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Use of Laser Carbon Dioxide Carbon Isotope Analysers in Agriculture

Joint FAO/IAEA Programme
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USE OF LASER CARBON DIOXIDE CARBON ISOTOPE ANALYSERS IN AGRICULTURE

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PREPARED BY THE
JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

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FOREWORD

The IAEA promotes nuclear and related technologies that help Member States improve food security and ensure sustainable agriculture. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture focuses on developing techniques that use nuclear and related technologies to increase the efficiency of resource use in agricultural soil, water and nutrient management practices.

Agricultural management practices that optimize soil organic carbon sequestration and water and nutrient use efficiency enhance food security and sustainable agriculture. These practices also promote climate-smart agriculture by making soils and plants more resilient to climate change and by reducing greenhouse gas emissions. Accurate measurements and data analysis are the first steps in ensuring proper evaluation and selection of such agricultural management practices. Information on how to perform such evaluations, however, often does not properly illustrate how to conduct each of the steps involved.

The present publication provides guidance for scientists, technicians and students in the use of laser CO_2 carbon isotope analysers to evaluate agricultural management practices and optimize soil organic carbon sequestration for sustainable and climate-smart agriculture. Laser CO_2 carbon isotope analysis is a relatively new technology that allows for real-time, in situ measurements of the concentration and carbon-13 signature of CO_2. Because this type of analysis is relatively simple and can provide robust data, it is increasingly being used to monitor and track CO_2 emissions.

This publication presents four standard operating procedures (SOPs) that provide guidance on the use of laser isotope analysis to conduct reliable measurements of CO_2 gas from soil or plant materials in ambient air or closed chambers. Two of the four SOPs (Creating Reference Gases for Gas Isotope Analysis and Data Management of Laser CO_2 Carbon Isotope Analyser Collected Measurements) were developed by the Joint FAO/IAEA Division; the remaining SOPs (Measurements Using the Laser CO_2 Carbon Isotope Analyser in Continuous Free Flow Mode and Injection Mode) clarify and illustrate existing procedures and include additional steps to enhance the accuracy and precision of CO_2 measurements. Additionally, an example study is described that uses these four SOPs to evaluate carbon loss from soils using a laser CO_2 carbon isotope analyser.

The Joint FAO/IAEA Division wishes to thank all contributors to its Soil and Water Management and Crop Nutrition Subprogramme and those involved in the preparation of this publication. The IAEA officer responsible for this publication was J. Chen of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture.

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1. INTRODUCTION

1.1. BACKGROUND

Laser CO_2 carbon isotope analysers are increasingly used to track CO_2 levels and trace the source of carbon emissions through isotope analysis. This relatively new technology provides many benefits for tracking and tracing changes in CO_2 emissions compared to older isotope technologies, such as continuous, robust measurements of changes in CO_2 concentration in real-time and the ^{13}C isotopic signature in CO_2. However, instructions on how to perform such evaluations are often limited. Member States require this guidance to improve soil management practices for sustainable and climate-smart agriculture using nuclear techniques. Isotope technology, such as the laser CO_2 carbon isotope analyser can be used to evaluate and select agricultural management practices that reduce CO_2 emissions and optimize soil organic carbon sequestration and water and nutrient use efficiency for food security and sustainable agriculture.

1.2. OBJECTIVE

This publication was developed to guide scientists, technicians and students in the use of a laser CO_2 carbon isotope analyser to track and trace CO_2 that, for example, can be emitted by soils. It contains novel protocols on how to create reference gases for calibration and quality control and how to manage data as well as how to enhance accuracy and precision of CO_2 measurements.

1.3. STRUCTURE

Four illustrated, step-by-step standard operating procedures (SOPs) on how to obtain measurements using a laser laser CO_2 carbon isotope analyser describe: (1) creating reference gases, (2) calibrating the laser CO_2 carbon isotope analyser, (3) sample measurements and (4) data correction. A fifth case study demonstrates how these protocols can be used to measure soil CO_2 emissions.

