest values were found under CT. The emissions were consistently higher in summer than in winter.

The mechanical root separation method indicated that only 2 to 4% of the whole N accumulated by the soybean plant was in the underground part, while estimates of 20 to 23% were obtained with the ^{15}N leaf labelling method. These results appear to be overestimated. Further studies to refine the technique and its application in tropical field conditions are needed.

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LONG-TERM EFFECTS OF CROPPING SYSTEMS AND FERTILIZATION ON CROP PRODUCTION, SOIL CHARACTERISTICS AND NITROGEN CYCLING IN THE GUINEAN AND SUDANIAN SAVANNAH ZONES OF BURKINA FASO (WEST AFRICA)

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Abstract

The long-term effects of annual fallow and two grain legumes, namely groundnut (*Arachis hypogea)* and cowpea (*Vigna unguiculata*) on soil N, fertilizer N recovery and yield of subsequent non-fixing crops were investigated in two ecological savannah zones of West Africa. The effects of fallow and groundnut on sorghum (*Sorghum bicolour*) and cotton (*Gossypium sp*) were studied using a 10-years (1993–2003) field experiment at the agronomic research station of Farakô-Ba (4^o 20' West, 11^o 6' North and 405 m altitude) in Guinean savannah zone Burkina Faso. A factorial 3x8 design in a split plot arrangement was used. Three crop rotations (cotton-groundnut-Sorghum, fallow-sorghum and mono cropping of sorghum) were used as first factor and 8 fertilizer treatments (mineral NPK fertilizer, NPK+crop residues, NPK+dolomite, PK+crop residues, PK+manure, PK+compost, PK and control) were applied as second factor. In the Sudanian savannah zone, a 5-year (1995–1999) field experiment was laid down at the research station of Kouaré (11^o 59' North, 0^o 19' West and 850 m altitude). A factorial of three cropping systems (cowpea-sorghum, fallow-sorghum and mono cropping of sorghum) as a first factor and 4 fertilizer treatments (mineral NPK fertilizer, NPK+dolomite, PK+manure and a control) as a second factor in a split plot design was used. In the Guinean savannah zone, grain yields of succeeding sorghum increased from 547 kg ha^{-1} in continuous sorghum to 912 and 1021 kg ha^{-1} in fallow-sorghum and cotton-groundnut-sorghum rotations respectively. Soils of fallow-sorghum and cotton-groundnut-sorghum rotations contained more mineral N at sowing. The N fertilizer use efficiency increased from 19% for the continuous sorghum to 32 and 51% for the fallow-sorgum and cotton-groundnut-sorghum respectively. Soil organic carbon increased from 0.36% in continuous sorghum to 0.39 and 0.54% in cotton-groundnut-sorghum and fallow-sorghum rotations respectively. Compared to the original soil, continuous sorghum and cotton-groundnut-sorghum rotations decreased soil organic carbon. Only fallow-sorghum rotation maintained soil organic carbon, exchange acidity and base saturation at same levels like those of original soil. Manure applications increased soil organic carbon, total N and available P. Except for the fallow-sorghum rotation, other rotations increased aluminum saturation and decreased soil pH compared to original soil. Manure or dolomite applications decreased exchange acidity and maintained soil pH and base saturation at same levels like those of original soil. Cotton-groundnut-sorghum rotation was the most efficient for the

Guinea savannah zone. In this cropping system, mineral NPK fertilizer plus one t ha^{-1} of dolomite can be recommended for cotton and sorghum. Alternatively mineral PK fertilizer combined with 3 t ha^{-1} cattle manure can be applied to sorghum. Only mineral NPK fertilizer can be used on groundnut. In the Sudanian savannah zone, sorghum grain yields increased by 75 and 100% when sorghum was rotated with fallow or cowpea respectively, compared to mono cropping of sorghum. Soils of fallow-sorghum and cowpea-sorghum rotations supplied 17 and 90% more N to succeeding sorghum compared to the mono cropping system of sorghum. The N fertilizer use efficiency increased from 17% for the continuous sorghum to 22 and 26% for the cowpea-sorghum and fallow-sorghum rotations respectively. Compared to the original soil, all cropping systems decreased soil organic carbon and increased aluminum saturation. All cropping systems decreased soil pH and increased aluminum saturation compared to original soil. Cowpea-sorghum rotation was the most efficient cropping system in the Sudanian zone. In this cropping system, mineral NPK fertilizer associated with one t ha^{-1} of dolomite or 3 t ha^{-1} of manure must be applied to sorghum. But only recommended mineral NPK fertilizer can be applied for cowpea.

1. INTRODUCTION

Crop yields in most agricultural systems of West Africa are very low due to a series of constraints and limiting factors. Despite their initial good agricultural productivity, crop yields decrease over time mainly due to soil fertility decline resulting from the low application of nutrient inputs [1, 2, 3]. Livestock production is not well-integrated with agricultural activities and crop residues are usually exported from the farm for household needs and animal feeding. In traditional systems, soil fertility maintenance was based on a relatively long fallow period (10–15 years) with natural bush vegetation regrowth followed by a short cropping period (3–5 years) [4]. However in recent years, increased population pressure has resulted in significant changes of the traditional bush-fallow systems. Long-term fallow systems are no longer practical and lands are continuously cultivated for long time leading to rapid soil fertility decline [5]. In this context, there is a need to develop and test short-term fallow systems that could be used as an appropriate alternative for soil fertility maintenance.

Despite the recognized need to apply chemical fertilizers to maintain soil fertility and obtain high crop yields, their use in West Africa is limited by a number of factors such as uncertainty of climatic conditions, in particular drought risk, land tenure, lack of financial resources, inefficient distribution systems, poor agricultural production and marketing-supporting policies and other socio-economic factors. Alternative, cost-effective and socio-economic viable technologies for improving and maintaining soil fertility and productivity must therefore be developed and pilot-tested before providing practical recommendations to the farmers. Among others, these include the integrated use of organic and inorganic nutrient sources. Thus, there is a need to increase the efficiency of the little applied fertilizer and enhance the addition of external nutrient inputs and recycling from other sources at the level of cropping systems.

In the Guinean savannah zone of Burkina Faso, sorghum, maize and cotton are the main basic crops cultivated by farmers. N_2-fixing legume crops, such as groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata*), are also important components of the cropping systems for the resource-poor farmers because they serve as food as well as cash crops. But cowpea is the most important legume crop used by farmers in the Sudanian savannah zone. The two legume crops (groundnut and cowpea) can eventually provide necessary financial resources to buy agricultural inputs, for instance fertilizers. Cropping systems including rotations of cereals with legumes have been studied in recent years and beneficial rotational effects of legumes on subsequent cereal yields have been reported for the Sahelian zone of West Africa [6, 7]. The main effect of legumes on succeeding crops is commonly attributed to an increase in soil N fertility status as a result of the N inputs from biological N_2 fixation [8]. However the N effect alone cannot completely explain the positive effects of legumes on the succeeding cereal crop yields. Little information exists on the long-term effects of crop rotations, in particular the effects of N_2-fixing legume crops, short-term fallow and selected fertilization treatments on soil fertility maintenance in the savannahs of the West African Sahel.

The main approach followed in this work is to improve the low soil fertility status through an integrated management of organic and inorganic nutrient sources. The contributions of N_2-fixing legumes in cropping systems or short-term annual fallow system and the effects of low-cost mineral amendments (phosphate rock and dolomite), and organic residues combined with low quantities of chemical NPK fertilizers were investigated. The purpose of this research was to identify novel and socio-economic viable management options for soil fertility improvement and sustained crop yields in smallholder farmer's systems of the West African Sahel.

2. MATERIALS AND METHODS

Long-term field experiments were carried out in two ecological savannah zones of the West African Sahel to study the effects of legume crops (groundnut and cowpea) and annual fallow on the yields of succeeding sorghum and cotton and the improvement of the soil fertility status. Cropping systems and fertilization treatments were selected for each agro ecological zone of Burkina Faso.

2.1. Long-term experiment in the Guinean savannah zone

The experiment was carried out over a period of 10 years (from 1993 to 2002) at the agronomic research station of Farakô-Ba (4^{o} 20' West, 11^{o} 6' North and 405 m altitude above sea level), located in the Guinean savannah zone of Burkina Faso. This agro-ecological zone has one rainy season per year, starting in May-June and ending in October. High variations of rainfall were observed both between years and within the years of experimentation (Table 1). In general, planting was done in June and harvesting in October.

The experiment was laid down in a 6-year old fallow field. The soil was an Ultisol of low fertility, representative of the study area. Soil samples were taken from the top 20 cm depth in the experimental plots in 1993 (first year) and 9 years after (2001) for laboratory analysis to assess changes in soil properties. Soil pH was measured in 1 N KCl using a 2:1 solution to soil ratio and exchangeable acidity as described by McLean [9]. Organic carbon was measured by the wet chemical digestion procedure of Walkley and Black [10]. Total N was determined by the Kjeldahl procedure [11]. Ca and Mg were determined by atomic absorption spectrophotometry, while K and Na were determined using flame photometry. Effective Cation Exchange Capacity of the soil (ECEC) was estimated as the total exchangeable bases. Available phosphorus was determined using Bray I method [12]. A factorial of 4 cropping systems and 8 fertilization treatments in split plot design with randomized block arrangement was used with four replications. Four cropping systems were used as first factor in the main plots: cotton-groundnut-sorghum and fallow-sorghum rotations were compared to mono cropping of sorghum, and continuous groundnut. Each main plot was split into 8 sub plots corresponding to the fertilization treatments, which were included as a second factor. As the rate of applied chemical fertilizers was relatively low, the fertilization treatments included other nutrient sources, which are locally available. The fertilization treatments were: NPK fertilizer, NPK+Crop Residues, NPK+Dolomite, PK+Crop Residues, PK+Manure, PK+Compost, PK alone and control (without any fertilization). Randomized

Fisher blocks arrangement was used. Rotation treatments were randomized in the main plots and fertilization treatments were randomized in the sub plots. Recommended rates of chemical fertilizers were applied to crops using urea, complex NPK fertilizer, triple super phosphate and potassium chloride. N, P, K and S nutrients were applied at sowing to groundnut at 14, 10, 11 and 6 kg ha^{-1} respectively, sorghum at 37, 10, 11 and 6 kg ha^{-1} and cotton at 44, 15, 17 and 9 kg ha^{-1}. At 40 days after sowing, complementary doses of 23 and 30 kg N ha^{-1} were applied as urea to sorghum and cotton respectively. For the manure and compost containing treatments, three t ha^{-1} of air-dried cattle manure or compost were applied. Cattle manure contained 1.8, 18.40, 0.31 and 0.16% of N, C, P and K while compost composition was 1.0, 15.5, 0.17 and 0.11% of N, C, P and K. of Dolomite was applied annually at a rate of 1 t ha^{-1} (249 and 114 kg ha^{-1} of Ca and Mg respectively).

Recommended improved varieties of groundnut (RMP-12), sorghum (Gnonfing) and cotton (FK 290) were sown at a planting density of 62,500 plants ha^{-1} for the three crops. Crop yields were measured at the end of each growing season. In the year 2001, dry matter production of sorghum was evaluated during the growth cycle at 15, 30 and 50 days after sowing. Soil mineral N (NH_4^+ + NO_3^-) was measured in composite soil samples taken from the 0–20 cm layer in the sorghum plots during the first two months of the season (at sowing, 8, 15, 29 and 46 days after sowing). Mineral N was extracted with 1M KCl solution and measured by a colorimetric method [13]. Statistical analysis was made using SYSTAT (version 8.0) software. A factorial procedure was used with cropping systems (main plots) as main factor and fertilizer treatment (sub plots) as second factor. Treatment means comparisons were made using Fisher's test.

2.2. Long-term experiment in the Sudanian savannah zone

The experiment was carried out over a period of 5 years (from 1995 to 1999) at the agronomic research station of Kouaré (11° 59' North, 0° 19' West and 850 m altitude), located in the Sudanian savannah zone of Burkina Faso. This agro-ecological zone has one rainy season per year, starting in May-June and ending in October. High variations of rainfall were observed both between years and within the years of experimentation (Table I). In general, planting occurred in June and harvesting in October.

TABLE I. MONTHLY AND SEASONAL RAINFALL (MM) DURING THE PERIOD OF EXPERIMENTATION AT FARAKÔ-BA (GUINEAN ZONE) FROM 1993 TO 2002 AND KOUARE (SUDANIAN ZONE) FROM 1995 TO 1999

Sites	Years	Months						Total
		May	June	July	August	September	October	
Farakô-Ba	1993	57	91	194	280	129	29	779
	1994	61	67	145	355	299	124	1051
	1995	78	226	225	237	149	61	976
	1996	157	120	173	170	230	70	919
	1997	323	185	158	171	168	79	1084
	1998	145	62	300	373	205	90	1175
	1999	80	112	168	246	192	119	916
	2000	60	172	236	309	241	41	1058
	2001	66	80	154	229	153	33	715
	2002	58	65	184	175	125	32	640
	Mean	109	118	197	255	189	68	931
Kouare	1995	27	69	150	372	138	50	806
	1996	113	173	92	210	165	26	779
	1997	97	90	121	141	164	57	670
	1998	89	171	222	244	178	90	994
	1999	91	89	151	178	228	34	771
	Mean	83	118	147	229	175	51	804

The soil was an Alfisol of low fertility, representative of the study area. Soil samples were taken from the top 20 cm depth in 1995 (first year) and 5 years after (1999) for laboratory analysis. The main soil characteristics were: weakly acid (pH = 5.5), sandy (75%) with low clay (11%) and organic carbon (0.50%) contents. Exchangeable Ca, Mg and K were low. As a consequence of low clay and organic C contents, ECEC was also very low (Table 5).

The experiment was laid down in an 8-year old fallow field. A factorial of 3 cropping systems and 4 fertilization treatments with four replications in split plot design in a randomized Fisher block arrangement was used. Three cropping systems (fallow-sorghum, cowpea-sorghum and mono cropping of sorghum) were used as first factor in the main plots. Recommended improved varieties of sorghum (ICSV-1049) and cowpea (KVX-61-1) were used at recommended planting density of 62 500 and 125 000 plants ha^{-1} respectively. Each main plot was split into 4 sub plots corresponding to the fertilization treatments, which were included as a second factor. The fertilization treatments were: mineral NPK fertilizer, NPK+Dolomite, PK+Manure and control (without any fertilization). Rotation and fertilization treatments were randomized in the main plots and sub plots respectively. Recommended rates of mineral fertilizers were applied to the crops using urea, complex NPK fertilizer, triple super phosphate and potassium chloride as described in the first experiment. Similar procedures to those described above for the first experiment were employed for statistical analyses.

2.3. Isotopic experiment

Using the ^{15}N isotopic dilution methodology (indirect approach), nitrogen fertilizer use efficiency (NFUE) was studied in the two agro-ecological zones. After eight seasons (1993–

2000) of cultivation in the Guinean zone and four seasons (1995–1998) in the Sudanian zone, NFUEs by succeeding sorghum were measured. At sowing fourteen kg N ha^{-1} were applied as urea with 5 atom % ^{15}N excess to the isotope micro plots of 3.2m × 1.6m (5.12 m^2) laid down in the main experimental plots. The total above ground parts of plants were harvested at physiological maturity. Plant samples were oven-dried at 60^o C during 72 hours, ground and total N and atom ^{15}N % excess were determined with an elemental analyzer coupled to a mass spectrometer at the IAEA Seibersdorf Laboratory. NFUEs were calculated using the percentage of N derived from fertilizer (Ndff) and the total N applied by fertilization treatment [14] (equation 1).

$$NFUE = (Ndff\text{-kg ha}^{-1} / N\ applied\ \text{-kg ha}^{-1}) \times 100 \quad (1)$$

3. RESULTS AND DISCUSSION

For each agro-ecological zone, the results on crop yields, soil and fertilizer N use by crops and changes in soil properties are presented and discussed.

3.1. Experiment in the Guinean savannah zone

3.1.1. Crop production

Sorghum, cotton and groundnut yields were affected by both fertilization and cropping system treatments (Table II). These effects were greatly influenced by cropping season (year effect). Although high variations in rainfall distribution were observed over the experimental period (Table I), significant correlations were not observed between crop yields and total rainfall. Crop yields were also not correlated with rainfall distribution pattern over the years indicating that rainfall variations alone didn't explain crop yield responses. High crop yield productions after fallow followed by a slow yield decrease during the subsequent five years have been sometimes observed in the Ultisols of this ecological zone [2]. Fauck *et al.* [15] explained this phenomenon by the evolution in the soil biological activity towards a new equilibrium leading to lower crop productivity.

3.1.1.1. Cotton and groundnut

Because cotton and groundnut were included in only one cropping system (cotton-groundnut-sorghum), only the effects of fertilizers are discussed (Table II). Fertilizer applications didn't affect groundnut grain yields. But the treatments NPK plus dolomite and PK application combined with manure had significant effects on groundnut haulm. Cotton yields were significantly increased by the combined application of NPK fertilizer and dolomite compared to NPK fertilizer alone, indicating that the correction of soil acidity (and aluminum toxicity) improve cotton yields [16, 17]. Seed yields of cotton were also high when PK fertilizer was applied with manure, probably because manure supplied enough nitrogen for cotton.

3.1.1.2. Sorghum

Fertilizer applications significantly increased sorghum grain yields during the nine years. This is likely explained by the original low fertility of the experimental soil [1, 2, 5]. Highest mean annual grain yields were obtained when mineral NPK fertilizer was associated with dolomite or when mineral PK fertilizer was associated with cattle manure. Despite the lack of mineral N, the combined application of PK fertilizer with manure was as effective as NPK fertilizer, indicating that the N provided by manure could be as effective as the N supplied by NPK fertilizer.

TABLE II. SORGHUM, COTTON AND GROUNDNUT YIELDS (KG HA^{-1}) AS AFFECTED BY FERTILIZATION AND CROPPING SYSTEMS AT FARAKÔ-BA OVER 10 YEARS (1993-2002)

	Sorghum	Cotton	Groundnut Grain	Groundnut haulms
Fertilization				
NPK +Dolomite	1125 ^{a}	1287 ^{a}	852	2688 ^{a}
PK+ Manure	1087 ^{ab}	1180 ^{b}	825	2595 ^{ab}
NPK+ Crop residues	1011 ^{c}	1087 ^{c}	730	2218 ^{cd}
PK+ Compost	965 ^{cd}	1035 ^{cd}	813	2381 ^{abc}
NPK Fertilizer	932 ^{de}	986 ^{cde}	660	1839 ^{de}
PK+ Crop residues	778 ^{f}	857 ^{f}	703	1992 ^{de}
PK fertilizer	632 ^{g}	773 ^{g}	664	1977 ^{de}
Control	473 ^{h}	656 ^{h}	564	2056 ^{cde}
Cropping systems				
Cotton-Groundnut-Sorghum	1021 ^{a}	943	735	2279
Fallow-Sorghum	912 ^{b}	na	na	na
Sorghum-Sorghum	547 ^{c}	na	na	na
Block	***	**	***	***
Rotation (R)	***	na	na	na
Fertilizer (F)	***	**	ns	**
Year (Y)	***	**	***	**
RxF	ns	na	na	na
RxY	***	na	na	na
FxY	***	ns	ns	ns
RxFxY	ns	na	na	na

*, **, ***: Significant at 0.05; 0.01 and 0.00 level; ns: Not significant (p>0.05).
Values affected by the same letter in the same column are not significantly different at p<0.05, according to Fisher's test.

With regard to the cropping systems, sorghum produced highest grain yields when groundnut was included in the cropping system (cotton-groundnut-sorghum) while the lowest yields were obtained in monocropping of sorghum. Fallow-sorghum rotation significantly increased sorghum grain yields compared to mono cropping of sorghum. Mean annual sorghum grain yield was increased by 1.7 and 1.9 times when sorghum was rotated with fallow and groundnut respectively. Beneficial effects of the inclusion of N_2-fixing legume crops on the production of succeeding non-fixing crops have been reported in many works, showing that legume-cereal rotations can increase cereal yields from 50 to 350% [6, 7, 17].

3.1.2. Soil mineral N

In all fertilization and crop rotation treatments, soil mineral N was relatively high at the start of the growing season but it decreased with time (Figs 1a and 1b).

The highest quantity of soil mineral N was found at sowing, thereafter it decreased with time, first slowly up to 30 days after sowing and afterwards very rapidly between 30 and 44 days after sowing. Very low quantity of mineral N (less than 10 kg N ha^{-1}) was measured at 44 days after sowing (DAS). It is evident that the N application as NPK fertilizer or manure supplied most of the initial mineral soil N. Moreover, in the control treatment (without fertilizer) it is likely that the first rains of the season stimulated the microbial activity leading to a high mineralization of soil organic matter resulting in an increase in mineral N. The decrease in soil mineral N found during the growing season in all treatments is likely due to a decline in organic N mineralization rates and increased N uptake/demand by plants, and also probably to losses by leaching and other N cycling processes.

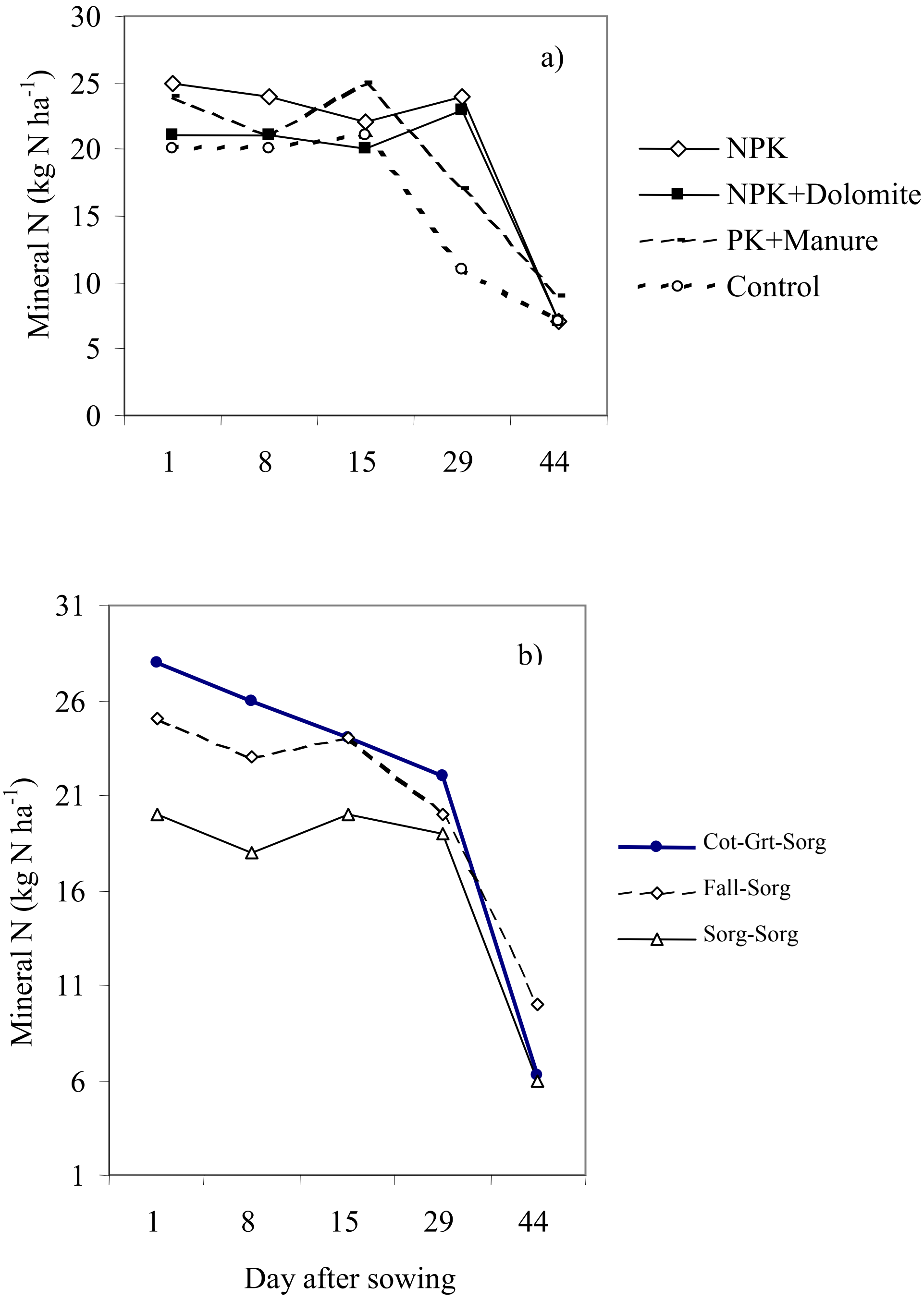

FIG.1. Soil mineral N as affected by (a) fertilizers and (b) crop rotations under sorghum during the first 44 days after sowing Cot, Cotton; Grt, Groundnut; Fall, Fallow; Sorg, Sorghum.

Cropping systems had significant effects on soil mineral N at sowing and interaction was not observed between the two factors ($P<0.05$). During the first 29 DAS, highest quantities of mineral N were observed in cotton-groundnut-sorghum rotation while lowest quantities were observed in monocropping of sorghum. The high content of mineral N in cotton-groundnut-sorghum rotations could be a consequence of the good quality of the groundnut residues. Similar results have been reported [6, 18, 19], showing that despite the exportation of legumes fodder from the field, the remaining residues and the below ground parts of groundnut can contribute to improve soil organic matter and thereby soil mineral N. Compared to continuous sorghum, the good quality of the groundnut residues probably supplied readily decomposable organic N resulting in increased levels of soil mineral N at the beginning of the next season. As reported by other researchers [2, 6, 7, 20, 21], soil mineral N is a good indicator to assess the N contributions of the precedent legumes. More mineral N was also found in the fallow-sorghum rotation than in the continuous sorghum. This may likely be explained by the higher biomass of organic residues produced by the natural fallow.

3.1.3. N cycling and N fertilizer use efficiency

Total N uptake by succeeding sorghum and N fertilizer use efficiency (NFUE) were significantly affected by cropping systems ($P<0.001$) and interactions were not observed between the two factors (Table III). The treatments NPK + dolomite and PK + organic manure showed higher total N uptake by sorghum than the treatment NPK fertilizer alone. Total N uptake increased from 48 kg N ha^{-1} in continuous sorghum to 104 and 119 kg N ha^{-1} when sorghum was rotated with fallow and cotton-groundnut respectively. The residual effects of rotation and fertilizers treatments were estimated by calculating the isotopic parameter N derived from residual effects (Ndfr) using sorghum-sorghum rotation or NPK fertilizer as reference treatments respectively. Fallow-sorghum and cotton-groundnut-sorghum rotations increased soil N supply from 56 to 71 kg ha^{-1} respectively. Compared to monoculture, sorghum took up 1.6 and 2.6 times more N from the fertilizer when it was rotated with fallow and cotton-groundnut respectively, leading to higher N fertilizer use efficiencies.

TABLE III. TOTAL N UPTAKE AND N FERTILIZER USE EFFICIENCIES (NFUE) OF SORGHUM AS AFFECTED BY CROPPING SYSTEMS AND FERTILISER APPLICATIONS AFTER 8 SEASONS OF CULTIVATION (1993–2001) AT FARAKÔ-BA AND AFTER 5 SEASONS OF CULTIVATION (1994–1999) AT KOUARE

Sites		Total N-uptake (kg ha^{-1})		NFUE
		Total	Ndfr	%
Farakô-Ba (Guinean zone)	Cotton-Groundnut-Sorghum	119^{a}	71	51^{a}
	Fallow-Sorghum	104^{abc}	56	32^{c}
	Sorghum-Sorghum	48^{d}	0	19^{d}
	NPK	78^{c}	0	37
	NPK+Dolomite	98^{b}	20	35
	PK+Manure	115^{a}	37	38
	Block	ns		ns
	Rotation(R)	***		***
	Fertilization(F)	**		ns
	RxF	ns		ns
Kouare (Sudanian zone)	Fallow-Sorghum	31^{b}	5	26^{a}
	Cowpea-Sorghum	48^{a}	22	22^{ab}
	Sorghum-Sorghum	26^{c}	0	17^{c}
	Test F	*		*

NFUE: Nitrogen fertilizer use efficiency; Ndff, Ndfs: N derived from fertilizer or soil respectively.
Ndfr: N residual from fertilizer or cropping system treatment in relation to respective control (NPK or sorghum-sorghum rotation).
*, **, ***Significant at 0.05; 0.01 and 0.00 level; ns: Not significant ($p>0.05$).
Values affected by the same letter in the same column are not significantly different at $p<0.05$, according to Fisher's test.

The N fertilizer use efficiency values were 51 and 32% for the cotton-groundnut and fallow treatments respectively compared to only 19% for sorghum monoculture. The effects of legumes on increasing NFUE have also been reported by others [22, 23]. Soils in the fallow-sorghum and cotton-groundnut-sorghum treatments also supplied 2.3 and 2.5 times more N to sorghum respectively, in comparison to sorghum monoculture. These results clearly indicate that high total N uptake values when sorghum was rotated with fallow or cotton-groundnut were due to the increased N supply from the two sources, the fertilizer and the soil. As shown by data on soil mineral N, legume residues provide more organic N leading to high soil mineral N for the succeeding crop [22].

3.1.4. Soil characteristics

After 8 years of cultivation (1993–2001), cropping system effects on soil properties were examined with reference to the original soil. Soil organic carbon was significantly influenced by crop rotations and fertilization (Table IV). Highest quantities of soil organic carbon were observed when sorghum was rotated with fallow while lowest quantities were obtained for monocropping of sorghum. The cotton-groundnut-sorghum rotation did not significantly increase soil organic carbon compared to monocropping of sorghum. Only fallow-sorghum rotation maintained soil organic carbon at same level of the original soil. Within fertilizer treatments, PK + manure applications increased soil organic carbon compared to NPK fertilizer alone. However, NPK + dolomite applications did not affect soil organic carbon compared to NPK fertilizer alone.

Soil total N was also affected by cropping systems and fertilizer applications (Table IV). All rotations decreased soil total N compared to original soil. Like organic C, highest quantities of total N were observed in soil when sorghum was rotated with fallow while lowest quantities were obtained in sorghum monocropping. But cotton-groundnut-sorghum rotation did not significantly increase soil total N compared to mono cropping of sorghum. PK +Manure applications increased soil total N compared to NPK fertilizer.

Soil acidity parameters (pH, exchangeable aluminum and exchangeable acidity) and exchangeable bases were affected by cropping systems and fertilization. Except for fallow-sorghum rotation, all cropping systems increased aluminum saturation and decreased soil pH compared to original soil. But only soils of fallow-sorghum rotations maintained exchangeable acidity and base saturation at same levels like those of original soil. Compared to mineral NPK fertilizer alone, combined applications of mineral fertilizer with manure or dolomite decreased exchangeable acidity and maintained soil pH and base saturation at same levels like those of original soil. On the contrary, NPK fertilizer alone tended to increase exchangeable acidity, lowering pH and base saturation.

Initial soil available P was very low, indicating that P deficiency is a main limiting factor for crop production in this soil [5]. Unlike organic C and total N, highest quantities of available P-Bray I were observed in soils with monocropping of sorghum and lowest quantities of P-Bray I were observed in fallow-sorghum rotation. This can be explained by the higher biomass production and P uptake in the rotation treatments compared to mono cropping. In the fertilization treatments, highest quantities of available P-Bray I were found when manure was applied combined with PK fertilizer. This effect was likely due to both the P supplied by the PK fertilizer and manure and the positive effects of organic manure on P availability [24].

TABLE IV. SOME SOIL (0-20 CM LAYER) CHARACTERISTICS OF THE EXPERIMENT AT FARAKÔ-BA (GUINEAN ZONE) AS AFFECTED BY FERTILIZERS AND CROP ROTATIONS AFTER 8 YEARS (1993-2000) OF CULTIVATION

	pH KCl	Org. Carbon (%)	P-BrayI mg P kg^{-1}	N-total mg N kg^{-1}	K^+ Cmol+ kg^{-1}	Ca^{++} Cmol+ kg^{-1}	Mg^{++} Cmol+ kg^{-1}	Al^{3+} Cmol+ kg^{-1}	EA Cmol+ kg^{-1}	ECEC Cmol+ kg^{-1}	Base Sat. (%)
Cropping systems											
Cotton-Ground-Sorg	4.9^b	0.39^b	6.2^b	373^c	0.17^b	0.87^c	0.45	0.08^a	0.16^a	1.82	81^b
Fallow-Sorghum	5.3^a	0.54^a	4.1^c	462^b	0.22^a	1.16^a	0.49	0.01^b	0.06^c	2.02	93^a
Sorghum-Sorghum	4.9^b	0.36^{bc}	7.9^a	372^c	0.21^a	0.87^c	0.42	0.08^a	0.15^a	1.83	79^b
Original soil	5.1^a	0.55^a	2.7^d	506^a	0.02^c	1.08^b	0.46	0.01^b	0.08^c	1.82	96^a
Fertilization											
NPK	4.5^d	0.41^{bc}	6.7^b	386^b	0.15^c	0.61^d	0.22^d	0.15^a	0.28^a	1.56^c	65^d
NPK+Dolomite	5.4^a	0.43^b	4.9^c	374^c	0.14^c	1.60^a	0.81^a	0.00^c	0.01^d	2.60^a	99^a
PK+Manure	5.4^a	0.51^a	10.3^a	459^a	0.35^a	0.97^b	0.46^b	0.00^c	0.04^c	1.88^b	96^b
Control	4.9^c	0.38^d	3.2^d	308^e	0.18^b	0.72^c	0.30^c	0.04^b	0.12^b	1.46^d	83^c
Block	*	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
Rotation(R)	***	***	***	***	*	*	ns	***	***	ns	***
Fertilizer(F)	***	*	***	*	***	***	***	***	***	***	***
RxF	*	ns	*	ns	ns	ns	ns	***	***	ns	***

EA: Exchangeable acidity; ECEC: Effective Cation Exchange Capacity.

*, **, ***Significant at 0.05; 0.01 and 0.00 level; ns: Not significant ($p>0.05$).

Values affected by the same letter in the same column are not significantly different at $p<0.05$, according to Fisher's test.

3.2. Experiment in the Sudanian zone

3.2.1. Crop production

High variations of both total rainfall and its distribution were observed during the five years (Table I). Total rainfall varied from 670 mm in year 1997 to 994 mm in 1998. But significant correlations were not observed between sorghum or cowpea yields and total rainfall. This indicated that factors other than rainfall affected crop production during the experimental period.

3.2.1.1. Cowpea production

Cropping system effects were not studied on cowpea. Only fertilizer effects can be discussed. Cowpea grain and haulms yields were affected by fertilization treatments and cropping season (Table V). Interaction was not observed between cropping season and both fertilization treatments or cropping systems, meaning that cowpea responses to fertilization or cropping system were not affected by factors related to cropping season. All fertilizer treatments increased production of cowpea haulms compared to the control (without fertilization), but no significant differences were observed between fertilization treatments. However significant differences in cowpea grain yields were found between fertilization treatments. Cowpea grain yields increased by 20% when dolomite was applied with NPK fertilizer compared to mineral NPK fertilizer alone. Grain yields of cowpea in the PK fertilizer + manure treatment were significantly higher than those of NPK fertilizer and control treatments.

3.2.1.2. Succeeding sorghum yields

The succeeding sorghum grain and stover yields were affected by fertilization treatments, cropping systems and cropping season (Table V, Figs. 2a and 2b). Interaction was not observed between cropping season and fertilization treatments or cropping system, indicating that cropping system didn’t influence sorghum responses to fertilization or cropping system.

In all fertilization treatments (except for control) sorghum grain yields increased over time from 1996 to 1998 (Fig. 2a), showing the positive effect of fertilizers on soil fertility replenishment because the initial soil fertility status was low [1, 2, 5]. Mineral NPK fertilizer applications doubled sorghum mean annual grain yields compared to the control treatment. Highest mean of sorghum grain yields were obtained when mineral NPK fertilizer was associated with dolomite. Similar results have been reported by Bado et al [16]. The combined application of PK fertilizer with manure was as effective as NPK fertilizer, indicating that the N provided by manure could be as effective as the N supplied by NPK fertilizer, as observed in the Guinean savannah zone too.

With regard to cropping systems, sorghum produced highest grain yields when fallow or cowpea were included in the cropping system (fallow-sorghum or cowpea-sorghum), while the lowest yields were obtained with continuous sorghum. Sorghum grain yield increases of 75 to 100% over that of sorghum monocropping were found when sorghum was rotated with fallow and cowpea respectively

TABLE V. SORGHUM AND COWPEA YIELDS (KG HA^{-1}) AT KOUARE AS AFFECTED BY FERTILIZATION AND CROPPING SYSTEMS DURING 4 YEARS (1996-1999)

	SORGHUM		COWPEA	
	Grain	Stover	Grain	Haulms
Fertilization				
NPK+ Dolomite	914 ^{a}	4489 ^{a}	611 ^{ab}	1228 ^{a}
PK+ Manure	746 ^{bc}	3769 ^{c}	649 ^{a}	1284 ^{a}
NPK	798 ^{b}	4205 ^{ab}	510 ^{c}	1028 ^{a}
Control	346 ^{d}	1611 ^{d}	265 ^{d}	380 ^{b}
Cropping systems				
Cowpea-Sorghum	938 ^{a}	4281 ^{a}	na	na
Fallow-Sorghum	819 ^{b}	3493 ^{b}	na	na
Sorghum-Sorghum	467 ^{c}	2912 ^{c}	na	na
Block	***	***	***	***
Cropping system (R)	**	**	na	na
Fertilization (F)	***	***	**	*
Year (Y)	***	***	**	**
RxF	ns	ns	na	na
RxY	ns	ns	na	na
FxY	ns	ns	ns	ns
RxFxY	ns	ns	na	na

*, **, ***Significant at 0.05; 0.01 and 0.00 level; ns: Not significant (p>0.05).
Values affected by the same letter in the same column are not significantly different at p<0.05, according to Fisher's test.
na: Not applicable.

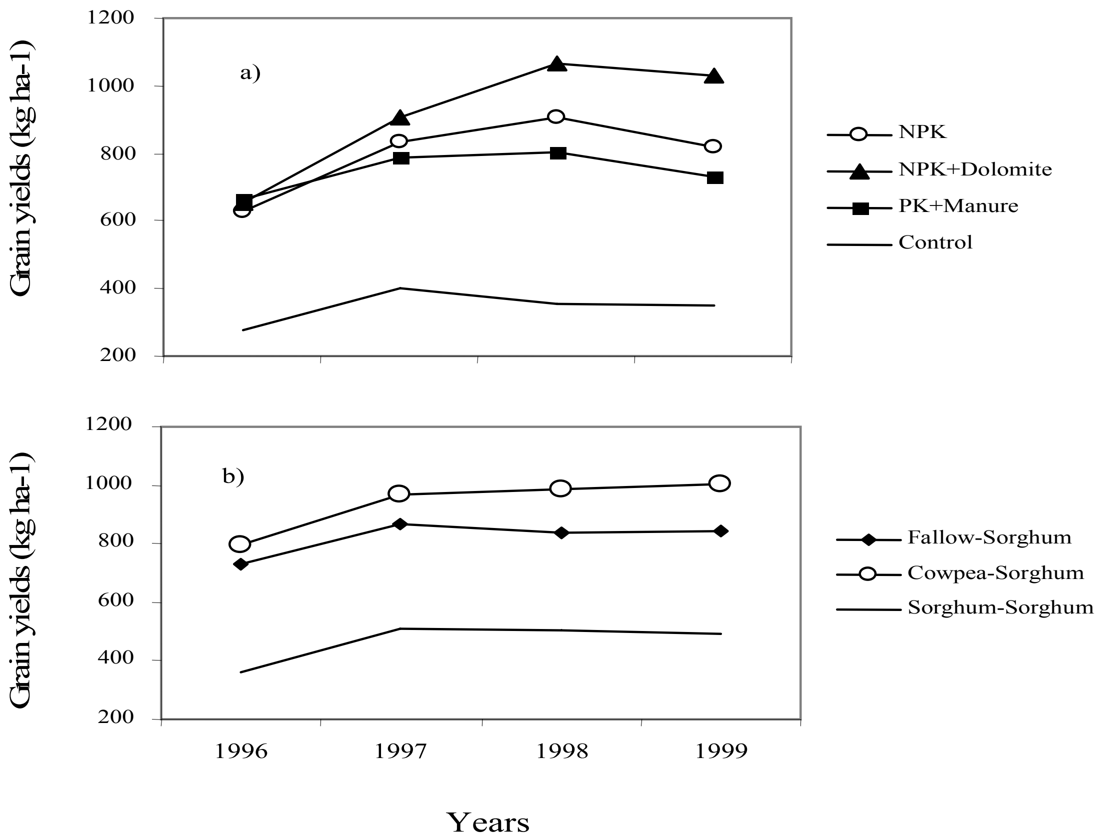

FIG.2. Sorghum grain yields (kg ha^{-1}) as affected by fertilization (a) and cropping systems (b) during four years (1996–1999).

3.2.2. N cycling and N fertilizer use efficiency

Utilizing the indirect approach of the ^{15}N isotope technique, the residual effects of rotation and fertilizers were calculated using sorghum-sorghum rotation or NPK fertilizer as reference (control) treatments respectively (Table III). Fallow-sorghum and cowpea-sorghum rotations increased the soil N supply by 5 and 22 kg N ha^{-1} respectively. Both total N uptake by succeeding sorghum and N fertilizer use efficiency (NFUE) were significantly affected by cropping systems ($P<0.05$). Total N uptake increased from 26 kg N ha^{-1} in continuous sorghum to 31 and 48 kg N ha^{-1} when sorghum was rotated with fallow or cowpea respectively. Compared to monocropping, sorghum absorbed more N from soil when it was rotated with fallow or cowpea. The highest quantities of N taken up by the succeeding sorghum crop were obtained under the cowpea-sorghum rotation. N uptake by sorghum under the cowpea-sorghum rotation was almost twice that of the mono cropping of sorghum. This relatively high availability of N for succeeding sorghum can be explained by the mineralization of organic N provided by cowpea residues [20]. The succeeding sorghum took also more N from fertilizer when it was rotated with fallow or cowpea than sorghum grown in monocropping. Thus, N fertilizer use efficiency increased from 17% to 22% and 29% when sorghum was rotated with cowpea or fallow respectively. These results clearly indicate that the high total N uptake when sorghum was rotated with fallow or cowpea was due to the increased N supply from the two sources, fertilizer and soil. Legume residues probably provided more and better quality organic N leading to higher content of soil mineral N for the succeeding crop [22].

3.2.3. Soil characteristics

After four years of cultivation (1995-1999), the soil characteristics were examined with reference to the original soil to assess fertilization and cropping system effects (Table VI). Soil organic carbon was significantly influenced by crop rotations. But fertilization didn't affect soil organic carbon. Compared to the original soil, all cropping systems decreased soil organic carbon. However soil organic carbon was highest when sorghum was rotated with fallow and lowest for cowpea-sorghum rotation and mono cropping of sorghum. No significant differences in organic carbon were found for cowpea-sorghum and mono cropping of sorghum. Soil total N was also affected by fertilization and cropping systems. As observed with organic carbon, all treatments (fertilization and cropping systems) decreased soil total N compared to original soil. Like organic C, highest total N was observed in soil when sorghum was rotated with fallow while lowest quantities were obtained in mono cropping of sorghum. But cowpea-sorghum rotation did not significantly increase soil total N compared to monocropping of sorghum. All fertilization treatments increased soil total N. This can be explained by a higher biomass production, leading to a larger amount of organic residues recycled in the soil. Manure also increased soil total N as a result of direct N supply or increase in the production of organic residues.

Compared to the original soil, significant effect of cropping systems was not observed on soil pH. However, all cropping systems increased Al^{3+} saturation. NPK fertilization increased Al^{3+} saturation whereas PK + manure decreased Al^{3+} saturation to a less extent. Only NPK plus dolomite applications prevented the increase of Al^{3+} saturation and the decrease of base saturation and soil pH. Conversely, cropping systems and fertilizer applications decreased base saturation compared to the original soil. Only dolomite applications prevented the decrease in base saturation through the supply of Ca and Mg. But soil ECEC was not significantly affected by fertilization or cropping systems.

TABLE VI. SOME SOIL (0-20 CM LAYER) CHARACTERISTICS AT KOUARE (SUDANIAN ZONE) AS AFFECTED BY FERTILIZERS AND CROP ROTATIONS AFTER 5 YEARS OF CULTIVATION (1995–1999)

	pH KCl	Org. carbon (%)	Total N mg N kg^{-1}	K^+ Cmol kg^{-1}	Ca^{++} Cmol kg^{-1}	Mg^{++} Cmol kg^{-1}	ECEC Cmol kg^{-1}	Base Sat. (%)	Al Sat. (%)
Cropping systems									
Fallow-Sorghum	5.3	0.39^b	360^b	0.11	1.43^b	0.44	2.10	95^b	3^a
Cowpea-Sorghum	5.2	0.29^c	294^c	0.09	1.09^c	0.53	1.84	94^b	3^a
Sorghum-Sorghum	5.3	0.29^c	335^c	0.14	1.07^c	0.35	1.68	94^b	3^a
Original soil	5.5	0.50^a	427^a	0.13	1.59^a	0.51	2.30	99^a	0^b
Fertilization									
PK+Manure	5.2^b	0.32	318^a	0.10	1.15	0.33	1.72	93^c	4^b
NPK	5.0^c	0.33	317^a	0.10	1.12	0.27	1.70	88^b	6^a
NPK+Dolomite	5.6^a	0.32	270^b	0.10	1.38	0.62	2.17	99^a	0^d
Control	5.3^b	0.32	237^c	0.15	1.13	0.54	1.91	97^a	2^c
Block	ns	***	ns	*	*	ns	*	ns	ns
Rotation (R)	ns	***	***	ns	*	ns	ns	*	*
Fertilization(F)	**	ns	*	ns	ns	ns	ns	**	**
RxF	ns	ns	ns	ns	ns	ns	ns	ns	ns

ECEC: Effective Cation Exchange Capacity.
*, **, ***: Significant at 0.05; 0.01 and 0.00 level; ns: Not significant ($p>0.05$).
Values affected by the same letter in the same column are not significantly different at $p<0.05$, according to Fisher's test.

4. CONCLUSIONS

In the two agro-ecological zones of the savannahs of Burkina Faso, soil fertility status can be improved by utilizing appropriate cropping systems and integrated soil fertility management practices of organic and inorganic nutrient sources. The inclusion of the two N_2-fixing grain legume crops (cowpea and groundnut) and low-cost amendments such as dolomite and organic manure combined with low quantities of chemical fertilizers provides viable management options for soil fertility maintenance in smallholder farmer's systems in the West African Sahel. A short-term (one year) natural fallow practice can be an alternative to the traditional long-term fallow system. However, it was less effective than legume-sorghum rotations in both agro-ecological zones studied. Legume-sorghum rotations, therefore, represent a highly appropriate technology to enhance soil fertility, to improve cereal crop productivity and sustainability of the cropping systems of smallholder farmers.

In the Guinean savannah zone, cotton-groundnut-sorghum rotation is the most efficient. In this cropping system, mineral NPK fertilizer combined with one ton ha^{-1} of dolomite can be used for cotton and sorghum fertilization. But mineral PK fertilizer associated with 3 tons ha^{-1} cattle manure can be applied as a low-cost and efficient fertilizer for sorghum. Mineral PK fertilizer can be used on groudnut. In the Sudanian savannah zone, cowpea-sorghum rotation was the most efficient. In this cropping system, mineral NPK fertilizer combined with one ton ha^{-1} of dolomite or 3 tons ha^{-1} of manure can be applied to sorghum. But only recommended mineral NPK fertilizer can be used for cowpea.

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IMPROVING SUSTAINABLE INTENSIFICATION OF CEREAL-GRAIN LEGUME CROPPING SYSTEMS IN THE SAVANNAHS OF WEST AFRICA: QUANTIFYING RESIDUAL EFFECTS OF LEGUMES ON MAIZE, ENHANCING P MOBILIZATION BY LEGUMES AND STUDYING LONG-TERM SOIL ORGANIC MATTER (SOM) DYNAMICS

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Abstract

Improved cereal-grain-legume systems, allowing farmers to use their land productively on a continuous basis, are being rapidly developed and adopted by small-scale farmers in the West African Moist Savannah. This paper summarizes work on several issues related to the improvement of productivity and sustainability of these intensified systems. A first study looked at the sustainability of several legume-maize cropping systems in a 5-year field trial at Sékou, Bénin. Fairly low maize yields were found in continuous maize cropping systems (maize/maize), poor response to N fertilizer beyond 45 kg N ha^{-1}, and no evidence that P and K were limiting crop yield. Over the last 5 years of the trial, maize/Mucuna relay cropping gave consistently a 2000 kg ha^{-1} yield increase relative to maize/maize cropping, and most of this yield gain was preserved even when Mucuna residues were removed from the plots when planting the next year's maize crop. Some yield gain, although far less than with maize/Mucuna, was observed in the maize/pigeon pea system. The maize/cowpea system offered no maize yield gain over maize/maize cropping. In a second study, enhanced isotopic methods to determine the plant available P allowed us to test the hypothesis that certain legume accessions can mobilize sparingly-available P. In one out of the 3 West-African Moist Savanna soils studied, we found that cowpea could access sparingly soluble soil P that is unavailable to maize. This mobilization of P was only observed when P deficiency occurred. These results confirm the P efficiency of some legume genotypes, which may lead to benefits of improved P availability by the incorporation of legumes in rotation systems. A third study, involving a 16-year continuous-cropping field experiment in Ibadan, Nigeria, provided information on long-term changes in soil organic matter carbon (SOC) contents in savannah soils with sandy top soil. In the control treatments with continuous maize and cowpea cropping without trees, SOC levels dropped from the initial 15.4 Mg C ha^{-1} to 7.3–8.0 Mg C ha^{-1} in 16 years (SOC content in 1700 Mg ha^{-1} equivalent soil mass). In the two continuously cropped alley cropping systems (Leucaena and Senna), the SOC levels dropped to levels between 10.7 and 13.2 Mg C ha^{-1}. The ^{13}C natural abundance technique yielded useful information to test the ROTHC-26.3 SOC model in sub-humid tropical conditions under a complex pattern of cropping systems.

1. INTRODUCTION

Increasing population in the West African Moist Savannah is forcing smallholder farmers to shorten or even abandon fallow periods, and to drastically change their farming methods. It offers challenges and opportunities to the research community to develop technological innovations that are acceptable to resource-poor farmers, and increase their income through sustainable intensification.

The West African Moist Savannah is defined as the lowland area (<800m) with a length of growing period between 5 and 9 months [1]. Lixisols dominate this agro-ecozone, and occur in association with Regosols, Plinthosols, Gleysols, and Leptosols [2]. Soils in this area generally have a favourable pH and cation status, but also have a low soil organic matter (SOM) content and a weak pH buffering capacity. Continuous cropping with application of commonly available NH_4-based fertilisers or urea and removal of crop residues may further reduce the SOM content, decrease the pH, and increase Mn to levels toxic for certain crops [3]. While most farmers in the Moist Savannah do use fertilizers, applied quantities are far below the recommended rates [4], often resulting in negative nutrient balances. Maize yields obtained by smallholder farmers are commonly in the range of 500–3500 kg dry matter ha^{-1} [5, 6, 7, 8], much lower than the theoretical yield level of 4500–8000 kg dry matter ha^{-1} [9] calculated with a crop growth simulation model for the situation where only rainfall and radiation limit crop growth. Nitrogen and phosphorous, in that order, are the main limiting nutrient elements for cereal production in the area [10].

During the last 10 years, the agricultural research community became increasingly convinced that in this setting, sustainable intensification could be achieved through improved cereal-grain-legume systems with the integrated use of organic inputs, fertilizers, and improved resilient germplasm [11, 12, 13]. Poor adoption of green manure crops and agroforestry systems, led to the breeding of grain legumes for dual-purpose. Long duration cowpea, soybean, and groundnut varieties with a high N-fixation capacity but low harvest index were developed thus yielding both more grain and more fodder/green manure (‘dual purpose’) than farmers’ traditional varieties. Some of these varieties had the added benefit of reducing the seed bank of the parasitic weed *Striga hermonthica*. These grain legume accessions are grown in rotation with drought-tolerant and N-efficient maize varieties.

These improved cereal-grain-legume systems met with rapid adoption by farmers [11], allowing them to use their land productively on a continuous basis. Several issues however, need further attention. First, a better understanding of the ‘rotation effects’, i.e. residual effects of the legume on the cereal crop other than N-effects, would allow further improvement of the cereal-grain-legume systems. Previous ^{15}N labelling work indicated that such rotational effects in soybean-maize rotations are considerable [14]. Secondly, there is a need to develop P-efficient grain legumes, as the performance of the legumes (and their symbiotic N-fixation) hinges on sufficient P supply. Breeding for P-efficient legume accessions, that make better use of fertilizer P and/or have the ability to mobilize less-available P, requires the development of suitable screening methods for P-use efficiency, and a better understanding of the P-acquisition mechanisms and/or responsible traits that can be improved through plant breeding. Thirdly, it is not yet clear if sufficient levels of soil organic matter (SOM) can be attained under these continuous cropping systems on tropical soils that have sandy top soils and hence a low capacity to protect SOM. Further work is needed to both improve our predictive capacity of long-term SOM changes in these soils. The present paper reports studies in each of these 3 issues above, and focus in particular on methodological aspects.

1.1. Residual effects of legumes in rotation with cereals

The commonly observed residual effects of the inclusion of a legume crop in a cropping system on a subsequent cereal crop can be due to many processes. First there is the possible positive effect due to the net N input in the soil from biological N fixation (provided the quantity of N fixed is larger than the quantity of N exported in grains or crop residues) and or due to the N-sparing effect [15]. Residual effects can also be due to reduction of the weed seed bank (including parasitic weeds such as *S. hermonthica* [16]), possible P mobilization and transfer of P to the subsequent cereal as discussed in the next section, changes of population structure of soil fauna and soil microbes (e.g. mycorrhizal fungi [17], or pathogenic organisms such as nematodes [17, 18], and the more obvious benefits related to the legume biomass that is returned to the soil (mulching, soil organic matter) improving soil physical, chemical and biological characteristics. While usually most attention is given to the N-effects, the non-N effects can be considerable too. Given the often observed low cereal yields and poor response to N-fertilizer obtained in the West-African savannah under continuous cereal cropping, better understanding the nature of the non-N residual effects of legumes could help us to identify some of the key limitations that cause low cereal yields. In the first part of this paper we report on results from a long-term trial in which maize yields in different legume-cereal systems were compared in order to quantify residual effects and to identify the mechanisms behind.

1.2. P mobilization: E- and L-values

The use of legumes in rotations may enhance P availability and crop yields of the subsequent cereal crop [19, 20]. P efficient legumes such as white lupin (*Lupinus albus*) and pigeon pea (*Cajanus cajan*) are characterized by rhizosphere processes (acidification of the rhizosphere and/or excretion of organic acids) that allow these species to mobilize P from sparingly soluble P pools [21, 22]. The P is taken up by the legume and partly returns to the soil through the residues as a high quality organic P source for the subsequent cereal crop.

^{32}P isotopic dilution techniques can be used to determine the capacity of plant species to take up P from sparingly soluble P sources. The use of isotopic methods to determine P availability is well established in P-fertile soils. However, the isotopically exchangeable P in soil suspensions (termed 'E-value') is difficult to assess in tropical soils, which are mostly P-deficient and P fixing (high sorption capacity) soils, with low P concentrations in the soil solution [23]. A method proposed by Maertens *et al.* [24] combines the determination of isotopic dilution with resin extraction to assess the E-value in these soils. The available P determined isotopically in plant growth experiments (termed 'L-value' from labile P), is often difficult to determine accurately because of interference by P from the seed. The aim of the second study was to develop and test enhanced methods to determine the E- and L-values for studies of P speciation in soils and P acquisition by plants, and to use these methods to find out to what extent grain legume accessions can mobilize sparingly soluble P sources.

1.3. Long-term SOM changes

SOM models, if properly validated against long-term data, offer the possibility of estimating changes in SOM levels under current and improved cropping systems without initiating new long-term experiments. Unfortunately, only a limited number of studies [25, 26, 27, 28, 29, 30] have tested SOM models against long-term data from tropical regions. However, scanty information on long-term soil organic carbon (SOC) dynamics hampers validation of SOC models in the tropics.

Many studies have used the ^{13}C natural abundance technique to generate quantitative information on long-term SOM dynamics in temperate and tropical regions (e.g. [31, 32, 33, 34]). However, very few researchers [35, 36] have used ^{13}C abundance data in combination with SOM models. Moreover, the use of the ^{13}C technique has been limited to complete transitions from pure C_3 to pure C_4 vegetation (or vice-versa). Tropical agro-ecosystems often involve a mixture or rotation of C_3 and C_4 plants.

The objectives of the third study were to (1) monitor SOC changes on a 16-year time scale in a field experiment under sub-humid tropical conditions involving cropping systems with mixtures of C_3 and C_4 plants, (2) investigate the usefulness of the ^{13}C tracer technique for such a mixed C_3-C_4 situation, and (3) use the experimental data to test the ROTHC SOM model [37, 38].

2. MATERIALS AND METHODS

2.1. Long-term trial on legume-maize rotations at Sékou, southern Bénin

A long-term trial was established in 1997 at Sékou, Bénin to investigate the response of maize yield to application of organic matter and/or urea. The Sékou site (6°37'N, 2°14'E) is situated in the Derived Savannah (length of growing period 7–9 months) on a Rhodi-Acric Ferralsol according to the WRB classification [39]. In 1999, 22 new treatments in 3 replications (blocks) were implemented in the trial to quantify the contribution to maize yield of in-situ produced organic matter, supplemented or not with N, P, and/or K. These treatments involved three cropping systems involving organic matter produced by a legume: (i) a maize-pigeon pea intercrop, in which a local long-duration pigeon pea (*Cajanus cajan*) variety is planted at the same time as the maize and allowed to grow during the second season and subsequent dry season, (ii) a maize-*Mucuna cochinchinensis* system, in which *Mucuna* is relay cropped, 6 weeks after planting maize, and allowed to grow during the complete second season and subsequent dry season, and (iii) a maize-cowpea rotation (maize in the long rain season, followed by a long-duration low-harvest index cowpea variety Ni-86-650-3 grown in the short rain season). Maize-maize systems were included as controls. Each year a hybrid maize (Oba super 2; 110-120 days duration) was planted in the first season in all treatments, while an open-pollinated maize variety (DMR; 90 days duration) was planted in the second season in the maize-maize system. The head crop maize was each year supplemented with fertilizer N, P, and/or K following Table I. All maize and legume residues were retained on the plots except for 3 treatments (Table I), one in each of the 3 legume-maize systems, where the legume residues were removed from the plots (cowpea residues were removed after the grain harvest; *Mucuna* and pigeon pea at the time of planting the subsequent maize crop). Land preparation consisted of loosening the top 5 cm of the soil with a hoe and removal of weed residues from the plots. The TSP, KCl, and 1/3 of the urea were broadcast at planting, while the remaining 2/3 of the urea was applied by banding (2 cm deep, 5 cm away from the maize) at 6 weeks after planting. Plot size was 6 by 6 m. Maize and cowpea were planted at a density of 75 cm between the lines (8 lines per plot) by 25 cm within the line and thinned to one plant per pocket. *Mucuna* and pigeon pea were planted on lines 75 cm apart in the middle between the maize lines, with an intra-row distance of 25 cm for *Mucuna* and 150 cm for pigeon pea. After maize planting, the plots were regularly weeded by hand (hoe), keeping weed residues on the plots.

TABLE I TREATMENT STRUCTURE OF THE SÉKOU LONG-TERM FIELD EXPERIMENT, BENIN

Cropping system from 1999 Onwards	Annual application rates from 1999 onwards			
	Legume residues removed?	N (as Urea) kg N ha^{-1}	P (as TSP) kg P ha^{-1}	K (as KCl) kg K ha^{-1}
Maize-maize	-	0	0	0
Maize-maize	-	0	10	30
Maize-maize	-	45	10	30
Maize-maize	-	90	10	30
Maize-pigeon pea intercrop	Yes	45	10	30
Maize-pigeon pea intercrop	No	45	10	30
Maize-pigeon pea intercrop	No	45	10	0
Maize-pigeon pea intercrop	No	45	0	0
Maize-pigeon pea intercrop	No	0	0	0
Maize-pigeon pea intercrop	No	0	10	30
Maize-cowpea rotation	Yes	45	10	30
Maize-cowpea rotation	No	45	10	30
Maize-cowpea rotation	No	45	10	0
Maize-cowpea rotation	No	45	0	0
Maize-cowpea rotation	No	0	0	0
Maize-cowpea rotation	No	0	10	30
Maize-*Mucuna* relay cropping	Yes	45	10	30
Maize-*Mucuna* relay cropping	No	45	10	30
Maize-*Mucuna* relay cropping	No	45	10	0
Maize-*Mucuna* relay cropping	No	45	0	0
Maize-*Mucuna* relay cropping	No	0	0	0
Maize-*Mucuna* relay cropping	No	0	10	30

At maize harvest, the total ear and stover fresh weight was determined on a net plot measuring 4 lines of 4-m length. Ear and stover subsamples were taken, weighed, and oven-dried (65°C). After drying, the ears were separated in grains and cobs, and the total dry matter (kg ha^{-1}) of grains, cobs and stovers was calculated. Subsamples of the 2003 maize yield components (grains, cobs, and stover) were analysed for N and P contents using hot acid digestion [40] followed by colorimetric analysis on a Technicon autoanalyser using the indophenol blue method [41] for N and the ascorbic acid method [42] for P. The 2003 samples were also analysed for K content using dry ashing followed by K analysis with a flame photometer. Statistical analysis of maize grain yields was made with a mixed model ANOVA using SAS [43].

Prior to the implementation of the legume-maize rotations, the experimental plots had been used to investigate the response of maize to application of organic matter and/or urea. In 1997, at the start of the trial, 18 treatments had been imposed, encompassing different quantities of nitrogen added in the form of urea (0, 22.5, 45, 67.5 or 90 kg N ha^{-1}), of organic matter (90 kg N ha^{-1} as *Leucaena leucocephala* or *Senna siamea* residues), or of a mixture of urea and residue (each at 45 kg N ha^{-1}). These 18 treatments received a blanket application of 30 kg P ha^{-1} as TSP in 1997. Another 4 treatments received no P or urea or organic amendment. Experimental details and results of the first year of the experiment and the initial soil properties were given by Vanlauwe *et al.* [44]. In 1998, all the plots had been cropped again with maize but without any addition of fertilizer to assess residual effects of the 1997 treatments. As no statistical significant residual effects were observed, the new treatments imposed in 1999 were randomly allocated to the 1997 treatments, with the only restriction that the 4 new treatments that receive no N, P and K were randomly allocated to the 4 treatments that did not receive TSP in 1997.

2.2. Plant-available phosphorous for maize and legumes in P-deficient soils: comparison of E- and L-values

The isotopically available P was determined in 3 soils from the northern Guinea savannah (NGS) in Nigeria: Danayamaka (11°21'N 7°51'E), Kasuwan Magani (10°24'N 7°42'E) and Shika (11°11'N 7°35'E). The topsoil was sampled (0-20 cm), dried and sieved (2 mm). All soils were low in available P (P concentration in soil solution < 0.006 mg P L^{-1}; Olsen-P < 2 mg P kg^{-1}).

E-values were determined in a laboratory incubation experiment. Soil was labelled with carrier free ^{32}P (20 MBq kg^{-1}) and the dilution of the added ^{32}P isotope was weekly determined using a method adapted from Maertens *et al.* [24].

To determine L-values, maize (Oba Super I), 2 cowpea cultivars (Dan-illa and IT-82D-716) and 2 soybean cultivars (TGx 1448-2E and TGM 1293) were grown in pots (2 kg soil per pot) in a nethouse. Treatments included a control, TSP addition at the equivalent rates of 15 and 45 kg P ha^{-1} and Togo PR addition at an equivalent rate of 45 kg P ha^{-1}. For maize, nitrogen was applied as NH_4NO_3 at an equivalent rate of 90 kg N ha^{-1}; and legumes were grown both with and without the N addition. A basal nutrient solution containing other macronutrients (Ca, Mg, K and S) and micronutrients (Fe, Cu, Zn, Mn, Co, Mo and B) was added to all pots. Soils were labeled with carrier free ^{32}P (500 kBq kg^{-1}). Plants were harvested 35 days after planting; L-values were calculated using Eq. (1):

$$L = \frac{D}{\left(\frac{^{32}P_{shoot}}{^{31}P_{dfsoil}}\right)} = \frac{D}{\left(\frac{^{32}P_{shoot}}{^{31}P_{shoot} - {}^{31}P_{dfseed}}\right)} \quad (1)$$

where L is the L-value [mg P kg^{-1} soil]; D the applied dose of ^{32}P [Bq kg^{-1} soil]; $^{32}P_{shoot}$ the activity in the shoot biomass [Bq g DM^{-1}]; $^{31}P_{shoot}$ the total amount of P in the shoot biomass [mg P g DM^{-1}]; the $^{31}P_{dfsoil}$ the amount of P in the shoot biomass taken up from the soil [mg P g DM^{-1}] and $^{31}P_{dfseed}$ the amount of P in the shoot biomass translocated from the seed [mg P g DM^{-1}].

To estimate the amount of P in the shoot that was translocated from the seed, a separate experiment was conducted in which plants were grown in washed P-free sand (2.5 kg per pot). Solutions of KH_2PO_4 with increasing P concentrations and known initial specific activity were added to the pots and plants were grown similarly as in the L value experiment. By measuring the specific activity in the biomass at harvest (35 DAP), the proportion of seed P in the shoot biomass could be calculated using Eq. (2):

$$^{31}P_{dfseed} = {}^{31}P_{shoot} \cdot \left(1 - \frac{SA_{shoot}}{SA_{in}}\right) \quad (2)$$

with SA_{shoot} the specific activity in the shoot biomass (ratio of ^{32}P over ^{31}P) [Bq mg P^{-1}] and SA_{in} the initial specific activity in the applied P solution [Bq mg P^{-1}].

2.3. Changes of soil organic carbon (SOC) and ^{13}C signature in a long-term trial at Ibadan, southwestern Nigeria

A field experiment was established in 1986 to compare *Leucaena leucocephala* and *Senna siamea* alley cropping (AC) systems with a control system without trees. The experiment was established on an Episkeleti-Ferric Lixisol (Chromic) [39] on the experimental farm of the International Institute of Tropical Agriculture (IITA) in Ibadan, SW-Nigeria (7°30'N, 3°54'E; derived savannah; length of growing period 7–9 months). The trial was set up as a randomized complete block design with 5 replications and 6 treatments: the above-mentioned 3 cropping systems with and without fertilizer. The experimental plots measured 19.5 by 10 m. Yearly additions of urea (120 kg N ha^{-1}), single super phosphate (40 kg P ha^{-1}) and muriate of potash (25 kg K ha^{-1}) comprised the fertilizer treatments. From 1994 onwards, these levels were decreased to 60 kg N ha^{-1}, 16.2 kg P ha^{-1}, and 15 kg K ha^{-1}. Full details of the management of this long-term experiment are given by Van der Meersch *et al.* [45] for the 1986–1990 period and Vanlauwe *et al.* [46] for the 1986-2002 periods.

While initially this trial aimed at providing information on the performance of the alley cropping systems and their N-cycling, the trial was later continued because it gives valuable insights into long-term soil processes such as SOM dynamics under tropical conditions. Since 1986, the experimental plots with AC systems and the control have been continuously cropped with maize during the first growing season (May–July) and cowpea during the second growing season (September-November), except for 1992 when the field was left fallow in the second season. Soil samples were taken in 1986 (0–5 and 5–10 cm depths, composite sample of 20 cores per plot), May 1997, and April 2002 (last two samplings at 0–5 and 5–15 cm depths, composite sample of 24 cores per plot). The 1986 soil samples were sieved through a 2-mm sieve and analyzed for % C using the Walkley and Black procedure. The 1997 and 2002 soil samples were sieved through a 4-mm sieve, ground with a ball-mill, and analyzed for % C and ^{13}C natural abundance simultaneously with a Europa Scientific ANCA-SL stable isotope mass spectrometer. The SOC content was expressed in Mg C ha^{-1} present in an equivalent soil mass of 1700 Mg ha^{-1}. Its equivalent thickness averaged 12 cm and varied slightly between sampling times and treatments due to bulk density differences. Average $\delta^{13}C$ values were calculated likewise for the same equivalent soil mass. The SOC contents and $\delta^{13}C$ values (1997 and 2002 data) were statistically analyzed with a mixed-model ANOVA considering cropping system, fertilizer level, and their interaction as fixed effects and replicates (blocks) as a random effect [43].

Changes in SOC levels and $\delta^{13}C$ were simulated using the ROTHC-26.3 model [37, 38] using the quantities of weed and crop residues and tree prunings, as observed over the years since the start of the trial, as input to the model. ROTHC-26.3 is a model of the turnover of organic carbon in soils on a time scale of years to centuries. It allows for the effects of clay content, temperature, moisture content, and plant cover on decomposition. Long-term averages of monthly mean air temperature, rainfall, and open pan evaporation for Ibadan were given as inputs to the model. Two sets of simulation runs were made: one with all decomposition rate constants set to the values fixed by the model authors [38]; and one with all decomposition rate constants doubled and all other parameters as set by the model authors. As the present model cannot calculate changes in $\delta^{13}C$ in the soil carbon, 13 separate simulations were made to calculate the build-up of carbon derived respectively from the maize, cowpea residues, and tree the 1st, 2nd, 3rd and 4th tree pruning, and the 7 weeding operations within a year. The simulated build-up of "new carbon", i.e. of carbon which entered the soil after the start of the experiment, was obtained by adding up the simulated SOC originating from each of these 13 plant litter inputs within a year. The $\delta^{13}C$ value of this "new carbon" at a given time was calculated as a weighted average (weighted by the quantities of C returned to the soil). Diels *et al.* [47] provided full details how the simulations were performed.

3. RESULTS AND DISCUSSION

3.1. Long-term trial on legume-maize rotations at Sékou, southern Bénin

The long-term trial at Sékou was designed to test to what extent the different legume-maize systems are able to improve yield and response to urea, compared to continuous maize cropping. While yields varied considerably across the years (Fig. 1), the effect of the cropping system was remarkably constant in time: Maize yields in the maize/*Mucuna* system were always about 2000 kg ha^{-1} higher, compared with the maize/cowpea, and maize/maize systems, which had very similar yields. Removing the *Mucuna* mulch from the maize/*Mucuna* plots at the time of planting for the next season crop decreased the maize yield by about 400–800 kg ha^{-1} only in the period 2000-2003 (*results not shown*), suggesting that the combined live-mulching, weed suppression ability, and rotational effects of the mucuna might cause a large part of the 2000 kg ha^{-1} yield gain over the maize/maize or maize/cowpea systems. The maize/pigeon pea (cajanus) system offered a maize grain yield advantage of about 800 kg DM ha^{-1} over maize/maize or maize/cowpea in 2001, 2002, and 2003, while there was no or little advantage in 2000 and 2004.

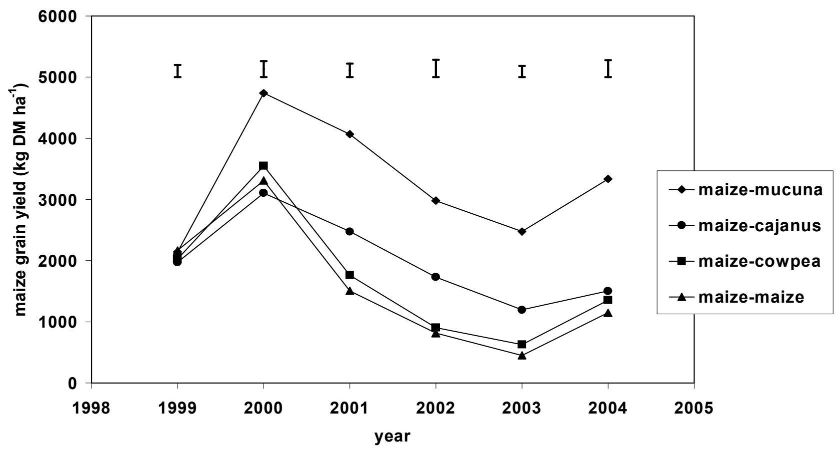

FIG.1. First season maize grain yields in the 4 cropping systems in the Sékou long-term trial. The data shown are averages of the "+R 0N 10P 30K" and "+R 45N 10P 30K" treatments in each system. Error bars indicate the standard errors of the treatment means.

The response to urea-N in the maize/maize system was non-linear from 2000 onwards, with little yield response beyond a urea application of 45 kg N ha^{-1} (Fig. 2). The urea-N recovery in 2003, as calculated from the N uptake in the above-ground biomass in these N response-curve treatments, was only 16% when 45 kg N ha^{-1} was applied, and as low as 10% when 90 kg N was applied.

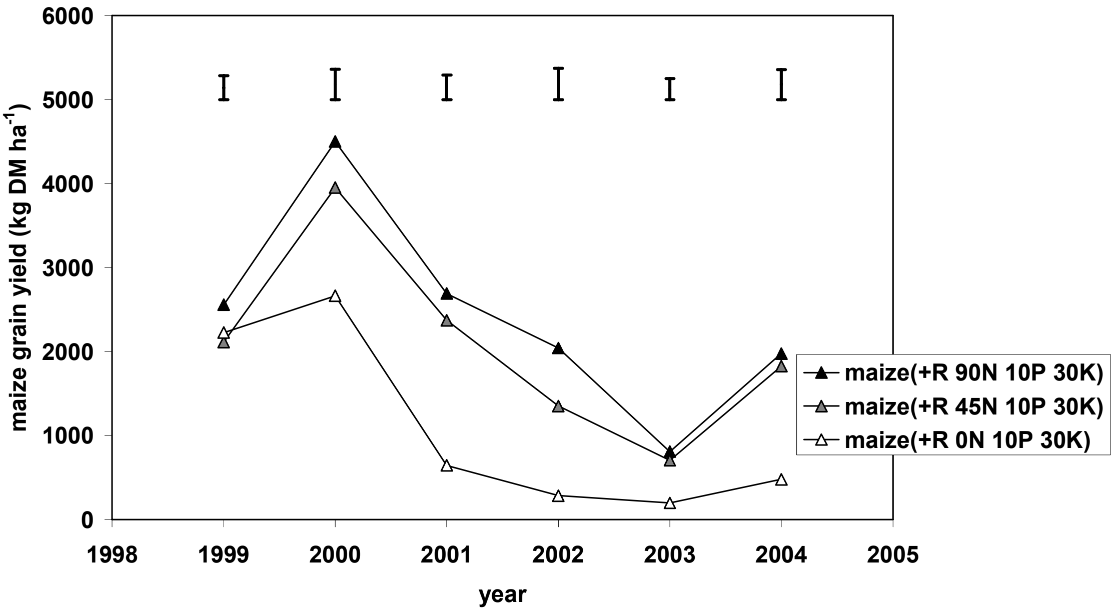

FIG.2. First season maize grain yields in N-response treatments (0, 45, and 90 kg N ha^{-1} as urea) in the maize/maize system in the Sékou long-term trial. The numbers in the treatment labels (e.g. 45N 10P 30K) indicate the fertilizer rate in kg N, P, K ha^{-1}; "+R" signifies that the legume residues were retained on the plots. Error bars indicate the standard errors of the treatment means.

To find out to which extent N, P, and K were limiting nutrients, 2003 maize grain yields in all treatments were plotted against the uptake of N, and P in the above-ground maize biomass. For N, data points from treatments with grain yields ranging between 0 and 1000 kg ha^{-1} fell close to the limit of maximum N dilution (and maximum N utilization efficiency). For treatments with higher yields, the points were located slightly away from the maximum dilution limit, indicating that another limiting factor came into play as well (Fig. 3). For P, the data points from the low-yielding plots were along the limit of maximum P accumulation, and fell slightly away from in higher yielding treatments (*graph not shown*). The closeness to the limit of maximum N dilution for the low-yielding treatments, among which were also the 45 urea-N and 90 urea-N treatments of the N response curve in the maize/maize system, indicated that N was the overruling, if not the only limiting factor. The fact that low N uptake efficiency was associated with high fertilizer N utilization efficiency is remarkable. The heavy rainfall at the beginning of the growing season (and probable leaching of N), in combination with lack of rainfall after the application of the second dose of urea (increasing risks of urea volatilization and preventing N uptake from the top soil), could explain this result. Nutrient analysis of the grains and stover samples for the other years will be carried out to confirm if this also happened in years with more favourable rainfall distribution.

The experimental treatments were also designed to study effects of P and K addition in the maize/legume systems. However, yield response to P and K fertilizer was small and variable over the years, and not consistent across the 3 systems. If P and K deficiencies developed over time, these should have been observed in the results of the most recent year (2004), with favourable rainfall distribution and good yields and hence high demand for P and K. The results, however, do not show evidence of response to P and K fertilizer, apart from a possible P-response in the maize/*Mucuna* system (Fig. 4).

Low fertilizer N use efficiencies as observed in the continuous maize cropping systems are a common problem in intensive cereal cropping in West Africa. The results indicated possible options to increase production in such continuous maize-based production systems. Over the last 5 years of the trial, maize/*Mucuna* relay cropping gave consistently a 2000 kg ha^{-1} yield increase relative to maize/maize cropping, and most of this yield gain was still achieved even when *Mucuna* residues were removed from the plots when planting the next year's maize crop. Although these benefits of *Mucuna* have been also demonstrated before, land-constrained farmers will not easily grow a crop that gives no direct returns (grain). Maize/*Mucuna* treatment was, therefore, rather included in the trial as a reference system with which some promising cereal-grain legume systems can be compared. The data, however, showed that including a grain legume such as cowpea in the maize/cowpea system offers no gain over maize/maize cropping. Some yield gain, although far less than with maize/*Mucuna*, was observed in the maize/pigeon pea (cajanus) system. In this trial we used a local long-duration pigeon pea variety that each year showed stem rot at the tree base and die-off during the dry season, thus leading to reduced soil cover and biomass production. Improved and local pigeon pea accessions are currently being screened to identify genotypes that combine high grain yield with good biomass production and that maintain effective ground cover until the end of the rainy season.

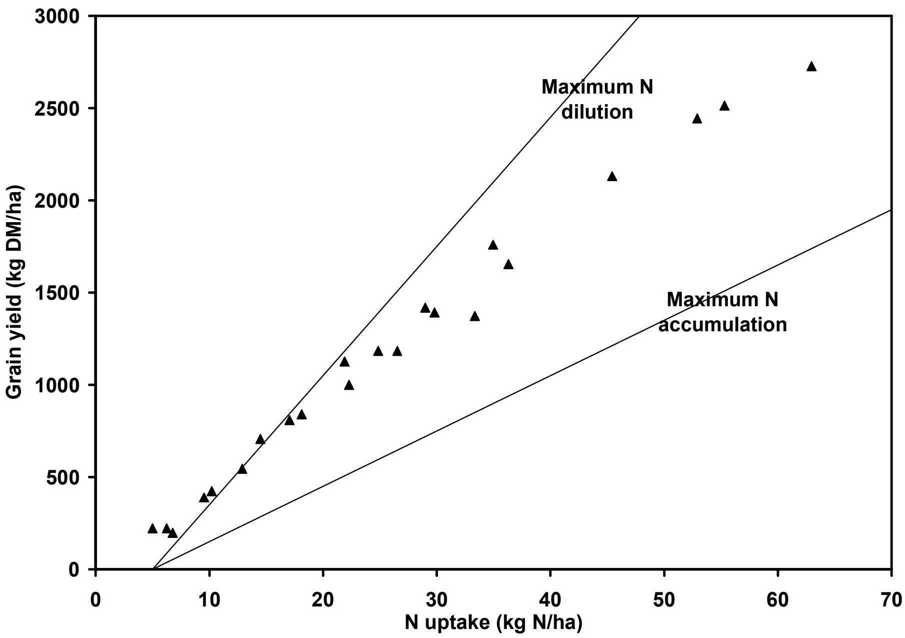

FIG.3. Plot of 2003 maize grain yield vs. N uptake in the above-ground maize biomass in all treatments at Sékou. The solid lines represent the limits of maximum N dilution and maximum N accumulation for maize according to Janssen et al. [48].

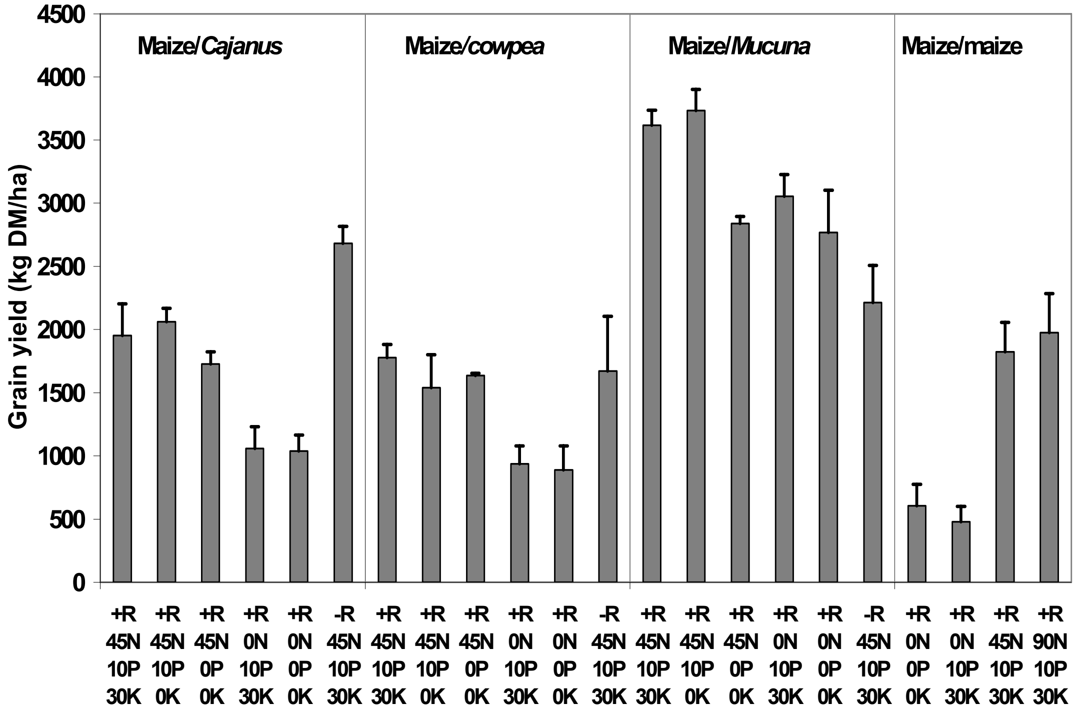

FIG.4. First season maize grain yields in Sékou (year 2004). Error bars indicate the standard error of the treatment means. The numbers in the treatment labels (e.g. 45N 10P 30K) indicate the fertilizer rate in kg N, P, K ha^{-1}; "+R" signifies that the legume residues were retained on the plots, while "-R" indicates they were removed.

3.2. Plant-available phosphorous for maize and legumes in P-deficient soils: comparison of E- and L-values

Relative shoot total P content was well correlated with the E-value (R^2 = 0.86 for maize and R^2 = 0.77 for cowpea), indicating that the E-value assessed by the method of Maertens *et al.* [24] correctly reflects treatment effects on both plant species in these soils (*graph not shown*).

Both maize and cowpea showed a response to the applied P as TSP (Fig. 5). Shoot dry weight and P uptake were significantly larger in TSP and PR treatments. The error on the L-value due to the seed P contribution was expected to be particularly large in the control treatments in view of the large quantity of P in the seed relative to the shoot total P at the time of sampling (Fig. 5).

The shoot P derived from the seed could be related to the total shoot P content by an exponential equation Fig. 6). This relationship allowed correction of L-values for seed P contribution. As such, L-values can be determined more accurately in soils where the isotopically exchangeable P is small and limiting for plant growth.

L- and E-values were correlated but not identical; L:E ratios varied between 1.0 and 2.1 for maize and between 1.2 and 3.3 for cowpea (Fig. 7). L-values for cowpea were only significantly larger than L-values for maize in the Kasuwan Magani soil for the control treatment and for the treatment with TSP addition at a rate equivalent to 15 kg P ha^{-1}. As such, the Kasuwan Magani soil contains sparingly soluble P, which could be accessed by the cowpea. At a rate equivalent to 45 kg P ha^{-1} however, L-values did not differ significantly between maize and cowpea, which suggests that the legume only mobilizes less available P when P deficiency occurs.

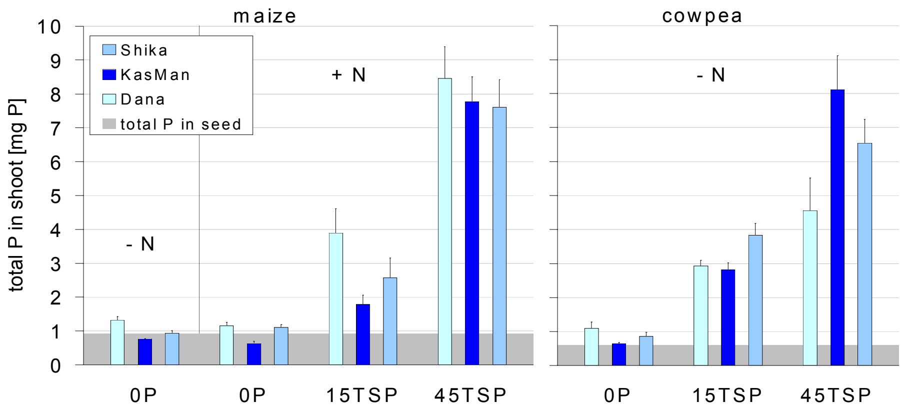

FIG.5. Shoot total P content for maize and cowpea at 35 days after planting in 3 soils as affected by TSP application at rates equivalent to 15 and 45 kg P ha^{-1}. Error bars represent 1 standard error of the mean value.

Although cowpea had more accessibility to soil P, the uptake of P was not increased. Other factors, such as the P concentration in the soil solution (P intensity) may have been limiting for plant growth. Rhizosphere processes such as organic acid exudation or pH changes may have caused sparingly soluble soil P to become exchangeable, but without significantly increasing the renewal of P concentration in the soil solution , which is necessary to raise P uptake. This suggests that raising P intensity through P fertilization may be essential to obtain adequate yields on the soils of the Nigerian NGS.

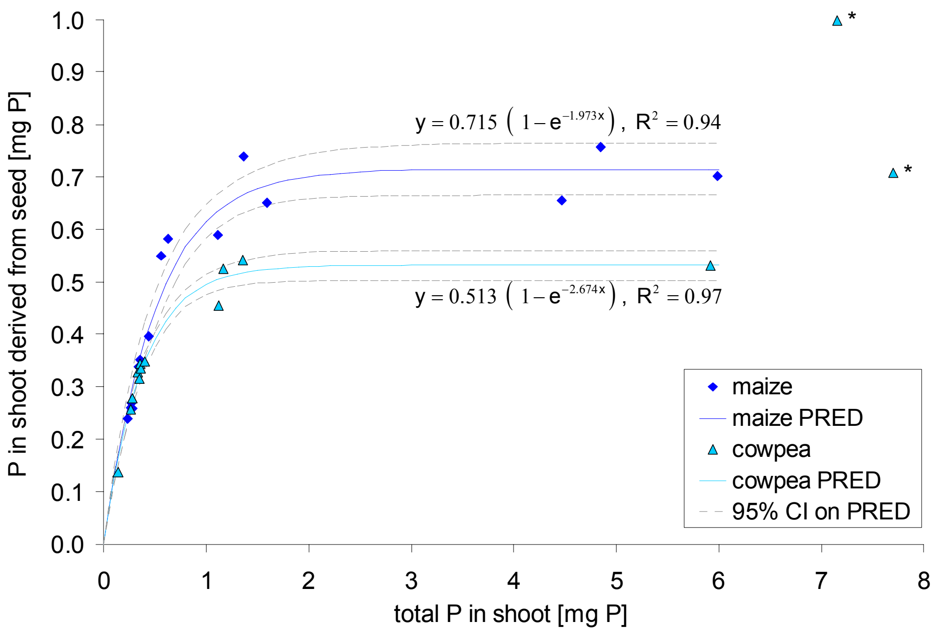

*FIG.6. P in shoot derived from seed as a function of shoot total P content for maize and cowpea. Full lines represent predicted values using non-linear regression; dotted lines represent upper and lower 95% confidence bounds for the mean predicted value. *Two data points for cowpea not considered in regression analysis.*

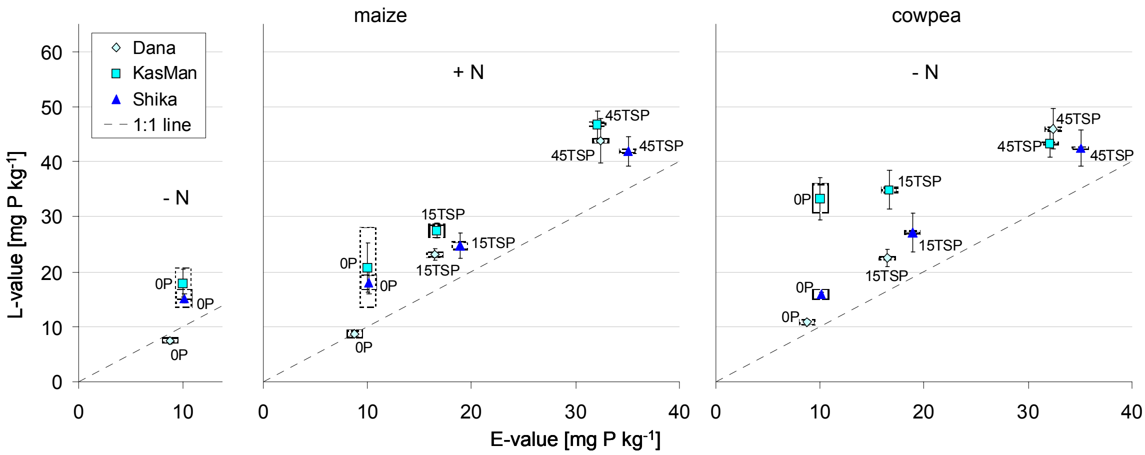

FIG.7. Comparison of E-values and L-values for maize and cowpea in 3 soils of the Nigerian NGS as affected by TSP application at rates equivalent to 15 and 45 kg P ha^{-1}. Error bars represent 2 standard errors of the mean L-value. Rectangular bars represent the average 95% confidence interval on the L-value, calculated using the upper and lower 95% confidence bounds for the mean predicted shoot P translocated from the seed.

3.3. Changes of soil organic carbon and ^{13}C signatures in a long-term trial at Ibadan, southwestern Nigeria

Soil organic carbon (SOC) levels declined in all treatments. The decline was most pronounced in the no-tree control treatments with continuous maize and cowpea cropping, where SOC levels dropped from the initial 15.4 Mg C ha^{-1} to 7.3–8.0 Mg C ha^{-1} in the 0–12 cm topsoil in 16 years (Table II). In the two continuously cropped alley cropping (AC) systems, one with *Leucaena leucocephala* and another with *Senna siamea* trees, SOC levels dropped to 10.7–13.2 Mg C ha^{-1}. Compared to the no-tree control treatments, an annual application of an additional 8.5 Mg ha^{-1} (dry matter) of plant residues, mainly tree prunings, led to an extra 3.5 Mg C ha^{-1} (~0.2% C) in the 0–12 cm top soil after 11 years, and 4.1 Mg C ha^{-1} after 16 years. The addition of NPK fertilizer had little effect on the quantities of above-ground plant residues returned to the soil, and there was no evidence that the fertilizer affected the rate of SOC decomposition.

The fact that both C_3 and C_4 plants returned organic matter to the soil in all cropping systems, but in different proportions, led to clear contrasts in the ^{13}C isotopic signatures ($\delta^{13}C$ values) in the SOC (Table II). This ^{13}C information, together with the measured SOC contents, was used to test the ROTHC model. SOC decomposition was very fast, illustrated by the fact that all decomposition rate constants in the model had to be doubled in order to simulate the measured contrasts in SOC contents and $\delta\ ^{13}C$ between the AC treatments and the no-tree controls (Table III, Fig. 8). It was hypothesized (1) that the pruning materials from the legume trees and/or the extra rhizodeposition from the tree roots in the AC treatments accelerated the decomposition of the SOC present at the start of the experiment (true C-priming), and/or (2) that the physical protection of microbial biomass and metabolites by the clay fraction on this site, having a sandy top soil, in which clay minerals are mainly of the 1:1 type, is lower than that assumed by the model.

TABLE II. TREATMENT MEANS OF OBSERVED SOC CONTENTS AND $\delta^{13}C$ VALUES FOR THE 1700 Mg ha^{-1} EQUIVALENT SOIL MASS (~0–12 cm) IN 1997 AND 2002 FOR THE 3 CROPPING SYSTEMS AT 2 FERTILIZER LEVELS (AFTER [47])

	Mg C ha^{-1}		δ ^{13}C ‰	
Cropping system	1997	2002	1997	2002
No-tree control -NPK	8.9	7.3	-22.3	-22.6
No-tree control+NPK	8.5	8.0	-22.4	-21.4
Leucaena AC –NPK	11.3	10.7	-24.4	-24.3
Leucaena AC +NPK	12.6	11.6	-24.8	-24.3
Senna AC –NPK	11.6	11.6	-24.8	-24.8
Senna AC +NPK	13.4	13.2	-24.6	-24.8
SED(1)	0.7	1.1	0.3	0.2
Treatment differences:				
AC-control(2)	3.5 ± 0.9	4.1 ± 1.4	-2.29 ± 0.35	-2.47 ± 0.24
Senna AC - *Leucaena* AC (3)	0.5 ± 1.0	1.2 ± 1.6	-0.10 ± 0.40	-0.53 ± 0.28

1 SED: Standard error of the difference.
2 Average difference between the four AC systems and the two control systems.
3 Average difference between the two *Senna* AC systems and the two *Leucaena* AC systems.

TABLE III. SIMULATED SOC STOCKS AND $\delta^{13}C$ VALUES IN THE 1700 MG HA^{-1} EQUIVALENT SOIL MASS (~0-12 CM) IN 1997 AND 2002 FOR THE 3 CROPPING SYSTEMS AT 2 FERTILIZER LEVELS. ALL DECOMPOSITION RATE CONSTANTS IN ROTHC WERE DOUBLED, WHILE ALL OTHER RATE CONSTANTS WERE LEFT UNALTERED [47]

	Mg C ha^{-1}		δ ^{13}C ‰	
Cropping system	1997	2002	1997	2002
No-tree control -NPK	11.0	10.3	-22.5	-21.7
No-tree control+NPK	10.6	9.9	-22.1	-21.2
Leucaena AC –NPK	15.9	15.2	-24.6	-24.7
Leucaena AC +NPK	16.0	15.4	-24.7	-24.9
Senna AC –NPK	14.6	13.0	-24.9	-24.7
Senna AC +NPK	15.6	13.8	-24.5	-24.5
Treatment differences:				
AC-control(1)	4.8	4.3	-2.4	-3.3
Senna AC - *Leucaena* AC (2)	-0.8	-1.9	0.0	0.2

(1) Average difference between the four AC systems and the two control systems.
(2) Average difference between the two *Senna* AC systems and the two *Leucaena* AC systems.

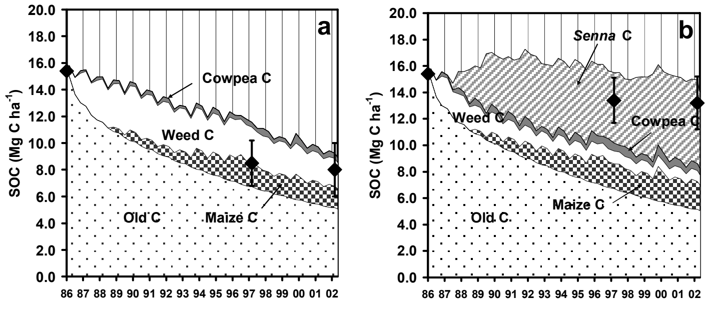

FIG.8. Simulated (lines) and observed (diamonds) SOC contents in the 1700 Mg ha^{-1} equivalent soil mass (~0–12 cm) in the no-tree control (with NPK) treatment (a) and the Senna AC system (with NPK) (b). Simulations were performed with doubled decomposition rate constants [47].

4. CONCLUSIONS

Developing improved technologies for sustainable intensification of cereal-grain legume systems for small-scale farmers in the West African moist savannah requires addressing several constraints in an integrated way. In this paper we presented research work done on three issues, with emphasis on methodological aspects. The work led to the following conclusions:

(1) Fairly low maize yields were found in continuous maize cropping systems (maize/maize), poor response to N fertilizer beyond 45 kg N ha^{-1}, and no evidence that P and K were limiting crop yield in the Sékou long-term trial. Over the last 5 years of the trial, maize/*Mucuna* relay cropping gave consistently a 2000 kg ha^{-1} yield increase relative to maize/maize cropping, and most of this yield gain was preserved even when *Mucuna* residues were removed from the plots when planting the next year's maize crop. Some yield gain, although far less than with maize/*Mucuna*, was observed in the maize/pigeon pea system. The maize/cowpea system offered no maize yield gain over maize/maize cropping.

(2) Enhanced isotopic methods to determine the plant available P allowed us to test the hypothesis that certain plant genotypes can mobilize sparingly-soluble P. In one out of the 3 soils studied, we found that cowpea could access sparingly-soluble soil P that is unavailable to maize but this mechanism was only observed when P deficiency occurred. These results confirm the superior P efficiency of some legume genotypes, which may lead to benefits of improved P availability by the incorporation of legumes in rotation systems.

(3) Soil organic carbon levels declined in all treatments in a 16-year field experiment in SW-Nigeria. The decline was most pronounced in the no-tree control treatments with continuous maize and cowpea cropping, where SOC levels dropped from the initial 15.4 Mg C ha^{-1} to 7.3–8.0 Mg C ha^{-1} in 16 years (SOC content in 1700 Mg ha^{-1} equivalent soil mass). In the two continuously cropped alley cropping (AC) systems (*Leucaena* and *Senna*), the SOC levels declined to levels between 10.7 and 13.2 Mg C ha^{-1}.

(4) In the same 16-year trial, the ^{13}C natural abundance technique yielded useful information to test SOM models in sub-humid tropical environment under a complex pattern of cropping systems, in which both C_3 and C_4 plants were intercropped/rotated. The obtained contrasting $\delta^{13}C$ values of the SOC resulted from the fact that the relative OM contribution of the C_3 and C_4 components differed significantly between the treatments compared.

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CASE STUDIES RELATED TO THE MANAGEMENT OF SOIL ACIDITY AND INFERTILITY IN THE WEST-AFRICAN MOIST SAVANNAH

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Abstract

Although the soil pH and base status of the soils in the West African Moist Savannah Zone (MSZ) are usually favourable, their buffer capacity is usually low, indicating that while soil acidity may not be a problem initially, inappropriate management of these soils may induce soil-acidity-related problems in the medium to long term. The current paper addresses 3 topics that are closely related to the management of soil pH (acidity) in the West African MSZ. A first experiment addressed the release of P from low reactivity phosphate rock (PR) by mixing it with various N fertilizers. Mixing ammonium-sulphate, urea, and calcium-ammonium nitrate with PRsubstantially enhanced the soil Olsen-P content, but not for soils with an initial pH above 5.5, while potassium nitrate did not change the Olsen-P content. Changes in soil pH could be predicted based on the production of nitrate from ammonium (nitrification) and the soil buffer capacity. A second experiment examined the changes in topsoil pH as affected by long term management based on the application of organic inputs derived from hedgerow trees (*Leucaena leucocephala* and *Senna siamea*), fertilizer, or both. Maize crop yields declined steadily over the 16 years studied, but the least so in the *Senna* + fertilizer treatment where in 2002 still 2.2 t ha^{-1} of maize were obtained. The fertilizer only treatment led to a yield of 0.4 t ha^{-1} in 2002, while the absolute control without any inputs yielded a mere 40 kg ha^{-1} in the same year. Nitrogen fertilizer use efficiency was usually higher in the *Senna* treatment compared to the control or the *Leucaena* treatment. Interactions between fertilizer and organic matter additions were negative for the *Leucaena* treatments in the first three years, and positive for the *Senna* treatment in the last 6 years. Trees had a positive effect on the maintenance of exchangeable cations in the topsoil. Exchangeable Ca, Mg and K - and hence ECEC - were only slightly reduced after 16 years of cropping in the tree-based systems, and even increased in the *Senna* treatments. Soil pH_{KCl} values decreased at least 0.5 units in the control and *Leucaena* treatments, but only slightly in the *Senna* treatments. In general, the soils that received fertilizer during the trial were more acid (0.2 to 0.3 units) than the ones not receiving fertilizer. From the above, the *Senna*-based alley cropping system with fertilizers is the more resilient one, both in terms of crop yields and soil fertility status. A third experiment evaluated the potential of deep-rooting hedgerow trees to recycle basic cations from the subsoil and increase the topsoil pH. Topsoil Ca content, effective cation exchange capacity, and pH were substantially higher under *Senna* than under *Leucaena leucocephala*, *Gliricidia sepium*, or the no-tree control plots in sites with soils having a Bt horizon rich in exchangeable Ca. It was shown that this effect was largely related to the recovery of Ca from the subsoil by the *Senna* trees. The lack of increase in Ca accumulation under the other species was related to (i) potential recovery of Ca from the topsoil itself and/or (ii) substantial Ca leaching. The accumulation of Ca in the topsoil under *Senna* had a marked effect on the topsoil pH, the latter increasing significantly compared with the *Leucaena*, *Gliridia*, and no-tree control treatments.

1. INTRODUCTION

Almost the entire moist savannah zone (MSZ) in West Africa is located in the lowland tropics (elevation <800 m). The lowland MSZ is characterized by a length of growing period of 150–270 days [1]. The MSZ has been subdivided into the Derived savannah (DS), Southern Guinea savannah (SGS), and Northern Guinea savannah (NGS) sub-zones (Fig. 1), each characterized by a specific range of lengths of growing period, decreasing from South to North [1]. Due to the heterogeneous biophysical and socio-economic characteristics of the sub-zones, the Eco-regional Program for the Humid and Sub-Humid Tropics of Sub-Saharan Africa (EPHTA) has identified benchmark areas in each of the sub-zones, in which impact-oriented research and development activities were concentrated [2]. Benchmark areas contain all the biophysical and socio-economic gradients found in the particular agro-ecozone to which they belong to and as such enable the extrapolation of technologies developed and tested in the benchmarks to the complete agro-ecozone.

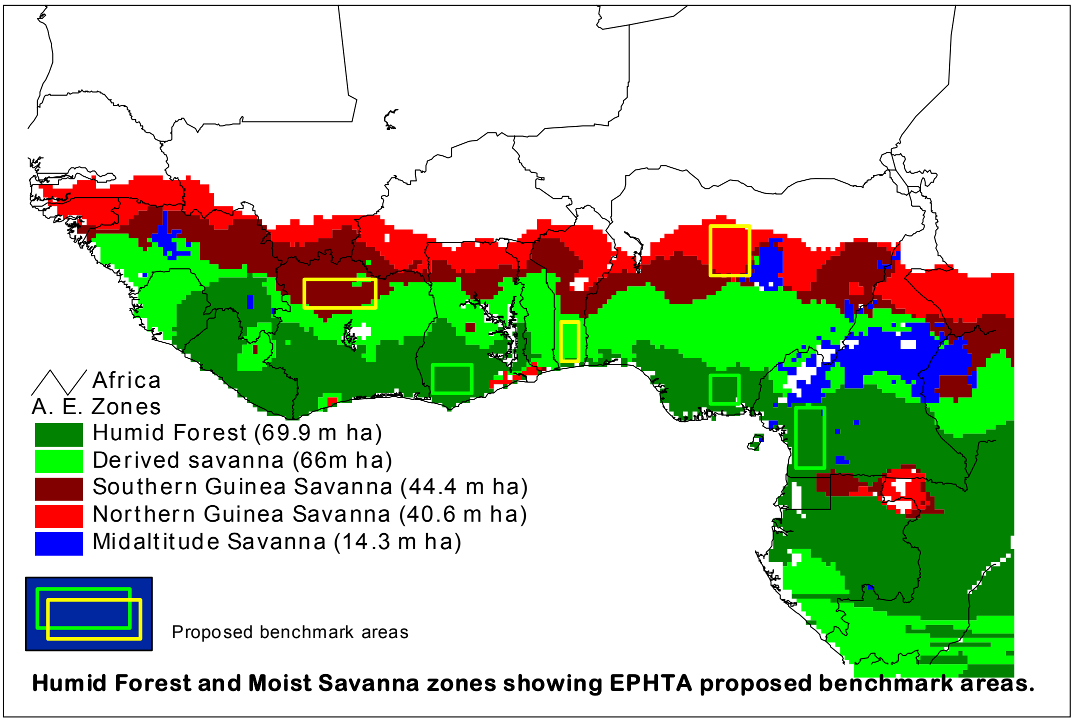

FIG.1. The various agro-ecozones of the Moist Savannah in West Africa following the EPHTA program [2], indicating the benchmark areas in each agro-ecozone.

Most of the soils in the MSZ belong to the 'Lixisol' soil association [3] and can often be differentiated following their position in the landscape. A typical toposequence consists of shallow and/or gravelly soil (Plinthosols or soils with a petroferric phase) on the interfluve crests, deeper soils (Luvisols or Lixisols) on the valley slopes and hydromorphic soils (Gleysols and Fluvisols or soils with gleyic properties) near the valley bottom [4]. Soils on the crest usually have an indurated plinthic layer at low depths restricting root development. Both the crest and slope soils usually have a sandy to loamy topsoil with weak surface structure, making them susceptible to erosion and surface crusting [5]. Soils near the valley bottom are usually heavier and more fertile, but gleyic properties near the soil surface may adversely affect crop growth by excess rainfall in absence of drainage [6]. The dominant soils in the southern part of Benin, Togo, and South-western Nigeria in the DS are deep red Ferralic Nitisols developed on sedimentary deposits, often referred to as 'terre de barre'. Jones and

Wild [5] already reported that K deficiency may appear rapidly after clearing the fallow vegetation on the 'terre de barre' soils. Although the area covered by these soils is rather limited, this region is densely populated and contributes significantly to agricultural production in the respective countries.

Although the soil pH and base status, enriched by seasonal dust deposits by Harmattan winds, of most soils in the West African MSZ are usually favourable (Table I), their buffer capacity is usually low (Fig. 2). This indicates that while soil acidity may not be a problem initially, inappropriate management of these soils may induce soil-acidity-related problems in the medium to long term. The current paper presents results from investigations addressing 3 topics that are closely related to the management of soil pH (acidity) and infertility in the West African MSZ. A first study addresses the release of P from a locally available phosphate rock (PR) of low reactivity, a second study examines the changes in topsoil pH as affected by long term management based on organic inputs, fertilizer, or both, while a third study evaluates the potential of deep-rooting hedgerow trees to recycle base cations from the subsoil and increase the topsoil pH.

TABLE I. SELECTED SOIL (0–10 cm) CHARACTERISTICS OF THE FIELDS IN THE DERIVED SAVANNAH AND THE NORTHERN GUINEA SAVANNAH BENCHMARK VILLAGES[a]. ADAPTED FROM VANLAUWE ET AL. [38]

	Derived savannah	Northern Guinea savannah	SE
Chemical characteristics			
Organic C (g kg^{-1})	9.3	6.3	0.4
Total N (g kg^{-1})	0.70	0.49	0.03
C-to-N ratio	13.2	12.9	0.3
Olsen-P (mg kg^{-1})	10.7	5.4	0.9
Ca^{2+} content ($cmol_c$ kg^{-1})	4.79	2.88	0.45
Mg^{2+} content ($cmol_c$ kg^{-1})	1.30	0.66	0.11
K^+ content ($cmol_c$ kg^{-1})	0.27	0.32	0.02
Exch. acidity ($cmol_c$ kg^{-1})	0.49	0.72	0.06
ECEC[b] ($cmol_c$ kg^{-1})	7.00	4.79	0.57
pH(H_2O)	6.7	6.0	0.1
pH(KCl)	5.2	4.9	0.1
Physical characteristics			
Gravel content (g kg^{-1})	10	4	2
Sand content (g kg^{-1})	785	584	14
Silt content (g kg^{-1})	104	288	9
Clay content (g kg^{-1})	111	128	10
Soil organic matter			
O2000 fraction (g kg^{-1})	0.09	0.04	0.01
O250 fraction (g kg^{-1})	0.19	0.17	0.01
O53 fraction (g kg^{-1})	0.27	0.38	0.03
POM[b] (g kg^{-1})	0.55	0.59	0.04

[a] Derived savannah: Zouzouvou and Eglimé (12 fields each); Northern Guinea savannah: Danayamaka (14 fields) and Kayawa (13 fields).
[b] 'ECEC': 'Effective Cation Exchange Capacity'; 'POM': 'Particulate Organic Matter'.
'SE': 'Standard Error.

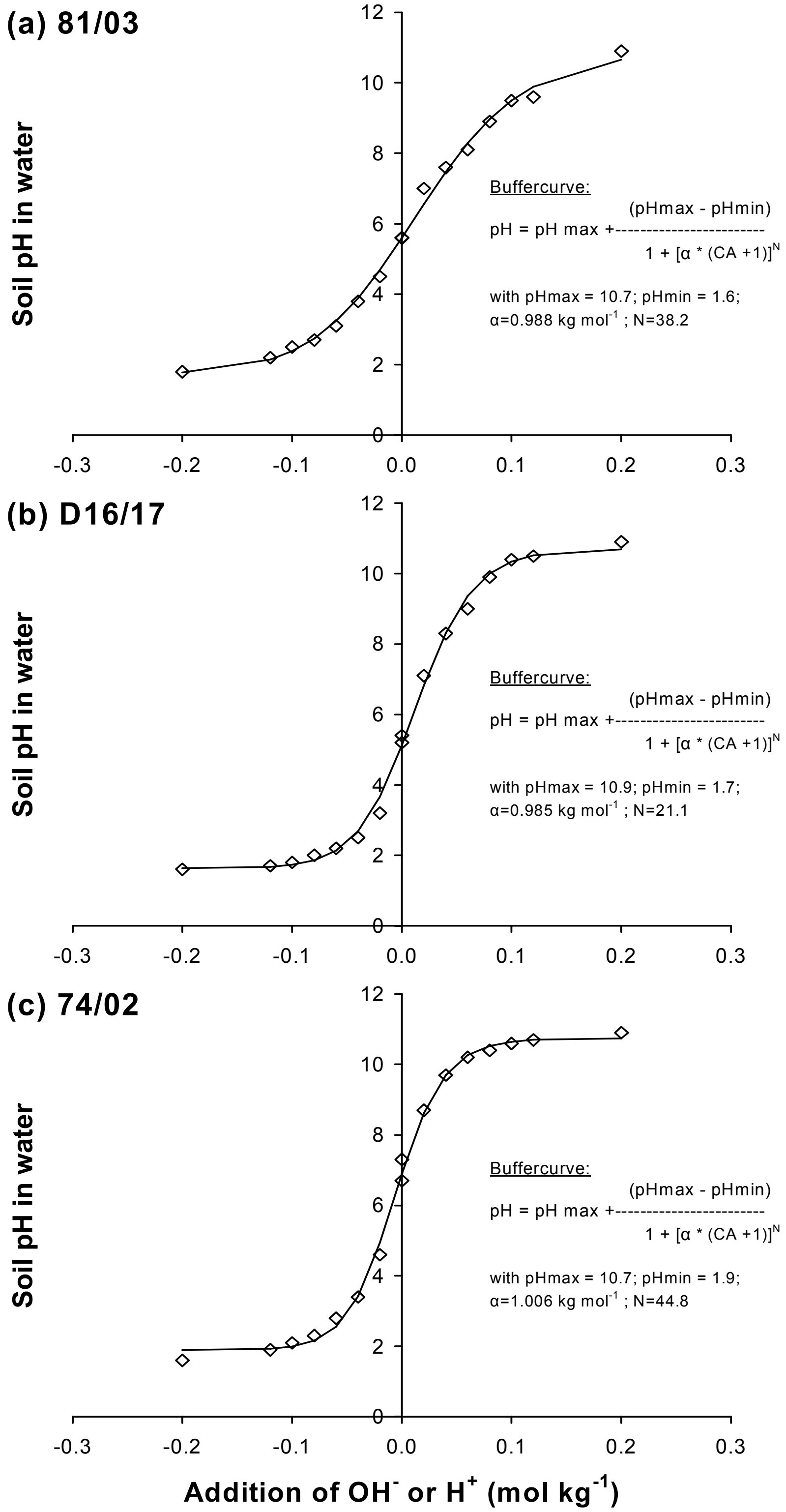

FIG.2. Soil buffer curves for 3 soils from the Northern Guinea savannah zone in Nigeria 'CA' means 'Charge Added'. The mathematical equation used to simulate the buffer curve is added to each graph.

2. AVAILABILITY OF P FROM TOGO PHOSPHATE ROCK AS AFFECTED BY ACIDIFICATION CAUSED BY VARIOUS NITROGEN FERTILIZERS

2.1. Introduction

Vast areas of Sub-Saharan Africa (SSA) experience moderate to acute P deficiency, which is for most regions the second most important plant nutrient after N. Soils in the West African MSZ usually contain quite large stocks of total P [7] but their availability for plants is low. Contrary to N, biological means to enhance the availability of P are limited. SSA contains a number of phosphate rock (PR) deposits [8] and consequently, substantial efforts have been made to use these local resources to address the P problems for increasing agricultural production. The major limitation with using these PRs is their low reactivity, i.e. solubility in standard chemical reagents. In fact, of all PR sources in SSA, only few of them such as the PR's from Tilemsi (Mali), Matam (Senegal), and Minjingu (Tanzania) have a solubility in 2% citric acid exceeding 10% (IFDC, unpublished results). Consequently, although crops show residual responses to application of most PR's minimally 3 years after application, their immediate agronomic effectiveness is too low to generate interest by smallholder farmers who usually have a shorter term view on returns to soil fertility management practices [9]. This warrants means to ameliorate in the short term (within 1 growing season) availability of P from most PR's, in particular under non-acid soil conditions. Most of these are related to decreasing the pH of the medium in which the rock phosphate is dissolving as protons favour the dissolution of rock phosphates, as shown by Hammond *et al.* [10], using fluoroapatite as an example:

$$Ca_{10}(PO_4)_6F_2 + 12\ H_2O \rightarrow 10\ Ca^{2+} + 6\ H_2PO_4^- + 2\ F^- + 12\ OH^-$$

Prolonged use of ammonium-based N fertilizers is often claimed to lead to soil acidification and consequently to reduce the soil base saturation status. Although this is undoubtedly true for ammonium sulphate, other sources of N fertilizer, such as urea have a lower soil acidification potential. Data on soil acidification through continuous use of N fertilizers show considerable declines in soil pH after continuous addition of ammonium-sulphate (AS) (Table II). This effect is less pronounced with urea and calcium ammonium nitrate (CAN). Thus, the acidifying capacity of certain N fertilizers could be used positively to increase the soil P status or to enhance the availability of P from PR when applied at relatively high rates in close contact with each other [11]. The objective of this work was therefore, to assess the P availability from Togo PR in presence of various forms of N fertilizer.

2.2. Materials and methods

A laboratory incubation study was carried out with 3 soils from the West African MSZ, having contrasting pH values and exchangeable cation exchange capacities (Table III; Fig. 2). The soils were (i) left untreated (control soils), (ii) amended with Togo PR (the equivalent of 90 kg P per ha or 396 mg PR per kg soil), or (iii) amended with Togo phosphate rock and potassium nitrate, urea, CAN, or AS at an equivalent rate of 120 kg N per ha. At 1, 2, 4, 8, and 16 weeks after application of these inputs, the soils were analyzed for ammonium-N and nitrate-N content [12], Available (Olsen)-P content [13], and pH (soil:water ratio of 1:2.5).

TABLE II. DIFFERENCES IN TOPSOIL ORGANIC C, TOTAL N, AND PH IN LONG-TERM EXPERIMENTS IN THE WEST AFRICAN MOIST SAVANNAH ZONE, AS AFFECTED BY APPLICATION OF VARIOUS FORMS OF N FERTILIZER. ADAPTED FROM VANLAUWE *et al.* [22]

Site (country)	Type of N fertilizer	Application rate	Duration	pH[c]		
		kg N ha^{-1}	years	Minus fertilizer	Plus fertilizer	Diffe-rence
(1) Zaria (Nigeria)	$(NH_4)_2SO_4$	24	15	6.0	5.4	-0.6
(2) Ife (Nigeria)	$(NH_4)_2SO_4$	134	7	6.3	5.2	-1.1
(3) Many sites[b] (Ghana)	$(NH_4)_2SO_4$	101-330	4-7	NA	NA	(-0.6)-(-0.2)
(4) Ibadan (Nigeria)	$(NH_4)_2SO_4$	150	5	5.8	4.5	-1.3
(5) Ife (Nigeria)	$(NH_4)_2SO_4$	69	14	4.4	3.6	-0.8
(6) Bouaké (Côte d'Ivoire)	Urea	160-200	20	6.0	5.5	-0.5
(4) Ibadan (Nigeria)	Urea	150	5	5.8	4.9	-0.9
(7) Ibadan (Nigeria)	Urea	60	14	5.7	5.4	-0.3
(5) Ife (Nigeria)	Urea	69	14	4.4	3.5	-0.9
(4) Ibadan (Nigeria)	CAN[a]	150	5	5.8	5.0	-0.8
(8) Mokwa (Nigeria)	CAN	31-188	12	5.0	5.0	0.0
(5) Ife (Nigeria)	CAN	69	14	4.4	3.9	-0.5

[a] 'NA': Not available; 'CAN': Calcium ammonium nitrate.
[b] Only a range of differences in absolute values is given for a series of 27 sites.
[c] Methods for pH determination: (1): soil:water 1:5, (2) and (8): 0.01 M $CaCl_2$, (3) and (5): unknown, (4) and (6): soil:water 1:1, (7) soil:water 1:2.5.

TABLE III. SELECTED CHARACTERISTICS OF THE SOILS USED

Soil	pH water	pH KCl	Organic C	Total N	Exch Ca	ECEC	Olsen-P	Sand	Silt	Clay
			g kg^{-1}		cmol$_c$ kg^{-1}		mg kg^{1}	g kg^{-1}		
81/03	5.4	5.0	5.8	0.5	1.8	2.9	5.6	474	408	119
D16/17	5.8	5.4	12.4	1.1	4.2	5.3	33.3	769	120	112
74/02	7.1	6.7	4.9	0.4	4.1	4.9	6.9	479	384	137

2.3. Results and Discussion

2.3.1. Results

Soil pH decreased greatly in the treatments with application of AS for most soils, followed by urea and CAN compared to the control and only PR treatments, with exception of soil 74/02 where little changes were observed (Fig. 3). This decrease in soil pH led to a substantial increase in Olsen-P after application of AS, urea, or CAN, only for the 81/03 soil, which had the lowest soil pH and buffering capacity at the start of the trial (Table III, Fig. 2). However Olsen-P values were only marginally higher in the treatment PR mixed with AS compared to those of PR application alone. In the urea treatments, soil pH initially increased, but only for a short period of time. Application of potassium nitrate hardly affected soil pH after 16 weeks. Prediction of the changes in soil pH after addition of the various N fertilizers, based on the nitrification data – conversion of ammonium-N to nitrate-N is accompanied by the release of two protons - and their soil buffer capacities, was quite successful, with most data points (calculated and measured) lying on or near the 1:1 line (Fig. 4).

2.3.2. Discussion

Mixing low reactivity Togo PR with ammonium-based N fertilizers increased the immediate P availability of the PR but only in soils with relatively low pH, low soil available P and low buffer capacity, as for the 81/03 soil with an initial soil pH of 5.4. Available (Olsen)-P contents were hardly enhanced after mixing Togo PR with AS for the D16/17 soil, which had an initial soil pH of 5.8 and high soil available P content or for the soil 74/02 with an initial soil pH of 7.1. Besides these basic theoretical considerations, practical limitations include the low accessibilities to PRs at the farm level, even in countries having large deposits, the disuse of AS as a fertilizer because of its acidifying properties, and the lack of financial resources and/or agronomic practices at the level of the small-scale farmer to ensure application of both inputs and in close contact.

3. LONG-TERM INTEGRATED SOIL FERTILITY MANAGEMENT IN SOUTH-WESTERN NIGERIA: CROP PERFORMANCE AND IMPACT ON THE SOIL FERTILITY STATUS

3.1. Introduction

Long-term trials dealing with soil fertility issues are scarce in West Africa and in the tropics as a whole. Although computer simulation models have been frequently used to evaluate the long-term impact of specific soil fertility replenishment strategies [14], data are needed to evaluate the performance of these simulation models. Especially the assessment of the effects of long-term soil management practices on the soil fertility status is lacking. Alley cropping was one of the first promising technologies dealing with soil fertility replenishment that was developed and tested at the International Institute of Tropical Agriculture (IITA) during the eighties and early nineties. In such cropping system, food crops are grown between hedges of preferably N_2 fixing trees [15]. These are regularly cut back to minimize tree-crop competition for water, nutrients, and light.

In 1986, a long-term alley cropping trial with *Leucaena leucocephala* and *Senna siamea* as hedgerow species was established at the IITA campus in Ibadan to study the long-term crop and tree performance and soil fertility characteristics with and without the input of chemical fertilizer. Major conclusions drawn from the first five years of the trial (1986–1990) were: (i) *Leucaena* produced most biomass and 20% of the applied N was used by the maize crop, (ii) maize yields were maintained in alley cropping, but the highest yields were obtained in the *Senna* system with fertilizer application, and (iii) the level of the major soil nutrients declined in the no-tree controls, but less so in the alley cropping systems. The current case study describes main results of this long-term alley cropping trial for the following 12 years (1991–2002). The objectives are (i) to evaluate the long-term biomass production of the hedgerows as affected by fertilizer addition, (ii) to evaluate the long-term production and stability of maize crop yields as affected by fertilizer addition, and (iii) to assess the impact of long-term application of prunings with or without fertilizer on selected soil fertility characteristics with a special focus on soil pH changes.

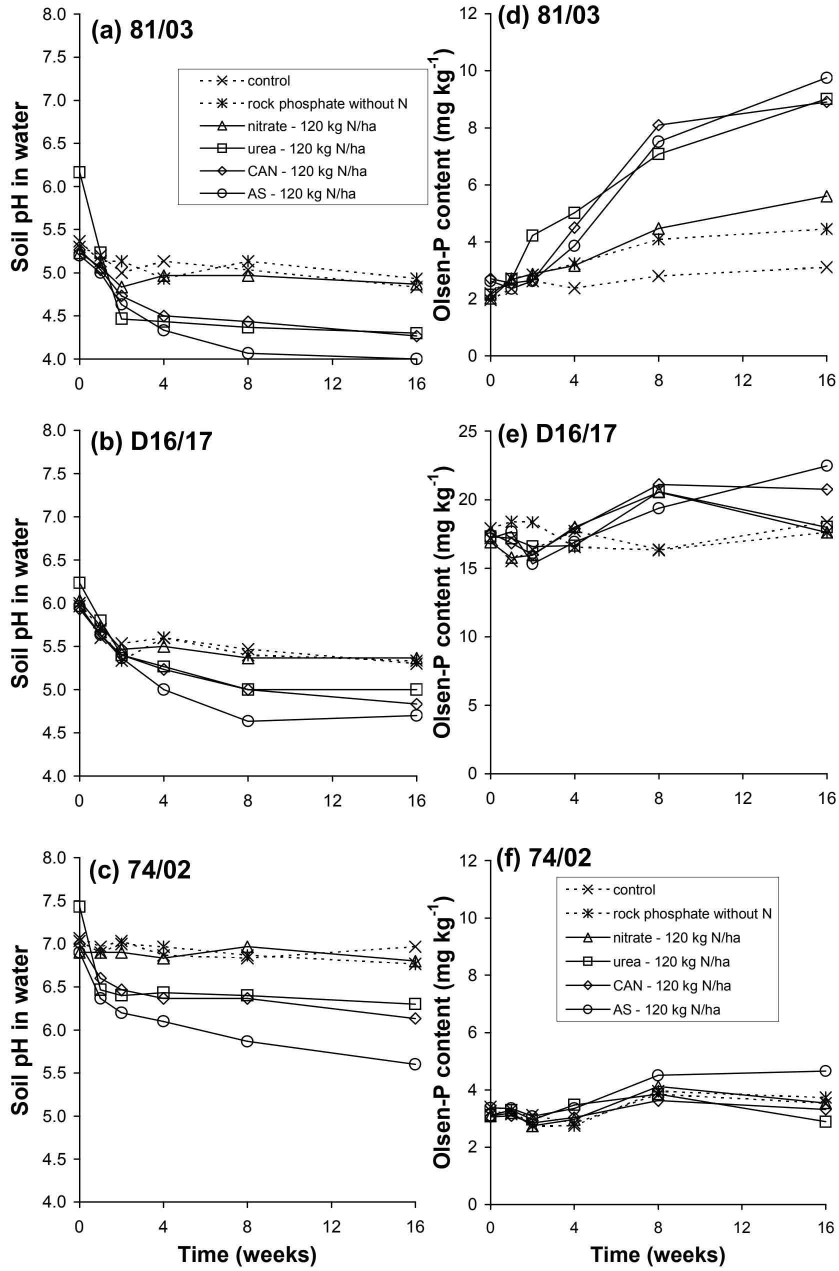

FIG.3. Changes in soil pH and Olsen-P content after application of Togo phosphate rock in presence or not of 4 sources of N fertilizer. CAN means calcium-ammonium-nitrate; AS means ammonium-sulphate. The nitrate fertilizer added was potassium-nitrate. The three soils used are the same as described in Table 3.

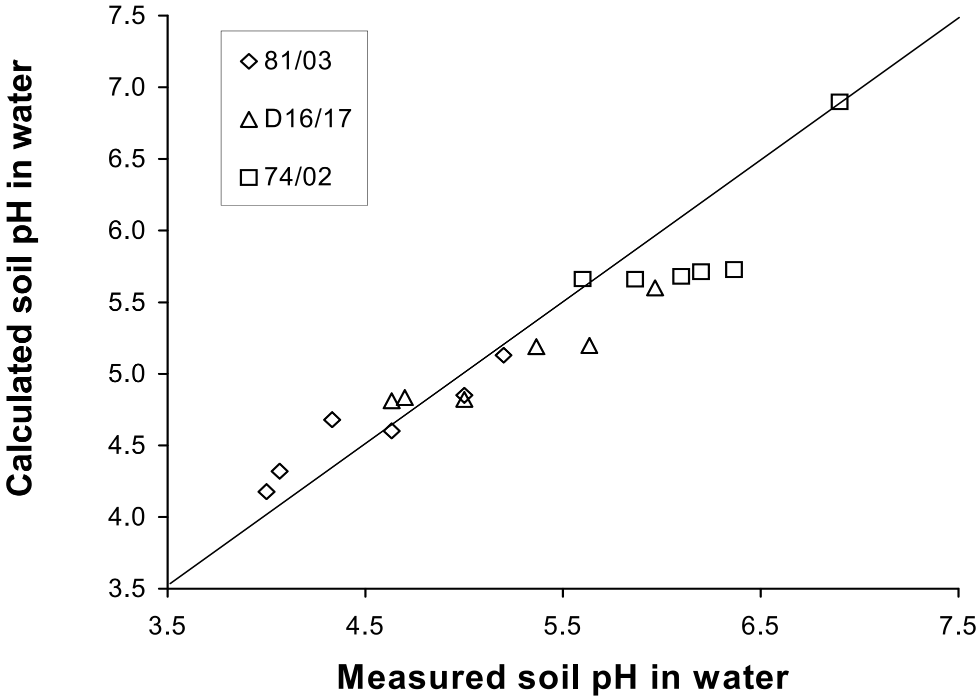

FIG.4. Prediction of the changes in soil pH after addition of the various N fertilizers based on the production of nitrate-N from ammonium-N (nitrification) and the soil buffer capacities. The straight line indicates the 1:1 line. The three soils used are the same as described in Table 3.

3.2. Materials and methods

The experiment was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Southwestern Nigeria (7°30'N and 3°54'E) on a Ferric Lixisol [3] with a maximal slope of 5%. A few plots belonging to the replicate in the lowest position are on a Dystric Regosol. The rainy season starts in March and ends in November, although scanty rainfall occurs until mid-December. The trial is a randomized complete block design with 6 treatments and 5 replicates, laid out perpendicular to the slope. The treatments are a no-tree control, alley cropping with *Leucaena leucocephala*, and alley cropping with *Senna siamea*, each treated or not with NPK fertilizer. Each alley cropping plot contained 5 hedgerows and 4 alleys, 4.5 m wide and 10 m long. *Leucaena leucocephala* is a fast-growing N-fixing legume tree, whereas *Senna siamea* is a non N-fixing legume tree with less vigorous growth characteristics.

Usually, at the start of the first season (April–May) the field was sprayed with herbicide and the hedgerows were pruned a first time. All prunings were applied on the soil surface and the maize was planted at a distance of 0.75 m between the rows and 0.25 m within the rows. At planting, one third of the N fertilizer (as urea), and the total amount of P (as triple super phosphate) and K (as muriate of potash) fertilizer were broadcast over the complete plot. In 1994 the yearly fertilizer N and P application rates were reduced from 120 to 60 kg N ha^{-1} and from 90 to 30 kg P ha^{-1} while K was continuously applied at 30 kg K ha^{-1}. At about 6 weeks after planting the maize, the hedgerows were pruned a second time. After maize harvest, the field was sprayed with herbicide and the hedgerows were pruned a third time. Cowpea was planted at the same density as the maize and sprayed 3–4 times with insecticides to avoid insect damage to the flowers or pods. At about 6 weeks after planting the cowpea crop, the hedgerows were pruned a fourth time. Usually the field was weeded thrice during the maize season and twice during the cowpea season. Maize and cowpea residues were retained on the plots.

Before the 2002 season, soil samples were taken at 0–5 and 5–15 cm depth, diagonally across the plots (24 cores per plot) and bulked per layer. The soil samples were analyzed for organic C [16], total N-Kjeldahl, Bray-1 P [12], pH (H20) (soil: water ratio of 1:2.5), pH (KCl) (soil: KCl solution 1*M* ratio of 1:2.5); ECEC [12], and texture [12].

The apparent fertilizer N use efficiency (NUE) or fertilizer N recovery was calculated as (equation 1):

$$NUE = \frac{MaizeN_F - MaizeN_{0F}}{N_{app}} * 100 \quad [\%] \qquad [1]$$

With $MaizeN_F$ and $MaizeN_{0F}$ the total amount of nitrogen taken up by fertilized and unfertilized maize plants respectively and N_{app} the amount of urea-N applied. Total N uptake comprises N uptake by the grains, cobs, and stover. All units are kg N ha^{-1}. Possible nitrogen interactions between the organic treatments and the inorganic fertilizer addition (N_{int}) were calculated as:

$$N_{\text{int}} = \left[MaizeN_{prunF} - MaizeN_{prun0F}\right] - \left[MaizeN_{contF} - MaizeN_{cont0F}\right] \ [\text{kg N ha}^{-1}] \qquad [2]$$

With $MaizeN_{prunF}$ the amount of nitrogen taken up by the fertilized, residue (*Senna* or *Leucaena*) treated maize, $MaizeN_{prun0F}$ the amount of N in the corresponding unfertilized stand, $MaizeN_{contF}$ the amount of nitrogen taken up by the fertilized control maize (no prunings) and $MaizeN_{cont0F}$ the unfertilized control. N_{int} so becomes the difference between the extra amount of N taken up in a residue (*Senna* or *Leucaena*) treated fertilized maize stand and the extra amount of N taken up in the only inorganically treated maize stand. Given the changes in both N rate and the maize variety during the experimental period (Table IV), fertilizer N recovery (NUE) and the organic/inorganic nitrogen interactions were analysed for three separate categories of data. We grouped together those years where variety TZSR-W was grown with 120 kg N ha^{-1} (years 1991–1993) in a first category, the years where the same variety was grown with 60 kg N ha^{-1} (years 1994–1996) in a second category and finally the years with variety Oba Super 2 and 60 kg N ha^{-1} (years 1997–2002) in a third category.

3.3. Results and Discussion

3.3.1. Results

Yearly pruning biomass production was generally higher for the *Leucaena* than for the *Senna* trees (Fig. 5). Application of fertilizer also slightly increased total yearly prunings biomass production. Maize yields have been declining over the years, and in an extreme case – the control treatment without fertilizers – even hitting a mere 44 kg ha^{-1} in 2002 (Fig. 6). Yields were sustained best in the *Senna* + fertilizer treatment with a yield of still about 2.2 t ha^{-1}of maize in 2002. Yields obtained in the *Leucaena* treatments with or without fertilizer or in the *Senna* treatment without fertilizer varied between 0.7 and 1.3 t ha^{-1}. Fertilizers alone could not sustain maize yields, and the final yield obtained after 16 years of continuous cropping was only 0.4 t ha^{-1}. The NUE results show rather low values in 1991 and 1992 (between 5 and 30%, irrespective the treatment), then increasing to maximal values between 46 and 73% in the years 1994 and 1995 and thereafter slowly decreasing to values between 14 and 40% in 2002 (Table IV). The first category 120V1, covering the years 1991–1993 showed extremely low NUE for the *Leucaena* systems (14%), and similar NUE values of around 32% for the control and *Senna* systems. For the second category 60V1 the NUE values were higher ranging from 50 to 68% but no statistical differences were found among the treatments. As from year 1997 onwards, for category 60V2, again significant treatment effects on the NUE values were observed. In line with the better sustained yields in the fertilized *Senna* system, also the highest NUE values were recorded, averaging 47%, compared to 28 or 30% for the control and *Leucaena* treatments respectively. Analysing for possible added nutrient interactions revealed that the treatments had only significant effects for the first and the last period or category of data (Table V). For 120V1, the first three years of the analysis, a negative interaction emerged, showing that (i) no added benefit was obtained with *Senna*, and (ii) a negative interaction was obtained with *Leucaena*. Introducing *Leucaena* in the system entailed that the increase in N-uptake by maize due to fertilizer addition decreased by about 23 kg N ha^{-1}. Again, in the category 60V1, no significant effects were seen, while in the third period or 60V2 category, (i) no interaction was found for *Leucaena*, while a positive interaction of about 10 kg N ha^{-1} was found for the *Senna* systems.

TABLE IV. APPARENT FERTILIZER N USE EFFICIENCY (NUE) AND INTERACTIONS BETWEEN THE ORGANIC TREATMENTS AND THE INORGANIC FERTILIZER ADDITION (NINT) VALUES FOR THREE CATEGORIES OF MAIZE N UPTAKE. ADAPTED FROM VANLAUWE *et al.* [27]

		Category	
Treatment	120V1	60V1	60V2
		NUE %	
Control	32.08	67.68	27.62
Leucaena	14.00	49.74	29.93
Senna	32.81	55.33	47.19
SED[a]	5.30**	10.38NS	4.84***
		Nint [kg N ha^{-1}]	
Leucaena	-21.70	-10.76	1.39
Senna	0.86	-7.41	11.74
SED[a]	5.15***	9.94NS	3.20**

120V1 = Maize variety TZSR-W with 120 kg N/ha; 60V1 = Maize variety TZSR-W with 60 kg N/ha; 60V2 = Maize variety Oba Super 2 with 60 kg N/ha.
NUE = Fertilizer nitrogen use efficiency, cf. equation 1.
Nint = nitrogen interaction, cf equation 2.
SED[a] = 'Standard Error of the Difference', NS = not significant, ***, ** and * significant at 0,1; 1 and 5% respectively.

The organic carbon in all treatments has decreased between 1986 and 2002 (Table V). Yet, this decrease is less dramatic in the treatments with trees due to the sustained inputs of pruning material over the 17 years. The changes in soil available (Bray)-P concentrations are rather more reflecting the fertilizer history of the plots than an effect of the trees. Soil pH reflected the treatments but more convincingly in the deeper layer than in the top layer. Alley treatments resulted in on average about 0.5 pH units higher pH values in the 5–15 cm layers compared to the control soils, both for pH_{H2O} and pH_{KCl}. These differences were not significant in the topsoil (0–5cm) for pH_{H2O} and slightly higher for pH_{KCl} in the alley systems.

In both layers the effect of fertilizer additions was significant and resulting in a decrease with 0.2 to 0.3 pH units as compared to the unfertilized treatments. Cationic bases (Ca, Mg and K) concentrations showed very significant responses to the alley treatments. For both depths and all three cations, alley treatments resulted in larger remaining stocks of these nutrients in soil after 17 years. This effect is especially obvious for Ca and more specifically in the *Senna* treatments. Testing for mean differences between the treatments with and those without fertilizers, they were negative for all the three nutrients and both depths. While there is no significant change in exchangeable acidity with the treatments, the differences in exchangeable cations in the alley treatments lead to higher ECEC values compared to the control soils and for both layers.

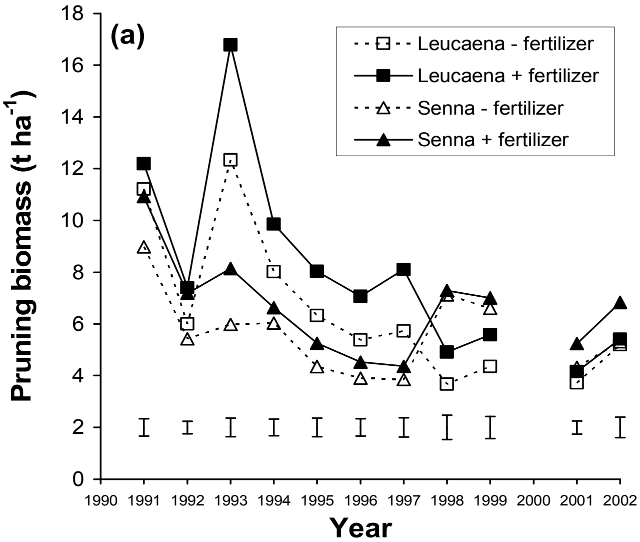

FIG.5. Evolution in pruning biomass added through prunings over 12 years between 1991 and 2002, except for missing data in 2000. Error bars indicate the SED between treatments for each year. Adapted from Vanlauwe et al. [27].

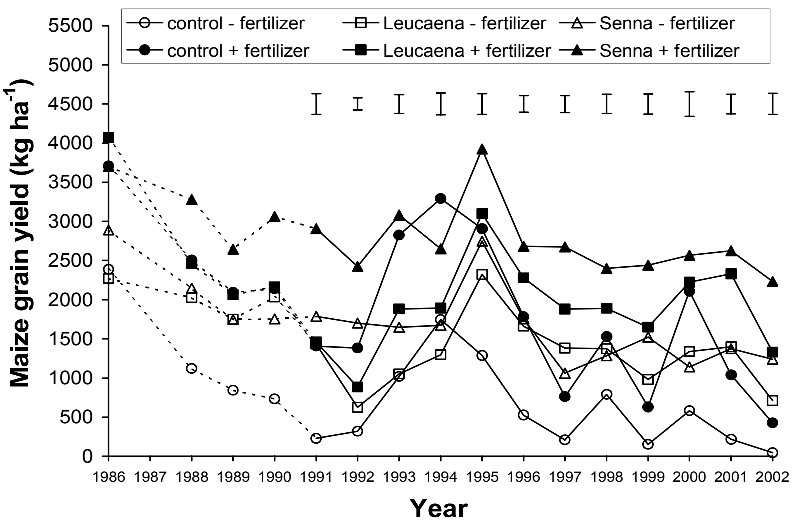

FIG.6. Maize grain yield obtained in the different treatments since the start of the trial in 1986 and up to 2002. Error bars are given from 1992 onwards indicating the SED between treatments for each separate year. Adapted from Vanlauwe et al. [27].

TABLE V. SELECTED SOIL CHARACTERISTICS AT TRIAL ESTABLISHMENT (APRIL 1986 DATA) AND BEFORE PLANTING THE 2002 MAIZE (APRIL 2002) FOR THE 0–5 cm AND 5–10 cm (APRIL 1986 DATA) AND 5–15 cm LAYERS (APRIL 2002 DATA). ADAPTED FROM VANLAUWE *et al.* [27]

	Organic C	Total N	pH (water)	pH (KCl)	Exch[a] Ca	Exch Mg	Exch K	Exch Acidity	ECEC[a]
	g kg^{-1}				cmol$_c$.kg^{-1}				
April 1986									
0 – 5 cm	12.41	1.45	5.7	5.6	2.75	0.73	0.45	0.04	4.1
5 – 10 cm	7.51	0.80	5.2	NA[a]	1.71	0.43	0.26	0.18	2.7
April 2002									
0 – 5 cm									
Control – F[a]	5.66	0.37	5.4	5.0	1.24	0.36	0.18	0.10	2.2
Control + F	5.94	0.48	5.3	4.8	1.62	0.38	0.18	0.07	2.6
Leucaena – F	9.02	0.81	5.3	4.9	2.08	0.58	0.30	0.23	3.5
Leucaena + F	10.02	0.82	5.2	4.9	2.26	0.60	0.32	0.20	3.7
Senna – F	10.01	0.78	5.8	5.4	3.42	0.56	0.32	0.03	4.7
Senna + F	10.58	0.84	5.4	5.2	3.32	0.48	0.32	0.03	4.5
SED[a]	1.11	0.10	0.1	0.1	0.27	0.05	0.04	0.13	0.3
[AC[a] vs control] +F	4.36^{***}	0.35^{***}	NS	0.2^{*}	1.17^{***}	0.16^{***}	0.14^{***}	NS	1.5^{***}
[AC vs control] – F	3.92^{***}	0.42^{***}	NS	0.2^{*}	1.51^{***}	0.21^{***}	0.13^{***}	NS	1.9^{***}
All + F vs – F	NS[a]	NS	-0.2^{**}	-0.2^{*}	NS	NS	NS	NS	NS
5 – 15 cm									
Control – F[a]	3.86	0.31	4.9	4.3	0.96	0.28	0.12	0.07	1.7
Control + F	4.22	0.35	4.6	4.1	1.20	0.24	0.12	0.13	2.0
Leucaena – F	5.24	0.44	4.9	4.4	1.24	0.34	0.20	0.20	2.3
Leucaena + F	5.56	0.47	4.7	4.2	1.34	0.34	0.22	0.17	2.4
Senna – F	5.72	0.43	5.9	5.5	2.18	0.38	0.20	0.07	3.1
Senna + F	6.72	0.53	5.5	5.1	2.18	0.32	0.22	0.07	3.1
SED	0.60	0.05	0.1	0.1	0.13	0.04	0.02	0.07	0.2
[AC vs control]+ F	1.92^{**}	0.15^{**}	0.5^{***}	0.6^{***}	0.56^{***}	0.09^{*}	0.10^{***}	NS	0.7^{***}
[AC vs. control]- F	1.62^{**}	0.12^{*}	0.5^{***}	0.6^{***}	0.75^{***}	0.08^{*}	0.08^{***}	NS	1.0^{***}
All + F vs - F	NS	NS	-0.3^{***}	-0.3^{***}	NS	NS	NS	NS	NS

[a] 'Exch' = 'Exchangeable'; 'ECEC' = Effective Cation Exchange Capacity'; 'F' = 'Fertilizer'; 'SED' = 'Standard Error of the Difference'; 'NA' = 'Not Available'; 'AC' = 'Alley cropping'; 'NS' = 'Not Significant'; '*', '**', and '***' = Significant at the 5, 1, and 0.1 %, respectively.

`[AC[a] vs. control] +F: contrasts tested between all fertilized AC systems versus fertilized control; [AC vs control] – F: idem for unfertilized systems; All + F vs. – F: contrasts between all fertilized versus all unfertilized systems.

3.3.2. Discussion

One of the most interesting features of the alley cropping trials liesin a better conservation of the limited reserves of exchangeable bases like Ca, Mg and K in highly weathered tropical soils as demonstrated in this long term trial. While this holds for all three nutrients studied here, the effects seem most striking for Ca, particularly in combination with *Senna*. In view of the absence of a fertilizer effect it must be concluded that the higher concentrations in the two soil layers under alley cropping are due to an enhanced adsorption (retention) by organic colloids in low buffer soil and reduced loss through leaching and a recycling of the captured nutrients through litter, root turnover and pruning additions. It has often been argued that trees in cropping systems serve as a kind of safety-net, preventing crop nutrients to leach beyond the rooting zone. The present results provide evidence for such mechanism, but more so for *Senna* than for *Leucaena*. This is further discussed in section 4.3 of the present paper.

A general conclusion is that the only system that reasonably resists resource degradation (most resilient) while still producing acceptable yields is the *Senna* + fertilizer system. Data of soil chemical characteristics, fertilizer N use efficiency, added benefits from the combination of organics and fertilizers and maize yields confirm this. The changes in N fertilizer rates in 1994 and maize cultivar grown since 1997 prevented a straightforward analysis of N use by maize in the different treatments. However, the grouping and analysis of the data in sets combining the same cultivar and fertilizer rate, revealed that in the first period examinedin this paper, apparent urea-N recovery increased between 1991 and 1993. A striking feature during this period was the apparent adverse effect of *Leucaena* trees on N recovery by maize. Despite the large supply of N in these systems, the maize crop does not seem to benefit greatly from it, most likely due to competition with the *Leucaena* trees. Both a low fertilizer N recovery (14%) by maize together with a negative organic/inorganic interaction confirm this effect.

The decrease in NUE observed as from 1993, coincides with the reduction in N rates from 120 to 60 kg N ha^{-1}. Between 1994 and 1997, apparently a transition period as far as N-dynamics is concerned occurs, where NUE values were not significantly affected by the respective treatments. The final 6year period then again points to the superior performance of the *Senna* + fertilizer system, with an average NUE of 47% and a positive organic/inorganic interaction estimated at about 12 kg N ha^{-1}. It should be noted that in this period, both fertilizer N use efficiency and N interactions of the *Leucaena* system were not significantly different from the control.

4. SENNA SIAMEA TREES RECYCLE CA FROM A CA-RICH SUBSOIL AND INCREASE THE TOPSOIL PH IN AGROFORESTRY SYSTEMS IN THE WEST AFRICAN DERIVED SAVANNA ZONE

4.1. Introduction

Trees in agroforestry systems in the tropics have been frequently hypothesized to recover nutrients and water from the subsoil because tree roots reach soil layers below the maximum rooting depth of annual crops [17, 18]. The so-called root safety-net zone is usually equated with that part of the soil profile from where trees recover substantial amounts of nutrients, not accessible to the associated food crop [18]. Although trees will certainly recover some of their nutrients and water from the subsoil at certain times in the year, regular pruning of trees may change their root distribution and hence the relative amount of roots in the subsoil. Vanlauwe *et al.* [19], for instance, showed that *Senna siamea* (Lam.) H.S. Irwin &

Barneby hedgerow trees that are regularly pruned accumulate a relatively higher amount of roots in the topsoil, compared with trees that are less regularly pruned.

Organic amendments can also increase soil pH based on their ash alkalinity [20]. This is an important attribute of organic resources in low input systems, which are still very common in sub-Saharan Africa, as removal of harvested products is leading to acidification [21]. Also in Integrated Soil Fertility Management (ISFM) strategies this attribute is important as most commercially available N fertilizers in sub-Saharan Africa (SSA) are ammonium-based and consequently tend to decrease soil pH upon nitrification of the ammonium [22]. Counteracting pH decrease is especially important in weakly buffered soils, as there are many in SSA. Tang and Rengel [23] stated that information on the effect of trees and shrubs on decreasing soil acidification in the field is limited. The objectives of this paper are: (i) to assess the impact of hedgerow tree species on topsoil base cation content for a wide range of trials in the West African derived savannah zone, (ii) to explore relationships between topsoil base cation accumulation and subsoil properties, and (iii) to relate base cation accumulation in the topsoil with changes in topsoil pH.

4.2. Materials and methods

In this study, various medium to long term alley cropping trials were included, all established at sites in the derived savannah zone of West Africa (Table VI). All trials were cropped at the time of sampling, except the Ibadan WB field trial, which was fallow since its establishment. All trials had at least the following treatments: a no-tree control, *Senna* alley cropping, and alley cropping with either *Leucaena* and/or *Gliricidia* (Table VI). *Senna* is a non-fixing legume tree, while *Leucaena* and *Gliricidia* are nodulating N-fixing trees. Pits were dug near the experimental sites and analysis of soil profiles showed that all but two of the sites had a clay accumulation horizon (Bt horizon) at about 40 cm soil depth (*data not shown*). The Arenosol in Amoutchou and the Cambisol on the non-degraded site in Niaouli did not show a substantial clay enrichment horizon. Soils with a clay accumulation horizon also showed a marked increase in soil exchangeable Ca and Mg contents with depth (Fig. 7). The exchangeable K content was below 0.2 $cmol_c$ kg^{-1} soil for all soil layers and sites (*data not shown*). The establishment and management of the trials are described in sufficient detail in other reports [24, 25, 26, 27]. The years of establishment varied between 1986 and 1992. Average yearly biomass input in the various treatments, either as prunings or as litterfall during fallow periods was presented in the other reports and varied between 1840 and 9240 kg dry matter ha^{-1} $year^{-1}$ for the *Senna* treatment and between 940 and 7442 kg dry matter ha^{-1} $year^{-1}$ for the *Leucaena* or *Gliricidia* treatments (Table VI).

Soils were sampled in all the trials at the start of the first season, before residue or fertilizer application, from the 0-10 cm layer. Various samples were bulked per treatment and replicate, air-dried, sieved through 2 mm, and analysed for effective cation exchange capacity (ECEC), exchangeable bases: Ca, Mg, and K content, and soil pH. Details of the various soil sampling schemes are given in the reports mentioned above. The hedgerows in the cropped trials were pruned 2 to 4 times every year. Samples of first prunings of a single year were collected in all sites, except in Sarakawa, and their ash alkalinity was determined using the procedure proposed by Pierre and Banwart [28]. The individual base cation contents of the prunings were not directly measured but the Organic Resource Database (ORD) (ftp://iserver.ciat.cgiar.org/webciat/ORD/) [29] was consulted to get appropriate estimates. Surprisingly, the variance of the various data compiled in the ORD was very low indicating that the estimated values are likely reasonably accurate. *Senna* leaves contained most Ca and least Mg and K, while *Gliricidia* leaves contained most Mg and *Leucaena* leaves most K.

TABLE VI. YEARLY ORGANIC INPUT APPLICATION RATES OF THE VARIOUS TREATMENTS WITH HEDGEROWS IN SEVERAL LOCATIONS OF THE DERIVED SAVANNAH OF WEST AFRICA. ADAPTED FROM VANLAUWE *et al.* [37]

Site	Prunings or litterfall	Years of assessment	Treatment	Dry matter input kg DM ha^{-1} yr^{-1}	Reference
Niaouli degraded field	Prunings (2 per year)	Average of 1994 to 1996	*Senna* *Leucaena* *Gliricica*	6030 2370 1750	Aihou *et al.* [24]
Niaouli non-degraded field	Same as above	Same as above	*Senna* *Leucaena* *Gliricidia*	2480 2990 940	Aihou *et al.* [24]
Glidji	Prunings (3 per year)	Average of 1995 and 1996	*Senna* *Gliricidia*	9240 2470	Tossah *et al.* [26]
Amoutchou	Same as above	Same as above	*Senna* *Gliricidia*	1840 5450	Tossah *et al.* [26]
Sarakawa	Same as above	Same as above	*Senna* *Gliricidia*	9750 4290	Tossah *et al.* [26]
Ibadan - D2 field	Prunings (4 per year)	Average of 1991 to 1999	*Senna* *Leucaena*	5806 7059	Vanlauwe *et al.* [27]
Ibadan – WB field	Litterfall	Average of 1992 and 1996	*Senna* *Leucaena*	7817 7442	Salako and Tian [25]

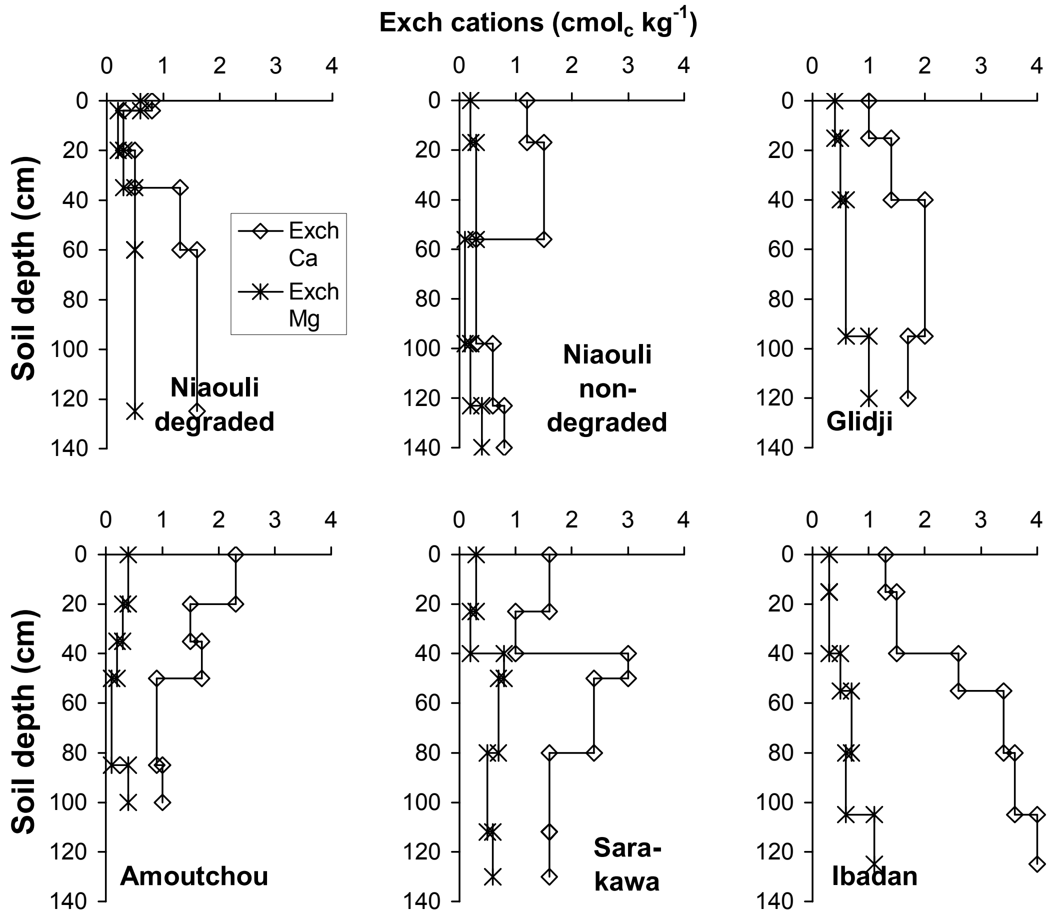

FIG.7. Exchangeable Ca content of the various soil layers up to 140 cm depth in the various target sites. Adapted from Vanlauwe et al. [37].

4.3. Results and Discussion

4.3.1. Results

In all sites, except in Amoutchou, the *Gliricidia* prunings had a significantly higher ash alkalinity than the *Leucaena* or the *Senna* prunings (Fig. 8). In both Ibadan fields, the *Senna* prunings had a significantly higher ash alkalinity than the *Leucaena* prunings. Ash alkalinity was highest in Glidji, and lowest on the Niaouli degraded site.

The ECEC of the topsoil was significantly higher in the *Senna* than in the control and *Leucaena/Gliricidia* treatments in the Glidji and both Ibadan trials (Fig. 9a). At the Sarakawa site, the *Senna* treatment had a larger ECEC than the control treatment. The exchangeable Ca content of the topsoil was significantly higher in the *Senna* than in the control and *Leucaena/Gliricidia* treatments in the Niaouli degraded field, in the Glidji field, and in both fields in Ibadan (Fig. 9b). In the Sarakawa site, the *Senna* treatment contained significantly more topsoil Ca than the control treatment. In the Niaouli non-degraded field and the Amoutchou field, the treatment effect was not significant, although in the Niaouli field this was due to the high site variability. Ca saturation was significantly higher in the Senna than in the other treatments in the Niaouli degraded and both Ibadan sites (Fig. 9c). In Sarakawa, the *Senna* treatment showed a larger Ca saturation than the control treatment. The topsoil pH was significantly higher in the *Senna* treatment than in the control and *Leucaena/Gliricidia* treatments in the Niaouli degraded field, and in the Ibadan D2 and WB fields (Fig. 10). In the Sarakawa site, the *Senna* treatment had a significantly higher topsoil pH than the control treatment, while in the Glidji site; this was true for the *Gliricidia* treatment. In the Niaouli non-degraded field and the Amoutchou field, treatment had no significant effect on topsoil pH, although in the Niaouli field this was due to the high site variability (Fig. 10). A highly significantly linear relationship was observed between the excess cation content of the topsoil relative to the no-tree control soil and the weighted Ca content of the 40–100 cm subsoil (Fig. 11).

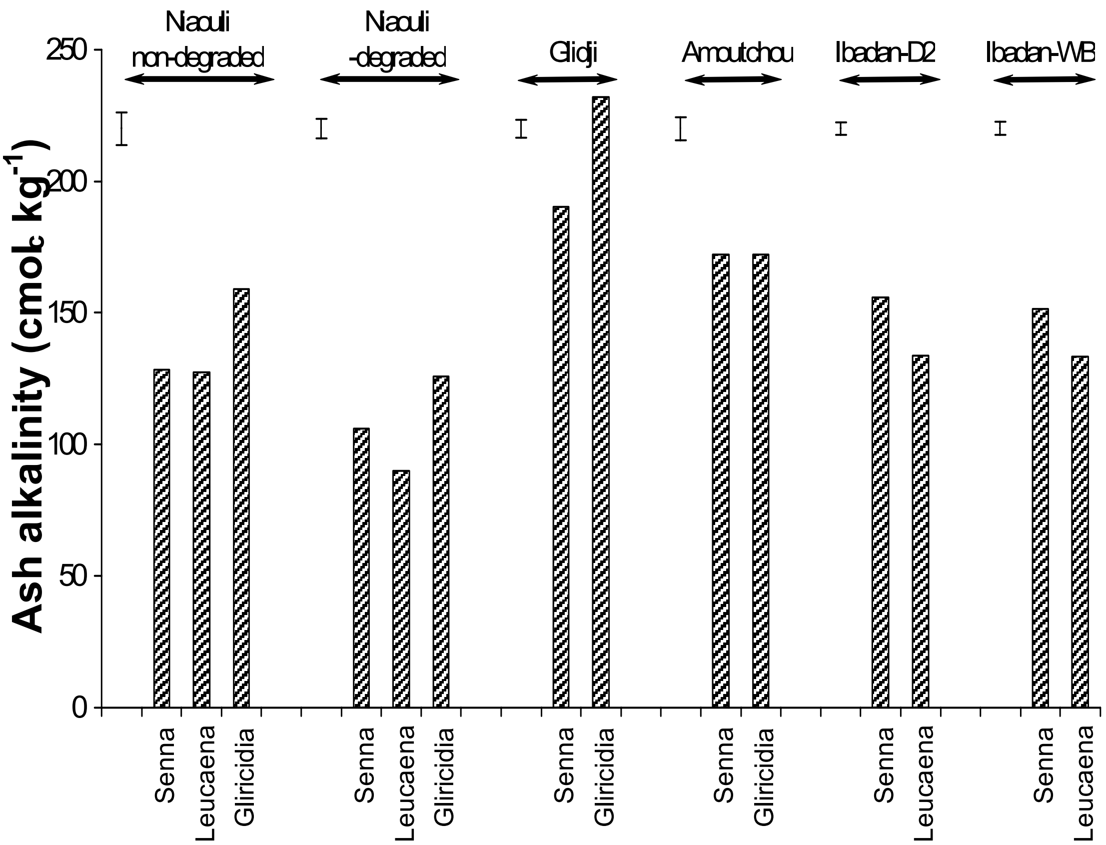

FIG.8. Ash alkalinity of the prunings obtained in the treatments used in this report. Error bars are Standard Errors of the Difference, calculated for each site. Adapted from Vanlauwe et al. [37].

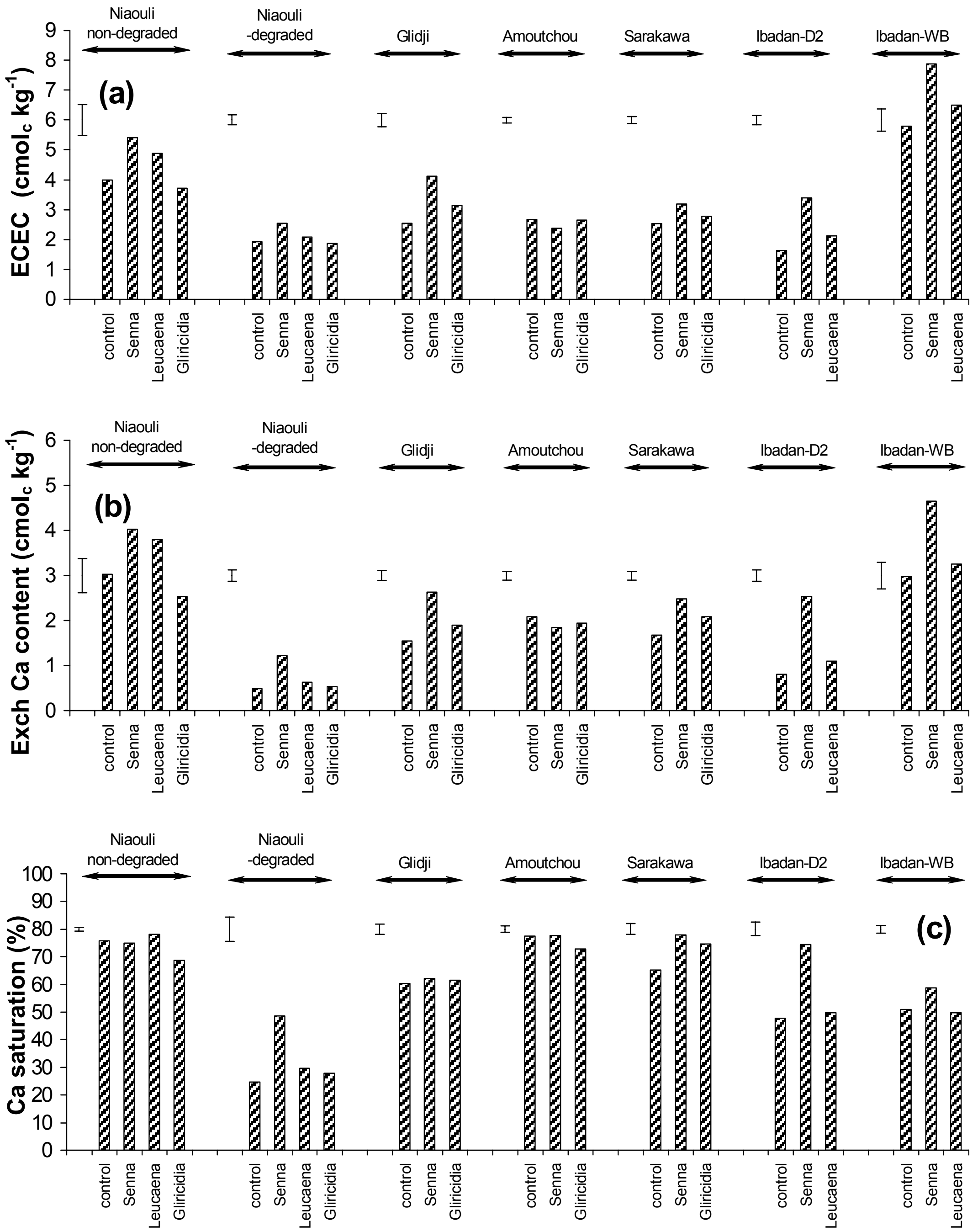

FIG.9. Effective cation exchange capacity (ECEC) (a) exchangeable Ca content (b) and Ca saturation of the ECEC (c) of the topsoil in the various trials included in this report. Error bars are Standard Errors of the Difference, calculated for each site. Adapted from Vanlauwe et al. [37].

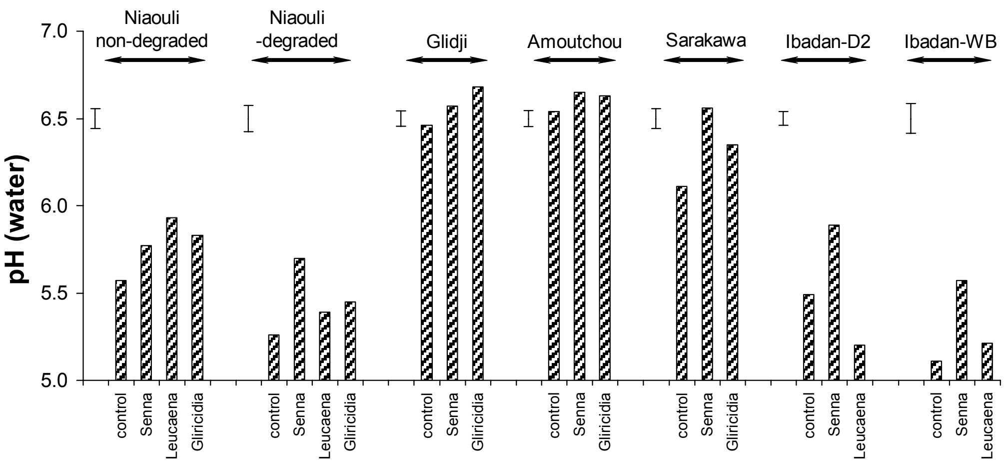

FIG.10. pH in water of the topsoil in the various trials included in this report. Error bars are Standard Errors of the Difference, calculated for each site. Adapted from Vanlauwe et al. [37].

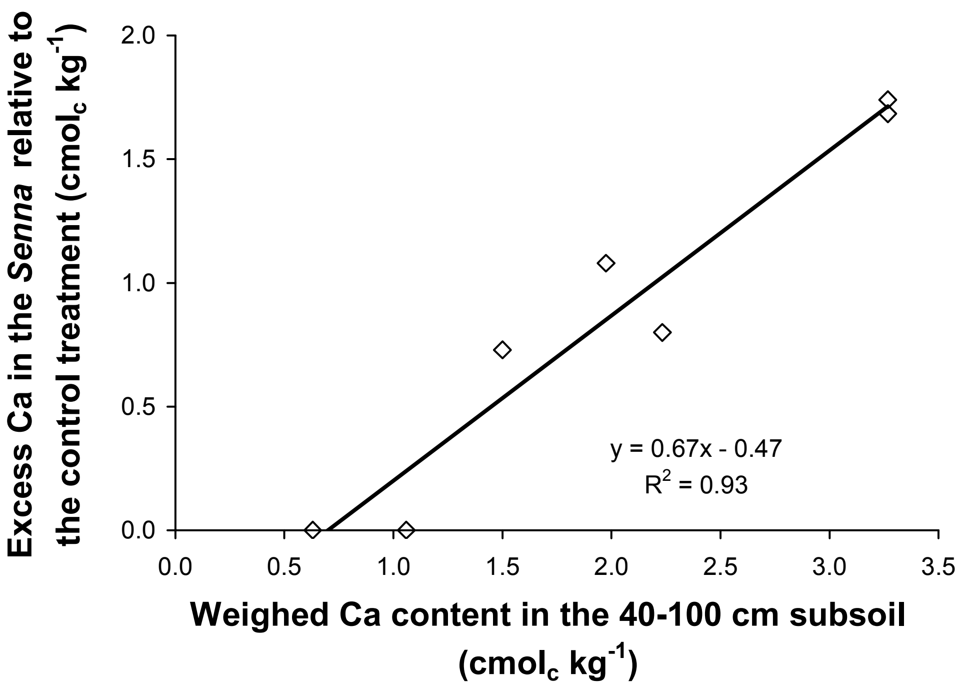

FIG.11. Relationships between the excess Ca content in the Senna treatment relative to the no-tree control treatment and the weighted Ca content of the 40-100 cm subsoil. Note that for the sites where the Senna treatment did not contain a significantly different amount of Ca than the control (i.e. the Amoutchou and the Niaouli non-degraded site), the excess Ca content was set to 0. Adapted from Vanlauwe et al. [37].

4.3.2. Discussion

In the studied trials, the *Senna* trees were recovering Ca from the subsoil and enriching the topsoil with Ca, but only on the sites with a Bt horizon and where exchangeable Ca content increased with soil depth. Evidence for this can be found in the relative enrichment of the cation exchange complex with Ca relative to the no-tree control for the degraded

Niaouli , Ibadan D2, and Ibadan WB sites. Such enrichment can only occur if the trees recover part of their Ca from layers beyond the topsoil that have a relatively higher Ca saturation. After all, if these trees would recover most of their Ca needs from the topsoil, then the topsoil Ca content would be at most as high as the topsoil Ca content of the no-tree control treatment. Moreover, not only does the relative enrichment with Ca increase but also the total ECEC, as a result of higher topsoil C contents after long term application of *Senna* residues indicating an even larger input of Ca from other than the topsoil layers [37]. On the two sites without Ca enrichment in subsoil horizons (the Amoutchou and Niaouli non-degraded sites), the topsoil enrichment does not occur and tree biomass production was severely restricted. *Senna* appears to be unable to compete with a crop (in the cropped alley cropping systems) or the weed vegetation (in the fallow plots) for topsoil nutrients. This is in sharp contrast with the *Leucaena* and *Gliricidia* trees that are fast-growing and may benefit more readily from periods with high topsoil moisture content and little demand for nutrients by the food crop. The accumulation of Ca in the topsoil of the *Senna* treatments varied for the different sites and appeared to be related to the Ca content of the clay accumulation horizon whereby sites without clay accumulation horizon (Amoutchou, Niaouli non-degraded) did not show any significant Ca accumulation in the topsoil.

The stocks of exchangeable Ca content of the topsoil depend on the Ca application rates (organic matter, fertilizer, and atmospheric deposition), the conversion to exchangeable forms, the ECEC of the topsoil, and the removal of Ca with harvested products and through leaching losses beyond the topsoil. While estimated Ca application rates in the *Leucaena* and *Gliricidia* treatments varied between 12 and 125 kg Ca ha^{-1} yr^{-1}, this did not result in an accumulation of Ca in the topsoil relative to the no-tree controls. This is certainly not the result of substantial differences in Ca removal, as Ca removal with maize grains is usually not exceeding 2 kg ha^{-1} per crop [30]. Delays in conversion of Ca from residue-bound into exchangeable forms also can not explain the lack of increase in the exchangeable Ca pool as *Leucaena* and *Gliricidia* prunings are known to release over 80% of their initial Ca content within 100 days under field conditions [31]. As the ECEC of the control and *Leucaena/Gliricidia* treatments is also not significantly different, this indicates that either all Ca applied is leached beyond the topsoil or that most of the Ca taken up by the *Leucaena* or *Gliricidia* aboveground biomass is recycled from the topsoil. As for leaching, this would require an equal amount of anions to leach, where nitrate would be the predominant one, most of it derived from the residue applications. Previous studies looking at N recovery of ^{15}N labelled *Leucaena* residues have shown that recovery of applied residue N was nearly 100% at 53 and 120 days after application, of which 5%, respectively, 3% was found in the mineral N pool of the top 100 cm of soil [32, 33]. This indicates that short-term leaching of freshly applied *Leucaena*-N is limited. However, N leaching may be delayed and the total N balance in such systems is a better indicator for the amount of N lost. The *Leucaena* topsoil (0–5 cm) was observed to contain about 400 kg N ha^{-1} more than the no-tree control [27] and this appears to be less than half of the amount of N added through N fixation during a 12-year period (about 7 ton dry matter ha-1 yr-1 applied as prunings with a minimal N content of 3.5% and a minimal proportion of N derived from fixation of 50% gives about 1400 kg N ha^{-1}). During that same period an estimated 250 kg N ha^{-1} was removed through maize grain harvests, indicating that indeed substantial losses of *Leucaena*-N may have occurred. Considering all the above, the fact that the topsoil Ca enrichment was not observed for the *Gliridicia* and *Leucaena* treatments is likely the result of a combination of topsoil recovery of Ca by those trees and leaching losses. Leaching of applied residue N is likely going to be less in the *Senna* treatment due to its less favourable organic resource quality [34] and the fact that the tree does not fix N, therefore *Senna* trees are likely mining the subsoil Ca content rather than recycling Ca leached beyond the root zone of the companion crops or weeds.

Ca uptake is associated with release of protons and decrease in soil pH for plants having a relatively large cation/anion uptake [23], as is the case with the current species (Fig. 4), while the amounts of alkalinity produced during decomposition of organic residues at or near the soil surface is related to the excess cation content of these residues [35]. One of the major processes increasing soil pH after application of organic resources is through the decarboxylation of organic anions, associated with cations in plant materials [36]. In this work, soil pH in the *Senna* treatments was observed to increase in the sites with a clay enrichment horizon relative to the control and *Leucaena* or *Gliricidia* treatments. This further supports the hypothesis that *Senna* withdraws most of its nutrients from the subsoil as otherwise the increases in soil pH, likely caused by the decomposition of cation-rich residues, would have been counteracted by proton release in the topsoil caused by cation uptake, as observed for the *Leucaena* or *Gliricidia* treatments. Obviously, subsoil recovery of Ca by the *Senna* trees can lead to subsoil acidification and decline in tree biomass production although to date the latter has not been observed for the *Senna* trees on the Ibadan D2 field, continuously maintained since 1986 [27].

5. CONCLUSIONS

Although the soil pH and base status of the soils in the West African Moist Savannah Zone (MSZ) are usually favourable, their buffer capacity is usually low, indicating that while soil acidity may not be a problem initially, inappropriate management of these soils may induce soil-acidity-related problems in the medium to long term. The current paper addressed 3 topics that are closely related to the management of soil pH (acidity) in the West African MSZ. Based on the above-presented results, the following conclusions can be reached:

(1) Mixing low reactivity phosphate rock with ammonium-based fertilizer may enhance the immediate availability of P from these rock phosphates but only for soils with a initial soil pH below 5.5 and a low buffer capacity.

(2) In a long term trial with inputs of tree-derived organic resources (*Leucaena leucocepahala* and *Senna siamea*) and fertilizer, alone or in combination, the *Senna*-based alley system with fertilizers was the more resilient one, both in terms of maintaining crop yields and soil fertility status. Nitrogen fertilizer use efficiency was usually higher in the *Senna* treatment compared to the control or the *Leucaena* treatment. Interactions between fertilizer and organic matter additions were negative for the *Leucaena* treatments in the first three years, and were positive for the *Senna* treatment in the last 6 years.

(3) In the above trial, trees had a positive effect on the maintenance of exchangeable cations in the topsoil. Exchangeable Ca, Mg and K — and hence ECEC — were only slightly reduced after 16 years of cropping in the tree-based systems, and even increased in the *Senna* treatments. Soil pH_{KCl} values decreased with at least 0.5 units in the control and *Leucaena* treatments, but only slightly in the *Senna* treatments.

(4) In a set of medium to long-term trials with various with various hedgerow trees, topsoil Ca content, effective cation exchange capacity, and pH were substantially higher under *Senna* than under *Leucaena leucocephala*, *Gliricidia sepium*, or the no-tree control plots in sites with a Bt horizon rich in exchangeable Ca. It was shown that this effect is largely related to the recovery of Ca from the subsoil under Senna trees.

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Use of Acid-Tolerant and P-Efficient Plant Genotypes

IDENTIFICATION AND CHARACTERIZATION OF ALUMINIUM-RESISTANT, PHOSPHORUS-EFFICIENT PLANT GENOTYPES ADAPTED TO TROPICAL ACID SOILS

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Abstract

In most tropical acid soils, aluminium (Al) toxicity and phosphorus (P) deficiency are the most important factors limiting plant growth and crop yields. One of the key elements of sustainable cropping systems on these soils is the integration of crops and/or crop cultivars with high Al resistance and P efficiency. Experimental activities on enhancing plant P efficiency concentrated on the characterization of the P mobilising capacity of different leguminous grain and cover crops, and their effect on P availability to less P-efficient cereals grown in mixed culture and in rotation. Fractionation of P in the rhizosphere soil revealed the capacity of some legumes to better use P from sparingly soluble soil P fractions than maize. Field experiments conducted on 2 sites in the Northern Guinea Savannah of Nigeria and accompanying green-house pot-experiments revealed a positive rotational effect of P-efficient cover crops on maize growth and grain yield with and without return of crop residues. This could be attributed to a better P supply to maize especially on the strongly P-fixing soil. Experimental work related to Al toxicity and resistance focussed on the development of a selection technique for Al resistance and thus adaptation to acid soils, the genetics of Al resistance in maize, the role of cell-wall and plasma-membrane characteristics, and organic acid metabolism in Al resistance in maize, and the characterisation of differences in Al resistance in common bean. A 15x15 cultivars diallel was evaluated under field conditions and in hydroponics for Al-induced callose formation. The statistical analysis clearly showed that the General Combining Ability (GCA) rather than the Specific Combining Ability (SCA) is of major importance for Al resistance. Al-induced callose formation was negatively correlated to yields on acid soils. This screening technique proved to be a powerful tool in the identification of germplasm with high combining ability for adaptation to acid Al-toxic soils. Among the cell-wall properties the pectin content and its degree of methylation appeared to be of particular importance as indicated by a positive correlation between Al injury of root apices and pectin content and its degree of methylation. Silicon-induced amelioration of Al toxicity could be related to an in-planta effect. Al-treated plants accumulated higher amounts of Si in the root apical cell walls than non Al-treated plants, thus reducing the amount of the readily exchangeable Al in the cell walls. The importance of plasma-membrane properties for Al resistance was indicated by experiments with Δ8-sphingolipid desaturase-transformed maize plants which were more affected by Al than the wild-type, and differences in the degree of desaturation of fatty acids between a resistant and a sensitive maize cultivar. The changes were small, but experiments with yeast transformed with a 9-acyl desaturase showed that a small change in the degree of desaturation led to a very strong increase in Al resistance.

1. INTRODUCTION

Acid soils cover 1.7 billion ha, approximately 43% of the world's tropical land area, 64% of tropical South America, 38% or tropical Asia and 27% of the tropical Africa [1]. Unfortunately, the problem of acidic soils is likely to increase, with increasing CO_2 levels in the atmosphere. In addition, more agricultural land in the developing countries is becoming acidic due to widespread use of ammonium-based nitrogenous fertilizers. In most tropical acid soils aluminium (Al) toxicity and phosphorus (P) deficiency are the most important factors limiting plant growth and crop yields [2]. One of the key elements of sustainable cropping systems on these soils is the integration of crops and/or crop cultivars with high Al resistance and P efficiency.

Ample information on physiology and genetics of resistance of maize to Al toxicity is available and advances have been achieved in developing acid soils-resistant cultivars as well. However, progress made has been slow because it has resulted from extensive and expensive field experimentations [3]. Data from trials in acidic soils are characterized by high experimental error, which reduces heritability and gains from selection. While field testing for yield evaluation of genotypes is indispensable, more precise information on mechanisms responsible for resistance, and efficient screening techniques would make the development of Al-resistant cultivars more focussed and efficient. Our general understanding of the physiology of Al toxicity and Al resistance has improved substantially during recent years. There are a number of excellent reviews summarising the state of knowledge and addressing knowledge gaps [4, 5]. Particular issues such as the relative importance of symplastic versus apoplastic lesions of Al toxicity remain a matter of debate [6] and especially [7] focused the attention on the role of the apoplast in Al toxicity regarding short-term inhibition of root elongation by Al. Screening techniques based on measurements of inhibition of root elongation, Al accumulation in roots visualized by haematoxylin staining and Al-induced callose formation have been developed and tested. However, their applicability in intensive breeding programs still needs to be proven.

Although Al toxicity is the most important factor limiting maize yields on acid soils other factors of the soil-acidity complex might be equally or even more important under certain conditions [2]. It is well established that P deficiency in addition to Al toxicity is highly limiting crop growth on large areas of tropical lands [8]. Under such conditions Al resistance and P efficiency are pre-requisites for adaptation of maize to these soils. It is not known so far, how Al resistance and P efficiency are physiologically and genetically related. The identification, development and use of Al-resistant and P-efficient germplasm do not only contribute to improved yields of that specific crop but also to the better performance of the cropping system, thus promoting the overall sustainability of the land-use system through reducing the external inputs needed for maintenance of its functioning. Long term sustainability may require the use of both strategies, i.e. acid soils-resistant cultivars and adapted agronomic measures. Little quantitative data are available so far which allow conclusions about the comparative advantages of either strategy or their combination.

The objectives of the studies reported here were (i) to evaluate the role of P-efficient cover crops for increasing the P efficiency of maize cropping systems on acid, low-available P soils in the tropics, (ii) to contribute to a better understanding of the physiological and molecular basis of Al toxicity and Al resistance with special emphasis on the role of the root apoplast, and (iii)-based on this understanding- to develop screening techniques facilitating and enhancing the breeding of maize cultivars with improved adaptation to acid tropical soils.

2. MATERIALS AND METHODS

2.1. P-use efficiency

2.1.1. Theoretical framework

The main interrelated processes governing the acquisition of soil and fertilizer P by crops are dissolution/precipitation and desorption/sorption (buffer capacity), transport primarily by diffusion, soil/root contact and biological P transformations. Dissolution/precipitation and desorption/sorption reactions are affected by chemical soil properties, and by temperature and soil moisture directly or indirectly through microbial activity. Transport of P to the plant primarily depends on the P diffusion coefficient [9], which is determined by soil temperature, moisture, tortuosity (bulk density) and buffer capacity. Root growth is decisive for establishing soil/root contact, and is primarily affected by soil physical properties [10], although chemical factors such as Al toxicity and Ca deficiency in acid soils may be even more important. Turnover of organic matter may lead to release or immobilisation of P dependent on soil microbial and faunal activity.

Plants may interfere with the above-mentioned processes either directly or indirectly through the modification of soil properties, thus enhancing P availability and uptake. It is especially important to clearly differentiate between those plant properties that contribute to a more efficient use of the plant-available soil P and those that mobilise P from less available soil-P fractions. In most soils, the transport of P to the root is the main limiting factor for P acquisition rather than root uptake of P [9]. Therefore, enhancing P transport and soil/root contact through lateral root formation [11], root-hair length [12] and reduced root diameter [13], or establishing a symbiosis with arbuscular mycorrhizal fungi which allows plant access to soil P up to several cm away from the root [14] are all mechanisms that will enhance plant P nutrition more than a highly efficient P transport across the plasma membrane. On another hand, processes such as the release of H^+ or OH^- [15], organic acid anions [16], the increase of reduction capacity [17], and rhizosphere phosphatase activity [18] will allow the plant to access poorly available inorganic and organic soil-P fractions and thus increase the pool of soil/fertilizer P which contributes to plant P nutrition.

Agronomic measures may affect P availability to crops either indirectly through the modification of soil properties or through direct crop impact on soil P dynamics. The selection and cultivation of crop cultivars with high P-uptake efficiency will enhance the depletion of plant-available P. If P efficiency is due to mobilisation of P from less available soil P fractions, then depletion of readily available soil P might be reduced. The return of crop residues will reduce the P export from the soil. The main advantage of crop residues, however, relies on their positive effects on soil properties [19]. Soil processes may be shifted to dissolution/desorption of P thus increasing the more readily available P fractions. In addition, improving the conditions for P transport and soil/root contact will enhance the P acquisition capacity of the crops. Also, in this context the accumulation of organic P fractions seems to be of special importance for the maintenance of soil-P availability. The return of crop residues or import of organic matter as mulch might be especially beneficial because the protection of the soil surface by mulching is especially important for the maintenance and improvement of soil physical properties, and the activity of soil organisms which not only reduces mechanical impedance, but also contributes to P mobilisation.

The most promising agronomic approach appears to be the integration of P-mobilising plant species such as cover crops into the cropping system as inter-crops or in rotationThere is good experimental evidence showing that some plant species such as *Lupinus albus* posses a remarkable ability to mobilise sparingly soluble soil P, even in excess of their own requirement [20]. In any case, such mobilised P could be made available to the main crop after decomposition of plant residues. The integration of P-efficient species into the cropping system could also improve the P acquisition of the main crop through enhancing root colonization by mycorrhizal fungi, P-mobilising and plant growth-promoting rhizobacteria [21].

2.1.2. Experimental work

In greenhouse pot-experiments, different legume species and maize were grown in an Alfisol from Zaria, pH 5.8 (H_2O) and 6.4 and 159 mg kg^{-1} resin-extractable P and total P, respectively or an Ultisol from Jos, pH 4.5, and 3.2 and 460 mg kg^{-1} resin-extractable P and total P, respectively, Northern Nigeria. Plants were grown in Mitscherlich pots containing

3 kg soil mixed with 2 kg sand and fertilized with 5, 10, and 50 mg P kg^{-1} soil. After the growing period (about 35–50 days depending on the growth rate of the species), plants were harvested and soil samples were taken from the rhizosphere and bulk soil. Phosphorus was analysed in shoots after dry ashing, while roots were incorporated into the soil. The different phosphate fractions (anion resin, $NaHCO_3$, and NaOH-extractable) in the rhizosphere and bulk soil were determined as above and acid phosphatase activity was assayed according to [22]. In the next season maize was planted in the pots after readjusting the N level. After 45 days maize plants were harvested and their shoot-P contents were determined.

Field experiments were conducted at two locations, Samaru near Zaria (Zaria soil) and Heipang near Jos (Jos soil), in northern Nigeria. The two locations have contrasting soil properties [23]. The main plots comprised the different legume species, maize/*Chamaecrista rotundifolia* and maize/*Cajanus cajan* mixed crops, as well as 2 sole maize treatments: maize + maize crop residues (*Zea* +r.) and maize with crop residues removed (*Zea* -r.). Plots were treated with either 0 or 30 kg P ha^{-1} as single super phosphate (SSP). All plots received a basal application of 10 kg S ha^{-1}, 50 kg K ha^{-1}as KCl, and 50 kg Mg ha^{-1}as $MgSO_4$. An additional 20 kg S ha^{-1} was applied to the sub-plots that did not receive SSP. Urea was supplied to the maize plots at 120 kg N ha^{-1} in two equal doses at planting and 6 weeks later, while the legume plots received 20 kg N ha^{-1} at 5 weeks after planting (WAP). Legumes were not inoculated. At maturity, grain yield, crop residues and P concentration were assessed. After harvesting the grain of each crop, the plant residues were incorporated into the soil except in the plots of the *Zea* -r. treatment. In the second season the sub-plots of the first season were further split into 2 with one half receiving a fresh application of 30 kg P ha^{-1} in Zaria and 60 kg P ha^{-1} in Jos as SSP irrespective of the first season P fertilization. This changed the experimental design to a split-split plot. Maize was the test crop on all plots. Urea was applied to all plots at 120 kg N ha^{-1} and KCl at 50 kg K ha^{-1}. After 5 weeks of plant growth, four soil samples from 0–15 cm depth were randomly taken from each sub-sub-plot along with roots (5–7 cm away from the plant stem). Roots were separated from the soil by washing and kept in 70% ethanol. Root-mycorrhizal infection was estimated following the method of [24]. After harvesting the maize plants, grain and stover yields and their P concentrations were assessed. Yield data were subjected to analysis of variance (ANOVA) using the GENSTAT V package for statistical analysis.

2.2. Al toxicity and resistance

Aluminium (Al) toxicity limits maize production on acid soils of the tropics. However, wide genetic variation exists in maize for Al resistance. The objective of this study was to characterize tropical maize lines and cultivars, from widely differing origin, and their diallel crosses for Al resistance in nutrient solution using callose formation as a physiological marker, and to study the inheritance and combining ability for Al resistance.

An open pollinated (OPV) diallel produced from maize genotypes from the breeding programs of IRAD, Cameroon; INRA, Guadeloupe; EMBRAPA, Brazil, and CIMMYT, Colombia widely differing in adaptation to soil acidity and Al resistance was evaluated for Al-induced callose production at early seedling stage in hydroponics culture. Al treatment induced callose formation in the root tip of all maize genotypes. Al-resistant and resistant x resistant crosses had lower callose formation compared to sensitive x sensitive or resistant x sensitive crosses.

For the diallel analysis, germinated seedlings of maize were pre-cultured in a continuously aerated nutrient solution for three to four days in a controlled climate chamber. The pH of the solution was step-wise reduced to 4.3 before Al treatment and it was maintained at this level during the treatment period. The plants were treated with 25 μM Al in the nutrient solution for 12 hrs. Then 1 cm root tips were collected and analyzed for callose content. The evaluation of the whole set of open pollinated diallel materials was completed in four series of experiments. In each series, two well-known cultivar checks, namely: ATP-Y (Al-resistant) and Lixis (Al-sensitive) were included. The line diallel was completed in one experiment facilitating the statistical analysis. Callose from root tips was extracted with 1 M NaOH for 30 min at $80^{o}C$ and quantitatively determined according to [25] using water blue as color reagent by a fluorescence spectrophotometer. Callose content was expressed as the percentage of that of Lixis in its respective series. Statistical analysis of the diallel data was done according to Griffing's Method 4, model-I; which involves only direct crosses without parents and reciprocal crosses and considers parents as fixed effect [26]. For this purpose a statistical-software, PZ14 (developed by Prof. Utz, Univ. of Hohenheim) was used.

For the pectin experiments, germinated maize seedlings were grown in nutrient solution for three days during which the pH of the solution was gradually decreased to 4.3. Then 5 mm root apices were collected in Eppendorf vials containing 96% ethanol. Cell-wall material was prepared as alcohol-insoluble residue (AIR) after homogenization of the root samples and two washing steps by ethanol. The AIR was divided into two parts, one for pectin and the other for methanol determination. Pectin assay was made according to [27] and [28] using galacturonic acid as standard. Since the methods available are not sensitive enough to determine the degree of methylation of the pectin extracted from root apices in maize, an immuno-histological study of cell-wall pectins in the maize root-apex in relation to Al toxicity has been developed**.** Two contrasting maize cultivars, ATP-Y (Al-resistant) and Lixis (Al-sensitive) were used in this study. Hand-sectioned thin sections of fresh root tips were directly fixed and loaded with a primary antibody. JIM7 and JIM5 are rat monoclonal antibodies that specifically bind to high-methyl-esterified or low-methyl-esterified epitopes of pectin respectively. They are used to localize the distribution of these epitopes in maize root sections. The primary antibody was washed away from the samples and then the section was exposed to a secondary antibody, antirat-IgG coupled with FITC. The samples were washed again, mounted on glass slides and observed under a confocal laser scanning microscope (CLSM).

Si has been reported to alleviate Al toxicity in maize [29]. The beneficial roles of Si are based on two aspects: solution chemical and in-planta mechanisms [30]. [31] suggested that an enhanced exudation of phenolic compounds leading to complexation of Al compounds and thus detoxification of Al is the mechanism responsible for the Si-mediated enhanced Al resistance in an Al-resistant maize cultivar. The majority of the work to study Si effects on plant Al resistance has focused on the whole roots and/or shoots system with relative long Al treatment periods, usually several days [32]. However, Al phyto-toxicity expresses within minutes and hours in the root apices [33]. Therefore, the objective of this study was to better understand short-term effects of Al on root injury with special emphasis on Al/Si interaction in the root apoplast, which is the primary target of Al [7, 34].

In order to study the effect of Si on Al resistance, seeds of an Al-sensitive maize cultivar 'Lixis' were germinated for three days and then transferred to a basic solution containing 500μM $CaCl_2$ and 8 μM H_3BO_3. Half the number of the plants was exposed to the basic solution supplemented with 1.4 mM H_4SiO_4. All experiments were conducted in a growth chamber under controlled environmental conditions. The silicic acid was prepared by passing potassium silicate through a column filled with cation exchange resin (Bio-Rad, AG 50W-X8, 100-200 mesh). The silicic acid solution was diluted into different concentrations immediately after preparation to avoid polymerization. One day after transplanting, the pH of the nutrient solution was stepwise adjusted to pH 4.3 within 12 hours, and then plants from both Si treatments were exposed to 0 and 25 µM $AlCl_3$ for 12 hours. At harvest, root tips were excised for Al or Si analysis or frozen immediately in liquid nitrogen for callose determination. The apoplastic sap of the root tips was collected by centrifugation, according to the method described by [35]. After getting the apoplastic sap, the samples were frozen at – 20^{o}C overnight. The symplastic 1 fraction was recovered from the frozen-thawed samples by centrifugation at 3000 g at 4^{o}C for 15 min. The residue was transferred to 2 ml Eppendorf vials and 1 ml of 95% ethanol was added. Then the sample was crashed with a Mixer mill. After centrifugation, the supernatant and pellet were separated, the pellet was washed with ethanol again, and the two supernatants together were called the symplastic 2 fraction. The pellet was the pure cell-wall material. Exchangeable Al was extracted from the pellet on Millipore. The cell-wall material was desorbed sequentially by 50 mM $BaCl_2$ (pH 4.3) for 5, 10, and 15 min, followed by desorption in 33 mM Na_3-citrate solution (pH 5.8) for 5, 10, and 15 min. The Al content in the BaCl2 or Na3citrate solutions was determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Callose from root tips was extracted and determined as described above. For Al analysis, the root tips or different fractions of root tips were wet digested with ultra pure concentrated HNO_3 at 135 ^{o}C for 25 min in a microwave. Al concentration in the solutions was quantified by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) or GFAAS. Silicon in the root tips or in different fractions of root tips were extracted by a mixture of 1M HCl and 2.3M HF (1:2 v/v), Si concentration was determined colorimetrically [36].

For the evaluation of Al toxicity at high pH, seedlings of the Al-sensitive maize cultivar 'Lixis' were grown in culture solution with 500 µM $CaCl_2$ and 8 µM H_3BO_3 in a growth chamber under controlled environmental conditions. One day after transplanting, the pH of the nutrient solution was adjusted step wise to the treatment target pH within 24 hours. Then the plants were exposed to 0 and 50 µM $AlCl_3$ for 8 to 36 hours. The solution pH was maintained at the target pH within ±0.1 by adding 0.1 M KOH or 0.1 M HCl. At harvest, 1 cm root tips from the primary root were excised for Al determination or frozen immediately in liquid nitrogen for callose determination. All experiments were conducted on a 16/8 h day/night cycle, 30/27^{o}C day/night temperature, 75% relative air humidity and a photon flux density of 230 µmol $m^{-2}s^{-1}$ photosynthetic active radiation at the plant level. The fractionation of Al in the root apices was performed similarly to the procedure described above for the Si experiments. Al was extracted from the cell wall on a Millipore filtration unit by a sequential procedure using solutions of 0.10, 0.25, 0.50, 1.0, 10.0 mM KOH, each for 10 min. After the KOH solution was acidified by HNO_3, Al contents in the KOH solution were determined by ICP-OES. Callose and Al in the root tips were quantified as described above. Aluminium localization in root cross sections was performed by morin staining. After 8 h Al treatment, 2-cm root tips from each treatment were excised and washed in the basic solution (Al-free) with the same solution pH as the treatment. Free-hand sections from the 1–3 mm zone behind the root apex were stained with 25 µM morin for 30 min at room temperature. After washing with distilled water, the sections were observed under a fluorescence microscope.

Binding of Al to negatively charged phosphate groups of phospholipids leads to a decrease of plasma membrane (PM) fluidity [37, 38] proposed that binding of Al induces a stronger association of membrane phospholipids and a higher packing density of phospholipids reducing membrane permeability. Furthermore, a rapid decrease of PM permeability following temporal contact with Al ions correlates to Al sensitivity among a variety of plant species [39]. After exposing the roots to Al, a higher proportion of phosphatidylcholine at the expense of phosphatidylethanolamine and a lower content of free sterols seem to be characteristic features of Al-resistant wheat lines [40]. These changes in lipid composition may lead to less ordered membranes and compensate for the Al-induced decrease in membrane fluidity. These studies suggest that specific changes in the lipid composition of the PM may contribute to maintain root growth under Al stress. The objectives of these studies were to evaluate the genetic modification of the plasma-membrane lipid composition on the Al sensitivity of plants.

Sphingolipids are abundant components of the plasma membrane. Due to their function as structural element and second messengers, sphingolipids could play a role in Al stress/resistance mechanisms. The sphingolipid desaturase gene from *Arabidopsis thaliana* was expressed in maize plants. In order to verify how Al affects the composition of the sphingoid bases in root tips of transgenic plants an experiment was done with the homozygous transgenic line 23/32/4, its parents, lines H99 and A188, and the varieties ATP Y and Lixis. Reciprocal crosses between maize lines A188 and H99 were performed. Following an osmotic pre-treatment of 24 h with sucrose (0.7 M), 11 to 13 d-old immature embryos were cultivated on modified N6 medium for one week. Immature embryos were bombarded with the constructs pUbiB5AT and p35S-PAT, and subsequently transgenic plants selected using Basta®. The open reading frame of the *Arabidopsis thaliana* Δ^8-LCB desaturase (GeneBank accession number AJ224161) ligated into the pGEM-T vector [41] was digested using the restriction enzymes *Asp*718 and *Eco*RI. The resulting filled-in *Asp*718/*Eco*RI-DNA fragment of the *A. thaliana* Δ^8-LCB desaturase (1366 bp) was ligated into the *Sma*I site of the plasmid pUbi.cas between the constitutively expressed *Ubiquitin*1 promoter and the *Nos* terminator from maize (*Zea mays*, L.) resulting in pUbiB5AT. The plasmid p35S-PAT contained the selectable marker gene phosphinothricin acetyl transferase (*pat*) from *Streptomyces viridochromogenes*. In order to multiply the seeds and obtain a population of homozygous T2 transgenic lines, transgenic T0 plants were grown to maturity and self-pollinated. Plants expressing the transgene were grown up to maturity and self-pollinated again generating a pool of T2 seeds. Homozygous T2 transgenic lines were identified by Basta® spraying of a representative part of the population. For the evaluation of their Al sensitivity uniform seedlings were transferred to nutrient solution [42]. The nutrient solution was exchanged every three days. Plants were kept under controlled environmental conditions (16/8h day/night, 27/25°C day/night, 230 µmol m^{-2} s^{-1}, and 75 ± 5% relative air humidity). Depending on the amount of material needed for subsequent analyses, plants were cultured from seven to 15 days in nutrient solution. One day before adding 25 µM Al, the pH of the nutrient solution was lowered stepwise to 4.3 and kept stable by the addition of HCl or KOH (0.1 N). After 12 h of Al treatment, root tips were collected for biochemical analyses. Callose was determined as described above. Complete roots or root tips were used for the sphingoid base analysis. Roots were immediately frozen in liquid nitrogen and stored at –80°C. To avoid thawing, the root tips were dried under vacuum at –20 °C. Twenty mg (d.w.) were submitted to strong alkaline hydrolysis using 10 % (w/v) aqueous Ba $(OH)_2$/dioxane (1:1, v/v) at 110° C for 24 h [43]. The long-chain bases (LCBs) were converted to 2,4-dinitrophenyl (DNP) derivatives extracted and purified by thin-layer chromatography according to [18]. The derivatized LCBs were analyzed by reversed-phase HPLC. Separation was achieved on a Multospher™ 100 RP 18-5 column (5 µm, 25 × 4.6 cm) with a concave gradient at a flow rate of 0.8 ml min^{-1} from 84% methanol/acetonitrile/2-propanol (10:3:1, v/v/v) and 16% water to 0% water in 55 min. Elution was monitored using an UV detector at 350 nm. Identification of the peaks relied on reference substances and on HPLC-MS analysis of the DNP-derivatized LCBs as described before [44]. Quantification of LCBs was based on the recovery of C14-D-*erythro*-sphingosine included as an internal standard.

3. RESULTS AND DISCUSSION

3.1. Enhancing P-use efficiency

The experimental activities concentrated on the characterization of the P mobilising capacity of different leguminous grain and cover crops, and their effect on P availability to less P-efficient cereals grown in mixed culture and in rotation. In a pot experiment the studied legumes differed in P efficiency (Fig. 1) and fractionation of P in the rhizosphere soil revealed the capacity of some legumes to better use P from sparingly soluble inorganic soil P fractions than maize (Table I). $NaHCO_3$-extractable organic soil P was negatively correlated with acid phosphatase activity in the rhizosphere (Fig. 2).

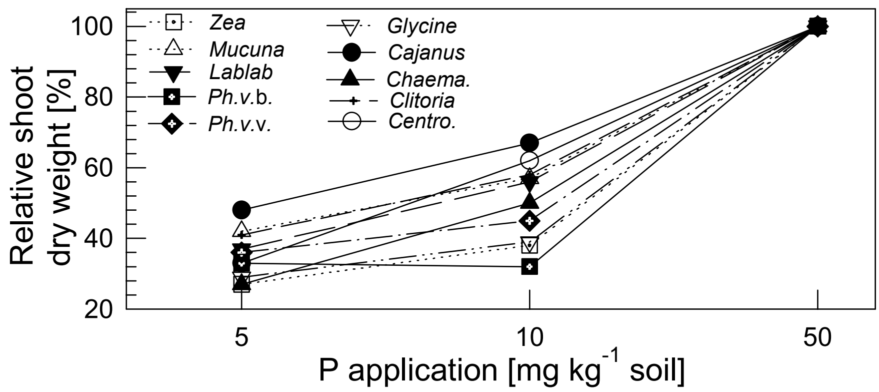

FIG.1. Relative shoot dry weight of different plant species grown in a pot experiment with Zaria soil, Northern Nigeria, as affected by P application (highest yield = 100%). (Zea mays, Zea; Cajanus cajan, Cajanus; Centrosema pubescens, Centro; Chamaecrista rotundifolia, Chaema; Clitoria ternatea, Clitoria; Glycine max, Glycine; Lablab purpureus, Lablab; Mucuna pruriens Mucuna; Phaseolus vulgaris (brown, Ph.v.b or variegated seeds, Ph.v.v.) [45].

TABLE I. PHOSPHORUS UPTAKE AND DEPLETION OF INORGANIC (Pi) AND ORGANIC (Po) EXTRACTABLE P FRACTIONS IN THE RHIZOSPHERE SOIL OF MAIZE AND 4 LEGUMES SPECIES GROWN IN P-TREATED (10 mg kg^{-1} soil) ZARIA SOIL, NORTHERN NIGERIA.

Species	P	P depletion [mg kg^{-1} soil]				
	Uptake	Resin	$NaHCO_3$		NaOH	
	[mg $plant^{-1}$]	P_i	P_i	P_o	P_i	P_o
Zea	7.02	4.40	2.44	-0.26	2.60	-4.90
Mucuna	9.00	2.77*	3.48	-0.16	4.60*	-2.90
Lablab	6.36	2.92*	3.92*	-2.25*	4.80*	-7.35*
Cajanus	7.35	4.17	4.92*	0.06	5.00*	-3.79
Clitoria	7.45	4.40	2.76	-0.09	6.60*	-6.55

An asterisk denotes significant differences between individual species and *Zea mays* within the same P fraction (t-test $P < 0.05$) [36]. Data on P depletion are obtained from the difference in extractable P concentration in the rhizosphere and the bulk soil (baseline). A negative depletion means an accumulation of P.