The first SOP describes how to mix CO_2 gases to create reference gases that are essential for ensuring accurate and precise CO_2 measurements and allow for comparison of measurements across times and different instruments. The second SOP clarifies and illustrates an example calibration of a laser CO_2 carbon isotope analyser using a reference gas in continuous free flow mode and further clarifies use of the instrumentation to measure concentration and $\delta^{13}C$ of CO_2 in this mode. The third SOP clarifies and illustrates an example calibration of a laser CO_2 carbon isotope analyser using a reference gas in injection mode and demonstrates use of the instrumentation to measure concentration and $\delta^{13}C$ of CO_2 in this mode. The fourth component describes how collected data should be evaluated and corrected prior to reporting results to ensure precision and accuracy. The last component of this publication is a case study that demonstrates how the laser CO_2 carbon isotope analyser can be used to measure CO_2 emissions from a soil incubation experiment. This combines the four SOPs to show how they may be used in an experiment to evaluate soil CO_2 emissions.

2. CREATING REFERENCE GASES FOR GAS ISOTOPE ANALYSIS

2.1. SCOPE AND FIELD OF APPLICATION

Laser CO_2 carbon isotope analysers should always be properly calibrated to ensure accurate measurements. One of the first steps when calibrating is to acquire standard reference gases that are used to check for instrument accuracy and precision. Reference gases with predetermined CO_2 concentration and ^{13}C-CO_2 isotopic signature within appropriate ranges (similar to that of sampled CO_2) are, however, not commercially available, requiring researchers to create alternatives. However, non-commercially made reference gases risk inaccuracies due to contamination with external CO_2 (such as CO_2 in the atmosphere or individual breath) that occurs during production or due to poor verification of the gas before use. One source of error can occur during the common use of gas bags to mix gases. This is likely due to weak seals that allow for CO_2 exchange with the atmosphere over time, prior to sampling on a laser CO_2 carbon isotope analyser. This risk can be reduced by making reference gases in larger batches that do not need to be made or verified often as well as by making and storing gases in a well-sealed steel line and tank. Atmospheric CO_2 also cannot be used as an alternative to stored CO_2 reference gases due to variation in the concentration and $\delta^{13}C$ of atmospheric CO_2 that can even vary by more than 100 ppm and several per mil $\delta^{13}C$ on a diurnal basis [1].

This section describes a sealed gas mixing line design in which steel gas tanks can be evacuated and filled with gases in order to mix and create a desired CO_2 reference gas (Figure 1. and Figure 2.). Example calculations are provided on how to determine how much of different gases to mix in order to create a reference gas with a desired CO_2 concentration and $\delta^{13}C$ value.

2.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Laser isotope analysers are frequently used in greenhouses and the field. So, for ease of transportation, reference gas tanks should not be too heavy, 50 L gas tanks are ideal for use in the lab and 10 L gas tanks are more practical for use in the field.

Gas tanks containing pressurized gas must be stored in well-ventilated areas and securely mounted. For secure operations, including transport to the field, it is recommended that reference gas tanks only be filled to a maximum of 10 bar.

2.3. PRINCIPLE

The described sealed gas mixing line (Figure 1. and Figure 2.) can isolate gases from the atmosphere and can be used to create CO_2 reference gases with a desired CO_2 concentration and $\delta^{13}C$ value without contamination. By mixing gases with known CO_2 concentrations and $\delta^{13}C$ at the right proportion (calculations described in section 2.7), CO_2 concentration and $\delta^{13}C$ can be diluted to the desired amount.