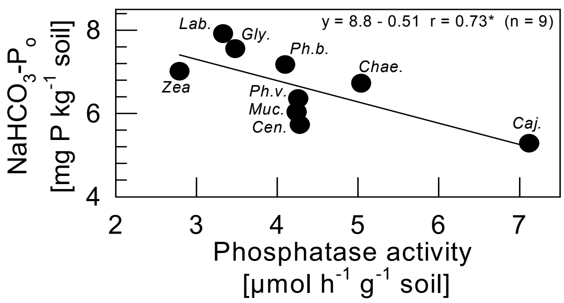

FIG.2. Relationship between $NaHCO_3$-extractable organic soil P ($NaHCO_3$-P_o) and acid phosphatase activity in the rhizosphere of different plant species grown in Zaria soil, Northern Nigeria, at 5 mg P kg^{-1} soil. For abbreviations of the species see legend to Fig. 1 [45].

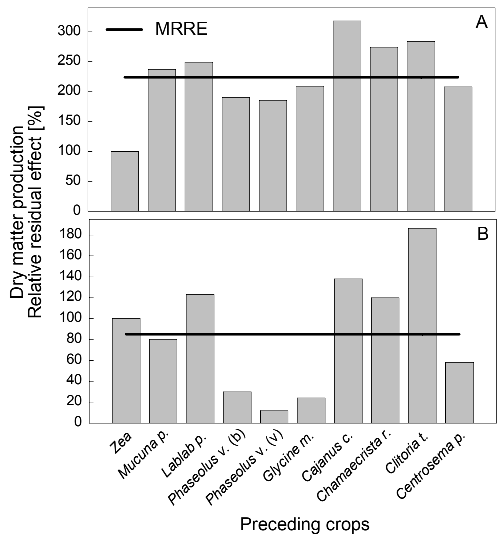

FIG.3. Relative residual effect of cover crops on dry matter production of the subsequently grown maize in rotation, pot experiment with Zaria (A) and Jos soil (B), Northern Nigeria. (MRRE, mean relative residual effect for all cover crops). For abbreviations of the species see legend to Fig. 1 [45].

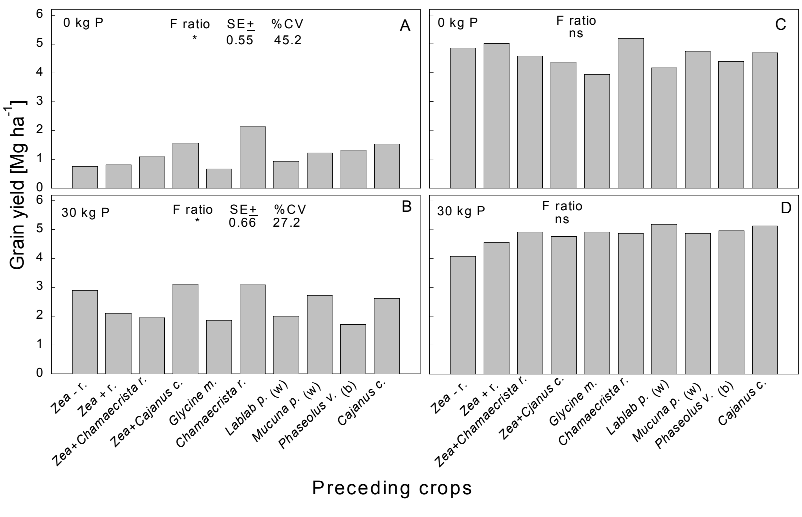

FIG.4. Effect of preceding crops fertilised with 0 and 30 kg P ha^{-1} as SSP on grain yield of the subsequently grown maize in rotation. Field experiment carried out in Jos soil, Northern Nigeria. Unfertilized (A and B) and freshly fertilized maize with 60 kg P ha^{-1} (C and D). SE: standard error of the difference of means between preceding crops. For abbreviations of the species see legend to Fig. 1 [45].

Further pot experiments (Fig. 3) and accompanying field experiments (Fig. 4) conducted on 2 sites (Zaria and Jos) in the Northern Guinea Savannah of Nigeria revealed a positive rotational effect of P-efficient cover crops on maize growth and grain yield with and without return of crop residues. This could unequivocally be attributed to a better P supply to maize especially on the strongly P fixing soil from Jos (Fig. 5). The P applied through the crop residues played an important role in this positive rotational effect. However, the residual effect was small compared to the fresh application of the water-soluble P fertilizer (single superphosphate, SSP). This clearly confirmed the need for fertilizer P application to improve the soil P status, in addition to the agronomic measures for sustainable crop production.

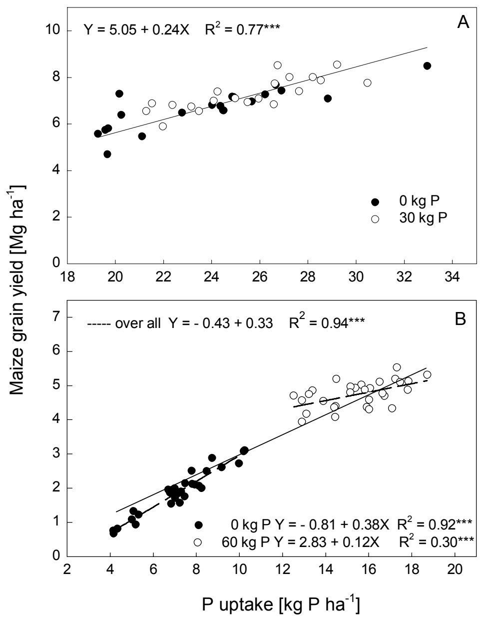

FIG.5. Relationship between total shoot P uptake and grain yield of maize grown in rotation with different cover crops on Zaria soil (A) and on Jos soil (B), Northern Nigeria [46].

Improved P acquisition by maize after cover crops may also be due to a modification of soil biological properties by the previous crop. In this context mycorrhizal infection rate might be of particular importance (Fig. 6). Results of the field study on the Luvisol of Zaria clearly show that mycorrhizal infection is significantly enhanced after most of the cover crops compared to maize after maize (-r).

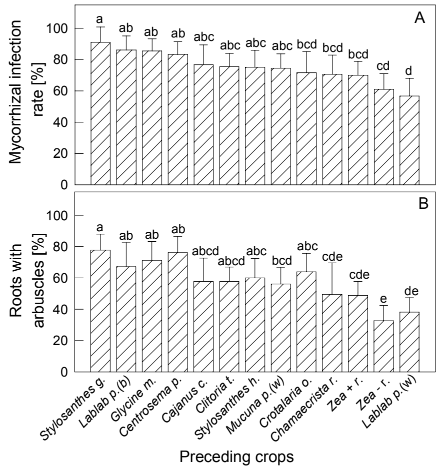

FIG.6. Effect of preceding crops on mycorrhizal infection (at five weeks after planting) of the subsequently grown maize in rotation on Zaria soil, Northern Nigeria. (A) Mycorrhizal infection rate, (B) Percent roots with arbuscles. Means with the same letters are not significantly different at the P > 0.05 level (Tukey's test). For abbreviations see legend to Fig. 1 [45].

As mentioned above, a substantial contribution in the use of soil P by cropping-systems components to the overall efficiency of the system mainly depends on their capability to mobilize sparingly plant available soil-P fractions. In these studies it was found that some leguminous cover crops well adapted to the humid savannah of West Africa are also capable of acquiring soil P fractions not accessible to maize (Table I). As reported with *Lupinus albus* [47], this capability seems related to an enhanced release of organic acid anions at limiting P supply, and an enhanced root-surface phosphatase activity [45] (Fig. 2). The P deficiency-induced synthesis and release of organic acid anions is primarily aimed at mobilising P to meet the plant's own P requirement. This assumption is supported by the demonstration of a strong depletion not only of less soluble but also of readily plant-available P fractions in the rhizosphere soil of P-efficient plant species (Table I).

Although [45] showed that *L. albus* improved the P nutrition and growth of subsequently grown wheat in a pot experiment, it appears more likely that a positive rotational effect of P-mobilising crops is mainly due to transfer of readily available P via the crop residues. This is shown by the significant positive relationship between P applied with the crop residues of different cover crops and yield of subsequently grown maize in the field experiment on the P-deficient and highly P-fixing Jos soil [46]. It is not the total amount of P returned to the soil in crop residues, but rather their C-to-P ratio [48], and the biodegradability of the organic matter [49] that appears to be decisive factors for possible residual P fertilisation effects of crop-residues. This has been also demonstrated for the high quality organic source *Tithonia diversifolia* by [50].

Crop-specific effects on soil micro-organisms may also affect P utilization in crop rotations. Enhanced mycorrhizal infection greatly contributed to the positive rotational effect of grain legumes on millet yields in the Sudano-Sahelian West Africa [21]. In the field experiment on the Luvisol soil of Zaria it was found that most cover-crop species significantly enhanced mycorrhizal colonization of subsequent maize (Fig. 6).

3.2. Aluminium toxicity and resistance

3.2.1. Inheritance of Al resistance in maize

The statistical analysis of the results of the maize OPV diallel experiment clearly show that the general combining ability (GCA) is of major importance for the selection criteria "Al-induced" callose formation as a indicator of Al sensitivity (Table II). The GCA effect, which determines the average performance of a parent in a series of crosses, was calculated for each parent (Fig. 7). Since higher callose formation is indicative of Al sensitivity, negative GCA is an indication of the genotypes' favorable contribution to the Al resistance of the crosses while the reverse is the case for genotypes with positive GCA effects.

TABLE II. ANALYSIS OF VARIANCE FOR AL-INDUCED CALLOSE FORMATION OF THE 13 × 13 MAIZE OPV DIALLEL [51]

Source	DF	MS	F
Crosses	77	200.89	
GCA	12	1011.38	19.73**
SCA	65	51.27	0.29^{ns}
Error	231	178.38	

** = significant at $\alpha = 0.01$; ns = non significant

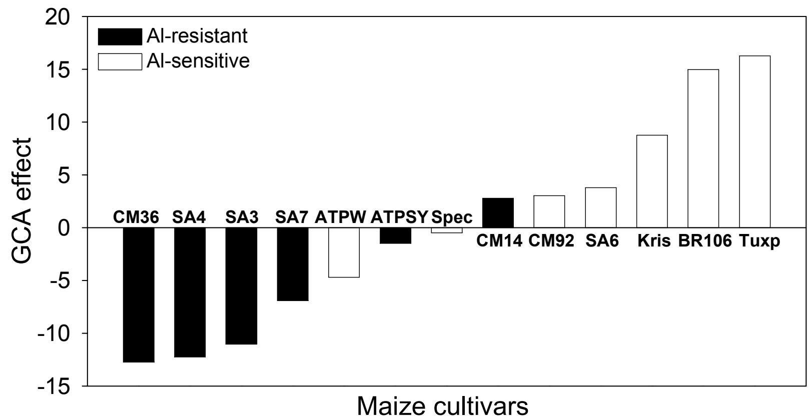

FIG.7. GCA effects of the 13 parental populations included into the maize OPV diallel based on Al-induced callose formation [51].

The parents as well as the crosses used in this experiment were also tested in the field at two locations (Cameroon and Guadeloupe) during two years and in Colombia during one year both on acid and non- acid soils. There was a significant and highly negative correlation between the GCA effect for grain yield and the GCA effect for callose formation (Fig. 8).

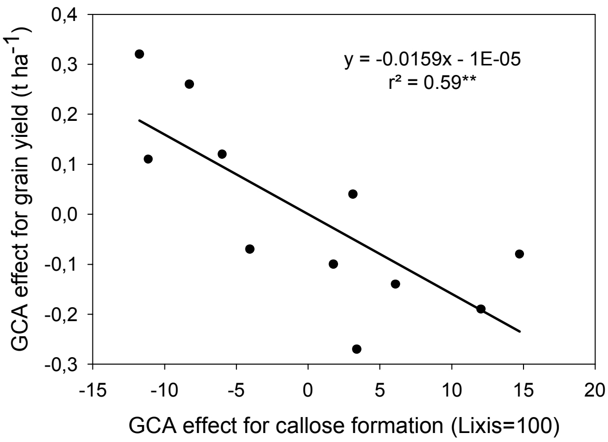

FIG.8. Relationship between GCA effects for grain yields on acid soils (field experiments) and for relative Al-induced callose formation (nutrient solution experiments of 11 maize OPVs) [51]. Field data from [52].

The evaluation of the performance of the diallel indicated that crosses among Al-resistant parents showed a better Al resistance compared to crosses between resistant and sensitive or among sensitive parents [53] reported similar observations after evaluating a diallel derived from 6 acid-soil tolerant (T) and 2 sensitive (S) parents. Yield on acid soil of TxT (3.0 t ha^{-1}) was greater than TxS (2.4 t ha^{-1}) and SxS (2.0 t ha^{-1}) suggesting polygenic inheritance [54] of Al resistance. Analysis of variance of the diallel crosses showed significant GCA effects while specific combining ability (SCA) effects were not significant indicating that additive genes have a more prominent role for Al resistance (Table II). The predominance of GCA effects for most characters of maize populations tested in the field under both acid and non-acid soil conditions has been reported [55, 52] Salazar, et al. [56] evaluated a diallel derived from acid-soil tolerant and sensitive populations in five acid soil environments and reported that non-additive gene effects were unimportant in the performance of the crosses. Similarly, [57] studied Al resistance of maize population and their F1 crosses in nutrient solution and observed that GCA variance explained most of the variation in relative root growth.

Additive genes can be exploited through recurrent selection, a breeding method that increases the frequency of favourable genes. Several studies indicate that selection of maize for tolerance to soil acidity and Al toxicity has been effective to increase both grain yield and other agronomic traits. [58] reported that selection was very effective for Al resistance using the nutrient solution technique. Similarly, [59] obtained gains in grain yield after four cycles of full-sib selection from a Composto Ampolo population on a soil with 45% Al saturation. Remarkable improvements for both Al resistance [60] and soil-acidity tolerance [61] were obtained through recurrent selection. Advanced cycles of selection showed better performance in grain yield and other agronomic traits indicating the valuable effect of recurrent selection.

The relationship between Al-induced callose content and relative grain yield of maize cultivars was weak for the environment average or even absent in some environments (data not shown). This might be due to the high variability of cultivar performance across locations and years as indicated by the significant interaction of cultivar × location × year [62]. This interaction suggests that Al was not the main growth-limiting factor on the acid soils across all environments. Soil moisture regime can be expected to influence the relative importance of Al toxicity. Under limiting moisture conditions, the Al effect on root growth may promote severe drought stress and N/Mg deficiencies since the root cannot reach the subsoil [62].

Since the cultivars respond differently to various environmental stresses, the identification of the best genotypes adapted to a specific stress under field conditions is difficult. Thus, the combination of field and laboratory studies (controlled environment) appears to be necessary. The Al-induced callose formation in root apices offers an attractive tool for a quick and non-destructive [63] screening for Al resistance. This study is a pre-requisite for further field studies on the adaptation of cultivars to acid Al-toxic soils.

3.2.2. The role of cell-wall characteristics in Al resistance

The general understanding of the physiology of Al toxicity and Al resistance has improved substantially during recent years. It is generally accepted that the root apex plays the major role in Al perception and response. Previous published work and results of the work performed particularly by our group strongly indicated that cell-wall properties of the root apex are involved in the perception of Al toxicity and in Al resistance [7].

Pectin contents of the root apices of the selected maize cultivars differed significantly (Fig. 9). These differences were dependent on whether the pectin content was expressed on the basis of root fresh weight or root length. The latter appears to be the more relevant parameter, because it better reflects the root surface in contact with Al. Although the most Al-resistant cultivar was characterized by lower pectin content than the Al-sensitive standard Lixis, there was no general relationship between pectin content and reported Al sensitivity/resistance.

Since the methods available to determine the degree of methylation of the pectin extracted from root apices in maize are not sensitive enough, an immuno-histological study of cell-wall pectins in the maize root-apex in relation to Al toxicity was utilised.

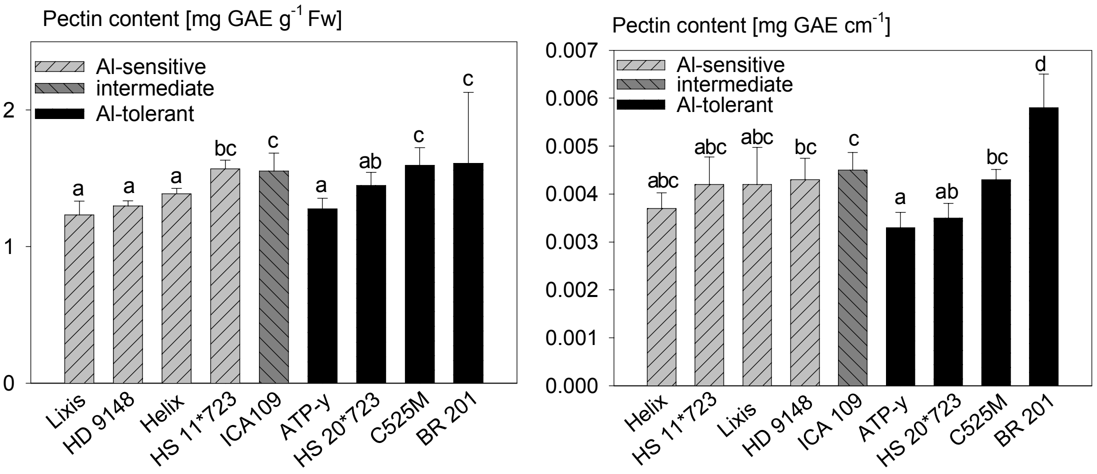

FIG.9. Pectin contents of 5 mm root apices of maize cultivars differing in Al sensitivity /resistance. Pectin content on the basis of root fresh weight (left) and on the basis of root length (right).

Representative images of root sections of ATP-Y and Lixis are shown in Fig. 10. In both cultivars, the cell walls of cortical cells are fluorescing whereas in the epidermis and the stele there is almost no fluorescence. The distribution and intensity of the fluorescence differ between the cultivars.

The rat monoclonal antibody JIM5 is specific for low-methyl-ester pectin. Similar to JIM7, the epitope of JIM5 is mainly located in the cortex of both in ATP-Y and Lixis (Fig. 11, A and B). The point of maximum fluorescence for ATP-Y is the junction points and the wall facing the intercellular space. There is brighter fluorescence in Lixis than in ATP-Y, which indicates that Lixis has a higher content of low-methyl-ester pectin than ATP-Y (Fig. 11, A and B).

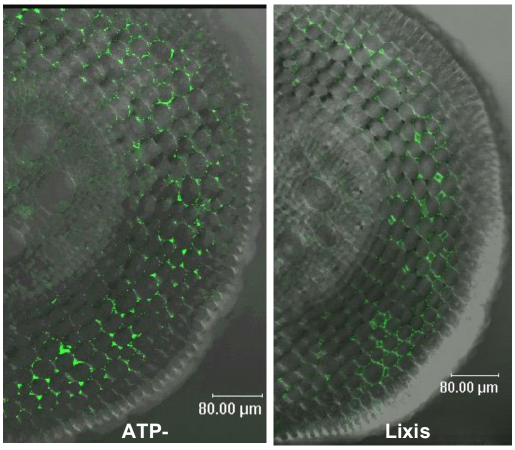

FIG.10. Distribution of high-methyl-ester pectin (JIM7 epitope) in root cross-sections of two maize cultivars ATP-Y and Lixis. Root sections were taken from 1–2 mm behind the tip. Maximum fluorescence is seen in the cortex [64].

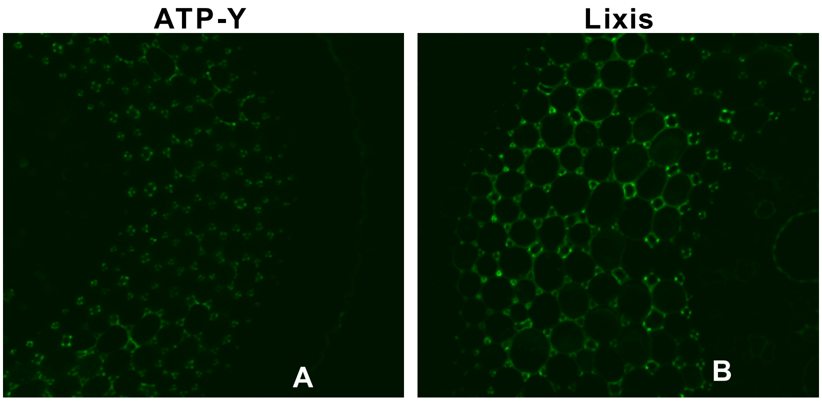

FIG.11. Immuno-localization of low-methyl-ester pectin (JIM5 epitope) in root cross sections of two maize cultivars ATP-Y and Lixis. Root sections were taken from 1–2 mm behind the tip [64].

3.2.3. The effect of Si on Al resistance

Aluminium-induced inhibition of primary-root growth was observed in all Si treatments. Silicon had a slightly positive effect on root growth under Al toxicity conditions, but only when Si was present in the solution during Al treatment period, Si pre-treatment did not enhance root elongation under Al toxicity conditions. In plants exposed to Si only during pre-culture Al-induced callose formation was not affected. In plants continuously treated with Si, callose formation was reduced indicating enhanced Al resistance of these plants (Fig. 12). Si treatment did not affect total Al contents of root apices.

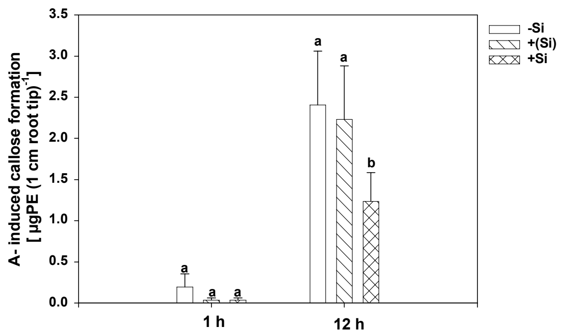

FIG. 12. Effect of silicic acid on Al-induced callose formation in root tips of maize cv Lixis supplied with or without 25 µM Al at pH 4.3. Plants were pre-cultured for 36 hours in the presence or absence of 1.4 mM Si and then were treated with 0 or 25 µM Al for 1 hour or 12 hours in the presence (+Si) or absence (+(Si)) of 1.4 mM Si. Error bars indicate standard deviations of the mean of 5 replicates [65].

In Al-treated plants, in more than 85% of the root-tips Al was detected in the cell-wall fraction confirming that the cell wall is the major site of Al localization after short-term Al treatment. There was no significant difference between –Si and +Si plants in the Al content in the mobile apoplastic fraction, the symplast, and the cell wall within the first 1 cm root apex. This indicates that the ameliorative effect of Si was not due to lower Al uptake into the root tip of maize. After 12h of Al treatment, only plants cultured in presence of Si had higher Si contents in the root apices. In these plants Al treatment led to higher Si accumulation (Fig. 13, bars at the left hand). Most of this Si accumulated in the cell wall, greatly changing the relative distribution of Si between apoplast and symplast (Fig. 13, circles at the right hand).

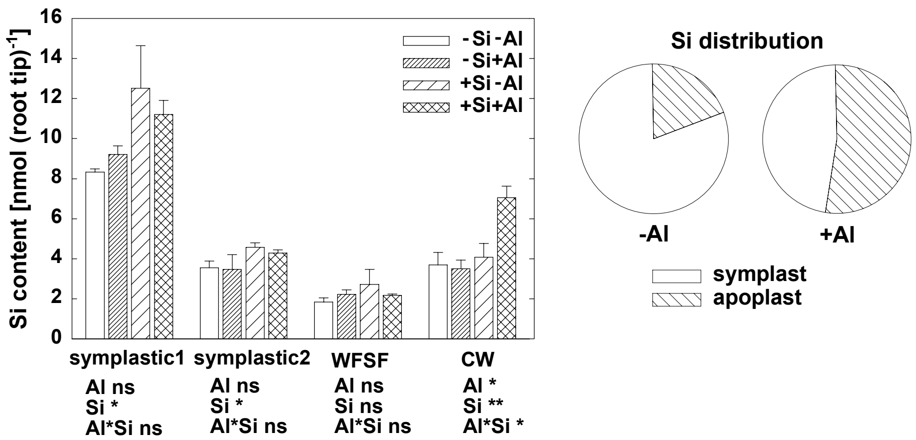

*FIG.13. The contents (left) and relative distribution (right) of Si in the symplast (symplastic 1 and 2), water free space fluid (WFSF) and cell walls (CW) of 1 cm root tips of maize cv Lixis as affected by Si and Al supply in a solution containing 500 µM $CaCl_2$ and 8 µM H_3BO_3, pH 4.3. Plants were pre-cultured for 36 h without or with 1.4 mM Si and then treated without or with 25 µM Al for 12 h in the absence or presence of 1.4 mM Si. Relative distribution after subtracting the background Al or Si contents in -Al or -Si treatments, respectively. Bars show standard deviation, n = 3. *, **, *** indicate significance at the $p < 0.05$, 0.01, and 0.001 level according to the F test. ns = non significant [65].*

This accumulation of Si in the cell walls in Al-treated plants affected the mobility of Al bound in the cell-wall fraction as revealed by the fractionated extraction of Al from the cell wall material. Different mechanisms are discussed, how Si could exert its positive effect on Al resistance. [66] proposed that esterification of cell-wall components by Si reduces the binding of Al to the cell wall. [31] suggested that an enhanced exudation of phenolic compounds is responsible for the Si-induced Al resistance in maize. Both mechanisms would lead to reduce Al concentrations in the apoplast. [30] proposed the formation of hydroxy-aluminium-silicate (HAS) in the apoplast, so that Al would be transferred into a non-phytotoxic form, without reducing the Al content. This conclusion is supported by the results of [67]. Using X-ray microanalysis, they showed the co-presence of Si and Al in epidermal cells of sorghum roots treated with both Al and Si. Later, in roots of Al and Si-treated wheat [68] found Al and Si co-localized in epidermal and hypodermal cells. In the experiments presented here, the total amount of Al in the cell wall, as well as in any other cell fraction was not changed by Si treatment [65]. But the exchangeability of the cell wall-bound Al changed. The easily exchangeable Al fraction was reduced by Si. Concomitant with this modification of Al binding we found a change in the cellular distribution of Si (Fig. 13). Al treatment shifted the cellular Si distribution from the cytoplasmic to the cell-wall fraction. These findings support the hypothesis that the formation of Al-Si complexes is responsible for the ameliorative effect of Si and are in agreement with the observation of [69], who detected Al and Si co-deposits in the outer tangential walls of the root epidermis of wheat. Her results suggested that although Si did not reduce the concentration of total Al, it might have reduced the concentration of biologically active Al within the cell wall. Our results were generally in accordance with these results. A possible explanation for this phenomenon could be the formation of HAS. High Al concentrations in the epidermis [70] and the relative high pH (compared to the bulk solution) on the root surface of the distal transition zone (DTZ) [71] will favor HAS formation, thus reducing the biologically active Al concentration.

Al toxicity has been well documented under acid soil conditions, where Al^{3+} is the most abundant monomeric Al species leading to rhizo-toxicity in plants, and it is generally believed to be the most toxic form (see reviews [4, 5]). However, Al toxicity is not only a plant growth and yield-limiting factor on acid soils, Al toxicity has also been reported in alkaline soils amended with alkaline fly ash [72, 73] and bauxite residue [74]. The comparison of Al toxicity at low pH (where predominant Al species in solution are Al $(H_2O)_6^{3+}$ and (Al^{3+})) and high pH (where predominant Al species is Al $(OH)_4^-$) appeared to be particularly suited to clarify the role of the apoplast versus the symplast in Al toxicity. It is expected to observe a contrasting behaviour of cationic and anionic Al in the root apoplast in spite of the confounding chemical processes in the root apoplast described by [75]. The present study focused on the effects of low-pH and high-pH solutions on Al uptake and distribution in the root apices, and on short-term Al rhizo-toxicity, as reflected by inhibition of root elongation and induction of callose formation.

Aluminium reduced root growth to similar levels at pH 8.0 and pH 4.3 although the monomeric Al concentration in the solution at pH 8.0 was four times lower than in the pH 4.3 solution (Table III).

TABLE III. ROOT LENGTH OF MAIZE SEEDLING AS AFFECTED BY AL TREATMENT AT DIFFERENT SOLUTION PH VALUES. 5-DAY-OLD SEEDLINGS WERE SUPPLIED WITH OR WITHOUT AL FOR 36 HOURS AFTER ADAPTATION TO DIFFERENT PH SOLUTIONS FOR ONE DAY

Culture solution pH	Al supply (µM)	Total root length (cm plant $^{-1}$)	Relative root length (%)
4.3	0	426	
	50	197	46
8	0	439	
	50	178	41
9	0	298	
	50	160	54
10	0	138	
	50	128	93

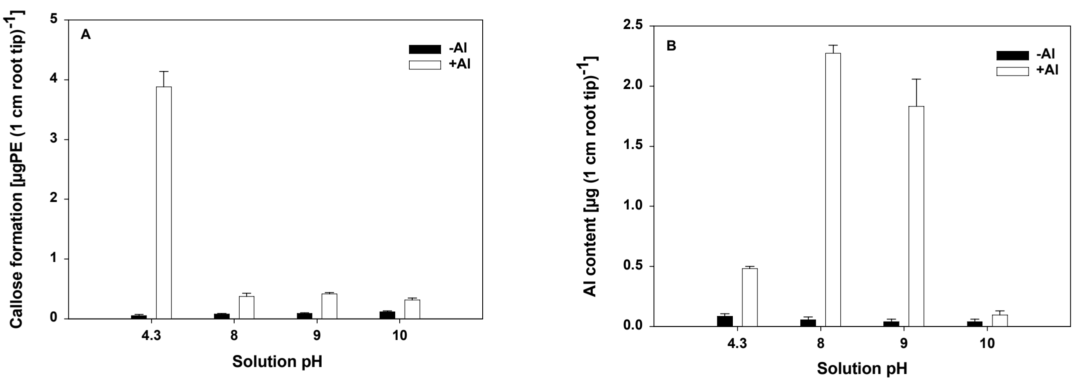

FIG.14. Aluminium effect on callose formation (A) and Al content (B) in 1 cm root tips of maize cv. Lixis at different solution pH values. pH-adapted plants were exposed to 0 or 50 µM $AlCl_3$ for 12 hours.

After 12 hours of Al treatment, Al content of the 1 cm root apices of the plants grown in solution at pH 8 was much higher than that at pH 4.3 (Fig. 14B). However, in contrast to pH 4.3, Al induced callose formation in the root apices only marginally, and root-tissue integrity was better maintained at pH 8 (Fig. 14A).

The largest fraction of the root-tip Al was recovered in the cell-wall fraction independent of the culture solution pH. A significantly slightly lower percentage of Al was recovered in the acid and base wash solutions but a higher percentage in the symplastic sap fraction in the root tips grown at alkaline pH. A sequential extraction of the isolated cell-wall material with increasing KOH concentrations suggests that most of the cell-wall Al was precipitated $Al(OH)_3$ in root tips exposed to Al at pH 8.0. This can be explained by a drastic pH reduction in the root apoplastic sap, which was only 0.3 units higher at bulk solution pH 8.0 compared to 4.3. The maintenance of an acidic apoplast at bulk solution pH 8.0 leads to the formation of cationic Al hydroxy species and $Al(OH)_3$ that induces root-growth inhibition. However this effect causes less plasma-membrane and cell damages than that when Al^{3+} is the dominating species at low solution pH. This finding can be interpreted as a circumstantial evidence.

3.2.4. The role of plasma membrane (PM) characteristics in Al resistance

In the maize transgenic plants the relation between the different types of sphingoid bases was drastically modified. There was a 10-fold increase in the amount of t18:1^{8t} that became the most abundant sphingoid base (45% of the total percent area, on average). On the other hand, the amount of t18:0 was reduced by half, reaching a level similar to t18:1^{8c} (20 to 30%). The less abundant sphingoid base in roots of transgenic plants was d18:2^{4t,8c} (15% of the total percent area). The results of the experiment to assess the total content of the sphingoid bases in root tips of the homozygous transgenic line 23/32/4, its parents, lines H99 and A188, and the varieties ATP-Y and Lixis are shown in Fig. 15.

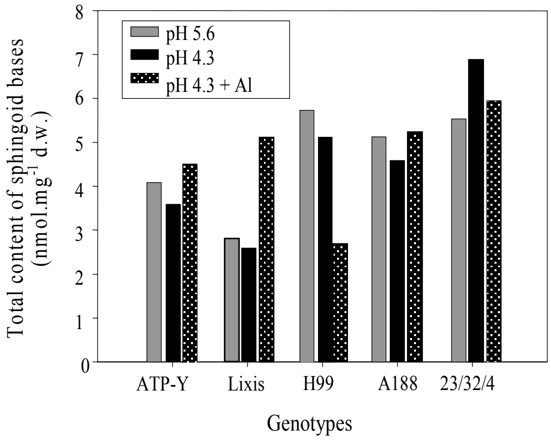

FIG.15. Total sphingoid base content of maize root tips (3 mm in length) [76].

Lowering the pH from 5.6 to 4.3 caused a slight reduction in the total sphingoid bases content of all genotypes, except for the transgenic line 23/32/4, where an increase from 5.5 to 7 nmol.mg^{-1}was observed. The Al treatment strongly affected the total sphingoid base content of the genotypes Lixis and H99. While a two-fold increase of the total amount of sphingoid bases was observed for Lixis (from 2.5 to 5 nmol.mg^{-1}), a reduction of one half was found for H99 (from 5 to 2.5 nmol.mg^{-1}). The over-expression of the *A. thaliana* Δ^8 (Delta (8))-sphingolipid desaturase gene did not increase the total sphingoid base content in the root tips of the transgenic line 23/32/4 (5.5 nmol.mg^{-1}) in comparison to the parental lines A188 (5 nmol. mg^{-1}) and H99 (5.8 nmol.mg^{-1}). The induction of callose formation in the root apices was used as a parameter to evaluate the effect of the transformation with the *A. thaliana* Δ^8-sphingolipid desaturase gene on the Al resistance of maize (Fig. 16).

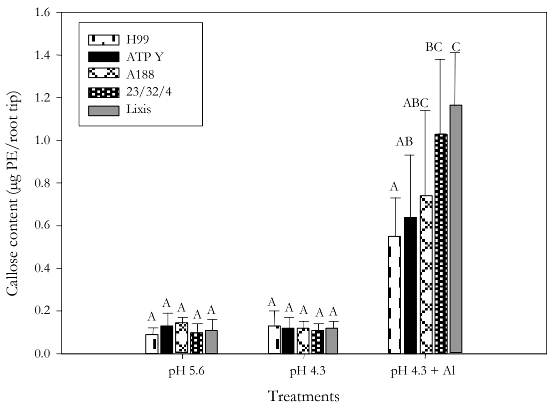

FIG.16. Callose content in root tips (0.5 cm in length) of maize cultivars and the transgenic line. Different capital letters indicate significant differences between the genotypes at each pH level [76].

The callose content in root tips of the transgenic line 23/32/4 was higher than in both parental lines, H99 and A188, though, due to high standard deviations, a statistical significant difference existed only in comparison with H99. Based on the callose content, the genotypes H99 and ATP-Y could be classified as Al-resistant, while the transgenic line 23/32/4 seems to be as sensitive to Al as Lixis. The over-expression of a Δ^8-sphingolipid desaturase seems to increase the Al sensitivity.

The analysis of the fatty acid composition of 3 mm apical root sections indicated (data not shown), that the degree of desaturation of fatty acids of the Al-resistant and the Al-sensitive maize genotypes was reduced in response to Al. This reduction was mostly due to a decrease in linoleic acid (18:2). The decrease in desaturation was more pronounced in the Al-sensitive than in the Al-resistant genotype. The changes were small (0.4% in the sensitive and 0.2% in the resistant cultivar), but experiments with yeast have shown that even small changes in fatty acid composition can have a pronounced effect on Al tolerance.

Yeast was transformed with a 9-acyl desaturase, which is the main desaturase in yeast and produces oleic acid (18:1) from stearic acid (18:0). Even so yeast is able to compensate changes in its fatty acid composition through a decrease of other unsaturated fatty acids (16:1), a small change in the degree of desaturation could be achieved through this transformation. This change in the degree of desaturation of 0.16% leads to a very strong increase in Al resistance (Fig. 17).

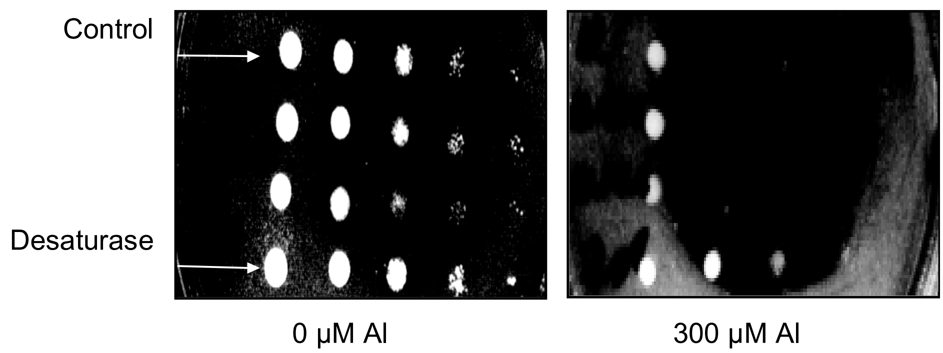

FIG.17. Growth of yeast transformed with 9 acyl desaturase or with an empty plasmid (control) on LPM (low phosphate medium) agar with or without Al. 10 µl of the yeast suspension in dilutions from 10^1 to 10^5 was placed on the agar and cultured at 28°C. Therefore, the assumption that an increase in Al resistance is achieved through an increase in unsaturated fatty acids in the membrane could be confirmed.

Seeds of wild type and mutants of *Arabidopsis thaliana* 'Columbia' differing in their fatty acid composition were screened for Al resistance on agar plates, containing different Al concentrations. Six mutants and the wild type were tested with 50 replicates for each Al concentration and genotype. Root length was analysed using the program 'Whinrhizo'. The results of root length of the mutants and the wild type are shown in Fig. 18.

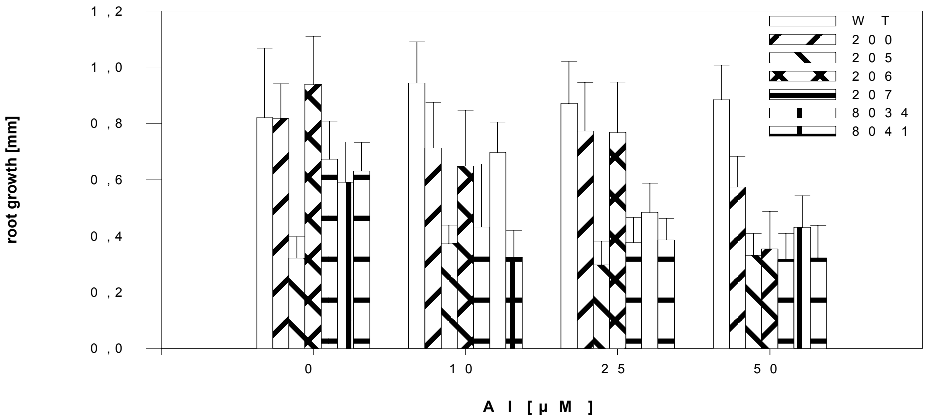

FIG.18. Effect of Al on root growth of Arabidopsis mutants, which show a decreased degree of saturation in their fatty acids. Root growth was measured after 24 h.

All mutants with increased degree of saturation in their fatty acids, with the exception of CS 205, showed an increase in their Al sensitivity (and a reduction in root growth) compared to the wild type. The root growth of mutant CS 205 was already reduced in the control treatment. This could be due to a greater pH-sensitivity of this genotype.

The results presented here support the hypothesis that the plasma membrane is involved in the perception and expression of Al stress. A decrease in chain length and/or degree of saturation of the fatty acids can increase the membrane fluidity. A change in the composition of the fatty acids is therefore, a possible way to counteract the negative effects of Al.

4. CONCLUSIONS

From these studies on the identification and characterization of Al-resistant, P-efficient plant genotypes adapted to tropical acid soils, the following can be concluded:

- Agronomic measures can contribute to increased P availability to crops. Among these, the application of organic matter sources such as green manure and crop residues to maintain or increase soil organic matter content and to enhance soil biological activity, and the incorporation into the cropping system of P-mobilising plant species are particularly beneficial. Such plant species may directly shift the equilibrium between sparingly soluble soil P fractions towards more plant-available forms. However, the main effect appears to be related to the recycling of the mobilised P via plant residues and enhanced root colonisation with P-mobilising rhizosphere micro-organisms of the crops subsequently grown in rotation. However, especially on P-deficient and strongly P-fixing soils these agronomic measures cannot substitute for the maintenance P fertilizer application.

- The mode of inheritance of Al resistance was predominantly through additive gene action. Results on both populations and inbred lines clearly show the potential of using Al-induced callose formation as a selection trait for adaptation to acid, Al-toxic soils**.** High correlation between *per se* value and general combining ability could greatly simplify the breeding schemes.

- The plant physiological studies have provided more evidence of the root apoplast involvement in Al toxicity and resistance. Particularly the use of pectin-specific antibodies for the localization of specific pectins differing in degree of methylation in the root apex appears promising in relation to Al localization in the root tissue. Maize genotypic differences in pectin contents and its degree of methylation were found. It can be concluded that Si treatment leads to the formation of hydroxy-aluminium-silicates (HAS) in the apoplast of the root apex thus detoxifying Al. The results on the relative phyto-toxicity of Al^{3+} versus $Al(OH)_4^-$ are interpreted as circumstantial evidence. The maintenance of an acidic apoplast at bulk solution pH 8.0 leads to the formation of cationic Al hydroxy species and $Al(OH)_3$ inducing root-growth inhibition. However this effect causes less plasma-membrane and cell damage than that when Al^{3+} is the dominating species at low solution pH. Moreover, the results confirm the possible involvement of plasma-membrane properties, in particular lipid composition on Al sensitivity and resistance. Modification of the membrane lipid composition clearly modulated Al resistance, although not always as predicted. Future work on more specific modulation of the plasma-membrane composition is needed to further elucidate the role of the plasma membrane properties on Al toxicity and resistance.

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COMPARISON OF THE ABILITY OF DIFFERENT PLANT SPECIES AND CORN HYBRIDS TO ACCESS POORLY-AVAILABLE SOIL PHOSPHORUS IN AN OXISOL OF THE CERRADO REGION, BRAZIL

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Abstract

The soils of the Cerrado region in Brazil have severe limitations for plant growth and crop production due to their high acidity and low natural fertility, in particular low phosphorus (P) availability. A series of experiments was carried out to make a comparative assessment of a large number of plant genotypes and identify the best adapted to these acid soils. In this paper the studies reported are related to the evaluation of a) twenty two (22) plant species and b) thirty (30) of the most recommended corn (*Zea mays* L.) hybrids for the Cerrado region with regard to their ability to absorb poorly-available soil phosphorus using the ^{32}P isotopic dilution technique. Two soils (Typic Dystrarox) of this region with contrasting management were employed: one soil was cultivated (and fertilized) for 20 years and another (natural) was kept under native vegetation. In the first experiment, species such as crotalaria, cowpea, soybean, stylosanthes, sunflower, peanut, millet and sorghum showed to have inefficient P uptake ability, while cotton, eucalyptus, rice, white lupin and pigeon pea were found to be highly P-efficient species. Black oat, corn, brachiaria, common bean, wheat, mucuna, tomato, triticale and barley were intermediate. In the second experiment significant differences in P uptake ability were also observed among corn hybrids in the cultivated soil. Seven genotypes were ranked as efficient, sixteen as intermedfiate and another seven as inefficient. Plant growth and P concentration of all corn hybrids grown in the natural soil with low fertility status were lower than those in the cultivated soil.

1. INTRODUCTION

The Brazilian savannah known as Cerrado has been the most important agricultural expansion area during the last two decades. The Cerrado region occupies approximately 2/3 of the country (2 037 600 km^2), of which 2/3 (136 Mha) is considered appropriate for food production [1]. Most of the soils are highly weathered Oxisols (46%) and Ultisols (15%), with serious limitations for crop production due to very low natural fertility, high acidity, high in Al saturation and high P fixation capacity. To control and mitigate these constraints to agricultural productivity, an integrated approach to crop, soil, water and nutrient management practices should be adopted [2].

Extensive research has produced technologies and strategies to overcome these soil fertility constraints. Among these, better fertilizer management practices and use of soil amendments are recommended [3]. However, a more sustainable strategy is to select plant genotypes more tolerant to soil acidity (Al toxicity) and more efficient in absorbing and utilizing P and other nutrients [4–7]. Although the use of crop genotypes tolerant to high soil acidity and low available P may not be so relevant for progressive farmers, for which liming and high P fertilization are common practices; certainly for most of the resource-poor farmers this practice would be a valuable alternative for obtaining better and sustained crop yields.

The ability of plants to grow in soils with low available P content can be attributed to several factors. Among them to the: (a) formation of proteoid roots and (b) secretion of organic acids (citric, pirocidic, malonic, oxalic, etc.), which solubilize "fixed" P. Parentoni et al working in acid soils of Brazil observed that corn genotypes considered efficient in absorbing P showed increased root length and weight under P deficiency [8].

There are several methodologies for the determination of plant P use efficiency. One of them is the use of the ^{32}P isotope dilution technique. Hocking *et al.* utilized this technique to compare several plant species for absorbing poorly available soil P [9]. The basic assumption of this technique is that when two or more plant species are grown in the same ^{32}P labeled soil and if they utilize the same pool of available P, then the specific activity of the plants should be similar. However, if one plant species absorbs a form of soil P (unlabelled) that is unavailable to other species, then the ^{32}P specific activity in that plant would be lower than that of the other species. The more efficient is the plant in absorbing less available soil P, the lower will be its specific activity [10].

In this research project a series of experiments was conducted to compare the ability of several plant species to access P from a tropical Oxisol from the Cerrado region using the ^{32}P isotope dilution technique. In a first experiment a number of plant species was evaluated for their ability to absorb poorly-available soil phosphorus. Further work was carried out with corn and soybean, the most important crops grown in the Cerrado. The studies with corn are only reported here.

2. MATERIALS AND METHODS

2.1. Experiment 1

A pot experiment was carried out in the greenhouse of the Center for Nuclear Energy in Agriculture (CENA), USP at Piracicaba, SP, Brazil.

Twenty two plant species cultivated in the Cerrado region were selected for the study (Table I). They included food, industrial and cover crops, pasture/forages and one tree species. They were grown in pots, each containing 1.5 kg of soil labeled uniformly with 6 MBq ^{32}P /pot.

The soil is a Typic Dystrarox, or Distrophyc red yellow Latosol, clayey, according to the Brazilian classification system [11] A bulk topsoil sample was collected from a farm in Planaltina, Goias state (Central Cerrado region) located at 15°14' latitude South and 47°42' longitude West, and 826 m above sea level. The soil used was under native vegetation. Selected characteritics are shown in Table II (refer to the column natural soil). Due to extremely low soil available P, an application of 200 mg P kg^{-1} soil was made utilizing the local Patos phosphate rock (PR) of very low reactivity (24.5% total P_2O_5; 4% citric acid soluble P_20_5 and negligible water soluble P).

TABLE I. PLANT SPECIES UTILIZED IN THE EXPERIMENT

Common name	Scientific name	Cultivar/hybrid
Peanut	*Arachis hypogea* L.	Tatu
Feijão bean	*Phaseolus vulgaris*	*Carioca Bico de Ouro*
Cowpea	*Vigna unguiculata*	*Branquinho*
White lupin	*Lupinus albus*	-
Tomato	*Lycopersicum esculentum* Mill.	*Sta Cruz Kade G*
Sunhemp	*Crotalaria juncea*	*IAC-KR1*
Velvet bean	*Mucuna aterrima*	-
Millet	*Pennisetum americanum*	*BN-2*
Cotton	*Gossypium herbaceum* L.	*IAC 280*
Black oat	*Avena strigosa Schieb*	-
Wheat	*Triticum aestivum*	*BRS-120*
Triticale	*Triticum secale*	*IAC-2*
Eucalyptus	*Eucalyptus grandis*	-
Brachiaria	*Brachiaria decumbens*	*Brizanta*
Stylosanthes	*Stylosanthes guianensis*	*Mineirão*
Soybean	*Glycine max* (L) Merr.	*Celeste*
Rice	*Oryza sativa* L.	*IAC-165*
Sunflower	*Helianthus annuus* Lin.	*IAC Uruguai*
Sorghum	*Sorghum vulgare* L.	*AG 2002*
Barley	*Hordeum vulgare* L.	*BR2*
Corn	*Zea mays* L.	*P30F33*
Pigeon pea	*Cajanus cajan* L.	*IAC fava L.*

TABLE II. SOME SELECTED CHARACTERISTICS OF THE EXPERIMENTAL SOIL

Soil	Natural*	Cultivated
OM (g kg^{-1})	22.55	22.55
S (mg dm^{-3})	1.20	2.50
P resin (mg dm^{-3})	5.40	26.70
P total (mg kg^{-1})	227	588
pH (H_2O)	5.02	6.11
pH ($CaCl_2$)	4.12	5.32
K ($mmol_c$ dm^{-3})	1.60	1.80
Ca ($mmol_c$ dm^{-3})	1.49	23.85
Mg ($mmol_c$ dm^{-3})	1.51	9.61
H+Al ($mmol_c$ dm^{-3})	65.81	26.05
SB ($mmol_c$ dm^{-3})	4.60	35.26
CEC ($mmol_c$ dm^{-3})	70.41	61.31
V%	6.50	57.50

*__Natural__ soil was under original Cerrado vegetation; **Cultivated** soil was under cultivation (and fertilized) over the last 20 years.

The experimental design was a complete randomized of 22 treatments with 4 replicates. All pots received basal nutrient solutions containing N, K, S, Ca, Mg, B, Cu, Zn, Mn and Mo to ensure adequate plant growth. The plants were harvested at 32 days after sowing, instead of 50 days as planned, as most plants showed stunted growth due to nutrient deficiencies.

The collected plants material were oven-dried (65°C) for 3 days, weighed, ground and digested (nitric-perchloric digestion) for determination of total P and ^{32}P activity. From the plant total P content and ^{32}P activity data, the specific activities of the treatments (plant species studied) were estimated. Comparisons were made, considering the white lupin (most P efficient) as reference plant species. The results were statistically analyzed and a treatment means comparison was made by the Tukey's test at 5% level.

2.2. Experiment 2

This study was aimed to compare grain yield potential and foliar P contents of twenty seven (27) corn hybrids grown in the cultivated (and fertilized) Typic Distrarox soil of the Planaltina, DF, Central Cerrado region. Selected characteristics are shown in Table II (refer to column cultivated soil). The list of corn hybrids utilized in this experiment is given in Table III. The crops were fertilized with NPK (90, 90 and 45 kg N, P_2O_5 and K_2O ha^{-1}, respectively).

TABLE III CORN HYBRIDS UTILIZED IN THE EXPERIMENT

Hybrid	Code	Hybrid	Code	Hybrid	Code
1- P30K7T	P1	10-DINA 766	D1	19- P30F89	P12
2- P3021	P2	11- AG6690	A2	20 - Z8420	Z1
3- P30F33	P3	12- P3071	P7	21- TORK	N1
4- P30207	P4	13- X1318J	P8	22- P3072	P13
5- AG6018	A1	14- X1268Z	P9	23- P3081	P14
6- C813	C1	15- C909	C2	24- Z8474	Z2
7- X1318H	P5	16- C333B	C3	25- C747	C4
8- P30F45	P6	17- P30F80	P10	26- Z8555	Z3
9- PopularX1	X1	18- P3041	P11	27- C929	C5

2.3. Experiment 3

Further experimental work was conducted to compare thirty corn hybrids most commonly grown in the Cerrado region (Table IV) for their P uptake ability under both natural and cultivated soils. White lupin was included as reference crop.

TABLE IV. MAIN CHARACTERISTICS OF THE CORN HYBRIDS EVALUATED

Hybrid	Type	Growth cycle	Plant height
P30F80	Single	Semi early	Medium
C909	Single	Early	Medium
PX1409K	Single	Semi Early	Tall
C333B	Single	Late	Medium
P30F88	Single	Semi Early	Medium
P3081	Single Modified	Super Early	Short
P3071	Triple	Early	Medium
DINA766	Single Modified	Semi Early	Medium
P3041	Triple	Semi Early	Medium
P3021	Triple	Semi Early	Medium
ZN8452	Single	Semi Early	Medium
AG7575	Triple	Semi Early	Medium
C747	Triple	Semi Early	Medium
DKB911	Single	Early	Medium
AG6018	Triple	Early	Medium
ZN8550	Single	Semi Early	Medium
PX1379F	Single	Semi Early	Medium
TORK	Single	Semi Early	Medium
ZN8410	Single	Semi Early	Medium
P30K75	Single	Semi Early	Short
AG9090	*	*	Medium
PX1359G	Single	Semi Early	Medium
P3027	Triple	Semi Early	Medium
PX1339F	Single	Early	Medium
P30F33	Single	Early	Medium
DINA657	Single Modified	Semi Early	Medium
ZN8471	Single	*	Medium
C813	*	*	Medium
P30F45	Single	Semi Early	Tall
ZN8420	Single	Early	Medium

The corn hybrids were grown in pots, each containing 1.5 kg of the soil either natural or cultivated (Table II) labeled uniformly with 6 MBq ^{32}P /pot. Four seeds of each corn hybrid were sown per pot and after 8 days thinned to 2 plants per pot. The remainder of the experimental procedures was similar to that described in Experiment 1.

3. RESULTS AND DISCUSSION

3.1. Experiment 1

During the experiment, plant growth was affected and visual symptoms of possible nutritional deficiencies were observed. At 18 days after sowing, cotton plants had some old leaves with chlorosis symptoms in the border, which became necrotic, probably were due to K deficiency, as this soil has very low K content. Corn plants started to show typical

P deficiency symptoms, i.e. reduced growth and purple bluish-dark green color. At 25 days after sowing, the leaves of cowpea showed necrotic points in the limbs and started to fall. These symptoms were apparently due to some plant disease. The prevailing high temperatures in the greenhouse have also likely affected plant growth.

Large differences in plant P contents were found between the plant species studied and plant P contents ranged from 0.44 to 2.39 g kg^{-1}. Among the plant species evaluated, cowpea presented the highest P content, 2.39 g kg^{-1}, followed by stylosantes (2.08 g kg^{-1}). Brachiaria, sorghum, velvet bean and barley were the plant species with least P content, varying from 0.44 to 0.73 g kg^{-1} P (Table V). These results are in agreement the work of Hocking *et al.* [8], showing that white lupin was the species with the highest accumulated P in the plant while canola was the least. In this study pigeon pea, sunflower and soybean showed intermediate values.

TABLE V. P CONTENTS OF PLANT SPECIES GROWN IN NATURAL OXISOL OF THE CERRADO

Plant specie	P (g kg^{-1})			
Crotalaria	1.36	c	d	
Cowpea	2.39	a		
Soybean	1.15	d	e	f
Stylosantes	2.08	b		
Sunflower	1.28	d	e	
Peanut	1.35	c	d	
Millet	1.18	d	e	f
Sorghum	0.67	h	i	
Oat	1.35	c	d	
Corn	0.83	g	h	
Brachiaria	0.44	i		
Common bean	1.63	c		
Wheat	1.23	d	e	
Velvet bean (Mucuna)	0.68	h	i	
Tomato	1.00	e	f	g
Triticale	1.20	d	e	f
Barley	0.73	g	h	i
White lupin	0.80	g	h	
Rice	0.93	f	g	h
Eucalyptus	0.99	e	f	g
Cotton	0.77	g	h	
Pigeon pea	0.92	f	g	h
F	71.79*			
CV(%)	8.27			

* Values followed by the same letter in the column do not differ significantly at p=0.05.

However the plant P content is not an indicator of their efficiency in absorbing less available soil P. The measured ^{32}P specific activities for the plant species studied are shown in Table VI. Large differences between plant species were found ranging from 27.8 to 150 dpm μg^{-1} P. Crotalaria and cowpea presented the highest specific activity, 150 and 142.6 dpm ^{32}P μg^{-1} P, respectively, while rice, eucalyptus, cotton and pigeon pea had the lowest specific activities, 44.2; 39.2; 35.3 and 27.8 dpm ^{32}P μg^{-1} P, respectively. The highest ^{32}P specific activities of crotalaria and cowpea, may suggest that these plants are not efficient in absorbing less available soil P.

TABLE VI. ^{32}P SPECIFIC ACTIVITIES (S) OF PLANT SPECIES GROWN IN NATURAL CERRADO OXISOL

Plant specie	S (dpm ^{32}P μg^{-1} P)	
Crotalaria	150.0	a
Cowpea	142.6	a
Soybean	136.5	a b
Stylosantes	130.9	a b
Sunflower	114.4	a b c
Peanut	104.2	b c d
Millet	104.0	b c d
Sorghum	100.4	b c d e
Oat	92.1	c d e f
Corn	87.2	c d e f g
Brachiaria	84.7	c d e f g h
Common bean	84.5	c d e f g h
Wheat	77.2	d e f g h i
Velvet bean (Mucuna)	66.5	e f g h I j
Tomato	65.5	e f g h I j
Triticale	57.5	f g h i J k
Barley	51.0	g h i j k
White lupin	49.2	h i j k
Rice	44.2	i j k
Eucalyptus	39.2	j k
Cotton	35.3	j k
Pigeon pea	27.8	k
F	28.15*	
CV(%)	14.13	

* Values followed by the same letter in the column do not differ significantly at p=0.05.

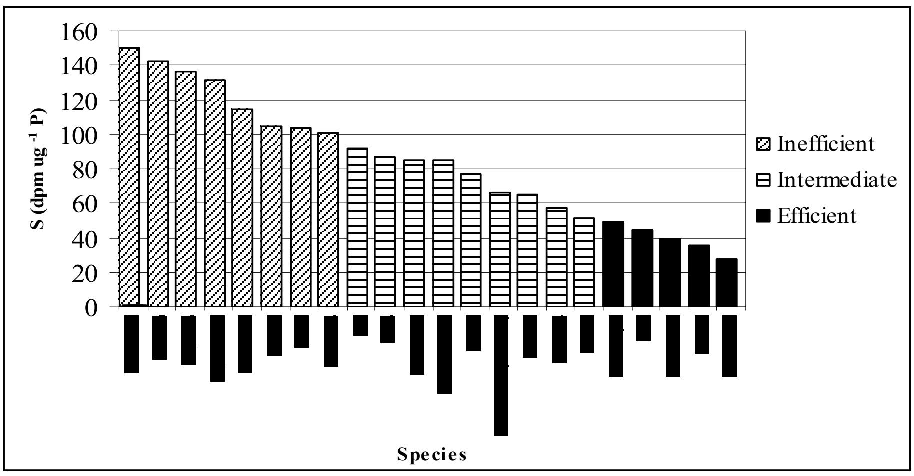

FIG.1. Classification of plant species according to P uptake ability from a natural Cerrado Oxisol.

Based on the specific activities obtained, the studied plant species were ranked in three categories (Fig. 1): Inefficient, those plant species with specific activities between 100–150 dpm µg^{-1} P (crotalaria, cowpea, soybean, stylosanthes, sunflower, peanut, millet and sorghum); Intermediate, those with specific activities between 50-100 dpm µg^{-1} P (black oat, corn, brachiaria, common bean, wheat, velvet bean, tomato, triticale and barley). Cotton, eucalyptus, rice, white lupin and pigeon pea, with specific activities of less than 50 dpm µg^{-1} P may be considered as efficient in absorbing available soil P.

Several practical implications are derived from these results. For the natural Cerrado soil, due to its inherent low available P, when not fertilized, crotalaria is certainly not a good option for green manuring. Similarly soybean, stylosanthes or sunflower will also not be adequate for rotation with corn. As these crops are inefficient for P uptake, they need to be fertilized, in other words their production levels are highly dependent on P fertilization. Velvet bean (mucuna) is probably a good option for green manure. Among the crops, cotton is a good option due to its high efficiency in absorbing low available P. Millet, black oat and wheat, are suitable crops to temperate climate and less efficient in absorbing P, thus they are not adequate for the Central Cerrado. The Eucalyptus was found to be a P-efficient tree species, therefore it may be a good option for reforestation purpose or timber production in the Central Cerrado region.

3.2. Experiment 2

The grain yields of the corn hybrids obtained in this experiment are presented in Fig. 2. The most productive hybrids P1, P2, P3, P4 and A1, yielded more than 9 t ha^{-1} of grain (9,282, 9,262, 9,224, 9096 and 9,066 kg ha^{-1} grain, respectively). The hybrids C4, Z3, C5 and Z1, with grain yields below 7.5 t ha^{-1} (7,457, 7,392, 6,722 and 5,469 kg ha^{-1} grain, respectively) were the less productive ones. With regard to the leaf P content (Fig. 3), the hybrids A2 and P5 presented the lowest leaf P content (2.49 g kg^{-1} P), while the hybrids P13, P4, P3 and Z2, had the highest leaf P content (3.19, 3.17, 3.15 and 3.13 g kg^{-1} P). The latter are probably the most adapted hybrids for the fertilized Cerrado soils.

These differences are a result of breeding and adaptation modifications, attributed to several factors such as differences in root morphology and abundance of root absorbent hairs, as well as physiological, biochemical and molecular alterations, rendering them more productive under the cultivated conditions of the Brazilian Cerrado.

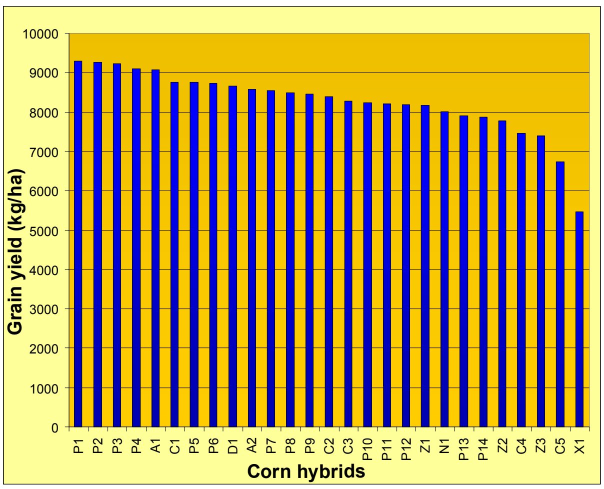

FIG.2. Grain yield of corn hybrids obtained in a fertilized Cerrado Oxisol.

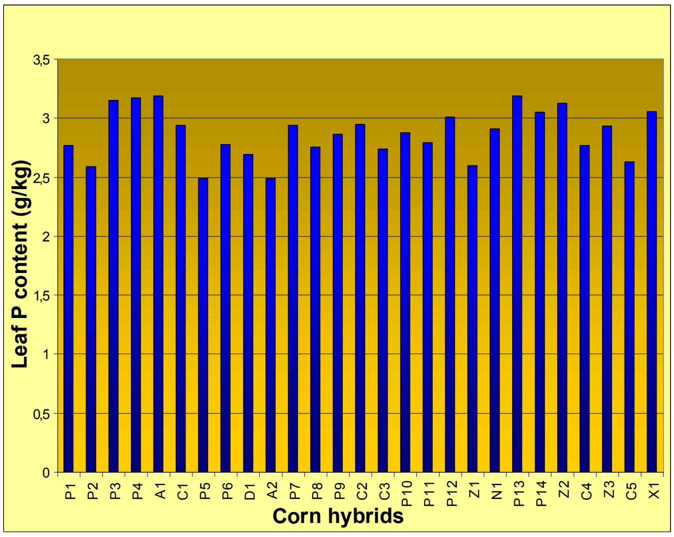

FIG.3. Leaf P content of corn hybrids in a fertilized Cerrado Oxisol.

In a follow up field experiment (data not shown) the response of corn to five increasing P rates (0, 30, 60, 90 and 150 kg P_20_5 ha^{-1}) was evaluated in the Central Cerrado area. The P application rate most commonly used by progressive farmers growing corn is 90 kg P_20_5 ha^{-1}. As expected, no statistical differences among the P rate treatments were found. The control (0 P) produced 8106 kg grain/ha and the maximum yield obtained was 8345 kg grain ha^{-1}. Thus, a farmer, if he had not applied any P fertilizer, would have saved about US$ 20/ha, i.e. the approximate cost of 90 kg of P_20_5 ha^{-1}.

3.3. Experiment 3

Plant growth and P concentration of all corn hybrids grown in the natural soil was lower than those in cultivated soil resulting from the inherent low fertility of the natural soil. The hybrids were listed in decreasing order of their specific activities to group them into three arbitrary categories: efficient, inefficient and intermediate as shown in Fig. 4. Among the hybrids evaluated, the ZN8420, P30F45, C813, ZN8471, DINA657, P30F33, and PX1339F, all single hybrids, except the DINA657, which is a single modified, were the most efficient to absorb less available soil P (Fig 4). Moreover they can be also considered suitable for the Cerrado region due to their earliness (Table IV). The less efficient hybrids were P30F80, C909, PX1409K, C333B, P30F88, P3081 and P3071.

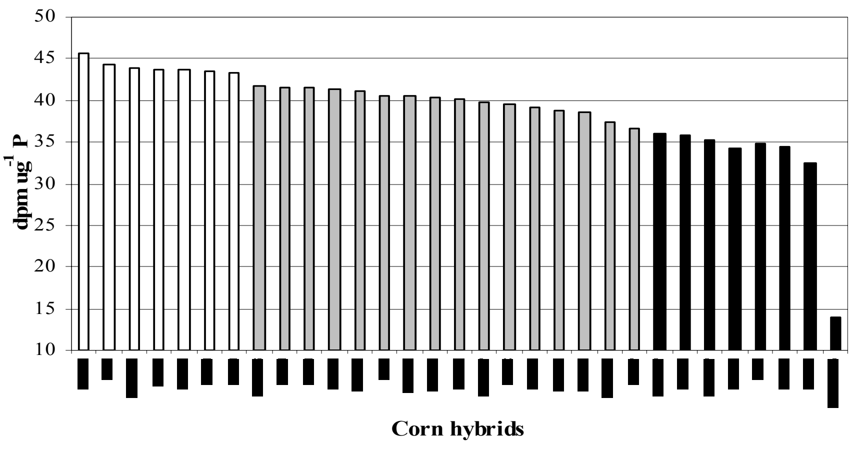

FIG. 4 Classification of corn hybrids grown in cultivated soil based on ^{32}P specific activity. ☐ *Inefficient* ▒ *Intermediate* ■ *Efficient*

4. CONCLUSIONS

From these studies, it can be concluded that there are ample genotypic differences in P use efficiency between commonly grown plant species and tested corn hybrids under the particular evaluation conditions. The most efficient ones such as cotton, eucalyptus, rice, white lupin and pigeon pea can be considered for cultivation in either natural or cultivated (fertilized) Oxisols of the Central Cerrado region. Significant differences in P uptake ability were also observed among corn hybrids in the cultivated soil. The P-32 isotopic technique proved to be an effective tool to compare the ability of different plant species and breeding materials to access poorly available soil P on Oxiols of Brazil.

ACKNOWLEDGEMENTS

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EVALUATING THE RESPONSE OF SELECTED COMMON BEAN GENOTYPES TO THE APPLICATION OF PHOSPHATE ROCK PRODUCTS IN ACID SOILS OF CUBA

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Abstract

Glasshouse experiments were carried out to evaluate the response of common bean (*Phaseolus vulgaris*) genotypes to the application of phosphorus (P) sources varying in solubility and to compare the agronomic effectiveness of local phosphate rock (PR) products in two acid soils (Acrisol and Rhodic Ferralsol) of Cuba. Recommended common bean genotypes were evaluated, i.e. BAT 477, DOR 364, DOR 390 and Censa were grown in the Acrisol and BAT 477, DOR 364, BAT 58 and CC 25-9(N) in the Rhodic Ferralsol. The ^{32}P isotopic dilution method was utilised to measure the P uptake from the following P sources: Trinidad de Guedes PR, a partially acidulated PR product (FPA 50) and water-soluble P fertilizers, i.e. single (SSP) or triple super phosphate (TSP), which were used as reference of comparison. A control (without P application) treatment was also included. Genotypic differences in dry matter production, total P uptake and in the plant P derived from the applied P sources were found, resulting in significant genotypic differences on the agronomic effectiveness of P sources studied. However, this effect varied with soil type. The direct application of Trinidad de Guedes PR was only efficient, i.e. % Pdff was higher than 30%, for DOR 364 in the Acrisol. The FPA 50 was efficient for all common bean genotypes in both soils. In the Acrisol on the average for the genotypes studied from 1,0 to 2,9 kg of P as FPA 50 were equivalent to 1 kg of P as SSP. In Rhodic Ferralsol, from 1,4 to 2,0 kg of P as FPA 50 were equivalent to 1 kg of P as TSP. The results obtained are useful for breeding programs aiming at obtaining common bean genotypes with high P use efficiency for areas having P deficient soils and where locally available cheap P sources of low (PR) or medium solubility (FPA 50) can be applied. Further field validation studies should be carried out in areas, which are representative of studied acid soils.

1. INTRODUCTION

Phosphorus (P) is an essential nutrient for plants and its deficiency in soils severely restricts crop yields. Tropical and subtropical soils are predominantly acidic and often extremely deficient in phosphorus. Moreover most of these soils posses a high phosphate sorption capacity. Therefore, substantial P inputs are required for optimum plant growth and adequate food and fiber production [1]. Manufactured water-soluble P fertilizers such as super phosphates are commonly recommended to correct P deficiencies, but most developing countries import these fertilizers, which are often in limited supply and represent a major outlay for resource-poor farmers. In addition, intensification of agricultural production in these countries necessitates the addition of P not only to increase crop production but also to improve soil P status in order to avoid further soil degradation. Hence, it is imperative to explore alternative P sources such as phosphate rocks [2]. Thus, in many countries a potential alternative P source to mostly imported and expensive water-soluble P fertilizers is the direct application of locally available phosphate rock (PR). The P availability from PRs to crops and therefore, their agronomic effectiveness when applied directly as P fertilizers depends on various factors and their interactions. The main factors include: (a) the physical and chemical properties of PRs; (b) soil and climatic factors; (c) plant species and cultivars; and (d) farming management practices. However, if inherent PR characteristics are not favourable for direct application, then it would be necessary to increase its effectiveness through biological and/or technological processes [2, 3].

Phosphate rocks of different origin were evaluated as P sources for common bean grown in Colombian soils. The best results were obtained with PRs having high citrate solubility and their agronomic effectiveness was similar as triple superphosphate (TSP) [4]. In another study several partially acidulated PR products were prepared to increase the solubility and agronomic effectiveness of low reactive PR sources. The PAPR at 40% with H_2SO_4 prepared from the low reactive Pesca of Colombia was as effective as TSP in increasing common bean grain yields in a high P sorption Andept soil [5]. It is also possible to improve the agronomic effectiveness of PR for direct application through biological means such as use of P-efficient genotypes with enhanced rizhospheric effect [2].

Several authors have reported on the significant role of P fertilization on the grain yield production of common bean, in particular the potential use of PRs in acid soils [6–9]. In Cuba, common bean, mainly the varieties of black grain, are an essential component of the diet of the local population. However, soil P deficiency, among others factors, limits severely the grain yields and the effectiveness of biological nitrogen fixation (BNF) in this grain legume crop. In a neutral Rhodic Ferralsol of Cuba the partial acidulation of Trinidad de Guedes PR increased grain yields of the common bean genotype BAT 304 [10]. However, there is little information on the response of common bean genotypes to the application of PR products in acid soils [11].

This study was carried out to evaluate the response of the common bean genotypes to phosphatic fertilizers varying in solubility and to compare the agronomic effectiveness of local PR products in two acid soils of Cuba using the ^{32}P isotopic technique [12]. The results will provide information on the P fertilizer use efficiency of selected common bean genotypes from locally available P sources of low to medium solubility. Moreover, this information would be useful for breeding programs aiming at obtaining common bean varieties with superior P use efficiency.

2. MATERIALS AND METHODS

Two glasshouse experiments were carried out in two representative acid soils of Cuba, i.e. an Acrisol (Experiment 1) and a Rhodic Ferralsol (Experiment 2).

2.1. Experimental treatments and design

The following specifications are common to both experiments. The experimental treatments consisted of a 4 x 4 factorial arrangement (4 common bean genotypes and 4 P fertilizer treatments) with a randomized complete block design and with 3 replications. Four common bean genotypes recommended for each location were selected. Experiment 1 was carried out in an Acrisol soil with BAT 477, DOR 364, DOR 390 and Censa whereas Experiment 2 in a Rhodic Ferralsol soil with BAT 58, BAT 477, CC 25-9(N) and DOR 364. The seeds of the studied common bean genotypes were obtained from the germplasm bank of the Experimental Station La Renée, except Censa that was provided by small farmers of Viñales,where this genotype is commonly cultivated. Three P-fertilizer sources were compared at 100 mg P kg^{-1} soil: (i) indigenous phosphate rock (PR) from the Trinidad de

Guedes deposit; (ii) a partially acidulated PR (PAPR) at 50% with H_2SO_4 (FPA 50); and (iii) single superphosphate (SSP) or triple super phosphate (TSP) were used as reference of comparison in the experiments 1 (Acrisol) and 2 (Rhodic Ferralsol) respectively. In addition a control treatment (without P) was included as reference of comparison.

2.2. Experimental procedures

The acid soils utilised are classified as Acrisol from Pinar del Rio province and Rhodic Ferralsol from La Habana province [13]. Some selected soil characteristics are shown in Table I. The Acrisol had lower pH, lower base saturation and lower P status than the Rhodic Ferralsol. The Rhodic Ferralsol is located in a region where the continous use of irrigation water with high contents of calcium has increased the soil pH. In addition, in the past this soil had received some NPK fertilization.

TABLE I. SELECTED SOIL CHARACTERISTICS[1]

	pH KCl	OM	Total N	Av.P	Ca	Mg	K	Na
		%		mg P kg^{-1}	cmol (+) kg^{-1}			
Acrisol	3.80	3.02	0.115	4.4	0.75	0.43	0.05	0.10
Rhodic Ferralsol	4.70	2.82	0.119	7.2	6.77	0.36	0.57	0.10

(1): pH, 1:2,5 soil:KCl ratio; OM, Walkley-Black method; Total N, Kjeldhal method; Available P, Bray - Kurtz I method; Exchangeable Cations, Schastschabel method.

The pots were filled with 1000 cm^3 of each soil. Six seeds were sown per pot and 4 days after emergence, seedlings were thinned to give 3 plants per pot. At sowing, all pots received a basal application of 20 ml of nutrient solution containing 100 mg N kg^{-1} of soil as urea (46% N) and 60 mg K kg^{-1} as KCl (60% K_2O).

Relevant data of the chemical composition, solubility indices and empirical formulae of the indigenous PR and the FPA 50 product used in the experiments are given in Tables II, III and IV respectively.

TABLE II. CHEMICAL COMPOSITION (%) OF TRINIDAD DE GUEDES PR AND PARTIALLY ACIDULATED PR

PR products[1]	P_2O_5	CaO	MgO	S	Fe_2O_3	Al_2O_3
PR	30.6	43.6	0.28	0.6	2.14	3.42
FPA 50	19.2	27.1	0.31	8.1	2.08	4.02

(1) PR: indigenous Trinidad de Guedes PR and FPA 50: Partially acidulated PR (PAPR) produced from Trinidad de Guedes PR with H_2SO_4 at 50% acidulation level. The FPA 50 fertilizer was produced by the Rayonitro Enterprise, Cuba.

TABLE III. SOLUBILITY (% OF TOTAL P_2O_5) TESTS IN CONVENTIONAL REAGENTS OF TRINIDAD DE GUEDES PR AND PARTIALLY ACIDULATED PR

	Water	Neutral Ammonium Citrate	2% Citric Acid	2% Formic Acid
PR	0.1	11.3	26.5	26.8
FPA 50	28.5	44.9	76.0	61.7

TABLE IV. EMPIRICAL FORMULA OF TRINIDAD DE GUEDES PR

Length of a axis (Å)	Empirical formula	Molar ratio CO_3 /PO_4
9,318	$Ca_{9.50}\ Na_{0.36}\ Mg_{0.14}\ (PO_4)_{4.71}\ (CO_3)_{1.29}\ F_{2.52}$	0,274

2.3. Isotopic method

In experiment 1 the pot capacity was 1,4 kg of Acrisol soil and the applied ^{32}P activity per pot was 2.96 MBq + 2 mg P kg^{-1}; whereas in experiment 2, the pot capacity was 1.2 kg of Rhodic Ferralsol soil and the applied ^{32}P activity per pot was 1.85 MBq + 5 mg P kg^{-1}.

The following formulas were used to evaluate isotopic data:

% Pdff = (S. A. Treatment with Fert. / S. A. Treatment. without Fert.) × 100;

S. A. (Specific Activity) = ^{32}P Activity (dpm)/mg ^{31}P;

Pdff = (% Pdff × P yield)/100;

% FUE (% Fertilizer Use Efficiency) = (Pdff / applied P dose) × 100.

A Value soil = [(1 - %Pdff) / % Pdff] × Applied P Dose;

A Value of the soil was estimated as the quantity of soil available-P expressed in equivalent units of water- soluble P fertilizer used as reference [12].

2.4. Experimental procedures for measuring plant response

Six weeks after sowing, the above-ground portions of the common bean plants were harvested. The plant materials were oven-dried at 65°C for72 hours, and weighted. Thereafter, the plant materials were cut in small pieces using scissors, ground in a mortar, ashed and dissolved in HCl. Total P was determined using the vanadomolybdate yellow method and the the ^{32}P activity was measured on an aliquot of the above samples by Cerenkov counting using a liquid scintillation analyzer. P uptake was calculated as mg P pot^{-1}.

The Relative Agronomic Effectiveness (RAE) for the PR products based on dry matter and P uptake values were estimated as follows:

$$RAE (\%) = [(Y_1 – Y_0)/(Y_2 – Y_0)] \times 100$$

where Y_1 = Dry matter or P uptake obtained with PR or FPA 50,Y_2 = Dry matter or P uptake obtained with the P fertilizer reference of comparison, i.e. SSP in experiment 1 and TSP in experiment 2, Y_0 = Dry matter or P uptake obtained with control [14].

2.5. Statistical analysis

The analysis of variance (ANOVA) was run according to the experimental design, in a 4 P treatments x 4 common bean genotypes factorial arrangement using the STATITCF software program. The treatments means were compared using the Newman – Keuls test. In all cases, the maximal probability of error was 5%.

3. RESULTS AND DISCUSSION

3.1. Shoot dry matter

Data in Tables V and VI show the dry matter yield of the common bean genotypes obtained in the Acrisol and Rhodic Ferralsol respectively. In both experiments there was a significant effect of genotypes on growth and dry matter production. In the Acrisol shoot dry matter of DOR 390 and Censa (4.90 and 4.77 g pot^{-1}, respectively) was significantly higher than that of BAT 477 (3.93 g pot^{-1}) while in Rhodic Ferralsol dry matter yields of BAT 58 and CC 25-9N (2.88 and 2.80 g pot^{-1}, respectively) were significantly higher than those of DOR 364 and BAT 477 (2.64 and 2.51 g pot^{-1}, respectively). The mean dry matter values for P sources in the Acrisol, though relatively higher than those of the Rhodic Ferralsol, were not statistically significant between them.

TABLE V. DRY MATTER YIELD (G POT^{-1}) OBTAINED WITH VARIOUS P SOURCES FOR COMMON BEAN GENOTYPES[1] GROWN IN AN ACRISOL

Acrisol	Control	PR	FPA 50	SSP	Genotypes means
BAT 477	3.82	3.50	4.02	4.40	3.93 b
DOR 364	4.08	4.35	4.96	4.21	4.40 ab
DOR 390	4.32	5.65	4.95	4.68	4.90 a
CENSA	4.39	4.62	4.98	5.11	4.77 a
P sources means	4.15a	4.53a	4.73a	4.60a	-

[1] Values followed by same letter within row (P sources) or within column (genotypes) are not significantly different ($p = 0.05$).

In addition, in the Rhodic Ferralsol (Table VI) the effect of P sources was significant; TSP and FPA 50 (3.00 and 2.97 g pot^{-1}, respectively) were significantly higher than PR and control treatments (2.62 and 2.25 g pot^{-1}, respectively). The dry matter response to P sources in this soil may be due to its relatively higher pH (less acidic) and better available P status (Table I). The increase in dry matter production was related to the degree of solubility of the P source but no statistical differences were found between FPA 50 and TSP. These results are in agreement with those from the past FAO/IAEA Phosphate project, where it was reported that the utilization of PR would be more effective in soils having low pH, low P availability, high cationic exchangeable capacity, low exchangeable Ca, and high content of organic matter [15].

TABLE VI. DRY MATTER YIELD (g pot^{-1}) OBTAINED WITH VARIOUS P SOURCES FOR COMMON BEAN GENOTYPES[1] GROWN IN A RHODIC FERRALSOL

Rhodic Ferralsol	Control	PR	FPA 50	TSP	Genotypes means
BAT 58	2.43	2.64	3.14	3.32	2.88 a
BAT 477	2.01	2.40	3.00	2.64	2.51 c
DOR 364	2.13	2.81	2.77	2.86	2.64 bc
CC 25-9N	2.41	2.64	2.97	3.17	2.80 a
P sources means	2.25 c	2.62 b	2.97 a	3.00 a	-

[1] Values followed by same letter within row (P sources) or within column (genotypes) are not significantly different ($p = 0.05$).

3.2. P uptake

P uptake data of the bean genotypes fertilized with various P sources are given for the Acrisol and the Rhodic Ferralsol in Tables VII and VIII respectively. Significant effects of genotypes and P sources were found in the Acrisol and only for P sources in the Rhodic Ferralsol. In the Acrisol the best P source was SSP and no differences were found between PR and FPA 50 whereas in the Rhodic Ferralsol TSP and FPA 50 were the best P sources and both were superior to PR. The P uptake response is related to the degree of solubility of the studied P sources. In both soils the water-soluble P fertilizers, i.e. SSP and TSP are the best P sources. In the strongly acidic Acrisol both PR products (PR and FPA 50) had similar performance whereas in the less acidic Rhodic Ferallsol TSP and FPA 50 showed similar performance. The effect of soil pH on PR dissolution is well documented [15–16]. There was a significant differences effect of the genotypes x P sources interaction in both soils (Tables VII and VIII). In the Acrisol P uptake of the Censa/SSP treatment (19.07 mg P pot^{-1}) was significantly higher than all other genotype/P source combinations. P uptake of DOR 390 and Censa was significantly greater when they were fertilized with SSP (12.82 and 19.07 mg P pot^{-1}, respectively) or FPA 50 (14.83 and 14.48 mg P pot^{-1}, respectively) than with PR (9.25 and 8.54 mg P pot^{-1}, respectively). It is noticeable that BAT 477, a bean genotype reported with good adaptability to tropical soils (acid and of low available P), showed the lowest P uptake in the control treatment (6.88 mg P pot^{-1}). On the contrary this genotype performs poorly in unfertilized acid soils. In the Rhodic Ferralsol P uptake of DOR 364/TSP treatment (13.56 mg P pot^{-1}) was significantly higher than all the other genotype/P source combinations. In addition, P uptake of BAT 477/FPA 50 (11.97 mg P pot^{-1}) or BAT 477/TSP (11.12 mg P pot^{-1}) was significantly higher than all other combinations, except the DOR 364/TSP treatment (13.56 mg P pot^{-1}). P uptake of CC 25-9(N) was greater than the others genotypes in the control treatment. This result is in agreement with [9] that reported the high P requirements of this common bean genotype for its growth.

TABLE VII. P UPTAKE (MG POT^{-1}) OF COMMON BEAN GENOTYPES[1] FERTILIZED WITH VARIOUS P SOURCES IN AN ACRISOL

Acrisol	Control	PR	FPA 50	SSP	Genotypes means
BAT 477	6.88 e	9.77 bcde	9.80 bcde	14.07 bc	10.13 b
DOR 364	9.22 cde	9.23 cde	12.37 bcd	12.46 bcd	10.82 b
DOR 390	9.85 bcde	9.25 cde	14.83 b	12.82 bcd	11.69 ab
CENSA	9.76 bcde	8.54 de	14.48 bc	19.07 a	12.96 a
P sources means	8.93 c	9.20 b	12.87 b	14.60 a	-

[1] Values followed by same letter within row (P sources) or within column (genotypes) are not significantly different (p = 0.05).

TABLE VIII. P UPTAKE (MG POT^{-1}) COMMON BEAN GENOTYPES[1] FERTILIZED WITH VARIOUS P SOURCES IN A RHODIC FERRALSOL

Rhodic Ferralsol	Control	PR	FPA 50	TSP	Genotypes means
BAT 58	7.32 d	8.54 cd	9.83 bcd	9.98 bcd	8.92
BAT 477	7.61 d	8.23 cd	11.97 ab	11.12 bc	9.73
DOR 364	7.38 d	9.78 bcd	10.10 bcd	13.56 a	10.20
CC25-9N	10.42 bcd	8.92 bcd	10.50 bcd	9.01 bcd	9.71
P sources means	8.18 b	8.86 b	10.60 a	10.92 a	

[1] Values followed by same letter within row (P sources) or within column (genotypes) are not significantly different (p = 0.05).

The low response of annual, in particular short growing season crops to direct application of PR and the increase of the agronomic effectiveness of PR through partial acidulation are in agreement with other reports. Several authors have demonstrated the possibility to use PR for the phosphatic nutrition of legumes and the importance of P for this crop in acid soils [6, 8].

In most cases the partially acidulated PR (FPA 50) was more effective than PR. This advantage of FPA 50 over PR is likely due to its higher P water-solubility and also better content of S (Table II). Many cultivated soils have been fertilized for years with TSP and currently they show low soil S status.

Extensive research was carried out at IFDC to determine if acidulation of PR of different origins could be used to prepare PR products having better agronomic effectiveness. These studies concluded that, under certain conditions, a partial acidulation is sufficient to reach such objective and the effectiveness of the products is comparable to that of water-soluble P fertilizers [5, 17]. Nevertheless, due attention should be given to the chemical composition of the original PR, in particular the content of accesory minerals such as Fe and Al oxides because they can immobilize a fraction of the dissolved phosphate.

Genotypic differences in physiological P use efficiency for common bean have been also reported and it is possible to select genotypes with high expression of this trait [18].

3.3. Relative Agronomic Effectiveness (RAE) of studied PR products

The RAE values of PR products obtained for the common bean genotypes grown in the Acrisol and Rhodic Ferralsol are given in Tables IX and X. In the strongly acid and very low P Acrisol the RAE values based on dry matter indicate that both FPA 50 and PR were P sources with variable efficiency in comparison to SSP. They were highly efficient for the genotypes DOR 390 and DOR 364 but poorly efficient for BAT 477. RAE values based on P uptake show that PR was not effective whereas FPA 50 showed intermediate effectiveness except for the genotype DOR 390 were it was as effective as SSP (Table IX).

In the acid and medium P Rhodic Ferralsol from the RAEs based on dry matter production, FPA 50 was an efficient P source for all common bean genotypes studied and PR only for DOR 364. The RAE values based on P uptake showed that PR was an inefficient P fertilizer and FPA 50 was only an efficient P source for BAT 477 and BAT 58 (Table X).

TABLE IX. RELATIVE AGRONOMIC EFFECTIVENESS (RAE) OF PR PRODUCTS FOR COMMON BEAN GENOTYPES GROWN IN AN ACRISOL

	RAE (%) (based on dry matter)		RAE (%) (based on P uptake)	
	PR	FPA 50	PR	FPA 50
BAT 477	0	34	40	41
DOR 364	208	677	0	60
DOR 390	369	175	0	168
CENSA	32	82	0	51

* RAE- SSP = 100%.

TABLE X. RELATIVE AGRONOMIC EFFECTIVENESS (RAE) OF PR PRODUCTS FOR COMMON BEAN GENOTYPES GROWN IN A RHODIC FERRALSOL

	RAE (%) (based on dry matter)		RAE (%) (based on P uptake)	
	PR	FPA 50	PR	FPA 50
BAT 58	24	80	46	94
BAT 477	62	157	18	124
DOR 364	93	88	39	44
CC25-9N	30	74	0	0

**RAE- TSP = 100%.

3.4. P fraction in plant derived from P sources

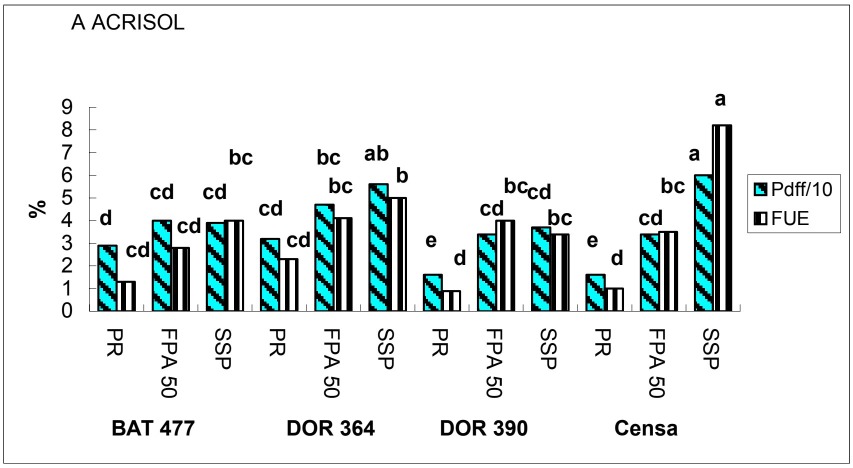
The determination of the fraction of P in the plant derived from fertilizer (Pdff) expressed as %Pdff for evaluating P fertilizers with the isotopic method (Figs 1A and 1B) has the advantage that this parameter is yield-independent [19]. The critical value to consider a P source as efficient has been set at 30% [12]. Following this criterion, in the Acrisol direct application of Trinidad de Guedes PR was only effective for DOR 364 because % Pdff was higher than 30% whereas FPA 50 and SSP were efficient P sources for all common bean genotypes tested (Fig. 1A). On another hand, the % Pdff values of FPA 50 and SSP for BAT 477 and DOR 390 are very similar. These results suggest that BAT 477 and DOR 390 have similar P acquisition mechanisms from P sources of medium to high solubility (SSP and FPA 50). In contrast, Censa and DOR 364 showed higher % Pdff values when they were fertilized with SSP than with FPA 50. In the Rhodic Ferralsol, the PR did not significantly supply P to the common bean genotypes evaluated because Pdff values were all lower than 30%. On the contrary, FPA 50 and TSP were effective P sources for all common bean genotypes studied (Fig. 1B). Thus, the results from both experiments suggest the existence of genotypic differences in the P utilization from phosphatic fertilizers of different solubility in these soils of different acidity and content of available P.

In a study to evaluate the effectiveness of PR sources of different origin as P fertilizer for soybean in acid soils of Thailand it was found that this legume crop did not respond to PR application [20]. In this work the local Trinidad de Guedes PR was only effective for DOR 364 in the Acrisol. These results would indicate that common bean is more sensitive to soil P status and P fertilizer application than soybean.

3.5. P fertilizer use efficiency (FUE)

The P fertilizer use efficiency (FUE) values are the coefficient of utilization or recovery of the applied P fertilizers expressed in percent (%). The FUE values revealed differences in the recovery of the applied P fertilizers. The FUE values were lower in the strongly acid and low P status Acrisol than those in the Rhodic Feralsol with higher pH and better P status. In the Acrisol for all common bean genotypes, FUE values were the lowest for PR and varied from 0.9% to 2.3%, intermediate for FPA 50 ranging from 2.8% to 4.1%, and the highest for SSP ranging from 3.0% to 8.2% (Fig. 1A). There were significant differences in FUE for bean genotypes: FPA 50 was a superior P source for DOR 364 (4.1%) and DOR 390 (4.0%) than SSP (3.0 and 3.4%, respectively). SSP was a better P source for BAT 477 (4.0%) and Censa (8.2%) than FPA 50 (2.8 and 3.5%, respectively).

In the Rhodic Ferralsol the magnitude of the FUE values was directly related to the degree of solubility of P sources: FUE $_{TSP}$ > FUE $_{FPA\ 50}$ > FUE $_{PR}$ (Fig. 1B). There were differences in the FUE for the two BAT common bean genotypes: FUE values of BAT 477 for PR, FPA 50 and TSP were two fold higher than those of BAT 58. As reported in this experiment, intra-specific variability in P-efficiency has been observed in common bean [21, 22].



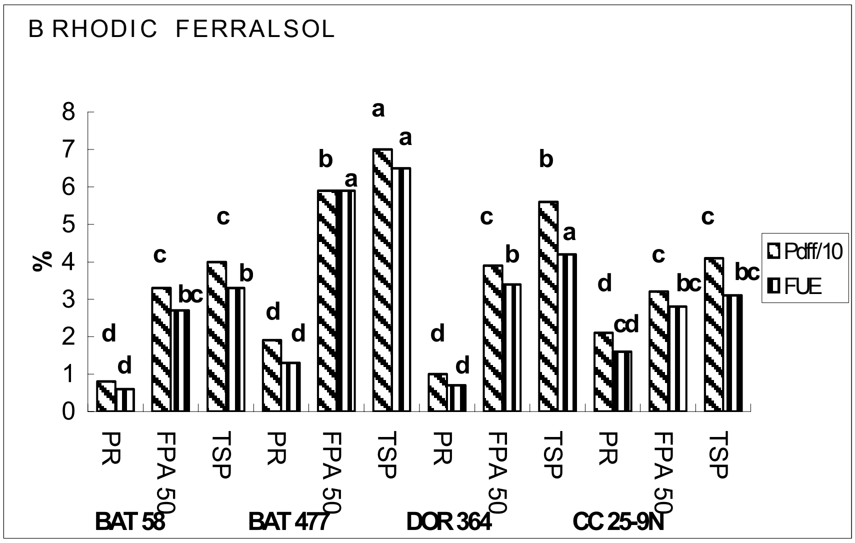


FIG.1. Phosphorus derived from the fertilizer (% Pdff) and P Fertilizer use efficiency (%FUE). Fig.1A. Acrisol and Fig.1B. Rhodic Ferralsol.

[1] *Columns shaded in the same way for each parameter (%Pdff and FUE) and followed by the same letter are not significantly different (p = 0.05).*

3.6. Substitution rates of the PR-based products

The substitution rates expressed as the kg of P as PR equivalent to 1 kg P as SSP or TSP were estimated for the PR products in both soils (Figs 2A and 2B). Whatever the soil, the Substitution Rates (SR) of PR was higher than those of FPA 50 for all common bean genotypes studied. In the Acrisol genotypic differences were found with regard to SR for PR and FPA 50; for BAT 477, DOR 364 and DOR 390 the SR values of PR were 1.6; 2.7, and 3.0 (kg P as PR equivalent to 1 kg P as SSP) while for Censa the SR value was 7.8. The SR for BAT 477, DOR 390 and DOR 364 were close to 1.0 indicating that FPA 50 was as effective as SSP (Fig. 2A). In the Rhodic Ferralsol for CC 25-9N the SR of PR was lower (2.6) than those of BAT 58 (7.7), BAT 477 (11.4) and DOR 364 (11.4). For all common bean genotypes, FPA 50 was almost as effective as TSP because the SR were similar ranging from 1.4 for BAT 58 to 2.0 for DOR 364 (Fig. 2B).

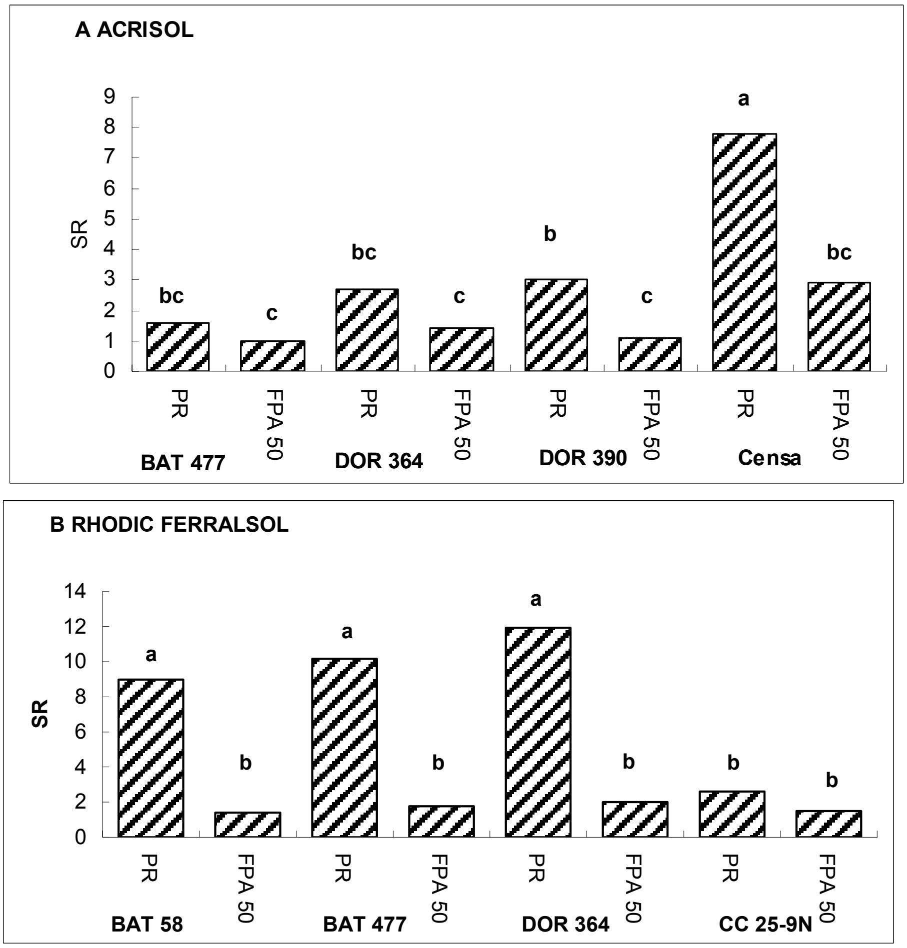


FIG.2. Substitution rates (SR) of the PR-based products. Fig 2A. Acrisol and Fig 2B. Rhodic Ferralsol[1].

[1] Columns with the same letter are not significantly different (p = 0.05).

The utility of the ^{32}P isotopic technique to establish genotypic differences and to evaluate the contribution of a PR to the P nutrition of cowpea genotypes grown in an Ultisol has been reported [23]. These authors reported that this technique enabled to make comparisons between the P uptake of the studied genotypes and to determine the equivalent amount of P as a PR source that show similar effectiveness to 1 unit of P as superphosphate.

In Chile utilizing the same technique, wheat genotypes were classified according to the substitution rates obtained for the local Bahia Inglesa PR [24].

The results obtained with FPA 50 in these experiments are very relevant for improving low P status of acid soils while increasing the grain yields of common bean in Cuba. In addition to its good performance, FPA 50 is a P fertilizer produced locally by partial acidulation of the indigenous Trinidad de Guedes PR, thus less expensive than imported P fertilizers. From the above, FPA 50 could be considered an alternative source to imported P fertilizers for common bean nutrition in the Acrisol soil.

4. CONCLUSIONS

In these greenhouse studies, genotypic differences in adaptation to acid soils and uptake of P coming from P fertilizers, in particular local PR-based products were found for common bean.

Overall, the Trinidad de Guedes PR showed low effectiveness in both studied acid soils. In both soils the water-soluble P fertilizers, i.e. SSP and TSP are the best P sources. FPA 50 showed intermediate and high effectiveness for all common bean genotypes in the Rhodic Ferralsol and Acrisol respectively, thus it can be used as a domestic substitute to the imported TSP.

In the Acrisol, the best common bean /P source combination was Censa /SSP and BAT 477 had the highest P fertilizer use efficiency. In the Rhodic Ferralsol, the better common bean /P source combination was DOR 364/TSP and CC 25-9(N) showed a high P requirement.

The ^{32}P isotopic technique was a valuable tool to evaluate the contribution of PR products to the P nutrition of common bean genotypes grown in acid soils and to establish genotypic differences in utilization from P sources.

These results should be further validated in field trials. Moreover, the results obtained demonstrate that it is possible through implementation of appropriate breeding programs to obtain common bean genotypes with superior P use efficiency for cultivation in acid P deficient soils and/or fertilized with local PR products.