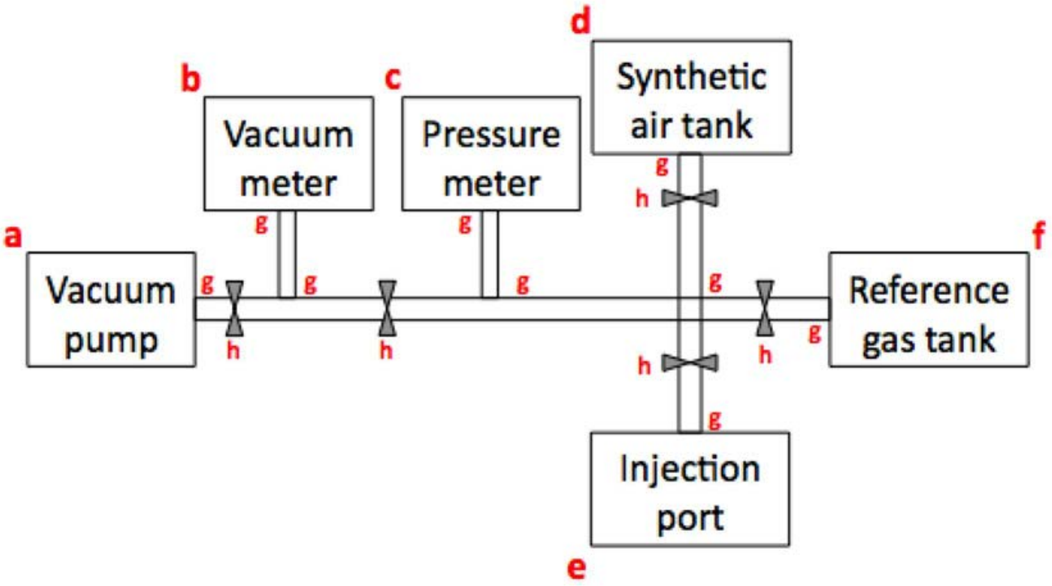


Figure 1. Diagram of the described sealed gas mixing line developed to mix gases. Letters next to each point are associated with equipment described in 2.5.

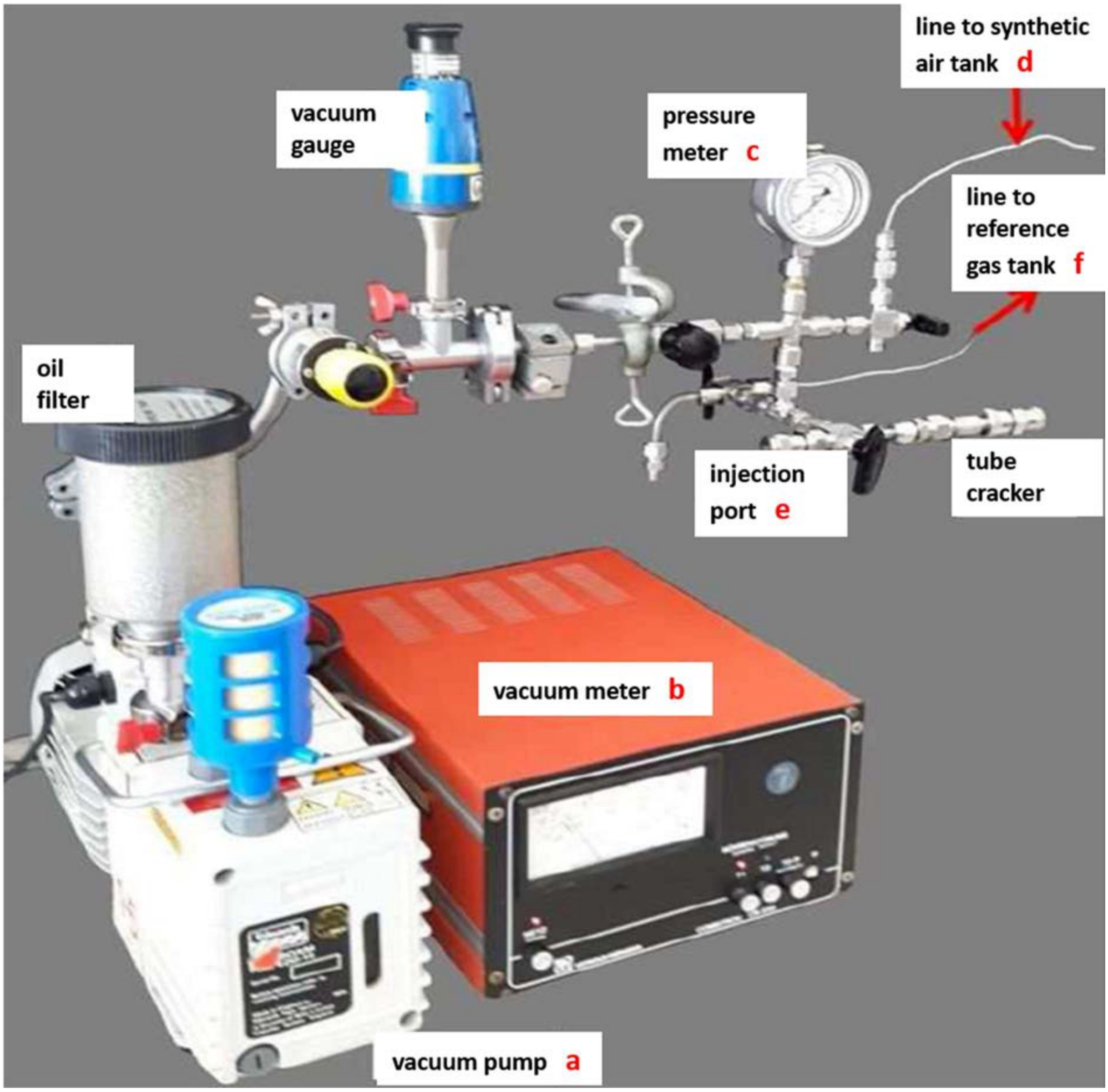


Figure 2. Picture of a sealed gas mixing line produced based on the sealed gas mixing line diagram in Figure 1. A "tube cracker" chamber that can be used to introduce gases that are in glass vials to the mixing line is also attached to the injection port in case a gas is introduced to the line by a glass vial rather than syringe.

2.4. REQUIRED GASES

- Synthetic air (without CO_2) containing N_2 and O_2 (with pressure regulator from 0 to 15 bar).
- Pure CO_2 at natural ^{13}C abundance ($\delta^{13}C$ varies depending on source, $\delta^{13}C$ of CO_2 must be determined).
- Pure CO_2 with high $\delta^{13}C$ ($\delta^{13}C$ varies depending on source).

2.5. EQUIPMENT

Letters a-h associated with each equipment piece are associated with Figure 1. Items i and j are not shown in Figure 1 as they are not attached to the sealed gas mixing line.

a) Rotary vacuum pump (pumping speed > 1 $m^3 h^{-1}$, ultimate vacuum better than 10^{-3} mbar)

b) Vacuum meter (10^{-3} to 1 mbar)
c) Pressure meter (0 to15 bar)
d) Synthetic air tank
e) Injection port (for CO_2 introduction by gas syringe)
f) Empty steel gas tanks (0.5 to 10 L bottles as desired, pressure regulator from 0 to 15 bar)
g) Stainless steel fittings and gas lines (length and diameter will vary depending upon the fittings of the pump, meters, valves and tanks)
h) Five-line valves
i) Gas tight syringe (100 mL)
j) Gas bag or gas vial with septum for needle gas exchange[1]

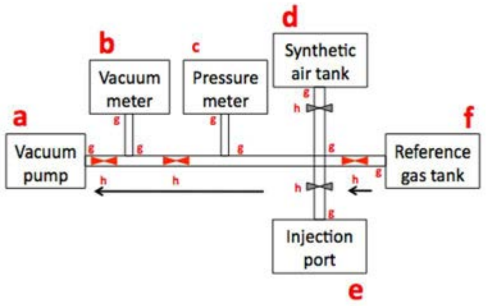
2.6. PROCEDURE

Reference gases can be created with the goal of having a certain CO_2 concentration, and/or with the goal of having a specific $\delta^{13}C$- CO_2 isotopic signature. In the first case, when the CO_2 concentration is of interest, a desired target CO_2 concentration is set. This value is obtained by diluting a pure CO_2 gas with CO_2-free synthetic air. The procedure for this method is described step by step here below, and the calculation for the dilution ratios can be found in section 2.7.2.