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PHOSPHORUS UPTAKE EFFICIENCY OF SORGHUM AND RICE GENOTYPES AS AFFECTED BY PHOSPHATE SOURCES OF VARYING SOLUBILITY

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Abstract

Greenhouse experiments were carried out to assess the phosphate uptake efficiency (EPa) by the aerial plant parts and associated plant parameters in sorghum (*Sorghum bicolor* L. Moench) and rice (*Oryza sativa)* genotypes with differential tolerance to aluminum. The following plant parameters were determined: acid phosphomonoesterase activity (APA), arbuscular mycorrhiza colonized root lenght (%CRL), soil-pH and rhizosphere-pH and P uptake efficiency. Two Ultisols of low pH and low phosphate availability from the savanna of Venezuela were employed. In a greenhouse experiment five sorghum cultivars, i.e. Chaguaramas III (Ch III), Chaguaramas VII (Ch VII), Dekalb-59 (D-59), Criollo-26 and Ismael were studied. In another experiment four acid tolerant rice cultivars of *Oryza sativa* (CT-81-1, Fonaiap 2000, Fedearroz y CT-102-1) and one cultivar of *Oryza glaberrima* susceptible to Al toxicity were compared. P fertilization treatments were: control (0P), Riecito rock phosphate (RRP) and triple superphosphate (TSP). Significant sorghum genotypic differences were found in the parameters evaluated enabling them to grow on the acid soils. Their response was differential depending on the P source and the interaction between source and genotype. The use of the Al-tolerant cultivars Ch III, Ch VII is a potential means to obtain high dry matter production for cattle feeding when pasture supply is low. The cultivars Ch VII, Ch III and Ismael showed higher P-uptake efficiency index (EPa) when fertilized with the local Riecito rock phosphate, thus this PR can be considered to increase soil P status and improve agricultural production in the acid soils of the savannah of Venezuela. Cultivars with higher P uptake efficiency, in particular Ch III and Ch VII (tolerant to Al toxicity) expressed mechanisms such as high % of CRL by arbuscular mycorrhiza and increases in rhizospheric pH whereas cultivars with lower P uptake efficiency such as D-59 (susceptible to Al toxicity) expressed higher APA. The acid tolerant rice cultivars (CT-81-1, Fonaiap 2000, Fedearroz 50, CT-102-1) showed increased rhizosphere-pH whereas the acid susceptible genotype (*Oryza glaberrima*) had lower rhizosphere-pH. All acid tolerant rice cultivars can be considered for cultivation in the savannahs of Venezuela.

1. INTRODUCTION

Agricultural productivity of highly weathered tropical acid soils is limited by their inherent low fertility, being high soil acidity (Al toxicity) and nutrient deficiencies, in particular phosphorus (P) the main limiting factors. In the Venezuelan central savannahs (Guárico, Apure and Cojedes states), the selection of appropriate agricultural management practices for optimal production depends to a great extent on their soil properties and socio-economic factors. In fact, nearly 70% of the Venezuelan soils present low soil fertility and acidity as primary (28%) or secondary (43%) limiting factors [1, 2]. In addtion, these tropical acid soils show important variations in calcium (Ca), magnesium (Mg), potassium (K), sulfur (S) and micronutrient availability, textural class and aluminum (Al) content in the exchange complex [3]. As a result, agricultural research attempts to develop technologies and strategies to overcome these limitations and increase crop productivity. P availability to plants, from soil and/or applied fertilizer, depends upon processes directly or indirectly affected by soil properties (adsorption, precipitation, dissolution, transport and mineralization) and plant characteristics. Therefore, improving the understanding of the processes controlling soil functioning is necessary to develop sustainable agricultural practices [4]. Plant properties, such as root morphology (root density, length, diameter and root hair abundance) and rhizosphere processes such as mycorrhizal symbiosis, exudation of organic compounds, protons (H^+) and hydroxide ions (OH^-), and acid phosphatase activity [5, 6] are all factors affecting P availability and uptake by plants.

Hedley et al. [7] postulated that external mechanisms in the rhizosphere determine P uptake efficiency in acid tolerant genotypes. Some of the plant mechanisms reported are the following: development of the root system [7-9], modification of soil pH mediated by the exudation of chelating agents and organic acids, which alter inorganic P solubilization [10–12], mineralization of organic P through the release of phosphatases [13–15], and symbiosis with mycorrhizal fungi [16, 17]. Several authors have reported that cultivars tolerant to Al toxicity develop greater root length [7, 15], have higher mycorrhizal [17], and greater ability to increase the pH in the rhizosphere [7].

Sorghum and rice are important cereals crops grown in the Venezuelan central savannahs. Grains are marketed for the agro-industry and the crop residues are used for cattle feeding during the dry season, when the pasture supply is low. The use of cultivars tolerant to Al toxicity is one of the proposed technologies to decrease production costs and increase benefit/cost ratios in systems where agroecological and socioeconomic conditions allow the use of such practices.. Plant tolerance to Al toxicity varies between species and within cultivars of the same species [18]. Sorghum genotypic differences in tolerance to Al have been found in Venezuela and those Al-tolerant cultivars are capable to grow under acid and low P fertility conditions [19]. In Venezuela rice, one of the basic products in the population's diet is traditionally cultivated under waterlogged soil conditions. However, in recent years, the cultivated area of upland rice has greatly increased due to the low and erratic rainfall regime. In this research project, pot experiments were conducted to evaluate the P uptake efficiency of selected sorghum and rice cultivars grown in acid P-deficient savannah soils of Venezuela and fertilized with P sources of varying solubility. Several plant parameters such as root morphology (length and surface), acid phosphomonoesterase activity (APA), root colonization by arbuscular mycorrhiza (AM), pH of the bulk soil and the rhizosphere and plant P uptake efficiency index were utilized as indicators.

2. MATERIALS AND METHODS

Two pot experiments were carried out in a greenhouse located at INIA-CENIAP, Maracay, Aragua state, Venezuela to determine the P uptake efficiency of selected sorghum and rice genotypes grown in acid P-deficient savannah soils of Venezuela and fertilized with P sources of varying solubility. The experiments were conducted in free-draining pots filled with 4 kg of air-dried soil. Table I shows some chemical characteristics of the experimental soils (Ultisols).

TABLE I. SOME CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL SOILS

Soil name (Locations)	Available P-Olsen ($mg\ kg^{-1}$)	Available K-Olsen ($mg\ kg^{-1}$)	Organic matter (Walkley and Black) ($g\ kg^{-1}$)	pH (soil/water 1:2,5)	Al saturation (%)
Espino (exp. 1)	2	22	10	5.0	57
Tapicito (exp. 2)	3	20	6	4.4	80

In experiment 1 a Typic Paleustult, kaolinite, isohyperthermic was employed and a Kandic Plinthustult, isohyperthermic in experiment 2. A bulk sample from the topsoil (0–20 cm) was taken from two locations, i.e. Espino (for experiment 1) and Tapicito (for experiment 2), both located in the Guárico state, Venezuela [2].

2.1. Germplasm material

In Experiment 1, five sorghum (*Sorghum bicolor* L. Moench) cultivars with reported differential tolerance to soil acidity (and Al toxicity) were selected. Four of them (D-59, Criollo-26, Ch III y Ch VII) are cultivars mainly grown for grain production and the remaining (Ismael) for forage. Cultivar D-59 is a US hybrid from DEKALB, selected by Guzmán and Puerta [20] and Rodríguez et al. [21] as sensitive to Al toxicity. Chaguaramas III (Ch III) and Chaguaramas VII (Ch VII) are Venezuelan hybrids. Ch III was selected as acid tolerant [20-, 22] whereas Ch VII showed tolerance to acid soils in field evaluation [23–25]. Hybrid Criollo-26 and the variety Ismael were developed and recently released by FONAIAP (currently INIA-Venezuela). From the latter, there is no information on their sensitivity or tolerance to Al toxicity.

In experiment 2, five rice cultivars, i.e. four acid tolerant cultivars of *Oryza sativa* (CT-81-1, Fonaiap 2000, Fedearroz and CT-102-1) and one cultivar of *Oryza glaberrima* (sensitive to Al toxicity) were employed.

2.2. P sources and fertilization

Seven seeds from each cultivar were sown in pots (experimental units). At emergence (one week later) plants were thinned out to 4 plants per pot. Soil moisture was maintained around 75% of field capacity. After thinning out the plants, a basal fertilization of 58.54 mg K pot^{-1} as potassium chloride (KCl -54%K_2O); and 117 mg N pot^{-1} as urea (46% N) was applied as solution. P fertilization was made at 97.56 mg P pot^{-1} in the form of Riecito rock phosphate (RRP-32% P_2O_5 total; NAC-soluble 12.29% P_2O_5; 39% CaO) or triple superphosphate (TSP-46% P_2O_5 total); according to the corresponding treatment, except for the control, without P.

2.3. Experimental design and treatments

Both experiments consisted of a factorial where genotype (cultivar) and P sources were the two factors arranged in a completely randomized design. In experiment 1, the factor sorghum genotype (cultivar) had five levels; and the factor P source three levels: 0P = control without P, TSP = triple super phosphate (water-soluble P fertilizer) and RRP = Riecito rock phosphate (of low solubility). All 15 treatments were replicated 4 times with a total of 60 experimental units.

In experiment 2, similar factors were studied. The factor rice genotype (cultivar) had five levels and the factor P source, three levels. All 15 treatments were replicated 3 times with a total of 45 experimental units.

2.4. Plant harvest and analyses

Whole plants were harvested at 45 days after planting (dap), during the phase of vegetative growth. Shoots and roots were separated. Shoots were washed with deionized water and oven-dried at 70°C for 48 h. After dry weight determination, samples were finely ground (<1mm) for chemical analyses. Plant samples were digested using a nitric-perchloric acid mix [26]. Plant P content was determined in the extract using the ammonium molybdate method [27].

In experiment 1, roots were carefully separated from the total soil volume (bulk soil) and placed on a sheet of white paper, using a paint brush and gentle removing the soil adhered to the roots (humidity of 6%) being described as “rhizosphere soil” [28]. After separating the rhizosphere soil, roots were placed on a 0.425 mm sieve and washed with tap water. Rhizosphere soil was used to evaluate acid phosphatase activity, APA [29, 30]. Rhizosphere pH (1:2.5 ratio, soil-water) was also determined. In each experimental unit to determine mycorrhizal root colonization approximately 1g of secondary roots were employed for the observation of typical arbuscular mycorrhizal (AM) structures using the trypan blue staining method [31]. Root colonization was quantified in the microscope at 10 X and expressed as percent colonized root length (% CRL) [32]. The root length pot^{-1} was determined [33, 34]. In experiment 1 the total root length pot^{-1} was determined; while in experiment 2, the root length pot^{-1} was determined in 3 sub samples of the harvested roots. After root separation from the soil mass, residual available soil P was analysed [35].

2.5. P uptake and P uptake efficiency index

Plant P content was used to calculate P uptake or accumulated P in the aerial part of the plant (Pa), using the following equation: Pa (mg/pot) = (% of plant P content/100) x (grams of aerial dry matter / pot) x 1000.

P uptake efficiency of the cultivars was calculated as plant aerial P efficiency (EPa) proposed by Fageria [36]. EPa refers to the ratio between the biomass (mg dry matter per pot) produced in the aerial parts and the P accumulated in the aerial biomass (mg P per pot).

2.6. Statistical analyses

Experimental data obtained were subjected to analysis of variance and means of treatments compared with Tukey´s test, $p \leq 0{,}05$.

3. RESULTS AND DISCUSSION

3.1. Experiment 1: Sorghum genotypic differences in P uptake and P uptake efficiency

Table II summarizes the main effects and interaction of the experimental treatments on the variables evaluated for the sorghum genotypes (cultivars). Results on shoot and root dry weight, accumulated P in the aerial biomass (Pa in mg.pot^{-1}), available soil P-(Olsen extractable) after cropping (P, mg kg^{-1}), bulk soil pH (soil-pH) and rhizosphere soil pH (rhizo-pH), acid phosphatase activity (APA, μmol PNP g^{-1}h^{-1}), and percent Arbuscular Mycorrhizal colonized root length (% CRL); root surface (m^2 pot^{-1}), total root length (m pot^{-1}) and P uptake efficiency (EPa) were analysed.

Table II shows the statistical significance of the sources of variation for the results obtained. The yield of aerial biomass dry weight (DW) had a highly significant response to the factors genotype (Gen) and P fertilizers (P fert) as well as the interaction of both, while acid phosphatase activity (APA) was only significant in response to genotype. Percentage of colonized root length (%CRL) was highly significant for the factor Gen, and significant in response to P fertilizer and the interaction of both factors. The variables available soil P (P-soil) and P content in plant aerial biomass (Pa) had highly significant differences in response to Gen, P fertilizers and the interaction of both factors. Bulk soil pH (soil-pH) was significantly different in response to P Fertilizer, while rhizospheric soil pH was significantly different in response to genotype, P fertilizer and the interaction of the two factors. Moreover, differences in P use efficiency index (EPa) were highly significant for all three sources of variation. The total root length had highly significant response to interaction GenxP fertilizer, while the variable root surface was significantly different in response to P fertilizer and significant to Gen xP fertilizer interaction.

TABLE II. ANOVA SUMMARY OF THE EVALUATED VARIABLES

Variables	Variation sources			CV (%)
	Gen.	P fert.	Gen x P fert.	
DW shoot (g pot^{-1})	**	**	**	4.862
APA (µmol PNP g^{-1} h^{-1})	**	NS	NS	28.036
CRL (%)	**	*	*	6.819
Available soil P-Olsen (mg kg^{-1})	**	**	**	14.133
Pa (mg.pot^{-1})	**	**	**	10.995
pH-soil (ratio soil:water 1:2,5)	NS	**	NS	2.742
pH-rhizo (ratio soil: water 1:2,5)	*	**	**	3.004
DW root (g pot^{-1})	NS	**	*	9.661
Total-root length (m pot^{-1})	NS	NS	**	12.99
Root surface (m^2 pot^{-1})	NS	*	**	9.818
EPa	**	**	**	13.040

** highly significant < 0.01; * significant < 0.05;NS not significant, CV= coefficient of variation.

3.1.1. Shoot dry weight (DW)

Dry matter accumulated in all sorghum cultivars evaluated had highly significant ($\propto$ = 1%) responses to P fertilization sources of high (TSP) and low (RRP) water solubility in a P-deficient acid soil. DW was significantly higher in cultivars Ch III and Ismael, followed by Ch VII, D-59 and Criollo 26 (Fig. 1). With respect to P fertilizer treatment, the cultivars D-59 and Ismael produced the same amount of DW depending on the P source: T3 (TSP) > T2 (RRP) > T1 (OP). Meanwhile, DW yield of cultivars Criollo-26 and Ch VII were as follows: T3 > T2 = T1, with no response to PR application. Finally, Ch III had a higher DW production in the control (without P) treatment (T1) than in the P fertilized treatments (TSP = RRP).

In the control (without P fertilization) treatment, DW values ranged between 3.94 and 8.72 g.pot^{-1}. In this treatment, DW production of cultivar Ch III (tolerant to Al toxicity) was significantly higher than for the other cultivars. Meanwhile, cultivar D-59 (susceptible to Al toxicity) had the lowest DW production (3.94 g.pot^{-1}) and it was not significantly different from Criollo-26. Cultivars Ch VII and Ismael accumulated the same amount of DW.

Production of DW under RRP fertilization ranged between 4.21 and 7.87 g.pot^{-1}. Cultivar Ismael accumulated a significantly higher ($\propto$ = 1%) DW followed by Ch III, Ch VII, D-59 and finally Criollo-26. The cultivar Ismael showed the greatest positive response to P fertilization with both P fertilizers of contrasting solubility (TSP) and (RRP). López et al. [15] and Ramírez and López [19] found that among three evaluated grain sorghum cultivars (D-59, Ch III and P-8225) with different Al toxicity tolerance in acid soils, under greenhouse conditions, Ch III had the highest productivity due to its high root development at 20 days after germination in the treatments with the lowest P availability (0P and RRP).

The superior performance in DW production shown by cultivars Ismael, Ch III and Ch VII could be attributed, to their better ability to take up P from the acid Ultisol and P accumulation in the aerial biomass and probably also to their higher % CRL.

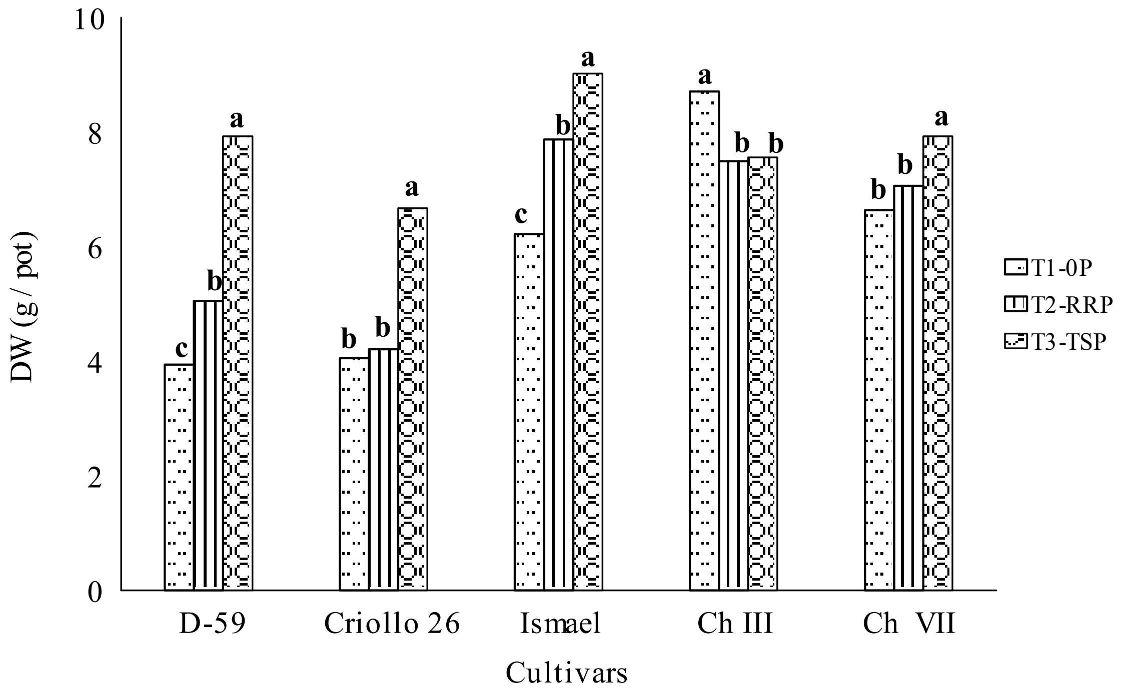

FIG.1. Effect of P fertilization on shoot biomass production (DW in g.pot^{-1}) in five sorghum cultivars. Means followed by the same letter are not significantly different (Tukey's test, p ≤ 0,05).

3.1.2. Acid phosphatase activity (APA)

APA (0.33 and 0.67 µmol PNP g^{-1}.h^{-1}) for the sorghum genotypes evaluated, are within the range previously reported for other crops (0.05 and 0.60 µmol PNP g^{-1}.h^{-1} h in onion and 0.03 and 1.19 µmol PNP g^{-1}.h^{-1} in wheat rhizosphere [9]). The APA values also agree with the range of values (0.38 and 0.48 µmol PNP g^{-1}.h^{-1}) reported for three sorghum cultivars (D-59, Ch III y P-8225) in Venezuela [15, 19]. It is noteworthy that cultivar D-59, susceptible to Al toxicity, has the highest APA values and the lowest % CRL in the absence of P fertilization, indicating that this cultivar may be able to use P fractions from acid soils through the production of P mineralizing enzymes.

The cultivars with the highest APA were Ch VII (Al toxicity tolerant), D-59 (Al toxicity susceptible) and Criollo 26, while the lowest APA was recorded for the cultivar Ismael (Fig. 2). The APA in the case of cultivars D-59 and Ismael could be related to the lower soil P availability (Fig. 4) since APA may be increased when P availability is low [37]. On the other hand, the higher soil P content (4.25, 6.25 and 15 mg kg^{-1} in 0P, RRP and TSP respectively) in the case of Ch VII did not seem to have inhibited APA; may be because these soil P values were still in the range of low P availability. The highest APA values in cultivars Ch VII and D-59 in the RRP and TSP treatments are in agreement with the higher values found by López et al. [15] for cultivars Ch III and D-59 fertilized with RRP (0.42 and 0.40 µmol PNP g^{-1}.h^{-1}) and TSP (0.42 and 0.46 µmol PNPg^{-1} h^{-1}) versus the control (0P) treatment (0.30 and 0.32 µmol PNP.g^{-1}.h^{-1}) respectively. These results suggest that some P addition is needed to stimulate APA in soils of low P-fertility status. Moreover, cultivars susceptible to Al toxicity have a higher APA than tolerant cultivars from the same species, under P stress [15]. This postulate is valid for D-59 (susceptible), but not for Ch VII (tolerant). This effect is probably related with a higher % CRL since AM hyphae could access P sources otherwise not available to the plants [38-39]. It is reported that APA increases in the rhizosphere of AM colonized cultivars such as 0.48 µmol PNP $g^{-1}.h^{-1}$ for onion and from 0.23 to 0.83 µmol PNP $g^{-1}.h^{-1}$ for onion and wheat respectively [40]. Furthermore, other processes such as organic P mineralization by the action of the acid phosphomonoesterases may occur resulting in the use of P forms after hydrolysis of organic P into inorganic P [41, 42]. The impact of microbial groups able to hydrolyze organic P on APA in the rhizosphere, occurred in AM colonized native plant species in savannahs and tropical forest [43].

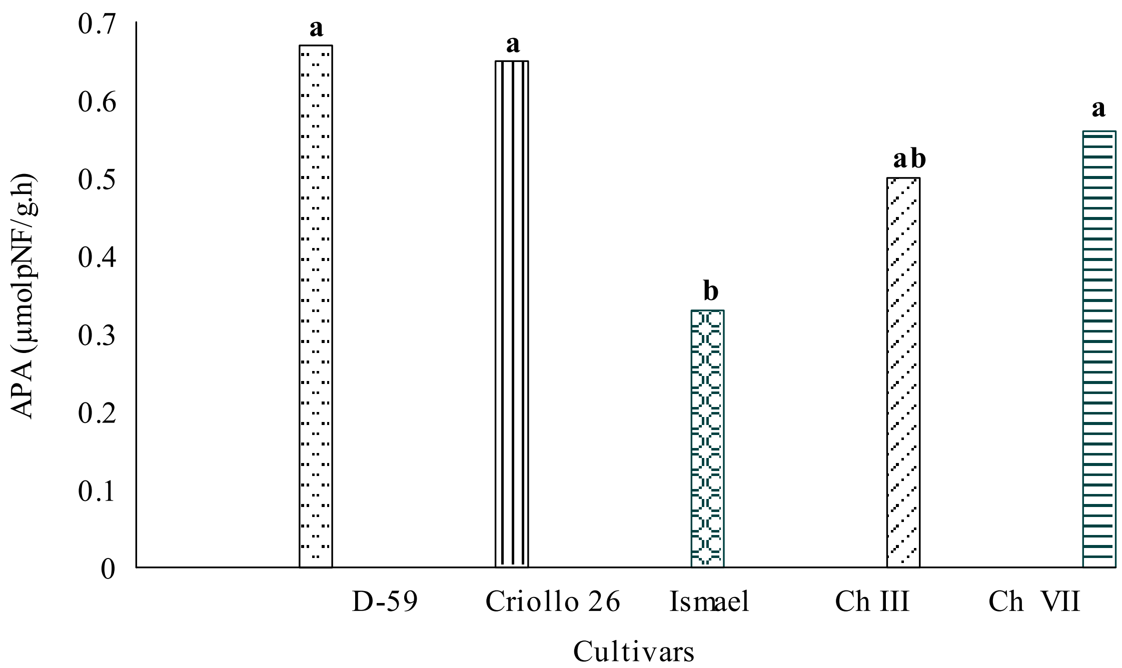

FIG.2. Acid phosphatase activity (APA in µmol PNP.$g^{-1}.h^{-1}$) in the rhizosphere of 5 sorghum cultivars with differing tolerance to Al toxicity. Means followed by the same letter are not significantly different (Tukey's test, $p \leq 0,05$).

3.1.3. Percentage of AM colonized root length (% CRL)

The CRL values ranged between 82.2 and 94.9%, which could be considered high because in a study with sorghum cultivars grown on an acid Ultisol after three years of conservative tillage the highest CRL reported has been 53% for cultivar Ch VII [43]. Such high % CRL could be a plant mechanism of P uptake when soil P availability is very low, as observed in cultivars Criollo 26, Ismael, Ch III and Ch VII in the treatment without P fertilization (T1-0P). In fact, P fertilization (T2-RRP and T3-TSP) did not affect this parameter between cultivars. AM colonization may have contributed to the higher efficiency of P uptake in the cultivars studied because the P uptake efficiency is from 1.3 to 7 fold higher in AM colonized plants than in their non-colonized counterparts [44].

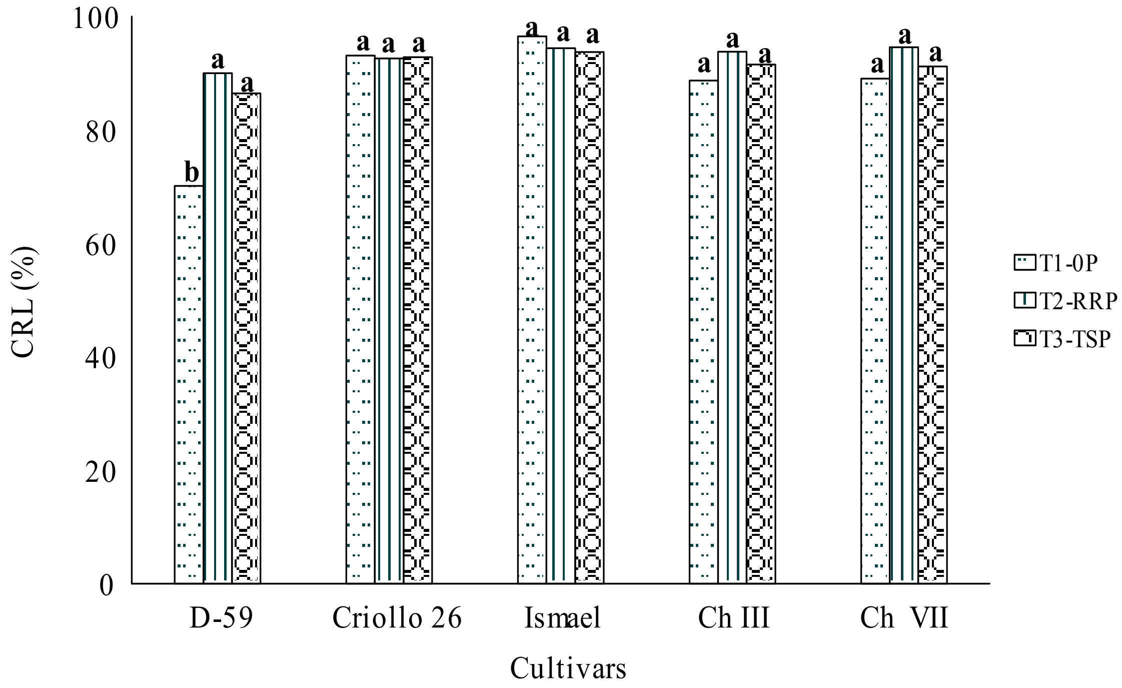

FIG.3. Effect of P fertilization on AM % of colonized root length (% CRL) in five sorghum cultivars. Means followed by the same letter within the same cultivar are not significantly different (Tukey's test, $p \leq 0{,}05$).

P fertilization significantly increased AM % CRL in cultivar D-59 (susceptible to Al toxicity). This result suggests that this cultivar was not able to mobilize soil fixed P forms since it has been demonstrated that small P additions in soils of very low P availability could stimulate AM colonization [45]. Meanwhile cultivars Criollo 26, Ismael, Ch III and Ch VII had high % CRL in all treatments, including T1-OP. This high % CRL could be a mechanism of these cultivars for increased P-uptake in this acid Ultisol as mentioned by Miyasaka and Habte [42]. The high % CRL in all sorghum cultivars indicates the high natural AM propagules abundance of the soil used. Furthermore, AM spore number in this soil (2050 spores/100 g soil) is rather high as compared to values reported for native Colombian savannahs (950 spores/100 g soil). This natural abundance of AM propagules would promote a rapid AM colonization of native species to cope with the low P fertility of these ecosystems [46].

In general, there were not differences in % CRL between cultivars (except for D-59), which shows their high mycotrophy and their potential to be used, along with AM, in agriculture.

3.1.4. Available soil P-(Olsen extractable)

At the end of the experiment, available soil P-Olsen (P-soil) in the control T1 treatment ranged between 2.5 and 4.25 $mg.kg^{-1}$ being significantly higher for cultivar Ch VII, followed by Ch III, Ismael, D-59 and Criollo 26. Cultivars Ch III and Ch VII (tolerant to Al toxicity) seem to have mechanisms to use sparingly soil P fixed forms present in acid soils, which agrees with the results of Pa (Fig. 5) and EPa (Fig. 7).

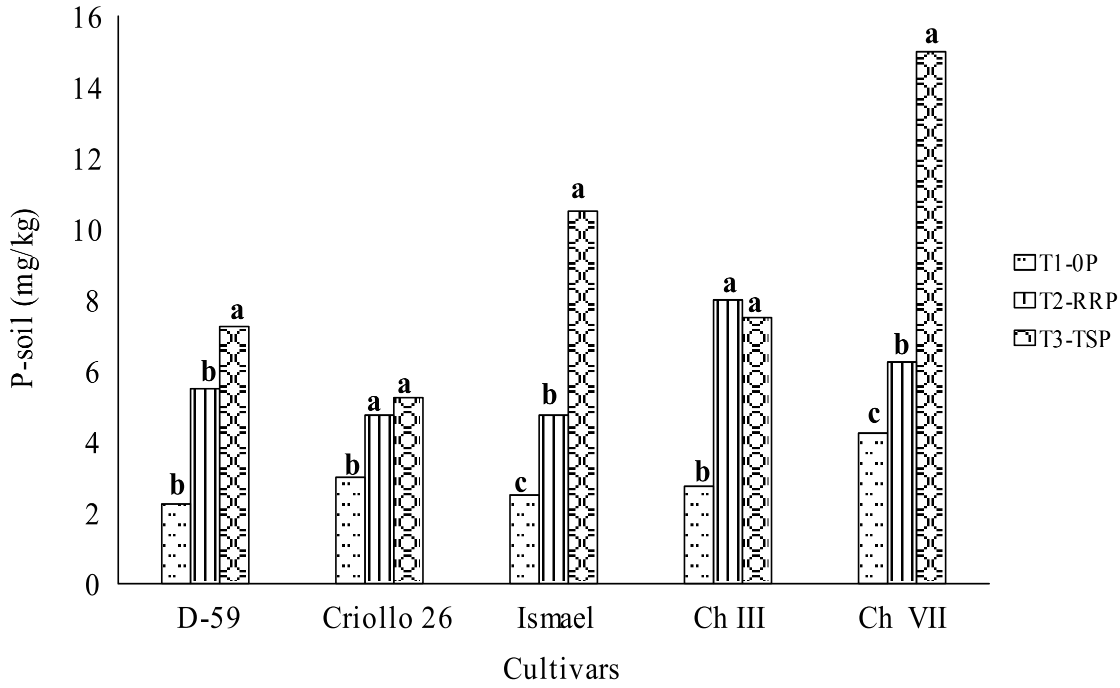

FIG.4. Effect of P fertilization on available soil P-Olsen (P-soil, in mg kg^{-1}) in five sorghum cultivars. Means followed by the same letter in the same cultivar are not significantly different (Tukey's test, p ≤ 0,05).

Levels of available soil P-(Olsen extractable P at the end of the experiment) varied depending on cultivar and P fertilization (T1, T2 and T3), as well as on the reactions occurring between the P (organic or inorganic) forms present in the soil and the applied P fertilizers. For cultivar D-59 available soil P was significantly higher for water-soluble P fertilizer (T3-TSP) compared to (T2-RRP) and (T1-0P), but not significantly differences were found between (T2-RRP) and the non-fertilized control (T1-0P). Available soil P for Criollo 26 and Ch III was not significantly different among fertilized treatments, but they were both significantly higher than T1-OP. However the mean values of Ch III were higher than those of Criollo 26. Finally, available soil P levels (Olsen extractable P at the end of the experiment) in Ch VII and Ismael followed the trend T3>T2>T1(Fig. 4). In all cases the application of the water-soluble TSP improved the soil P status, and the locally available RRP was equally effective P source as TSP to improve soil P status for Ch III and Criollo 26, the overall effect being better for Ch III.

3.1.5. P in plant aerial biomass (Pa)

P uptake was significantly different among cultivars, depending on P fertilizer source. Pa values ranged between 4.16 and 6.71 mg P pot^{-1}. Cultivars Ismael, Ch III and Ch VII had a significantly higher Pa than D-59 and Criollo 26 (Fig. 5).

P uptake efficiency by the cultivars was influenced by the P source (Fig. 5). All cultivars responded to TSP application. Criollo 26 and to some extent Ch VII showed a similar Pa regardless of fertilizer source (RRP and TSP) but significantly higher than that of the control (0P). Pa in Ismael followed the trend T3>T2>T1 according to P fertilization (P rate and source solubility). RRP application did not contribute to P nutrition of Ch III and D-59 cultivars, because Pa of this treatment was equally low as the control.

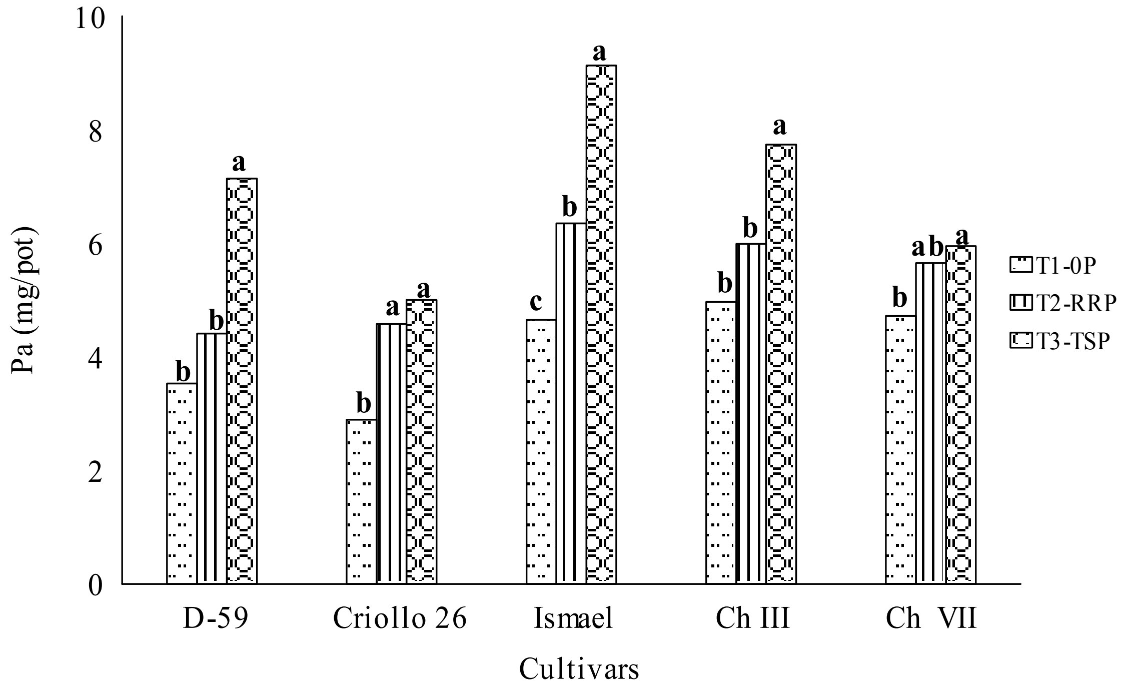

FIG.5. Effect of P fertilization on plant aerial biomass P (Pa, in mg pot^{-1}) in five sorghum cultivars. Means followed by the same letter within the same cultivar are not significantly different (Tukey's test, p≤ 0,05).

3.1.6. Soil-pH andrhizosphere- pH

At the end of the experiment the pH of the bulk soil in the treatments ranged between 4.54 and 4.74 with no significant pH differences between cultivar or P fertilization. Rhizosphere pH values ranged between 4.33 and 5.36 (Fig. 6). Cultivars showed differential changes in rhizosphere pH to P fertilization. In general rhizosphere pH values of cultivar D-59 were lower than those of other genotypes. For this cultivar the values were similarly low for RRP and 0P treatments but increased with TSP. Criollo 26 and Ismael belong to the second category where the values were low at 0P but increased with P fertilizer application, either RRP or TSP. In the third category (Ch VII and Ch III), the rhizosphere pH values were the highest and similar for all P fertilizer treatments, including the control 0P.

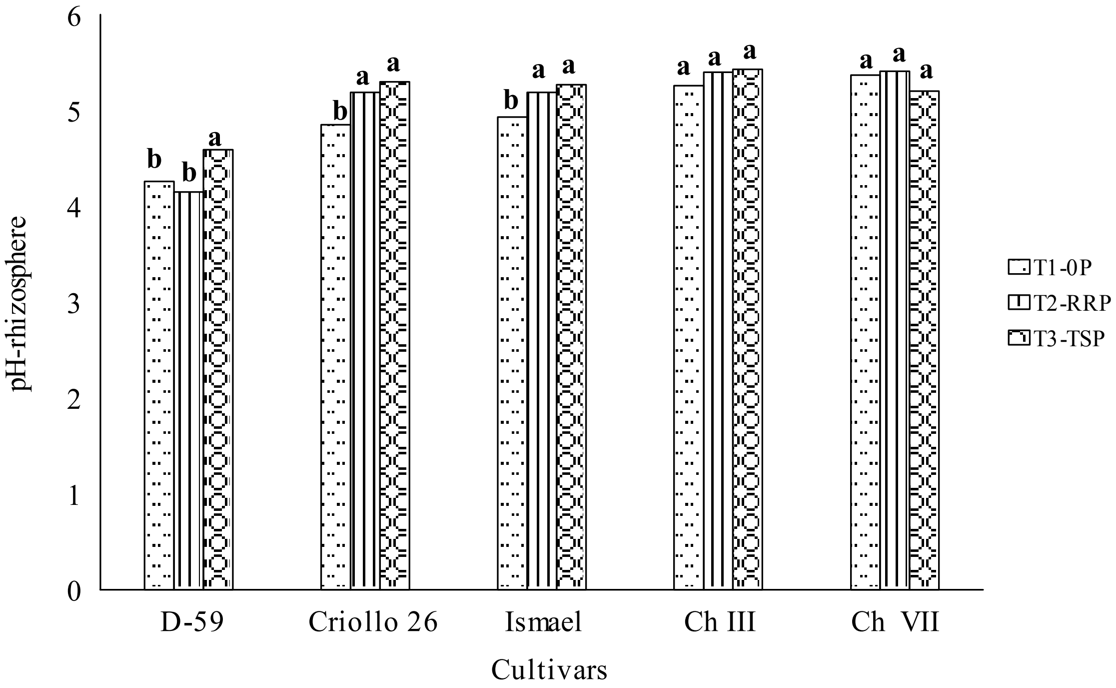

FIG.6. Effect of P fertilization on the rhizosphere pH of five sorghum cultivars. Means followed by the same letter within the same cultivar are not significantly different (Tukey's test, p ≤ 0,05).

Changes in rhizosphere pH, either increase or decrease, have been reported for plant species able to efficiently absorb P, such changes have been associated with the presence of different P-fixed soil forms [42]. For instance, P-Ca increases as pH decreases. The decrease in pH-rhizosphere of cultivar D-59 in the RRP treatment seems to be related to the former mechanism since it has been previously reported in other plant species able to efficiently absorb P [42]. Whilst increases in pH-rhizosphere in plant species able to efficiently absorb P has been related to the presence of soil P-Al and P-Fe and adaptation to acid soils [42]. This seems to be the case for the cultivars (Ismael, Criollo, Ch III and Ch VII) where pH-rhizosphere increases were found in T1 but in particular Ch III and Ch VII.

3.1.7. Plant P-uptake efficiency index (EPa)

EPa was significantly different among cultivars with values that ranged between 1122 and 1338 (Fig. 7). According to this index cultivar efficiency was as follows: Ch VII = Ch III > Ismael > Criollo 26 = D-59. EPa in Ch VII, Ismael and D-59 was independent of fertilizer P source (TSP and RRP) or from soil (0P control)(Fig. 7). Criollo 26 had a higher efficiency with the water- soluble (TSP) than with the P source of low solubility (RRP). Ch III and Criollo 26 showed highest EPa in soil P (0P, without P fertilizer) (Fig. 7).

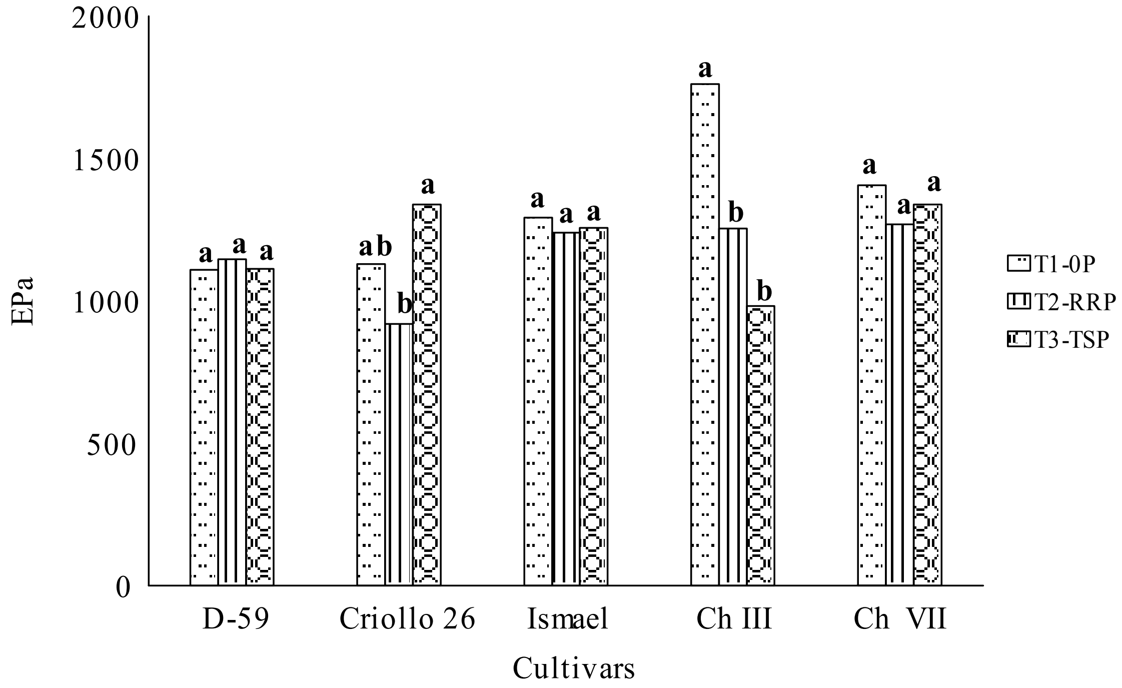

FIG.7. Effect of P fertilization on plant P uptake efficiency index (EPa) in five sorghum cultivars. Means followed by the same letter are not significantly different (Tukey's test, p ≤ 0,05).

A positive correlation ($r^2 = 0.88$) between dry weight production in plant aerial parts and the P efficiency index for the control treatment (0P) was found (Fig. 8). This demonstrates that the cultivars with higher P uptake efficiency are able to grow better in acid soil conditions and thus to accumulate more dry matter than those cultivars with the lower P uptake efficiency.

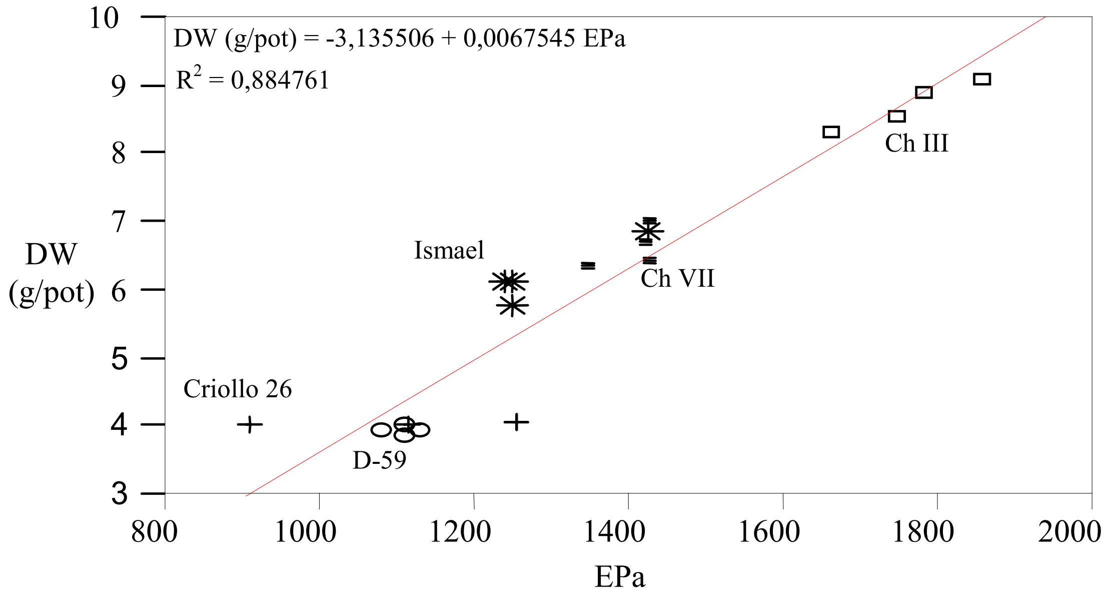

FIG.8. Linear correlation between aerial biomass dry matter production (DW) and the P uptake efficiency index (EPa) in the control treatment.

The results suggest that the plants can directly or indirectly affect the processes governing P acquisition from soil or fertilizer through physiological processes and modifications in soil properties [5]. This likely explains the different mechanisms expressed by the sorghum cultivars to increase P availability and uptake in acid Ultisol from the soil and applied P fertilizers of contrasting solubility. Moreover, the selection and use of highly P uptake efficient cultivars could improve overall plant P availability, which can only be achieved with the combined application of P maintenance levels in soils with a low P sorption capacity [5].

3.2. Experiment 2: Rice genotypic differences in P uptake and P uptake efficiency

Table III summarizes the main effects of the experimental treatments on the variables evaluated for the rice genotypes. Results on accumulated P in the aerial biomass (Pa in mg.pot^{-1}), available soil P-Olsen after cropping (P-soil, mg kg^{-1}), bulk soil pH (pH-soil) and rhizosphere soil pH (pH-rhizo), acid phosphatase activity (APA, µmol PNP g^{-1}.h^{-1}), and percentage of AM colonized root length (%CRL); root surface (m^2 pot^{-1}) and total root length (m pot^{-1}).

TABLE III. ANOVA SUMMARY OF THE EVALUATED VARIABLES

Variables	Variation sources			CV (%)
	Gen.	P fert.	Gen x P fert	
DWshoot (g pot^{-1})	NS	NS	NS	9.165
APA (µmol PNF g-^{1}h^{-1})	NS	NS	NS	53.998
CRL (%)	1	1	1	1
P-soil (mg kg^{-1})	NS	NS	NS	15.507
Pa (mg pot^{-1})	NS	NS	NS	11.528
pH-soil (ratio soil-water 1:2,5)	NS	NS	NS	2.180
pH-rhizo (ratio soil:water 1:2,5)	**	NS	NS	2.268
DW root (g pot^{-1})	1	1	1	1
Total-length root (m pot^{-1})	NS	NS	NS	20.800
Root surface (m^2 pot^{-1})	NS	NS	NS	24.825
EPa	NS	*	NS	9.082

** highly significant < 0.01; * significant < 0.05; NS not significant, CV= coefficient of variation. 1 = not determined.

Table III shows the sources of variation of the results obtained. The yield of aerial biomass dry weight (DW); acid phosphatase activity (APA), soil P (P-soil) and P in plant aerial biomass (Pa), bulk soil pH (pH soil), rhizosphere pH (pH-rhizo), total length root and root surface show no significant response to the experimental treatments (genotype, P fertilizer and their interactions), while rhizospheric soil pH was significantly different in response to genotype.

Soil rhizosphere pH was significantly different among the cultivars. The rice species *Oryza glauberrima* decreased rhizosphere pH significantly, while the rice cultivars of the species *Oryza sativa* increased rhizosphere pH. The root morphology (length, surface) increased in some cultivars and P fertilizer treatments, but there were not statistically significant differences.

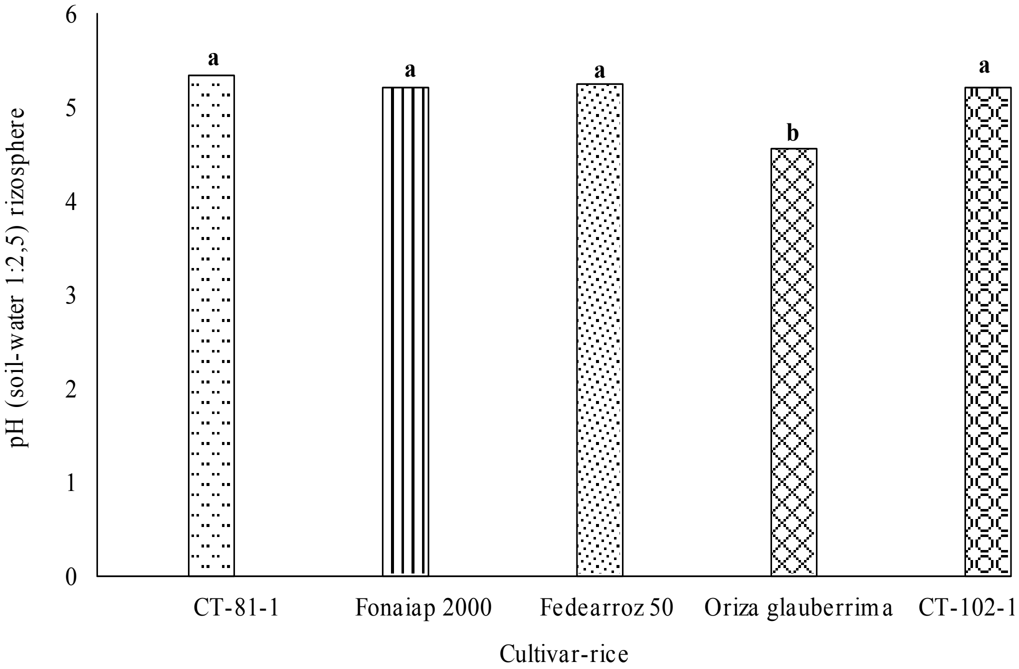

FIG.9. Effect of P fertilization on rhizosphere pH in five rice genotypes. Means followed by the same letter are not significantly different (Tukey's test, p ≤ 0,05).

4. CONCLUSIONS

Two greenhouse experiments were carried out to determine the P uptake efficiency of selected sorghum and rice genotypes grown in acid savannah Ultisols of Venezuela.

Significant sorghum genotypic differences were found in the parameters evaluated enabling them to grow better on acid soils. Their response was dependentupon the P source and the interaction between P source and genotype.

The use of the sorghum cultivars Ch III, Ch VII tolerant to Al toxicity is a potential means to obtain high dry matter production for cattle feeding when pasture supply is low. Also, to reduce production costs and to increase benefit/cost ratios while promoting the sustainability of the agricultural production systems.

Cultivars with higher P uptake efficiency, in particular Ch III and Ch VII (tolerant to Al toxicity) expressed mechanisms such as high % CRL by arbuscular mycorrhiza and increases in rhizospheric pH whereas cultivars with lower P uptake efficiency such as D-59 (susceptible to Al toxicity) expressed higher APA.

The acid tolerant rice cultivars (CT-81-1, Fonaiap 2000, Fedearroz 50, CT-102-1) increased rhizosphere-pH whereas the acid susceptible genotype (*Oryza glaberrima*) decreased rhizosphere-pH. All acid-tolerant rice cultivars can be considered a potential alternative for cultivation in the savannahs of Venezuela.

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Ameliorating Soil Acidity and Infertility of Tropical Acid Soils

LIMING EFFECT ON THE AGRONOMIC EFFECTIVENESS OF PHOSPHATE SOURCES VARYING IN SOLUBILITY APPLIED TO UPLAND RICE AND SOYBEAN GROWN ON AN ACID ULTISOL

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Abstract

A greenhouse study was conducted over a 3-year period to investigate the effect of liming on the relative agronomic effectiveness (RAE) of P sources varying in solubility in three cropping sequences. The P sources were (1) a highly reactive Sechura phosphate rock (PR) from Peru, (2) a partially acidulated medium-reactive Huila PR from Colombia at 50% acidulation with H_2SO_4 (PAPR), and (3) a commercial-grade triple superphosphate (TSP). An acid Ultisol that was unlimed (pH 4.8) and limed to pH 5.6 and 6.6 was used in the three experiments. The first two experiments were carried out over the same period involving upland rice-upland rice-upland rice and soybean-upland rice-soybean cropping sequences. The three P sources were evaluated in terms of RAE based on dry matter yield and changes of soil pH, available P, and exchangeable Ca and Al in these two experiments.. The third experiment was conducted over a two-year period to evaluate the effect of freshly applied P sources (1st year) and their residual effect on on biological nitrogen (N) fixation (BNF) by soybean using ^{15}N isotopic dilution technique. Soybean residue was then compared with urea as an N source for the following upland rice. The results show that the use of Sechura PR will not only provide available P nutrient, but also reduce potential Al toxicity for upland rice and soybean contributing to sustainable crop production and BNF in the legume crop, especially for its residual P effect, in this acid Ultisol. The use of possibly more cost-effective Huila PAPR can be also an alternative P source to TSP for this soil due to its good agronomic performance. However, the effect of liming on the soil properties should also be carefully determined and monitored since liming can significantly reduce the agronomic effectiveness of PR. This is due to an increase in soil pH and exchangeable Ca that reduces PR dissolution and soil available P. In the third experiment, P was more limiting than N in influencing upland rice yield in both unlimed and limed soils. When P was applied, the highest yields were obtained using urea. N availability from soybean residue was inferior to urea for the following upland rice indicating low net N mineralization of the soybean residue, probably due to its relatively high C/N ratio.

1. INTRODUCTION

Phosphate (P) fertilizers and lime are often required for sustainable crop production on tropical acid Ultisols and Oxisols because of their low available P and potential Al toxicity [1, 2]. However, both inputs are costly to crop growers, especially to resource-poor farmers in the tropics. Use of alternative P fertilizers such as indigenous phosphate rock (PR) or partially acidulated PR (PAPR) rather than the use of conventional water-soluble P fertilizers may be a more cost-effective means of supplying P under these conditions [3, 4, 5]. Whenever possible, liming is practiced to control Al toxicity in the acid Ultisols and Oxisols. However, the Ca released can also reduce the P availability from the PR products. Although a considerable amount of research work has been conducted on the use of PR and PAPR, little research has been conducted to investigate the effect of liming on the agronomic effectiveness of PR and PAPR. Thus there is a need to investigate the interaction of lime and PR or PAPR for crop production in acid soils.

The purpose of this paper is to present a summarized report on a 3-year greenhouse experiments carried out to study the effect of liming on the agronomic effectiveness of three P sources varying in water and citrate solubility applied to upland rice and soybean grown in crop sequences.

2. MATERIALS AND METHODS

The three P sources used were unground, as-received Sechura PR (Peru); granular PAPR produced from Huila PR (Colombia) with H_2SO_4 at 50% acidulation level; and a commercial-grade, granular triple superphosphate (TSP). Their total P content and solubility indices are shown in Table I.

TABLE I. TOTAL P AND SOLUBILITY INDICES OF THE P SOURCES

	P_2O_5, %		
P Source	Total	Water soluble	NAC soluble[a]
Sechura PR	30.6	-	7.5
Huila PAPR	17.3	8.9	1.7
TSP	46.2	38.4	7.8

[a] NAC = Neutral ammonium citrate.

A *Typic Hapludult* Hartsells silt loam with pH 5.0; organic matter 1.7%; ECEC 5.1 cmol/kg; exchangeable Al 1.0 cmol/kg; and available P 1.3 mg P/kg by the iron oxide impregnated paper strip method (P_i test) [6] was used in the experiments. The soil pH was adjusted by incubating with or without powdered calcitic limestone at rates of 0, 1.25, and 2.50 g/kg for 4 weeks to pH 4.8, 5.6 and 6.6. In total, three greenhouse experiments were conducted.

2.1. Experiment 1. Upland Rice—Upland Rice—Upland Rice

The P sources were mixed with one unlimed and two limed soils at 0, 25, 75, and 150 mg P/kg (4 kg per pot). Rates of N and K were 150 mg N/kg as urea and 100 mg K/kg as KCl. All other nutrients were added at adequate levels so that P was the only nutrient limiting plant growth. Upland rice was planted by seeding and grown to maturity. After harvesting, soil samples were collected from the treatments of 0 and 150 mg P/kg for chemical analyses (pH, Excangeable Ca, exchangeable Al and available P by the Pi-test). Upland rice was planted during the next 2 years to monitor the residual P effect without adding P. All other nutrients were applied at the same rates as the first year except N was added at 200 mg N/kg and the crop was grown to maturity.

2.2. Experiment 2. Soybean—Upland Rice—Soybean

The P sources were mixed with one unlimed and two limed soils at 0, 25, 75, and 150 mg P/kg (4 kg per pot). Rates of N and K were 20 mg N/kg and 100 mg K/kg as KCl. Inoculated seeds of soybean were planted. Due to power failure in the greenhouse 2 months after planting, overheating killed the plants; therefore, only the total aboveground dry-matter yield was measured at that time. After that, the soils were planted with upland rice during the second year and soybean during the third year to assess the residual P effect. All other nutrients were applied at the same rates as the first year except N was applied at 200 mg N/kg for upland rice and the crops were grown to maturity.

2.3. Experiment 3. Biological N Fixation (BNF) in Soybean Followed by Upland Rice

The same unlimed and two limed soils were labeled with an aqueous solution of $K^{15}NO_3$ (16% atom ^{15}N excess) at a rate of 8.5 mg N/kg soil. Sucrose was also added at a 14:1 (C:N) ratio to enhance immobilization of the applied ^{15}N labeled fertilizer. The soils were thoroughly mixed and potted, watered to field capacity, and incubated for 2 months. All P sources were then mixed with the unlimed and limed soils at 0 and 150 mg P/kg; whereas, Huila PAPR was excluded in the two limed soils for nodulating soybean plants due to insufficient quantity of the material available for the study. All other nutrients without N were added at adequate levels. For non-nodulating soybean plants, only TSP at 150 mg P/kg was applied. Similar problem of power failure reported above in Experiment 2 occurred and the soybean plants were cut 2 months after planting. The concentration of N in the combined tissue samples of soybean roots, stems, and leaves was determined as 1.8% N. Two additional identical sets of pots without the use of ^{15}N were also prepared for soybean plants that were also cut 2 months after planting because of power failure. Upland rice was then grown following soybean to maturity in the three identical sets of soil pots during the second year without additional P added. In the first set the soils received no N while the other two sets the soils were treated with ground combined soybean residues and urea, respectively, to provide 75 mg N/kg rate. All other nutrients were added as previously described.

The percentages of plant N derived from the air (% Ndfa) for soybean of the first year were calculated by the following equation as described by Chien *et al.* [7]:

$$\%\ \text{Ndfa} = \left[1 - \frac{\text{Atom}\ \%\ ^{15}\text{N excess (nodulating)}}{\text{Atom}\ \%\ ^{15}\text{N excess (non - nodulating)}}\right] \text{x}\ 100 \qquad [1]$$

The total amounts of N derived from the air (Ndfa) were calculated as

$$\text{Ndfa} = \%\ \text{Ndfa x total N uptake} \qquad [2]$$

2.4. Statistical Analysis

In this study, grain yields of upland rice and soybean in Experiments 1 and 2 or dry-matter yield of soybean in Experiment 2 obtained with each P source were fitted to the following semi-log response function as described by Chien *et al.* [8]:

$$Y_i = b_o + b_i \ln X_i + e_i,\ X_i \geq 1 \qquad [3]$$

where Y_i is the yield obtained with source i, X_i is the P rate applied, b_o is the common intercept (i.e. $X_i = 1 \rightarrow 0$), b_i is the regression coefficient, and e_i is the error term of the fitted model.

To compare the relative agronomic effectiveness (RAE) of Sechura PR or Huila PAPR with respect to TSP, the RAE was defined as

$$\text{RAE, \%} = (b_i/b_{TSP}) \text{ x } 100 \qquad [4]$$

where b_i is the regression coefficient of Sechura PR or Huila PAPR. Thus, RAE of TSP is 100%. To compare treatment effects among P sources, the standard error (SE) of estimate for b_i was used to evaluate whether a given P source was statistically different from other P sources in terms of increasing grain yield of upland rice or dry-matter yield of soybean.

In Experiment 3, calculated LSD $_{(0.05)}$ values were used to detect any significant differences in grain yield of upland rice (after soybean) that was obtained with each P source treated with no N, soybean residue or urea.

3. RESULTS AND DISCUSSION

3.1. Experiment 1. Upland Rice—Upland Rice—Upland Rice

The grain yield responses of upland rice to P application over a three-year period are shown in Figs 1 to 3 respectively. A significant P effect on rice grain yield was observed in unlimed and two limed Hartsells soils during the 3-year period. Without P added, there was almost no grain yield indicating the soil used was very deficient in P as evidenced by its low available P status (Pi-P = 1.3 mg P/kg).

The calculated RAE values for all P sources in all three upland rice crops are shown in Table II. For the first crop, TSP was more effective than Sechura PR and Huila PAPR in the unlimed soil as well as the limed soils. Liming the soil from pH 4.8 to 6.6 drastically reduced the RAE of Sechura PR from 88% to 44%, indicating a negative effect on PR agronomic effectiveness in the year of application. Liming is known to raise soil pH and increase exchangeable Ca that can result in a reduced PR dissolution [8]. For the second crop, Sechura PR was as effective as TSP in the unlimed soil and the soil limed to pH 5.6, and was even more effective (RAE = 113%) than TSP in the soil limed to pH 6.6. For the third crop, Sechura PR was statistically as effective as TSP in the unlimed and limed soils, although its RAE values were 11% and 15% higher than TSP in the unlimed soil and the limed soil at pH 5.6, respectively. The good residual P effect of Sechura PR for the second and third crops was due to its high reactivity and the decline of the liming effect on soil pH (and exchangeable Al levels) over time.

Although Huila PAPR was as effective as or more effective than Sechura PR for the first crop, it was less effective than Sechura PR for the second and third crops except for the second crop in the limed soil at pH 5.6. Huila PAPR was less effective than TSP for all the crops regardless of whether or not lime had been applied except for the second crop in limed soil at pH 5.6. Possible explanations are (1) most of water-soluble P in Huila PAPR was taken up by the first crop and was also fixed by the soil so that less P was available for the second and third crops, (2) lower water solubility of Huila PAPR compared to TSP, and (3) since the reactivity of Huila PR is lower than that of Sechura PR, the contribution of Huila PR in Huila PAPR would be expected to be less than Sechura PR.

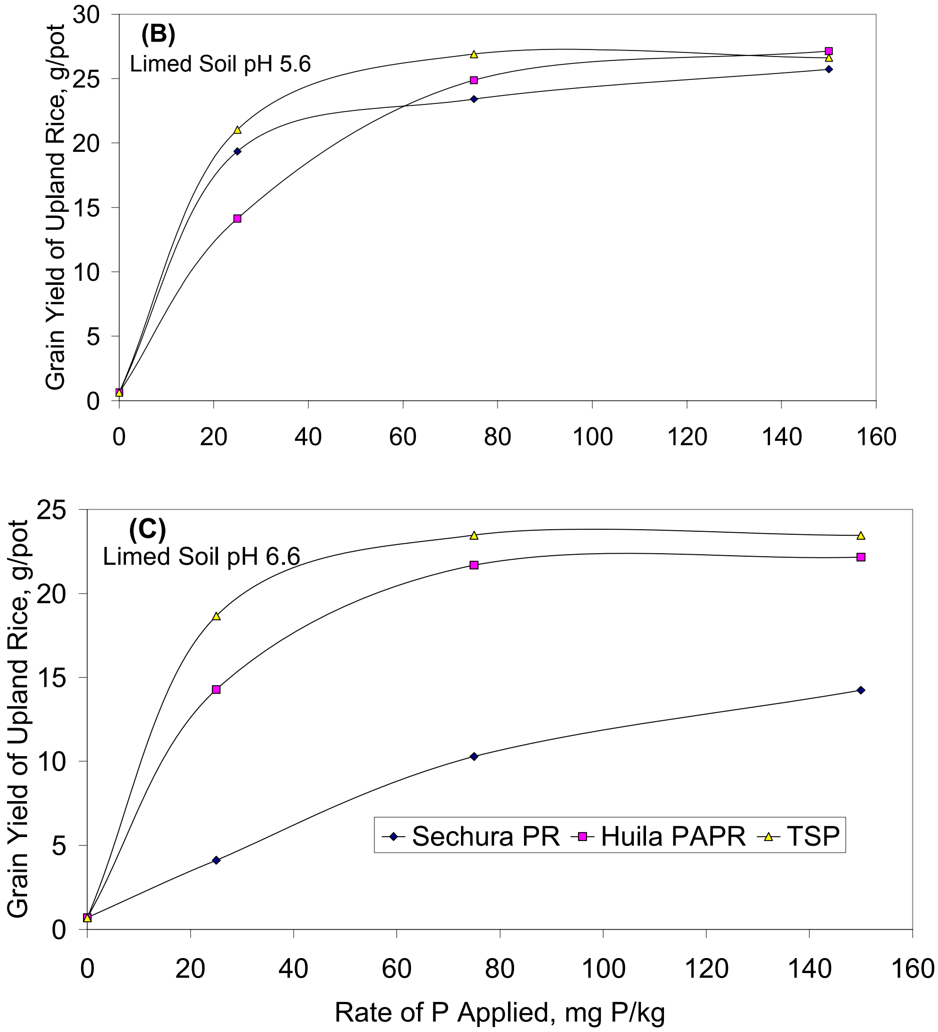
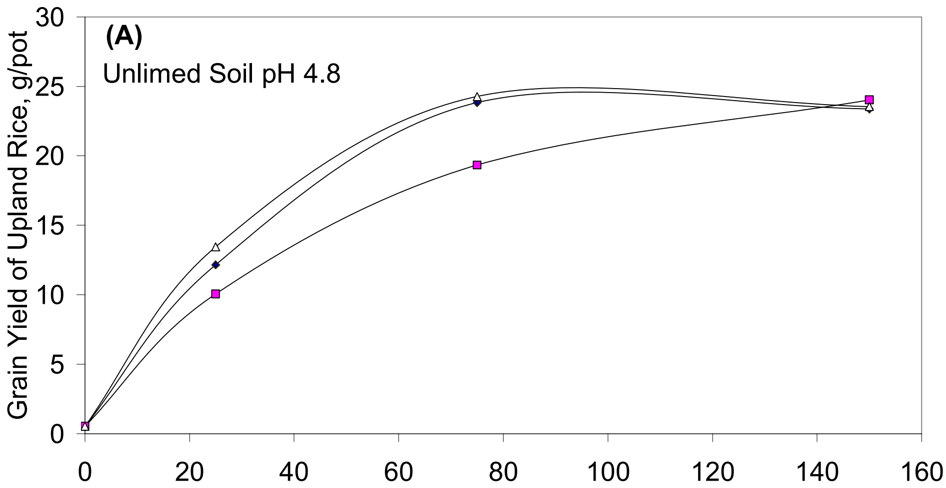


FIG.1. Grain yield of the first upland rice in upland rice-upland rice-upland rice in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.



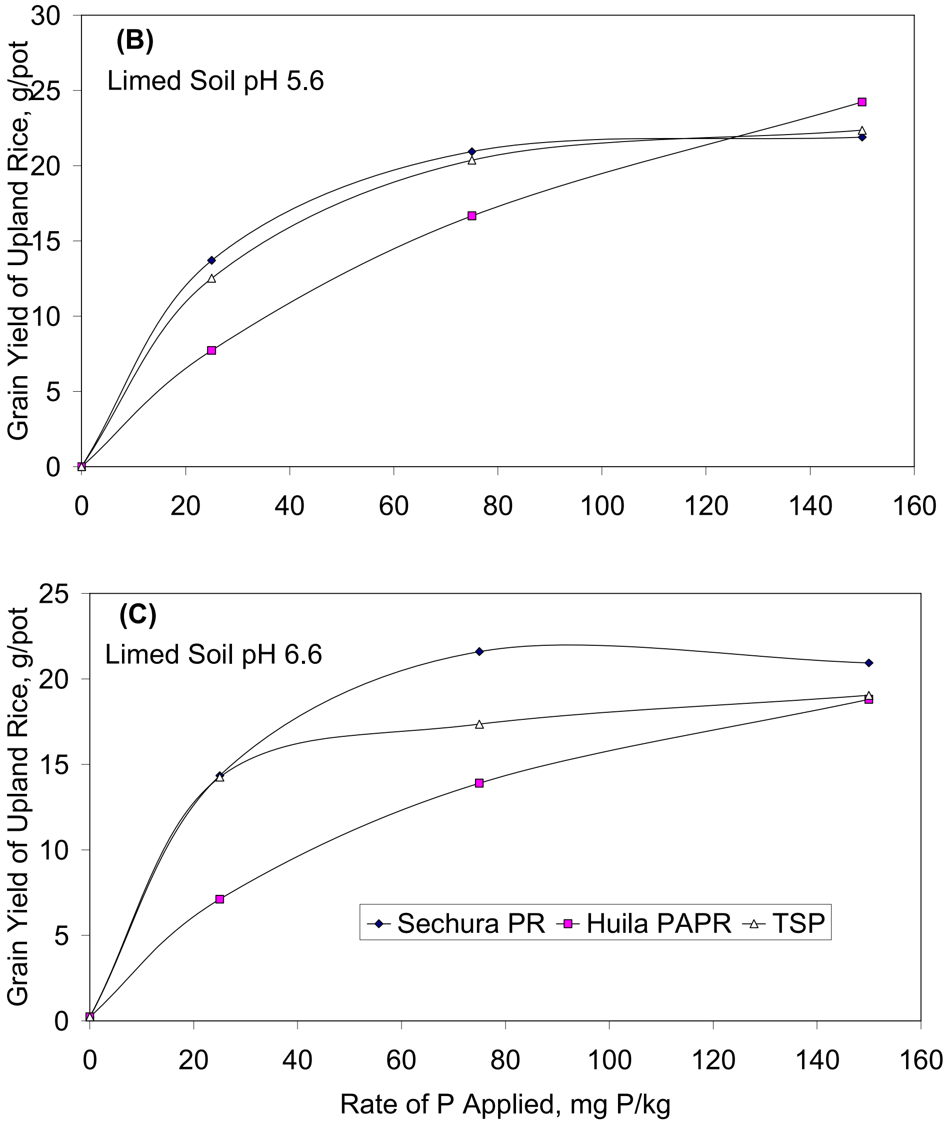


FIG.2. Grain yield of the second upland rice in upland rice-upland rice-upland rice in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.

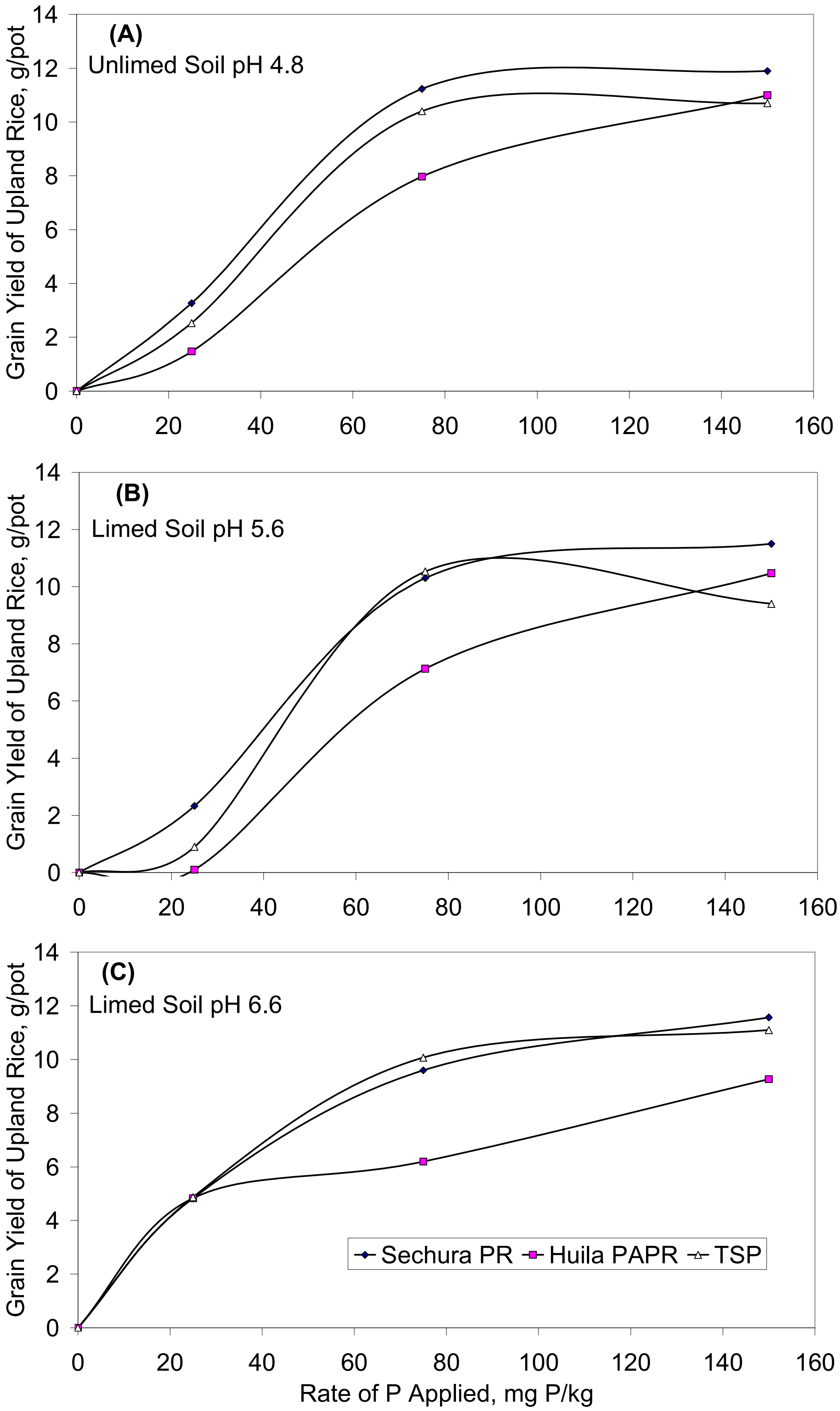


FIG.3. Grain yield of the third upland rice in upland rice-upland rice-upland rice in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.

TABLE II. CALCULATED RAE VALUES OBTAINED WITH P SOURCES FOR UPLAND RICE - UPLAND RICE - UPLAND RICE IN THE INITIAL UNLIMED SOIL (PH 4.8) AND TWO LIMED SOILS (PH 5.6 AND PH 6.6)

	RAE[a], %		
P Source	First Upland Rice	Second Upland Rice	Third Upland Rice
	pH 4.8		
Sechura PR	88 B	97 A	111 AB
Huila PAPR	86 B	89 B	87 C
TSP	100 A	100 A	100 BC
	pH 5.6		
Sechura PR	92 B	102 A	115 A
Huila PAPR	91 B	91 A	88 B
TSP	100 A	100 A	100 A
	pH 6.6		
Sechura PR	44 C	113 A	A00 A
Huila PAPR	89 B	81 C	60 B
TSP	100 A	100 B	100 A

[a] *Values with the same letter in each column for each soil pH level are statistically not different (p = 0.05).*

After cropping with upland rice, the pH of all the soils treated with 0 and 150 mg P/kg except Sechura PR in the unlimed soil decreased as compared with that before cropping (Fig. 4). The decrease in soil pH during cropping was probably due to soil acidification by annual application of 150 mg N/kg. All P sources had higher soil pH values than the check. For unlimed soil and limed soils, Sechura PR tended to result in a higher pH than Huila PAPR and TSP suggesting the highly reactive Sechura PR has a relatively greater liming effect in terms of pH changes than Huila PAPR and TSP. No significant difference in pH was found with Huila PAPR and TSP.

Available P as measured by the P_i test in the soils following cropping with upland rice showed a decrease in available Pi-P for all P sources, especially for TSP due to soil P-fixation (Table III). However, Pi-P of TSP after each cropping was still greater than Sechura PR and Huila PAPR due to its high water solubility (Table I). Available Pi-P of Sechura PR was significantly lower in the limed soil at pH 6.6 than that in the unlimed soil and limed soil at pH 5.6. For example, available Pi-P of Sechura PR decreased from 7.6 mg P/kg in the unlimed soil at pH 4.8 to 3.2 mg P/kg in the limed soil at pH 6.6 (Table III) after first crop. This significant decrease in available P_i-P was in agreement with a significant decrease of grain yield in the limed soil at pH 6.6 (Fig. 3).

All P sources increased pH compared to the check (Fig. 4) and thus increased exchangeable Ca and decreased exchangeable Al in the unlimed soil as compared with the check after first cropping with upland rice (Table IV). Sechura PR was more effective than Huila PAPR and TSP in reducing exchangeable Al whereas no significant difference was observed between Huila PAPR and TSP. Both Sechura PR and Huila PAPR consistently showed higher exchangeable Ca than the check throughout the cropping periods. After the third crop, only Sechura PR still showed a reduction in exchangeable Al compared to the check. Thus, use of a highly reactive PR at high P rate in acid tropical soils, may have the benefit of providing available P, raising soil pH, increasing exchangeable Ca and reducing exchangeable Al. This is particularly important for the strategy for one-time application of a high reactive PR source at a high dosage (as a capital investment) to capture its long-term residual P effect as compared to annual application of water-soluble P.

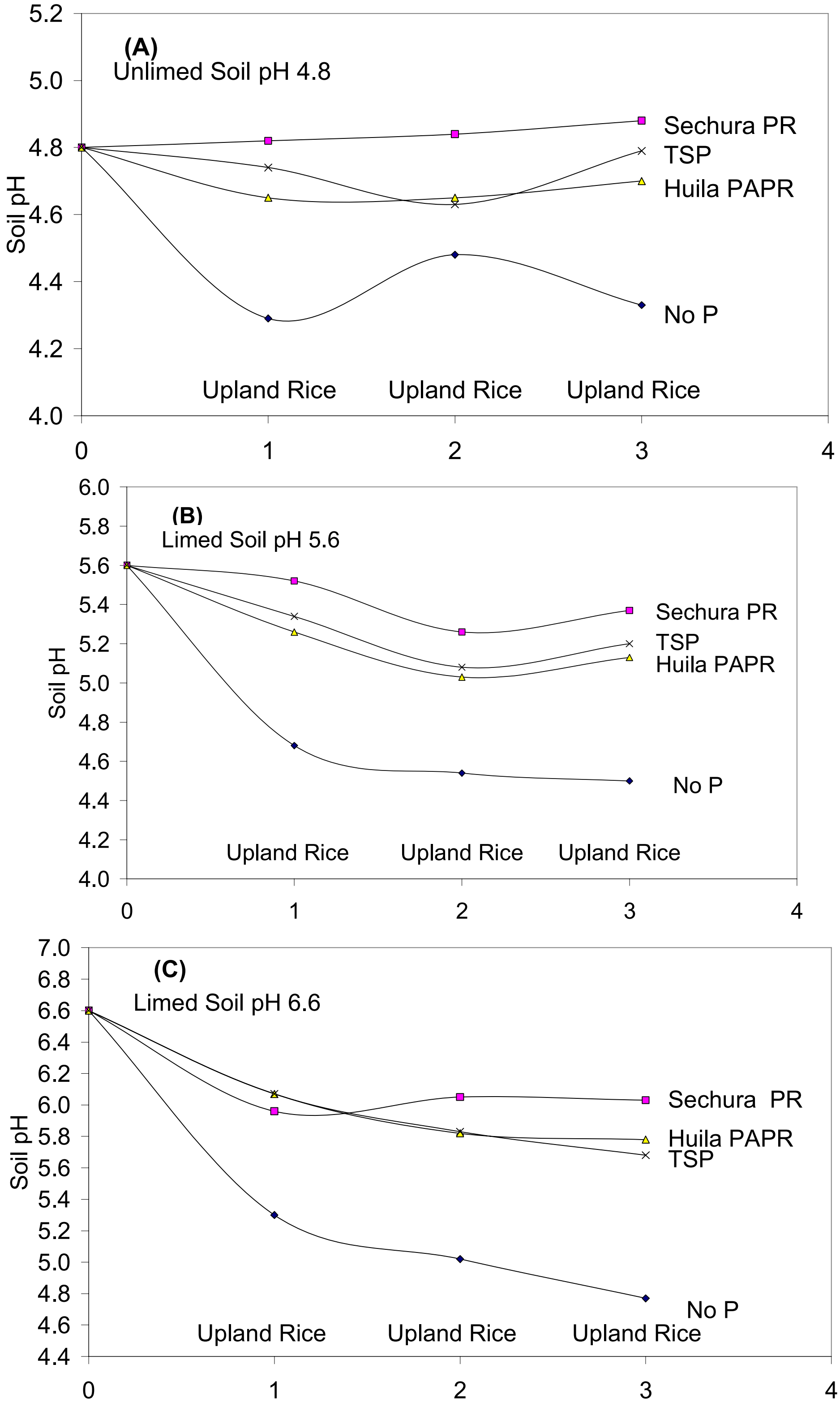


FIG.4. Soil pH before and after upland rice-upland rice-upland rice at 0 and 150 mg P/kg in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6), and (C) limed (pH 6.6) soils.

TABLE III. AVAILABLE SOIL PI-P OBTAINED WITH P SOURCES AT 150 MG P/KG AFTER UPLAND RICE - UPLAND RICE - UPLAND RICE IN THE INITIAL UNLIMED SOIL (PH 4.8) AND TWO LIMED SOILS (PH 5.6 AND PH 6.6)

P Source	First Upland Rice	Second Upland Rice	Third Upland Rice
		Soil pH 4.8	
No P	2.0	2.8	1.2
Sechura PR	7.6	7.1	5.7
Huila PAPR	8.9	6.0	4.4
TSP	21.3	13.3	8.4
		Soil pH 5.6	
No P	1.5	3.7	1.7
Sechura PR	8.8	7.1	5.5
Huila PAPR	7.2	5.3	3.9
TSP	22.0	12.1	7.5
		pH 6.6	
No P	1.7	2.4	1.2
Sechura PR	3.2	4.0	3.2
Huila PAPR	8.4	5.6	3.9
TSP	22.4	13.1	7.9
($LSD_{0.05}$)	(4.6)	(2.1)	(0.7)

TABLE IV. SOIL EXCHANGEABLE CA AND AL OBTAINED WITH P SOURCES AT 150 MG P/KG AFTER UPLAND RICE - UPLAND RICE - UPLAND RICE IN THE INITIAL UNLIMED SOIL (PH 4.8)

P Source	First Upland Rice	Second Upland Rice	Third Upland Rice
		Exchangeable Ca, cmol/kg	
No P	2.3	2.1	2.0
Sechura PR	2.9	2.4	2.5
Huila PAPR	3.1	2.6	2.6
TSP	2.5	1.9	1.8
($LSD_{0.05}$)	(0.4)	(0.3)	(0.5)
		Exchangeable Al, cmol/kg	
No P	1.9	1.9	1.7
Sechura PR	1.3	1.5	1.5
Huila PAPR	1.6	1.8	1.7
TSP	1.7	1.8	1.8
($LSD_{0.05}$)	(0.2)	(0.1)	(0.4)

In summary, the results from this experiment clearly showed that the highly reactive Sechura PR had a high residual P effect for the second and third upland rice even though the initial soil was limed to pH 6.6 before the first upland rice. On the other hand, PAPR produced from medium-reactive Huila PR could not provide effective residual P effect for the third upland rice in the limed soil at pH 6.6. Thus raising soil pH of acid Ultisols by liming should be carefully adjusted and monitored to maximize the utilization of PR and PAPR for upland rice production. It should be pointed out, however, that a better criterion to determine the liming requirements is the reduction of the exchangeable Al (% Al saturation) in the soil in relation to the Al tolerance of the crop and variety to be utilized.

3.2. Experiment 2. Soybean—Upland Rice—Soybean

There was a significant response of soybean-upland rice-soybean to P application with all P sources in the unlimed and two limed soils (Figs 5–7). Without P added, all the yields were very low indicating soil available P was very deficient for soybean and upland rice.

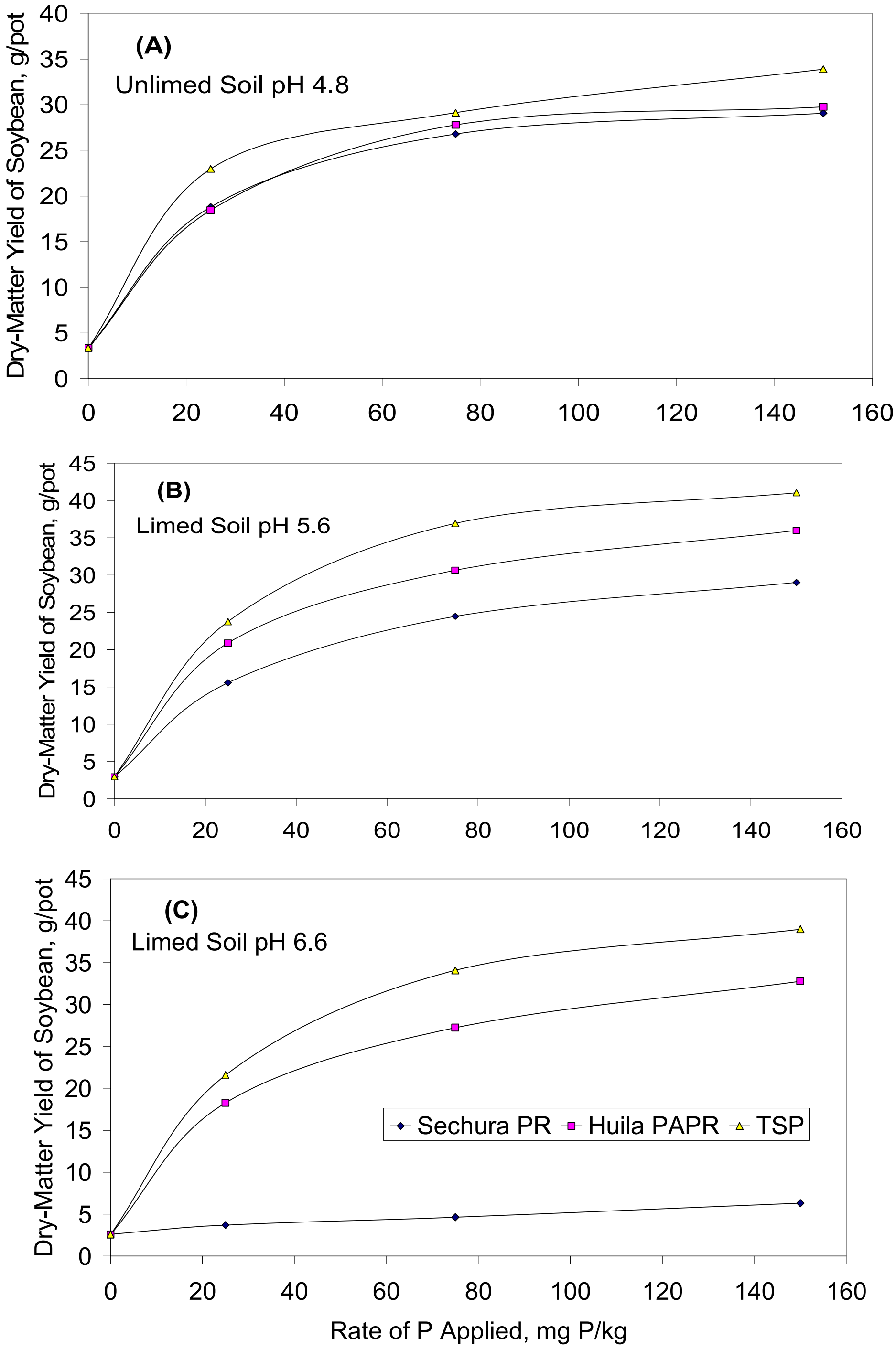


FIG.5. Grain yield of the first soybean in soybean-upland rice-soybean in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.

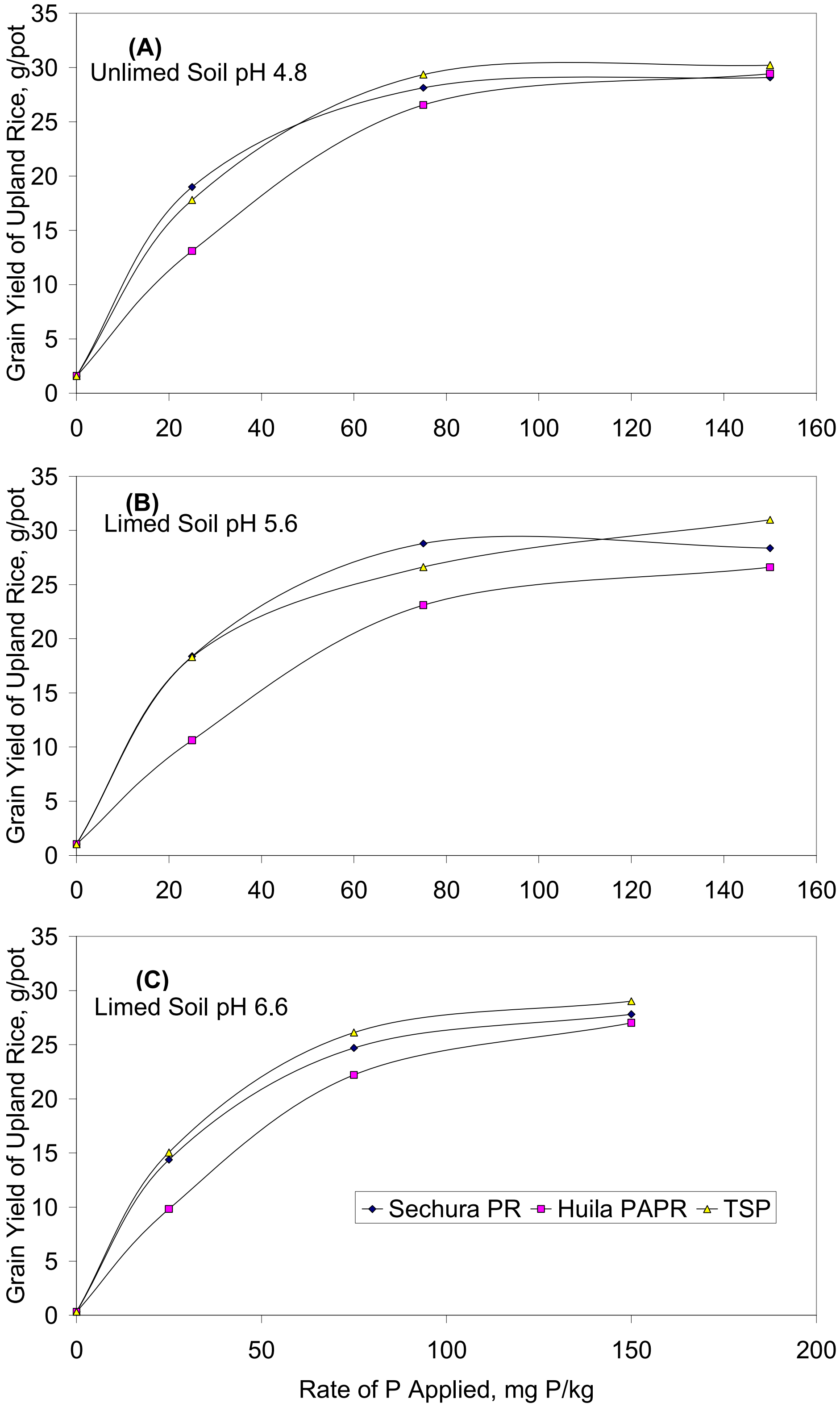


FIG.6. Grain yield of the second upland rice in soybean-upland rice-soybean in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.

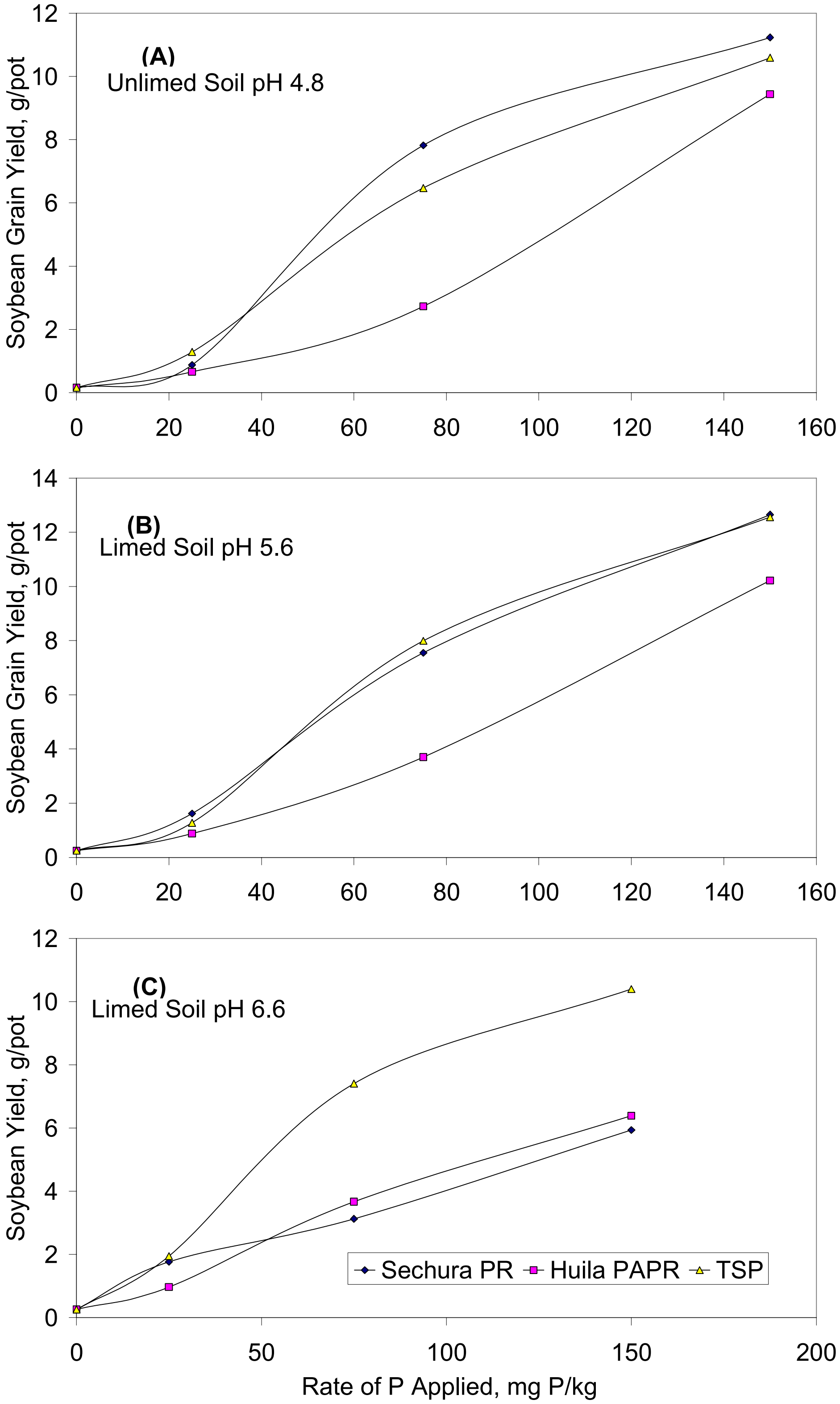


FIG.7. Grain yield of the third soybean in soybean-upland rice-soybean in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6) and (C) limed (pH 6.6) soils.

The calculated RAE values of soybean-upland rice-soybean obtained with all P sources are shown in Table V. For the first crop (soybean) in the unlimed soil (pH 4.8), the agronomic effectiveness of the P sources was as follows TSP > Sechura PR = Huila PAPR. In the two limed soils (pH 5.6 and 6.6), the order follows TSP > Huila PAPR > Sechura PR, indicating a negative effect of liming on PR dissolution. For example, when the soil was limed to 6.6, the RAE of Sechura PR for the first soybean was only 5%. For the second crop (upland rice), Sechura PR was as effective as TSP and more effective than Huila PAPR. For the third crop (soybean), Sechura PR was as effective as TSP whereas Huila PAPR was less effective in the initial unlimed and limed soil at pH 5.6. However, both Sechura PR and Huila PAPR were only about 50% as effective as TSP for this crop grown on the soil initially limed at pH 6.6.

Considering the calculated initial RAE values for upland rice (Table II) and soybean (Table V), it is noted that upland rice was more efficient than soybean in utilizing P from Sechura PR in the limed soils. In the limed soil at pH 6.6, RAE of Sechura PR was only 5% for the first soybean compared to 44% for the first upland rice and 53% for the third soybean. It should be pointed out that in the unlimed soil Sechura PR was as effective as TSP and more effective than Huila PAPR for the third soybean. The good performance of Sechura PR compared to TSP for the second upland rice in all the soils and the third soybean in the unlimed and limed (pH 5.6) soil was most likely due to its high reactivity and also due to more P fixation of TSP by the soil. The reason for Huila PAPR being less effective than Sechura PR in supplying residual P to the second upland rice and the third soybean in the unlimed and limed soils was probably due to lower reactivity of Huila PR than that of Sechura PR.

TABLE V. CALCULATED RAE VALUES OBTAINED WITH P SOURCES FOR SOYBEAN - UPLAND RICE - SOYBEAN IN THE INITIAL UNLIMED SOIL (PH 4.8) AND TWO LIMED SOILS (PH 5.6 AND PH 6.6)

P Source	RAE[a], %		
	First Soybean	Second Upland Rice	Third Soybean
		pH 4.8	
Sechura PR	85 B	98 AB	109 A
Huila PAPR	88 B	90 B	71 B
TSP	100 A	100 A	100 A
		pH 5.6	
Sechura PR	65 C	99 A	97 A
Huila PAPR	85 B	80 B	68 B
TSP	100 A	100 A	100 A
		pH 6.6	
Sechura PR	5 C	95 A	53 B
Huila PAPR	81 B	86 B	55 B
TSP	100 A	100 A	100 A

[a] Values with the same letter in each column for each soil pH level are statistically not different ($p = 0.05$).

After cropping with soybean-upland rice-soybean, the pH of all soils treated with 0 and 150 mg P/kg decreased after each cropping (Fig. 8) except in the initial unlimed soil treated with PR sources after the third soybean (Fig. 8A). One possible explanation for the increased soil pH of PR treatments after the third soybean compared to that after the second upland rice was rhizospheric acidification due to BNF of soybean that dissolved PR and thus raised soil pH. The results of soil pH in the soybean-upland rice-soybean system were similar to the upland rice-upland rice-upland rice system. Namely, the order of pH values follows Sechura PR > Huila PAPR = TSP > check suggesting the highly reactive Sechura PR has a greater liming effect in terms of pH increase than Huila PAPR and TSP.

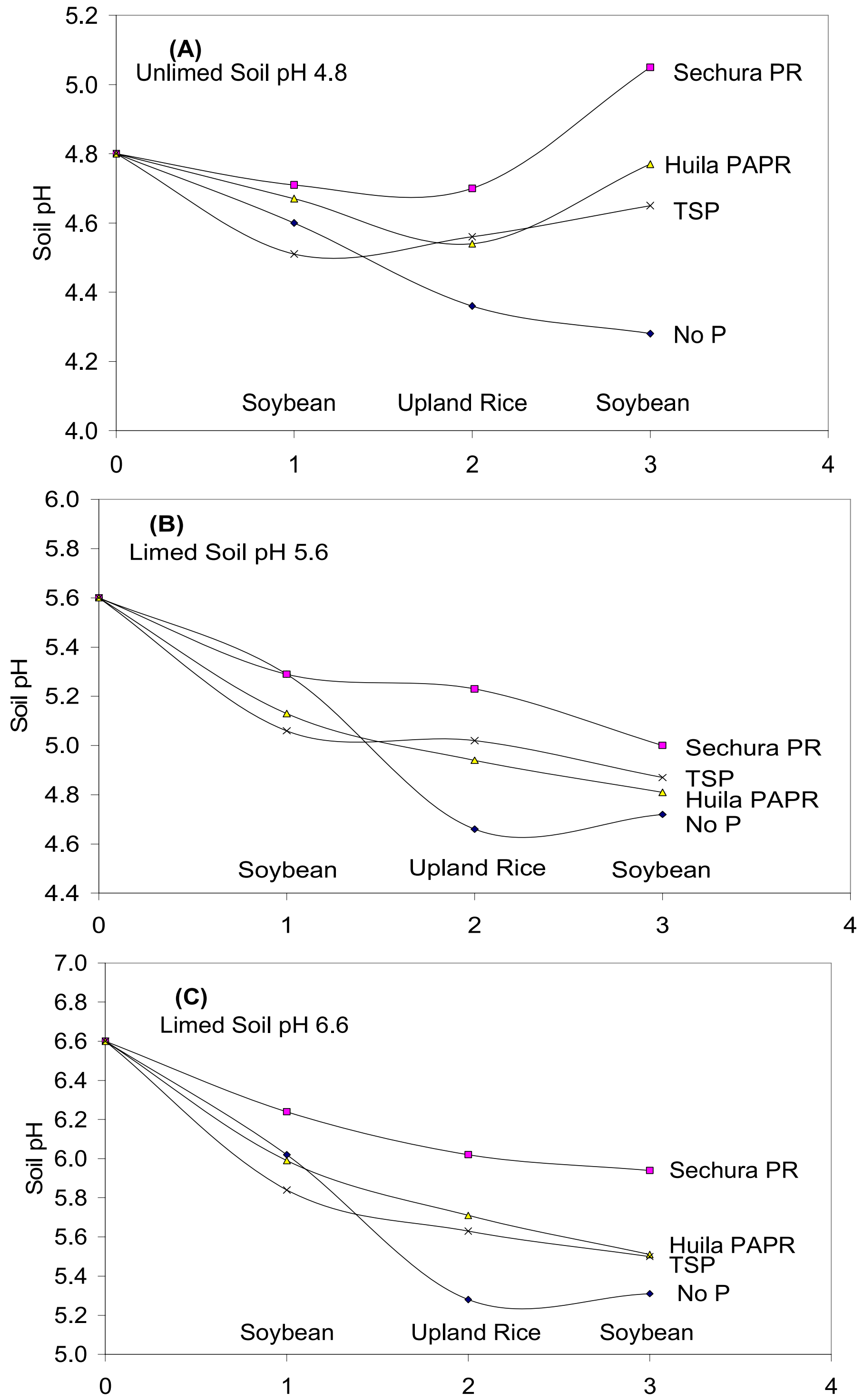


FIG.8. Soil pH before and after soybean-upland rice-soybean at 0 and 150 mg P/kg in initial (A) unlimed (pH 4.8), (B) limed (pH 5.6), and (C) limed (pH 6.6) soils.

3.3. Experiment 3. Biological N Fixation (BNF) in Soybean Followed by Upland Rice

Data of dry-matter yield of aboveground and root of soybean obtained with check and P sources at 150 mg P/kg are shown in Table VI. Nodulating plants produced higher total dry-matter yield than non-nodulating plants in unlimed and two limed soils treated with TSP, averaging about three times more. The non-nodulating plants showed a distinctive yellow color during the growth indicating N deficiency. In the unlimed soil at pH 4.8, the order of total dry-matter yield follows: TSP > Sechura PR = Huila PAPR > check. In the limed soil at pH 5.6, TSP was also the best source and Sechura PR was more effective than the check. In the limed soil at pH 6.6, Sechura PR was only slightly more effective than the check, and both were much less effective than TSP in increasing dry-matter yield.

TABLE VI. DRY-MATTER YIELD OF NODULATING AND NON-NODULATING SOYBEAN IN UNLIMED AND LIMED SOILS

	Dry-Matter Yield, g/pot				
Plant Part	Check	Sechura PR	Huila PAPR	TSP	TSP[a]
		Unlimed Soil at pH 4.8			
Aboveground[b]	1.9	25.0	25.6	29.2	9.7
Root[c]	0.6	6.0	6.4	8.4	3.1
(Total)[d]	(2.5)	(31.0)	(32.0)	(37.6)	(12.8)
		Limed Soil at pH 5.6			
Aboveground[b]	2.2	28.5	-	43.5	10.3
Root[c]	0.7	6.1	-	8.2	3.3
(Total)[d]	(2.9)	(34.6)	-	(51.7)	(13.6)
		Limed Soil at pH 6.6			
Aboveground[b]	1.6	4.7	-	42.0	14.6
Root[c]	0.5	1.3	-	7.8	4.0
(Total)[d]	(2.1)	(6.0)	-	(49.8)	(18.6)

[a] Non-nodulating variety.
[b] $LSD_{0.05} = 5.5$
[c] $LSD_{0.05} = 1.7$
[d] $LSD_{0.05} = 6.8$

Based on the ^{15}N isotopic dilution technique, the fraction of N derived from air (Ndfa) by BNF was calculated for soybean with P sources at 0 and 150 mg P/kg (Table VII). Data show that essentially no BNF took place with the check in all the soils. There was also no BNF with Sechura PR in the limed soil at pH 6.6 due to poor P availability at this high pH. The total amounts of Ndfa by BNF in soybean were calculated for each P source as shown in (Table VIII). For Sechura PR, amounts of Ndfa in the unlimed soil at pH 4.8 and limed soil at pH 5.6 were about the same. In the unlimed soil at pH 4.8, Sechura PR and Huila PAPR were about the same in BNF but both were less effective than TSP. For TSP, liming had a positive effect on BNF as evidenced by higher amounts of Ndfa in the two limed soils than that in the unlimed soil. Irrespective of liming and P source, P availability to nodulating soybean was a critical factor to the plant growth (dry matter yield) and the occurrence of nitrogen fixation.

In the unlimed soil at pH 4.8, the RAE values of Sechura PR and Huila PAPR were 87% and 89%, respectively, with respect to TSP (RAE = 100%) in BNF (data not shown). The RAE value of Sechura PR decreased to 52% in the limed soil at pH 5.6 and 0% in the limed soil at pH 6.6. However, it should be pointed out again that both dry matter yield and the actual amount of Ndfa with Sechura PR did not decrease with the change in soil pH from 4.8 to 5.6. No comparisons can be made for Huila PAPR as there were no dry matter data.

TABLE VII. FRACTION OF N DERIVED FROM AIR (% NDFA) IN THE ABOVEGROUND OF SOYBEAN TREATED WITH P SOURCES AND LIMING

Ndfa (%)[a]			
Check	Sechura PR	Huila PAPR	TSP
Unlimed Soil at pH 4.8			
0	82.5(2.8)	82.7(2.8)	78.1[b]
Limed Soil at pH 5.6			
0	81.4(2.0)	-*	91.2(2.6)
Limed Soil at pH 6.6			
0	0	-*	87.9(2.7)

[a]( ) values in brackets denote standard deviation.
[b] Only one replicate available during ^{15}N analysis.
* No data available.

TABLE VIII. AMOUNTS OF N DERIVED FROM AIR (Ndfa) IN WHOLE SOYBEAN PLANT (ABOVE-GROUND + ROOT) TREATED WITH P SOURCES AND LIMING

Ndfa, mg N/pot[a]		
Sechura PR	Huila PAPR	TSP
Unlimed Soil at pH 4.8		
459(105)	472(153)	528[b]
Limed Soil at pH 5.6		
486(56)	-*	929 (208)
Limed Soil at pH 6.6		
0	-*	931(284)

[a]( ) values in brackets denote standard deviation.
[b] Only one replicate available during ^{15}N analysis.
* No data available.

The grain yields for upland rice after soybean are shown in Table IX. Without added P, grain yields were very low for all N sources in the unlimed and limed soils, indicating P was limiting upland rice yield more than N. When P was applied, the highest yields were obtained using urea. Decomposing soybean residue provided some N as upland rice dry matter yields were increased over the no N treatment, but still the yield levels were much less than those obtained using urea. Net N mineralization of the soybean residue was probably limited due to the relatively low N content of the soybean residues (hence relatively high C/N ratio, which was estimated to be about 22). There was essentially no difference in grain yield response to urea and incorporated soybean residue across all P sources TSP and Sechura PR had similar effects on grain yield. No data are available for Huila PR.

TABLE IX. GRAIN YIELD OF UPLAND RICE AFTER SOYBEAN IN SOILS TREATED WITH SOYBEAN RESIDUE AND UREA (75 MG N/KG) AND UNTREATED (NO N), IN THE INITIAL UNLIMED (pH 4.8) AND TWO LIMED SOILS (pH 5.6 AND 6.6)

		Grain Yield of Upland Rice[b]			
Soil pH[a]	N Source	No P	Sechura PR	Huila PAPR	TSP
		(g/pot)			
4.8	No N	1.4 B	4.1 D	4.6 C	4.7 E
	Residue	3.4 A	6.2 C	6.5 B	7.2 C
	Urea	1.1 B	15.1 AB	13.3 A	14.6 B
5.6	No N	1.3 B	3.7 D	-*	5.9 D
	Residue	0.8 BC	6.2 C	-*	7.9 C
	Urea	0.7 BCD	13.7 B	-*	16.1 A
6.6	No N	0.1 D	4.2 D	-*	4.9 DE
	Residue	1.0 B	6.5 C	-*	8.0 C
	Urea	0.2 CD	15.7 A	-*	15.1 AB

[a] Soil pH before soybean crop.
[b] Values with the same letter in each column are statistically not different ($p = 0.05$).
* No data available.

4. CONCLUSIONS

A greenhouse study was conducted over a 3-year period to investigate the effect of liming on the initial and residual relative agronomic effectiveness of P sources varying in solubility for cropping sequences of upland rice and soybean. The results show that use of a highly reactive indigenous PR such as Sechura PR (Peru) will not only provide available P nutrient for crop growth, but also reduce potential Al toxicity to upland rice and soybean and increaseN inputs through biological fixation in soybean, thus contributing to sustainable crop production. The study shows that Sechura PR had a good residual P effect, in an acid Ultisol with no significant differences in soybean dry matter yield between limed and unlimed soil. The use of possibly more cost-effective P fertilizers such as PAPR produced from a medium-reactive Huila PR (Colombia) can be an alternative P source to water-soluble P fertilizers such as TSP for this soil due to their good agronomic performance. However, to achieve the beneficial effects of liming on soil properties and plant growth in acid soils the applied rates should be carefully determined and changes in soil properties monitored since liming can significantly influence the dissolution of the applied PR and the released available P, thus reducing the agronomic effectiveness of natural and modified PR products due to an increase in soil pH and exchangeable Ca.

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IMPROVING GRAIN YIELD AND NITROGEN FIXATION OF COMMON BEAN GROWN IN AN ACRISOL FROM CUBA

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Abstract

Field experiments were carried out to evaluate the effect of selected management practices such as the use of phosphate rock (PR), partially acidulated phosphate rocks (PAPRs) and soil liming, on improving grain yields and biological nitrogen fixation (BNF) of common bean (*Phaseolus vulgaris*) grown in an strongly acid Acrisol from Cuba. In Experiment 1 the agronomic effectiveness of the indigenous Trinidad de Guedes PR and FPA 50, a PAPR from Trinidad de Guedes PR with H_2SO_4 at 50% acidulation level were evaluated as P sources for the common bean genotypes, BAT 477, DOR 364, DOR 390 and Censa. In Experiment 2 the response of the CC 25-9N common bean genotype to the increasing application rates (40, 80 and 120 kg P_2O_5 ha^{-1}) of two P sources, i.e. triple super phosphate and C 40, was determined at two liming levels. C40 was a PAPR from the indigenous Trinidad de Guedes PR with H_2SO_4 at 40% acidulation level. In Experiment 3 the effect of soil liming on grain yield and BNF of BAT 58, BAT 304, BAT 477, DOR 364, DOR 390, Judía Roja Camagüeyana (JRC) and Censa common bean genotypes was studied. The most effective P sources to increase grain yields of the studied bean genotypes were the water-soluble superphosphate in experiments 1 and 2. Both partially acidulated PR products, FPA 50 and C 40, showed intermediate agronomic effectiveness and they increased grain yields over the PR and control (without P) treatments. Soil liming increased grain yields and BNF of the common bean genotypes tested. Based on an economic analysis of profit and value/cost ratios, single superphosphate was the P source that gave the highest economic profit for all common bena genotypes while FPA 50 was efficient for DOR 390 and Censa. Lime application increased the economic profit of all bean genotypes. Genotype BAT 304 showed adaptability to soil acidity and gave the highest profit in limed soil.

1. INTRODUCTION

Phosphorus (P) is an essential nutrient for plant and its deficiency in soils severely restricts crop yields. Tropical and subtropical soils are predominantly acidic and often extremely deficient in phosphorus. Moreover most of these soils posses a high phosphate sorption capacity. Therefore, substantial P inputs are required for optimum plant growth and adequate food and fiber production [1]. Manufactured water-soluble P fertilizers such as super phosphates are commonly recommended to correct P deficiencies, but most developing countries import these fertilizers, which are often in limited supply and represent a major outlay for resource-poor farmers. Moreover intensification of agricultural production in these countries necessitates the addition of P not only to increase crop production but also to improve soil P status in order to avoid further soil degradation. Hence, it is imperative to explore alternative P sources [2].

Leguminous crops, in particular common bean (*Phaseolus vulgaris*) are very important protein sources for the local populations in many parts of the world. Nevertheless, grain yields are low in many agricultural production systems due to a series of limiting factors such as low water availability, nitrogen and phosphorus deficiencies, and aluminium toxicity in acid soils [3].

Research conducted in tropical and subtropical areas is aimed at developing technologies and strategies to increase agricultural production by overcoming these soil infertility and acidity problems through the adoption of an integrated approach to crop, soil, water and nutrient management practices. Among these, improved fertilizer management practices and use of soil amendments are recommended [4]. Also, a more sustainable strategy would be to select plant genotypes tolerant to soil acidity (Al toxicity) and more efficient in absorbing and utilizing P and other nutrients [5].

In this context, a series of field experiments was carried out to assess the effect of selected management practices such as soil liming and P fertilization on grain yield and nitrogen fixation in recommended common bean genotypes grown in an Acrisol from Cuba. A simple economic analysis of these management practices was also made to identify those cost-effective strategies that increase grain yields of common bean in acid soils of the country.

2. MATERIALS AND METHODS

Three field experiments were conducted at the Experimental Station Viñales located at 22°37' latitude N and 83°43' longitude W, in the province Pinar del Rio, Cuba.

2.1. Soil and PR based products characteristics

The experimental soil was an Acrisol [6] and some selected characteristics are shown in Table I.

TABLE I. SOME SOIL CHARACTERISTICS[1] OF THE ACRISOL

	pH (KCl)	OM	Total N	Av.P	Ca	Mg	K	Na
		%		mg P kg^{-1}	cmol (+) kg^{-1}			
Acrisol	3.80	3.02	0.115	4.4	0.75	0.43	0.05	0.10

(1): pH, 1:2,5 (Soil:KCl solution ratio); OM (Walkley-Black method); Total N (Kjeldhal method); Available P (Bray - Kurtz I method); Exchangeable Cations (Schastschabel method).

The studied P fertilizers were locally available phosphate rock (PR) products, i.e. the indigenous Trinidad de Guedes PR and two partially acidulated PR (PAPR) products derived from this PR. Relevant data of the particle size distribution of the indigenous Trinidad de Guedes PR are given in Table II. Chemical composition and solubility indices of the PR products utilised in the experiments are shown in Tables III and IV, respectively.

TABLE II. PARTICLE SIZE DISTRIBUTION OF TRINIDAD DE GUEDES PR (IN %)

Particle size		%
> 1 mm		98.7
< 1 mm	> 0,63 mm	97.7
< 0,630 mm	> 0,200 mm	94.7
< 0,200 mm	> 0,160 mm	16.2
< 0,160 mm	> 0,100 mm	12.9
< 0,100 mm	> 0,063 mm	0.6
< 0,063 mm		0.2

TABLE III. CHEMICAL COMPOSITION OF TRINIDAD DE GUEDES PR AND PARTIALLY ACIDULATED PR PRODUCTS (IN % WEIGHT BASIS)

PR products[1]	P_2O_5	N	CaO	MgO	S	Fe_2O_3	Al_2O_3
PR	30.6	-	43.6	0.28	0.6	2.14	3.42
C 40	29.0	1.25	35.0	0.24	6.2	2.59	2.59
FPA 50	19.2	-	27.1	0.31	8.1	2.08	4.02

(1) PR: Indigenous Trinidad de Guedes PR. C 40: Partially acidulated Trinidad de Guedes PR with H_2SO_4 at 40% acidulation + monoammonium phosphate (MAP). The C 40 fertilizer was produced by Technifert Enterprise, Saint Malo, France. FPA 50: Partially acidulated Trinidad de Guedes PR with H_2SO_4 at 50% acidulation level. The FPA 50 fertilizer was produced by Rayonitro Enterprise, Matanzas, Cuba.

TABLE IV. SOLUBILITY TESTS IN CONVENTIONAL REAGENTS (% OF TOTAL P_2O_5) OF TRINIDAD DE GUEDES PR AND PARTIALLY ACIDULATED PR PRODUCTS

	Water	Neutral Ammonium Citrate	2% Citric Acid	2% Formic Acid
PR	0.1	11.3	26.5	26.8
C 40	13.5	31.9	55.8	50.3
FPA 50	28.5	44.9	76.0	61.7

2.2. Experiment 1. Agronomic effectiveness of PR derived products for common bean genotypes

The experimental design was a factorial of 4 common bean genotypes x 4 P-fertilizer treatments arranged in randomized complete block with 3 replications. The three P-fertilizer sources studied were: the indigenous Trinidad de Guedes PR; FPA 50 (a PAPR product derived from this PR) and single super phosphate (SSP), which was used as reference P-fertilizer. In addition, a check (no P fertilizer application) treatment was also included. The applied P rate for all P fertilizer sources was 90 kg P_2O_5 ha^{-1}. Four common bean genotypes (BAT 477, DOR 364, DOR 390 and Censa) were selected for this experiment.

2.3. Experiment 2. Liming effect on the agronomic effectiveness of P fertilizer sources

The experimental design was a factorial of 2 P sources x 3 P levels plus P-check x 2 liming levels arranged in randomized complete block design with 3 replications.

Liming was the main factor evaluated in this experiment at two levels: limed and unlimed soil. The applied rate of $CaCO_3$ was 2,450 kg ha^{-1} and it was estimated based on the neutralization of 0,5 units of hydrolytic acidity (Y_1) first extraction, using the following formula [7]:

$$Y = (\text{kg } CaCO_3 \ (ha^{-1}) = 8647 - 988 \text{ pH KCl}$$

The P fertilizer sources studied were: C 40 (a PAPR product) and triple super phosphate (TSP), which was used as a reference water-soluble P-fertilizer. They both were applied at three P rates, i.e. 40, 80 and 120 kg P_2O_5 ha^{-1}. A check (without P fertilization) was also included. The common bean cultivar CC 25-9N was used in this experiment.

The experimental procedures described below were common for experiments 1 and 2. Plot sizes were 4.2 m × 3 m and they were surrounded by a border of 0.5 m of untreated soil. Each plot had 6 rows. The seeding spacing within the row was 0.05 m. At planting the seeds of all common bean genotypes were inoculated with the 6bIII *Rhizobium* strain and received a basal fertilization of 15 kg N ha^{-1} as urea and 60 kg K_2O ha^{-1} as KCl. Ten plants of four central rows were harvested at R6 – R7 (at about 75 days after planting) and at R9 (about 90 days after planting) growth stages for P uptake and grain yield determination. Total P concentration in shoot and grain was determined by the vanadomolybdate yellow method. P uptake (kg P ha^{-1}) in grains was obtained by calculation using P concentration and grain yield.

The Relative Agronomic Effectiveness (RAE) values for the PR products were estimated as RAE (%) = $[(Y_1 - Y_0)/(Y_2 - Y_0)] \times 100$, where Y_1 = Grain yield or P uptake obtained with PR or FPA 50,Y_2 = Grain yield or P uptake obtained with SS, Y_0 = Grain yield or P uptake obtained with check (no P added) [8].

2.4. Experiment 3. Agronomic response and biological nitrogen fixation (BNF) of common bean genotypes as affected by liming

The experimental design used was randomized complete blocks in a 2 × 7 factorial arrangement (2 lime treatments and 7 nodulating or fixing common bean genotypes). Each treatment was replicated three times. Seven common bean genotypes, i.e. BAT 58, BAT 304, BAT 477, DOR 364, DOR 390, Judía Roja Camagüeyana (JRC) and Censa were evaluated for BNF capability. NN A285, a non-nodulating (non-fixing) common bean genotype was used as reference crop. The treatments were unlimed and limed with calcium carbonate ($CaCO_3$). As mentioned above for experiment 2, the applied amount of $CaCO_3$ was 2,450 kg ha^{-1}.

The ^{15}N isotope dilution technique (A value approach) was utilised to measure the amount of N biologically fixed [9]. In the A value technique – different N rates are applied to the reference and fixing crops. A higher rate is given to the reference crop when the soil N supply could be insufficient to ensure a normal growth and development of the reference crop, and this consequently, could also affect the accuracy in the estimation of the BNF of fixing crops. The applied doses and enrichments of the ^{15}N-tagged fertilizer were 50 kg N ha^{-1} as urea 2.5 at. % ^{15}N excess and 15 kg N ha^{-1} as urea 5 at. % ^{15}N excess to the reference (non-fixing) and fixing crops, respectively.

The following formulae were used to estimate nitrogen fixation [9]:

$$\% \text{ Ndff} = (\text{atom } \%^{15}N \text{ excess in plant/atom } \%^{15}N \text{ excess in fertilizer}) \times 100 \qquad (1)$$

$$n\ (\%\ Ndff\ NF/\%\ Ndfs\ NF) = \%\ Ndff\ F/\%\ Ndfs\ F \quad (2)$$

$$\%\ Ndff + \%\ Ndfs + \%\ Ndfa = 100 \quad (3)$$

$$\text{“Fixed” } N_2 = (\%\ Ndfa \times N \text{ yield of fixing crop}) / 100$$

$$A \text{ value} = ((100 - \%Ndff)/ \%Ndff) \times N \text{ dose applied with the fertilizer} \quad (4)$$

A value: quantitative measure of the available amount of a soil nutrient in terms of a fertilizer standard, in this case, urea.

The plot sizes were 4.2 m × 3 m = 12.6 m^2 and they were surrounded by a border of 0.5 m of untreated soil. Each plot had 6 rows (with a row distance of 0.70 m). The isotopic plots had 2.8 m^2 (four rows, with a row distance of 0.70 m = 2.8 m × 1 m of length). Prior to seedling, the common bean seeds were inoculated with the peat-based 6bIII *Rhizobium* strain. All plots received a basal fertilization of 60 kg K_2O per ha as KCl (60% K_2O) and 90 kg P_2O_5 per ha as TSP (45% P_2O_5). Ten plants of four central rows were harvested at R9 growth stage (about 90 days after planting) to determine grain yield. The plant samples were oven-dried at 65°C during 72 hours, finely ground using a Wiley mill and analyzed for total N and the N isotopic ratio [9].

2.5. Statistical and economic analysis

The analysis of variance (ANOVA) was run according to the experimental design of each experiment using the STATITCF software program. The treatment means were compared using the Newman – Keuls test.

An exponential response function was used to describe the relationship between grain yield and applied P rates of C40 and TSP.

The economic analysis of each fertilization/liming treatment was calculated using the following formulas and costs:

$$EP = (\text{Yield, kg ha}^{-1}).\ (\text{Price, USD kg}^{-1}) - \text{Cost of fertilization/liming (USD ha}^{-1})$$

Where EP is the Economic Profit and V/C (Value/Cost ratio) = EP/Cost of fertilization. Reference of comparison was the control treatment.

Estimated costs utilised in the evaluation: Common bean grain = 0.415 USD kg^{-1}; KCl = 0.21 USD kg^{-1}; Urea=: 0.18 USD kg^{-1}; Ammonium sulphate = 0.11 USD kg^{-1}; TSP = 0.241 USD kg^{-1}; SSP= 0.090 USD kg^{-1}; C40 = 0.100 USD kg^{-1}; FPA 50 = 0.086 USD kg^{-1}; *Rhizobium* inoculant = 13 USD ha^{-1} and calcium carbonate = 24.50 USD ha^{-1}.

3. RESULTS AND DISCUSSION

3.1. Experiment on the agronomic effectiveness of PR derived products for common bean genotypes

3.1.1. Grain yield of common bean genotypes

Grain yield data of the common bean genotypes as affected by the P fertilizer sources are displayed in Table V. The effect of P fertilizer sources on grain yield production was statistically significant. The means of grain yield for P fertilizer sources was as follows: TSP

(1120 kg ha^{-1}) > FPA 50 (656 kg ha^{-1}) > PR (299 kg ha^{-1}) = control (203 kg ha^{-1}). The grain yield production of the bean genotypes was related to the solubility of the P fertilizer sources and no significant differences were found between PR and control treatments (Table V). Thus the local Trinidad de Guedes PR was ineffective in this experiment. In spite of favourable soil (acidity and P deficiency) and PR characteristics (high degree of substitution of carbonate for phosphate), for PR dissolution, the low PR effectiveness was likey due to the short-term duration (3 months growth cycle) of the common bean crop. PR needs time for dissolution, thus it is not effective for short-term duration crops while it often shows a high residual effect for subsequent crops. Generally, the PRs are effective when the PR have high reactivity and applied in optimum conditions of soil (low P bioavailability, low Ca, acidic soil, among others) and crop (long-term duration or crop rotation, high P use efficiency) [10, 11].

Significant differences were found for the interaction genotypes and P sources studied. The genotype DOR 364 fertilised with single super phosphate (SSP) produced significantly higher grain yield than all others common bean genotypes /P fertilizer treatments. This differential response of common bean genotypes was also observed in genotypes developed with the same traits, like DOR 364 and DOR 390 (resistance to the Virus ‘Mosaico Dorado’) that produced highest grain yields (1420 and 995 kg grain ha^{-1} respectively), when they were fertilized with single super phosphate (Table V). Grain yield of the common bean genotypes fertilized with SSP and DOR 390 and Censa fertilised with FPA 50 were significantly greater than that of the PR and control treatments.

TABLE V. GRAIN YIELD[1] (KG HA^{-1}) OF COMMON BEAN GENOTYPES GROWN IN AN ACRISOL AND FERTILIZED WITH VARIOUS P SOURCES

	Control	PR	FPA 50	SSP	Genotypes Means
BAT 477	240	343	636	1012	558
DOR 364	193	341	616	1420	543
DOR 390	272	267	640	995	642
CENSA	108	244	734	1051	534
P Fertilizers Means[1]	203 c	299 c	656 b	1120 a	-

[1] Values followed by same letter are not significantly different according to the Newman – Keuls test (p = 0,05).

3.1.2. P uptake of common bean genotypes

Tables VI and VII show the P uptake of the studied common bean genotypes at R6 – R7 and R9 growth stages representing their plant and grain P uptake respectively. It can be observed that the P uptake of DOR 364 fertilized with single super phosphate (SSP) was significantly greater than all others common bean/fertilizer treatments at both R6 – R7 and R9 stages. All common bean genotypes fertilized with SSP or FPA 50 had significantly higher P uptake than that of PR and Control treatments. No statistically significant differences were found between Control and PR treatments. Genotypes DOR 364 and DOR 390 had higher P uptake than Censa and BAT 477 at both stages of growth.

TABLE VI. PLANT P UPTAKE (KG HA^{-1}) AT R6 – R7 STAGE OF COMMON BEAN GENOTYPES GROWN IN AN ACRISOL AND FERTILIZED WITH VARIOUS P SOURCES

	Control	PR	FPA 50	SSP	Genotypes means[1]
BAT 477	1	2	7	10	5 a
DOR 364	1	2	8	11	6 a
DOR 390	1	1	5	8	4 b
CENSA	1	2	5	6	4 b
Fert. P means[1]	1 d	2 c	6 b	9 a	-

[1] Values followed by same letter within row (P fertilization) or within column (genotypes) are not significantly different according to the Newman – Keuls test (p = 0,05).

TABLE VII. GRAIN P UPTAKE (KG HA^{-1}) AT R9 STAGE OF COMMON BEAN GENOTYPES GROWN IN AN ACRISOL AND FERTILIZED WITH VARIOUS P SOURCES

	Control	PR	FPA 50	SSP	Genotypes means[1]
BAT 477	1	2	3	5	3
DOR 364	1	1	2	6	3
DOR 390	1	1	3	5	3
CENSA	1	1	3	5	2
Fert. P means[1]	1 c	1 c	3 b	5 a	-

[1] Values followed by same letter are not significantly different according to the Newman – Keuls test (p = 0,05).

3.1.3. *Relative Agronomic Effectiveness of PR products*

The average RAE values based on total P uptake increased from 10% with PR to 60% with FPA 50 and those based on grain yield from 10% with PR to 51% with FPA 50 (Table VIII). For all common bean genotypes, RAE values of FPA 50 based on either total P uptake or grain yield were higher than those of PR. Thus the local Trinidad de Guedes PR was ineffective and the partially acidulated PR product FPA 50 was medium effective in this experiment. According to the average RAE's values, the common bean genotypes had the ability to utilize the P coming from FPA 50 and PR in the following order: Censa > BAT 477 > DOR 390 > DOR 364.

TABLE VIII. RELATIVE AGRONOMIC EFFECTIVENESS (RAE, %) OF THE PR PRODUCTS BASED ON TOTAL P UPTAKE AND GRAIN YIELD OF SELECTED COMMON BEAN GENOTYPES[1]

Genotype	Total P uptake		Grain yield	
	PR	FPA 50	PR	FPA 50
BAT 477	18	62	13	51
DOR 364	8	53	12	35
DOR 390	0	54	0	51
CENSA	15	69	14	66
Mean Value	10	60	10	51

Total P uptake: P uptake in shoot+ P uptake in grain (kg P ha^{-1}); RAE of SSP = 100%.

The results obtained are in agreement with other studies in that the partial acidulation of PR is a well-established technology for increasing the agronomic effectiveness of low to medium reactive PRs [2]. In Thailand the efficiency of Lamphun PR was also increased when it was mixed with triple super phosphate [12]. In Oxisols and Ultisols of Indonesia direct application of a local PR at P rates between 100 and 200 P_2O_5 kg ha^{-1}, was effective in most cases but for a crop rotation including rice, corn, soybean and mungbean [13].

3.1.4. Economic analysis

The economic profit (EP) and value/cost ratios (numbers on top of the bars) of the bean genotypes fertilized with various P sources are presented in Fig. 1. For all common bean genotypes, single super phosphate was the P source that gave the highest economic profit and value/cost ratios. In this experiment the fertilization with SSP is very cost-effective and attractive due to the high grain yield response. The FPA 50 could be a promising domestic substitute because this PAPR produced acceptable grain yields and economic profits. However, the indigenous (unacidulated) Trinidad de Guedes PR was not cost-effective.

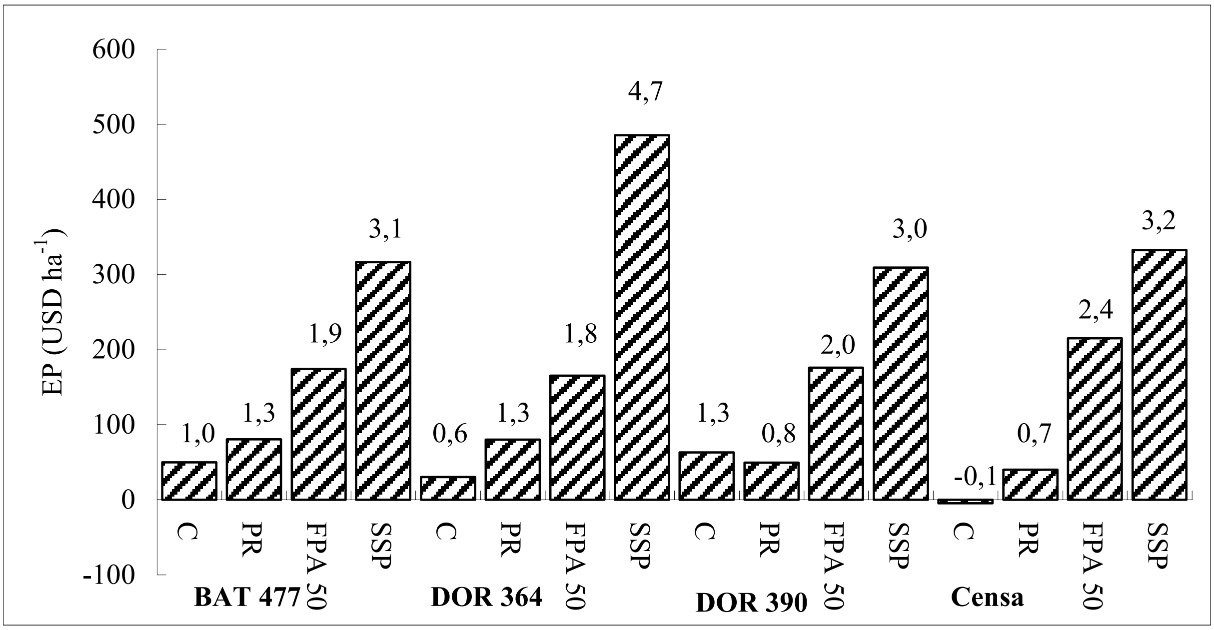

FIG.1. Economic analysis of the effect of PR based products on grain yields of four common bean genotypes (values on top of bars are V/C ratios).

It has been reported that the V/C ratio value should be at least 2 to consider a treatment with good economic return [14]. Following this criterion, SSP for all common bean genotypes and FPA 50 for DOR 390 and Censa were P-efficient sources.

3.2. Experiment on the liming effect on the agronomic effectiveness of P sources

3.2.1. Effect of liming and P fertilization on common bean grain yield

Significant differences in grain yield of the common bean cultivar CC 25-9N were found for the main P fertilization and liming effects but not for their interaction (Table IX). The average grain yield for all treatments increased from 693 kg ha^{-1} in the unlimed soil to 870 kg ha^{-1} in the limed soil. The response to liming in this soil is likely due to two possible effects: (i) increase in soil pH neutralizing soil acidity, in particular reducing Al saturation and, (ii) provision of Ca to correct this nutrient deficiency.

The average grain yield of the treatment fertilized with the water-soluble TSP at the highest P rate (120 kg P_2O_5 ha^{-1}) was 1103 kg $ha^{-1}$and it was significantly higher than all other P fertilizer treatments. Although the differences were not statistically significant, bean grain yields increased with the applied P rate and the degree of solubilization of P fertilizers. The P fertilization means were ranked as follows: 120 kg P_2O_5 ha^{-1} > 80 kg P_2O_5 ha^{-1} > 40 kg P_2O_5 ha^{-1} and TSP > C40, respectively.

TABLE IX. GRAIN YIELD (KG HA^{-1}) OF COMMON BEAN CC 25-9N AS AFFECTED BY LIMING AND P FERTILIZATION

P fertilization treatments[2]	Unlimed soil	Limed soil	P Fertilization (means)[1]
Control (0)	501	644	572 b
C40 (40)	557	775	666 b
TSP (40)	622	834	728 b
C40 (80)	588	833	710 b
TSP (80)	815	876	846 b
C40 (120)	759	934	847 b
TSP (120)	1012	1195	1103 a
Liming (means)[1]	693 b	870 a	-

[1] Values followed by same letter within row (liming) or within column (P fertilization) are not significantly different (p = 0,05). [2] For the P fertilization treatments, values in brackets are kg P_2O_5 ha^{-1} applied of each P source.

The grain yield responses of the common bean cultivar CC 25-9N to the application of increasing P rates of C40 and TSP in the absence (unlimed) and presence (limed) of $CaCO_3$ are shown in Figs 2 and 3 respectively. It can be seen that grain yield response due to TSP application was higher than C40 in the unlimed soil for all P rates whereas in the limed soil the grain yield was very similar for both P sources when applied at 40 and 80 kg P_2O_5 ha^{-1}.

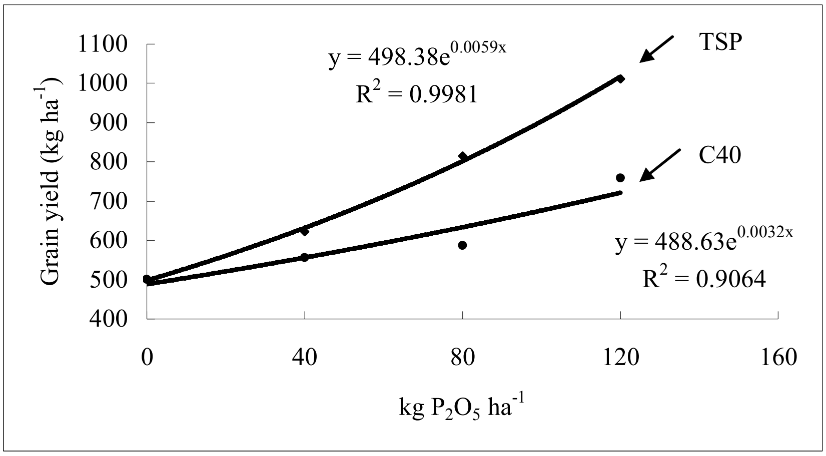

FIG.2. Grain yield response of CC 25-9(N) common bean genotype to increasing P rates of C40 and TSP in the unlimed Acrisol soil.

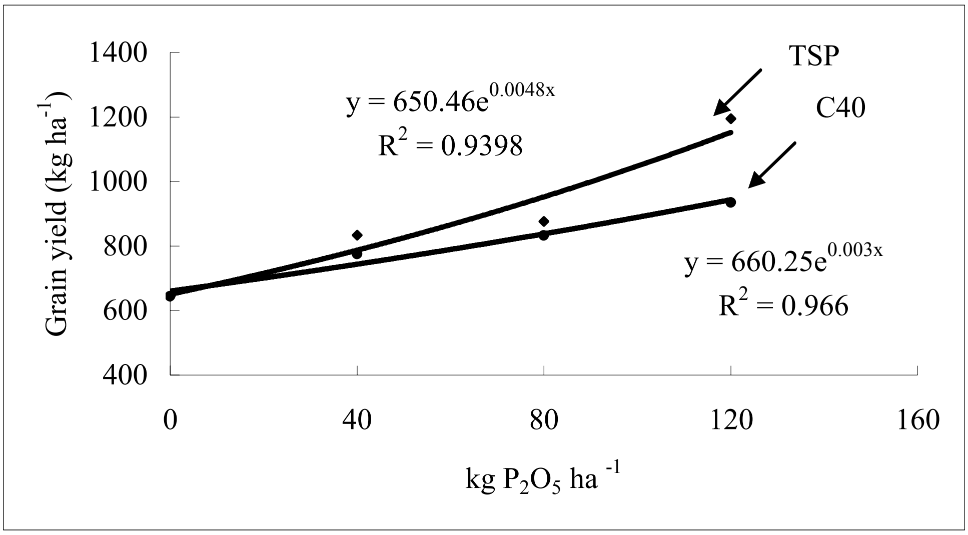

FIG.3. Grain yield response of CC 25-9(N) common bean genotype to increasing P rates of C40 and TSP in the limed Acrisol soil.

From the results it can be inferred that soil acidity and P deficiency are the main yield-limiting factors for common bean. The correction of both, through liming and P fertilizer application enabled to greatly increase grain yield. The use of the new PR-based fertilizer C 40 as domestic substitute to imported TSP is a promising practice.

3.2.2. Effect of liming and P fertilization on P uptake of common bean

Significant differences in P uptake of the common bean cultivar CC 25-9N were found for the main P fertilization effects but not for liming and the interaction P fertilization x liming (Table X). The highest P uptake (6 kg P ha^{-1}) was obtained with TSP applied at 120 kg ha^{-1} and the lowest (3 kg P ha^{-1}) with the control. However, there were not statistical differences in P uptake for both P sources (TSP and C40) when applied at the same P rate. These results demonstrate that the locally produced P fertilizer C40 is an effective P source that can be employed as domestic substitute to the imported TSP fertilizer.

TABLE X. P UPTAKE IN GRAIN (KG HA^{-1}) OF COMMON BEAN CC 25-9N GROWN IN AN ACRISOL AS AFFECTED BY LIMING AND P FERTILIZATION

P fertilization treatments[1]	Unlimed soil	Limed soil	P Fertilization (means)
Control (0)	3	3	3 c
C40 (40)	3	4	4 bc
TSP (40)	4	4	4 bc
C40 (80)	3	4	4 bc
TSP (80)	5	4	5 ab
C40 (120)	5	4	4 bc
TSP (120)	5	6	6 a
Liming (means)	4	4	-

[1] *For the P fertilization treatments, values in brackets are kg P_2O_5 ha^{-1} applied of each P source.*

[2] *Treatment means within the column (P fertilization) followed by same letter are not significantly different (p = 0,05).*

As the soil was P-deficient, the P application using TSP or C 40 enabled to increase P uptake. The liming did not contribute to increase P uptake. The main effect of liming in this soil, as it has been mentioned above, is the supply of Ca to correct this nutrient deficiency and to increase soil pH neutralizing soil acidity, in particular reducing Al saturation.

3.2.3. Relative Agronomic Effectiveness of local C40 in a limed and unlimed Acrisol

The estimated RAE values based on P uptake and grain yield of the common bean cultivar CC 25-9N for the local PR product C40 are shown in Table XI. The average RAE values based on P uptake increased from 33% in unlimed soil to 78% in limed soil and those RAEs based on grain yield from 41% in unlimed soil to 68% in limed soil.

For all C40 treatments, RAE values based on grain yield and P uptake obtained with limed soil were much higher than those of unlimed soil except for C40 applied at 120 kg P_2O_5 ha^{-1} in the limed soil. These results could be due to the beneficial effects of liming on increasing soil pH and reducing Al saturation, thus increasing soil P availability. These effects can be better observed comparing the changes in RAEs based on P uptake for increasing P rates of C40 in both limed and unlimed soil at 40 and 80 kg P_2O_5 ha^{-1}. However, liming reduced P availability of C40 applied at 120 kg P_2O_5 ha^{-1}. Liming also increased RAEs based on grain yield at 40 and 80 kg P_2O_5 ha^{-1} but not at 120 kg P_2O_5 ha^{-1}. These results are in partial agreement with those obtained by [15], who reported that the neutralization of 0,5 hydrolitic acidity units had a synergystic effect on the application of Maardu PR to increase the yield of barley.

TABLE XI. RELATIVE AGRONOMIC EFFECTIVENESS (RAE, %)[1] OF C40 APPLIED AT THREE LEVELS IN AN UNLIMED AND LIMED ACRISOL SOIL

Treatment	P uptake		Grain yield	
	Unlimed soil	Limed soil	Unlimed soil	Limed soil
C40 (40)	0	100	46	69
C40 (80)	0	100	28	81
C40 (120)	100	33	50	53
Mean values	33	78	41	68

RAE values were calculated for C 40 at each P rate of application using TSP as reference.

3.2.4. Economic analysis

The economic profit (EP) and value/cost ratios (numbers on top of the bars) of the bean genotype CC 25-9N fertilized with two P sources at increasing P rates in limed and unlimed soil are given in Fig. 4. The highest economic profits were obtained with TSP applied at 120 kg P_2O_5 ha^{-1} in limed and unlimed soil. The application of 40 and 80 kg P_2O_5 ha^{-1} as C40 in limed soil produced similar EP to TSP at the same P rates. The V/C values showed different trends according to the P sources, however on the average they increased with lime application. Highest V/C ratios of 5.6 and 6.0 were obtained in the limed soil for TSP and C40 at 40 kg P_2O_5 per ha respectively. The control plus lime has also a high V/C indicating that soil acidity (Al toxicity) and Ca deficiency were more growth-limiting in this studied Acrisol and had greater influence on bean grain yield production than P deficiency.

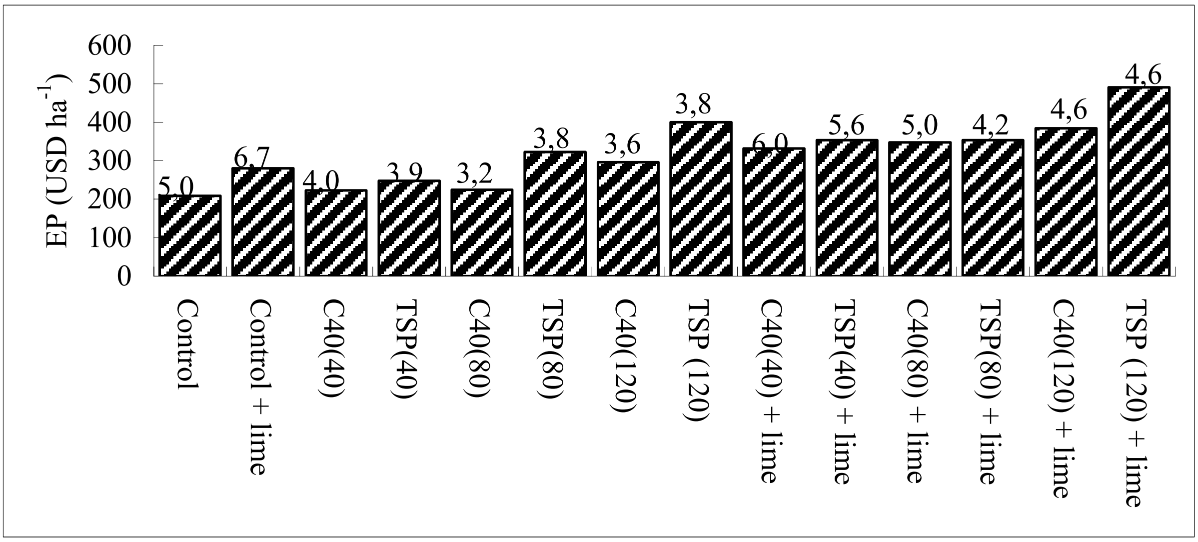

FIG.4. Economic profit (USD ha^{-1}) obtained for CC 25-9(N) common bean genotype grown in an Acrisol as affected by liming and P fertilization (values on top of the bars are V/C ratios).

These results have important practical implications as follows: (i) liming is a necessary management practice for the strongly acid Acrisol soil used, (ii) the C 40, which is a PAPR locally produced from the Trinidad de Guedes PR can be a potential domestic substitute to replace the costly and imported TSP.

3.3. Liming effect on the agronomic response and biological nitrogen fixation (BNF) of common bean genotypes

3.3.1. Grain yield of common bean genotypes

Grain yield of selected common bean gentotypes in unlimed and limed Acrisol are shown in Table XII. There were significant differences in grain yield for liming and genotypes effects. The average grain yield of the bean genotypes in limed soil (1408 kg ha^{-1}) was significantly higher than in unlimed soil (720 kg ha^{-1}). The common bean genotypes studied showed variable response to lime application but the interaction genotype x liming was not statistically significant. On another hand, the average grain yield of BAT 304 (2406 kg ha^{-1}) was significantly higher that those of remaining common bean genotypes studied. This genotype showed good yield potential under both unlimed and limed soil conditions.

It was noted that in the unlimed soil some genotypes such as BAT 304 and BAT 58 showed good grain yield (above the genotype average), thus demonstrating some adaptability to soil acidity. These common bean genotypes can be recommended when lime is unavailable. On another hand, genotypes such as BAT 304, BAT 58, DOR 390 and BAT 477, in this ranking, were responsive to lime application, producing grain yields above 1000 kg ha^{-1}. These results provide experimental evidence that some common bean genotypes can have better growth and express a higher production potential in the limed Acrisol.

TABLE XII. GRAIN YIELDS (KG HA^{-1}) OF SELECTED COMMON BEAN GENOTYPES IN UNLIMED AND LIMED ACRISOL SOIL[1]

Common bean Genotypes	Unlimed soil	Limed soil	Means of Genotypes
BAT 58	896	1628	1262 b
BAT 304	1546	3266	2406 a
BAT 477	465	1201	833 bc
DOR 364	415	652	534 c
DOR 390	652	1351	1001 bc
JRC	544	846	695 bc
CENSA	523	915	719 bc
Means of Liming	720 b	1408 a	-

[1] Values followed by same letter within row (liming) or within column (genotypes) are not significantly different (p = 0,05).

3.3.2. *Total N uptake of common bean genotypes*

There were statistically significant differences in total N uptake for the genotypes and liming effects as well as the interaction genotype x liming. The average total N uptake of BAT 304 was significantly higher than all other common bean genotypes (Table XIII). The average total N uptake of the genotypes studied in limed soil (48 kg N ha^{-1}) was significantly higher than that in unlimed soil (22 kg N ha^{-1}). All common bean genotypes increased the total N uptake when the soil is limed, in particular BAT 304, BAT 58 and BAT 477. BAT 304 had the highest N uptake in both unlimed (49 kg N ha^{-1}) and limed (114 kg N ha^{-1}) soil. However, total N uptake of other common bean genotypes studied was very low in both unlimed and limed soil, for instance DOR 364 (12 and 19 kg N ha^{-1}) and Judía Roja Camagüeyana (15 and 22 kg N ha^{-1}), respectively.

TABLE XIII. TOTAL N UPTAKE (KG HA^{-1}) OF SELECTED COMMON BEAN GENOTYPES IN UNLIMED AND LIMED ACRISOL SOIL[1]

Genotypes	Unlimed soil	Limed soil	Means of Genotypes
BAT 58	27	62	44 b
BAT 304	49	114	82 a
BAT 477	15	41	28 bc
DOR 364	12	19	15 c
DOR 390	20	42	31 bc
JRC	15	22	18 c
CENSA	16	35	26 bc
Means of Liming	22 b	48 a	-

[1] Values followed by same letter within row (genotypes) or within column (liming) are not significantly different (p = 0,05).

3.3.3. *Biological nitrogen fixation in common bean genotypes*

Nitrogen derived from air (%Ndfa) and the amounts of fixed N (Kg N ha^{-1}) in the common bean genotypes studied are given in Tables XIV and XV respectively. There were statistically significant differences in %Ndfa and fixed N for genotypes and liming effects as well as the interaction genotype x liming (Tables XIV and XV).

The average %Ndfa for all genotypes in limed soil was significantly higher (37.8%) than in unlimed soil (26.7%). The average %Ndfa of DOR 390 (43.9%), DOR (39.7%) and BAT 304 (37.3%) were superior to those of other bean genotypes. The reported %Ndfa for the common bean genotypes in this experiment are within the normal range (less than 50%) found for common bean but in general low for grain legumes [16].

In the limed Acrisol soil, %Ndfa of DOR 390 (46.8%), DOR 364 (45.6%) and BAT 304 (44.7%) were the highest. In the unlimed soil, %Ndfa of BAT 58 (14.9%), BAT 477 (17.5) and Judía Roja Camagüeyana (18.4%) were the lowest. It is interesting to note that some genotypes such as DOR 390 and to some extent DOR 364 showed an intermediate capacity to fix nitrogen from the air in both unlimed and limed soil.

TABLE XIV. PER CENT NITROGEN DERIVED FROM AIR (%NDFA) IN COMMON BEAN GENOTYPES IN UNLIMED AND LIMED ACRISOL SOIL[1]

Genotypes	Unlimed soil	Limed soil	Means of Genotypes
BAT 58	14.9	26.4	20.7 d
BAT 304	29.8	44.7	37.3 b
BAT 477	17.5	34.1	25.8 cd
DOR 364	33.7	45.6	39.7 ab
DOR 390	40.9	46.8	43.9 a
JRC	18.4	34.8	26.6 cd
CENSA	31.4	32.1	31.8 c
Means of Liming	26.7 b	37.8 a	-

[1]Values followed by same letter within row (genotypes) or within column (liming) are not significantly different (p = 0,05).

Genotypic differences in the amount of fixed N were found for the bean genotypes in unlimed and limed soil (Table XV). The average fixed N for all genotypes in limed soil was significantly higher (18.5 kg ha^{-1}) than in unlimed soil (5.9 kg ha^{-1}). Average amount of fixed N in BAT 304 (33.1 kg ha^{-1}) was superior to all other genotypes studied. In the limed soil, fixed N in BAT 304 (51.1 kg ha^{-1}) was significantly higher than all other common bean genotypes. In unlimed soil, the amounts of fixed N were rather low ranging from 2.6 kg ha^{-1} (BAT 477) to 15.2 kg ha^{-1} (BAT 304).

TABLE XV. FIXED NITROGEN (KG N HA^{-1}) BY COMMON BEAN GENOTYPES IN UNLIMED AND LIMED ACRISOL SOIL[1]

Genotypes	Unlimed soil	Limed soil	Means of Genotypes
BAT 58	4.0	16.3	10.1 b
BAT 304	15.2	51.1	33.1 a
BAT 477	2.6	14.4	8.5 b
DOR 364	3.8	8.3	6.1 b
DOR 390	8.1	20.0	14.0 b
JRC	2.7	7.7	5.1 b
CENSA	4.9	11.5	8.2 b
Means of Liming	5.9 b	18.5 a	-

[1] Values followed by same letter are not significantly different (p = 0,05).

The results obtained in this experiment are in agreement with those reported by Hardarson *et al*. [16] who evaluated the ability of common bean genotypes to fix N from air in several countries of Latin America. For example, in Goiania, Brazil the %Ndfa of 17 common bean genotypes ranged from 12 to 25% while the higher quantities of N fixed reported were 11–12 kg N ha^{-1}. In Piracicaba, another region of Brazil, the highest %Ndfa and the N fixed were 51–53% and 32–45 kg N ha^{-1}. In Chile, evaluating bean genotypes of various growth cycles, the %Ndfa ranged from 35 to 55% whereas the highest amount of N fixed was 62 kg N ha^{-1}.

These results provide experimental evidence that there are genotypic differences in both %Ndfa and amount of fixed N for the common bean studied with regard to their response to liming. Liming had a differential effect on plant growth, biomass production and grain yield and therefore, on the amount of N fixed in the bean genotypes. This information is very valuable for bean breeding improvement programs when focusing on tolerance to soil acidity. Also, to formulate practical recommendations regarding the utilization of common bean genotypes with better grain production in Acrisol soil for limed and unlimed conditions.

3.3.4. Economic analysis

The economic profit (EP) and V/C ratios of the selected bean genotypes in limed and unlimed soil are given in Fig. 5. Lime application increased the economic profit of all bean genotypes. BAT 304 showed the highest economic profits in limed and unlimed soil. This common bean genotype was developed for tropical acid soils having some adaptation capacity to acidity (aluminum toxicity) and soil P deficiency. The others BAT common bean genotypes tested, BAT 58 and BAT 477, had much lower economic profit than BAT 304. The EP of the common bean genotypes with resistance to the 'Mosaico Dorado' virus showed contrasting results. DOR 390 had higher EP values whereas DOR 364 showed low EP.

In unlimed soil, mainly BAT 304 and BAT 58, and to a less extent DOR 390 showed good EP and V/C ratios. Consequently, these bean genotypes could be recommended when lime is unavailable. On the other hand, JRC and Censa genotypes must be limed for achieving acceptable EP and V/C values.

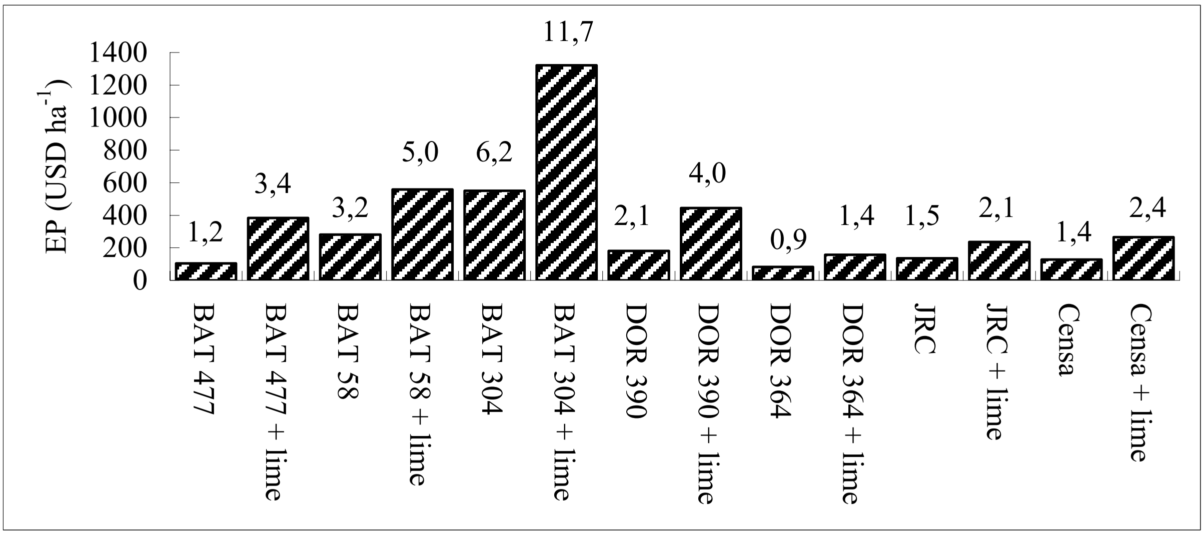

FIG.5. Economic analysis of the effect of liming on grain yields of seven common bean genotypes (values on top of the bars are Value/Cost ratios).

From the economic analysis it may be concluded that the genotype BAT 304 can be recommended for use in the zone, in particular if lime is applied to obtain the highest EP. The implementation of this simple study is critical to facilitate the adoption of new technologies and management practices by the farmers.

4. CONCLUSIONS

From Experiment 1, the most effective P source for increasing grain yield of the bean genotypes in the Acrisol was the water-soluble SSP. Direct application of the local Trinidad de Guedes PR was ineffective and the locally produced FPA 50 had intermediate effectiveness. The fertilization with SSP is very cost-effective and attractive P source. The FPA 50 could be a promising domestic substitute of SSP depending on the bean genotype utilised.

From Experiment 2, the water-soluble TSP was the most effective P source for the CC 25-9(N) common bean genotype grown in the limed and unlimed Acrisol. The C40 had intermediate effectiveness in unlimed Acrisol and it was almost as effective as TSP in the limed Acrisol, thus it could be considered a potential domestic substitute to replace the costly and imported TSP. Soil liming was necessary to increase grain yield of CC 25-9(N) common bean genotype in the strongly acid Acrisol and the highest economic profits were obtained when TSP or C40 were applied at 40 and 80 kg P_2O_5 per ha in the limed soil.

In Experiment 3, the common bean genotypes BAT 304, BAT 58 and DOR 390 had high grain yields and intermediate nitrogen fixation capacity. Liming increased grain yield and biological nitrogen fixation of all common bean genotypes. Lime application increased the economic profit of all bean genotypes studied. Genoype BAT 304 showed good adaptability to soil acidity and also the highest EP in limed soil. BAT 58 and DOR 390 genotypes showed similar EP increases under both limed and unlimed soil.

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DEVELOPMENT AND TESTING OF A PHOSPHATE ROCK DECISION SUPPORT SYSTEM (PRDSS) FOR DIRECT APPLICATION

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Abstract

An expert system, the Phosphate Rock Decision Support System (PRDSS), was developed to provide users with the option to compare the agronomic and economic feasibility of direct application of phosphate rock (PR) with commercially available water-soluble phosphate (WSP) fertilizers. The PRDSS predicts the relative agronomic effectiveness (RAE) of PR with respect to WSP for the initial PR application. The RAE as predicted by the PRDSS depends on: (1) PR sources as quantified by PR solubility determined using the second extraction with neutral ammonium citrate (NAC_2); (2) soil pH; (3) crops as they influence the rhizosphere, root quantity and distribution, uptake of Ca, crop duration, Al toxicity/tolerance, etc.; (4) soil P-fixation capacity; (5) soil texture; (6) soil organic matter content; (7) Al saturation; (8) moisture and rainfall regime; and (9) free calcium carbonate content of the PR source. The current version as presented here is ready for future web application. The model does not predict the cumulative effect of annual PR application or the residual effect of PR. These features will be included in the next version of the PRDSS. The testing of the current version of PRDSS with available data from new and previously conducted field and greenhouse trials, in general, gave good agreement between observed and predicted RAE. As expected many of these trials were conducted with very different objectives; hence, all input information required for testing the PRDSS was not available. To further test the PRDSS with complete input data and also to generate more information for long-term PR use and residual effect, field trials are being conducted in six countries. In future, the linkage of PRDSS output with results from P simulation models would extend the applicability of the PRDSS beyond its current capability to the prediction of crop yield and P application rates for a given yield target.

1. INTRODUCTION

Phosphorus is an essential plant nutrient and its deficiency severely restricts crop yields. Tropical and subtropical soils are predominantly acidic and often extremely P-deficient with high P-fixation (sorption) capacities. Therefore, substantial P inputs are required for optimal plant growth and adequate food and fiber production. P deficiency also results in low biological nitrogen fixation (BNF) and poor yields in legumes and other N-fixing plants. Hence, without adequate P application, additions of organic matter, other nutrients, and inclusion of legumes in cropping systems would not enhance crop productivity and improve soil fertility.

The need to develop and use indigenous raw materials as alternative sources to provide plant nutrients for food production is critical for developing a sustainable agriculture in developing countries, particularly in sub-Saharan Africa where there is (1) negative soil nutrient balance – mining of nutrients [1] and (2) very low fertilizer consumption – for example, use of phosphate (P) fertilizers is only 2.5 kg P_2O_5 ha^{-1} compared with 32 kg P_2O_5 ha^{-1} in Asia and Latin America. Fortunately, developing countries in sub-Saharan Africa, Asia, Latin America, and Oceania are endowed with phosphate rock (PR) deposits, for example, Togo, Zimbabwe, South Africa, and Senegal have a large commercial-scale mining of PR. Others like Burkina Faso, Tanzania, Mali, and Nigeria are to a limited extent using their PR deposits as a source of P nutrient.

A key question is: Can these indigenous PR deposits provide the P requirements for P-depleted soils in sub-Saharan Africa and elsewhere? Results from research demonstrate that the direct application of PR can be an effective alternative to the use of more expensive water-soluble phosphate (WSP) fertilizers for crop production under certain soil, climate, and crop conditions. Direct application of PR may provide the essential P nutrient in adequate amounts to crops grown in tropical and subtropical regions. However, agronomic effectiveness of PR for improving soil P status and crop P nutrition depends on a host of factors ranging from PR characteristics; soil, crop and climate conditions; farm management, size of the PR deposit; cost of mining; grinding and distribution; social, economic and environmental impact; and governmental policy. All of these can be better integrated through a decision support system. With the aim to assess agronomic effectiveness of PR sources for direct application, IFDC and the Joint FAO/IAEA Division of Nuclear Techniques have been cooperating since 2002 to develop a phosphate rock decision support system – PRDSS. The paper describes the development and testing of the PRDSS.

2. MATERIALS AND METHODS

2.1. Methodology

The development and testing of the PRDSS have been made utilizing primarily results from field trials conducted by IFDC in sub-Saharan Africa and Latin America, selected field trials from past FAO/IAEA projects, and a wide range of sites, crops, soil types, and PR reactivity from other published and unpublished sources. These preliminary results were then used to develop and modify relationships in the PRDSS. The expert system is based on our current understanding of the main agronomic factors that affect the PR response. The current version of the model incorporates the effect of PR sources (PR solubility), soil pH, soil P-fixation capacity, soil texture (sand and clay content), organic matter, type of crop, moisture/rainfall regime, aluminum (Al) saturation, and crop genotype effects on Al toxicity (Fig. 1). This paper describes the key equations/relationships used in developing the decision support tool. The focus of the model is on developing relationships that apply globally; hence, wherever possible the relationships are based on sound biophysical effects. However, many of the relationships are based on the empirical/statistical approach. A field validation of the model under a wider range of conditions is needed. Further calibration and revisions of these relationships are envisaged.

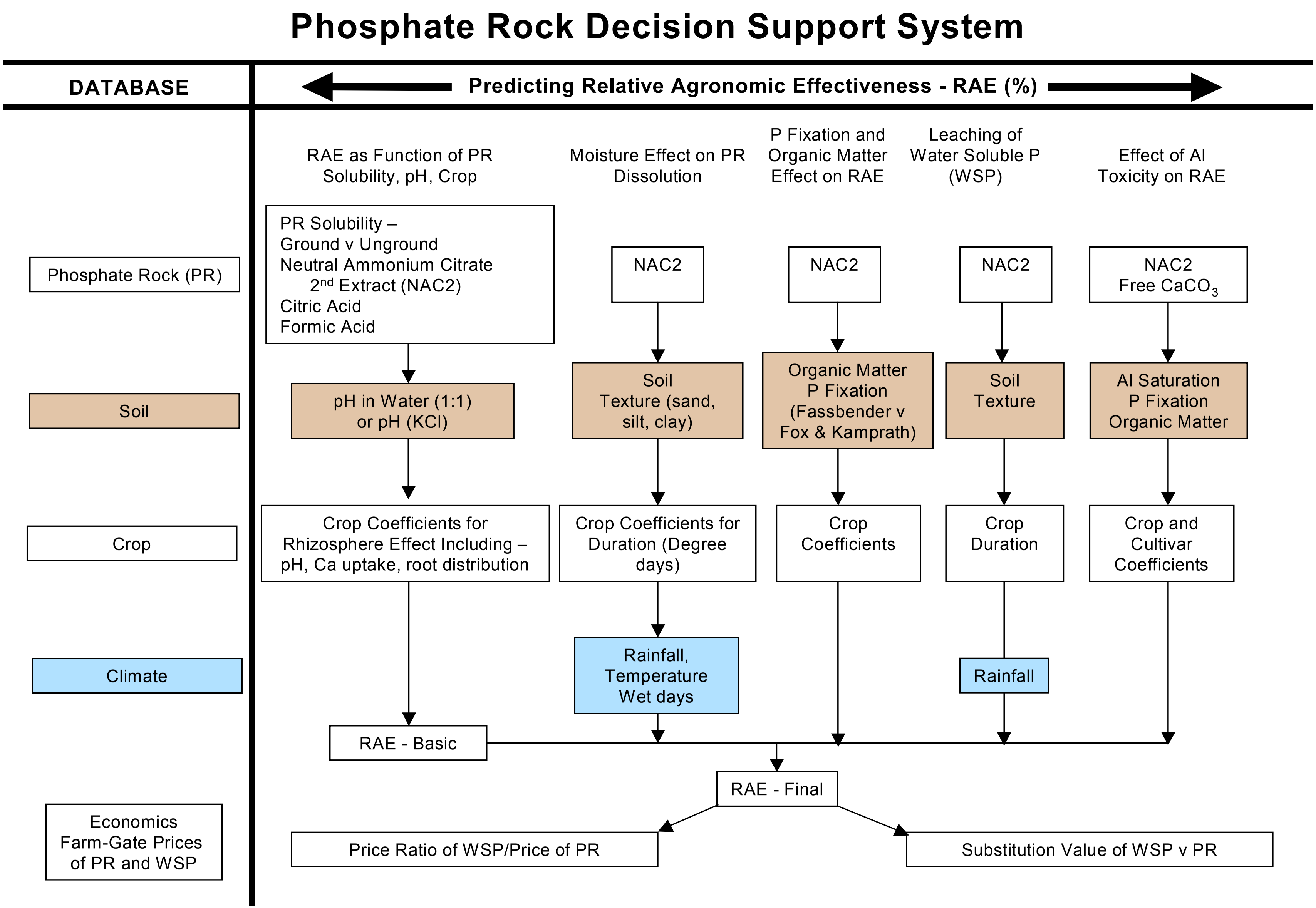

FIG.1. Schematic diagram linking databases to the expert system in the PRDSS.

A diagram of the components of the PRDSS is shown in Fig. 1. The basic information is obtained from databases on PR, soil, crop and climate. The PRDSS is programmed in Delphi (Borland Delphi Version 5.0). The PRDSS predicts the relative agronomic effectiveness (RAE) of PR with respect to a water-soluble P fertilizer (WSP) as follows:

$$\text{RAE (\%)} = \frac{\text{Marginal crop response to PR}}{\text{Marginal crop response to WSP}} \times 100$$

Or

$$= \frac{\text{Yield with PR} - \text{zero - P yield}}{\text{Yield with WSP} - \text{zero - P yield}} \times 100$$

The PRDSS uses the farm-gate price of WSP and PR to determine when PR may be an economically a better choice than WSP (Fig. 1). The model also generates a substitution value index of WSP with PR based on response function to WSP and PR application. The substitution value index thus could be used to determine the relative amount of PR needed to achieve the same target yield as with WSP.

2.2. Model Assumptions

The PRDSS predicts RAE for the initial or first season application of PR. This version does not predict the cumulative effect of PR application or its residual effect. The model also cannot predict requirements of P application rates or diagnose whether soil P status is a yield limiting factor. Therefore, the following criteria must be met for determining the suitability of data and conditions for the best use of PRDSS:

- Crop is highly responsive to P application. In other words, there is a significant difference between the crop yields with and without P application.
- Other growth conditions/management practices such as supply of water and nutrients, and pests control are not main limiting factors of crop performance.
- The agronomic effectiveness of PR and WSP sources is compared within the non-plateau (responsive) component of the response curve.

Also, if absolute yields are low then the chances are that one or both of the above criteria are not being met. As a quality check, the PRDSS users are encouraged to compare the yields of at least the check (zero-P) and high P rate treatments.

3. RESULTS

3.1. Basic RAE Prediction

A prototype PRDSS for estimating initial response to PR application with respect to WSP fertilizers was developed predominantly based on soil pH and PR solubility [2]. The basic RAE prediction in the current version of the model requires soil pH, PR solubility as determined using second extraction with neutral ammonium citrate (NAC_2), and crop rhizosphere effect on PR solubility and P uptake (Fig. 1). This approach thus includes key parameters and improves the usefulness of PRDSS for many users who may not have complete data. The basic RAE prediction approach will also be most appropriate for web users.

3.1.1. PR Solubility

The agronomic performance of PR for direct application is mostly dependent on the reactivity of the rock as determined by the PR solubility using the second extraction data from neutral ammonium citrate [3]. The PRDSS uses the PR solubility of finely ground rock to estimate the basic RAE. The model will, however, allow users to input PR solubility of moderate to high-reactive unground PR. Although the agronomic effectiveness of moderate to high-reactive unground PR sources is similar to their finely ground forms [4, 5], the PR solubility greatly depends on the particle size of PR used. If a PRDSS user inputs the PR solubility (NAC_2) for moderate to high-reactive unground PR (> 4% P_2O_5 rock with NAC_2), the relationship presented in Fig. 2 is used to obtain the finely ground PR solubility as input to the PRDSS. For less reactive rocks the above relationship cannot be used; hence, the model will not be able to predict the agronomic effectiveness for such rock in the unground form. It is also impractical and uneconomic to use low reactive PR in unground form.

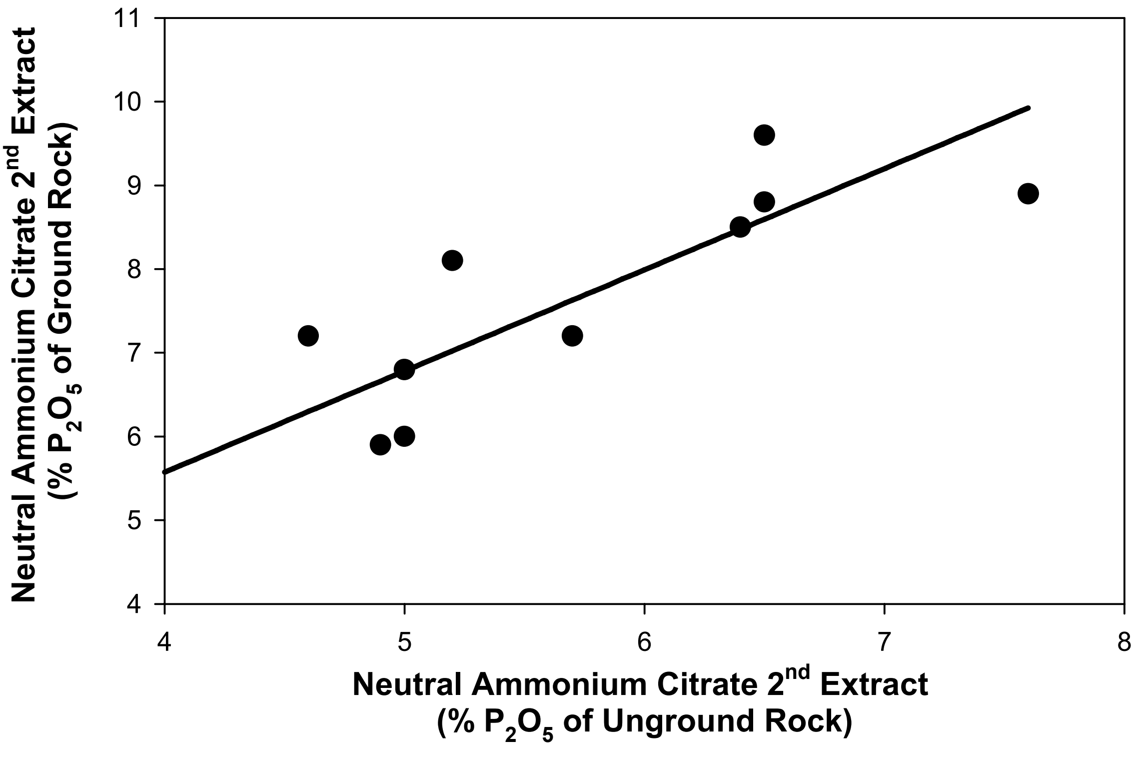

FIG.2. Estimating PR solubility of moderate-to-high reactive PR from their unground PR solubility using 2nd extract of neutral ammonium citrate (NAC_2).

3.1.2. Soil pH

The agronomic effectiveness of a PR is dictated by soil properties, particularly the soil pH. Other soil properties that influence PR performance are soil texture (proportion of sand, silt, and clay content), organic matter content, soil P-fixation capacity, Al saturation, exchangeable Ca, and soil P status. With the exception of exchangeable Ca and soil P status, the current version of PRDSS uses the above properties to estimate the agronomic effectiveness of a given rock. Therefore, the user of the PRDSS must have access to these soil properties. The exchangeable Ca effect is included indirectly in the model through soil pH. However, in the next version of the model that predicts the long-term and residual effects of

PR application, the effect of calcium saturation on RAE will be included, especially if a soil has been limed. Since the soil solution P content is rarely high enough to influence PR dissolution [6], the model does not incorporate the effect of "available" soil P on RAE. However, it may be necessary to incorporate this effect in future versions if high initial soil available P provides a "starter" effect on PR utilization and defines the response to PR application.

The generic effect of soil pH and PR reactivity on RAE was based on a series of regression equations for similar soil types. Greenhouse and field experimental results from sub-Saharan Africa, South America, and Asia, including both published and unpublished data, were used to develop the relationship shown in Fig. 3. The model uses these series of equations describing the changes in RAE as a function of PR solubility and soil pH values and the interpolative function from the Delphi program to generate a continuous relationship for all soil pH values.

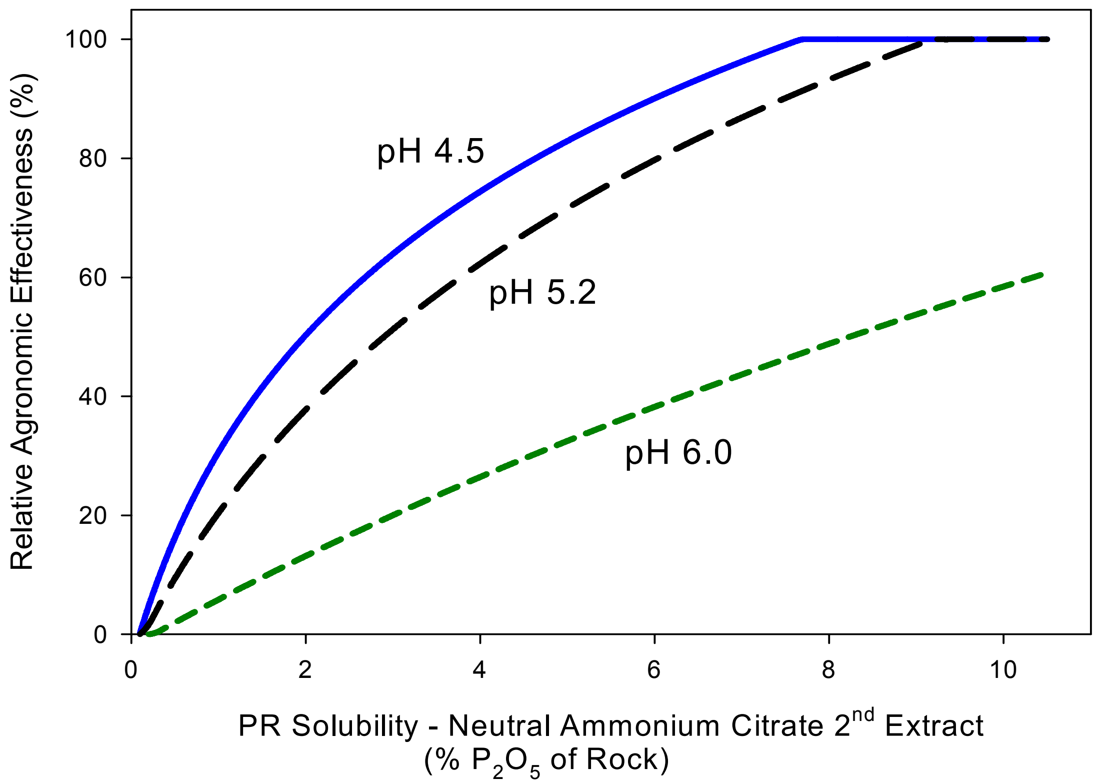

FIG.3. Effect of PR solubility and soil pH (without the crop effect) on the relative agronomic effectiveness of PR.

3.1.3. Crop Rhizosphere Effect

The dissolution, uptake, and resulting agronomic effectiveness of PR are dependent on the crop's growth cycle (duration), it's rooting system, and the effect of the crop physiology and associated microbes on the rhizosphere (rooting zone). The crop rhizosphere effect is the third factor after PR solubility and soil pH that the PRDSS uses to determine the RAE of PR (Fig. 1). A crop may influence the rhizosphere [6, 7, 8, 9] due to:

- Rooting system: Type, quantity and distribution of roots. Greater root contact (volume and density) is more critical for P (less mobile) than N. Within P sources, low solubility PR sources will be less available for uptake than WSP with crops having lower rooting density. A longer duration (late growth cycle) crop not only helps PR performance by providing more time for PR dissolution but also greater quantities of roots come in contact with PR [10].

- The cation-anion balance of the crop. For example, to counter the effect of BNF, plants release H^+ ions to the soil. Inhibition of nitrification can also result in preferential uptake of NH_4^+ and, hence, release of H^+ to maintain a neutral charge. In either case the release of H^+ or HCO_3^- would modify the rhizospheric pH and thus influence the PR dissolution [11, 12].

- Certain crops such as canola or rape seed (Cruciferae family, in general), pigeon pea, and lotus are known to exudate organic acids – malic, oxalic, or citric acid – to the rhizosphere. As expected these crops tend to receive better performance from PR application due to reduced pH and chelation of Ca^{2+} with organic acids around the roots [11, 12].

- Higher Ca demand by some legumes [13]. Higher uptake of Ca^{2+} would promote PR dissolution because of reduced soil pH.

The crop rhizosphere, soil pH, and PR solubility relationship on RAE are presented in Fig. 4 for maize and canola. Most PR sources are as effective as WSP on a wide range of soil pH when canola is grown instead of maize. Even on neutral soils most rocks will perform well (RAE > 60%) with canola.

3.2. Moisture Effect on RAE

The RAE as estimated from soil pH, PR reactivity, and crop rhizosphere effect may be reduced because of limited PR dissolution when soil moisture or rainfall is inadequate (Fig. 1). The PRDSS assumes that if rainfall is limiting crop growth, then that limitation will be overcome by the farmer before he/she applies P fertilizer including PR. In case of limiting rainfall/moisture, the PRDSS further assumes that water stress effects on crops grown using WSP fertilizers and PR will be similar. However, inadequate rainfall will influence dissolution of PR more than that of WSP.

3.2.1. PR Reactivity

Limited rainfall will also have a more drastic impact on the dissolution of less reactive PR. As evident from Fig. 5, the moisture requirement index increases as the basic RAE (Fig. 1) decreases; thus, the threshold rainfall needed for PR dissolution will increase. The minimum value for threshold rainfall is set at 450 mm. The threshold rainfall concept is based on Gillard [14] and Rajan [6]. The PR dissolution and, consequently, the RAE of rock are unaffected as long as the growing season rainfall exceeds the threshold rainfall.

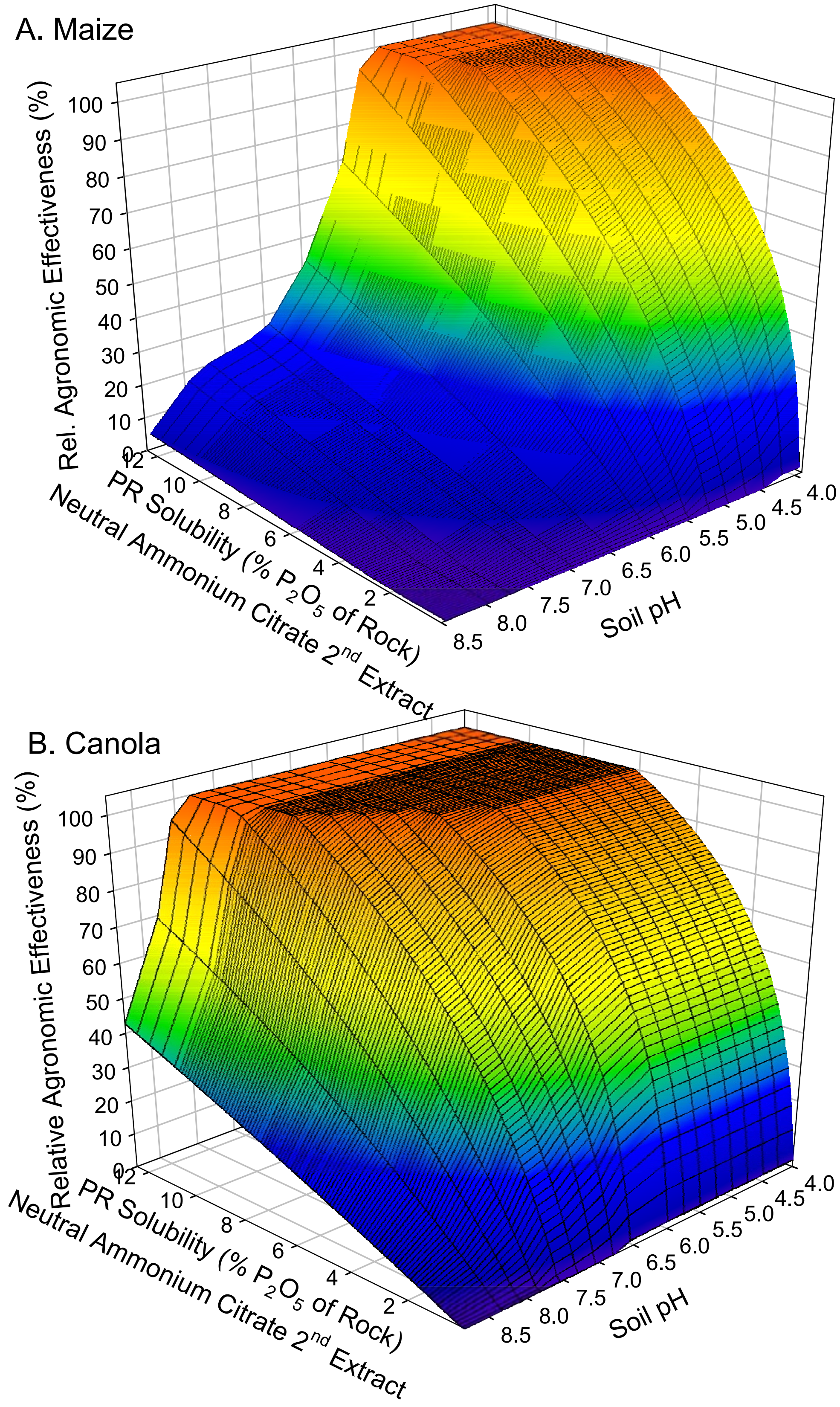

FIG.4. The relationship between PR solubility, soil pH, and crop rhizosphere on RAE for (A) maize and (B) canola.

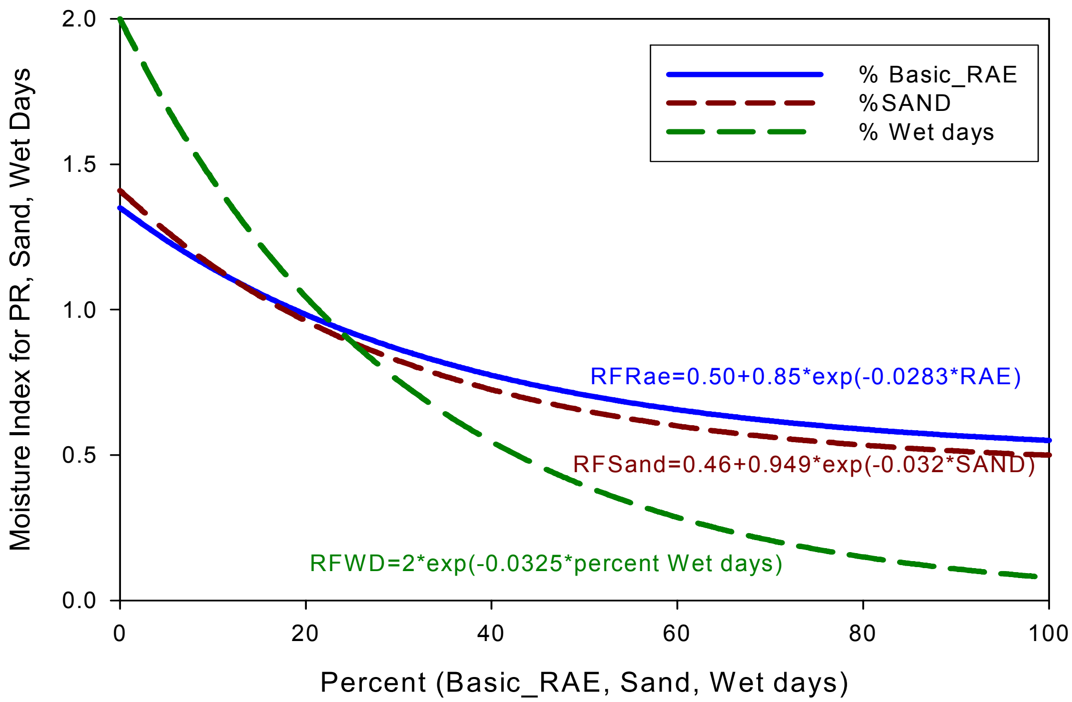

FIG.5. Effect of basic_RAE (%), sand (%) and wet days (%) on moisture requirement index for PR dissolution.

3.2.2. Weather and Crop Duration

To estimate the moisture effect on PR dissolution and RAE, the PRDSS requires: (1) the growing season rainfall amount, (2) duration (total number of days) from the time that PR is applied until crop harvest – this will depend on crop's growth cycle and temperature, and (3) the number of rainy (wet) days as input. The PRDSS uses a weather generator, MARKSIM [15], to assist users, particularly those from the tropics who do not have access to actual weather data to make the best use of PRDSS. The MARKSIM generates rainfall based on a third order Markov model that is especially adapted for the tropics. It estimates the temperature and rainfall pattern of a given place with the user-input of latitude, longitude, and planting date. The outputs of interest for the PRDSS users are the growing season rainfall amount, number of wet days during the season, and crop duration.

As the percentage of wet days (number of wet days/growth duration) increases, the moisture requirement index for PR dissolution decreases, resulting in lower threshold rainfall required for PR dissolution (Figs. 5 and 6). For sites with poor rainfall distribution PRDSS requires higher amounts of rainfall for PR dissolution.

3.2.3. Soil Texture

The algorithm developed to incorporate the effect of soil moisture as a function of soil texture on PR dissolution and leaching potential for WSP (discussed later in section 3.4) is based on relationships used for estimating water-holding capacity in the DSSAT model [16]. As evident from Figs 5 and 6, the moisture requirement index and the threshold rainfall required for PR dissolution are also dependent on soil texture (predominance of sand or clay content), with less water needed for sandy soils.

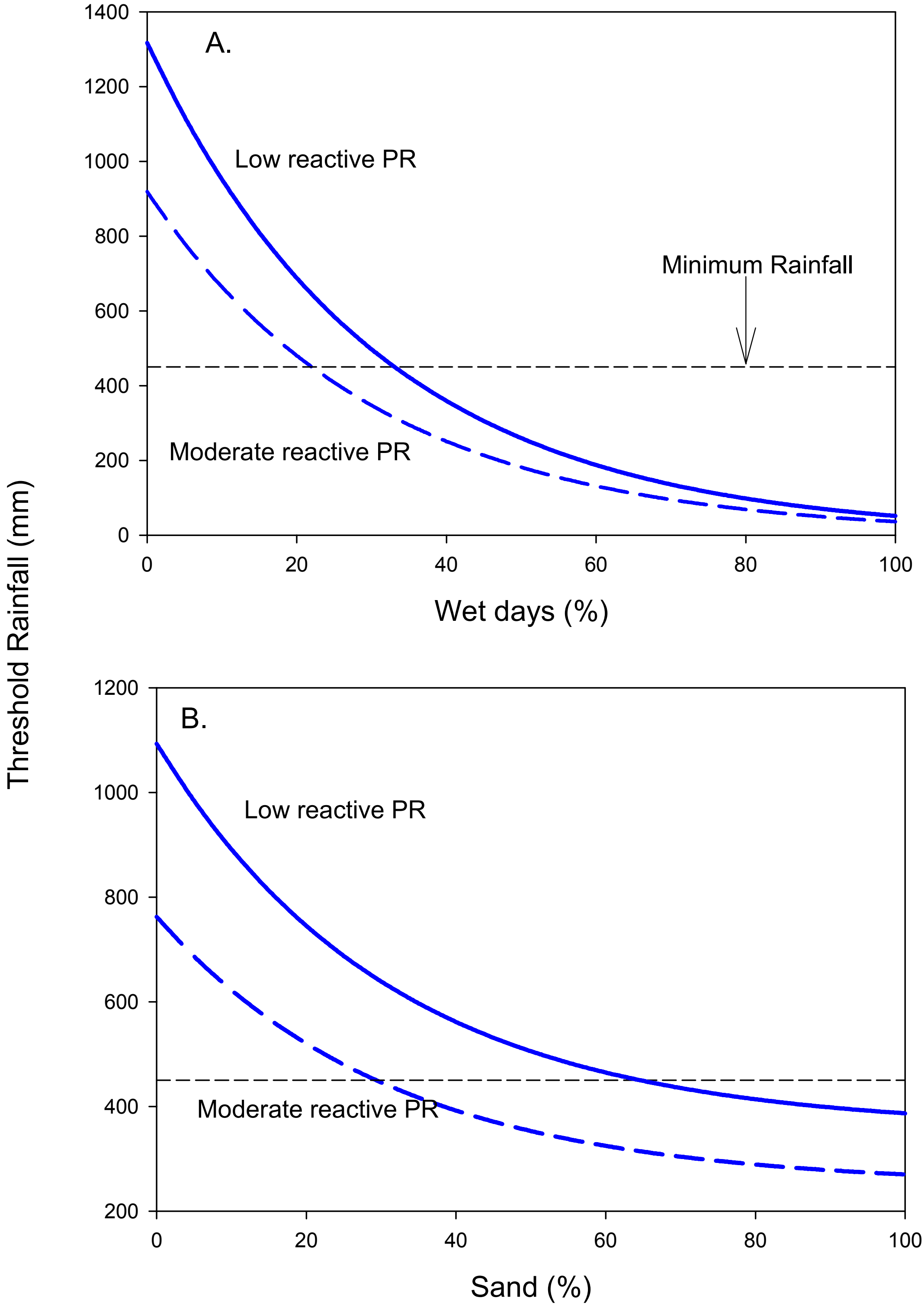

FIG.6. Effect of (A) percentage of wet days and (B) soil texture (% sand) on the threshold rainfall requirement for PR dissolution (minimum threshold rainfall = 450 mm).

Thus, if there are fewer rainy days, lower PR reactivity, and heavier textured soils, PRDSS will need more rainfall for PR dissolution. If the actual rainfall is less than the threshold value, the agronomic performance of PR will be reduced relative to WSP, resulting in a lower RAE for the rock. Under similar soil and climatic conditions, shorter duration crops/varieties may have lower RAE because of less rainfall. As it has been mentioned earlier, a short duration (early growth cycle) crop may also have lower RAE because (1) it offers less time for PR to dissolve, (2) it may have less root density, and (3) there is less direct contact of PR with roots. The effect of organic matter on soil moisture is not included here; however, its combined effect on agronomic effectiveness of PR is included in the PRDSS and presented in the next section.

3.3. Organic Matter and P-Fixation Effect on RAE

Soil organic matter (or soil organic carbon), in general, may have a positive effect on RAE, while high soil P fixation would result in reduced RAE (Fig. 1).

3.3.1. Soil Organic Carbon

The function that captures the effect of soil organic carbon to modify the PR reactivity is presented in Fig. 7. In most soil types, the model uses a scaler factor ranging from 0.8 to 1.1 to modify the RAE of a given PR. In volcanic ash soils (Andisols) where the organic matter is more tightly bound, the effect on RAE is less significant ranging from 0.8 to 1.03. The scaler value combines the following beneficial effects that organic matter has on PR dissolution:

- Improves moisture availability for PR dissolution.
- Improves cation exchange capacity (CEC). Indirectly this also lowers Ca saturation.
- Organic acids promoting PR dissolution.
- Chelation with Ca^{2+} to enhance PR dissolution.
- Reduce soil P-fixing capacity.

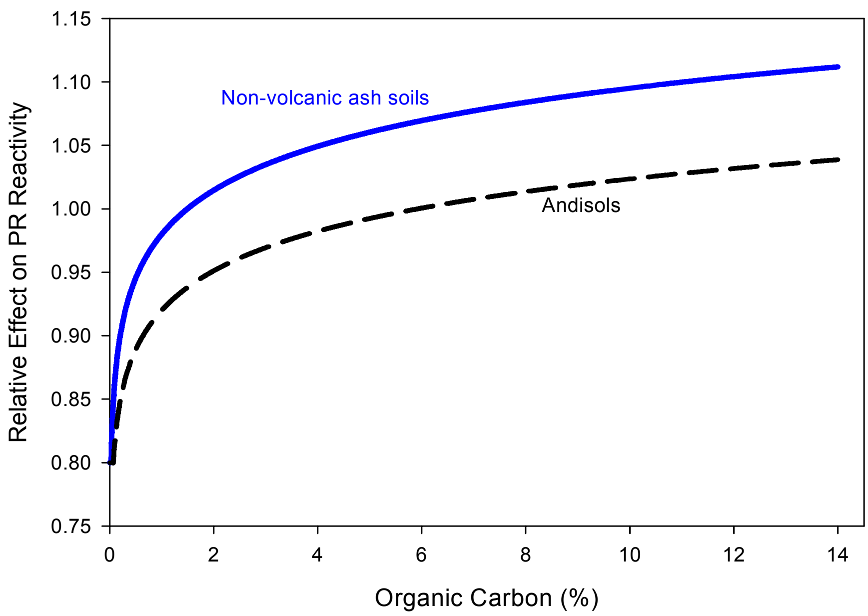

FIG. 7. Effect of soil organic carbon on PR reactivity for volcanic ash soils (andisols) and all the other soils.

However, in reality several soil factors are associated with the above changes, e.g., CEC, hence the data used in developing the relationship presented in Fig. 7 may be representing more than the organic matter effect. The effect of soil organic matter in reducing Al toxicity effect is discussed in Section 3.5.

3.3.2. Soil P-Fixation Capacity

It is generally postulated that the agronomic effectiveness of PR is directly related to the extent that it dissolves in the soil. However, with increasing soil P-fixing capacity while the dissolution of PR increases, the availability of P for crop uptake decreases [17]. Hence, with increasing soil P-fixation capacity, the RAE of a rock decreases. The degree of P fixation for a given PR is also influenced by its solubility. The agronomic performance of reactive rocks is generally reduced only on soils with high P fixation. On the other hand, for low reactive PRs (e.g. Togo PR with NAC_2 = 4.1% P_2O_5 of rock), their RAEs will be reduced even in soils with soil P-fixation capacity as low as 30% (Fig. 8). Unfortunately, PRs with low-to-moderate reactivity are most prone to higher percentage reduction in their agronomic effectiveness across a wide range of P-fixing soils.

The P-fixation effect on PR agronomic effectiveness is also modified by the crop species and cultivars grown. Greenhouse and field results indicate that legumes and cereals differ in their response to P fixation, with the legumes showing reduced RAE at a lower soil P-fixing capacity than cereals but having less drastic decline in RAE with increasing P fixation (lower slope). Nonetheless, more field data are needed for further refinement of crop effect on P fixation and RAE.

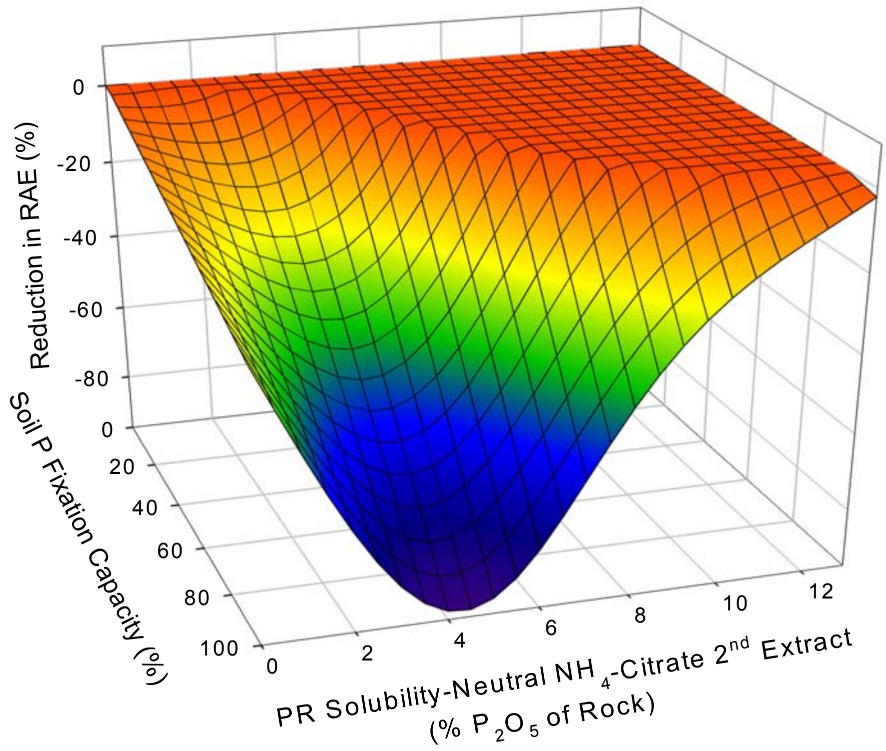

FIG.8. Combined effect of soil P fixation and PR solubility on reduction in RAE (%).

3.4. Leaching of Water-Soluble P

The leaching of soluble P from WSP fertilizers and PR in the PRDSS is based on algorithm adapted from the CERES-N model [18] and Phosphate Rock Expert System for Pastures [14]. The key factors influencing the leaching and hence the RAE of a PR are (1) the total amount of rainfall from the time P is applied until harvest, (2) soil texture with input of either sand or clay content, and (3) the PR solubility as determined by NAC_2. As expected, with increasing rainfall more soluble P leaching will occur, thus the leaching potential of WSP will increase from 1.0 (no leaching) to > 1.0 (Figs 9–10). Lighter-textured soils with high sand content or low clay content (Fig. 9) will have proportionately higher P leaching from WSP sources.

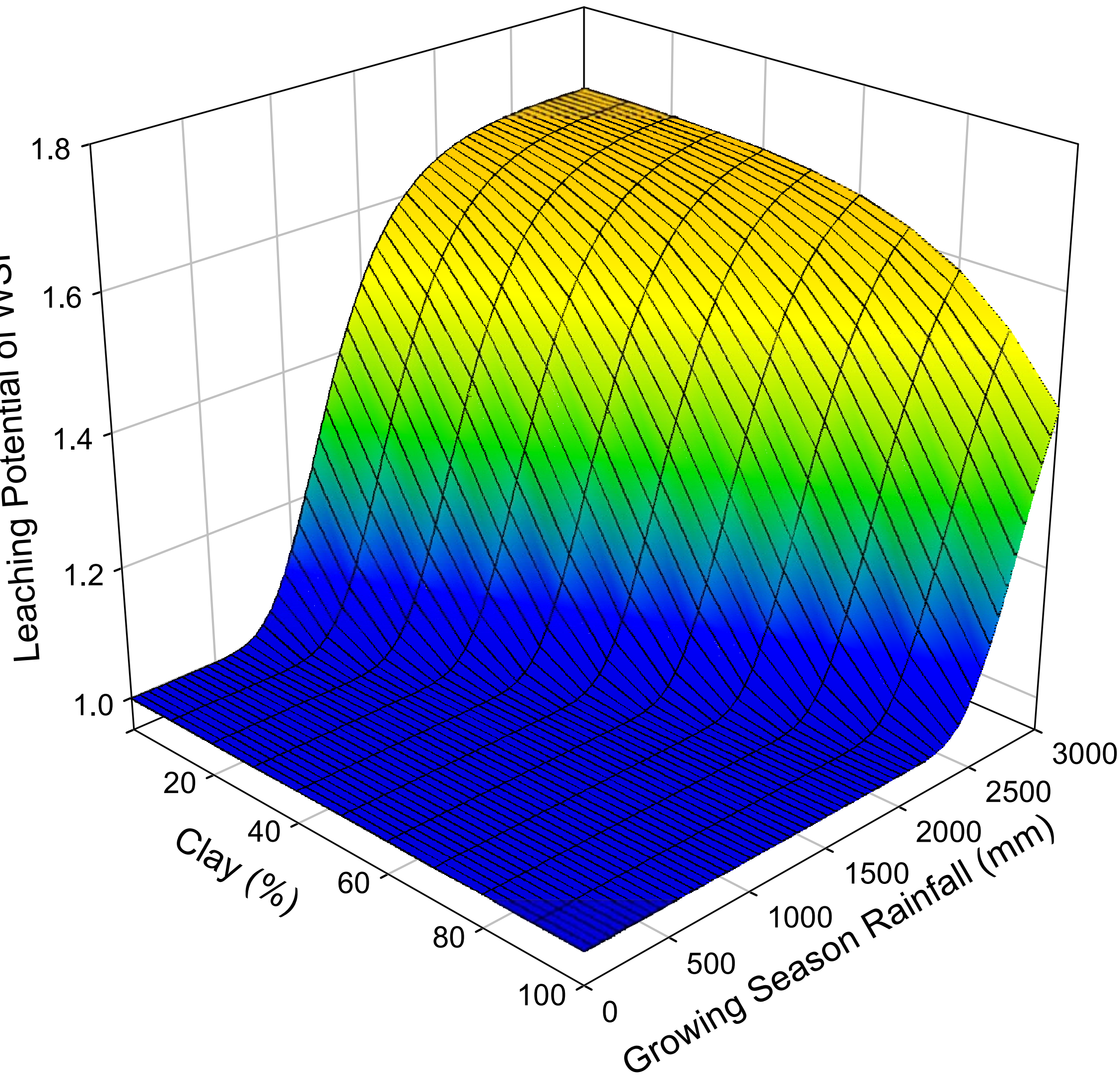

FIG.9. Leaching potential of water-soluble phosphate (WSP) with Togo PR as influenced by clay content and growing season rainfall.