If a reference gas with a desired $\delta^{13}C$- CO_2 isotopic signature and a specific CO_2 concentration is needed, then the reference gas is created in two steps. First, CO_2 gas having a known, high $\delta^{13}C$-CO_2 signature is diluted with CO_2 gas at natural abundance. The calculation for these dilution ratios can be found in section 2.7.3. To perform the mixing, the same step by step procedure below can be followed, with the following substitutions:

- Pure CO_2 gas at natural abundance will take the role of "synthetic air"
- A CO_2 tank with high $\delta^{13}C$ will replace the "CO_2 gas source"

In the second step of making this reference gas with a desired $\delta^{13}C$- CO_2 isotopic signature and a specific CO_2 concentration, this pure CO_2 gas with the target $\delta^{13}C$ is diluted to the desired CO_2 concentration, again using the target CO_2 concentration procedure here below.

Step	Procedure	
Step 1 **Evacuate reference gas tank**	Connect the reference CO_2 gas tank[f] (without pressure regulator) to the gas line. Open vacuum pump[a], vacuum meter[b] and reference gas tank[f] line valves (in red). Also open reference gas tank[f]. Close valves to injection port[e] and synthetic air tank[d].	

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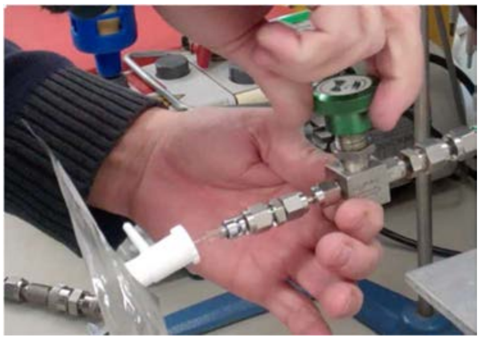
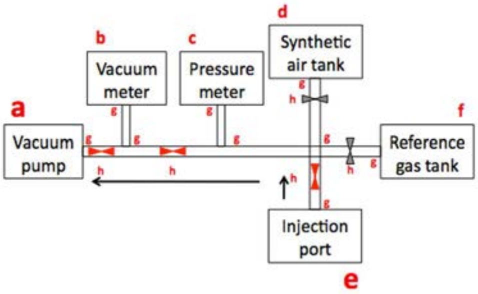
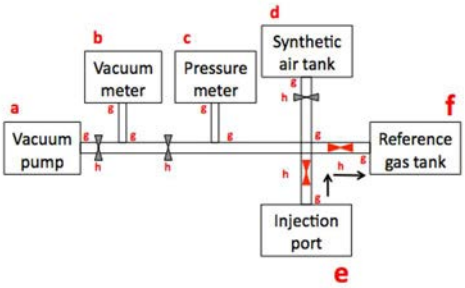
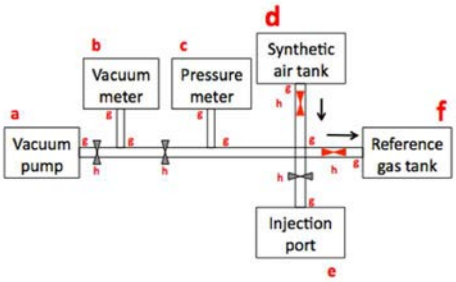
[1] Gas bags can be purchased from for example Restek Co., PA, USA, vials from Supelco, USA

	Turn on vacuum pump[a] and run until pressure (as read on the vacuum meter[b]) is lower than 5×10^{-2} mbar to evacuate the reference gas tank and line. Depending on the pump, running time may last 1-2 hours. Close all line valves and the reference gas tank[f] main valve, turn off the vacuum pump[a].	
Step 2 **Evacuate gas bag or vial**	Attach a syringe needle to injection port[e]. Attach gas bag or vial[j] to needle.	
	Open gas line valves (in red) for the vacuum pump[a] and injection port[e]. Keep all other valves closed. Turn on the vacuum pump[a] and evacuate to 5×10^{-2} mbar. Then close all valves and remove the bag or vial[j] from the injection port[e].	
Step 3 **Fill gas bag or vial**	Fill the evacuated gas bag or vial[j] with a pure CO_2 gas source. Take gas syringe[i] and flush it by taking CO_2 from bag or vial[j] and injecting it into open air 2-3 times then finally pull the desired volume of CO_2 gas source into the syringe (see calculation in 2.7.2).	