The leaching of soluble P results in lower yields from WSP sources; consequently, the RAE of the PR improves proportionately as additional soluble phosphate from WSP fertilizers is leached. The highest potential increase in RAE of a rock due to leaching of WSP will occur under high rainfall conditions and light-textured soils. Hence, PR application may have added benefits over WSP fertilizers on light-textured soils of humid and sub-humid regions. These gains in RAE will be most prominent with low reactive PR (Fig. 10). Thus, less reactive PRs will have proportionately the greatest increase in RAE due to leaching of WSP fertilizers. In practical economic and environmental terms, it would be more appropriate to use high- to moderately-reactive PR under high leaching conditions to minimize P leaching and achieve profitable yields.

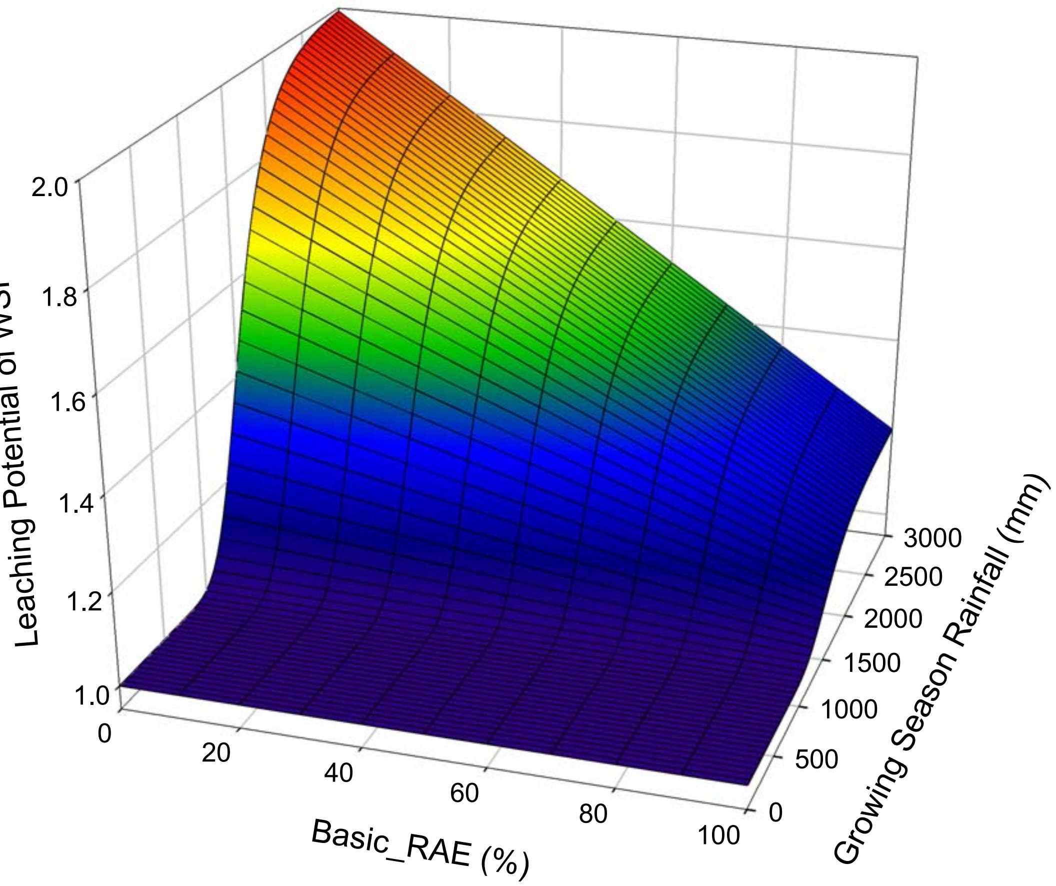

FIG.10. Leaching Potential of Water-Soluble Phosphate With Respect to Basic RAE and Growing Season Rainfall for Coarse-Textured Soil (sand = 60% or clay = 18%).

3.5. Effect of Soil Al Toxicity on RAE

The best response to PR use is obtained on soils with low pH, in general, below pH 5.2. In some soils, however, low pH is also associated with high exchangeable Al and, hence, reduction in crop growth and yield due to Al toxicity. The predicted RAE for these soils will then be higher than the actual RAE. The PRDSS as a decision support tool now captures the effect of Al saturation and crop tolerance on reduction in RAE due to Al toxicity.

3.5.1. Al Saturation

PRDSS users now have the option to enter the Al saturation of their soils. For most PR and crops the Al effect will be significant only when Al saturation is > 15%–20%. In soils with significant amounts of exchangeable Al, the P-fixation capacity may be correlated with the Al saturation effect. Hence, it is important not to over-estimate the reduction in RAE under such conditions. High P fixation in soils may be associated with high content of Fe, Al, or both. The PRDSS corrects for the double accounting of P fixation effect on RAE by estimating effective aluminum saturation. Likewise, organic matter tends to ameliorate the Al toxicity effect on crops and, hence, soils with higher organic matter content would help improve the yield of a crop and increase to some extent the RAE. Overall the organic matter effect, based on data from greenhouse and field trials in Indonesia and Latin America, will reduce the effective Al saturation and, hence, the Al toxicity effect on a crop.

3.5.2. PR Solubility Effect on Al Toxicity

Results from Smalberger and coworkers [19] (unpublished data) showed that higher PR solubility generally results in less Al toxicity effect on crop. Hence, the RAE of a given crop will increase with PRs of higher reactivity, due to both the higher availability of P and reduced Al toxicity. This relationship as incorporated in the PRDSS is shown in Fig. 11 for a soil with effective Al saturation of 45%.

3.5.3. Crop-Specific Al Tolerance

As shown in Fig. 11, the degree of reduction in RAE not only increases with rocks of low PR solubility, but it also changes with crop genotypes (species and cultivars). For example, with Togo PR (NAC_2 = 4.09% P_2O_5 of rock) the estimated reduction in RAE is 50% and 18%, for wheat and maize, respectively. The crop genotype effect is most pronounced with PRs of low solubility. The model thus captures the Al toxicity x PR solubility x crop genotype interactions.

3.5.4. Free-Calcium Carbonate

Several PR sources may contain variable amounts of free $CaCO_3$; however, many of these rocks have low PR solubility, thus in general their agronomic effectiveness on soils with high Al saturation is not high. Since high Al saturation is associated with low pH (<5.2), these rocks will still play a key role in providing available Ca and reducing exchangeable Al and hence Al toxicity. The PRDSS database on PR sources also now includes their free $CaCO_3$ content. The model uses the free $CaCO_3$ content to modify the Al toxicity effect and hence the RAE.

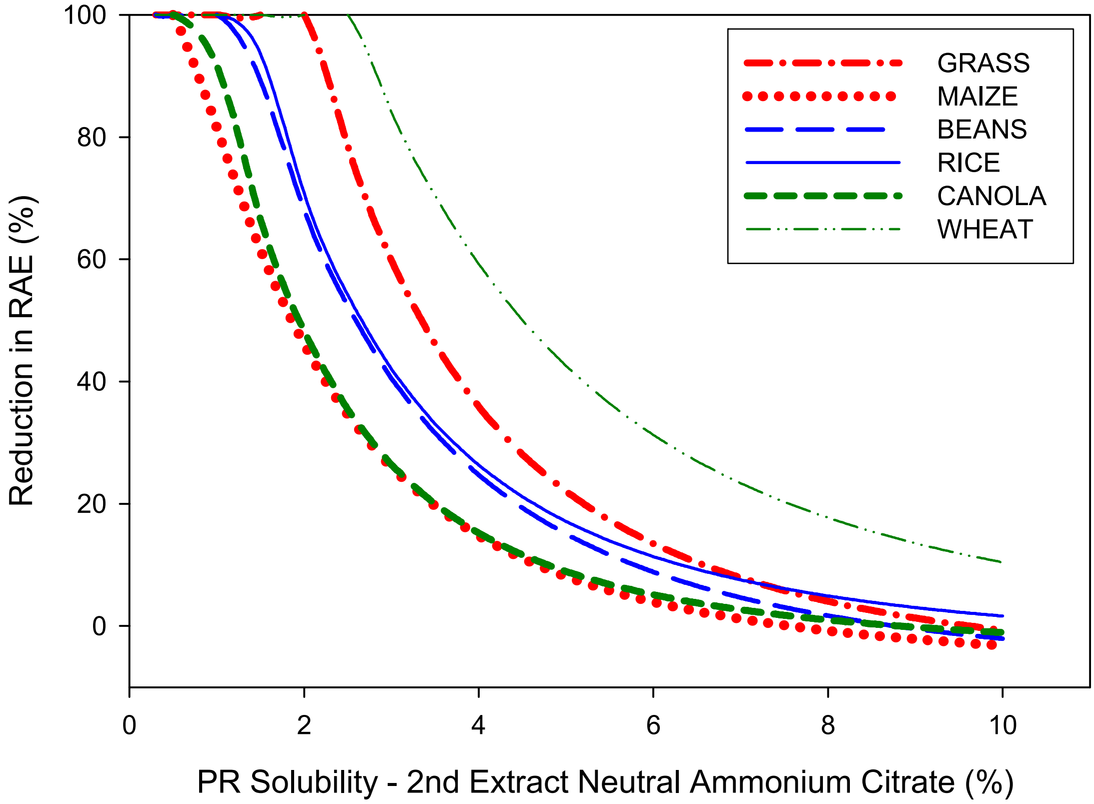

FIG.11. Effect of PR solubility and crop species on reducing relative agronomic effectiveness (RAE) due to 45% effective Al saturation.

The interaction between PR solubility x free $CaCO_3$ content x Al toxicity (or reduction in RAE) is illustrated in Fig. 12. For example, Jhamarkotra rock with a PR solubility of 0.4% will show no improvement in RAE in spite of its high free $CaCO_3$ content (38.4%). However considering Huila PR (PR solubility of 4.3% and free $CaCO_3$ of 13.4%) at 45% Al saturation (Fig. 12) and without taking into account the free $CaCO_3$ effect, its RAE will be reduced by 12% but once the model accounts for the free $CaCO_3$ effect, the reduction is only 6% - the reduction in RAE is almost halved. The liming effect of free $CaCO_3$ on Al saturation and RAE is also dependent on the PR solubility.

3.6. Model Testing

Most field and greenhouse trials were conducted with very different objectives and hence they do not have complete input information for testing of the PRDSS, particularly on crop duration, rainfall, and some of the soil properties. The test results need to be carefully interpreted because several errors are introduced due to (1) estimation of inputs, (2) missing inputs, and (3) inherent errors of the model equations because they are based on regression and empirical and functional relationships. The results from the complete testing of PRDSS with data from field and greenhouse experiments are summarized in Fig. 13. As evident from the results, in spite of the above limitations, the overall performance of the PRDSS is promising.

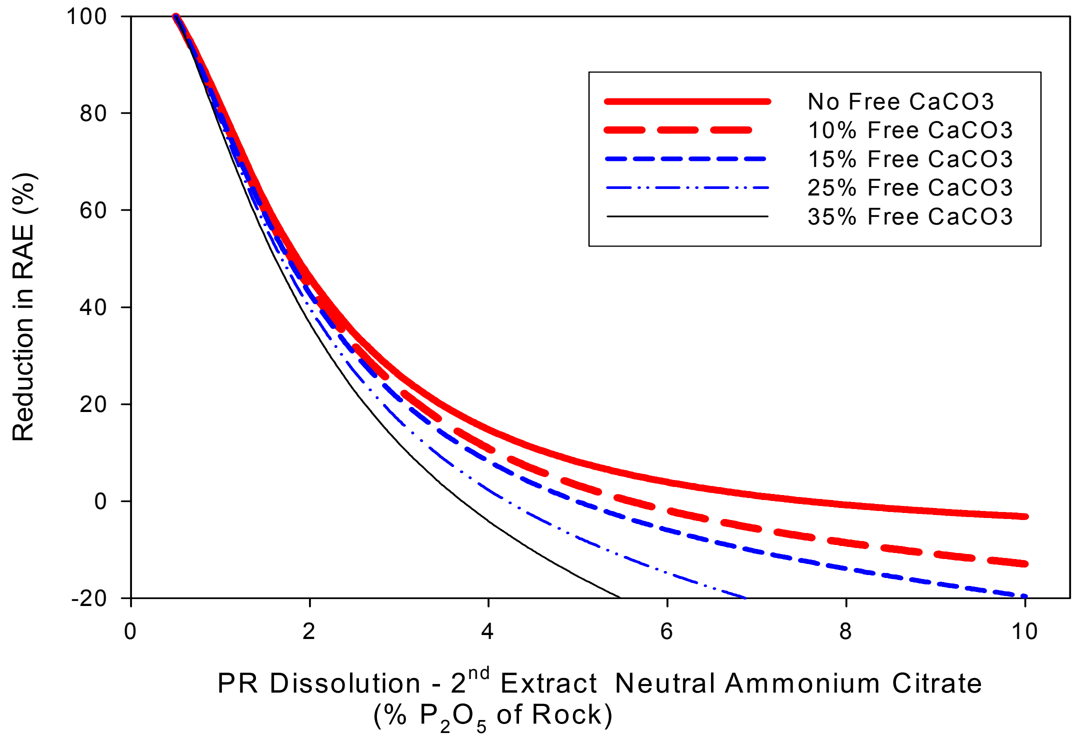

FIG.12. Effect of free-calcium carbonate content of PR on RAE for maize grown on a soil with 45% effective Al saturation.

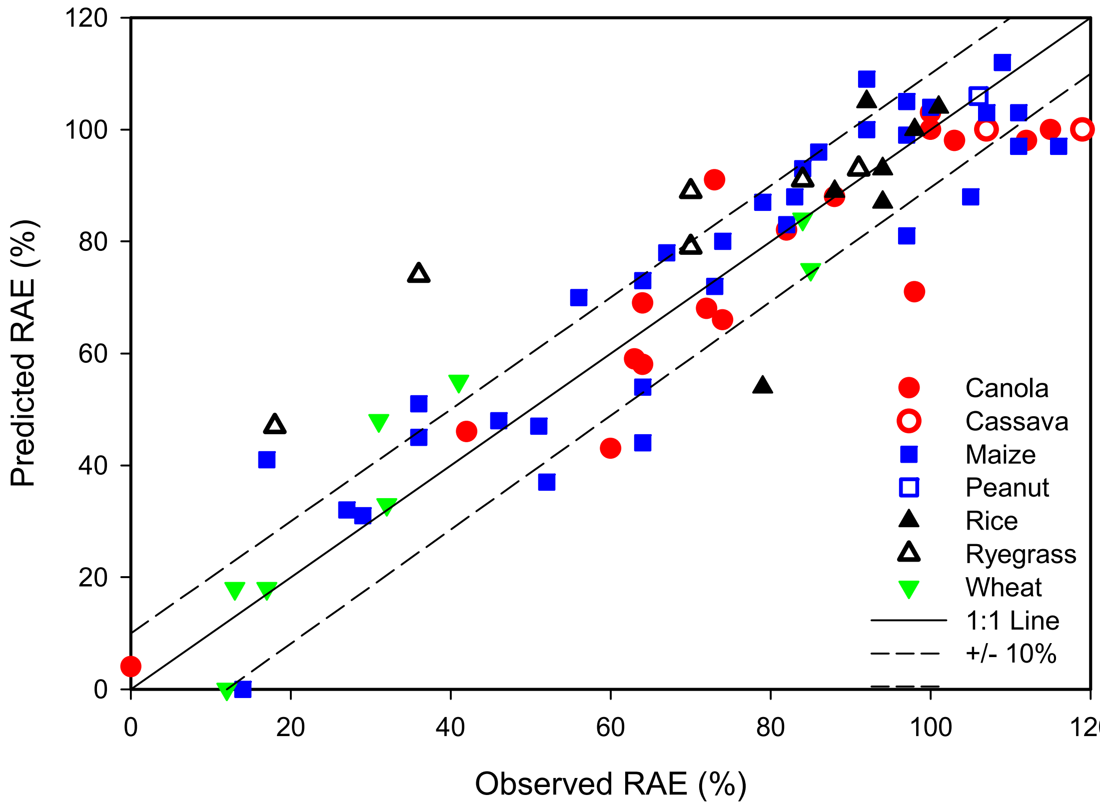

FIG.13. Performance of PRDSS against field and greenhouse experiments.

4. DISCUSSION

The key inputs of the initial PRDSS are PR solubility, soil pH, and crop species. The PRDSS prediction based on these inputs forms the base-or the basic-RAE. The base-RAE will be best suited for web users, because the RAE of PR and its economic feasibility for direct application are evaluated with minimum input. The developed PRDSS version reported here has expanded capability and offers the options of including effects of rainfall, soil texture, organic matter, Al saturation, P fixation, and crop species effect related to P fixation and Al toxicity. The model now considers factors that affect PR dissolution, P fixation, P leaching, and Al toxicity.

The RAE values predicted by the PRDSS reflect the agronomic effectiveness of the various PR sources relative to WSP (e.g., TSP); however, these should be supplemented by an economic analysis before rational choices can be made. The following calculation based on Engelstad and coworkers [20] provides an estimate of the relative economic effectiveness (REE):

$$\text{REE} = \text{RAE} \times \frac{\text{Price of TSP/kg P}}{\text{Price of PR/kg P}}$$

If the REE exceeds 100 in this calculation, the PR would be preferable to TSP. For example, if the price ratio is 2.0 and the RAE value of the PR is greater than 50, the PR in question may be used. However, if the RAE value were less than 50, WSP would be a better choice. This analysis does not consider the possibility of lower yields from use of PR. In addition, RAE is based on the responsive portion of the yield curve.

The PRDSS use is still limited to the initial application of PR; however, the next version will include residual and long-term effects of PR application and the possible lime effect of PR source, and also the effect of lime application. Thorough testing of PRDSS with good quality field data remains a priority. During the development and testing of PRDSS, it was found that while much PR research has been done, the type of data needed for developing and testing the model is scarce. This prompted the setting of field calibration and testing with FAO/IAEA support in Argentina, Brazil, Burkina Faso, Malaysia, Tanzania, and Vietnam. To further promote the use of PRDSS in decision making, the version as presented in this report will also be made available to web users through the FAO-IAEA website. At a later stage, predicting yield and the quantity of PR needed for achieving that yield will also be explored using the P simulation model within the Decision Support System for Agrotechnology Transfer (DSSAT). Using the DSSAT-PRDSS linkage users will obtain simulated yield response for WSP rates from DSSAT and RAE from PRDSS, and thus estimate the PR application rates and the corresponding yields.

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ENHANCING THE AGRONOMIC EFFECTIVENESS OF A LOW REACTIVE LOCAL PHOSPHATE ROCK TO IMPROVE AGRICULTURAL PRODUCTIVTY IN A LOW P STATUS OXISOL OF THE CENTRAL CERRADO, BRAZIL

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Abstract

A series of studies was carried out to investigate technologies to enhance the agronomic effectiveness of the locally available Patos phosphate rock (PR) for direct application to corn, a major food crop and Eucalyptus, a tree commonly grown for timber and paper mill production in the Cerrado region. The specific objectives of this research were to evaluate the effects of a water-soluble phosphatic fertilizer such as triple superphopshate (TSP) when applied mixed with the low reactive Patos PR on PR-phosphorus utilization by corn and Eucalyptus, in a tropical Typic Dystrarox soil of the Cerrado region using ^{32}P isotopic techniques. Technologies studied included a physical means by mixing the local Patos PR with TSP in different proportions and a biological one by inoculating the plants with endomycorrizhae. Granular TSP, finely powdered Patos PR and TSP + PR (both finely powdered and mixed with slight humidity) were applied to plastic pots containing 1.5 kg soil, previously labeled with 10 MBq ^{32}P carrier-free/pot. Additional treatments with ^{32}P labeled TSP were included. In the first experiment with corn the treatments were: TSP (0, 50, 100 mg P kg^{-1} soil), Patos PR (200, 400 and 800 mg P kg^{-1} soil) and TSP + PR (50 + 200 mg P kg^{-1} soil, 50 + 400 mg P kg^{-1} soil, 50 + 800 mg P kg^{-1} soil, and 100 + 400 mg P kg^{-1} soil). The treatments of second corn experiment were: TSP (0, 30, 60, 90, 120 and 150 mg P kg^{-1}soil); Patos PR (400 mg P kg-^{1} soil); TSP + PR (30, 60, 90, 120 and 150 mg P kg^{-1} soil, 1:1 ratio) and some treatments were inoculated with mycorrhiza. Corn plants were grown and harvested 40 days after seeding for total P and ^{32}P activity analysis. The combined application of TSP with Patos PR increased slightly the utilization of P from PR by the corn plants (0.49 to 1.71%). This slight increase in attributed to the low reactivity of Patos PR as a source of P for corn even when it was mixed with TSP. Increasing TSP proportion in the TSP + Patos PR mixture (1:1) did not show any advantage, as a reduction on the utilization of TSP-phosphorus by the plants in the presence of Patos PR was found. The mycorrhiza increased the P uptake, but this enhancement effect was very small. In the third experiment *Eucalyptus urophylla, Eucalyptus grandis,* and the hybrid *E. urophylla x E. grandis* were used. Plants were grown in plastic pots containing 1 dm^{-3} of soil, labeled with 3.7 MBq ^{32}P carrier free. Harvesting was done 55 days after planting and analysed for total P and ^{32}P activity. The dry-matter yield response of the Eucalyptus genotypes to P sources followed the order TSP = (TSP + PR)> PR and the P uptake response in the following order: (TSP + PR)> TSP>P. The increase in P uptake from PR due to TSP was 217.4% for *E. urophylla*, 235.7% for *E. grandis* and 28.7% for *E. urophylla* x *E. grandis*, indicating a significant enhancement effect of TSP on the agronomic effectiveness of the Patos PR. The hybrid *E. urophylla* x *E. grandis* was the most efficient genotype on utilizing soil P and *E. grandis* the most demanding species in P fertilizer.

1. INTRODUCTION

The Brazilian savannah known as the Cerrado region occupies approximately 2/3 of the country (2 037 600 km^2), of which 2/3 (136 Mha) is considered suitable for food crop production. Most of the soils are highly weathered Oxisols (46%) and Ultisols (15%), with serious limitations for crop production due to very low natural inherent N and P fertility, high acidity (high Al and low base saturation) and high P sorption (fixation) capacity. Therefore substantial nutrient inputs are required for optimum plant growth and adequate food, fiber and timber production. Although annual rainfall ranges from 700 to 2000 mm, it may also be a limitation to crop production due to non-uniformity in its distribution throughout the year. There is a variable period of 4 to 6 months of dry season and recurrent dry spells during the rainy season associated with high evapo-transpiration rates. Besides, the soils have high permeability with low water holding capacity. Another problem is the limiting effective rooting depth of many crops as result of Al toxicity and/or Ca deficiency in subsurface layers [1].

Although past reports indicated that Cerrado soils may not have capability to sustain an intensive agriculture due to all these constraints, approximately 10 Mha (5% of total Cerrado area) and 35 Mha have been cropped to annual crops and improved pastures respectively during the last three decades. It is reported that it is possible to increase 4.5 fold the actual Cerrado food production (to 94 millions t of grains and 4 million t of meat) through proper intensification of these already cultivated lands, i.e., without any further expansion of the cultivated area [2].

The constraint of soil P deficiency can be overcome, at least partly, by the application of manufactured water-soluble P fertilizers, but Brazil import these fertilizers, which are often in limited supply and represent a major outlay for small resource-poor farmers. P fertilizer consumption in Brazil is almost 3 percent of the total world consumption. It is known that under certain soil and climatic conditions the direct application of phosphate rocks (PR) may be an agronomic and economically sound alternative to the more expensive superphosphate. Brazil has important PR reserves but mainly of igneous origin and low inherent reactivity. Current strategies taken in the country have been to either import water-soluble P fertilizers or reactive PRs, or treat indigenous PRs at high temperatures in a mixture with basic slags to produce thermophosphates [3].

The P availability from PRs to crops and therefore, their agronomic effectiveness when applied directly as P fertilizers depends on a number of factors and their interactions. The main factors include: a) the inherent physical and chemical properties of PRs; b) soil and climate factors; c) plant species and the cropping system; and d) farming management factors. Although the direct application of PR may be an alternative to reduce the cost of supplying P to the crops, PRs with low to medium reactivity often do not respond as water-soluble P fertilizers in terms of yield increases for single season food crops [4, 5, 6, 7]. Experimental field evidence has shown that mixing medium reactive PR sources with a water-soluble P fertilizer, e.g. triple superphosphate (TSP) enhances the efficiency of P utilization from the PR by the crop. In a greenhouse study Chien *et al.* measured the enhancement effect of mixing a water-soluble P fertilizer (TSP) on the P availability from medium reactive central Florida PR using P-32 isotopic techniques [8]. This effect was attributed to an increased early plant root development, as induced by the water soluble P, thus enabling the plant to use P from the PR more effectively than could a plant treated with PR alone. Another approach to enhance P availability from PR sources is the use of biological means. Numerous studies have shown the positive effect of vesicular-arbuscular mycorrhiza (VAM) on P uptake from PRs by the plants [9, 10, 11]. However there is no information on the quantitative estimation of P uptake from PR by corn enhanced by these technologies in the acid soils of the Cerrado region. The cultivated area with Eucalyptus plantations for timber and paper mill products covers more than 2.5 Mha in the Cerrado region [12]. The initial P demand by young Eucalyptus appears to justify the recommended rates of water-soluble P fertilizers, but this practice increases the establishment costs of the plantations. Under these conditions phosphate rock (PR) application becomes an interesting economic option [13]. Genotypic differences in P use efficiency have been reported for Eucalyptus [14]. However little is known about the actual P demand by Eucalyptus at young stages (establishment of the plantation) and its ability to access P from sparingly P sources such as PR.

As most Brazilian PRs have low inherent reactivity, a series of studies was carried out to study technologies to enhance the agronomic effectiveness of a local PR source when directly applied to corn, a major food crop and Eucalyptus, a tree commonly grown for timber and paper mill production in the Cerrado region. The objectives of this research were to evaluate the effects of TSP, when applied mixed with Patos PR, a low reactivity PR from Brazil on PR phosphorus utilization by corn and Eucalyptus, in a tropical Oxisol of the Cerrado region using ^{32}P isotopic techniques. Technologies in study included a physical means by mixing the local Patos PR with triple superphosphate (TSP) in different proportions and a biological one by inoculating the plants with endomycorrizhae. Due to the possible interactions between water soluble P from TSP, soil P and PR-P, ^{32}P was utilized as tracer to distinguish the supply of P from these various sources.

2. MATERIALS AND METHODS

Three pot experiments were conducted between 2000 and 2004 at the greenhouse of the Center for Nuclear Energy in Agriculture (CENA), University of Sao Paulo (USP), Piracicaba, SP, Brazil.

A clayey (sand = 276 g dm^{-3}; silt = 324 g dm^{-3} and clay = 410 g dm^{-3}) Typic Dystrarox (Red Yellow Latosol) of low available soil P [15] was collected from the arable layer in an area under natural vegetation of the central “Cerrado” at Planaltina, State of Goiás, Brazil, located at 15º 14' South latitude and 47º 42' of West latitude and 826 m above sea level. The main soil chemical and physical properties were analyzed according to standard analytical methods of the Agronomic Institute of Campinas [16] and [17]. Selected characteristics of the experimental soil are shown in Table I.

TABLE I. SOME CHARACTERISTICS OF THE EXPERIMENTAL SOIL FROM CERRADO

pH (water)	5.02
pH ($CaCl_2$)	4.12
Organic matter (g dm^{-3})	22.55
P (resin, mg dm^{-3})	5.40
K (mmol$_c$ dm^{-3})	1.60
Ca (mmol$_c$ dm^{-3})	1.49
Mg (mmol$_c$ dm^{-3})	1.51
S (mg dm^{-3})	1.20
H+Al (mmol$_c$ dm^{-3})	65.81
Sum of bases (mmol$_c$ dm^{-3})	4.60
CEC (mmol$_c$ dm^{-3})	70.41
V%	6.50

2.1. Experiment 1

The experiment consisted of the following treatments: Triple Superphosphate (TSP) at two P rates and Patos Phosphate Rock (PR) at three P rates were applied alone and in mixture at variable P ratio (Table II). A set of additional treatments using ^{32}P labeled TSP in mixture with Patos PR was also included (Table III).

TABLE II. TREATMENTS OF EXPERIMENT 1

Treatments	P rate (mg P kg^{-1})
T_1	0
T_2	50 TSP
T_3	100 TSP
T_4	200 PR
T_5	400 PR
T_6	800 PR
T_7	50 TSP+200 PR
T_8	50 TSP+400 PR
T_9	50 TSP+800 PR
T_{10}	100 TSP+400 PR

TSP = Triple Superphosphate ; PR = Patos Phosphate Rock

TABLE III. ADDITIONAL TREATMENTS OF EXPERIMENT 1

Treatments	P rate (mg P kg^{-1})
T_{11}	50 TSP (^{32}P) + 200 PR
T_{12}	50 TSP (^{32}P) + 400 PR
T_{13}	50 TSP (^{32}P) + 800 PR

TSP (^{32}P)=^{32}P Labelled Triple Superphosphate

Granular TSP (48.46% total P_2O_5; 47.70% P_2O_5 neutral ammonium citrate soluble P and 41.51% P_2O_5 water-soluble P); finely powdered Patos PR (24.12% total P_2O_5 and 4.03% P_2O_5 soluble in 2% citric acid) and the mixture TSP+PR (mixed with slight humidifying) were added and mixed with the soil (1.5 kg in plastic pots) previously labeled with 10 MBq ^{32}P carrier free/pot. In the additional treatments (T_{11}, T_{12} and T_{13}) ^{32}P labeled TSP was employed. Four seeds of the corn hybrid P30F33, of good performance in a previous experiment were sown per pot and a week later they were thinned to 2 plants per pot. At 12 days after seeding, a basal fertilization of 100 mg N kg^{-1} and 114 mg S kg^{-1}, as ammonium sulphate, 100 mg kg^{-1} K as KCl, micronutrients (B, Cu, Mn, Zn and Mo), Ca as $CaCl_2$ and Mg as $MgCl_2$ was applied. At forty days after seeding the above-ground parts of the plants were cut. The plant samples were oven-dried at 70 ^{o}C, weighed and digested (nitric + perchloric acid) for total P and ^{32}P activity analysis [18, 19]. Based on the ^{32}P isotopic dilution method, the proportions of P taken up by the plants from the soil, PR and ^{32} P-labeled TSP were calculated according to Chien *et al.;* Muraoka; and Zapata and Axmann [8, 20, 21]. The results were analysed statistically and the means comparison was made using the Tukey's test at 1% level [22].

2.2. Experiment 2

Using similar materials and experimental procedures as previously described for experiment 1, a follow-up greenhouse experiment with the treatments listed in Table IV was carried out. The main differences with the previous experiment were increasing P application rates, the use of a 1:1 P ratio in all the TSP+PR treatments and the inclusion of mycorrhizal inoculation in the last set of treatments. Soil labeling was made by adding 10 MBq carrier free ^{32}P per pot containing 2.5 kg soil. The mycorrhizal inoculation was made applying 20 g of substrate with *Glomus* clawn obtained from Soil and Plant Nutrition Department (ESALQ-USP).

TABLE IV. TREATMENTS OF EXPERIMENT 2

Treatment	P rate (mg P kg^{-1} soil)	P source	Mycorrhiza
T_1	0 (Control)	-	-
T_2	30	TSP	-
T_3	60	TSP	-
T_4	90	TSP	-
T_5	120	TSP	-
T_6	150	TSP	-
T_7	400	Patos PR	-
T_8	30	TSP+PR (1:1)	-
T_9	60	TSP+PR (1:1)	-
T_{10}	90	TSP+PR (1:1)	-
T_{11}	120	TSP+PR (1:1)	-
T_{12}	150	TSP+PR (1:1)	-
T_{13}	0	-	+
T_{14}	30	TSP	+
T_{15}	60	TSP	+
T_{16}	120	TSP	+
T_{17}	30	TSP+PR	+
T_{18}	60	TSP+PR	+
T_{19}	120	TSP+PR	+
T_{20}	400	PR	+

2.3. Experiment 3

Three Eucalyptus genotypes, namely two species (*E. urophylla, E. grandis*) and one hybrid *(E. urophylla x E. grandis*), which are commonly grown in Brazil were used in the experiment. Seeds were supplied by the "Departamento de Ciências Florestais" (ESALQ-USP), Piracicaba, SP. The P sources studied were Triple superphosphate (TSP) and Patos de Minas (Brazil) PR, both finely ground (0.15 mm or 100 mesh) and the mixture TSP + PR. For each cultivar four P treatments were laid down: 1) Reference or standard for the ^{32}P technique (without P), 2) 100 mg P dm^{-3} as TSP, 3) 400 mg P dm^{-3} as PR and 4) 50 mg P dm^{-3} as TSP + 400 mg P dm^{-3} as PR. Some chemical characteristics (total P, soluble P in neutral ammonium citrate, citric acid and water) of the phosphatic sources are listed in Table V [23].

The experimental design was a 2-factor complete factorial (3 cultivars and 3 P sources plus one reference) with a total of 12 treatments arranged in a completely randomized design with 3 replications. The ^{32}P isotopic indirect approach was used to assess the P availability from the phosphates sources [20, 21]. Plants were grown in plastic pots lined with polyethylene bag and filled with 1 dm^{-3} of soil. A 100 mL aliquot solution containing 3.7 MBq ^{32}P was added per pot in order to obtain sufficient activity in the plant material and left drying for two days. The ^{32}P-labelled carrier solution was prepared by adding the total activity required for the experiment as ^{32}P carrier-free to a KH_2PO_4 carrier solution containing 50 mg P L^{-1}. The soil was homogenized together with each phosphatic source according to the experimental treatments. A basal fertilization of 55 mg dm^{-3} N as urea, 52 mg dm^{-3} K as

K_2SO_4 and calcium and magnesium as $CaCO_3$ and $MgCO_3$, proportion 4:1, was applied in order to raise the level of nutrients to 1 $cmol_c\ dm^{-3}$. The pots were incubated during seven days keeping the soil moisture to 70% of the field capacity. Calcium and magnesium carbonates were applied here as nutrient (calcium and magnesium) sources rather than soil amendment (raise pH), because the available information suggests that Eucalyptus is tolerant to aluminum and grows well under low soil pH [13]. Six of five days-old seedlings grown in washed sand of each Eucalyptus genotypes were transplanted to each pot. Five days later, three seedlings were left to growth in each pot keeping the soil moisture content equivalent to 70% of the field capacity. A micronutrients solution [24] containing Mn, Fe, Cu, Zn, B and Mo was added to each pot in aliquots of 15 mL when the plants were 2, 4, and 6 weeks old. Harvesting was done by cutting the above-ground part of the plants at 55 days after planting. Plant samples were washed with deionized water, oven-dried at 70°C and weighed. The dried plant material was ground in a Wiley mill and digested in a nitro-perchloric acid mixture. The total P concentration was determined using the ammonium molybdate method [18] and the ^{32}P activity by Cerenkov counting in a Liquid Scintillation analyser [19]. Based on the ^{32}P isotopic dilution method, the proportion of P in plant from soil, TSP, and PR were calculated according to [8, 20-21]. Statistical analysis of the data was made with a SAS system package [22].

TABLE V. SOME CHEMICAL CHARACTERISTICS OF PHOSPHATE SOURCES USED

Indices	TSP	PR
	--------------- % --------------	
Total P	19.65	10.04
P NAC	19.21	0.66
P CA	17.47	1.75
P H_2O	87.00	-

P NAC = P soluble in neutral ammonium citrate; P CA = P soluble in 2% citric acid and P H_2O = P soluble in water.

3. RESULTS AND DISCUSSION

3.1. Experiment 1

Due to the very low available soil P utilized in this study (5.4 mg P kg^{-1} soil), which is typical of the virgin Cerrado soils, the response of the crop to TSP application was evident (Table VI). The dry matter (DM) production increased from 1.96 g (control) to 11.25 and 16.11g for the rates of 50 and 100 mg P kg^{-1} soil, respectively. It was also found that the increases in both DM and P content practically resulted in doubling the amount of P taken up by the crop, when the P rate was increased from 50 to 100 mg P kg^{-1} soil as TSP. The P in the plant derived from the fertilizer reached 97.1% at 100 mg P kg^{-1} soil as TSP.

In spite of the low soil P availability, the application of higher P rates of PR (200, 400 and 800 mg P kg^{-1} soil) resulted only in slight increases in DM weight and plant P content. Only little more than a two-fold increase over the DM weight of the control treatment was obtained for the highest P application rate (800 mg P kg^{-1} soil) and this DM yield did not represent even half of that obtained with the lowest TSP application rate.

TABLE VI – EFFECT OF P FERTILIZER SOURCES ON DRY MATTER WEIGHT, P UPTAKE AND FERTILIZER P UTILIZATION EFFICIENCY BY CORN PLANTS

Treatment	DM /pot	P Content	P upt /pot	P_{pdfF}	P_{pdfF}	P_{pdfPR}	P_{pdfTSP}	FPUE (%)		
	(g)	(mg/g)	mg	(%)	mg	mg	mg	Total	PR	TSP
Control	1.96	0.80	1.58	-	-	-	-	-	-	-
50TSP	11.25	0.96	10.77	95.31	10.26	-	10.26	13.69	-	13.69
100TSP	16.11*	1.29	19.42	97.10	19.02	-	19.02	12.57	-	12.57
200PR	2.53	0.91	2.30	64.22	1.48	1.48	-	0.49	0.49	-
400PR	3.29	0.91	3.00	77.42	2.32	2.32	-	0.39	0.39	-
800PR	4.39	0.86	3.79	84.09	3.18	3.10	-	0.26	0.26	-
50TSP+200PR	12.80	1.03	13.68	96.07	12.66	5.14	7.80	3.38	1.71	7.80
50TSP+400PR	12.36	1.11	13.68	96.40	13.18	6.36	7.65	1.95	1.06	7.65
50TSP+800PR	14.38	1.13	16.19	96.66	15.65	8.13	7.44	1.23	0.68	7.45
100TSP+400PR	16.05	1.33	21.30	97.63	20.78	-	-	2.78	-	-

DM = Dry Matter; PpdfF= P in the plant derived from fertilizer
PpdfPR = P in the plant derived from PR (phosphate rock)
PpdfTSP = P in the plant derived from TSP (Triple Superphosphate)
FPUE (%) = Fertilizer P use efficiency
* One replicate was disregarded because one plant had been damaged by insects (grasshopper) at initial development stages.

In comparing the efficiency of both P sources when applied alone, large differences in the coefficients of utilization were found. On the average the utilization efficiency was 13% for the water-soluble P source (TSP) and less than 0.5% for the Patos PR. The combined application of two sources (TSP and PR), in the mixture, increased the DM weight and plant P content compared to the PR treament alone. This effect, however, was rather small and would not be attributed only to the priming effect of the water-soluble TSP; because these increases were not higher than those obtained when the highest rate of TSP was applied alone. However, the mixing of P sources increased considerably the P in the plant derived from PR: 1.48 mg to 5.14 mg P at 200 mg P kg^{-1}; 2.32 to 6.36 mg, at 400 mg P kg^{-1}; and 3.18 to 8.13 mg at 800 mg P kg^{-1} as PR (Table VI and Fig. 1).

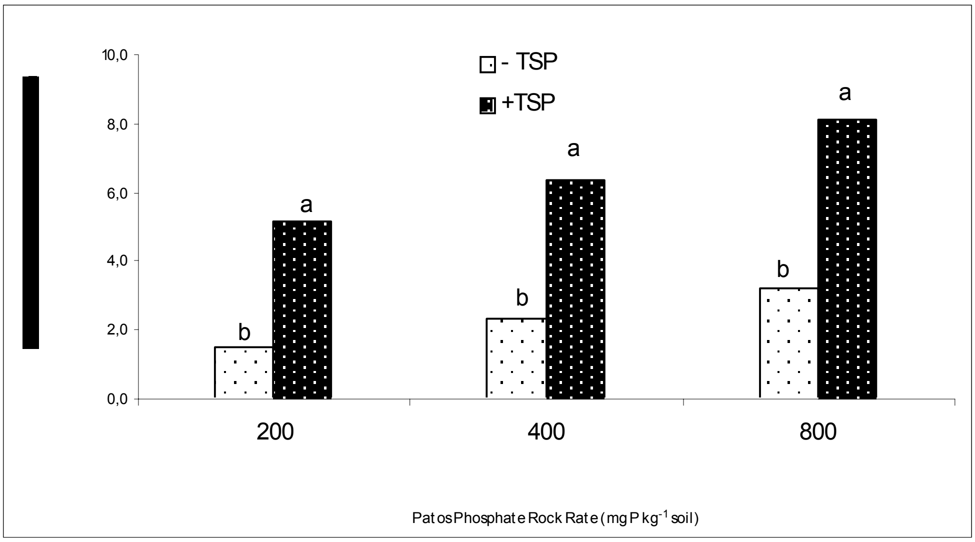

FIG.1. Effect of triple superphosphate on Patos rock phosphate P absorption by corn plants (Values followed by different letters within the P rate are statistically different, Tukey's test 1%).

In comparing the coefficients of fertilizer P utilization efficiency (FPUE) obtained for the P sources in the mixtures, the enhancement effects were also significant (Fig. 2). These results are in agreement with those reported by Chien *et al.* on mixing P sources [8]. However, these increases ranging from 0.68% to 1.71% are rather small for the applied PR rates, and none statistically significantly different. It should be noted that the P rates of the sources was different and the P ratios (P-TSP: P-PR) employed in this study were too high (1:4 and above). According to the work of Chien *et al.* a more balanced 1:1 P ratio would be ideal [8].

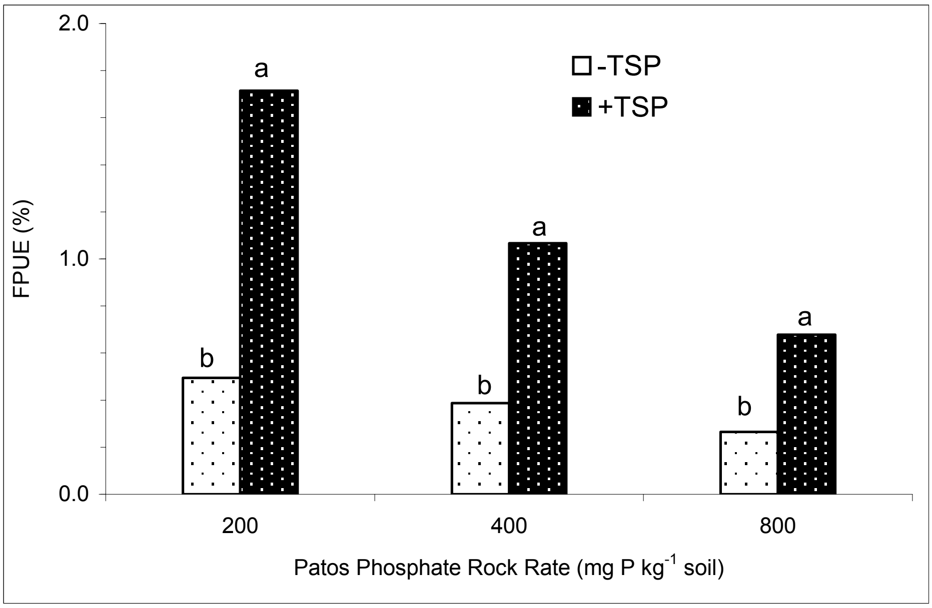

FIG.2. Effect of TSP fertilizer on efficiency of Patos PR phosphorus utilization by corn plants (Values followed by different letters within each P rate are statistically different, Tukey's test 1%).

3.2. Experiment 2

The response in dry matter weight and the amount of total P taken up by the corn plants for all the P fertilizer treatments are presented in Figs. 3 and 4 respectively. The maximum DM yield was obtained at 90 mg P kg^{-1} as TSP soil whereas the total P uptake increased up to the maximum application rate (150 mg P kg^{-1} as TSP).

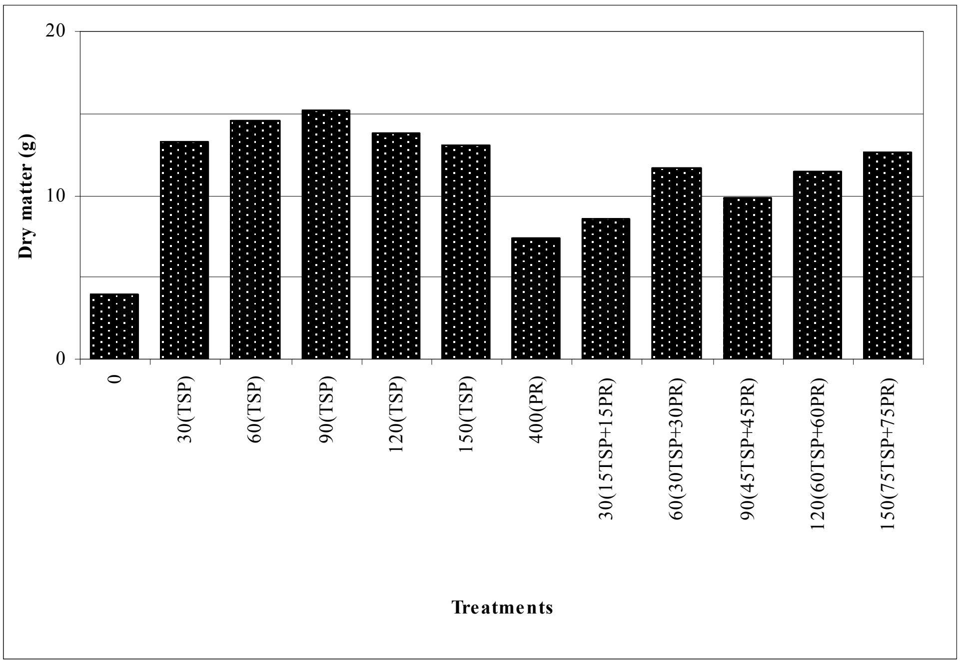

FIG.3. Effect of P application rates as triple superphosphate (TSP), Patos phosphate rock (PR) and TSP+PR on corn plant dry matter weight (DM).

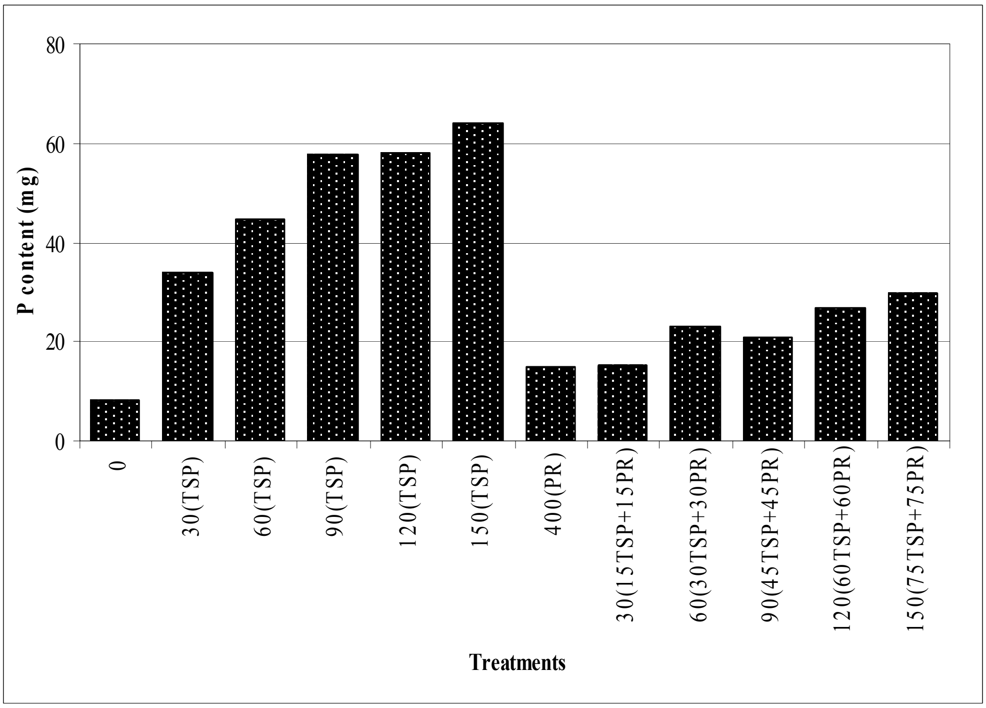

FIG.4. Effect of P application rates as triple superphosphate (TSP), Patos phosphate rock (PR) and TSP+PR on total P uptake (mg) by corn plants.

Because the soil had low available P and high P fixing capacity it was not expected that the maximum DM yield would be obtained with the application of 90 mg kg^{-1} P as TSP. It should be noted that in a previous experiment the corn hybrid used in this study was one of most efficient among 30 corn genotypes recommended for the Cerrado soils.

The TSP and PR mixtures when applied at the same P rates as the TSP alone and at 1:1 P ratio, did not improve the PR phosphorus utilization by the plants. In experiment 1, a slight increase in PR efficiency was reported when the mixture was applied at the P ratio of 4 and above. In this experiment a decrease in P uptake was found, as shown in Fig. 4. The same effect can be observed in the response curves shown in Figs 5, 6 and 7 where the response curves of the mixtures are always below those of the TSP applied alone. This effect can be seen in Figs. 8 and 9. In the Fig. 8, when comparing the treatment 30 mg P kg^{-1} soil as TSP with treatment with the same P rate applied in mixture, i.e. 50% TSP and 50% PR, there was a considerable reduction in dry matter, P content and P_{pdff} , all statistically significant at $p = 0.05$.

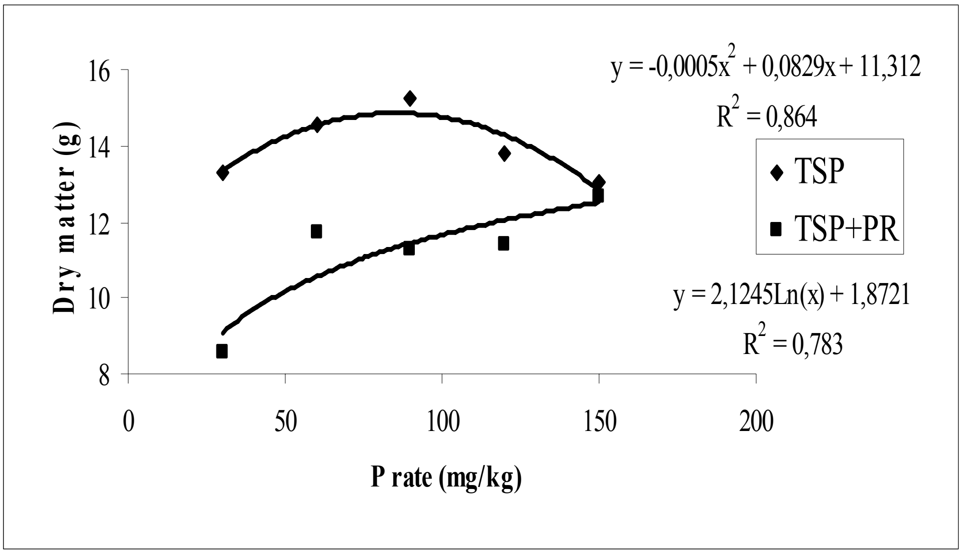

FIG.5. Effect of P applied as TSP or TSP + PR on corn plant dry matter weight (g).

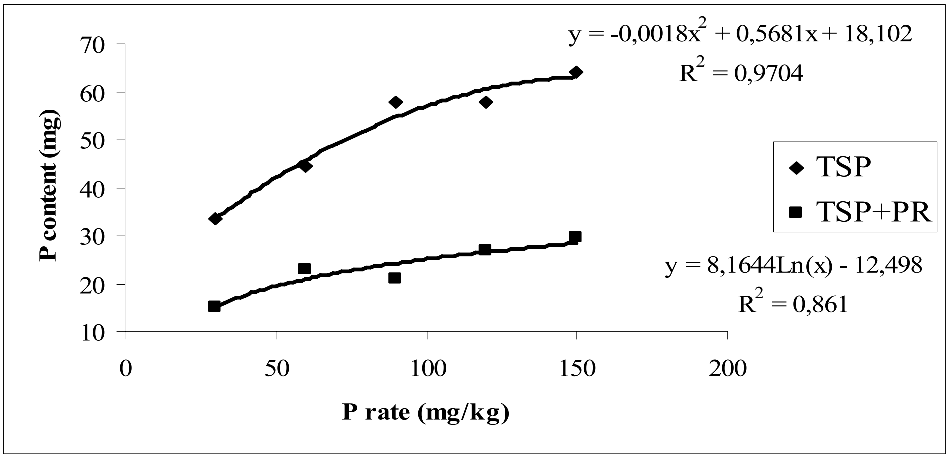

FIG.6. Effect of P applied as TSP or TSP + PR on corn plant P content (mg pot $^{-1}$).

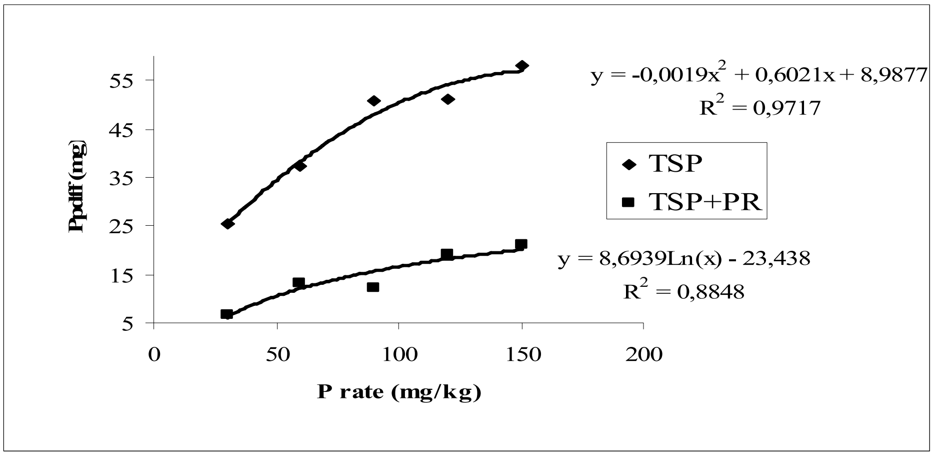

FIG.7.Effect of P applied as TSP or TSP + PR on corn plant P derived from fertilizer (Ppdff).

In Fig. 8 when comparing the treatment 30P as TSP with 60 P as mixture TSP + PR (1:1 P ratio), although the dry matter weight was not statistically different, the total P uptake and the P_{pdff} were significantly reduced in the mixture treatment 60 (30 TSP + 30 PR). Similar results were observed when comparing the treatment 60 TSP with treatment 30TSP + 30PR, 60TSP+60PR and 75TSP+75PR (Fig. 9). This reduction in P availability occurred probably due to insolubilization of the water-soluble phosphorus released from the TSP by reacting with PR, when the P ratio of the mixture is 1:1. Moreover when measuring the pH of the solution prepared with the TSP alone and in mixture TSP + PR, the following pH values were obtained: 3.44, 3.15, 2.99, 2.90 and 2.82 at 30, 60, 90, 120 and 150 mg P kg^{-1} as TSP respectively and 3.52, 3.32, 3.28, 3.11 and 3.11 for the equivalent P rates in the mixture TSP+PR. This small pH increase observed when mixing the two P sources also likely contributed to decrease the P availability in the mixture.

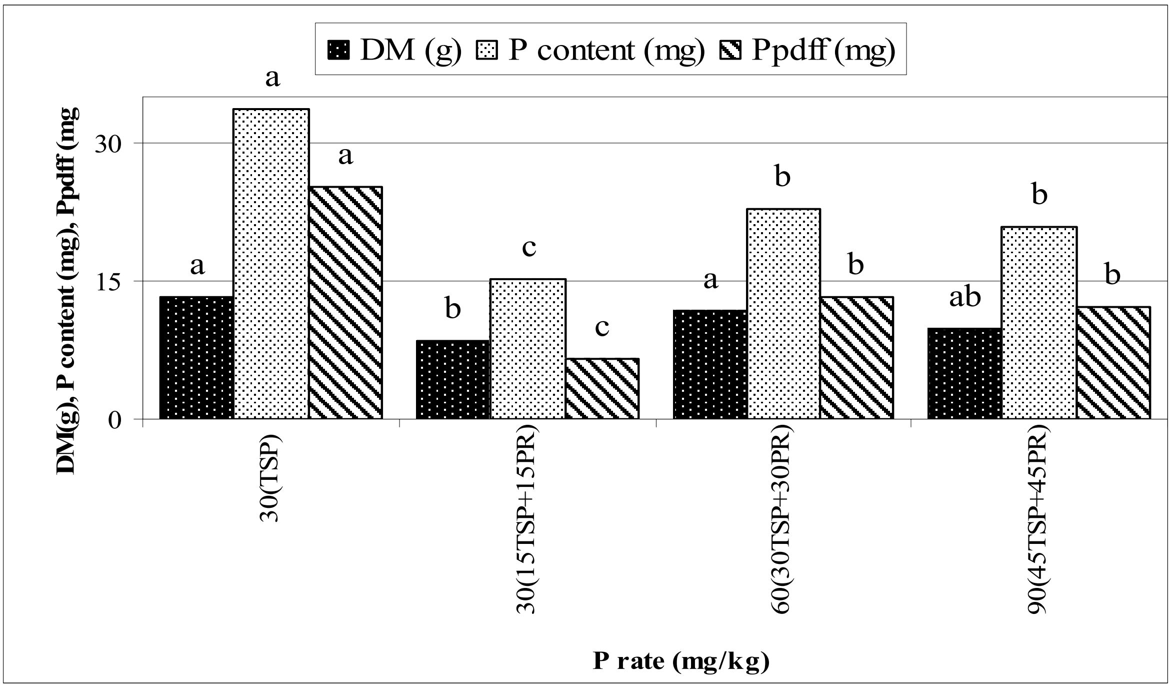

FIG.8. Effect of triple superphosphate + Patos PR on corn plant dry matter (DM) weight (g), P content (mg pot^{-1}) and P in the plant derived from fertilizer (Ppdff).

The mycorrhiza effect on the P uptake was observed in all treatments fertilized with TSP, but not in the unfertilized control (Fig. 10). Although the mycorrhiza increased the P uptake by corn, no significant differences were found in the 60 mg P kg^{-1} as TSP treatment. This also occurred for the 30 mg P kg^{-1} in mixture (15TSP + 15PR). Considering the application of PR alone (400 mg P kg^{-1} soil), there was also effect of mycorrhiza on its P availability as the P uptake increased significantly from 14.74 to 22.11 mg P (Fig. 10). However these uptake values represent only 0.6 and 0.96% P utilization of the applied PR.

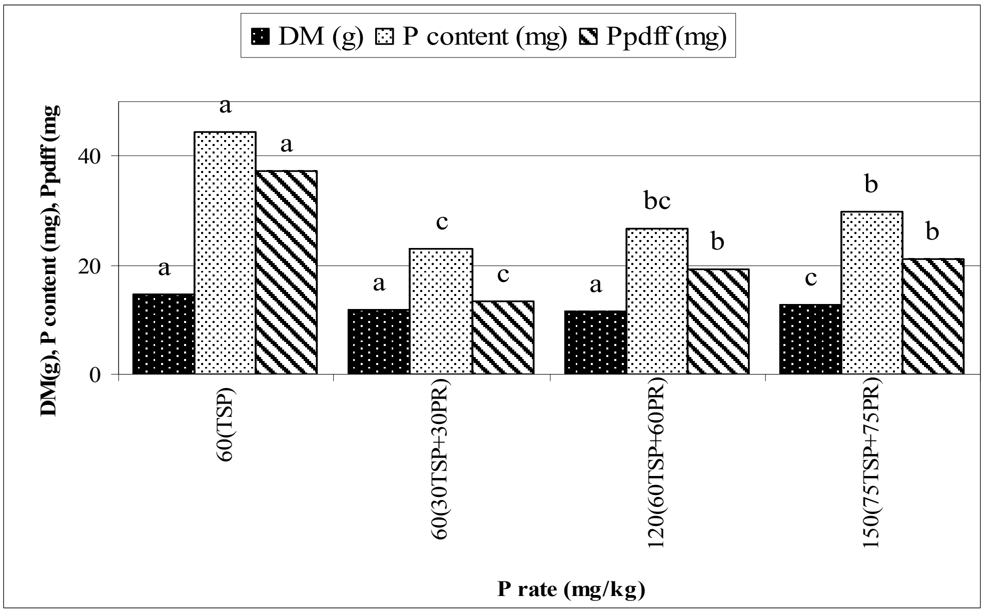

FIG.9. Effect of triple superphosphate + Patos phosphate rock on corn plant dry matter (DM) weight (g), P content (mg pot^{-1}) and P in the plant derived from fertilizer (Ppdff).

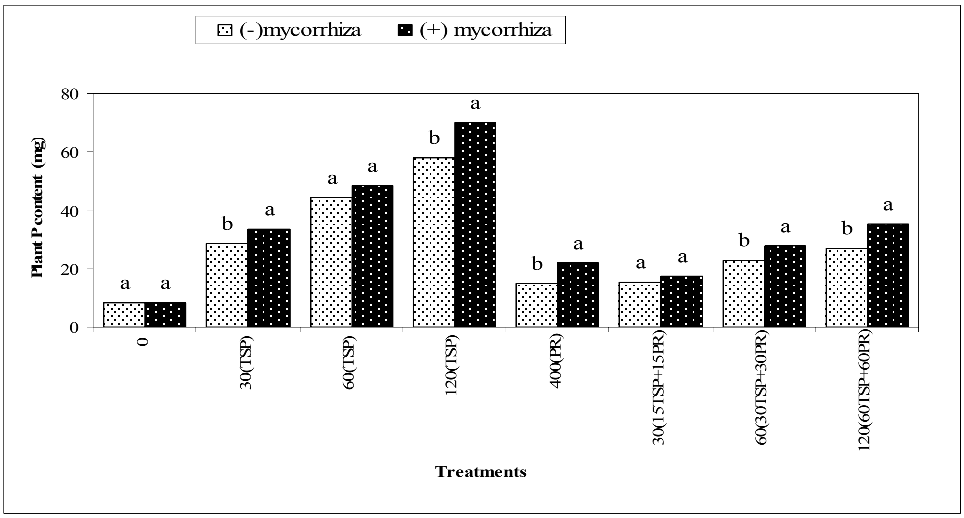

FIG.10. Effect of mycorrhiza on P uptake by corn fertilized with different rates of triple superphosphate (TSP), Patos phosphate rock (PR) or TSP+PR.

3.3. Experiment 3

Dry-matter yield and P uptake of the Eucalyptus genotypes as affected by the P sources are shown in Tables VII and VIII. Both dry matter and P uptake were significantly increased with the P application. The extremely low dry-matter yield and P uptake in the standard treatment (without P addition) confirm the very low P availability of the experimental soil and the great sensitivity of Eucalyptus to soil P deficiency.

The effectiveness of P sources in terms of increasing dry-matter yield followed the order (PR + TSP) = TSP > PR for both species and the hybrid. The P uptake increased in the following order (PR + TSP) > TSP > PR. The hybrid *E. urophylla x E. grandis* was the best in dry-matter yield and P uptake from all P sources.

TABLE VII. ABOVE GROUND DRY-MATTER WEIGHT (G POT^{-1}) OF THREE EUCALYPTUS GENOTYPES AS AFFECTED BY P SOURCES

Species	Standard	TSP	PR	(TSP + PR)
E. urophylla	0.090 A b	2.58 B a	0.57 C c	2.49 B a
E. grandis	0.065 B c	2.47 B a	0.74 B b	2.60 B a
E. u x E. g	0.085 A c	3.14 A a	0.91 A b	3.20 A a

Values followed by the same letters (Capital letters in columns between species, and small letters in row between P source) are not significantly different (P< 0.05), as determined by Tukey's multiple range test. *E.*= (*Eucalyptus*), *u*= (*urophylla*), *g*= (*grandis*).

TABLE VIII. PHOSPHORUS UPTAKE (MG P POT^{-1}) BY THREE EUCALYPTUS GENOTYPES AS AFFECTED BY P SOURCES

Species	Standard	TSP	PR	(TSP + PR)
E. urophylla	0.042 A d	2.91 B b	1.06 B c	4.29 B a
E. grandis	0.025 B d	2.75 B b	1.43 A c	4.58 B a
E. u x E. g	0.050 A d	4.01 A b	1.60 A c	5.50 A a

Values followed by the same letters (Capital letter in columns between species, and minuscule letter in row between P source) are not significantly different (P< 0.05), as determined by Tukey's multiple range test.

The differences in dry-matter yield and P uptake found between applied P sources are mainly related to their solubility (Table VI). The "Patos de Minas" PR utilized in this work, has low reactivity due to its low solubility in chemical extractants, with the main phosphate mineral in the form of fluor-carbonate-apatite or a mixture of fluorapatite and carbonate-apatite [23]. The solubility of this PR is facilitated by isomorphic substitution of phosphate for carbonate or fluorine in its structure [5]. On another hand, the low efficiency of PR in acid and loamy soils of low available P are due to sorption (fixation) reactions of P limiting its P availability and utilization by plants [3-5].

The P uptake from TSP and PR applied alone was calculated from the fractions PdfTSP, and PdfPR [20–21] and from each P source in the mixture (TSP + PR). The data are shown in the Table IX.

TABLE IX. P UPTAKE (MG P POT^{-1}) BY TWO SPECIES OF EUCALYPTUS AND THE HYBRID FROM TSP AND PR APPLIED ALONE OR TOGETHER WITH TSP

Species	P_{TSP}	P_{PR}	$P_{PR\ (TSP + PR)}$	$P_{TSP(TSP + PR)}$
E. urophylla	2.13 B a	0.69 B c	2.19 B a	1.21 B b
E. grandis	2.54 A b	1.23 A c	4.13 A a	0.11 C d
E. u x E. g	1.40 C b	0.80 B d	1.03 C c	2.18 A a

Values followed by the same letters (Capital letter in columns between species, and minuscule letter in row between P source) are not significantly different (P< 0.05), as determined by Tukey's multiple range test.

The P uptake from PR in the presence of TSP was higher than that from PR it is applied alone. The increase in P uptake from PR due to TSP effect was 217.3% for *E. urophylla*, 235.7% for *E. grandis* and 28.7% for *E. urophylla x E. grandis*, indicating an enhancement effect of TSP on the P availability and agronomic effectiveness of the Patos PR. This effect can likely be explained by the higher P rate in the mixture (TSP + PR) treatment in relation to others treatments and a "priming effect" of the TSP promoting a greater root development of the seedlings and enabling the plant to use P from PR more effectively than could its with PR alone [8]. *E. grandis* was found to be the species with higher use of P from PR and TSP. This species was more responsive to high P rates of application than other five species indicating to be more P demanding for its growth and development [14].

4. CONCLUSIONS

The combined application of TSP when mixed with Patos PR at P ratios of 1:4 and above increased the utilization of P from the PR by the corn plants. However, the agronomic effectiveness of this PR was still low due to its low reactivity. The Patos PR proved to be ineffective and it may not be used directly as P source to corn in the Central Cerrado soil, even when used in powdered mixtures with a water-soluble P source. Increasing the proportion of TSP in the powdered mixture TSP + Patos PR to a P ratio of 1:1 did not improve the P availability from PR, on the contrary it was found a reduction on the P utilization from TSP by the plants. Mycorrhizal inoculation increased the P uptake by the corn plants, but this enhancement effect was very small. Further field investigations are needed to study this enhancement effect by compaction or granulation of the mixture TSP and Patos PR at several P rates and increasing the proportion of water-soluble phosphate to PR.

The TSP enhanced the P uptake from PR by the seedlings of *E. urophylla, E. grandis, and E. urophylla x E. grandis* when they were applied together. The hybrid *E. urophylla* x *E. grandis* was the most efficient genotype on utilizing soil P and *E. grandis* the most demanding species in P fertilizer.

ACKNOWLEDGEMENTS

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IMPROVING AGRICULTURAL PRODUCTIVITY IN THE SAVANNAH OF TABASCO STATE, MEXICO: I. MANAGEMENT OF MAIZE AND SORGHUM PRODUCTION SYSTEMS

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Abstract

A series of studies was carried out to evaluate the effects of selected biological components of agrosystems, in particular mycorrhizae, earthworms, and *Trichoderma* on sorghum and maize crop production in an acid Ultisol of the savannah of Huimanguillo, Tabasco state, Mexico. Isotopic techniques using ^{15}N and ^{32}P were used to assess N and P use efficiency of sorghum transgenic lines (citrate over-producers) under nutrient-limited conditions. No significant differences in ^{15}N-fertilizer use efficiency between the lines tested (L8, a citrate over-producer) and a commercial genotype were found. The commercial line and a negative control (L18-2 transgenic line), showed the highest N derived from the fertilizer as well as P uptake derived from rock phosphate. The combined application of the legume green-manure *Mucuna pruriens* and earthworms (*Balanteodrilus pearsei*) had a positive effect on N and P uptake and maize yields (78 g grain plant^{-1} vs. 26 g grain plant^{-1} for the control). Inoculation of maize with *Trichoderma* enhanced the effect of N fertilization and the N-uptake efficiency was highest when the fertilizer N was applied at sowing. Both *Trichoderma* strain C4 and the commercial inoculant "Tricom" gave the highest values of N-fertilizer use efficiency (43 and 38%, respectively), which was equivalent to a 2.4-fold increase over that found when fertilizer was applied alone. In a field evaluation of maize genotypes, the genotype VS-536 yielded 2.35 Mg grain ha^{-1} and highest fertilizer N efficiency when 100 kg P_2O_5 ha^{-1} of the reactive Baja California PR was applied, suggesting that this genotype could be used in the local production system. Interestingly, inoculation with mycorrhizae did not have positive effects on the fertilized maize. Overall, these data will contribute to establish better management practices for increasing soil fertility and improving crop production in the acid, unfertile savannah soils of Mexico.

1. INTRODUCTION

Acid soils in Mexico occupy about 1.3×10^7 ha, which corresponds to 6.7% of the surface area and 14% of the total cultivated land. Most acid soils are located in the southeast of Mexico, including the states of Campeche, Chiapas, Oaxaca, Tabasco, and Veracruz. The main savannah ecosystem in the country, known as the savannah of “Huimanguillo” and “Plan Balancan-Tenosique” is located in the state of Tabasco [1]. These acid soils are considered marginal to agricultural production due to serious constraints on sustainable agricultural production.

Agronomic research in this zone has focused on addressing the main constraints that affect soil fertility and decrease crop productivity, including the availability of nutrients (N, P, Ca, Mg) and the presence of toxic elements (Al) [2]. These programmes have generated a wealth of information on sustainable use of soils in the state of Tabasco [1]. In these savannah areas, corn and sorghum are the most important food crops. Nitrogen (N) is the key nutrient to crop production in the tropics: it is the most mobile element but also the most easily depleted nutrient in the soil. Moreover, due to crop intensification in the subhumid tropics, long fallow periods to restore the organic matter and N status are no longer sustainable. Thus N inputs are necessary to sustain high crop yields in intensive and continuous crop production systems [3]. As N is the most costly nutrient especially for smallholder farmers, efforts to develop an integrated N management system and increase plant N-use efficiency can have large benefits [4].

Despite the available information from research, an integrated approach to crop, soil, water and nutrient management has not been applied to the cropping systems of the zone. It is of paramount importance that the technologies developed should be socially viable, enviroment-friendly and cost-effective to ensure their adoption by the farmers. Molecular biology is an important tool in biological and agricultural research. One aspect of gene manipulation in plants has focused on over-producing organic acids that are directly involved on nutrient uptake [5]. Other studies have shown the beneficial effects of mycorrhizal fungi, especially in increasing phosphorus (P) uptake in acid soils with low P availability [6] as well as from applied phosphate rock [7, 8]. Mycorrhizae have also synergistic effects on N uptake [9]. Inoculation of maize plants with *Trichoderma* increases crop yields because the fungus acts both as a biocontrol agent and also as a stimulator of plant growth [10].

In this context, the use of sorghum and maize genotypes with high nutrient (N and P) use efficiency and good agronomic performance, complemented with soil management practices that include the use of beneficial microorganisms, could contribute substantially to increased productivity and sustainability of the acid savannah soils. Part I of this work deals with the assessment of the effects of selected biological components of sorghum and maize agrosystems, in particular mycorrhizae, earthworms, and *Trichoderma* on improving soil fertility and crop production in an Ultisol of the savannah of Huimanguillo, Tabasco state, Mexico. Isotope ^{15}N and ^{32}P techniques were utilized to assess these effects.

2. MATERIALS AND METHODS

A series of greenhouse and field experiments were carried out in a strongly acidic Ultisol collected from the savannah of Huimanguillo, Tabasco state, Mexico [1].

Selected characteristics of the experimental soil are shown in Table I. This topsoil (0–20 cm) is very rich in organic matter (5.2%), probably because of the poor microbial activity occurring at this low pH [11]. The K, Ca and Mg contents are considered low [12].

TABLE I. SOME CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE EXPERIMENTAL TOPSOIL (ULTISOL)

Characteristic	Content
Textural class:	Sandy loam
Clay (%):	21
Silt (%):	11
Sand (%):	68
pH_{water} (1:2):	4.6
Organic matter (%):	5.2
P-Olsen (mg kg^{-1} soil):	6.0
Exch. Cations ($cmol_+$ kg^{-1} soil)	
K	0.30
Ca	2.20
Mg	0.50
Na	0.04
Cat. Exch. Cap. ($cmol_+$ kg^{-1} soil)	8.20

2.1. Greenhouse experiments

2.1.1. Experiment 1: N uptake from N-fertilizer by transgenic lines of sorghum (Sorghum bicolor)

The Department of Genetic Engineering of CINVESTAV-Irapuato Unit has generated plants that overproduce organic acids to overcome Al toxicity in acid soils [5]. Some transgenic lines of sorghum that overproduce citrate were evaluated in terms of N and P uptake using ^{15}N (Exp. 1) and ^{32}P (Exp. 2) isotopic techniques.

In this study five sorghum genotypes were used. Two transgenic lines that overproduce citrate [L8 (CS++) and L15 (CS+)], and a transgenic line [L18-2 (CS-)] that does not overproduce citrate were kindly provided by the Department of Genetic Engineering of CINVESTAV, Irapuato Unit. Two commercial varieties (a wild-type and one recommended for acid soils) were also included. Seeds were sown in pots containing 2 kg of soil. The experiment was conducted on the typical Ultisol described above (Table I). P fertilizer as phosphate rock (PR) from Baja California was applied at two levels: (1) with PR (80 kg P_2O_5 ha^{-1}) and (2) without PR. The chemical characteristics of this highly reactive PR were a content of 26% total P_2O_5 and 13.5% P_2O_5 ammonium citrate solubility [13]. The pots were irrigated every third day with 70 mL pot^{-1} of distilled water. A completly randomized experimental design (with four replicates) was used, and the experiment was conducted in a greenhouse at CINVESTAV, Irapuato Unit, from August 30 to October 30, 2000.

At sowing, urea labeled with 1% atom ^{15}N excess was applied at the recommended rate for this crop of 180 mg N kg^{-1} of soil [14] in aqueous solution. At 60 days after sowing (DAS), the fresh shoot weight was determined, then the shoots were oven-dried at 70°C for 72 h. Total-N (1016 digestion and 1002 distillation units, TECATOR) and $^{15}N/^{14}N$ isotopic ratios were determined by the Kjeldahl method and Rittenberg oxidation method using sodium hypobromite [15] and an optical emission spectrometer (NOI6-e PC, FAN) respectively [16]. The calculations for estimating the N recovery in the plant from ^{15}N labelled fertilizer (N derived from the fertilizer and N fertilizer efficiency) were performed according to Zapata [17]. The experimental data were statistically analyzed following standard procedures for analysis of variance, and the differences between mean values were determined at $p \leq 0.05$ by HSD Tukey test, using the SAS system [18].

2.1.2. *Experiment 2: P uptake from rock phosphate by transgenic lines of sorghum (Sorghum bicolor)*

The objective of this experiment was to assess the efficiency of sorghum transgenic lines on the uptake of P derived from phosphate rock using the ^{32}P isotopic dilution technique. Similar procedures to those described above for Experiment 1 were used including the same transgenic lines, experimental management, biological variables, experimental design and statistical analysis, etc. The experiment was conducted on the typical Ultisol described above (Table I). Phosphate rock (PR) from Baja California, sieved to 100 mesh was used as the P source. Pots were used as the experimental units, and each received 100 mg P_2O_5 kg^{-1} of soil as PR [2] and a control without PR. The ^{32}P labelled solution as orthophosphoric acid was applied at a rate of 1 mL of 5 mg KH_2PO_4 L^{-1} solution and with a specific activity of 3.7 MBq ^{32}P kg^{-1} soil (Muraoka, 2000 personal communication). The isotopic dilution method was used to determine the P uptake derived from PR [17]. At 60 DAS, total-P was determined both using the vanado-molybdate method [19, 20], and the ^{32}P activity was measured by Cerenkov counting in a liquid scintillation analyser [17]. The experiment was laid out in a greenhouse at CINVESTAV, Irapuato Unit from October 27–30 to December 28, 2000. Estimates of the P derived from PR were performed according to Zapata [17]. The experimental data were statistically analyzed following standard procedures for analysis of variance, and the difference between mean values was determined at $p \leq 0.05$ by HSD Tukey test, using the SAS system [18].

2.1.3. *Experiment 3: Effect of a native tropical earthworm (Balanteodrilus pearsei) and added green manure (Mucuna pruriens) on the formation of mycorrhizae and maize yield*

In this experiment the objective was to evaluate the effect of a native tropical earthworm (*Balanteodrilus pearsei*) and *Mucuna pruriens* residues added as green manure on the formation of mycorrhizal vesicles and hyphae as well as maize yield.

The soil, endogenic earthworms and also green manure *M. pruriens*, were collected in a maize field near to the village “Tamulté de las Sabanas”, 30 km east of Villahermosa city. This particular agroecosystem has been continuously cultivated for the past 30 years, without irrigation, tillage or pesticides (Ortiz-Ceballos and Fragoso, in press). A sandy clay-loam Fluvisol [21] was collected from the A horizon (0–20 cm), air-dried and passed through a sieve (2–6 mm) before being screened for visible organic fragments and small native earthworms. It was stored in burlap bags until use. Some soil characteristics were: 4.5% organic matter, 0.25% total N; pH (H_2O) 5.7, 41.5% sand, 26.8% clay and 31.6% silt. Earthworms of the species *B. pearsei* were collected by hand-sorting from the soil. Juveniles of similar size and weight were separated and placed in containers filled with moisturized soil, transported to the laboratory, and stored at room temperature until use. Stems and leaves of *M. pruriens* (green manure) were collected and air-dried for 72 h and stored in bags until use. The experiment was conducted in a greenhouse of the Instituto de Ecología, A.C., Jalapa, Veracruz, from February 23 to August 3, 2002. A 2 × 2 factorial (the factors were *B. pearsei* and *M. pruriens*) in complete randomized design with ten replicates was used. Four treatments were studied: 1) soil without either *M. pruriens* or *B. pearsei* (-Mp & -Bp); 2) soil without *M. pruriens* nut with *B. pearsei* (-Mp & +Bp); 3) soil with *M. pruriens* and without *B. pearsei* (+Mp & -Bp); and 4) soil with both *B. pearsei* and *M. pruriens* (+Bp & +Mp). The pots were plastic containers (19 L) with holes covered with a fine mesh to allow free-drainage and to prevent the entry or exit of earthworms and plant roots. Twelve kg (air dry weight, DW) soil were placed in each plastic pot and watered to field moisture capacity (42% H_2O). On February 23, stems and leaves of *M. pruriens* (106 g DW equivalent to 15 Mg DW ha^{-1}) were added at the surface of each bucket of the corresponding treatment. One week after *M. pruriens* addition, 31 juveniles of *B. pearsei* were introduced to the buckets (equivalent to 438 individuals m^{-2}). Total worm fresh weight (FW) added to each pot was recorded. The initial weight of worms varied from 1.7 to 1.9 g (equivalent to 24–27 g m^{-2}), but the total initial earthworm abundance and biomass were not different between the two worm treatments (Tukey´s HSD test, $p \leq 0.05$). On April 3, five certified maize seeds (H-7375) were sown 2 cm deep in each pot. Two weeks after sowing, seedlings were thinned and only the most vigorous seedling was kept in the buckets and grown for 120 days without chemical fertilizer or pesticide. The buckets were kept clean by manual weeding every week. Plants were watered every second to fourth day to field moisture capacity (42%).

Plant height, air and soil temperature were recorded each week during the crop cycle. Plants in all buckets were harvested 120 days after sowing. The litter of *M. pruriens* remaining at the surface was removed, oven-dried (63ºC for 48 h) and weighed. *B. pearsei* were collected by hand-sorting the soil, counted, weighed (fresh), and freeze dried. Maize plants (roots, stalks, leaves, spikes, cobs and grain) were collected separately, oven-dried (6ºC, 72 h) and weighed. The soil in each bucket was mixed thoroughly, subsamples taken, and air-dried. Subsamples of soil, plant components, earthworms and litter were sieved (1 mm) and analysed for total N using a microKjeldahl method. Roots were separated manually by sorting the soil. A small amount of roots from still living plants were taken to assess vesicular-arbuscular mycorrhizal (VAM) colonization and vesicle formation [22]. Differences among treatments were determined using a general linear model with *B. pearsei* and *M. pruriens* as the main effects. Differences among means were determined using a Tukey´s HSD mean separation tests with a $p \leq 0.05$ significance level. All results were statistically analysed using the SAS system [18].

2.1.4. Experiment 4: Effect of Trichoderma inoculation and N fertilization on nitrogen uptake and fertilizer N recovery by maize (Zea mays L.*) grown in an Ultisol*

The objective of this greenhouse study was to assess the effect of different strains of the fungus *Trichoderma* on the uptake of ^{15}N-labelled urea by maize grown on an Ultisol using ^{15}N-isotopic techniques.

Maize cultivar VS-486 was sown at 2 cm deep in pots containing 10 kg of a strongly acidic Ultisol from the Tabasco savannah. Selected characteristics of the experimental soil are described above (Table I). Two seeds were sown per pot and one week after germination, only the most vigorous maize seedling was kept. Three *Trichoderma* strains were evaluated: (1) C4 strain, (2) C13 strain (donated by Dr. Alfredo Herrera from "Lab. Expresión Génica de Hongos", CINVESTAV-Irapuato Unit) and (3) a multi strain commercial product TRICOM (*Trichoderma harzianum* and *T. viridae*) (Tricom, Nocon, S.A. de C.V., Edo. México, México). The experiment was conducted at two levels of fertilization: (1) fertilized and (2) non-fertilized (*Trichoderma* strains alone), as well as uninoculated and a full control (uninoculated and non-fertilized). The experiment was laid down on August 23, 2004 and harvested on December 11, 2004. A completely randomized experimental design with four replicates was used and the experimental units were inoculated at a rate of 6 g inoculum pot^{-1} of a wheat-bran substrate containing approximately 2×10^4 conidia g^{-1}.

At sowing, the pots were fertilized with an equivalent rate of 80 kg P_2O_5 ha^{-1} as triple superphosphate and 40 kg K_2O ha^{-1} as KCl [2]. The urea fertilizer (commercial and ^{15}N-labelled fertilizer) was applied at a rate of 60 kg N ha^{-1}, which is equivalent to 50% of the recommended rate for maize in the region [2]. This N-fertilizer rate was split in two equal applications: (1) 50% N applied at sowing and (2) 50% N applied at 45 DAS. ^{15}N-urea containing 1 % atom ^{15}N excess was applied to pots in the same way as the commercial fertilizer. Pots were kept clean by manual weeding every week and irrigated approximately each 2–3 days with 450 mL pot^{-1} of distilled water. At physiological maturity (approximately 110 DAS), plants were harvested and fresh and dry shoot and root weight was determined. Total-N and $^{15}N/^{14}N$ isotopic ratio were analysed using the procedures described above in Exp. 1 [16]. Calculations for estimating the N-recovery in the plant from ^{15}N labelled fertilizer were performed according to Zapata [17]. In addition, the colonization of maize roots by *Trichoderma* was recorded according to Phyllips and Hayman [22]. Experimental data were statistically analyzed following standard procedures for analysis of variance, and the differences between mean values were determined at $p \leq 0.05$ by the HSD Tukey test, using the SAS system [18].

2.2. Field Experiments

The field experiments were carried out at the farm "Ejido Tierra Nueva 2nd Section" (93º 15′ N and 17º 30′ W), in the savannah of Huimanguillo, Tabasco, Mexico. The experimental area had been cultivated during the last ten years with maize using traditional management practives (three years under production and one year natural fallow).

The field experiments were conducted on a typical Ultisol (Table I) under rainfed conditions. The climate of the region corresponds to Am type according to Koppen system, i.e. warm humid with summer rainfall [2]. The average precipitation was 2,271 mm $year^{-1}$, of which 1,600 mm during the growing season and the average annual temperature of 26ºC [23] (Table II).

2.2.1. Experiment 5: N uptake and recovery from N-fertilizer by selected genotypes of maize (Zea mays L.*)*

In this experiment the objective was to assess the N uptake and recovery from the applied fertilizer by selected maize genotypes using the ^{15}N isotopic technique.

Five lines of maize, including three commercial lines recommended for acid soils: VS-486, VS-536, C-343, and and two wild types: WT_1 (sharp corncob) and WT_2 (wide corncob) were sown at a density of 62,500 plants ha^{-1}. These genotypes were selected on the basis of their good agronomic performance (Barron, 2001 personal communication). N-fertilizer application was equally split: (1) 50% at plant emergence (about 5 days afer sowing, DAS), and (2) 50% at 35 DAS. N uptake and recovery from each appplication were evaluated. The experiment was laid out on a randomized block experimental design with four replicates, except for the VS-536 genotype, which was used as a border crop and replicated fourteen times. The experimental plot had an area of 80 m^2 $treatment^{-1}$ $replicate^{-1}$ (10 rows of 0.8 m width and 10 m long) and the yield plot had 51.2 m^2 $treatment^{-1}$ $replicate^{-1}$ (8 rows of 0.8 m width and 8 m long). At germination, a basal fertilization of 80 kg P_2O_5 ha^{-1} as triple superphosphate and 40 kg K_2O ha^{-1} as KCl was made [2]. The experiment was sown on July 15–16, 2001 and harvested on December 8-10, 2001.

TABLE II. AVERAGE RAINFALL, TEMPERATURE AND EVAPORATION IN THE STUDY ZONE AT EJIDO TIERRA NUEVA

Month	Rain	Temperature		Evaporation
		Max.	Min.	
	(mm)	----------------(^oC)----------------		(mm)
January	95.7	28.4	14.2	2.8
February	210.2	29.5	17.7	4.3
March	18.5	32.5	16.1	4.9
April	29.7	35.9	33.7	6.1
May	99.3	34.7	19.7	5.3
June	134.6	35.2	20.6	6.4
July	230.8	35.3	19.7	5.5
August	210.3	31.5	19.8	3.9
September	402.0	34.7	19.7	5.3
October	363.2	31.5	19.5	4.6
November	246.3	30.3	16.9	8.2
December	230.9	27.8	17.1	7.4
	2271.5	32.3	19.6	64.7

Average values from the last 4 years.

Urea (commercial and ^{15}N-fertilizer) was applied at a rate of 120 kg N ha^{-1}, which is the recomended rate for the crop in the region [2]. N-fertilizer application was fractionated: (a) 50% N was applied at germination, and (b) the remaining 50% N was applied at 35 DAS. The ^{15}N-urea with 1% atom ^{15}N excess was applied to microplots of 6.4 m^2 stage^{-1} replicate^{-1} (four rows of 0.8 m width and 2 m long). At 120 DAS (physiological maturity), the fresh and dry grain and straw yield, total-N and ^{15}N/^{14}N ratio were determined following procedures similar to those described above. The experimental data were statistically analyzed following standard procedures for analysis of variance, and treatment mean differences were determined at $p \leq 0.05$ by HSD Tukey test, using the SAS system [18].

2.2.2. Experiment 6: Effect of mycorrhizal inoculation and P application as phosphate rock on yield and N uptake by maize

The objective of this experiment was to investigate the effects of mycorrhizal inoculation and P application as phosphate rock (PR) on yield and N-fertilizer uptake by maize. N fertilizer uptake and recovery were measured using ^{15}N isotopic techniques.

The VS-536 maize genotype was sown at a density of 62,500 plants ha^{-1} on the Ultisol described in Table I. The experimental treatments received a basal fertilization of 120 and 70 kg ha^{-1} of N and K_2O as urea and KCl, respectively. The N-fertilization rate was split in two applications: (1) at sowing, 50 kg N ha^{-1}, and (2) at 35 DAS, 70 kg N ha^{-1}. Three P application rates were studied: (1) 100 and (2) 200 kg P_2O_5 ha^{-1} as PR from Baja California, described above in Experiment 2, and (3) a control without P application. Mycorrizhae was studied at two levels: (1) mycorrhizal inoculation and (2) uninoculated control. The experimental design was a factorial of three P fertilizer application rates and two mycorrhizal treatments arranged on a randomized block design with four replicates. The experiment was sown on July 15–16, 2001 and harvested on December 8–10, 2001. The inoculum of mycorrizhal fungi (*Glomus intraradices* in sterilized soil and sorghum roots with 2 spores g^{-1} was applied at a rate of 1.5 kg inoculum ha^{-1}. Due to erratic rainfall at the start of the growing season, a single application of the urea fertilizer (70 kg N ha^{-1}) labelled with 1% atom ^{15}N excess was made at 35 DAS to the microplots of 6.4 m^2 $treatment^{-1}$ $replicate^{-1}$ (four rows of 0.8 m width and 2 m long). At 120 DAS (physiological maturity), the fresh and dry straw and grain yield, total-N and $^{15}N/^{14}N$ ratio were determined using the procedures described for Experiment 1. The experimental data were statistically analyzed following standard procedures for analysis of variance, and the treatment means differences were determined at $p \leq 0.05$ by HSD Tukey test, using the SAS system [18].

3. RESULTS AND DISCUSSION

3.1. Greenhouse experiments

3.1.1. Experiment 1: N uptake from N-fertilizer by transgenic lines of sorghum (Sorghum bicolor)

Significant differences ($p \leq 0.05$) in terms of dry matter yield were observed for the PR treatment (1.56 g dry matter pot^{-1}) versus the no PR treatment (0.87 g dry matter pot^{-1}) (Table III). Similar results were observed for other variables measured: total N uptake, percent Nitrogen derived from the fertilizer (%Ndff) and % N-fertilizer use efficiency. These results are in agreement with other studies that showed an increase in dry matter yield as a result of amending acid soils low in P with reactive PR [24].

In this study, the commercial line and the transgenic, negative-control line (L18-2 line), yielded the highest values of total-N and N derived from fertilizer. Most likely, this result is due to a general adaptative mechanism that increased the performance of these genotypes in acid soils. In contrast, wild-lines always accumulated the least N. In general, the % N fertilizer use efficiency or recovery values are rather low for a greenhouse experiment.

Under the experimental conditions, these preliminary results may indicate that there are no beneficial effects of citrate overproduction of the sorghum transgenic lines on N uptake from the fertilizer. It is important to keep in mind however that the lines tested were not genetically identical, that the experiment was of short duration and that the disturbance of the soil rich in organic matter in the greenhouse may have negatively affected their N-uptake from the fertilizer. Studies conducted on tobacco have shown that overproduction of organic acids results in better Al tolerance by lowering the penetration of Al into the roots, an effect that is not reflected in an increase of dry matter production [5].

3.1.2. Experiment 2: P uptake from phosphate rock by transgenic lines of sorghum (Sorghum bicolor)

Significant differences were observed ($p \leq 0.05$) for the factors “lines” and the “PR-lines” interactions (Table IV). No significant differences in dry matter and total P yield were found for the PR factor. Line L18-2 (CS-) showed the highest values (28.5%) of P derived from PR, whereas the commercial line showed the lowest values. Lines L8 (high citrate overproduction) and L15 (low citrate overproduction) showed similar results in terms of P derived from PR (22% and 19%, respectively). Under the experimental conditions, these preliminary results suggest that there are no beneficial effects of citrate overproduction in relation to the P uptake derived from PR. Other studies in acid soils have shown that plants secrete significant quantities of organic acids, yet do not acidify their rhizosphere. Nevertheless, their ability to access Al and Fe phosphates, which are the predominant forms of phosphates in acid soils, is enhanced [27].

TABLE III. DRY MATTER AND TOTAL N YIELD, AND NITROGEN UPTAKE FROM THE ^{15}N LABELLED FERTILIZER BY TRANSGENIC LINES OF SORGHUM GROWN UNDER GREENHOUSE CONDITIONS IN AN ACID ULTISOL FROM THE "SAVANNAH OF HUIMANGUILLO", TABASCO, MEXICO

Treatment	Dry Matter	Yield Total-N	N derived from Fertilizer	N-Fertilizer use efficiency
	------------------------(mg pot^{-1})†------------------------			-------(%)†-------
With Phosphate Rock:				
L8 (CS++)	1.42±0.16	40.1±3.6	25.4±2.7	14.1±1.5
L15 (CS+)	1.51±0.20	42.7±4.4	27.5±3.4	15.3±1.9
L18-2 (CS-)	1.63±0.12	48.4±3.8	30.6±2.9	17.0±1.6
Wild Type	1.34±0.19	36.5±5.1	23.4±3.4	13.0±1.9
Commercial	1.92±0.05	51.7±1.9	32.6±1.8	18.1±1.0
Without Phosphate Rock:				
L8 (CS++)	0.63±0.05	21.5±2.3	14.7±2.1	8.2±1.2
L15 (CS+)	0.61±0.04	19.0±1.3	12.0±0.8	6.7±0.5
L18-2 (CS-)	1.22±0.21	38.1±5.8	23.4±4.1	13.0±2.3
Wild Type	0.45±0.05	14.0±0.7	8.9±0.5	4.9±0.3
Commercial	1.45±0.38	38.3±8.4	27.5±6.0	15.3±3.4
CV (%):	14.46	12.4	14.0	14.02
HSD (p≤0.05)‡:				
P-Rock (PR)	0.11	2.8	2.1	1.1
Line (L)	0.17	4.4	3.2	1.8
PR x L	0.25	6.3	4.6	2.5
tc (p≤0.05):				
+PR vs. -PR:				
L8 (CS++)	13.53	21.50	17.80	17.6
L15 (CS+)	9.49	11.19	9.36	9.41
L18-2 (CS-)	2.51	2.25	2.10	2.12
Wild Type	9.18	8.31	8.70	8.73
Commercial	2.28	2.62	1.39	1.27

† Means values of four replicates ± SD, ‡ Significantly different at $p \le 0.05$ Tukey test; tc values ≥ 2.77 (5 degrees of freedom and p ≤ 0.05) are significantly different.

3.1.3. *Experiment 3: Effect of a native tropical earthworm and Mucuna pruriens added as green manure on the formation of mycorrhizal vesicles and maize yield*

The results of *B. pearsei* (Bp) and *M. pruriens* (Mp) treatments on mycorrizhal infection are shown in Table V. In this study it was found that *B. pearsei* alone and in combination with *M. pruriens* showed significative differences on hyphae and vesicles formation. As the earthworms use the soil fungus as food source, it was hypothesized that the earthworm could have an influence on the infection and activity of mycorrhizae in symbiosis with plants.

TABLE IV. DRY MATTER AND TOTAL P YIELD AND P UPTAKE FROM PHOSPHATE ROCK BY TRANSGENIC LINES OF SORGHUM GROWN UNDER GREENHOUSE CONDITIONS IN ACID SOIL FROM “SAVANNAH OF HUIMANGUILLO”, TABASCO, MEXICO

Treatment	Yield Dry Matter	 Total-P	 P derived from PR
	-------------------(mg pot^{-1})†	-------------------	----------(%)†----------
With Phosphate Rock:			
L8 (CS++)	1.49±0.27	6.4±1.1	21.7±6.4
L15 (CS+)	1.01±0.08	6.4±0.4	18.8±6.0
L18-2 (CS-)	1.11±0.23	7.4±1.6	28.5±4.1
Wild Type	1.56±0.18	10.3±1.2	20.1±3.5
Commercial	1.42±0.07	10.5±1.5	5.4±1.4
Without Phosphate Rock:			
L8 (CS++)	1.40±0.18	9.4±1.3	-
L15 (CS+)	1.35±0.28	9.0±2.0	-
L18-2 (CS-)	0.76±0.17	5.4±1.1	-
Wild Type	1.33±0.35	9.4±2.7	-
Commercial	1.20±0.11	8.6±1.1	-
CV (%):	16.74	18.4	33.4
HSD ($p \leq 0.05$)‡:			
P-Rock (PR):	NS	NS	-
Line (L):	0.22	1.6	12.4
PR x L:	0.31	2.2	-
tc ($p \leq 0.05$):			
+PR vs. -PR:			
L8 (CS++)	0.47	3.58	
L15 (CS+)	2.99	2.92	
L18-2 (CS-)	2.20	1.93	
Wild Type	1.26	24.00	
Commercial	6.38	1.91	

† Means values of four replicates ± SD, Significantly different at $p \leq 0.05$ Test Tukey; tc values ≥ 2.77 (5 degrees of freedom and $p \leq 0.05$) are significantly different.

TABLE V. EFFECT OF THE INTERACTION *M. pruriens* AND *B. pearsei* ON MYCORRHIZAL HYPHAE AND VESSICLE FORMATION IN MAIZE

Factor *M. pruriens*	 *B. pearsei*	Hyphaes	Vesicles
		(%)	
-	+	40 a	13 a
+	-	62 ab	32 ab
+	+	64 ab	45 b
-	-	84 b	35 b

Values in the column with different letters are statistically different (Tukey´s, $p \leq 0.05$).

In general, the results of this study show that *B. pearsei* reduce the mycorrhizal colonization of the plants. The control treatment (-*B. pearsei* and -*M. pruriens*) showed the highest values of colonization by mycorrhizae (84%) vs. –*M. pruriens* and +*B. pearsei* treatment (40% and 13% for hyphaes and vesicles, respectively). In addition, the

+*M. pruriens* and +*B. pearsei* showed similar values to the control (Table V). These results contrast with those from another study, which showed an increase in the mycorrhizal colonization [28].

In comparing the effect of the treatments on maize dry matter yield, the +Mp and +Bp treatments showed the highest values in terms of total biomass and grain yield (Table VI). The control -Bp & -Mp and +Bp & -Mp treatments showed the lowest dry matter produced and they were not significantly different.

TABLE VI. EFFECT OF *M. pruriens* AND *B. pearsei* ON MAIZE DRY MATTER YIELD

	Treatment			
Component	-Bp & +Mp	+Bp & +Mp	-Bp & -Mp	+Bp & -Mp
	g DW plant^{-1}			
Root	43.5 b	53.8 b	29.3 a	26.0 a
Culmo	88.0 b	79.7 b	78.2 b	59.9 a
Leaf	33.9 b	36.1 b	24.8 a	25.0 a
Spikle	2.3 b	2.2 ab	1.8 a	1.8 a
Joloche	19.9 b	21.0 b	13.6 a	14.2 a
Corncob	16.3 b	19.8 b	10.5 a	11.6 a
Grain	57.0 b	77.7 c	25.5 a	30.7 a
Biomass	260.6 b	290.2 b	183.5 a	169.2 a

Values in the column with different letters are statistically different (Tukey's, $p \leq 0.05$).

3.1.4. *Experiment 4: Effect of Trichoderma inoculation on N uptake by maize (Zea mays* L.*) grown on an Ultisoil*

The effects of *Trichoderma* inoculation on shoot, root and total biomass of maize and root colonization are shown in Table VII. The highest values, both in terms of shoot and root dry weight were obtained when *Trichoderma* inoculants were applied ($p \leq 0.05$). Biomass (shoot+root) production was also increased. The C13 strain plus N-Fertilizer treatment gave the highest biomass yield (60 g biomass plant^{-1}), however the biomass of the fertilized control was higher than that of the inoculated treatment alone. All treatments assayed showed a high root colonization of about 100%. The identification of inoculated and native strains of *Trichoderma* or other fungi is in progress. These results indicate a positive interaction between inoculation and the N fertilizer application. *Trichoderma* is known to reduce disease severity and enhance plant growth under field conditions [10].

The results of total-N uptake, N-fertilizer yield and N- fertilizer efficiency are shown in Table VIII. Significant differences were observed ($p \leq 0.05$) for the strain factor when ^{15}N-fertilizer was applied at both phenological stages. Although there were significant statistical differences ($p \leq 0.05$) in the effects of inoculation treatment (Table VII), differences in terms of total-N yield were not found. The N fertilizer yield and N- fertilizer use efficiency were slightly higher at sowing application than when fertilizer N was applied at 45 DAS. The C4 and Tricom plus N-fertilizer treatments showed the highest N-fertilizer yields (279 and 285 mg plant^{-1}, respectively) and N-fertilizer use efficiency (43 and 38%, respectively) when the ^{15}N-fertilizer was applied at sowing. The N-fertilizer use efficiency in the inoculated and fertilized treatments was 2.4 higher than the fertilized alone treatment. The use of ^{15}N isotopic techniques provided direct evidence that *Trichoderma* increases N uptake by maize. In general, most variables recorded in this study had a positive effect on yield and N uptake by maize inoculated with *Trichoderma*, indicating the feasibility of using *Trichoderma* inoculants on maize grown in acid soil conditions.

TABLE VII. EFFECT OF *Trichoderma* INOCULATION ON BIOMASS, SHOOT AND ROOT YIELD AND ROOT COLONIZATION OF MAIZE GROWN IN AN ULTISOL

Treatment	Shoot	Dry Weight Root (g plant^{-1})	Biomass	Colonization (%)
Control*	23.8±4.3	10.7±2.9	34.5±7.0	85
Fertilized	30.9±1.3	9.6±0.4	40.4±1.7	80
C4	29.5±4.0	8.9±1.5	38.5±5.1	100
C4+N-Fertilizer	39.4±0.7	17.6±6.7	56.9±7.1	100
C13	29.5±3.5	8.2±2.7	37.7±5.6	100
C13+N-Fertilizer	46.6±2.8	13.5±2.2	60.1±3.3	100
Tricom	27.2±5.2	6.6±2.0	33.7±7.1	100
Tricom+N-Fertilizer	40.4±2.2	8.0±2.4	48.4±2.0	100
CV (%):	9.9	30.0	12.4	ND
Probability P>F:				
Strains (S)	0.000	0.008	0.001	ND
Timing N fert. (F)	0.000	0.004	0.000	ND
S x F	0.034	0.024	0.048	ND
HSD Tukey (p≤0.05):				
Strains (S)	4.6	4.3	7.5	ND
Timing N fert. (F)	2.4	2.3	3.9	ND
S x F	4.9	4.5	7.9	ND
F x S	6.5	6.1	10.6	ND

*Treatment non-inoculated- and non-fertilized; ND=Not determined.

3.2. Field Experiments

3.2.1. *Experiment 5: N uptake and recovery from N-fertilizer by selected genotypes of maize (Zea mays* L.*)*

The grain yield data were calculated based on a harvest index of 0.37 [29] due to damages caused in the experimental field by rodents. Significant differences ($p \leq 0.05$) among the studied genotypes were observed (Table IX). The results of straw and grain yield of the genotypes showed statistically significant differences. The VS-486 and WT_2 (wide corncob) genotypes had the highest straw yield values (3,215 and 2,956 kg straw ha^{-1}, respectively). However, the WT_2 (wide corncob) genotype showed heterogeneous growth because this genotype is not genetically stable. The grain yield estimates are higher than the mean regional yield 1.5 Mg ha^{-1} [30].

TABLE VIII. EFFECT OF *Trichoderma* ON TOTAL N YIELD AND N FERTILIZER YIELD AND N-FERTILIZER USE EFFICIENCY OF MAIZE GROWN IN AN ULTISOL

Treatment	Growth stage (das)	Total-N (mg plant^{-1})	N-fertilizer yield (%)	N-fertilizer yield (mg plant^{-1})	N-fertilizer use efficiency (%)
Control*		90±13			
Control Fertilized	0	409±56	26±1.0	112±20	17
	45	356±66	30±0.9	106±26	16
C4+N-Fertilizer	0	516±29	54±0.9	290±11	43
	45	449±69	32±0.1	145±22	22
C13+N-Fertilizer	0	519±27	49±0.5	252±13	38
	45	503±84	45±0.6	227±20	35
Tricom+N-Fertilizer	0	501±33	57±1.0	286±20	44
	45	448±64	36±0.5	160±32	25
CV (%):		13.6	1.9	12.6	13
Probability P>F:					
Strains (S)		0.001	0.000	0.000	0.000
Timing N fertilizer (F)		0.069	0.000	0.000	0.000
S x F		0.839	0.000	0.000	0.000
HSD Tukey ($p \leq 0.05$):					
Strains (S)		85.9	1.1	34.0	5.2
Timing N fertilizer (F)		NS	0.6	18.0	2.8
S x F		NS	1.2	36.0	5.5
F x S		NS	1.5	48.1	7.4

* Treatment non-inoculated and non-fertilized; NS=Not significant difference (Tukey´s, $p \leq 0.05$).

TABLE IX. STRAW AND GRAIN YIELD BY MAIZE GENOTYPES GROWN IN AN ULTISOIL OF THE SAVANNAH "HUIMANGUILLO", TABASCO STATE, MEXICO

Genotype	Yield: Straw (Mg ha^{-1})†	Yield: Grain (Mg ha^{-1})†	Yield: Total (Mg ha^{-1})†
VS-486	3.22a	2.35a	5.57
C-343	1.73c	1.26c	2.99
VS-536	2.79b	2.03b	4.82
WT_1 (sharp corncob)	2.69b	1.97b	4.66
WT_2 (wide corncob)	2.96ab	2.16ab	5.12
Mean:	2.71	1.98	
CV (%):	7.5	7.5	
Probability P>F:	0.01**	0.01**	
HSD ($p \leq 0.05$):	0.43	0.31	

† Values with different letters in the column are statistically different (Tukey´s, $p \leq 0.05$); ** means values statistically different at $P<0.01$.

Total N uptake, fertilizer N yield and N fertilizer use efficiency of the maize genotypes for the two split application of fertilizer N are given in Table X. Significant differences were observed ($p \leq 0.05$) for the genotypes when ^{15}N-fertilizer was applied at both phenological stages.

TABLE X. TOTAL NITROGEN UPTAKE AND N-FERTILIZER USE EFFICIENCY BY MAIZE GENOTYPES GROWN IN AN ULTISOIL OF THE SAVANNAH "HUIMANGUILLO", TABASCO STATE, MEXICO

Genotype	Yield Total-N	N-Fertilizer	N-fertilizer use efficiency
	---------------(kg ha^{-1})---------------		(%)
	1st N-fertilizer application (60 kg N ha^{-1} at seedling)		
VS-486	32.2a	4.6a (15)	9.3a
C-343	13.1c	2.0c (15)	3.9c
VS-536	20.9bc	3.0bc (14)	5.9bc
WT_1 (sharp corncob)	25.4ab	4.4a (18)	8.8a
WT_2 (wide corncob)	29.2a	4.1ab (14)	8.3ab
CV (%):	16.5	19.6	19.9
Probability P>F:	0.0001**	0.0002**	0.0003**
HSD (p≤0.05):	7.9	1.4	2.8
	2nd N-fertilizer application (60 kg N ha^{-1} at 35 das)		
VS-486	29.6a	5.1a (17)	7.3a
C-343	14.2b	1.9c (13)	2.6c
VS-536	22.2ab	3.4b (15)	4.9b
WT_1 (sharp corncob)	23.8ab	3.8ab (16)	5.4ab
WT_2 (wide corncob)	27.6a	3.6b (14)	5.2ab
CV (%):	22.7	20.1	20.0
Probability P>F:	0.0106**	0.0006**	0.0006**
HSD (p≤0.05):	10.9	1.5	

† Values in the column with different letters are statistically different (Tukey´s, p ≤ 0.05); ** means values statistically different at P<0.01; Values in brackets are the % N derived from fertilizer.

The VS-486 genotype showed highest values of total-N (32 kg N ha^{-1}), N-fertilizer (4.6 kg N ha^{-1}) and N-fertilizer use efficiency (9.3%) when the ^{15}N-fertilizer was applied at sowing. Similar results were obtained for this genotype at 35 DAS, whereas the C-343 genotype showed the lowest values of these variables. N Fertilizer use efficiency of the first split N-fertilizer application (at sowing) was higher than that of the split applied at 35 DAS. Although the N-fertilizer use efficiency obtained for both split applications in this study were in general low, they are similar to those reported in other studies conducted in tropical areas [31]. Further investigations aiming at increasing the N-fertilizer use efficiency are needed.

3.2.2. *Experiment 6: Effect of mycorrhizal inoculation and P application as phosphate rock on yield and N-uptake by maize*

The effects of mycorrhizal inoculation and PR application on straw and grain yield of maize are shown in Table XI. The highest grain and straw yields were obtained in the treatment with no mycroorhizal inoculation (-mycorrhizae) and PR application at 100 kg P_2O_5 ha^{-1}. No significant differences in maize yield were found between the control and the highest PR rate. Both straw and grain yield data were signficantly lower in the inoculated treatments than those of the uninoculated ones. The same mycorrhizal inoculum was tested in greenhouse conditions using different soils and no positive effects were observed. It is very likely that the producers did not monitor the quality of the commercial inoculum utilized (Peña-Cabriales and Vera-Núñez, 2000 unpublished data). Other studies conducted in these soils have shown a positive effect of the reactive PR from Baja California on corn yield [13, 32].

TABLE XI. EFFECT OF P FERTILIZATION AS PHOSPHATE ROCK AND MYCORRHIZAL FUNGI INOCULATION ON GRAIN AND STRAW YIELD OF MAIZE GROWN IN AN ULTISOIL OF THE SAVANNAH “HUIMANGUILLO”, TABASCO STATE, MEXICO

Factor	P-Rate ($kg\ P_2O_5\ ha^{-1}$) 0	100	200	Mean†
		Grain yield --------------------($kg\ ha^{-1}$)--------------------		
-Mycorrhizae	2,266	3,641	2,172	2,693a
+Mycorrhizae	1,657	2,031	2,188	1,959b
Mean†	1,961b	2,836a	2,180b	
CV (%):		19		
Probability P>F:				
Mycorrhizae (M)		0.001**		
P-Rate (P)		0.004**		
M x P		0.009**		
HSD (p≤0.05):				
Mycorrhizae (M)		391		
P-Rate (P)		583		
M x P		825		
		Straw yield --------------------($kg\ ha^{-1}$)--------------------		
-Mycorrhizae	3,399	5,462	3,259	4,040a
+Mycorrhizae	2,485	3,047	3,283	2,938b
Mean†	2,942b	3,992a	3,270b	
CV (%):		8		
Probability P>F:				
Mycorrhizae (M)		0.001**		
P-Rate (P)		0.004**		
M x P		0.009**		
HSD (p≤0.05):				
Mycorrhizae (M)		587		
P-Rate (P)		876		
M x P		1239		

† Values with different letters within the column (mycorrhiza) and the row (P rates) are statistically different (Tukey´s, p ≤ 0.05); ** means values statistically different at P<0.01.

Significant differences were observed (p ≤ 0.05) on the effect of mycorrhizae and P application rate as phosphate rock (PR) from Baja California on N-uptake. When the mycorrhizal inoculation was applied, total N and N fertilizer yield and the N-fertilizer use efficiency (in grain and straw) were lower than in the non-mycorrhizae treatment (Table XII). This is contrary to the results obtained with legumes, where mycorrhizae seem to enhance BNF and increase yields [9].

TABLE XII. EFFECT OF P FERTILIZATION, AS PHOSPHATE ROCK AND MYCORRHIZAL FUNGI INOCULATION ON TOTAL N AND FERTILIZER N YIELD AND FERTILIZER N USE EFFICIENCY IN GRAIN AND STRAW OF MAIZE GROWN IN AN ULTISOL OF THE SAVANNAH "HUIMANGUILLO", TABASCO STATE, MEXICO

Treatment	Yield				
	Total-N		N-Fertilizer		N-Fertilizer use efficiency
	----------------(kg ha^{-1}) ----------------				----(%)†----
	Grain	Straw	Grain	Straw	
-Mycorrhizae:					
0 P (Control)	31.4	27.2	6.6	6.6	24.5
100 P	53.0	48.4	12.5	12.5	46.3
200 P	30.8	29.9	7.3	7.3	29.4
+Mycorrhizae:					
0 P	24.4	25.5	5.4	5.4	25.3
100 P	28.9	26.5	6.6	6.6	26.0
200 P	31.7	25.9	7.3	7.3	26.6
CV (%):	25	31	26	26	27
Probability P>F:					
Mycorrhizae (M)	0.01**	0.09**	0.011**	0.011**	0.04**
P-Rate (P)	0.02**	0.20**	0.009**	0.009**	0.04**
M x P	0.03**	0.03**	0.024**	0.024**	0.04**
HSD (p≤0.05):					
Mycorrhizae (M)	7.4	7.4	1.7	1.7	7.1
P-Rate (P)	11.1	11.1	2.6	2.6	10.5
M x P	15.7	15.7	3.7	3.7	14.9

† Total N-fertilizer use efficiency (grain+straw); ** means values statistically different at P<0.01.

4. CONCLUSIONS

In greenhouse experiments using ^{15}N and ^{32}P isotopic techniques sorghum transgenic lines (citrate overproducers) did not show superior N and P use efficiency in an Ultisol. Combined application of earthworms (*B. pearsei*) and the legume green-manure mucuna (*M. pruriens*) enhanced both N- and P-uptake by maize and it could be considered as a promising agricultural practice to increase maize yield in the acid savannah soils of Tabasco.

From the studies on N uptake by maize, the genotypes VS-536, VS-486, H-Z30 and the wild type "Dosmesano" showed better fertilizer N-efficiency and increased yield when 100 kg P_2O_5 ha^{-1} of the reactive Baja California PR are applied. Interestingly, mycorrhizal inoculation did not have a positive effect when the maize crop was fertilized.

Differences among *Trichoderma* strains were found. Strains C4 and Tricom plus N-fertilizer treatments produced the highest values of N-fertilizer yield and % N-fertilizer use efficiency when the N-fertilizer was applied at the seedling stage. The % N-fertilizer use efficiency in the inoculated and fertilized treatments was 2.4 higher than the fertilized alone treatment.

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IMPROVING AGRICULTURAL PRODUCTIVITY IN THE SAVANNAH OF TABASCO STATE, MEXICO: II. MANAGEMENT OF NITROGEN-FIXING LEGUMES

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Abstract

A series of experiments was carried out in a strongly acidic (with high Al and Fe) and infertile Ultisol to enhance the N inputs derived from biological nitrogen fixation (BNF) through the legume-*Rhizobium* symbiosis and to draw recommendations on the best agronomic practices needed to intensify sustainable maize production of the subtropical savannahs of Mexico. A large diversity of native *Rhizobium* strains with variable N fixation capacity exists for use with legume species of agricultural interest in the savannah of Tabasco. Of the black common bean genotypes evaluated, "N. Sahuatoba, DOR 448, TPL 18 and TPL 16" showed the best potential in terms of BNF (% NdfA and fixed N) and they can be recommended for the savannah of Tabasco. *Cajanus cajan* and *Canavalia ensiformis* are the multipurpose legumes with greatest potential in terms of BNF and biomass yield, specially when P fertilized, for the acid Ultisols of the Tabasco region. These legumes can be grown in monoculture and in association with maize and they can be recommended as green manures to reduce the need for chemical N fertilizers. The growth, biomass production and recovery of fertilizer N by selected maize genotypes were better when grown as monoculture than in association with legumes. The wild type "Dosmesano" was superior to the other maize genotypes tested. Further studies are needed to increase the N inputs from the *Rhizobium*-legume symbiosis to contribute to improving productivity and sustainability of the agricultral production on the acid savannah soils of Mexico.

1. INTRODUCTION

Nitrogen (N) is the key production factor but also the most mobile and the most easily depleted nutrient in cultivated soils of the savannah of Mexico. Due to crop intensification, long fallow periods to restore the organic matter and N status are no longer sustainable. Rather, N fertilizer or biological sources of N are required for optimal plant growth and food and fiber production [1]. As N fertilizer is expensive and often beyond the means of smallholders, efforts need to be made to either increase the efficiency of N fertilizer usage or to develop integrated N management systems where the N inputs from organic residues and biological N fixation are included.

Biological nitrogen fixation (BNF) is an important process in N cycling in natural and agroecosystems. It is the main natural mechanism by which the gaseous form of the element (N_2) is converted into forms readily available to plants. Thus, the N inputs from BNF represent an important source of the N for increasing and maintaining crop yields. Amongst the many BNF processes, the symbiosis between legumes and root nodule-bacteria has received particular attention due to the significance of these plants to world's food, feed and protein production. Legumes are found throughout the world, but the greatest variety grows in the tropics and subtropics, and there is a wealth of untapped legume germplasm potential. This is demonstrated in Mexico, where villages often have their own varieties of common bean (*Phaseolus vulgaris*) [2, 3].

Part II of this work reports on a series of studies conducted to assess BNF and enhance the N inputs derived from the legume-*Rhizobium* symbiosis in various ways and to draw recommendations on the best management practices of the nitrogen-fixing legumes to intensify sustainable agricultural production of the subtropical savannahs of Mexico.

2. MATERIALS AND METHODS

A series of greenhouse and field experiments were carried out in a strongly acidic Ultisol from the savannah of Huimanguillo, Tabasco state, Mexico [4]. Selected characteristics of the experimental soil are shown in Table I. This soil has high organic matter (5.2%) because the microbial activity is low at ph 4.6 [5]. The base (Na, K, Ca and Mg) contents are considered low [6].

TABLE I. CHEMICAL AND PHYSICAL CHARACTERISTICS OF THE ULTISOIL

Characteristic	Content
Textural class	Sandy loam
Clay (%):	21
Silt (%):	11
Sand (%):	68
pH_{water} (1:2):	4.6
Organic matter (%):	5.2
Available P-Olsen (mg kg^{-1} soil):	6.0
Exch. Cations ($cmol_+$ kg^{-1} soil):	
K	0.30
Ca	2.20
Mg	0.50
Na	0.04
Cat. Exch. Cap. ($cmol_+$ kg^{-1} soil)	8.20

2.1. Greenhouse experiments

2.1.1. Experiment 1: Isolation of native Rhizobium strains from selected legume species

Since the field experimental site is distant from our laboratory (approximately 1700 km), a preliminary experiment was conducted on the typical Ultisol described above (Table I) at the greenhouse of the CINVESTAV-Irapuato Unit from July 15 to October 30, 2002 to isolate native *Rhizobium* strains from multi-purpose legume species grown in the savannah of Tabasco. The legume species studied were (local names in Spanish are given in brackets): (1) *Mucuna deeringiana* (Mucuna blanca), (2) *Canavalia ensiformis* (Frijol espada), (3) *Vigna umbellata* (Frijolin), (4) *Cajanus cajan* (Gandul), (5) *Dolichos lablab*

(Dolicos), (6) *Arachis pintoi* (Cacahuatillo) and (7) *Crotalaria juncea* (Crotalaria). Seeds of these legumes were sown in pots containing 12 kg Ultisol soil, each legume species as experimental units under a complete randomized design with 10 replicates. The pots were watered with distilled water every 3–4 days to maintain the soil humidity at 75% of field capacity.

At the pre-flowering stage, all nodules from each legume species (10 plants) were collected and stored in tubes [7]. Approximately 20% of these nodules were processed using standard methods [7] and the *Rhizobium* strains were isolated and characterized in terms of antibiotic resistance [8]. Multidisks (Multidisco^{TM}, Sanofi) for Gram-negative bacteria with 12 antibiotics were used: (1) Amikamicine (30 µg), (2) Ampicillin (10 µg), (3) Cephalothin (30 µg), (4) Ceftriaxone (30 µg), (5) Chloranphenicol (30 µg), (6) Dicloxacillin (1 µg), (7) Enoxacin (10 µg), (8) Erythromycin (15 µg), (9) Gentamycin (10 µg), (10) Netilmicin (30 µg), (11) Penicillin (10 U) and (12) Trimetoprim-Sulphametoxazol (25 µg). Each isolate was plated out on yeast-mannitol-agar (YMA) with the multidisks and incubated at 29^{o}C for 24–48 h, before the antibiotic resistance/susceptibility patterns were recorded [9].

2.1.2. Experiment 2: BNF potential of black common bean genotypes (Phaseolus vulgaris L.*) grown in an Ultisol*

The BNF capacity of common bean genotypes of black grain color was assessed using the ^{15}N-isotopic dilution technique ("A"-value approach). Sorghum (*Sorghum bicolor*) and a non-nodulating common bean cv. NN DOR 364 were used as the reference crops [10]. The experiment was conducted in a greenhouse at the CINVESTAV-Irapuato Unit, from February 12 to June 1, 2004. Twenty eight black common bean genotypes obtained from the common bean breeding program of the Postgraduate College were sown in pots containing 4 kg of soil (Ultisol) from the Tabasco savannah (Table I). To avoid problems of cross-contamination, the treatments were set up in two sections of the greenhouse (Exp. A: 1–13 genotypes and Exp. B: 14–28 genotypes). Two *Rhizobium* inoculants and a control were evaluated: (1) A cocktail of BR-835, CFN-299 and BR-864 strains; (2) *R. tropici* strain CIAT-299 (donated by Dra. Esperanza Martinez from CIFN-UNAM) and (3) A non-inoculated control. In case of the second inoculant, the common bean genotypes were inoculated with a suspension containing 10^9 CFU mL^{-1} of *Rhizobium tropici* growing in yeast-mannitol (YM) liquid media (1 mL pot^{-1}). The experimental design was completely randomized with four replicates. The pots were irrigated every third day with 70 mL pot^{-1} of distilled water. At sowing, the common bean genotypes and reference crops were fertilized with 80 kg P_2O_5 ha^{-1} as triple super phosphate and 40 kg K_2O ha^{-1} as KCl. The legume and reference crops received 20 and 60 kg N ha^{-1} respectively as ^{15}N labeled urea (10 atom % ^{15}N excess), which was applied in solution [11]. At the grain ripening stage, the fresh shoot weight was determined, then the shoots were oven dried at 70^{o}C for 72 h. Total-N (1016 digestion and 1002 distilling units, TECATOR) and ^{15}N/^{14}N ratio were determined by the Kjeldahl method and Rittenberg oxidation using sodium hypobromite [12] and an optical emission spectrometer (NOI6-e PC, FAN) respectively [13]. Calculations for estimating the N-recovery using ^{15}N labeled fertilizer and N-derived from atmosphere (% NdfA) was performed using the "A"-value method [10]. The experimental data were statistically analyzed following standard procedures for analysis of variance, and the treatment means differences were determined at $p \leq 0.05$ by HSD Tukey test, using SAS system [14].

2.2. Field Experiments

The field experiments were conducted at the farm "Ejido Tierra Nueva 2$^{nd.}$ Section" (93º 15′ N and 17º 30′ W), in the savannah of Huimanguillo, Tabasco, Mexico. The experimental area had been cultivated during the last ten years with maize under a traditional management cycle (three years under production and one year fallow). The field experiments were conducted on a typical Ultisol (Table I) under rain fed conditions. The climate of the region corresponds to Am type according to the Koppen system, i.e. warm humid with summer rainfall [15]. The average precipitation was 2,271 mm year^{-1} and average annual temperature was 26^{o}C [16] (Table II).

TABLE II. AVERAGE RAINFALL, TEMPERATURE AND EVAPORATION IN THE EXPERIMENTAL AREA

Month	Rain (mm)	Temperature Max.	Temperature Min.	Evaporation (mm)
		----------------(°C)----------------		
January	95.7	28.4	14.2	2.8
February	210.2	29.5	17.7	4.3
March	18.5	32.5	16.1	4.9
April	29.7	35.9	33.7	6.1
May	99.3	34.7	19.7	5.3
June	134.6	35.2	20.6	6.4
July	230.8	35.3	19.7	5.5
August	210.3	31.5	19.8	3.9
September	402.0	34.7	19.7	5.3
October	363.2	31.5	19.5	4.6
November	246.3	30.3	16.9	8.2
December	230.9	27.8	17.1	7.4
	2271.5	32.3	19.6	64.7

Average values from the last 4 years

2.2.1. Experiment 3: BNF in herbaceous legumes for multiple uses in agricultural production systems

A field experiment using the ^{15}N-isotopic dilution technique (“A”-value approach) [10] was carried out to assess the BNF potential of several herbaceous legumes for multiple uses (Table III) in agricultural production systems of the savannah from Tabasco State, Mexico.

TABLE III. LEGUMES AND REFERENCE CROPS USED IN THIS STUDY

Species	Common name (Spanish)
Legumes:	
Mucuna deeringiana	Mucuna blanca
Canavalia ensiformis	Frijol espada
Vigna umbellata	Frijolin
Cajanus cajan	Gandul
Dolichos lablab	Dolicos
Arachis pintoi	Cacahuatillo
Crotalaria juncea	Crotalaria
Reference crops:	
Zea mays	Commercial line VS-486
Zea mays	Commercial line VS-536
Sorghum bicolor	Cultivar Esmeralda

Species		Common name (Spanish)
	Phaseolus vulgaris, Non-Nod.	Variedad No Nodulante DOR 364
	Brachiaria brizantha	Cultivar Insurgentes
	B. decumbens	Cultivar Chontalpo
	B. humidicula	Cultivar Humidicula

The experiment was laid-down using a split plot experimental design with four replicates under field conditions. The main plots were assigned to P fertilizer rates and the subplots to legumes and reference crops. At one-two weeks after sowing the legumes, maize, and sorghum; and at two months after transplanting the *Brachiaria* grasses, the crops were fertilized with two P levels: (1) no fertilized control and (2) 80 kg P_2O_5 ha^{-1} as triple super phosphate. In addition, all treatments were fertilized with 40 kg K_2O ha^{-1} as KCl. ^{15}N labeled urea was applied at 80 kg N ha^{-1} (1 atom % ^{15}N excess) and 25 kg N ha^{-1} (10 atom % ^{15}N excess) to micro plots of 3.6 m^2 $treatment^{-1}$ $replicate^{-1}$ (three rows of 0.8 m width and 1.5 m long) for reference (non-fixing) and legumes (fixing) crops respectively. The experimental plot was 48 m^2 $treatment^{-1}$ $replicate^{-1}$ (ten rows of 0.8 m width and 6 m long) and the yield plot of 34.8 m^2 $treatment^{-1}$ $replicate^{-1}$ (eight rows of 0.8 m width and of 4 m long).

At the following phenological stages: (1) pre-flowering, (2) flowering, and (3) grain ripening, the fresh shoot and grain yield, total-N and $^{15}N/^{14}N$ ratio (the two last parameters were only measured at stage (3) were determined with similar procedures to those described above for Exp.2. Acetylene reduction activity was also measured at these phenological stages [17]. Legume and reference crop species were sown on July 16, 2002, except the grasses *Brachiaria brizantha*, *B. decumbens* and *B. humidicula,* which were planted on June 1st, 2002. Harvest of maize was made on December 12, 2002 whereas the legumes species were harvested on January-February, 2003. The experimental data were statistically analyzed following standard procedures for analysis of variance, and the difference between mean values was determined at p≤0.05 by HSD Tukey test, using the SAS system [14].

2.2.2. Experiment 4: BNF in multiple use herbaceous legumes grown in monoculture and in association with maize crop

The main objectives were: 1) to evaluate the biological nitrogen fixation in three legume species *Canavalia ensiformis* (L_1) *Cajanus cajan* (L_2) and *Mucuna deeringiana* (L_3) selected for their good nodulation and N-fixation in previous experiments when grown in monoculture and in association with maize and 2) to compare the N uptake and N fertilizer use efficiency by maize genotypes VS-536 (M_1), a wild type “Dosmesano” (M_2) and H-Z30 (M_3) grown in monoculture and associated with legumes (Table IV).

TABLE IV. EXPERIMENTAL TREATMENTS OF THE MAIZE GROWN IN MONOCULTURE AND IN ASSOCIATION WITH LEGUMES

Monoculture		Associated*		
Maize**:	Legumes:			
VS-536 (M_1)	*C. ensiformis* (L_1)	M_1+L_1	M_1+L_2	M_1+L_3
WT “Dosmesano” (M_2)	*C. cajan* (L_2)	M_2+L_1	M_2+L_2	M_2+L_3
H-Z30 (M_3)	*M. deeringiana* (L_3)	M_3+L_1	M_3+L_2	M_3+L_3
	P. vulgaris non nod DOR 364 (R_1)			

* Intercropped/associated crops. ** In addition to R_1, the maize genotypes were also used as reference crops.

The maize and legume crops were sown at a density of 40,000 and 13,300 plants ha^{-1}, respectively in both systems. At 5-15 days after sowing, the legumes, maize, and reference crops were fertilized with 80 kg P_2O_5 ha^{-1} as triple super phosphate and 40 kg K_2O ha^{-1} as KCl. The legumes and reference crops were fertilized with 20 and 60 kg N ha^{-1} as urea, respectively. ^{15}N-urea fertilizer was applied at 20 kg N ha^{-1} with 10 atom % ^{15}N excess and 60 kg N ha^{-1} with 1 atom % ^{15}N excess to micro plots of 4.5 m^2 $treatment^{-1}$ $replicate^{-1}$ (three rows of 1 m width and 1.5 m long) for the legume and reference crops respectively. The experimental plot was of 60 m^2 $treatment^{-1}$ $replicate^{-1}$ (ten rows of 1 m width and 6 m long) and a yield plot of 32 m^2 $treatment^{-1}$ $replicate^{-1}$ (eight rows of 1 m width and of 4 m long). The experimental design was a randomized block with four replicates. The experiment was sown under field conditions on August 2003 and harvested on June 2004. When the maize was mature (at about 120 days after sowing, DAS) and when the legumes flowered (at 140 DAS), the fresh and dry matter (shoot and grain) yield was determined. Procedures similar to those described previously (Exp. 2) were followed for the analyses of total-N and $^{15}N/^{14}N$ isotopic ratio.

3. RESULTS AND DISCUSSION

3.1. Greenhouse experiments

3.1.1. Experiment 1: Isolation of native strains of Rhizobium from selected legume species

One hundred and fifteen rhizobial isolates were obtained from the legumes studied. Based on their antibiotic resistence/susceptibility, these isolates were grouped into ten different patterns, approximately two patterns per legume species (Table V).

TABLE V. ANTIBIOTIC RESISTENCE/SUCEPTIBILITY PATTERNS OF *Rhizobium* ISOLATES FROM THE LEGUME SPECIES STUDIED

Legume species	Nodules number (No. $plant^{-1}$)*	Isolates (No.)	Pattern
Arachis pintoi	696	1	Ara-I (1)
Cajanus cajan	116	18	Caj-II (13)†
			Caj-III (5)
Canavalia ensiformis	228	6	Can-IV (2)
			Can-V (4)
Crotalaria juncea	76	17	Cro-VI (5)
			Cro-VII (12)†
Vigna umbellata	104	24	Vig-VIII (5)
			Vig-IX (19)†

* Average values of four plants sampled; † Dominating pattern; Roman number means pattern code assigned arbitrary; Arabic numbers in brackets represent the isolates per pattern.

As there is only limited information on the diversity of *Rhizobium* for these legumes [18], the results of this study provide useful information on the availability of native strains with variable nitrogen fixation capacity in symbiosis with legume species of agricultural interest.

3.1.2. Experiment 2: BNF potential of black common bean genotypes (Phaseolus vulgaris L.*) sown in an Ultisol*

The results of biomass, total N yield and N derived from the atmosphere (%NdfA) and N fixed (mg plant^{-1}) are shown in the Tables VI and VII for the genotypes 1–13 and 14–28 respectively. Significant differences were observed between the twenty-eight bean genotypes tested. The bean cultivars included in this study were genotypes adapted to the savannah conditions. The estimated values of % NdfA, using the non-nodulating NN DOR 364 as reference crop, ranged from 0 to 46%. Sahuatoba genotype showed the highest % NdfA values in all treatments (45% in average). However, average N fixation capability of the studied common bean genotypes can be considered low, compared to other grain legumes. In fact, more than 50% of the tested genotypes showed % NdfA values lower than 25%. In the control treatment (nodulated by native rhizobia) DOR 488, TPL16 and TPL18 genotypes showed a relatively high % NdfA (38% on the average). Inoculation with a cocktail of rhizobial strains only increased the % NdfA in the TPL-20, N. Altiplano and N. Cotaxtla genotypes whereas inoculation with the CIAT-299 strain increased the % NdfA in the gentoypes N. Huasteco 81, N-Nico/UCR-55 and N.8025. This low success from the inoculation could be likely due to ecological interactions with native *Rhizobium* strains as demonstrated in different studies carried out in Mexico. These results highlight the need to conduct follow-up studies on the relative influence of the main limiting factors (biotic and abiotic) that influence BNF in acid soils.

TABLE VI. BIOMASS, TOTAL N YIELD AND %NDFA IN VARIOUS BLACK COMMON BEAN GENOTYPES GROWN IN AN ULTISOL (EXP. A)

Genotype	Inoculum	Biomass	NdfA	Total-N yield	N-Fixed
		(mg plant^{-1})	(%)	(mg plant^{-1})	
1. Jamapa	Cocktail	587	24	18	4
	CIAT-299	682	28	24	7
	Control	387	23	12	3
2. N-Veracruz	Cocktail	281	26	9	2
	CIAT-299	660	11	21	2
	Control	325	21	10	2
3. N-Cotaxtla	Cocktail	521	38	18	7
	CIAT-299	709	22	24	5
	Control	537	30	18	6
4. N-Tacana	Cocktail	907	32	27	9
	CIAT-299	834	22	25	5
	Control	596	34	20	7
5. N-Tropical	Cocktail	508	23	18	4
	CIAT-299	422	31	14	5
	Control	309	24	13	3
6. N-Medellín	Cocktail	431	26	17	5
	CIAT-299	440	25	14	5
	Control	406	30	14	4
7. N-Nico/UCR-55	Cocktail	537	30	17	5
	CIAT-299	686	37	22	8
	Control	750	26	25	7
8. DOR-448	Cocktail	453	33	15	5
	CIAT-299	647	25	20	5

Genotype	Inoculum	Biomass (mg plant^{-1})	NdfA (%)	Total-N yield	N-Fixed
				(mg plant^{-1})	
	Control	401	39	13	5
9. DOR-454	Cocktail	309	25	12	3
	CIAT-299	701	27	20	5
	Control	697	33	26	9
10. N.8025	Cocktail	846	25	30	8
	CIAT-299	722	38	22	8
	Control	1217	29	39	11
11. ICTA Ligero	Cocktail	455	25	17	4
	CIAT-299	554	21	19	4
	Control	871	23	28	6
12. ICTA JU 93-15	Cocktail	484	16	15	2
	CIAT-299	523	35	15	5
	Control	539	31	16	5
13. ICTA JU 95112	Cocktail	576	14	18	2
	CIAT-299	644	12	17	2
	Control	618	21	19	4
	Probability P>F:				
	Genotype (G)	0.000	0.000	0.007	0.000
	Inoculum (I)	0.329	0.004	0.226	0.004
	G x I	0.410	0.000	0.455	0.126
	HSD (Tukey p≤0.05:				
	G	463.43	11.97	16.43	5.52
	I	N.S.	2.68	N.S.	1.24
	G x I	N.S.	13.70	N.S.	N.S.
	I x G	N.S.	20.73	N.S.	N.S.

NS = Not statistically significant; NdfA = Nitrogen derived from the atmosphere

TABLE VII. BIOMASS, TOTAL N YIELD AND %NDFA IN VARIOUS BLACK COMMON BEAN GENOTYPES GROWN IN AN ULTISOL (EXP. B)

Genotype	Inoculum	Biomass (mg plant^{-1})	NdfA (%)	Total-N yield	N-Fixed
				(mg plant^{-1})	
14. TLP-16	Cocktail	1131	22	33	7
	CIAT-299	857	19	24	5
	Control	862	38	28	10
15. TLP-18	Cocktail	772	27	25	7
	CIAT-299	315	26	11	3
	Control	629	38	23	9
16. TLP-20	Cocktail	524	32	18	6
	CIAT-299	498	26	19	5
	Control	658	24	22	5
17. NEPA 68	Cocktail	597	8	21	2
	CIAT-299	950	28	31	9
	Control	717	26	27	7
18. NEPA 69	Cocktail	680	18	23	4
	CIAT-299	695	25	24	6

Genotype	Inoculum	Biomass (mg plant^{-1})	NdfA (%)	Total-N yield	N-Fixed
				(mg plant^{-1})	
	Control	501	-	16	-
19. ICTA JU 91-37	Cocktail	291	25	9	2
	CIAT-299	579	-	18	-
	Control	709	-	17	-
20. DOR 678	Cocktail	613	0.3	21	0
	CIAT-299	541	1.4	19	2
	Control	634	10	20	2
21. DOR 685	Cocktail	383	1.6	16	1
	CIAT-299	878	7	30	2
	Control	644	28	26	10
22. ICTA JU 93-1	Cocktail	1045	16	33	5
	CIAT-299	590	19	17	4
	Control	869	14	34	5
23. CUT-68	Cocktail	559	19	19	4
	CIAT-299	635	12	17	2
	Control	612	20	19	4
24. CUT-53	Cocktail	699	18	23	4
	CIAT-299	602	22	22	6
	Control	536	23	19	4
25. ICTA JU 97-1	Cocktail	451	12	14	2
	CIAT-299	555	8	19	1
	Control	591	8	19	2
26. N. ALTIPLANO	Cocktail	633	29	21	6
	CIAT-299	606	0	20	0
	Control	571	5	17	2
27. N. SAHUATOBA	Cocktail	696	44	21	9
	CIAT-299	895	45	29	13
	Control	1422	46	46	19
28. N. HUASTECO81	Cocktail	791	0.8	24	0
	CIAT-299	712	26	23	6
	Control	748	4	25	3
	Probability P>F:				
	Genotype (G)	0.000	0.000	0.007	0.000
	Inoculum (I)	0.329	0.004	0.226	0.004
	G x I	0.410	0.000	0.455	0.126
	HSD (Tukey p≤0.05)				
	G	463.43	11.97	16.43	5.52
	I	N.S.	2.68	N.S.	1.24
	G x I	N.S.	13.70	N.S.	N.S.
	I x G	N.S.	20.73	N.S.	N.S.

NS = Not statistically significant; NdfA = Nitrogen derived from the atmosphere

3.2. Field Experiments

3.2.1. *Experiment 3: BNF in herbaceous legumes with multiple uses in agricultural production systems*

The results of nodulation and biomass (shoot, grain and total) by legumes species and reference crops are shown in Table VIII. Significant differences in terms of nodulation and biomass were observed ($p \leq 0.05$) for the main effects of P fertilizer rate and legume species. The data of the *Brachiaria* grasses, sorghum and those of the legumes *Mucuna* and *Dolichos* were discarded because of their poor germination and growth. In general, higher nodule numbers and dry weight (DW) were found in the P fertilized treatment than the control without P fertilizer. The highest number of nodules and acetylene reduction activity (data not presented) in all legume species was observed at flowering. Dry weight (DW) of nodules was highest in *C. cajan* followed by C. *ensiformis*>*A. pintoi* and *V. umbellata*. Similar values were obtained in terms of dry matter, except that C. *ensiformis* (12.2 Mg ha^{-1}) yielded the highest total dry matter (Table VIII). The values obtained in this study are higher than those reported in the literature for these legumes [19].

TABLE VIII. NODULATION AND BIOMASS PRODUCTION BY LEGUMES SPECIES AND REFERENCE CROPS

Treatment	Nodules*†		Dry Matter Yield†		
			Shoot	Grain	Total
	No. $plant^{-1}$	DW (mg $plant^{-1}$)	---------(Mg ha^{-1})---------		
+Phosphorus:					
C. ensiformis	57±11	500±82	6.5±0.3	5.7±0.2	12.2±0.5
V. umbellata	62±14	210±74	1.7±0.2	0.8±0.1	2.5±0.3
C. cajan	49±26	758±132	9.6±0.1	1.7±0.0	11.3±0.0
A. pintoi	530±116	434±142	3.4±0.1	-	3.4±0.1
C. juncea	86±14	492±206	1.8±0.1	0.9±0.0	2.6±0.2
Z. mays VS-486	-	-	3.8±1.4	1.96±0.7	5.8±2.2
Z. mays VS-536	-	-	4.1±0.7	2.80±2.2	6.9±1.2
Non-nod bean DOR 364	-	-	0.41±0.1	0.55±0.1	0.96±0.1
-Phosphorus:					
C. ensiformis	48±7	409±40	6.36±0.2	5.26±0.2	11.62±0.0
V. umbellata	25±4	48±29	1.42±0.1	0.65±0.0	2.07±0.1
C. cajan	30±7	596±21	7.49±0.1	1.66±0.0	9.15±0.2
A. pintoi	301±25	117±17	2.29±0.2	-	2.29±0.2
C. juncea	18±4	29±19	1.47±0.2	0.88±0.1	2.35±0.3
Z. mays VS-486	-	-	4.13±0.3	1.87±0.1	6.01±0.4
Z. mays VS-536	-	-	3.19±0.8	1.37±0.3	4.55±1.1
Non-nod bean DOR 364	-	-	0.25±0.1	0.33±0.1	0.58±0.1
CV (%):	33	31	4	5	5
Probability (P>F):					
Phosphorus (P):	0.040**	0.080	0.002**	0.029**	0.002**
Crop (C):	0.000**	0.000**	0.000**	0.000**	0.000**
P x C:	0.001**	0.065	0.000**	0.029**	0.001**
HSD (p≤0.05)‡:					

Treatment	Nodules*†		Dry Matter Yield†		
	No. plant^{-1}	DW (mg plant^{-1})	Shoot	Grain	Total
			---------(Mg ha^{-1})---------		
Phosphorus (P):	67	NS	0.16	0.12	0.22
Crop (C):	48	135	0.21	0.15	0.34
P x C	68	NS	0.30	0.21	0.48

† Means values of four replicates ± SD; *Nodules values (number and dry weight plant-1) at flowering stage; ** Values statistically different; ‡ Significantly different at p ≤ 0.05 HSD's Tukey test; NS = Not statistically different.

A non nodulating common bean (*Phaseolus vulgaris)* cultivar DOR 364 and maize were employed as reference crops to estimate BNF. Significant differences were observed ($p \leq 0.05$) for the effect of P fertilization and legume species. In general, the highest values of N-fixed and N-yield by legumes species were observed with the P fertilized treatment vs. control without P application (Table IX). Similar trends to those of the dry matter yield were found. The N-fixed values followed the ranking *C. ensiformis*>C. *cajan*>*A. pintoi*>*V. umbellata*>*C. juncea*. Thus the results of this experiment demonstrate that *C. ensiformis* and *C. cajan* with 260–370 and 133–170 kg fixed N kg ha^{-1} respectively, have very good potential for use as green manures in the Ultisols of the savannah of Tabasco, specially when they are P-fertilized. Investigators working in other parts of the world have reported similar results with other legume species for use as fodder or cover crops by resource-poor farmers [19, 20].

TABLE IX. N-FIXED AND TOTAL N-YIELD IN LEGUMES SPECIES AND TOTAL N IN REFERENCE CROPS

Treatment	Total-N	N-Fixed		
		Shoot	Grain	Total
		(kg ha^{-1})		
+Phosphorus:				
C. ensiformis	454±20	122±14 (82)	244±20 (77)	366±34
V. umbellata	68±10	25±6 (66)	28±3 (47)	53±8
C. cajan	197±8	117±7 (82)	53±2 (89)	170±8
A. pintoi	83±3	55±2 (65)	-	55±2
C. juncea	70±7	6±0.01 (20)	15±2 (85)	21±2
Z. mays VS-486	114±43	-	-	-
Z. mays VS-536	90±32	-	-	-
Non-nod bean DOR 364	22±0.5	-	-	-
-Phosphorus:				
C. ensiformis	339±15	64±14 (68)	194±17 (82)	258±16
V. umbellata	45±3	4±0.1 (27)	12±4 (49)	16±4
C. cajan	164±7	84±5 (84)	49±1 (87)	133±6
A. pintoi	54±4	28±3 (57)	-	28±3
C. juncea	55±4	5±1.4 (30)	20±4 (56)	25±4
Z. mays VS-486	78±14	-	-	-
Z. mays VS-536	54±16	-	-	-
Non-nod bean DOR 364	13±0.7	-	-	-
CV (%):	6	15	11	11

Treatment	Total-N	Shoot	Grain	Total
		N-Fixed (kg ha^{-1})		
Probability (P>F):				
Phosphorus (P):	0.000**	0.005**	0.002**	0.000**
Crop (C):	0.000**	0.000**	0.000**	0.000**
P x C:	0.000**	0.000**	0.001**	0.000**
HSD (p≤0.05) ‡:				
Phosphorus (P):	2	10	4	9
Crop (C):	12	9	11	15
P x C:	17	13	15	22

† Means values of four replicates ± SD. Data in brackets represent the %NdfA; ** Values statistically different at p<0.01; ‡ Significantly different at *p ≤ 0.05* HSD Tukey' s test.

3.2.2. Experiment 4: BNF in herbaceous legumes with multiple uses when grown in monoculture and in association with maize

Biomass production, total-N yield and N-fixed in the legumes studied are shown in Table X. In terms of biomass, significant differences were observed (p ≤ 0.05) for the factor legumes. *C. cajan* presented the highest values of biomass for both systems (monoculture and in association) followed by *C. ensiformis* and *M. deeringiana*. In addition, biomass yield of the legumes increased when they were grown in association with maize but total N yield decreased. This increase in biomass (and %NdfA) was probably due to improved soil conditions, particularly to the lowered content of available Al and Mn as it has been reported by Rebafka *et al*. [21]. Biomass production and %NdfA of the legume species grown in monoculture were higher than those observed in Experiment 3. In this experiment *C. cajan* both in monoculture and in association produced the highest biomass, total and fixed nitrogen followed by *C. ensiformis* and to a less extent by *M. deeringiana.* These values are similar to those reported by Sanginga [22]. Theoretically, these N inputs may be sufficient to reduce, if not substitute, the need for chemical N fertilizers of the cereal crops. Previous studies have shown that corn production can be increased up to 50% when *Mucuna deeringiana* or *Canavalia ensiformis* are used as green manures. Unfortunately, this increase in productivity cannot be maintained over long periods [1] suggesting that other factors and interactions influence the potential of this agricultural practice. Thus there is a need for assessing and monitoring the actual N inputs in farmer's fields over long periods.

TABLE X. BIOMASS, TOTAL-N YIELD AND N-FIXED IN LEGUMES SPECIES GROWN IN MONOCULTURE AND IN ASSOCIATION WITH MAIZE

Treatment	Biomass (Mg ha^{-1})	Total-N yield (kg ha^{-1})	NdfA (%)	N-Fixed (kg ha^{-1})
Monoculture:				
C. ensiformis (L_1)	10.15	340	88	298
C. cajan (L_2)	14.98	597	81	495
M. deeringiana (L_3)	10.15	222	40	121
Association:				
L_1 + VS-536 (M_1)	9.45	273	90	250
L_2 + M_1	16.80	402	90	363
L_3 + M_1	11.83	211	79	162
L_1 + Dosmesano (M_2)	15.75	560	89	501

Treatment	Biomass ($Mg\ ha^{-1}$)	Total-N yield ($kg\ ha^{-1}$)	NdfA (%)	N-Fixed ($kg\ ha^{-1}$)
$L_2 + M_2$	18.69	660	92	611
$L_3 + M_2$	12.60	344	86	297
L_1 + H-Z30 (M_3)	13.51	469	90	242
$L_2 + M_3$	14.84	548	90	499
$L_3 + M_3$	14.49	223	70	148
CV (%)	23.8	26.8	15.6	40.7
Probability P>F:				
Legume (L)	0.023	0.000	0.000	0.000
Association (A)	0.184	0.008	0.004	0.006
L x A	0.741	0.728	0.040	0.812
HSD (Tukey $p \leq 0.05$):				
Legume (L)	3.99	129.14	11.17	122.93
Association (A)	NS	164.13	14.20	156.25
L x A	NS	NS	24.59	NS
A x L	NS	NS	22.34	NS

NS = Statistically no significant at $p \leq 0.05$ HSD's Tukey test; % NdfA = % Nitrogen derived from the atmosphere.

Biomass production, total-N yield, N derived from the fertilizer and fertilizer N recovery (N-fertilizer use efficiency) by maize genotypes are shown in Table XI. In general terms, significant differences were only found ($p \leq 0.05$) for the factor association vs. monoculture.

TABLE XI. BIOMASS, TOTAL-N YIELD AND N DERIVED FROM THE FERTILIZER AND N-FERTILIZER USE EFFICIENCY BY MAIZE GENOTYPES GROWN IN MONOCULTURE AND IN ASSOCIATION WITH LEGUMES

Treatment	Biomass ($Mg\ ha^{-1}$)	Total-N yield ($kg\ ha^{-1}$)	N-derived from fertilizer (%)	N-fertilizer use efficiency (%)
Monoculture:				
VS-536 (M_1)	2.94	37	35	22
Dosmesano" (M_2)	4.20	47	47	38
H-Z30 (M_3)	2.15	27	36	15
Association:				
C. cajan (L_1) + M_1	2.21	17	72	20
C. ensiformis (L_2) + M_1	1.71	12	32	6
M. deeringiana (L_3) + M_1	2.70	13	46	10
$L_1 + M_2$	3.11	22	24	9
$L_2 + M_2$	2.86	16	34	10
$L_3 + M_2$	1.89	12	35	8
$L_1 + M_3$	3.79	32	24	12
$L_2 + M_3$	2.13	17	53	15
$L_3 + M_3$	2.78	25	39	16
CV (%)	29.88	40.27	33.20	36.77
Probability P>F:				

Treatment	Biomass (Mg ha^{-1})	Total-N yield (kg ha^{-1})	N-derived from fertilizer (%)	N-fertilizer use efficiency (%)
Maize (M)	0.261	0.243	0.832	0.364
Association (A)	0.222	0.006	0.013	0.003
M x A	0.55	0.878	0.001	0.075
HSD (Tukey p≤0.05):				
Maize (M)	NS	NS	NS	NS
Association (A)	NS	19.68	14.53	11.25
M x A	NS	NS	25.17	NS
A x M	NS	NS	22.87	NS

NS = Statistically not significant at p ≤ 0.05 test Tukey; ^{15}N-Fertilizer = N derived from the ^{15}N-labeled fertilizer.

The maize genotypes in monoculture presented the highest values of biomass, total-N yield, N derived from the fertilizer and N-fertilizer use efficiency. The ranking of these variables were wild type “Dosmesano” > VS-536 > H-Z30 in monoculture. It is interesting to observe that the wild type “Dosmesano” presented the highest values of N-fertilizer use efficiency in monoculture (38%). However, the yield and N-fertlizer use efficiency of maize genotypes declined when they were grown associated with the legumes species. The low values of N-fertilizer efficiency (average 12% - Table XI) could be due to competition for water, nutrients and light by legume species as suggested by Badaruddin and Meyer [23].

4. CONCLUSIONS

A large diversity of native *Rhizobium* strains with variable N fixation capacity exists for use with legume species of agricultural interest in the savannah of Tabasco.

Cajanus cajan and *Canavalia ensiformis* are the multipurpose legumes with greatest potential in terms of BNF and biomass yield, specially when P fertilized (over 10 Mg ha^{-1} and 150–315 kg N ha^{-1}), for the acid Ultisols of the Tabasco region. They can be grown as monoculture and in association with maize as green manures to reduce the need for chemical N fertilizers.

Of the black common bean genotypes evaluated, “N. Sahuatoba, DOR 448, TPL 18 and TPL 16” showed the best potential in terms of BNF (20–46% Ndfa and 5–19 fixed N plant^{-1}) and they can be considered for the savannah of Tabasco.

The growth, biomass production and N fertilizer use efficiency of the studied maize genotypes was better when grown as monoculture (25% FNUE) than in association with legumes (10% FNUE). The wild type “Dosmesano” was superior (4.20 Mg ha^{-1}) to the other tested maize genotypes (1.71–3.79 Mg ha^{-1}).

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HOW DO COWPEA AND GROUNDNUT IMPROVE SOIL N FERTILITY AND YIELDS OF SUCCEEDING SORGHUM CROP IN THE GUINEAN SAVANNAH ZONE OF BURKINA FASO (WEST AFRICA)?

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Abstract

Field experiments were carried out in a weakly acid (pH H_2O 6.5) Ultisol at Farakô-Ba (4^o 20' West, 11^o 6' North and 405 m altitude) in the Guinean savannah zone of Burkina Faso to study the beneficial effects of cowpea (*Vigna unguiculata*) and groundnut (*Arachis hypogea*) on grain yield of succeeding sorghum. The first experiment was a factorial of three crop rotations (cowpea-sorghum, groundnut-sorghum and sorghum-sorghum) as first factor and five fertilizer treatments (NPK fertilizer, NPK+ dolomite, NPK+ manure, NK+ phosphate rock and a control) as second factor in a split plot design with a randomised block arrangement and four replications. Soil mineral N, nematode infection and fertilizer N recoveries were studied during three years (2000 to 2002). Biological nitrogen fixation (BNF) in cowpea and groundnut was measured in the second year (2001) with ^{15}N isotopic dilution method using a non-fixing cowpea as reference crop. Groundnut fixed 8 to 23 kg N ha^{-1} and the percent of N derived from the atmosphere (% Ndfa) varied from 27 to 34%. Cowpea fixed 50 to 115 kg N ha^{-1} and the Ndfa varied from 52 to 56%. Compared to mineral NPK fertilizer alone, legumes fixed more nitrogen from the atmosphere when NPK fertilizer was combined with dolomite or manure. Compared to water-soluble phosphate, phosphate rock increased BNF by cowpea. Highest responses to N fertilizer applications were found when sorghum was rotated with legumes and the lowest in mono cropping of cereals. Compared to mono cropping of cereals (maize-sorghum or sorghum-sorghum), sorghum produced 2.9 and 3.1 times more grain yields when it was rotated with groundnut or cowpea respectively. In a second experiment, the response of a succeeding sorghum to N fertilizer, nitrogen fertilizer equivalencies (NFE) of the two legumes and nematode infection in the soil and roots of the succeeding sorghum were studied. The NFE of groundnut (35 kg N ha^{-1}) was higher than that of cowpea (25 kg N ha^{-1}). However, soil and succeeding sorghum roots were most infected by nematodes in cowpea-sorghum rotation while groundnut in the rotation decreased nematode infections. The high NFE of groundnut compared to cowpea indicated that positive effect of groundnut on nematode suppression was accounted in NFE as N effect. A better use of N fertilizer was observed in legume-sorghum rotations. In continuous sorghum, fertilizer N use efficiency (NUE) was 20% whereas NUEs in cowpea-sorghum and groundnut-sorghum rotations were 28 and 37% respectively. The soils of legume-sorghum rotations contained more mineral N (40–50% more than the monocropping control), thus increasing soil N supply to succeeding sorghum compared to mono cropping of sorghum. The highest total N uptake by sorghum was found in legume-sorghum rotations. It was estimated from the response curves that 51 and 58 kg N ha^{-1} should be applied to sorghum when it was rotated with groundnut or cowpea respectively to achieve optimum grain yields of 2,000 kg ha^{-1}. Recommended NPK fertilizer should be applied to the two legumes. Best results were obtained when

legumes received NPK fertilizer plus dolomite or manure. But the local phosphate rock can be used as source of P on groundnut.

1. INTRODUCTION

Nutrient deficiencies, in particular nitrogen (N) and phosphorous (P) are the main soil fertility constraints limiting crop yields in West Africa. Despite the recognized need to apply nutrient inputs such as chemical fertilizers for obtaining high crop yields, their use by smallholders in this region is limited by several factors such as the lack of capital investment and financial credit, inefficient distribution systems, poor supportive policies and other socio-economic factors. Efficient and socio-economic affordable technologies for improving and sustaining soil fertility and productivity are therefore, necessary. N_2-fixing grain legume crops such as groundnut (*Arachis hypogea* L.) and cowpea (*Vigna unguiculata* (L) Walp) are reported to improve soil fertility and contribute to the sustainability of cropping systems as a consequence of N inputs supplied via biological nitrogen fixation [1, 2]. This could be demonstrated in cropping systems where legume residues are recycled in the soil. However at the end of each rainy season, after grain harvesting, legume residues are also exported for animals feeding. Thus, the total N yield and the actual amount of nitrogen fixed by legume crops do not reflect their actual contribution to improving soil nitrogen status. The N input effect of legume crops on the succeeding cereal crops could be overestimated when the amount of N fixed in the above-ground is accounted. N_2-fixing legumes may also provide nitrogen to the subsequent crops through the fallen senescent leaves and below ground parts. However, the N effect alone cannot completely explain the positive effects of legumes on the succeeding cereal crop yields. The quantity of nitrogen fixed by legumes can be measured but itis difficult to accurately assess the quantity of nitrogen supplied by the legume to the succeeding cereal in legume-cereal rotations. In the savanna areas of West Africa there is little information on the actual contributions of N_2-fixing legume crops in term of quantity of nitrogen supplied to the subsequent non-fixing crop..

In the *Rhizobium*-legume symbiosis, both partners, the microsymbiont *Rhizobium* and the host plant (legume) are affected by the inherent soil fertility status and fertilizer applications, if any. Itis also well known that many legume plants can efficiently use P from sparingly P forms through their rhizosphere abilities to dissolve P strongly fixed by Fe and Al in mineral acid soils [3, 4]. Also, P from natural phosphate rock applied to P deficient soils should be efficiently used by legumes for improving their growth and the inputs from biological nitrogen fixation (BNF). Soil acidity (and Al toxicity) is another factor limiting crop production in weakly acid soils with low buffer capacity and thus affecting biological nitrogen fixation by legume crops. Soil acidity correction can be made using local agro mineral resources such as dolomite or organic manures. Thus, less expensive locally available resources (indigenous phosphate rocks, dolomite and/or organic manures) can be applied to increase the actual inputs from BNF by legumes in cropping systems, thus improving soil fertility status and increasing cereal grain yields in the Guinean savannah of Burkina Faso.

The purpose of this research was: a) to measure biological nitrogen fixation in cowpea and groundnut, two commonly grown grain legumes and quantify their actual N contributions to improve soil N fertility status and b) to assess the performance of a sorghum crop when grown in crop rotations with the before-mentioned grain legume crops. Integrated soil fertility management practices including cheap locally available organic and inorganic nutrient sources were also investigated to sustain productivity of weakly acid soils with low buffer capacity in the Guinean savannah of Burkina Faso.

2. MATERIALS AND METHODS

Two field experiments were carried out at the agronomic research station of Farakô-Ba (4^o 20' West, 11^o 6' North and 405 m altitude above sea level), located in the Guinean savannah zone of Burkina Faso. This agro ecological zone has one rainy season per year, starting in May-June and ending in October. Annual rainfall was 1058 mm during the year 2000 and 715 mm in year 2001, of which 999 mm and 649 mm rain fell during their respective cropping seasons (Table I). In general, planting dates occurred in June and harvesting was made in October. The experiments were laid down on a six years old fallow area.

TABLE I. MONTHLY, AND TOTAL ANNUAL RAINFALL (MM) DURING THE EXPERIMENTAL PERIOD (2000- 2002)

Years	Months					Total
	June	July	August	September	October	
2000	172	236	309	241	41	999
2001	80	154	229	153	33	649
2002	65	184	175	125	32	581
Means	16	191	238	173	35	743

The experimental soil was an Ultisol, a weakly acid sandy soil with low clay and organic carbon contents. At the start of the two experiments (year 2000), soil samples were taken from the top 20 cm depth. Soil pH was measured in 1 N KCl using a 2:1 solution to soil ratio and exchangeable acidity was measured as described by McLean [5]. Organic carbon was measured by the wet chemical digestion procedure of Walkley & Black [6]. Total nitrogen was determined by the Kjeldahl procedure. Exchangeable bases (Ca, Mg, K and Na) were displaced with ammonium acetate. Ca and Mg were determined by atomic absorption spectrophotometry and K and Na by flame photometry. Effective Cation Exchange Capacity of the soil (ECEC) was calculated as the total exchangeable bases. Available phosphorous was determined using Bray I method [7]. The main characteristics of the soil are shown in Table II. The soil is a sandy loam, slightly acid, and very low fertility status. Available P (P-Bray I), Ca, Mg and exchangeable K were very low. As a result of its low clay and organic C contents, ECEC of the soil was very low.

TABLE II. MAIN PHYSICO-CHEMICAL CHARACTERISTICS OF THE TOPSOIL (0-20 CM)

Clay (%)	7		
Sand (%)	74	pH H_2O	6.5
Silt (%)	19	pH KCl	5.6
Organic C (%)	0.61	Ca^{++} (cmol$^+$ kg^{-1} soil)	1.08
Total N (mg kg^{-1})	409	K^+ (cmol$^+$ kg^{-1} soil)	0.02
Total P (mg kg^{-1})	69.8	Mg^{++} (cmol$^+$ kg^{-1} soil)	0.46
Avail.-P Bray I (mg kg^{-1})	5.6	ECEC (cmol+ kg^{-1} soil)	1.82
Available K (mg kg^{-1})	531		

A factorial of 3 × 6 experimental treatments in split plot experimental design with a randomized block arrangement and four replications was utilized during three seasons (2000 through 2002). Improved varieties of sorghum (Sariaso 2), groundnut (RMP-12) and cowpea (KVX-61-1) recommended by the National Agronomic Research Institute (INERA) for the Guinean savannah zone were sown with planting densities of 62 500 and 125 000 plants per hectare for groundnut and cowpea respectively. Recommended planting density of 62 500 plants per hectare was used for sorghum. Crops (cowpea, groundnut and sorghum) were sown in the main plots as first factor. Each main plot was split in 6 sub plots for various fertilization treatments (PK, NPK, NPK + dolomite, NPK+ manure, NK + phosphate rock and a control, natural fallow without fertilizer), which were employed as second factor. Legume treatments were randomized in the blocks and fertilization treatments were randomized in the sub plots. Legume crops were not inoculated. Chemical fertilizers were applied at rates of 14 kg N ha^{-1}, 10 kg P ha^{-1} and 11 kg K ha^{-1} to the two legumes using complex NPK fertilizer, triple super phosphate and potassium chloride. For NK+ phosphate rock treatment, the same dose of phosphorous (10 kg P ha^{-1} supplied by a water-soluble phosphate) was applied in the form of phosphate rock. Local phosphate rock of Burkina Faso (11.5% total P; 34.5% CaO; 8.02% P_2O_5 soluble in NAC; 48.48% P_2O_5 soluble in formic acid; 3.1% Al_2O_3 soluble in HCl and 3.4% Fe_2O_3 soluble in HCl) was used. To the manure treatments, three tons ha^{-1} of air-dried cattle manure was applied. Cattle manure contained 1.8, 18.40, 0.31 and 0.16% of N, C, P and K. In dolomite containing treatment, 1 t ha^{-1} of dolomite (249 and 114 kg ha^{-1} of Ca and Mg respectively) was used. Except urea, all fertilizers were applied at sowing. Nitrogen fertilizers were split-applied to the sorghum plots: 14 kg N ha^{-1} with NPK fertilizer at sowing and 23 kg N ha^{-1} at 40 days after sowing (DAS).

Biological nitrogen fixations (BNF) in the two legumes, N recoveries, soil mineral and nematode infections were evaluated during the second season (2001) of cultivation. In the nitrogen fixation study, ammonium sulphate with 10 atom % ^{15}N excess was applied at 9.7 kg N ha^{-1} to isotope micro plots laid-down in the four fertilization subplots of the legumes at sowing. The shoots of legumes were harvested at physiological maturity. Plant samples were oven-dried at 60 °C during 72 hours; ground and their total N and atomic % ^{15}N excess were analysed using an elemental CN analyzer coupled to an isotopic ratio mass spectrometer at the IAEA Seibersdorf Laboratory. Nitrogen fixed by legumes was calculated using the percentages of ^{15}N excess in N_2-fixing legume and non-fixing cowpea variety (IC-1) (Pemberton, 1990) as reference test crop (equation 1) according to the isotope dilution method [8].

$$\text{N-fixed (kg ha}^{-1}) = \left(1 - \frac{\%\ ^{15}\text{N a.e N-fixing legume}}{\%\ ^{15}\text{N a.e Non-fixing legume}}\right) \times \underset{\text{N-fixing legume}}{\text{Total N (kg ha}^{-1})} \qquad (1)$$

Soil mineral nitrogen ($NH4^+ + NO3^-$) was measured during the first two months of the second season (2001). Soil samples were taken in the first 20 cm layer at sowing, 9, 20, 30 40 and 53 days after sowing in sorghum plots. Mineral N was extracted with 1M KCl solution and measured by a colorimetric method [9]. Fertilizer and soil nitrogen recoveries by sorghum were studied by isotopic dilution method on the three rotations and three fertilization regimes. Fourteen kg N ha^{-1} of urea fertilizer with 5 atom % ^{15}N excess were applied at sowing in the isotope micro plots of 3.2 m × 1.6 m (5.12 m^2) delimited in the sub plots. The above-ground plant parts was harvested at physiological maturity. Plant samples were oven-dried at 60°C during 72 hours, ground and analyzed for total N and ^{15}N % atomic excess by an elemental analyzer coupled to a mass spectrometry at the IAEA Seibersdorf Laboratory. Nitrogen Fertilizer Use Efficiency (NFUE) was calculated using the amount of N derived from fertilizer (Ndff) and the total N applied by fertilization treatment [10] (equation 2).

$$NFUE = Ndff(kg\ ha^{-1})/N\ applied\ (kg\ ha^{-1}) \times 100 \qquad (2)$$

Soil and root samples of sorghum were removed at 60 and 90 days after sowing for nematode infection assessment. Soil and sorghum root nematodes were extracted and measured by the methodology described by Seinhorst [11]. Zero values and high coefficient of variation were obtained, particularly on sorghum root infections by nematodes. Thus, a logarithmic function (equation 3) was used for data transformation before statistical analysis [12].

$$Y = \log x + 1 \qquad (3)$$

Where X was the number of nematodes and Y the transformed value.

In a second experiment, Nitrogen Fertilizer Equivalency (NFE) was estimated for the two legumes during two consecutive seasons (2000 and 2001). The concept of NFE is used to assess the effective N contribution of legume crop to the succeeding non-fixing sorghum. When a legume is rotated with a non fixing-crop, the NFE of this legume is defined as the quantity of N fertilizer needed to produce the surplus of yields compared to mono cropping of non-fixing crop [13]. The surplus yields of the succeeding crop due to rotation with legume are used to estimate the equivalent of N fertilizer needed to achieve this yield increase. According to the experimental method, cereal-legume rotations were set during the first season and randomized within the blocks. During the second year, sorghum response to N fertilizer was studied. Nitrogen response curves were used to estimate the NFE of legume.

During the first season (year 2000), a simple randomized block experiment of four treatments (groundnut-sorghum, cowpea-sorghum, maize-sorghum and sorghum-sorghum) with four replications was employed. Similar procedures regarding the fertilizer rates and sources and varieties of sorghum and legumes as described for the first experiment were utilized. In addition, improved variety of maize (*Zea mays*) SR22 was included in cropping system (maize-sorghum). Maize was fertilized with 60 kg N ha^{-1}, 10 kg P ha^{-1} and 11 kg ha^{-1} K using NPK fertilizer and urea. Two cereal rotations (sorghum-sorghum and maize-sorghum) were used as reference rotations for legume's NFE assessment. During the second season (year 2001), sorghum was sown on all plots and the effect of the previous crop on the succeeding sorghum responses to N fertilizer applications were studied. At sowing a basal fertilization of 10 kg P ha^{-1} and 11 kg K ha^{-1} was applied to sorghum using triple super phosphate and potassium chloride respectively. Each main plot was split into five sub plots corresponding to five increasing N rates (0, 20, 40, 60 and 80 kg N ha^{-1}), which were applied as urea. Nitrogen fertilizer treatments were randomized in the sub plots. Then, the experimental design became a factorial of 4 × 5 treatments in a split plot arrangement with four replications. Nitrogen response curves were used for recommended N fertilizer determination using a linear-plateau model [14] and NFEs assessment was made according to Hesterman et al [13].

3. RESULTS AND DISCUSSION

3.1. Legume yields and biological nitrogen fixation

3.1.1. Cowpea and groundnut yield production

On the average (over 2 seasons) cowpea showed better grain yield responses to the fertilization treatments than groundnut (Table III).

Without fertilizer application (control), groundnut grain yields were very low. PK fertilization alone didn't increase groundnut grain yields. However, NPK fertilizer significantly increased grain yields indicating that in spite of the groundnut capability to fix atmospheric N, a low quantity of starter chemical N fertilizer is necessary to improve groundnut yields in this soil. Compared to NPK fertilizer alone, the addition of soil amendments such as dolomite or manure didn't significantly increase groundnut grain yields. Unlike grain yields, PK fertilizer increased groundnut shoot yields. Compared to NPK fertilizer alone, the combined application of organic and chemical fertilizer (NPK +Manure) increased groundnut shoot yields. Nevertheless, dolomite application with NPK fertilizer didn't affect groundnut shoot yields compared to NPK fertilizer alone.

Cowpea grain productions were also very low when it was grown without fertilizer application (control) (Table III). Unlike the groundnut, PK fertilization alone increased cowpea grain yields during the two years. Manure or dolomite applications had significant effects on cowpea shoot yields.

3.1. N accumulation and biological nitrogen fixation in cowpea and groundnut

Total N yields of the two legumes and N derived from atmosphere (Ndfa) are presented in Table IV. Cowpea accumulated almost twice more N than groundnut. Total nitrogen yields in the above ground parts of groundnut and cowpea averaged about 31 and 59 kg N ha^{-1} respectively, of which 14 and 32 kg N ha^{-1} were derived from the atmosphere in groundnut and cowpea respectively.

From the estimations using the isotopic data, groundnut and cowpea showed 42 and 52% Ndfa. But fertilizer applications affected total N yields and the amount of N fixed in the two legumes. The total N yield of groundnut ranged from 26 to 35 kg N ha^{-1} depending on the fertilizer applications (Table IV). Highest quantities of nitrogen were fixed by groundnut when NPK fertilizer was applied combined with dolomite or manure and significant differences were not observed between these two treatments. Similar results have been reported by others workers, indicating that without inoculation as it was the case in this experiment, groundnut can fix 19 to 63% of its total N from the atmosphere [15, 16, 17, 18]. With appropriate inoculation techniques, groundnut can fix 47 to 78% of its total N from the atmosphere [16].

TABLE III. GROUNDNUT AND COWPEA YIELDS (KG HA^{-1}) AS AFFECTED BY FERTILISER APPLICATIONS DURING TWO YEARS (2000 AND 2001)

Year	Fertilization	Groundnut Grain	Groundnut Shoots	Cowpea Grain	Cowpea Shoots
2000	NPK	958^{a}	3153^{ab}	1139^{bc}	2143^{c}
	NPK + Dolomite	994^{a}	2790^{ab}	1203^{ab}	2930^{b}
	NPK +Manure	889^{a}	3358^{a}	1307^{a}	3350^{a}
	NK+PR*	862^{a}	2892^{b}	835^{d}	1621^{d}
	PK	622^{b}	2723^{b}	1040^{c}	1907^{e}
	Control	679^{b}	2502^{bc}	777de	1351^{f}
	Block	*	ns	ns	*
	Fertilization (F)	**	*	*	***
2001	NPK	1142	3919^{b}	885^{bc}	2606^{bc}
	NPK + Dolomite	1188	4055^{b}	957^{ab}	2982^{ab}
	NPK +Manure	963	4773^{a}	973^{a}	3345^{a}
	NK+PR*	994	3186^{cd}	601^{e}	1559^{e}
	PK	879	3450^{c}	821^{cd}	2325^{cd}
	Control	900	2874^{e}	407^{f}	1209^{f}
	Block	ns	***	**	***
	Fertilization (F)	ns	***	***	***
Means (two years)	NPK	1050^{ab}	3 536^{b}	1 012^{bc}	2 375^{c}
	NPK + Dolomite	1 091^{a}	3 423^{bc}	1 080^{ab}	2 956^{ab}
	NPK +Manure	926^{bc}	4 066^{a}	1 140^{a}	3 348^{a}
	NK+PR*	928^{b}	3 039de	718^{e}	1 590^{e}
	PK	751^{ef}	3 087^{d}	931^{d}	2 116^{cd}
	Control	790^{e}	2 688^{f}	592^{f}	1 280^{ef}
	Block	ns	***	**	***
	Fertilization (F)	*	***	***	***
	Year (Y)	*	**	*	*
	Interaction F x Y	ns	ns	ns	ns

*, **, ***: Significant at 0.05; 0.01 and 0.00 level; ns: Non significant ($p>0.05$).
Values affected by the same letter in the same column are not significantly different at $p<0.05$, according to Fisher's test.

Total N yield of cowpea ranged from 50 to 68 kg N ha^{-1} depending on the fertilizer applications (Table IV). As observed with groundnut, cowpea took highest quantities of N from atmosphere when NPK fertilizer was applied in combination with dolomite or manure (N fixed was 40–46 kg N ha^{-1} corresponding to 62–67% Ndfa) and no significant differences were observed between the two treatments. The lowest quantities of N came from atmosphere were found when NPK fertilizer alone or NK+ phosphate rock were applied. The application of NK + phosphate rock as P source had a significant increase on the percentage of N derived from atmosphere over the NPK alone. Similar results indicated that cowpea can fix from 50 to 75% of its total nitrogen from the atmosphere [19, 20].

TABLE IV. FERTILIZATION EFFECTS ON TOTAL NITROGEN YIELD AND NITROGEN FIXATION ESTIMATES (NDFA) IN GROUNDNUT AND COWPEA DURING 2001 USING ^{15}N ISOTOPIC DILUTION METHOD

		Total nitrogen yield (kg N ha^{-1})	Ndfa (kg N ha^{-1})	Ndfa (% of total N)
Groundnut	NPK	27 ^{cd}	-	-
	NPK + Dolomite	34 ^{ab}	15 ^{ab}	43 ^{ab}
	NPK +Manure	35 ^{a}	16 ^{a}	45 ^{a}
	NK+PR*	26 ^{c}	10 ^{c}	37 ^{c}
	Mean	31	14	42
Cowpea	NPK	53 ^{c}	20 ^{d}	37 ^{d}
	NPK + Dolomite	63 ^{ab}	40 ^{ab}	62 ^{ab}
	NPK +Manure	68 ^{a}	46 ^{a}	67 ^{a}
	NK+PR*	50 ^{cd}	22 ^{c}	43 ^{c}
	Mean	58	32	52

* Phosphate rock

Values affected by the s.ame letter in the same column are not significantly different at $p<0.05$, according to Fisher's test.

-: Missing data.

Biological nitrogen fixation in the two legumes studied was affected by fertilizer applications because of the beneficial effects of the nutrients supplied on both plant and microbes. Considering the very low fertility of the experimental soil, it was expected that nutrients supplied by fertilizers even at low application rates would promote plant legume growth and rhizobial activities leading to increases of legume biomass and N_2 fixation [17]. For instance, in this study Ndfa in cowpea increased from 37% (NPK fertilizer treatment) to 43% (NK fertilizer plus phosphate rock as source of P). This may be likely explained by both the superior use efficiencies of sparingly P sources by legumes and the beneficial effects of Ca and other nutrients supplied by the phosphate rock [3, 21, 4]. The beneficial effects of phosphate rock and dolomite can be attributed to their double role on controlling soil acidity and supplying nutrients for both legumes and Rhizobia. Legume total N yields were positively correlated to the amounts of N fixed from the atmosphere (Fig. 1) indicating that 94% of the variations in total N yields of legumes were due to variations in biological nitrogen fixation, which was partly affected by inherent soil fertility status and nutrients supplied by the fertilization treatments.

3.2. Succeeding sorghum yields

During the first season (in absence of rotation effect), only the effects of fertilizers were measured. The rotation effects were assessed during the last two years (2001 and 2002). Sorghum yields were affected by fertilizer applications and rotations (Table V). But during the last two years, interactions were not observed between the two factors indicating that rotation effects were not influenced by fertilizer applications and conversely. Without fertilizer applications, sorghum grain yields were very low during the last two years. But in the first year, sorghum produced high yields even when fertilizers were not applied. This is probably due to the positive effects of the natural fallow in the first year of cultivation. Chemical NPK fertilization alone increased sorghum grain yields because of the inherent low soil fertility status, leading to good responses to fertilizer applications [22, 23, 24]. But NPK fertilizer plus manure produced the highest sorghum yields over the three years. Similar results on the beneficial effects of the combined application of chemical and organic fertilizers on crop yields have been reported, though they are usually attributed to the role of organic materials on both soil acidity correction and source of nutrients [25, 26, 27].

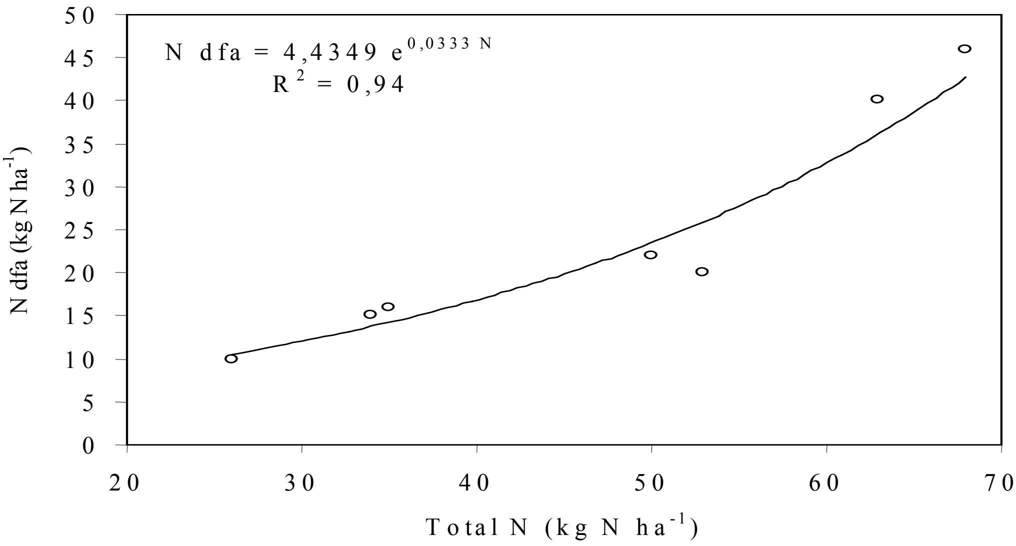

FIG.1. Relationships between grain legumes (cowpea and groundnut) total nitrogen yield (kg N ha^{-1}) and amount of nitrogen derived from atmosphere (kg N ha^{-1}) estimated using the ^{15}N isotopic dilution method in the Guinean savannah zone of Burkina Faso
** Each point represents the mean of four values*

With regard to the crop rotations, continuous cultivation of sorghum produced the lowest yields during the two years (Table V). But sorghum produced highest yields when it was cultivated in rotation with groundnut or cowpea, and the effects of legume-sorghum rotations increased over time. In year 2001, groundnut-sorghum and cowpea-sorghum rotations increased sorghum grain yields by 60 and 96% respectively compared to continuous sorghum. In 2002, groundnut-sorghum and cowpea-sorghum rotations produced 2.9 and 3.1 times more grain yields respectively than mono cropping of sorghum. Beneficial effects of N_2-fixing legumes on succeeding crops have been reported by many workers [28, 1, 29]. In the semi-arid zone of West Africa, Bationo and Ntare [2] found that cowpea increased pearl millet yields from 58 to 100%. The positive effects of legume on the yield increase of the succeeding crop are mainly attributed to BNF inputs leading to soil fertility improvement for the succeeding non-fixing crops. Although the N effects of legumes are likely to occur, other effects related to the legumes themselves such as soil and fertilizer N utilization can interact with these N-effects.

TABLE V. EFFECTS OF CROP ROTATIONS AND FERTILIZATION TREATMENTS ON SUCCEEDING SORGHUM GRAIN YIELDS (KG HA^{-1}) DURING THREE YEARS (2000 TO 2002)

		-----------Years----------			--------Means-----	
		2000	2001	2002	Kg ha^{-1}	% of control
Rotations	Groundnut-Sorghum	na	1626 b	1826 ^{b}	1726 ^{b}	209
	Cowpea-Sorghum	na	1995 a	1987 ^{a}	1991 ^{a}	241
	Sorghum-Sorghum	na	1018 c	636 ^{c}	827 ^{c}	100
Fertilization	NK+PR	1416 ^{b}	1221 ^{e}	1357 ^{d}	1331 ^{d}	141
	NPK	1487 ^{b}	1553 ^{bc}	1574 ^{b}	1538 ^{b}	163
	NPK +Dolomite	1493 ^{b}	1627 ^{b}	1464 ^{bc}	1528 ^{bc}	162
	NPK +Manure	1779 ^{a}	1876 ^{a}	1933 ^{a}	1863 ^{a}	197
	PK	1096 ^{c}	1387 ^{d}	1340 ^{de}	1274 ^{de}	135
	Control	1092 ^{c}	864 ^{f}	881 ^{f}	946 ^{f}	100
	Block	**	***	***	***	
	Rotation (R)	na	***	***	***	
	Fertilization (F)	***	***	***	***	
	Interaction (RxF)	na	ns	ns	ns	

*, **, ***: Significant at 0.05; 0.01 and 0.00 level; ns: Not significant (p>0.05).
Values affected by the same letter in the same column are not significantly different at p<0.05, according to Fisher's test; na: Not applicable.

3.3. Soil mineral N, total N uptake and N fertilizer use efficiency

Soil mineral N measurements at the start of the growing season (at 1 and 7 days after sowing) of the first year (2001) indicated that available soil N varied with the cropping systems (Table VI). For all crop rotation treatments, soil mineral N decreased rapidly during the season (data not shown). The first rains of the season induced a boost of microbial activity leading to a "mineralization flush" of soil organic nitrogen and increases in mineral N. Soil mineral N decline with time was due to the occurrence of other N cycling processes such as mineralization/immobilization turnover, N uptake by plants, N losses by leaching and other processes.

In spite of those variations, soil mineral N at the start of the growing season was affected (P<0.05) by crop rotations (Table VI). But soil mineral N was not affected by fertilizer applications and interaction was not observed between these two factors. Compared to continuous sorghum, the soils under cowpea-sorghum and groundnut-sorghum rotations provided 2.3 and 2.6 times respectively more N to succeeding sorghum (Table VI). However, significant differences were not observed between cowpea-sorghum and groundnut-sorghum rotations. After the first week, no differences were observed between rotations. Shumba [30] also found an increase of 32 kg N ha^{-1} of soil mineral N in cowpea-maize rotation compared to continuous maize cultivation. Similar results have been reported by Bationo and Ntare [2]. The residual effects of rotation and fertilizers (Ndfr) on increasing N yields were also calculated using either the sorghum-sorghum rotation or the NPK fertilizer as control treatments respectively. Cowpea-sorghum and groundnut-sorghum rotations increased N supply by soil from 59 to 73 kg ha^{-1} respectively. At the same time, legume-sorghum rotations increased N fertilizer use efficiency (NFUE) compared to continuous sorghum (Table 5).

Nitrogen fertilizer use efficiency increased from 20% in continuous sorghum to 28 and 37% when sorghum was rotated with cowpea and groundnut respectively. As a consequence of soil mineral N and NFUE increases, total N uptake was also affected by rotations (P<0.001) and fertilizer applications (P<0.001). But, no interaction was observed between the two factors.

TABLE VI. FERTILIZATION AND LEGUME-SORGHUM ROTATIONS EFFECTS ON SOIL MINERAL N, N FERTILIZER USE EFFICIENCY (NFUE) AND TOTAL N UPTAKE BY THE SUCCEEDING SORGHUM CROP IN 2001

	Soil mineral N (kg ha^{-1})		NFUE	Total N uptake(Kg ha^{-1})	
	at sowing	7 DAS	%	Total	Ndfr
Rotations					
Groundnut-Sorghum	64 ^{a}	52 ^{ab}	37 ^{a}	121 ^{a}	73
Cowpea-Sorghum	57 ^{ab}	58 ^{a}	28 ^{b}	107 ^{b}	59
Sorghum-Sorghum	42 ^{c}	45 ^{c}	20 ^{c}	48 ^{c}	0
Fertilization					
NPK	53	48	30	82 ^{bc}	0
NPK+Dolomite	50	43	23	85 ^{b}	3
NPK+Manure	58	59	32	110 ^{a}	28
Block	**	**	**	***	
Rotation (R)	**	**	ns	***	
Fertilization (F)	ns	ns	ns	***	
Interaction RxF	ns	ns	ns	ns	

*, **, ***: Significant at 0.05; 0.01 and 0.00 level; ns: Not significant (p>0.05).

Values affected by the same letter in the same column are not significantly different at p<0.05, according to Fisher's test.

Ndfr: Increasing of soil mineral N compared to control treatment (sorghum-sorghum rotation or NPK fertilizer).

DAS: Days after sowing.

These data provide experimental evidence that a high N uptake in the legume-sorghum rotations may be explained by the effectiveness of legume-sorghum rotations to provide more N to succeeding sorghum compared to continuous sorghum and the better use of the applied N fertilizer thus promoting better cereal plant growth and higher yields. Legume residues probably provided more organic N leading to high content of mineral N in the soils for the succeeding crop [31, 32]. Despite the exportation of legumes shoots, the remaining crop residues and the below ground part of legumes can substantially improve organic matter and soil mineral N of this topsoil of low fertility status. Moreover, the good quality of the legume residues likely contributed to supply more soil mineral N in the legume-sorghum rotations at the beginning of the next season. Giller *et al.* [33] has estimated that 15 to 20% of the nitrogen of legumes is recycled for the succeeding crop by legume residues.

3.4. Legume effects on sorghum responses to N fertilizer

The results of the first experiment posed a pertinent question. Do the N effects of legumes alone explain the yield increases observed in legume-cereal rotations? To further elucidate the legume effects, a second experiment was carried out to study the response of succeeding sorghum to N fertilizer application and the legume influence on nematode infection in soil and sorghum roots.

The results indicated that succeeding sorghum grain yields were significantly affected by both rotations and N fertilizer applications (data not shown). The highest response of sorghum grain yield (kg ha^{-1}) to rates of N fertilizer applications (kg N ha^{-1}) was observed when sorghum was rotated with legumes (Fig. 2).

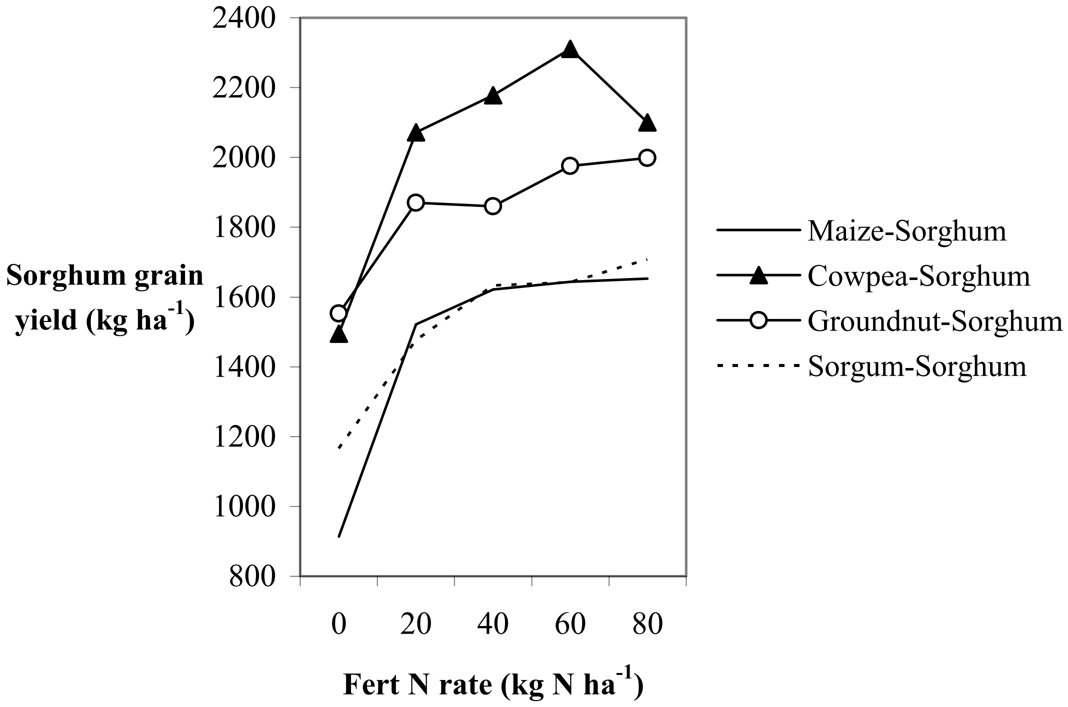

FIG.2. Effects of crop rotations on succeeding sorghum responses to nitrogen fertilizer applications in 2001.

The corresponding N response equations for sorghum grown under the four rotations were estimated as follows:

Sorghum-Sorghum: $Y = -0.114\ N^2 + 15.38\ N + 1184$, $R^2 = 0.97^{**}$
Maize-Sorghum: $Y = -0.2276\ N^2 + 26.20\ N + 969$, $R^2 = 0.93^{**}$
Cowpea-Sorghum: $Y = -0.2765\ N^2 + 29.37\ N + 1495$, $R^2 = 0.97^{**}$
Groundnut-Sorghum: $Y = -0.0828\ N^2 + 11.61\ N + 1586$, $R^2 = 0.97^{**}$

Where Y is the grain yield of sorghum (kg ha^{-1}) and N is the applied dose of N fertilizer (kg N ha^{-1}). Sorghum productivities were low and very similar in mono cropping of sorghum or when it was rotated with maize. This suggested that specific factors related to mono cropping of sorghum like allelopathy was not observed.

The linear-plateau model [14] was used to estimate N fertilizer requirements of succeeding sorghum according to the cropping systems. The results indicated that N fertilizer requirements for optimum yields varied with cropping systems. In maize-sorghum and sorghum-sorghum rotations, N fertilizer rates of 35 to 40 kg N ha^{-1} should be applied for obtaining 1610 and 1640 kg ha^{-1} grain yield of sorghum respectively. The succeeding sorghum showed better response to N fertilizer in legume-sorghum rotations and N fertilizer rates could be increased for achieving optimum sorghum yields. In groundnut-sorghum and cowpea-sorghum rotations, N fertilizer rates of 51 and 58 kg N ha^{-1} must be applied for achieving sorghum grain yields of 1963 and 2268 kg ha^{-1} respectively.

3.5. Fertilizer N equivalency and nematode infection

Succeeding sorghum and maize response curves to N fertilizer applications (Fig. 2) were used to estimate fertilizer N equivalency (FNE) of the two legumes using maize-sorghum and sorghum-sorghum rotations as control treatments. The NFEs of cowpea were 25 and 26 kg N ha^{-1} when maize-sorghum and continuous sorghum were used as reference rotations respectively. The NFEs of groundnut were 32 and 35 kg N ha^{-1} when maize-sorghum and continuous sorghum were used as reference rotations respectively.

An analysis of the response curves indicated whether the response was due entirely to N or others factors. For instance, the response curves of maize-sorghum and sorghum-sorghum rotations were paralleled and never reach the ones from the cowpea-sorghum or groundnut-sorghum rotation (Fig. 2), indicating that factors other than N may have played a role on legume benefits [28]. The converging curves, which do not intersect those of maize-sorghum and groundnut-sorghum rotations, show a response to N as well as other factors. The positive effects of legumes can be attributed to the N supplied by legumes as well as the improvement of soil biological and physical properties [34]. Legumes can affect other factors such as dissolution of occluded P and highly insoluble calcium bounded phosphorus by legume root exudates [35]. Other advantages of crop rotations include soil conservation [36], organic matter restoration and pest and disease control [28].

The results from the first study showed highest available soil N, fertilizer N use efficiency and highest yields when sorghum was rotated with cowpea compared to groundnut. However, in this experiment the contrary was observed. The NFE of cowpea (average 25 kg N ha^{-1}) was lower than that of groundnut (average 35 kg N ha^{-1}). Thus, another factor was likely influencing the sorghum response to N fertilizer. Data on nematode infection in soil and roots of succeeding sorghum are presented in Table VII.

TABLE VII. SOIL AND SUCCEEDING SORGHUM ROOT INFECTIONS BY NEMATODES UNDER COWPEA-SORGHUM, GROUNDNUT-SORGHUM AND CONTINUOUS SORGHUM AT 30 AND 90 DAYS AFTER SOWING (DAS)

Time	Rotation	Nematodes (number/g)	
		Soil	Roots
30 DAS	Groundnut-Sorghum	294 ^{c}	12 ^{c}
	Cowpea-Sorghum	4507 ^{a}	449 ^{a}
	Sorghum-Sorghum	2623 ^{b}	232 ^{ab}
	Block	ns	ns
	Rotation (R)	**	**
	Nitrogen(N)	ns	ns
	RxN	ns	ns
90 DAS	Groundnut-Sorghum	905 ^{c}	4 ^{c}
	Cowpea-Sorghum	5880 ^{a}	109 ^{a}
	Sorghum-Sorghum	3360 ^{b}	69 ^{ab}
	Block	ns	ns
	Rotation (R)	**	**
	Fertilizer (F)	ns	ns
	RxF	ns	ns

*, **Significant at 0.05 and 0.01 level; ns: Not significant (p>0.05).
Values affected by the same letter in the same column are not significantly different at p<0.05, according to Fisher's test.

In total five nematode groups were identified in the soil but the two most important were Helicotylenchus (55%) and Scutelonema (34%). At 30 DAS sorghum roots were mainly infected by Pratylenchus (80%) and Scutelonema (20%) and at 90 DAS mainly by the Pratylenchus group (data not shown). Nematode infections were not affected by fertilizer application (Table VII). They were only affected by crop rotations and interaction between the two factors was not observed meaning that only rotation treatments were responsible for nematode population variations. Cowpea increased soil and sorghum root infection by nematodes while groundnut decreased nematode infection. The soil of the cowpea-sorghum rotation contained 1.5 to 2 times more nematodes than the soil of the sorghum mono cropping treatment. In contrast, the soil of groundnut-sorghum rotation contained from 17 to 19 times less nematodes than that of the sorghum monocropping. Alvey *et al.* [37] also found that compared to continuous cultivation of cereals, groundnut-cereal rotations can reduce nematode populations from 60 to 80%. Diop *et al.* [38] reported that groundnut was an effective crop which can be used to reduce nematode infections while Riekert and Henshaw [39] showed that cowpea increased nematode infections. But little is known about nematode-induced damages by cowpea in West Africa. Despite the high infection levels, nematode didn't significantly affect sorghum yields in the cowpea-sorghum rotations, probably because of the better N supply in a N-deficient soil than the other cropping systems and the high N uptake resulted in good growth of the succeeding sorghum in the cowpea-sorghum rotation.

As it was hypothesized, nematode infection gave more information on the differential effects of legumes on increasing yields of the succeeding sorghum crop. Soils in both cowpea-sorghum and groundnut-sorghum rotations provided more N for succeeding sorghum compared to monocropping of sorghum. A better use of N fertilizer and good response to N fertilizer was particularly observed in cowpea-sorghum rotations leading to higher N uptake and yields of succeeding sorghum compared to groundnut-sorghum rotations. Although in the absence of N fertilizer, the nitrogen fertilizer equivalency (NFE) of groundnut was slightly higher than that of cowpea. But sorghum produced highest yields in cowpea-sorghum rotation when N fertilizer was used. This apparent contradiction pointed out a weakness of the concept of NFE in the assessment of N effect of legumes. The NFE is an estimation of N effect of legume crops on the succeeding non-fixing crop in the absence of N fertilizer applications. Without N fertilizer applications, sorghum yields were higher in groundnut-sorghum rotation than those of cowpea-sorghum rotations. In the absence of N fertilizer applications, suppression of nematode infection by groundnut may explain the better yields of sorghum in groundnut-sorghum rotations and the higher NFE values of groundnut indicated that groundnut effectiveness on nematode reduction is probably accounted as N effect in the NFE assessment, leading to an overestimation of N effect of groundnut.

4. CONCLUSION

These experiments provided a better understanding of the contributions of the two N_2-fixing legume crops (cowpea and groundnut) to improving soil N fertility status and increasing grain yields of succeeding sorghum in a weakly acid Ultisol of the Guinean savannah of Burkina Faso. Despite the exportation of legume shoots, the two legume crops had significant N effect on succeeding sorghum due to organic N supply by remaining legume residues (fallen senescent leaves and below ground parts). In addition, cowpea-sorghum and groundnut-sorghum rotations improved N fertilizer use efficiencies (NFUE) of the succeeding sorghum. These legume effects on the supply and availability of both organic and inorganic N may explain the higher N accumulation and grain yields of the succeeding sorghum. But some differences occurred between the two legumes. Compared to groundnut, cowpea had the highest N effects and NFUE, resulting in a higher productivity of the succeeding sorghum.

The nematode infections observed in the cowpea-sorghum rotation were higher than those in the groundnut-sorghum rotation. Despite the high infection levels, nematode did not significantly reduce sorghum yields in cowpea-sorghum rotation. Groundnut had less N effects compared to cowpea. In addition, the N effects of groundnut could be overestimated by the concept of N fertilizer equivalency (NFE) due to the reduction in nematode infection.

An integrated management including legume-sorghum rotations, chemical fertilizers and local nutrient sources can improve N balance and decrease soil acidity, leading to system productivity improvement. In addition to the recommended doses of PK fertilizers, it was estimated that some 51 to 58 kg N ha^{-1} should be applied to achieve optimum average grain yields of 2000 kg ha^{-1} when sorghum is grown in legume-sorghum rotation. Additional applications of 1 t ha^{-1} of dolomite or 3 t ha^{-1} of manure are recommended on sorghum and cowpea. The local phosphate rock from Burkina Faso could be used as source of P on groundnut.

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NITROGEN UTILIZATION FROM UREA AND GREEN MANURE RESIDUES BY CORN GROWN UNDER NO TILL IN SOUTHERN CERRADO, BRAZIL

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Abstract

Field experiments were carried out in Selviria, Mato Grosso do Sul state in the Brazilian southern Cerrado, during 2 consecutive years, to evaluate the N use efficiency from the urea fertilizer and green manures (millet and crotalaria) by corn grown in a red Latosol under no tillage system. The experimental design was completely randomized blocks with 24 treatments (incomplete factorial 5×3×2): 5 fertilizer N rates (0, 30, 80, 130 and 180 kg N ha^{-1}), 3 fallow systems: natural and green manure (millet and crotalaria) and 2 split application times of fertilizer N (at seeding and topdressing at 4 or 8 leaves stage). The green manures and urea were labeled with ^{15}N. The highest grain productivity were obtained in the crotalaria-corn sequence during both years followed by natural fallow-corn and millet-corn, the fallow systems differing statistically in the first year, and only when N was applied at the 8 leaves stage in the second year. The crotalaria provided more N to corn than fallow and millet in both years and the corn grain yield response to N rates was fitted to a quadratic function. The fertilizer N application at 4 leaves stage resulted in higher grain productivity in all three fallow systems in the first year, and significant differences were found for natural fallow-corn and millet-corn systems when N was topdressed at 8 leaves stage. The grain productivity data were fitted to quadratic functions with regard to the fertilizer N application rates. The maximum technical efficiency for grain productivity was estimated to be achieved at the N rates of 148, 117 and 161 kg N ha^{-1} and 171, 160 and 174 kg N ha^{-1} for the natural fallow-corn, crotalaria-corn and millet-corn during the first and second year respectively. The FNUE was, on the average of 2 years, 53%, 49% and 44% for the corn grown as succeeding crop to crotalaria, fallow and millet, respectively. The application of increasing fertilizer N rates resulted in a quadratic response of the utilization by corn of the N from crotalaria and millet. The N utilization from the crotalaria and millet was (on the average of two years) 15.6% and 7.6%, respectively. Although millet is often used as cover crop/green manure for corn in this region, the results of this study showed that the millet did not show any advantage in terms of corn grain productivity compared to the natural fallow system.

1. INTRODUCTION

The savannah areas of Brazil known as Cerrado are the largest ecosystem after the Amazonian Forest and have great importance in the national and world agricultural scenarios, being an important reserve for the biodiversity. They cover an extension of approximately 204 Mha of potential arable land, thus showing the highest potential for expanding agricultural production. Studies indicate that it is possible to incorporate to the productive system up to 127 Mha using appropriate technology and still maintaining 38% of the savannah as natural reserve [1]. However, the soils of Cerrado, in natural conditions, are highly weathered and present several limitations to agricultural production due to their inherent low fertility, in particular high acidity and aluminum saturation, low content of most of plant nutrients and organic matter, low cation exchange capacity, and high P fixing capacity.

Corn is one of the main crops in terms of cultivated area for grain production in Brazil (13 Mha), being responsible for the introduction and consolidation of no-till system in the region of Cerrado. Brazil is the third largest corn-producing country in the world, following USA and China but it is not self-sufficient, thus importing part of its consumption, except during the year 2003. The average national corn grain yield was 3.53 t ha^{-1} in 2003, although most of large farmers (500–1000 ha) in the Cerrado region often obtain more than 7 t ha^{-1} grain yield.

Owing to the large demand of nitrogen (N) by corn, and the low available soil N of the cultivated Cerrado soils, high rates of chemical N fertilizers must be applied to ensure high grain productivity. Recently the high cost of fertilizer N combined with the increasing environmental concern on the pollution of water resources and the atmosphere have prompted the search for sustainable and cost-effective alternatives to obtain at least a partial substitution of those inputs.

Corn cultivation under no-tillage system succeeding to a fallow period with green manure crops, mainly legumes, has been shown to be a promising alternative to supplementing N inputs to the system [2]. The absence of ploughing and the maintenance of crop residues over the soil surface result in a significant increase of soil organic matter with time, due to the regulated turnover rate, mainly because of higher nitrogen and carbon quantities. The flux of nitrogen release is influenced by the quality of the crop residues, in particular the C/N ratio, which assumes an important role on the immobilization and mineralization turnover of soil nitrogen and, consequently, on the nitrogen availability to plants [3]. Therefore, it is important to determine the source, quantity, application time of the green manure crops and the utilization efficiency of this nitrogen source by the corn crop grown in this region.

The objective of this work was, therefore, to evaluate the nitrogen utilization by the corn crop from the urea when applied alone at several rates and times of application and combined with green manure crops, i.e. crotalaria (*Crotalaria juncea*) and millet (*Pennisetum americanum*). The urea and green manure N utilization by corn was evaluated using ^{15}N isotope techniques.

2. MATERIALS AND METHODS

This field study was carried out in Selviria, Mato Grosso do Sul state (some 600 km from Piracicaba), Brazil. The experimental site was located in the humid tropics at 51° 22' longitude W; 20° 22' Latitude S, and 335 m altitude, under Aw climatic conditions (Koeppen). The average annual rainfall was 1370 mm and the mean annual temperature: 24.5°C. The soil is a clayey distrophic red Latosol (LVd), previously under Cerrado vegetation and cropped for 19 years in the conventional system of soil management and under no till over the last 5 years [4].

2.1. Experimental design

The experimental work consisted of two field trials carried out over two consecutive (2001/2002 and 2002/2003) growing seasons. The experimental design was Completely Randomized Blocks with 24 treatments (incomplete factorial 5×3×2) with 4 replications, consisting of three fallow systems: natural fallow and two green manure/cover crop treatments (crotalaria and millet); five fertilizer N rates (0, 30, 80, 130 and 180 kg N ha^{-1}) and two split applications of fertilizer N (at seeding and topdressing at 4 leaves or 8 leaves stage respectively) (Table I). All treatments, except the control, received a basal N (30 kg N ha^{-1}). The main plots consisted of 8 rows of corn spaced 80 cm each other and 7 m length. The ^{15}N fertilizer was applied to the 1.0 × 2.4 subplots.

The experimental area after the harvest of first season trial was left under natural regrowth fallow, i.e. without cover crop and corn was sown again to evaluate N residual effect from millet, crotalaria and fertilized corn.

TABLE I. EXPERIMENTAL TREATMENTS

Treatment No.	N rate kg ha^{-1}	Fertilizer N ----------Time of application----------			Fallow/Green Manure
		Seeding	4 leaves	8 leaves	
T1	0				Fallow
T2	30	30*			Fallow
T3	80	30	50*		Fallow
T4	80	30		50*	Fallow
T5	130	30	100*		Fallow
T6	130	30		100*	Fallow
T7	180	30	150*		Fallow
T8	180	30		150*	Fallow
T9	0				Crotalaria*
T10	30	30*			Crotalaria*
T11	80	30	50*		Crotalaria*
T12	80	30		50*	Crotalaria
T13	130	30	100*		Crotalaria*
T14	130	30		100*	Crotalaria
T15	180	30	150*		Crotalaria*
T16	180	30		150*	Crotalaria
T17	0				Millet*
T18	30	30*			Millet*
T19	80	30	50*		Millet*
T20	80	30		50*	Millet
T21	130	30	100*		Millet*
T22	130	30		100*	Millet
T23	180	30	150*		Millet*
T24	180	30		150*	Millet

* Labeled urea or green manure. The treatments that received labeled urea and green manure had separate subplots within the respective plots.

2.2. Field experimental procedures

2.2.1. Green manure cultivation

Crotalaria and millet were sown mechanically in the beginning of the rainy season (September of 2001 and 2002) for both trials respectively. Crotalaria was sown with a seeding density of 30 to 40 seed /m^2, in 40 cm spaced rows, and millet, BN-2 variety, in 17 cm spaced rows, at the rate of 25 kg ha$^-$following recommended practices [5–6]. The above ground crotalaria and millet biomass was cut mechanically at the beginning of flowering stage (on 30 November 2001 and 25 November 2002, 1st and 2nd trial, respectively).

2.2.2. ^{15}N labeling of millet and crotalaria plants

^{15}N labeled millet and crotalaria plants were obtained by growing them simultaneously in a area adjacent to the main experiment, and applying 30 kg N ha^{-1} as urea-^{15}N (10.02% atom ^{15}N excess) in three equal splits at 20, 34 and 48 days after germination (1^{st} application to the soil; 2^{nd} application ½ to the soil and ½ foliar application and 3^{rd} application only foliar spraying). The ^{15}N labeled plants were harvested at the same time as the unlabelled plants. Samples were taken for chemical and isotopic ^{15}N analysis.

2.2.3. Main field experiments (corn crop)

Pioneer 30F80, a semi-early single corn hybrid was sown on December 5, 2001 (1^{st} trial) and on November 28, 2002 (2^{nd} trial). At seeding 375 kg ha^{-1} of NPK compound fertilizer (8-28-16) was applied to all treatments, except to the control treatment, 5 cm below the seed row.

The ^{15}N fertilizer (urea-^{15}N at 2.366% atom ^{15}N was applied in a 1.0 × 2.4 m subplot inside the main plots (the same N rate was applied to the main plot). The topdressing of N fertilizer was done on December 28, 2001 and January 14, 2002 at 4 leaves and 8 leaves, respectively during the first growing season. In the second year trial the urea fertilizer was top-dressed at 4 leaves stage on December 20, 2002 and January 6, 2003 at 8 leaves stage.

In the green manuring subplots, unlabeled millet or crotalaria plant material was substituted by equivalent amount of ^{15}N labeled plants (4.665% atom ^{15}N and 2.331% atom ^{15}N, for millet and crotalaria respectively). The ^{15}N labeled plants were cut in small pieces, as the unlabeled plants were cut mechanically.

Leaf samples were collected at flowering and the following elements were analyzed at CENA [7]: N (Kjeldhal), P (colorimetrically), K (flame photometry), S (turbidimetry), B (azomethine-H colorimetry), and Ca, Mg Cu, Fe, Mn and Zn by Atomic Absorption Spectrometry (AAS). Based on the results of the analyses, the nutritional status of the cultivated plants (corn, millet and crotalaria) was evaluated [8]. For the evaluation of millet and crotalaria production 3 m of central rows in the main plots were considered. Corn plants were taken from 6 central rows, leaving out the two outer rows and 1 m of row at both extreme of the central rows. Corn plant samples were separated in leaves, stalks, grains and cobs for chemical and ^{15}N analysis made at CENA. The following parameters were also determined in 1^{st} year and 2^{nd} year experiments: Plant stands, number of grains per cob, plant height, corn ear insertion height, weight of 1000 grains (transformed to 13% moisture content), grain yield and grain N and ^{15}N contents.

From the ^{15}N data the Npdff (nitrogen in the plant derived from fertilizer, or green manure) in % and quantity and the % fertilizer N use efficiency (FNUE) and the coefficient of N utilization from the green manure were calculated.

2.3. Soil Chemical Analysis

Soil samples were taken from the soil profile at 0–10, 10–20 and 20–40 cm depth layer, for chemical analysis to assess the soil fertility status and provide PK fertilizer recommendations [9].

Each year prior to the start of the corn growing season (August 2001 and August 2002) following local recommendations (Comissão de Fertilidade do Solo-CFSRS/SC 1995), selected soil characteristics (pH, OM, P, K, Ca, Mg, H+Al) were analyzed according to [10]. The micronutrients Cu, Fe, Mn and Zn were analyzed using the DTPA method [11]. The results of the soil chemical characteristics prior to the start of the first year experiment (2001/02) are shown in Table II.

Total N content was determined in soil samples collected in between rows at 0–10, 10–20 and 20–40 cm depth, at corn flowering stage (February 1, 2002 and January 22, 2003) according to [12]. Inorganic forms of nitrogen (NH_4^+ and NO_3^-) were also measured in the same soil samples, extracting with KCl 2M [13].

TABLE II. SOIL CHEMICAL CHARACTERISTICS PRIOR TO THE START OF THE FIRST YEAR EXPERIMENT (2001/2002 SEASON)

Soil Depth	pH $_{(CaCl2)}$	Total N	O.M.	P (resin)	Ca	Mg	K	H+Al	S	Cu	Fe	Mn	Zn
cm		g kg^{-1}	g dm^{-3}	mmol$_c$ dm^{-3}						mg dm^{-3}			
0-10	5.04	0.89	21.10	11.93	17.70	15.71	2.3	22.13	6.45	2.5	16.0	20.2	0.34
10-20	4.78	0.75	19.23	6.00	11.49	7.41	1.0	23.50	6.03	2.4	16.7	16.2	0.30
20-40	4.59	0.49	11.77	2.73	7.78	6.55	0.7	19.56	7.07	1.4	8.3	4.5	0.10

2.4. Statistical Analysis

The data were submitted to analysis of variance, utilizing the SAS statistical program applying F test, and the comparison of means was made by Tukey's test (at 5 and 1% level). In addition correlation analysis between individual variables and grain yield and treatment means comparison by t test (at 5 and 1% level) and regression analysis, was made considering the factorial for 5 rates of fertilizer N (0, 30, 80 130 and 180 kg N ha^{-1}), two times of N application (topdressing at 4 leaves and 8 leaves stage) and the 3 fallow/green manure systems (natural fallow, millet and crotalaria).

The maximum efficiency for grain productivity was obtained by setting the first derivative of the yield function to zero and solving for N: $y = c + bx - ax^2$. The factors were: 5 rates of fertilizer N (0, 30, 80 130 and 180 kg N ha^{-1}), mean values of the 2 times of N application (topdressing at 4 leaves and 8 leaves stage) and 3 fallow/green manure systems (natural fallow, millet and crotalaria).

3. RESULTS AND DISCUSSION

3.1. ^{15}N Labeled crotalaria and millet

It can be observed in Table III, that the dry matter yield of the crotalaria was superior to the millet, in both years (9770 kg ha^{-1} of crotalaria and 7370 kg ha^{-1} of millet, during the first year and 8610 kg ha^{-1} crotalaria and 7835 kg ha^{-1} millet, during the second year). Moreover, the crotalaria presented greater N concentration, consequently a higher amount of total N content and potentially mineralizable N in the green manure biomass. The crotalaria as a legume plant had also better quality, i.e. a low C/N ratio. In Brazil, the *Crotalaria juncea* produced 31% more biomass than millet and 108% more biomass than the spontaneous weeds grown in the natural fallow area [14]. The plant materials labeled with ^{15}N for substituting unlabeled plants in the subplots, presented enrichments of 1.965 and 4.299 atoms % ^{15}N excess in the first year for crotalaria and millet respectively whereas in the second year, the enrichments were 1.882 and 2.416 atoms % ^{15}N excess for crotalaria and millet, respectively.

These ^{15}N enrichment values are high enough to study N dynamics in the soil-plant system, according to [15]. The applied amount of green manure residues contained a nitrogen rate equivalent to 169 and 68.5 kg N ha^{-1}, for crotalaria and millet respectively in the first year experiment and 189 and 79.1 kg ha^{-1}, in the second year.

Although both crops received the same amount of the same ^{15}N labeled nitrogen fertilizer (equivalent to 30 kg N ha^{-1} as urea with 10.02% atom ^{15}N), the crotalaria plants were much less enriched, probably due to the dilution by the atmospheric N fixed symbiotically and also by producing more biomass than millet. Considering their biomass produced (on the average 9.2 and 7.6 t ha^{-1} for crotalaria and millet respectively) and total N average concentrations (19.6 g kg^{-1} and 9.7 g kg^{-1} for crotalaria and millet, respectively) the nitrogen contents of crotalaria and millet are 180 kg N ha^{-1} and 74 kg N ha^{-1} respectively.

Several weeds, mainly "picão preto", "trapoeraba", "capim colonião" and "corda de viola" (Brazilian common names) had grown on the natural fallow plots. The mean dry matter weight of these weeds was 2.67 Mg ha^{-1}and their N content was 31 kg N ha^{-1}.

TABLE III DRY MATTER YIELD, TOTAL N CONCENTRATION, ^{15}N ENRICHMENT AND TOTAL N CONTENT AND C/N RATIO OF CROTALARIA, MILLET AND NATURAL VEGETATION OF FALLOW PLOTS (1ST AND 2ND YEAR EXPERIMENT)

Fallow/Green manure crop	DM yield Kg ha^{-1}	Total N conc. g kg^{-1}	^{15}N atom % excess	N content kg ha^{-1}	C/N ratio
1st experiment					
Crotalaria	9770	17.3	1.965	169	19.2/1
Millet	7370	9.3	4.299	68.5	42.3/1
Natural fallow	2490	11.3		28.1	37.3/1
2nd experiment					
Crotalaria	8610	21.9	1.882	189	16.2/1
Millet	7835	10.1	2.416	79.1	46.6/1
Natural fallow	2860	11.9		33.9	34.3/1

The elemental composition of the green manure crops did not show much differences in P, K, S, Mn and Zn concentrations but crotalaria contains more Ca, Mg and Cu and millet more Fe. (Table IV). The P, K, Ca, Mg, S, Cu, Fe, Mn and Zn content in the corn leaves taken at flowering (2001/02 trial) (data not shown) are within the adequate level for the corn crop, according to [8].

TABLE IV. ELEMENTAL COMPOSITION (% DRY MATTER) OF THE CROTALARIA AND MILLET AT HARVEST (1^{ST} AND 2^{ND} YEAR EXPERIMENT)

	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
	----------------------g kg^{-1}----------------------					---------------mg kg^{-1}---------------			
1st Experiment									
Crotalaria	3.3	24.6	9.9	5.4	1.9	12.3	133.1	72.5	27.1
Millet	3.2	26.3	4.3	2.9	1.5	6.2	653.7	93.5	31.7
2nd Experiment									
Crotalaria	2.7	25.9	9.6	4.7	1.6	10.1	197.4	59.9	23.9
Millet	2.2	26.9	3.8	2.8	0.8	6.3	317.1	56.7	24.7

3.2. Crotalaria and Millet as green manures for corn

In the first year, the millet residues supplied a relatively small amount of nitrogen to corn crop, on the average less than 5 kg ha^{-1} (Figs 1 and 2). It is very likely that this low N supply from this N source was due to the much higher C/N ratio in comparison to crotalaria, which supplied between 21.8 to 31.3 kg ha^{-1} N to the corn crop. The highest value was obtained when 100 kg N ha^{-1} was applied as fertilizer. The % N utilization by corn from millet and crotalaria ranged from 3.2% to 5.9% and 12.9 to 28.5%, respectively.

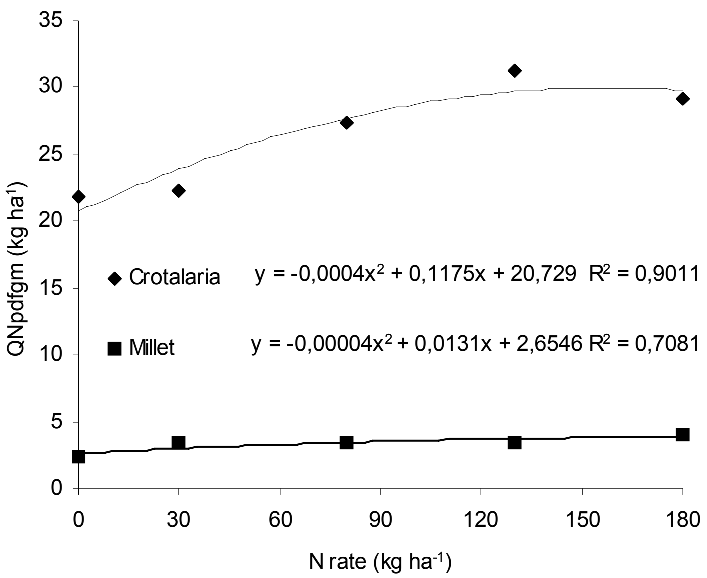


FIG.1. Nitrogen (kg N ha^{-1}) in corn plant derived from millet and crotalaria (1^{st} year experiment).

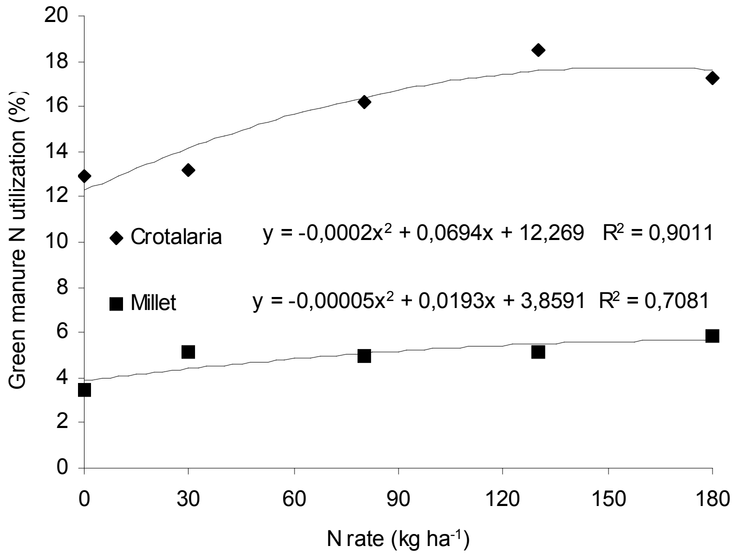


FIG.2. Green manure-N utilization (%) by corn crop (1st year experiment).

In the second year (Figs 3 and 4) the crotalaria also supplied more N to the corn, the amounts varying from 21.2 to 37.6 kg N ha^{-1}, which were equivalent to 11 to 19% N utilization. For the millet the amounts supplied varied from 4.9 to 8.1 kg ha^{-1} of N, equivalent to 7 to 12% N utilization. Increasing the added N rate as green manure resulted in a higher N utilization by the corn crop, probably due to increased activity of chemotrophic microorganisms of the soil.

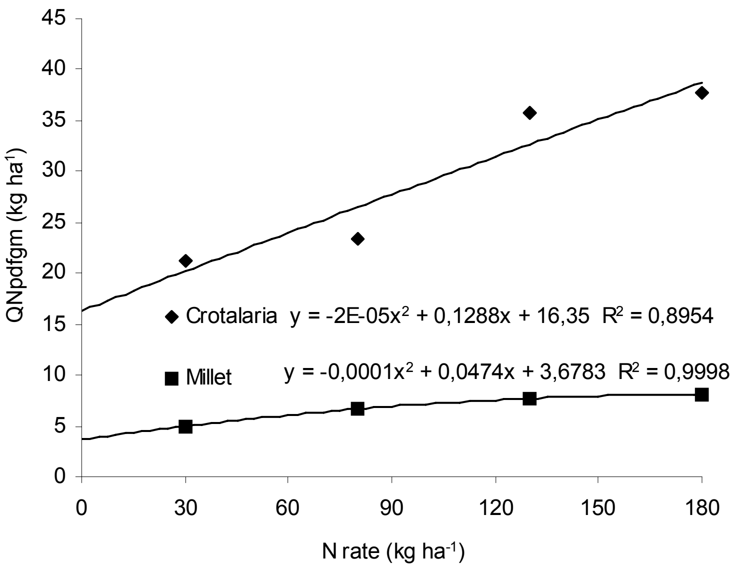


FIG.3. Nitrogen (kg N ha^{-1}) in corn plant derived from millet and crotalaria (2nd year experiment).

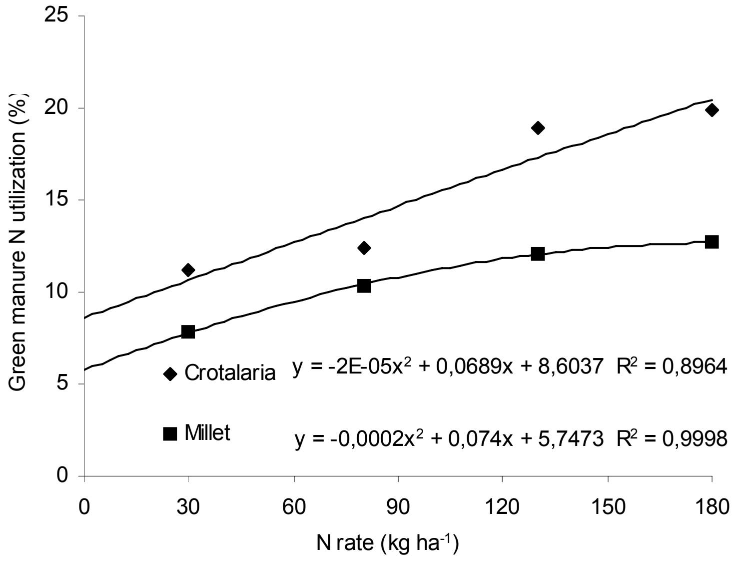


FIG.4. Green manure-N utilization (%) by corn crop (2nd year experiment).

The amounts of N (kg N ha^{-1}) taken up from the applied fertilizer N by corn in the three systems studied (natural fallow-corn, crotalaria-corn and millet-corn) was adjusted to a quadratic equation. The highest values were observed in crotalaria-corn system and the lowest in the millet-corn system (Figs 5 and 6). The estimated coefficient of fertilizer N utilization or fertilizer N use efficiency (% FNUE) decreased, in all three systems, with increasing rates of fertilizer nitrogen, except for millet, in which the maximum utilization was obtained when 100 kg N was applied, but with smaller utilization for higher rate, as it was for the lower rates (30 and 50 kg N ha^{-1}). These results explain what it was found for grain yield.

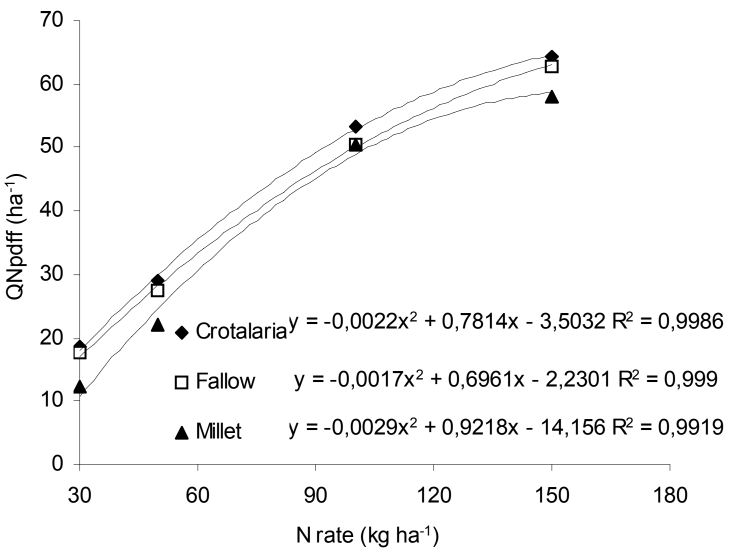


FIG.5. Nitrogen (kg N ha^{-1}) in corn plant derived from the applied fertilizer (1st year experiment).

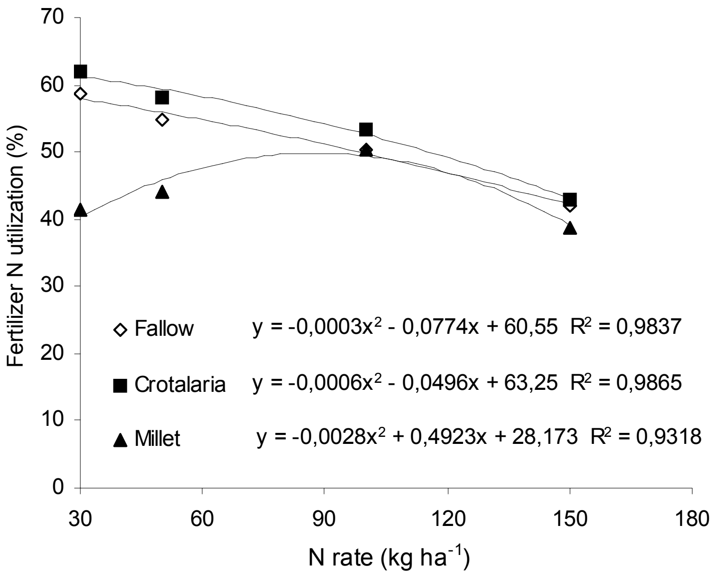


FIG.6. Fertilizer N utilization or fertilizer N use efficiency (1st year experiment).

The highest amount of nitrogen (kg N ha^{-1}) in corn plant derived from the fertilizer (QNpdff) in the 2nd year experiment (Fig. 7) was observed in corn grown after crotalaria and the least after millet. As in the previous year, increasing N rate increased the QNpdff values reducing, however, the fertilizer N use efficiency (FNUE) (Fig. 8). The FNUE ranged from 40 to 60%.

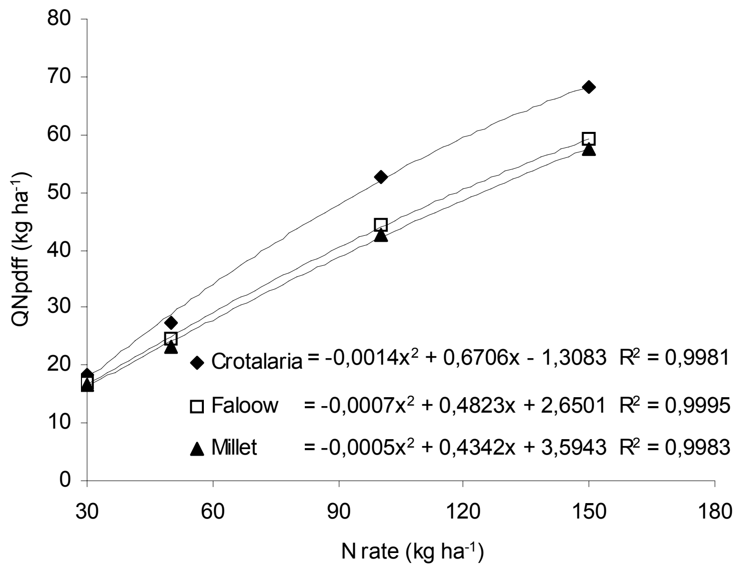


FIG7. Nitrogen (kg N ha^{-1}) in corn plant derived from fertilizer (2nd year experiment).

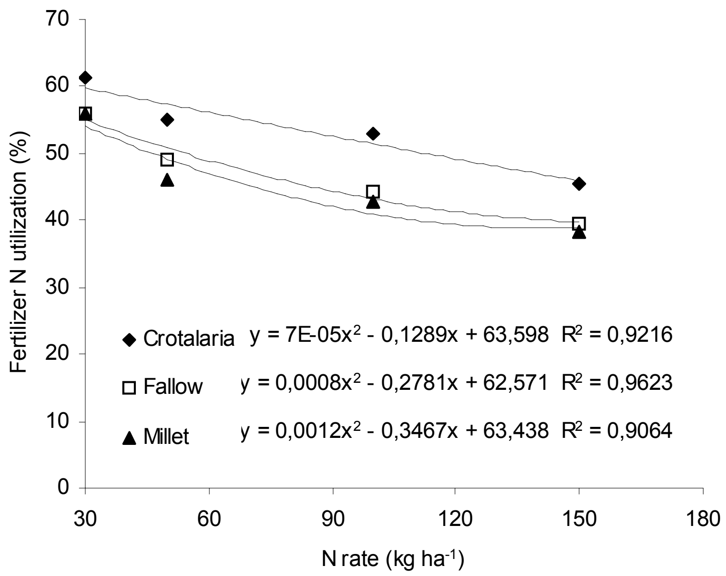


FIG. 8. Fertilizer N utilization or fertilizer N use efficiency (2nd year experiment).

3.3. Corn grain yield and related parameters

The mean values for plant height, height of corn cob insertion, leaf N content, number of grains per cob, weight of 1000 grains and grain productivity (1st and 2nd year experiment) are presented in Table V. In the comparisons of the first and second year experiment, there was not much differences between grain productivity of the same treatment in different years. But, in most of the treatments, the evaluated plant and yield components were greater in the second year experiment. There was significant interaction between N rate and cover crops systems for grain productivity in the first year experiment (Table V). The partitioned data were fitted to quadratic functions and the maximum efficiency of the N rates was obtained for 148, 117 and 161 kg N ha^{-1} in the fallow-corn, crotalaria-corn and millet-corn, respectively (Fig. 9).

TABLE V. EFFECT OF FERTILIZER N RATE AND APPLICATION TIME ON CORN YIELD PARAMETERS UNDER DIFFERENT FALLOW SYSTEMS, (1ST AND YEAR 2ND EXPERIMENT)

Source of variation		Plant height (m)	Cob insertion height (m)	Leaf N content (g kg^{-1})	Grain per cob (g)	Weight of 1000 grains (g)	Grain yield (kg ha^{-1})
Fertilizer N rates (kg N ha^{-1})							
At seeding	Top dressing			1st year experiment			
0		2.06	1.08	15.9	482.5	264.91	4936*
30		2.14	1.14	17.,4	466.3	269.4	5920
30	50	2.25	1.21	22.1	505.7	286.6	7235
30	100	2.28	1.24	23.2	505.7	285.2	7738
30	150	2.27	1.24	24.0	518.1	274.4	7491
N application times							
4 leaves		2.21 a	1.19 a	20.5 a	494.9 a	276.8a	6756 a
8 leaves		2.20 a	1.17 a	20.5 a	496.3 a	275.4 a	6571 b

Source of variation		Plant height (m)	Cob insertion height (m)	Leaf N content (g kg^{-1})	Grain per cob (g)	Weight of 1000 grains (g)	Grain yield (kg ha^{-1})
Fallow systems							
Crotalaria		2.31 a	1.26 a	21.7 a	513.3 a	290,4 a	7790*
Natural fallow		2.17 b	1.17 b	20.1 b	494.1 ab	269.3 b	6339
Millet		2.14 b	1.13 c	19.7 b	479.5 b	268.7 b	5862
				2nd year experiment			
0		2.22	1.18	22.5*	446.6	274.8	4602*
30		2.26	1.18	23.1	460.3	280.8	5709
30	50	2.31	1.22	25.9	498.1	297.5	7213
30	100	2.34	1.22	26.7	530.6	309.8	7860
30	150	2.36	1.25	27.5	530.7	310.4	8222
N application time							
4 leaves		2.31a	1.21a	25.3*	499.2 a	293.0 a	6810*
8 leaves		2.29a	1.21a	25.0	487.3 a	296.0 a	6632
Fallow systems							
Crotalaria		2.37 a	1.27 a	26.7*	512.9 a	304.3 a	7755*
Natural fallow		2.30 b	1.24 b	24.7	491.4 b	290.1 b	6345
Millet		2.22 c	1.23 c	24.0	475.6 b	289.5 b	6068

The values followed by same letter in the column, within the same year, do not differ statistically (Tukey's test 0.05). * Significant interaction.

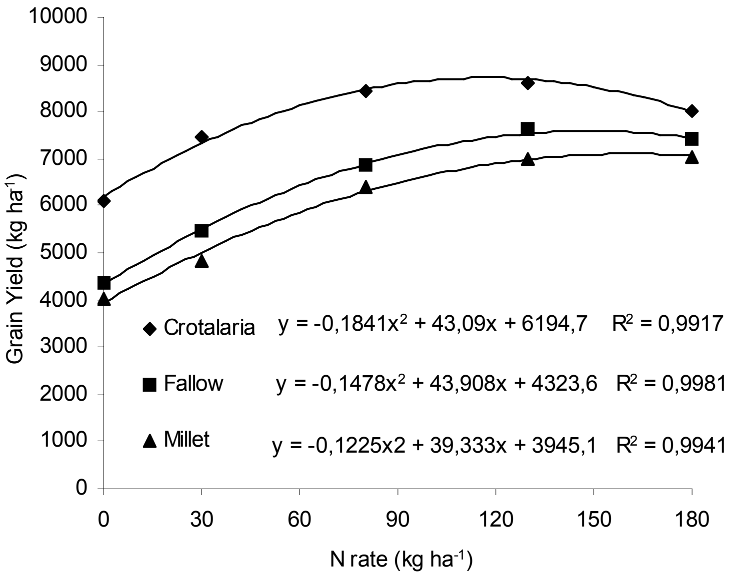


FIG.9. Productivity of corn (grain yield) in three cover systems: fallow, millet and crotalaria, mean of values when N was applied at 4 and 8 leaves stage (1st year experiment).

The data for all variables in the second year were fitted to a quadratic equation, except for leaf N content and grain productivity data (Table V), which were partitioned for the significant interaction between N application times and fallow/green manure crop systems

(Table VI). The maize grain yield in the crotalaria-corn system was followed by natural fallow-corn system and both were better than millet-corn sequence (Fig. 10). Timing of N application did not show great effect on grain yield in the crotalaria-corn system. The superiority of the grain yield in the natural fallow-corn system to that in millet-corn was found significant only when N was applied at 8 leaves stage. This is likely due to higher activities of chemo-organotrophic microorganisms occurring in the initial phase of corn development, which may have caused temporary N immobilization, as reported by some authors [2, 16].

TABLE VI. PARTITIONING OF THE SIGNIFICANT INTERACTION BETWEEN FALLOW SYSTEMS AND TIMING OF FERTILIZER N APPLICATION FOR LEAF N CONTENT AND GRAIN PRODUCTIVITY OF CORN (2ND YEAR EXPERIMENT)

Fallow/green manuring treatments	Leaf N content (g kg^{-1})		Grain yield (kg ha^{-1})	
	4 leaves	8 leaves	4 leaves	8 leaves
Crotalaria-corn	26.7 a	26.7 a	7693 a	7818 a
Natural fallow-corn	24.7 b	24.8 b	6400 b	6281 b
Millet-corn	24.5 b	23.6 c	6338 b	5798 c

The values followed by the same letter in the column do not differ statistically (Tukey's test 5%).

TABLE VII. EFFECT OF DIFFERENT RATES AND TIMES OF APPLICATION OF FERTILIZER NITROGEN ON GRAIN YIELD OF CORN GROWN AFTER NATURAL FALLOW CROTALARIA AND MILLET UNDER NO-TILLAGE SYSTEM (1ST AND 2ND YEAR EXPERIMENT)

Treatments ------Times of N application------				Corn grain yield kg ha^{-1}	
	Sowing	4 Leaves	8 Leaves	Natural fallow-corn	
				1st year	2nd year
1	0			4367	4111
2	30			5452	5262
3	30	50		7088	6987
4	30		50	6619	6689
5	30	100		7588	7580
6	30		100	7547	7363
7	30	150		7410	8097
8	30		150	7360	7944
				Crotalaria-corn	
9	0			6110	5723
10	30			7457	6821
11	30	50		8131	8167
12	30		50	7733	8337

	Treatments ------Times of N application------			Corn grain yield kg ha^{-1}	
	Sowing	4 Leaves	8 Leaves	Natural fallow-corn	
				1st year	2nd year
13	30	100		8645	8826
14	30		100	8774	9032
15	30	150		8321	8927
16	30		150	8565	9176
				Millet-corn	
17	0			4032	3972
18	30			4849	5046
19	30	50		6619	6863
20	30		50	6179	6238
21	30	100		7455	7826
22	30		100	6530	6932
23	30	150		7522	7976
24	30		150	6522	7213

It may be observed in the Figs 10 and 11 that the grain productivity for all N rates and application times was highest in the sequence crotalaria-corn, followed by the natural fallow and lowest in the sequence millet-corn. For the three fallow systems and the two N application times (at four and eight leaves), the data were fitted to quadratic functions in relation to the rates of fertilizer N. However, it may be noted that the largest differences among times of application of N and smaller productivities of grains in the sequences millet-corn and natural fallow-corn occurred when N was applied at the eight leaves stage. This certainly is related to the lack of N in the initial phase of development of the crop, due to the immobilization of soil mineral N, as mentioned before.

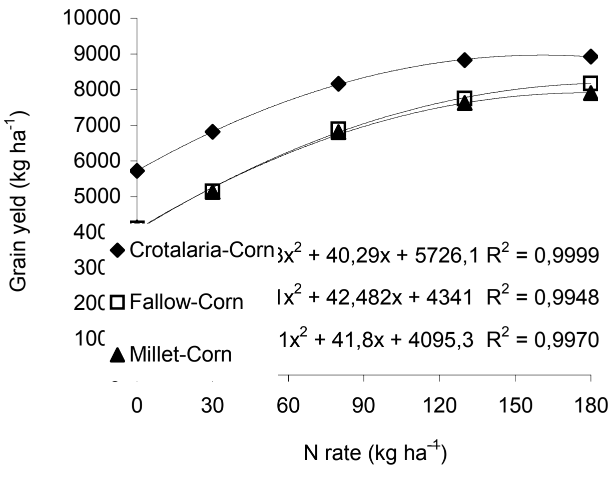


FIG.10. Productivity of corn (grain yield) in three cover systems: fallow, millet and crotalaria, when N was applied at 4 leaves stage (2nd year experiment).

In the relation to the previous year experiment, there were not many discrepancies among the grain productivity data for the same treatment in different years. But, in most treatments, the evaluated plant and yield components were greater in the second year experiment. For the grain productivity, the highest difference was 180 kg N ha^{-1} in the corn. Considering the mean values of grains productivity with fertilizer N application oat the 4 and 8 leaves stages, the maximum efficiency would have been obtained with 171, 160 and 174 kg N ha^{-1} for the fallow-corn, crotalaria-corn and millet-corn in the second year, respectively.

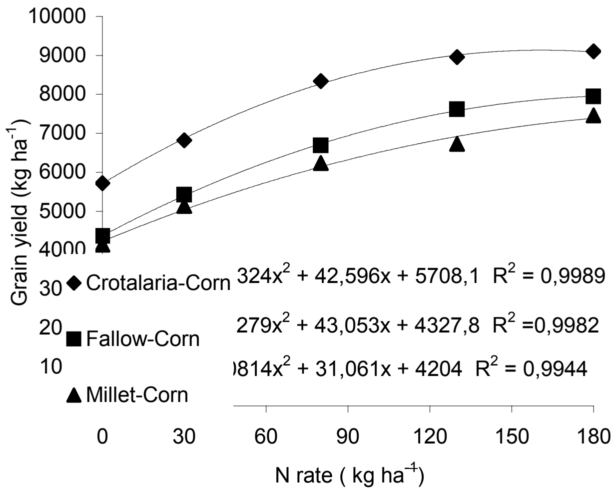


FIG. 11. Corn grain yield in three cover systems (fallow, millet and crotalaria) when N was applied at 8 leaves stage (2nd year experiment).

Positive and significant correlations between corn grain productivity and all evaluated plant components and production were found in both years (Table VIII). The correlations with plant height and corn cob insertion height are likely linked to the fact that N play important role in the cellular division and expansion, besides being constituent of chlorophyll molecule, influencing consequently the plant photosynthetic process with direct relationship to the increase in the foliar area, and the carbohydrate translocation and accumulation in the corn grains ([17–18].

The number of grain per cob and the grain mass are components directly related to the productivity of grains, which probably, is related to the largest leaf N content, leading to a better grain filling.

TABLE VIII. SIMPLE CORRELATIONS OF GRAINS PRODUCTIVITY VERSUS THE PLANT AND CORNCOB HEIGHT, LEAF N CONTENT, NUMBER OF GRAINS PER COB MEAN WEIGHT OF 1000 GRAINS, (1ST AND 2ND YEAR EXPERIMENT)

Experiment	Plant height	Corn cob insertion height	Leaf N content	Number of grain per cob	Weight of 1000 grains
1st year	0.95**	0.93**	0.79**	0.76**	0.80**
2nd year	0.87**	0.80**	0.96**	0.93**	0.91**

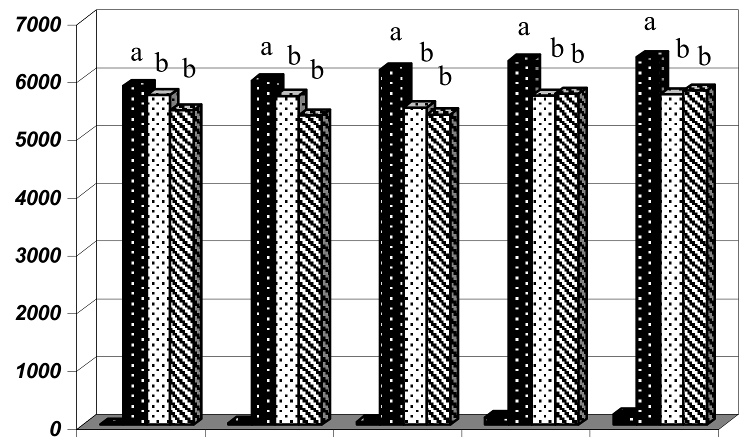
Note: * ** denote significant at 5 and 1% level, respectively, by t test.

3.4. Residual effect of N from the applied fertilizer and green manure crops

The highest corn productivities after the first season experiment were obtained in the crotalaria treatment and they were statistically superior to the fallow and millet treatments, which did not differ among them (Fig. 12).

The best results of corn production obtained with crotalaria demonstrate that a significant fraction of N and other nutrients contained in its residues still benefited subsequent corn crop. According to [19], the efficiency of green manure N utilization rarely exceeds 20% in the first season after its application, which probably may have favored the crotalaria treatment by supplying more N and other macro and micronutrients in labile organic forms becoming readily available. This premise is also valid for the millet, as it was demonstrated in the Fig. 1, though only a small amount of N from the millet residues was found in the corn cultivated in sequence.

Nitrogen applied as urea in the first crop probably had insignificant effect on the corn grain productivity of the subsequent year since hhere were not great differences among the treatments that received different rates of N in the previous year. This effect may be explained by the relatively high utilization of the applied fertilizer N by the first crop after its application and to the possible losses of N by volatilization, leaching and denitrification [20]. It is also likely that a fraction of the remaining N may have interacted with soil organic N and transformed to more stable forms of the organic matter.



	1	2	3	4	5
N rate (kg ha-1)	0	30	50	130	180
Crotalaria-Corn	5866	5960	6150	6306	6365
Millet-Corn	5714	5695	5496	5702	5725
Fallow-Corn	5443	5355	5372	5736	5792

N rate (kg ha^{-1})

FIG.12. Residual effect of fertilizer N and green manures on corn productivity.
Note: The values followed by same letter do not differ statistically (Tukey's test 5%).

4. CONCLUSIONS

The highest grain yields of corn were obtained in crotalaria-corn sequence during both years followed by natural fallow-corn and the lowest were found with millet-corn. Corn grain yields between fallow systems were statistically different in the first year, and only when N was applied at the 8 leaves stage in the second year.

The crotalaria provided more N to corn than fallow and millet in both years and the corn grain yield response to N rates was fitted to a quadratic function.

The N application at 4 leaves stage resulted in higher grain productivity in all three fallow systems in the first year, and significant differences were found for natural fallow-corn and millet-corn systems when N was topdressed at 8 leaves stage.

The grain productivity data were fitted to quadratic functions with regard to N rate. The maximum efficiency would have been obtained with 148, 117 and 161 kg N ha^{-1} and 171, 160 and 174 kg N ha^{-1} for the fallow-corn, crotalaria-corn and millet-corn in the first and second year respectively.

The application of increasing fertilizer N rates resulted in higher amount of plant N derived from fertilizer (QNpdff) and reduced fertilizer N utilization efficiency (FNUE). The FNUE was, on the average of 2 years, 53%, 49% and 44% for the corn grown as succeeding crop to crotalaria, fallow and millet, respectively.

The application of increasing fertilizer N rates resulted in a quadratic response of the utilization by corn of the N from crotalaria and millet. The N utilization from the crotalaria and millet was (on the average of two years) 15.6% and 7.6%, respectively.

Although millet has often been used as cover crop/green manure for corn crop, results of two years of the present study showed that the millet did not show any advantage in terms of corn grain productivity compared to the natural fallow system.

ACKNOWLEDGEMENTS

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RESPONSE OF PROMISCUOUS SOYBEAN TO RHIZOBIAL INOCULATION AND FERTILIZATION TREATMENTS AND THEIR EFFECTS ON SUBSEQUENT MAIZE YIELDS IN DEGRADED "TERRE DE BARRE" IN BENIN

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Abstract

Poor adoption of green manures and agroforestry systems for the sustainable intensification of agricultural production in the moist savannah of West Africa, and the low contribution of the traditional grain legumes such as groundnuts, cowpea and common bean have prompted the search for alternative socio-economic solutions for the smallholders such as the development the N_2 fixation of promiscuous soybean to increase food production and improve soil fertility status, in particular in the degraded lands. Twenty one and fifteen farmers' fields were selected in 2001 and 2002 respectively and again in 2002 and 2003 for the trials. Each farmer's field represented one replication. The ^{15}N isotope dilution method was used to assess symbiotic N_2 fixation of the IITA promiscuous soybean variety TGX 1448 2E and its response to inoculation and fertilization. In both years 2001 and 2002, the application of 20 kg N did not affect nodulation, biomass production and N accumulation of soybean uninoculated in 2001, or inoculated in 2002. However, inoculation produced the highest nodule number and nodule weight in 2002. The highest values of biomass production and N accumulation were found with soybean that received poultry manure in 2001 and 100 kg N ha^{-1} as urea in 2002. The highest biomass and N accumulation in 2001 was 1600 kg ha^{-1} and 41 kg N ha^{-1} with soybean amended with poultry manure. Shoot N production in 2002 averaged only 25 kg N ha^{-1}, while the average N accumulation in soybean seed was 64 kg N ha^{-1}. The best percentage of N derived from atmosphere (54%) amounting only 13 kg N ha^{-1}was obtained with soybean fertilized with 20 kg N ha^{-1} and inoculated treatment. These values are too low indicating that soybean cultivated in the study area is far from satisfying its N requirements through N fixation. The N balance calculated on the basis of the amount of N fixed removed in the grain is negative (-48 kg N ha^{-1}). In the season 2002, it was observed that maize yields in the plots previously cropped to soybean did not show any significant difference from the plots previously cropped to maize. Significant increase occurred only when plots were previously grown to soybean and fertilized with poultry manure being 5124 and 2311 kg DM ha^{-1} for maize shoot and grain respectively. Total N yield in shoots was significantly higher in plots previously cropped to soybean than those of maize. The contribution of soybean to maize yield was not significantly different from the contribution of maize-to-maize in 2003. Soybean development in West Africa is a promising technology that has multiple benefits. However substantial inputs and important investments are required to remove severe soil constraints affecting its growth and grain yield potential before these benefits can be fully achieved.

1. INTRODUCTION

Poor adoption of green manure crops and agroforestry systems for the sustainable intensification of agricultural production in the moist savannah of West Africa (MSWA) has led to the development of alternative but attractive technologies for the smallholders. Among these, particular attention has been paid to the breeding of grain legumes for dual-purpose. Long duration cowpea, soybean, and groundnut varieties with a high N-fixation capacity but low harvest index were developed thus yielding both more grain and more fodder/green manure ("dual purpose") than farmers' traditional varieties. Some of these varieties have added benefits such as being promiscuous in nodulation, waiving the need to use inoculants to nodulate and having the capacity to reduce the seed bank of the parasitic weed *Striga hermonthica* by stimulating suicidal *S. hermonthica* seed germination. These grain legume accessions are grown in rotation with drought-tolerant and N-efficient maize varieties to improve sustainable agricultural production in the MSWA. Mpepereki *et al.* [1] demonstrated that the benefits of soybean over traditional grain legumes commonly grown by smallholders, such as groundnut (*Arachis hypogea* L.), cowpea (*Vigna unguiculata* L.) and common bean (*Phaseolus vulgaris* L.) include lower susceptibility to pests and disease, better grain storage quality and a large foliar biomass, which gives a better soil fertility benefit to subsequent crops.

Acording to Mpepereki and Pompi [2] the traditional grain legumes have failed to respond consistently to inoculation with commercial rhizobial strains. Among these multipurpose grain legumes, soybean responds well to rhizobial inoculation and has a higher N fixation capacity even in fairly marginal soils [3–4]. Although soybean is a relatively new promising crop for smallholders in most Africa countries, its development is still problematic because of the lack of technical support programs similar to what is found in other parts of world.

Manushwar *et al.* [5] reported that organic manure should be applied along with the rhizobial culture during seed inoculation due to its benefits on improving locally the soil physical conditions and providing an appropriate environment for *Rhizobium* establishment and nodule growth required for adequate N fixation in soybean [6].

Many studies have emphasized the need for rhizobial inoculants to help increasing soybean production and economic profits in USA, Brazil, and Australia [7-8], but the adoption of this technology by smallholder, subsistence farmers has lagged behind in African countries [9].

Mpepereki *et al.* [1] indicated also that the use of promiscuous soybean varieties represent a highly appropriate technology for cultivation of soybeans by smallholder farmers, whereas use of varieties with greater yield potential together with rhizobial inoculants is an appropriate technology for commercial production of soybeans by farmers who have ready access to agricultural inputs. Osunde *et al.* [10] studied the response to rhizobial inoculation by promiscuous soybean varieties in Nigeria and suggested that it is pertinent to know, prior to the introduction of a promiscuous soybean cultivar, if the symbiotic association with indigenous rhizobia satisfies the optimum yield potential of the cultivar at a given location or whether inoculation can better promote nodulation and N_2 fixation. Other authors have reported that the “promiscuous” soybean cultivars developed by the IITA breeding programme nodulate freely with indigenous rhizobia, thus waiving the need to inoculate with *B. japonicum*. They also observed that the selection for promiscuity was based on the number of nodules formed [11, 12]. Sanginga *et al.* [13] summarizing the results from studies carried out in IITA concluded that the selected lines were not tested for their response to inoculation. Recent studies have shown that responses to inoculation by the promiscuous soybeans can be still obtained [14, 15, 13]. It would not be possible to have a cultivar that nodulate effectively with indigenous rhizobia in all locations [13].

Agronomic results from studies conducted in the north Guinean savannah are abundant to confirm that maize yields increase when grown in crop rotations with soybean compared to maize grow after maize [16, 17]. In Nigeria, soybean is being increasingly cultivated within the savannah agro-ecological zone, where it is well adapted [18], especially with the introduction of the promiscuous soybean cultivars developed by IITA. Studies conducted in the region with a soybean-maize (*Zea mays* L.) rotation have shown that the net N contribution of the soybean crop to the soil increased with growth duration, and ranged between -8 and 47 kg N ha^{-1} [19, 20, 21]. Carsky *et al.* [22] observed that even when the stover was exported, maize grain yield increase following soybean that was given a basal application of 20 kg N ha^{-1} was similar to that from 40 kg N ha^{-1} applied to maize preceded by maize.

Many factors have been postulated to explain these results including enhanced N availability following soybean and other rotational effects such as a reduction of diseases and higher mycorrhizal colonization rate and diversity [23]. Sanginga *et al.* [24] reported that although various ^{15}N methodologies have been used to trace the fate of N from leguminous plant material incorporated into the soil [25] and to estimate the availability of N to a crop after a legume compared to a crop after a non-legume [26], there is no conclusive evidence whether the beneficial effect of legumes to subsequent crops is due to a N contribution from fixation or a net ‘rotational’ contribution, which is a complex effect combining N availability, disease and weed control, and improvement in soil structure [27–28].

In Benin the interest for soybean cultivation by the smallholders is increasingly growing since the cotton’s marketing crisis persists. However, limited information is available on the nitrogen fixation capacity of soybean and its actual contribution to soil fertility improvement in the degraded soils known locally as ‘terre de barre’. The objectives of this study were, therefore: (i) to evaluate the response of the promiscuous soybean variety TGX 1448 2E to rhizobial inoculation and poultry manure and (ii) to assess its rotational effects using the ^{15}N isotopic techniques in soybean-maize rotations grown in “terre de barre”, southern Benin.

2. MATERIALS AND METHODS

2.1. Site description

The experiments were conducted in farmers’ fields at Adingningon, southwest of Bohicon at 07° 08’ N, 02° 02’ E and 150 m above sea level, and at approximatively 130 km from Cotonou. The soils of “Terre de barre” are classified as red Ferralitic Nitisols. They are also called Nitosols in the FAO system [29] or “*sols ferrallitiques moyennement désaturés appauvris”* [30]. The experimental soil is slightly acid and of sandy textural class in the topsoil overlying an acid sandy clay loam subsoil. The topsoil (0–15 cm) had very low fertility status, as shown by some selected soil characteristics (Table I).

TABLE I. SOME PHYSICO-CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL (0-15 CM) AT ADINGNINGON

Sand (%)	Silt (%)	Clay (%)	pH (KCl)	Org.C (%)	Total N (%)	C:N ratio	Bray I Available P (mg kg^{-1} soil)
85	5	10	5.3	0.53	0.058	9.14	1.9

2.2. Climate and rainfall

The experimental site is characterized by a subequatorial humid hot climate with a bimodal rainfall distribution. Rainy seasons start from March to July and from September to November. Data were obtained from from the nearest Bohicon weather station. Total annual rainfall averaged over the last 20 years between 1000 and 1200 mm. The annual rainfall recorded in Bohicon station was 850 mm in 2001 and 1408 mm in 2002. The average daily temperature is 27°C with the lowest at 22°C during July and the highest at 36°C in March.

2.3. Selection of the farmers' fields

Fields trials were carried out during cropping seasons of 2001–2003 on twenty one farmers' fields in 2001 and fifteen farmers' fields in 2002. Farmers who had been collaborating with previous soil management project were invited to a meeting and volunteered to take part in the trials. These farmers made small plots of 240 m^2 available for the research.

2.4. Soybean trial

2.4.1. Evaluation of biological nitrogen fixation in soybean

During the 2001 and 2002 cropping seasons, promiscuous soybean cultivar TGX 1448 2E was cultivated to measure N_2 fixation in soybean using ^{15}N isotopic techniques. Maize variety DMR was used as reference non N-fixing crop

In 2001, the experimental treatments were: control (without inoculation and fertilization), soybean fertilized with 20 kg N ha^{-1} as urea, soybean amended with 8 t ha^{-1} of poultry manure, and maize crop fertilized with 100 kg N ha^{-1} as urea. The poultry manure had 2.3% and 1.3% average total N and total P contents respectively.

In 2002, three soybean treatments were grown as follows: soybean fertilized with 20 kg N ha^{-1}, soybean fertilized with 100 kg N ha^{-1}, soybean fertilized with 20 kg N ha^{-1} and inoculated with peat based inoculant containing an approximate density of 10^7 viable rhizobia per seed [31]. One additional treatment planted with maize fertilized with 100 kg N ha^{-1} was used as reference non N-fixing crop for the N fixation estimates.

In both years 2001 and 2002, the experimental design was a randomized complete block where each farmer' field represented one replication. Each main plot measured 10 m × 3.75 m. On 11 May 2001 and 25 April 2002, soybean and maize were planted. In each plot, two soybean seeds were planted per hole and thinned to 1 plant after emergence. The planting space was 75 cm between rows and about 5 cm within plants. Three maize seeds were planted per hole and thinned to 2 plants after emergence with spacing of 75 cm and 30 cm between and within rows. Within the plots (soybean or maize) that received urea N, a confined micro plot was market out using metal roofing sheets 20-cm deep and 5 cm above, and was left bare during the experimental period. The roofing sheets were arranged in such a way that soybean or maize roots could not go outside the micro plot, while other roots were prevented from entering.

Microplots of soybean measured 2.25 m × 1 m = 2.25 m^2 and were labelled using a solution of urea enriched with 5 atom % ^{15}N excess at 20 kg N ha^{-1} while the microplot planted with maize measured 1.5 m × 1 m = 1.5 m^2 and was fertilized at 100 kg N ha^{-1} with a solution of urea enriched with 1 atom % ^{15}N excess. Split applications of urea were made at 2 and 5 weeks after planting (WAP) soybean and maize. A uniform rate of 30 kg P_2O_5 ha^{-1} and 30 kg K_2O ha^{-1} as ordinary super phosphate and potassium chloride were applied to all plots by incorporation along the sowing lines at emergence of the crops.

2.4.2. Soil sampling for rhizobial counting

Rhizobial populations for each field were enumerated by the most probable number (MPN) methods using spreading out – dilutions on petri dishes in 2002. Soil samples for the MPN assays were taken at the planting time from the fields planted with soybean, and adjacent areas (maize and weed cover of *Imperata cylindrica*). One composite sub-sample (25g of soil) in each 3 groups per farmer's field sampled in 2001 was used for the initial dilution (25g of soil in 100 ml of distilled water was shaken at least for 24 hours). A five-fold dilutions series with four replicates per dilution were used [32]. One ml of each dilution was added to the gelose nutritive Petri dishes [0.5g of Yeast extract; 10g of mannitol; 0.5g K_2HPO_4; 0.2g of ($MgSO_4$ $7H_2O$); 0.1g of NaCl; 15g of agar agar dissolved in one litter of distilled water]. The Petri dishes were incubated at 28°C and checked every day for colonies observation. Number of colonies was counted and rhizobial population was determined using corresponding tables [9]. Analysis of variance was performed on the number of rhizobia per gram of oven-dry soil (100°C, 24 h) and after log transformation of these data. A constant of 1 was added to all data before log transformation to avoid computer calculation of log 0 for soils where no rhizobia were detected.

2.4.3. Plant sampling for nodulation

Four soybean plants were sampled in the microplots at pod filling initiation that occurred at approximately 10 weeks after planting soybean. Nodule number and nodule fresh weight were recorded.

2.4.4. Harvest, chemical and isotopic analyses

At about 18 weeks after planting soybean and maize, plant biomass and grain were harvested in a pre-determined area of 4.5 m^2 within the main plots. Harvests were made by gathering all above-ground plant material within the innermost of the confined micro plots and also on the unlabelled plots. Harvested soybean and maize plant samples were chopped into 10 to 20 mm pieces and sub sampled, and about 500 g fresh weight were oven-dried at 70°C before grinding to pass through a 0.5 mm sieve. For the last harvest, plant samples were separated into reproductive (grains) and vegetative parts (shoots) before sub-sampling. The sub samples were pulverized with a ball mill and analyzed for total N and N isotope ratios were determined with the Automatic Nitrogen Analyser (1500 Carlo Erba), coupled to a SIRA mass spectrometer at the IAEA Seibersdorf Laboratories [33]. The proportion of N derived from atmosphere was calculated using the isotope dilution equation [34]. Fixed N_2 values in soybean were estimated using maize as reference crop. N fixation estimates were only made in 2002.

2.5. Maize trial

Eight farmers' fields were selected in 2002 and 10 in 2003. In both years, the previous soybean and maize plots were sown to maize, variety DMR. Seeds were sown on ridges at a within-row spacing of 75 cm between ridges and with 30 cm within plants. At three weeks after planting maize, a uniform application of 40 kg N ha^{-1} as urea form was applied to all plots. Maize plants were harvested at maturity, and similar procedures described above for soybean were followed in sample preparation and analyses of total N and N isotope ratios.

2.6. Statistical analysis

Data were subjected to analysis of variance using "PROC-CORR" and "PROC GLM" [35] to determine the statistical differences between the main effects of treatments and their interactions. Correlation studies were also made. Specific pair-wise comparisons of treatment levels were done using the Duncan test or the Least Significant Difference (LSD) test at $p = 0.05$.

3. RESULTS

3.1. Soybean trial

3.1.1. Rhizobial density

Rhizobial counts obtained in 2002 ranged between 1 and 18 cells g^{-1} dry weight soil, depending on the plot history and the farmers' field (Table II).

TABLE II. RHIZOBIAL COUNTS AS AFFECTED BY PLOT HISTORY FOR SOYBEAN GROWN IN TEN FARMERS' FIELDS AT ADINGNINGON, SOUTHERN BENIN IN 2002

Plot history	Field number										
	2	3	5	6	7	8	10	14	17	21	Means
(No.g^{-1} dry weight soil)											
Imperata weeds	5	6	6	7	8	5	6	8	10	7	7±1
Soybean	13	12	7	16	14	6	10	5	14	18	12±3
Adjacent fallow field	4	3	2	1	3	5	2	4	3	6	3±1
Means	7±4	7±4	5±3	8±5	8±5	5±3	6±4	6±3	9±5	10±6	

The results showed that indigenous rhizobia capable of nodulating soybean varied with farmers' field (average range of 5–10 cells g^{-1} soil) and were affected by the plot history in the study area, which is characterized by the regular planting of legumes such as cowpea, groundnut and bambara nut in the past cropping sequences (Table III). Lowest counts were found in an adjacent fallow field and the highest in the soybean cropped plots whereas intermediate counts were observed in the plots with *Imperata* weeds (Table II).

3.1.2. Nodulation

Nodule number and nodule dry weight of soybean are presented in Table IV. Nodulation was observed in all farmers' fields during both years 2001 and 2002 and it was related to the plots history and to the size of indigenous rhizobial population ($r = 0.43$; $p = 0.05$; $n = 99$). Nodule number varied between and within farmers' fields but it was not statistically affected by the applied treatments in 2001. However, in 2002 the rhizobial inoculation treatment influenced significantly number and fresh weight of nodules. Nodule number ranged from 0 to 300 and it was mostly dependent on the farmers' fields and the applied treatment (Table IV). Nodule fresh weight was the highest when the soybean plots were rhizobial inoculated and received 20 kg N ha^{-1}. Nodule fresh weight was 1.25 g $plant^{-1}$ when the uninoculated soybean received 20 kg N.ha^{-1} and declined to 0.62 g $plant^{-1}$ when the uninoculated soybean received 100 kg N ha^{-1}. In 2002, nodule number was highly related to soybean fresh root weight ($r = 0.49$; $p = 0.05$; $n = 33$). This effect was not observed in 2001.

TABLE III. CROP SEQUENCE HISTORY FROM 1997 TO 2001 OF FARMERS' FIELDS THAT WERE USED FOR THE EXPERIMENTS IN 2002

Farmers' fields	Season	1997	1998	1999	2000	2001
FF 1	1	groundnut	groundnut	groundnut	groundnut	groundnut
	2	groundnut	groundnut	groundnut	groundnut	groundnut
FF 2	1	groundnut + sorg	groundnut + sorg	groundnut	cowpea	groundnut + sorg
	2	sorghum	sorghum	groundnut	cowpea	sorghum
FF 3	1	cowpea	fallow	fallow	fallow	fallow
	2	groundnut	fallow	fallow	fallow	fallow
FF 4	1	cowpea	cowpea	groundnut	cowpea – sorgh	groundnut
	2	fallow	fallow	fallow	sorghum	groundnut
FF 5	1	groundnut – sorgh	cowpea – sorghum	groundnut	groundnut	Bambara nut
	2	sorghum	sorghum	Bambara nut	cowpea	fallow
FF 6	1	groundnut + cowp	groundnut + cowp	groundnut + cowp	groundnut + cowp	fallow
	2	groundnut + cowp	groundnut + cowp	groundnut + cowp	groundnut + cowp	fallow
FF 7	1	fallow	sorgh – groundnut	sorgh – groundnut	fallow	sorgh – groundnut
	2	fallow	sorghum	sorghum	fallow	sorghum
FF 8	1	fallow	fallow	fallow	fallow	fallow
	2	fallow	fallow	fallow	fallow	fallow
FF 9	1	groundnut	groundnut + sorgh	groundnut	fallow	groundnut + sorgh
	2	sorghum	sorghum	groundnut	fallow	sorghum
FF 10	1	cowpea	groundnut	groundnut	cowpea	cowpea – sorghum
	2	groundnut	cowpea - maize	groundnut	groundnut	sorghum

TABLE IV. EFFECT OF N FERTILIZER, POULTRY MANURE AND RHIZOBIAL INOCULATION ON SOYBEAN NODULATION, BIOMASS AND GRAIN PRODUCTION AT ADINGNINGON IN 2001 AND 2002

Year	Treatment	Nodule number (No.plant^{-1})	Nodule weight (mg plant^{-1})	Shoot dry weight (kg ha^{-1})	Grain yield (kg ha^{-1})
2001	0 kg N	28 (1 – 104)	1.0	737	560
	20 kg N	31 (2 – 112)	1.2	522	462
	Poultry manure	36 (8 – 188)	1.3	1600	1381
	LSD (5%)	ns	ns	432	348
2002	20 kg N	43 (0-141)	1.25	2465	942
	100 kg N	31 (1-123)	0.62	3068	1219
	20 kg N + Inoc	112 (18-300)	2.67	2173	804
	LSD (5%)	47	0.98	ns	400

3.1.3. Soybean biomass and grain production

Biomass production of soybean was higher in 2002 than in 2001. This could be explained by the earlier planting dates and the better rain distribution during 2002. In 2001, only the soybean plots remained in the farmers' fields while the other crops were harvested earlier. The pressure of wild animals (rabbit and other rodents) was high on the green soybean pods. However, shoot dry weight was statistically affected only in 2001 but not in 2002 (Table IV). The highest shoot biomass production (1600 kg ha^{-1}) in 2001 was found with the application of poultry manure while the best shoot biomass (3068 kg ha^{-1}) in 2002 was obtained with the application of 100 kg N ha^{-1} of urea fertilizer.

In 2001 no differences in grain yield were found between the control and the soybean fertilized with 20 kg N ha^{-1}. Soybean grain yield was significantly improved with the application of poultry manure (1381 kg ha^{-1}). In 2002 the application of 100 kg N ha^{-1} of urea fertilizer increased significantly ($p<0.05$) the grain yield (1219 kg ha^{-1}) when compared with the fertilized 20 kg N ha^{-1} plus rhizobial inoculation treatment (804 kg ha^{-1}). No significant differences in grain yield were found between the applications of 20 and 100 kg N ha^{-1} of urea fertilizer, although the latter treatment produced 129% more grain than the 20 kg N treatment.

No response in grain yield was obtained to the rhizobial inoculation (treatments 20 kg N ha^{-1}and 20 kg N ha^{-1}plus Inoc.).

3.1.4. Total N accumulation, N_2 fixation and N balance

The highest soybean N accumulation in 2001 was 41 kg N ha^{-1} (only shoots) when soybean was amended with poultry manure. An average of 15 kg N ha^{-1} (only shoots) was accumulated in the remaining treatments. Shoot N accumulation in 2002 averaged only 25 kg N ha^{-1} for all treatments whereas the average seed N accumulation was 64 kg N ha^{-1}. The highest grain N (75.6 kg ha^{-1}) was accumulated in the treatment that was fertilized with 100 kg N ha^{-1}. No significant differences in total N accumulated in both shoot and grain were observed between the treatments 20 kg N ha^{-1}and 20 kg N ha^{-1}plus Inoculation.

The average percentage of N derived from atmosphere (Ndfa) in soybean was 45% equivalent to an amount of 11 kg N ha^{-1} (Table V). The highest nitrogen fixation values (54%) were obtained when the soybean was rhizobial inoculated and received 20 kg N ha^{-1} and the lowest Ndfa (37%) when the uninoculated soybean received 100 kg N ha^{-1}. Intermediate Ndfa values (45%) were found when the uninoculated soybean received 20 kg N ha^{-1}. The average %Ndfa values obtained in this study are within the range of values commonly reported for soybean. However, the amounts of fixed N are too low indicating that optimum growth and grain yield production of soybean in the study area is seriously affected by local limiting factors. A rough N balance indicates a negative balance (average inputs of 11 kg N ha^{-1} and outputs of 64 kg N ha^{-1} exported in the grain), thus soybean is far from satisfying its N requirements through N fixation.

TABLE V. TOTAL N UPTAKE, PERCENT AND AMOUNT OF N DERIVED FROM ATMOSPHERE IN SOYBEAN USING ^{15}N ISOTOPE DILUTION TECHNIQUES OF SOYBEAN GROWN AT ADINGNINGON IN 2001 AND 2002

Year	Soybean (S) treatment	Shoot N (kg N ha^{-1})	Grain N (kg N ha^{-1})	^{15}N isotope dilution technique	
				Percent Ndfa (%)	Amount Ndfa (Kg N ha^{-1})
2001	S + 0 kg N	17.3	nd	nd	nd
	S + 20 kg N	12.6	nd	nd	nd
	S + Poultry manure	41.1	nd	nd	nd
	LSD (5%)	11.3			
2002	S + 20 kg N	22.5	59.3	45	10
	S + 100 kg N	28.9	75.6	37	11
	S + 20 kg N + Inoculation	24.3	58.3	54	13
	LSD (5%)	ns	ns	nd	nd

3.2. Maize trial

3.2.1. Maize shoot, grain production and N accumulation

In the year 2002 and 2003, maize was grown as subsequent crop to assess the residual effect of the past soybean treatments established in 2001 and 2002. The previous soybean and maize treatments had different effects on the shoot biomass production, grain yield and total N accumulated of the subsequent maize crop. Maize grown after maize fertilized with 100 kg N ha^{-1} gave lower yields than maize after soybean that was fertilized with 20 kg N ha^{-1}. However, significant maize dry matter and total N yield increases occurred only when plots previously grown with soybean that received poultry manure (Table VI). The increases in maize shoot production over the maize after maize ranged from 1.3% for maize after previous soybean that received 20 N to 76% for the treatment with soybean previously treated with poultry manure. Highest increases were observed when maize grain yields were considered, ranging between 25% and 147% for the same soybean treatments mentioned previously. The N yield in maize was also highest on plots previously grown to soybean. The mean value of N uptake in maize after maize was on average 38.3 kg N ha^{-1} while the one in maize after soybean averaged 108.5 kg N ha^{-1}, this representing an increase of 183% in 2002 (Table VI).

The results obtained in 2003 when the maize was grown on previous soybean treatments but fertilized with 40 kg N ha^{-1} are completely different. The highest shoot biomass production was found in previously inoculated soybean plot (2659 kg DM ha^{-1}), although no significant differences existed between the different treatments. Maize in previously cropped soybean plots accumulated on average 52 kg N ha^{-1} in the shoots while maize after maize plot had only 42 kg N ha^{-1}.

With regard to the maize grain yield, the mean value of previously soybean plots was 826 kg DM ha^{-1} against 591 kg DM ha^{-1} for maize after maize plots. The corresponding figures for the grain N accumulation were respectively 15.6 and 8.9 kg N ha^{-1} for the previous soybean plots and the maize after maize plots (Table VI).

TABLE VI. EFFECT OF PREVIOUSLY SOYBEAN AND MAIZE PLOTS ON MAIZE DRY MATTER PRODUCTION AT ADINGNINGON IN 2002 AND IN 2003

Year	Previous soybean (PrS) treatments	Maize shoot		Maize grain	
		DM yield (Kg ha^{-1})	N yield (kg N ha^{-1})	DM yield (Kg ha^{-1})	N yield (kg N ha^{-1})
2002	PrS + 0N	2352	60.2	748	10.6
	PrS + 20N	2954	70.0	1171	15.6
	PrS + Manure	5124	136.1	2311	33.1
	Maize + 100 N	2915	31.4	937	6.9
	LSD (5%)	937	19.1	503.6	8.6
2003	PrS + 20N + 40 kg N	1877	39.9	985.8	16.8
	PrS + 100N+ 40 kg N	2176	53.5	639.7	12.2
	PrInoc S + 20N+ 40 kg N	2659	63.7	851.2	17.9
	Maize + 100N+ 40 kg N	1800	42.4	591.2	8.9
	LSD (5%)	ns	ns	ns	ns

4. DISCUSSION

This study indicated that there is a need to develop appropriate technologies such as improved residue management and N-fixing grain legumes, which will enable most farmers in the sub-Saharan Africa to manage better their limited financial and economic resources, contribute to improve soil fertility and raise the productivity of the maize-grain legume systems in a sustainable manner. Soybean development in West Africa is a promising technology that has multiple benefits such as improving household nutrition, source of cash income, and supply of N inputs, which can contribute to improve soil fertility and to the sustainability of the cropping systems. Thus soybean can be utilised to replenish soil nutrients and contribute to arrest land degradation occurring in “terre de barre” in southern Benin. However, important investments are required to remove severe soil constraints affecting growth and grain yield before these benefits can be fully achieved.

Although, in this study promiscuous soybean variety TGX 1448 2E had been amended with high level of chicken manure, N fertilizer or rhizobial inoculated, our results indicated that N in shoot biomass and grain yield of soybean averaged 25 and 64 kg N ha^{-1} respectively. The mean percentage of N derived from atmosphere ranged from 37 to 76% amounting on average to only 11 kg N ha^{-1} (Table V). These values are low indicating that soybean cultivated in the study area is unable to satisfy its N requirement through N fixation. The simple N balance calculated on the basis of the difference between the amount of N fixed and that removed in the grains is negative (-48 kg N ha^{-1}). Similar findings were reported by Sanginga et al [20] when soybean can be expected to fix less than 50 kg N ha^{-1} in infertile or strongly acid soils. In these cases the N contribution of soybean to the soil-plant system is much lower than that of leguminous trees and cover crops.

Further research on the BNF capacity of soybean is required to promote its development in the country. The way forward to improve soybean production in Africa is still underway. The lack of financial resources by smallholder farmers is a serious constraint to the use of rhizobial inoculants. However, it should be pointed out that many programs were built in Sub Sahara Africa for the development of the promiscuous soybean varieties [1]. A breeding programme aiming at producing higher yielding lines able to nodulate with indigenous rhizobia [36] was initiated in Tanzania, but it appears to have been discontinued at an early stage. IITA approach was to develop high-yielding, adapted soybean germplasm able to nodulate with indigenous rhizobia. This strategy was found to be fundamentally different to that used in Zambia, where varieties were simply selected from those available on the basis of their yield potential and ability to nodulate promiscuously with rhizobia indigeous in soils in which they were grown [37]. The results obtained in this study with the promiscuous soybean line TGX 1448 2E agree with those reported by other authors [1]. They stated that although the breeding programme at IITA achieved early success in identifying genotypes that could nodulate with indigenous rhizobia in Nigerian soils, transferring this trait has proven perhaps more complex than initially imagined. The selected 'promiscuous' varieties were found to nodulate with a rather more restricted range of indigenous rhizobia than expected [7], leading research to conclude that nodulation promiscuity is a more complex genetic trait than initially envisaged, and that susceptibility to infection by different rhizobia is not necessarily conferred by the same genes. The degree of promiscuity observed for any genotype in a given set of soils undoubtedly depends on their compatibility with the rhizobia present. Following the approach adopted in Zimbabwe and Zambia, identification of soybean varieties with better agronomic performance will be reinforced in Benin because recent studies have shown that some lines had better agronomic performance than the promiscuous TGX 1448 2E studied here (data not shown). Another issue addressed in this research was the compatibility of the soybean varieties and rhizobial inoculation. Few trials conducted on farmer's fields in the "terre de barre" using three rhizobial strains and three soybean varieties including the promiscuous TGX 1448 2E (data not shown) indicated significant response of soybean to rhizobial inoculation. It is reported that the IITA lines classified as promiscuous are dependent on rhizobial inoculation and nodulate poorly or ineffectively with indigenous strains. This was confirmed by the response to inoculation (nodulation and highest Ndfa % in the year 2002) while the highest shoot and grain yield response was obtained with the application of 100 kg N because N inputs from fixation (amount of kg N) were too low. Research on more efficient strains compatible with selected soybean lines and agronomic practices to improve the very low fertility status of these degraded soils is needed to optimise the contribution of N fixation in soybean in the cropping systems cultivated in the "terre de barre".

The mean maize yield in plots previously cropped with soybean was 826 kg DM ha^{-1} against 430 kg DM ha^{-1} for maize after maize plots. The corresponding figures for the grain N accumulation were respectively 15.6 and 6.2 kg N ha^{-1} for the previous soybean plots and the maize after maize plots (Table VI). Maize yields increases following soybean were not too important because of low N contribution of the legume crop. Residual N values ranging between 10 and 24 kg ha^{-1} representing 14% to 36% of the maize total N were also reported by Sanginga *et al.* [20] using ^{15}N labelling and N difference methods. These authors concluded that the biological nitrogen fixation benefit to non-legumes due to the inclusion of legumes in a cropping system is small indeed compared to the level of N inputs needed for high maize yields [38]. It was concluded that the improvement of N contribution in the legume and cereal crops depend of the level of mineral fertilizers that could be added to and organic N recycled in the system.

5. CONCLUSIONS

Theres is an urgent need to develop viable technologies for improving food security of small holder farmers in the moist savannah of West Africa, in particular in degraded soils.

Using appropriate BNF technologies such as N-fixing legumes, soybean in this study and rhizobial inoculants does not provide sufficient N to increase soil fertility status and improve maize crop productivity.

BNF may need to be supplemented with N fertilizer application and other locally available N sources such as manures and crop residues. Another complementary approach that can contribute to make cost-effective small application of inorganic fertilizer N inputs would be to investigate and select crop genotypes with high N use efficiency under low N soil fertility.

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ESTIMATING BIOLOGICAL NITROGEN FIXATION POTENTIAL OF TROPICAL LEGUMES GROWN IN ACID SAVANNAH SOILS OF VENEZUELA USING ^{15}N-ISOTOPIC TECHNIQUES

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Abstract

The main limiting factors of agricultural production in the tropical acid savannah soils of Venezuela are the toxicity of aluminium (Al) and the deficiencies of phosphorus (P) and nitrogen (N). Sustainable and cost-effective N-replenishment strategies, in particular for smallholders can rely on the potential inputs from the biological N_2- fixation (BNF) in legumes, thus contributing to the overall N economy of system and reducing the needs for N-fertilizer to be applied to cereals or grasses. Preliminary experiments were carried out to estimate the BNF potential in various tropical legumes grown in a Typic Paleustult of the savannah of Venezuela using ^{15}N-isotopic techniques. In the field experiment pigeon pea (*Cajanus cajan)* showed high BNF (79% of its total N) with a positive N balance in the soil-plant system. Thus it is a promising grain legume for inclusion in cop rotation systems in the tropical acid savannah soils of Venezuela. In a greenhouse study, the nitrogen fixation estimates in soybean and indigosphera were 68–76% and 62–71% Ndfa respectively indicating their potential for inclusion as N contributors to crop rotation systems.. Field experiments should be carried out to confirm this potential, to establish an accurate N balance and assess the N contribution of soybean and indigosphera to the cereal crop grown in rotation or as green manure/cover crop in the savannah areas of Venezuela.

1. INTRODUCTION

In Venezuela, the savannah areas known as “Los Llanos” cover some 28 Mha. These tropical lowlands are described as the main agricultural frontier of the country. However, about 70% of arable lands present severe limitations of soil acidity and low phosphorus (P) status. They are fragile, i.e. susceptible to rapid degradation and considered as marginal lands with low agricultural productivity [13].

Toxicity of Al and deficiencies of P and nitrogen (N) are often the main limiting factors of agricultural production on these tropical acid savannah soils. Research conducted in the country is aiming at developing technologies and strategies to overcome these soil fertility constraints. The high cost of N fertilizers together with an increasing concern on the protection of water and air resources have defined the search for sustainable and socio-economic viable alternatives to obtain at least a partial substitution of those external inputs. Sustainable N-replenishment strategies on tropical acid savannah soils, particularly for smallholders can rely on the potential inputs from the biological N_2- fixation (BNF) of legumes, reducing the needs for N-fertiliser. It is postulated that a combination of indigenous phosphate rocks with legumes could be a cost-effective means of providing both P and N for sustainable crop production of cereal-legume systems on the tropical savannah areas [3].

Legumes represent a key technology in the development of sustainable agriculture in the tropics due to their capacity to fix atmospheric nitrogen in symbiosis with *Rhizobium*. They can contribute substantially to the overall N economy of the system and increase the productivity of other crops when incorporated into agro-ecosystems, either as intercrops or as a crop within a rotation. [12, 14]. Several legume species can be included as green manures/cover crops in farming systems and their benefits depend on the amount of biologically fixed N and the proportion of residual N left over for subsequent crops [16]. The amount of N that grain legumes could contribute to rotation crops is smaller than that of green manure legumes, because much of the N is removed in the grain. However, the grain legumes are often much more attractive to farmers, because they contribute directly to the household food supply, cash income and crop diversification [14].

Several methods have been developed to measure biological N_2 fixation (BNF) and the choice of a particular method depends on several factors such as the type and site of the experiment, the available resources and the species and system in question [7]. The application of the isotopic (^{15}N) techniques as a tool to assess nitrogen fixation has mainly focused on grain legumes due to their nutritional importance. Such crops are the main source of proteins and carbohydrates in many parts of the world. More recent studies have applied the ^{15}N isotopic technique to measure BNF in woody, forage and wild legume species that can be used as green manure/ cover crops for soil conservation and improvement of soil fertility status [6].

Limited information is available on the potential inputs of nitrogen fixation from grain legumes and green manures for developing sustainable cropping systems in the savannah areas of Venezuela. The objective of this study was therefore, to estimate the BNF potential in various tropical legumes grown on acid savannah soils of Venezuela using ^{15}N-isotope techniques.

2. MATERIALS AND METHODS

The research program consisted of two experiments, which were carried out in the field and greenhouse respectively to assess BNF in various tropical legumes species grown on acid savannah soils of Venezuela.

2.1. Field experiment

2.1.1. Experimental site

The field experiment was conducted at the location Espino in the savannah of the Guárico state (8º 33' 59'' N; 66º 05' 10'' W), Venezuela. Mean annual rainfall ranges between 800 and 1000 mm, distributed in 5 to 6 months. The rains are erratic, and of high intensity and short duration.

The soil was a Typic Paleustult, kaolinite, isohyperthermic, loamy sand with the following chemical characteristics (0-20 cm): pH (soil/water 1:2.5): 5.0; organic matter (Walkley and Black): 10 g kg^{-1}; available P-Olsen: 2 mg kg^{-1}, available K-Olsen: 22 mg kg^{-1} and Al saturation (extracted with 0,5 M $BaCl_2$, 1:10): 57%.

2.1.2. Treatments

The legume N-fixing species studied was pigeon pea (*Cajanus cajan*) cv. Aroita and sorghum (*Sorghum bicolour* cv. Chaguaramas VII) was used as the non-N_2-fixing reference crop. At 15 days after planting (DAP) the pigeon pea plots were treated with 15 kg N ha^{-1} as ^{15}N-labelled ammonium sulphate (10 atom % ^{15}N excess) 100 kg P ha^{-1} as partially acidulated phosphoric rock –Riecito (11.94% P-total and NAC-soluble 12.29% P_2O_5) and 45 kg K ha^{-1} as potassium chloride (KCl-60% K_2O). The sorghum (reference crop) plots received 120 kg N ha^{-1} as ^{15}N-labelled urea (5 atom % ^{15}N excess); 100 kg P ha^{-1} as partially acidulated Riecito-phosphoric rock (11.94% P-total and 12.29% P_2O_5 NAC-soluble) and 60 kg K ha^{-1} as potassium chloride (KCl-60% K_2O)). The ^{15}N labelled fertilizers were applied as solutions.

2.1.3. Experimental design

The experimental plots (5.4 m^2 and 8.4 m^2 for pigeon pea and sorghum respectively) were arranged in a randomized complete design with six replicates, of which three received ^{15}N-labelled fertilizers as above indicated.

2.1.4. Harvest and plant analyses

The plants were harvested at 100 days after planting by gathering the above-ground plant material. Plant samples were taken from a central area of 1 m^2 of the ^{15}N-labelled plots. All plant samples were separated into grain and stover, oven-dried at 65 °C, weighed and finely ground. Samples were analysed for total N content using the semi-micro Kjeldahl procedure. The ^{15}N enrichment in plant samples was determined using a NOI-6 PC emission spectrometer [11]. The estimation of biological fixation nitrogen was made using the ^{15}N-isotope technique, A-value approach [2, 9, 4].

2.2. Greenhouse experiment

2.2.1. Experimental treatments

The legumes species studied were two grain legumes, soybean (*Glycine max* L.) and pigeon pea *(Cajanus cajan*), and two green manure legumes: sunhemp (*Crotalaria juncea* L.) and indigosphera (*Indigosphera lespediciodes*). The N-fixing legumes were inoculated with specific *Rhizobium* or *Bradyrhizobium* strains for each legume. The inoculants were applied in solution at seedling emergence. Maize (*Zea mays*), sorghum (*Sorghum bicolour* L. Mohen) and a non-inoculated soybean (*Glycine max.* L.) were used as non-N_2-fixing reference crops.

The experiment was carried out in a greenhouse located at INIA-CENIAP, Maracay-Venezuela, in free-draining pots filled with 4 kg of soil (Typic Paleustult, loamy sand, kaolinite, isohyperthermic). A bulk topsoil (0–20cm) sample was taken from Espino, Guárico state, Venezuela, (from the same location as the field experiment described above). The soil was air dried, mixed, homogenized and sieved (<4 mm). Applications of ^{15}N-labelled ammonium sulphate (10 atom % ^{15}N excess) at 14.63 mg N/pot were made to the legumes, and 117.07 mg N/pot to the reference crops. All pots received 97.56 mg P/pot as partially acidulated Riecito-phosphoric rock (11.94% P-total and NAC-soluble 12.29% P_2O_5) and 58.54 mg K/pot as potassium chloride (KCl-60% K_2O).

2.6. Experimental design

The experimental design was a randomised complete design of seven treatments (four legume treatments and three reference crops) with five replicates.

2.7. Harvest and plant analyses

The entire plants were harvested at 45 days after planting (DAP), and separated into shoots and roots. All plant samples were oven-dried at 65°C, weighed and finely ground. Plant samples were analysed for total N content and ^{15}N enrichment using the methods described above for the field experiment. Biological fixation nitrogen was determined according to ^{15}N-isotope technique, A-value approach [9, 4]. Both, nitrogen derived from the atmosphere (%Ndfa) and amount of fixed N were estimated.

3. RESULTS AND DISCUSSION

3.1. Field experiment

The dry matter yield of sorghum was 2584 kg ha^{-1} with a total N accumulation of 41 kg ha^{-1} in the harvested biomass. The total N cumulated in the grain was 30 kg ha^{-1}, thus indicating that the quantity of nitrogen exported by the grain represents almost 75% of the total N in the plant. Thus sorghum had a high nitrogen harvest index in this experiment. Dry matter yield of pigeon pea was 2823 kg $ha^{-1}$with a grain yield of 739 kg ha^{-1} and a relatively low total N uptake of 62 kg ha^{-1}. In this case the quantity of nitrogen removed in the grain was 24 kg.ha^{-1} (equivalent to 40% of the total N uptake). The N harvest index in this legume is smaller than in other grain legumes such as soybean, which can export in the grain over 70% of its total N, sometimes leaving a negative N balance in the system [5]. It should be noted that the pigeon pea plants were harvested at 100 days after planting in this study.

The relative distribution of N sources in pigeon pea (fertilizer, nitrogen fixation, and soil) and sorghum (fertilizer and soil) is shown in Table I. The highest proportion of nitrogen in the pigeon pea (79% of the total N in the plant) was derived from the air (biological nitrogen fixation) and it was equivalent to 49 kg N ha^{-1}. Thus pigeon pea is a legume with high potential BNF capacity but with limitations for biomass production under the experimental conditions affecting the N inputs from BNF. Slightly lower BNF values (62%) and (55–67%) for pigeon pea have been reported by Mafongoya [14] and Gathumbi [7] respectively in field experiments in Kenya. In this study, the growth and biomass production of pigeon pea were likely affected by high acidity (and Al saturation) and very low Ca and P supply of the Ultisol.

TABLE I THE RELATIVE DISTRIBUTION OF NITROGEN SOURCES IN PIGEON PEA AND SORGHUM GROWN IN AN ULTISOL FROM VENEZUELA

Legume and reference crop	Total N (kg ha^{-1})	Ndff (%)	Ndff (kg ha^{-1})	FNR (%)	Ndfs (%)	Ndfs (kg ha^{-1})	Ndfa (%)	Ndfa (kg ha^{-1})
Pigeon pea	62.1±8.2	2.9±0.4	1.8±0.2	11.8±1.6	18.3±2.4	11.4±2.7	78.8±2.7	48.8±5.6
Sorghum	40.8±4.5	55.5±3.9	22.5±1.5	18.8±1.2	44.5±3.9	18.2±3.5	0.0±0.0	0.0±0.0

Values are means of three replicates ± standard deviation. Ndff: Nitrogen derived from fertilizer, Ndfs: Nitrogen derived from soil, Ndfa: Nitrogen derived from air, FNR: Fertilizer N recovery.

The N contribution made by legumes in agro-ecosystems is linked to the recycling and decay of fallen litter, crop residues, and below ground parts (roots, nodules) [14]. The contribution of N_2-fixation from grain legumes to the overall N balance of a soil can be roughly summarized as the amount of N derived from N_2-fixation less the amount of N exported in grain [7] considering that the remaining parts are left for N cycling in the soil. In this study the pigeon pea showed a positive N balance, providing estimated N inputs into the cropping system of 24 kg N ha^{-1}, equivalent to 50% of total amount of N derived from fixation over a period of 100 days.

3.2. Greenhouse experiment

The sunhemp produced the lowest quantity of dry matter (5.8 ± 1.1 g pot^{-1}) and the pigeon pea the highest value (7.4 ± 1.6 g pot^{-1}). In case of the non-N_2 fixation reference crops used, the sorghum showed higher dry matter production (14.9 g pot^{-1}) than maize (12 g pot^{-1}) and soybean non-inoculated soybean (6.6 g pot^{-1}). With regard to the total N content in the plants, soybean (115.5 ± 15.4 mg N pot^{-1}) and indigosphera (100 ± 17.4 mg N pot^{-1}) accumulated more N than pigeon pea (85.6 ± 18.4 mg N pot^{-1}) and sunhemp (75.6 ± 13.3 mg N pot^{-1}). For the reference crops, maize (84.6 ± 16.0 mg N pot^{-1}) had the lowest N compared to with non-inoculated soybean (103 ± 13.5 mg N pot^{-1}) and sorghum (121.4 ± 18.1 mg N pot^{-1}).

In general these values are lower than those reported by other authors. Okito [15], indicated accumulation of dry matter for soybean and sunhemp (grown in pot of 4 kg soil and harvested at 90 DAP) of 40–77 g pot^{-1} and 72–112 g pot^{-1}, respectively. In this study the low growth and biomass production was likely due to not only the early harvest of the plants (at 45 DAP), but also to the high acidity and inherent low fertility of the soil in which the plants were grown, even considering that P and K fertilizer inputs were added. Gathumbi [7] reported that pigeon pea plants at 60 DAP contained 45% of the dry matter and and 42% total N accumulated at harvest time (at 90 DAP). Thomas [17] reported in a study in the savannah of Colombia that the amount of N fixed by forage legumes in low fertility acid savannah soils are dependent on legume growth, soil type and level of fertilization.

To obtain a more reliable assessment of the BNF potential of the legumes studied, the %Ndfa was calculated using each of three reference crops separately (Table II). In this experiment BNF estimations in the legumes studied were different according to the non-N_2 fixing reference crop used. Very similar Ndfa estimates were obtained using non-inoculated soybean and maize. However, Ndfa values were overestimated with the sorghum. The magnitude of the differences was dependent on the legume species. In soybean and indigosphera the differences in Ndfa estimates were 7-10%, however for pigeon pea and sunhemp (legume with the smallest N_2-fixation) these differences were much higher (9–15%).

These results confirm the relevance of the choice of the reference crop to get reliable Ndfa estimates. The use of an inadequate reference crop can result in erroneous BNF estimates, especially when the level of N fixation is low. When the N fixation level is high, the errors involved in utilising an inadequate reference crop are generally smaller [10]. The use of several control crops produces a range of different BNF estimates in the N2-fixing crop. The extent of this range gives a measure of the accuracy of the estimates [1].

The soybean showed the highest proportion of Ndfa (68-76%), followed by indigosphera (62–71%), while pigeon pea and sunhemp had lower percentages with 52–64% and 38–53%, respectively. In this greenhouse experiment the BNF estimates in pigeon pea were in general average for reported values [7, 14] but lower than those obtained in field conditions (described above under 3.1). Overall these BNF values obtained are in agreement with those reported by other authors under similar conditions. España *et al.* [5] reported 54-67% Ndfa for soybean grown in the savannah of Venezuela. The same authors found 88% Ndfa in indigosphera grown in field conditions (data not shown) indicating that it is a suitable legume for green manure in acid soils of this savannah area. In another study conducted in Cuba, BNF estimates of 27 and 39% were reported for sunhemp using maize and sorghum respectively as reference crops [16].

TABLE II ^{15}N ENRICHMENT OF LEGUMES AND REFERENCE PLANTS AND ESTIMATES OF THE PROPORTION OF N DERIVED FROM THE AIR (% NDFA) IN LEGUMES USING THREE DIFFERENT REFERENCE CROPS GROWN FOR 45 DAYS IN POTS OF 4 KG

	Atom % ^{15}N excess	Estimation of % Ndfa of the legumes with three reference crops		
		Maize	Sorghum	Soybean N[b]
Grain legumes				
Soybean	0.170	68 a[a]	76 a	68 a
Pigeon pea	0.239	52 c	64 c	52 c
Green manure legumes				
Sunnhemp	0.351	38 d	53 d	38 d
Indigosphera	0.231	62 b	71 b	63 b
Reference plants				
Maize	3.670			
Sorghum	3.479			
Soybean Non-inoculated	2.983			

Values are means of five replicates. [a] Those followed by the same letter are not significantly different according to Tukey's Test (P=0.05). [b] Soybean non-inoculated.

The proportions of N derived from BNF, soil and fertilizer in each legume, estimated using the reference crops are shown in Fig. 1A for the grain legumes and in Fig. 1B for the legume green manures.

As mentioned above, an assessment of the contribution of N_2-fixation to the overall N balance of a soil can be roughly estimated as the amount of N derived from N_2-fixation less the amount of N exported in grain [7] considering that the remaining parts are left for N cycling in the soil. The data shown in the figures allow a more accurate N balance having data on the actual amount of the N taken up from the soil by the legumes. In this balance the N derived from the fertilizer is very low and it can be disregarded since the ^{15}N labelled fertilizer has been mostly applied as a tracer for labelling purposes. Thus, if a legume species is not efficient in N fixation, it will take relatively more N from the soil. The actual amount of N exported from the soil by the legume will depend on its biomass produced and total N uptake. The legume species and cultivars generally vary with respect to both the efficiency N_2 fixation (% Ndfa) and the total N yield, which determine the amount of fixed N [8]. Follow up field experiments including promising legume species in cereal-based (maize and sorghum) cropping systems should be carried out to collect reliable data on both the %Ndfa and fixed N (kg N ha^{-1}) to establish an accurate N balance and assess the actual N contribution to the subsequent cereal crop.

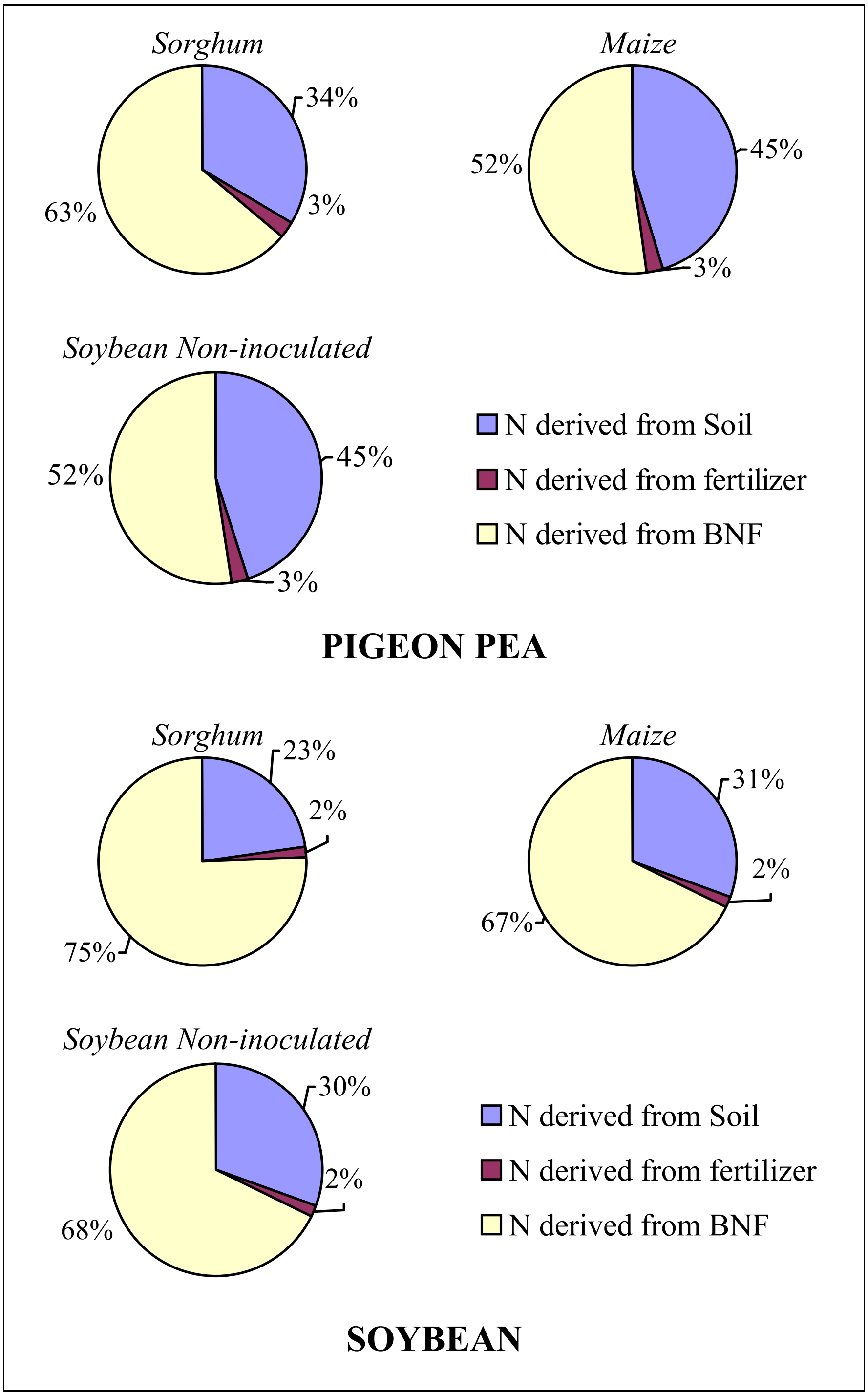

FIG.1A. Estimates of N derived from BNF, soil and fertilizer using the A-value approach in two grain legumes utilising three non- N2-fixing reference plants. (Values are means of five replicates).

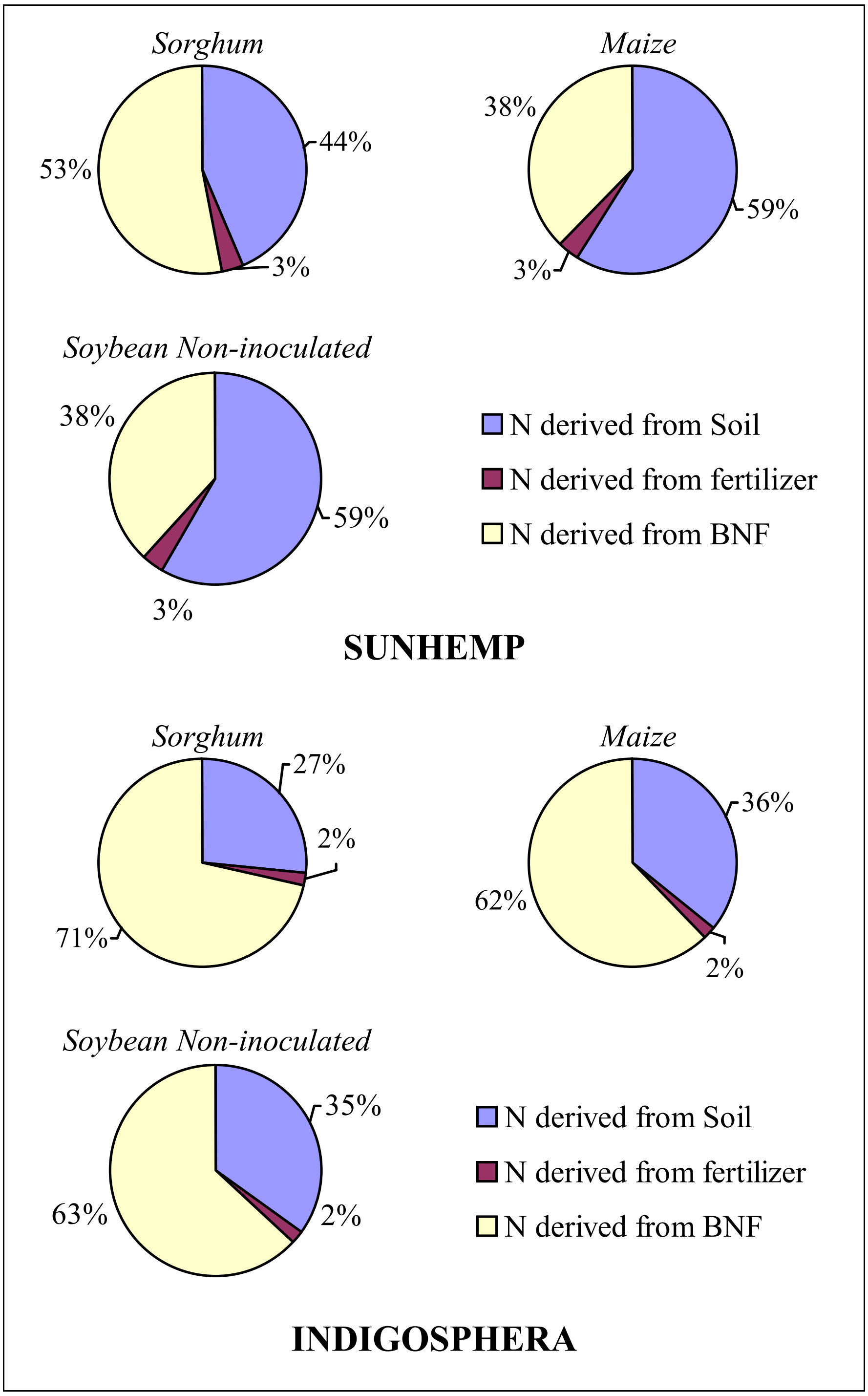

FIG.1B. Estimates of N derived from BNF, soil and fertilizer using the A-value technique in two green manure legumes utilising three different Non- N2-fixing reference plants. (Values are means of five replicates).

4. CONCLUSIONS

From the field experiment it can be concluded that pigeon pea (*Cajanus cajan)* showed high BNF (79% of its total N) with a positive N balance in the soil-plant system. Thus it is a promising grain legume for inclusion in crop rotation systems in the acid savannah soils of Venezuela. Further studies are needed to remove constraints for increasing biomass and grain yield production.

In the greenhouse soybean and indigosphera showed good BNF potential (68–76% and 62–71% Ndfa respectively) for inclusion either in rotation with cereals or as green manures respectively in the tropical savannah areas of Venezuela. Follow up field experiments should be carried out to establish an accurate N balance and assess the actual N contribution to the subsequent cereal crop.

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