Step 4 **Adding CO_2 to reference gas tank**	Switch injection port[e] to a septum to receive CO_2 gas injections.	
	Open line valves (in red) for injection port[e] and evacuated reference gas tank[f]. Also open main valve on the reference gas tank[f]. Inject the desired volume of source CO_2 (see calculation in 2.7.2) into the injection port[e] with a gas tight syringe[i]. Remove needle[i] then close injection port[e] valve.	
Step 5 **Adding synthetic air CO_2 to mixed gas tank**	Open the synthetic air gas tank and synthetic air gas tank line[d] valve (in red) and set outgoing pressure to 10 bar. Let gas line equilibrate with the mixed reference gas tank[f] for 15 minutes.	
	Close the synthetic air tank[d] valve and check if the reading of the pressure meter[c] on the gas line drops. If so, open the synthetic air tank[d] valve and add more gas. When the reading remains constant at 10 bar, close all valves, including those on gas tanks.	

Step 6 **CO_2 gas verification**	Confirm mixed CO_2 reference gas[f] CO_2 concentration, $\delta^{13}C$ and $\delta^{18}O$ values by submitting samples for analysis on separate instrumentation.	

2.7. CALCULATIONS

The following calculations are to determine the amounts of the different CO_2 gases to produce a reference gas with the desired CO_2 concentration and isotopic signature.

2.7.1 Calculating the volume of gas that can be held in a filled tank.

When injecting CO_2 from a gas bag with a syringe, the CO_2 is at atmospheric pressure in the syringe. Therefore, to obtain the volume ratios of CO_2 and synthetic air to be mixed, we need to know the equivalent volume of synthetic air at atmospheric pressure. The following calculation determines this equivalent volume.

Use Ideal Gas Law:

$$PV = nRT \tag{2.1}$$

Where: P = pressure; V = volume; n = number of gas moles; R = gas constant; T = absolute temperature

Assuming that T is kept constant (along with n and R), then:

$$P_1V_1 = P_2V_2 \tag{2.2}$$

Where: P_1 = pressure of compressed gas in tank (bar); V_1 = volume of tank (L); P_2 = atmospheric pressure (~1.0 bar); V_2 = Equivalent volume of gas at atmospheric pressure (L)

EXAMPLE to determine the equivalent volume V_2 of gas at atmospheric pressure:

If a tank has a volume of 10 L and is filled to a maximum pressure of 10 bar, then:

$$10\ bar \times 10\ L = 1.0\ bar \times V_2$$

therefore $V_2 = 100\ L$

Where: V_2 is the equivalent volume of gas that would be present at Standard Temperature and Pressure (STP)

2.7.2 Calculating the volume of CO_2 gas and synthetic air to add to the reference gas tank to reach a desired reference gas CO_2 concentration

1 ppm CO_2 is equivalent to 1 $\mu L\ L^{-1}$ CO_2.

The volume of CO_2 gas to be added to the reference tank, can be calculated by using the equivalent volume of gas in ambient air pressure from section 2.7.1 and the desired CO_2 concentration of reference gas:

$$V_{add} = V_2 \times [CO_2]_{desired} \tag{2.3}$$

Where V_{add} the volume of pure CO_2 gas that should be added to the reference gas tank; V_2 the equivalent volume at atmospheric pressure as calculated in section 2.7.1 and $[CO_2]_{desired}$ the target concentration of the reference gas in ppm (equal to $\mu L\ L^{-1}$).

EXAMPLE to determine how much pure CO_2 gas to add to a reference gas tank to create a reference gas with a CO_2 concentration of 1000 ppm (=1000 $\mu L\ L^{-1}$):

If a 10 L volume tank holds 100 L of gas (at 10 bar with tank standing at STP) when filled and a pure (100%) CO_2 gas is added, then:

$$V_{add} = 100\ L \times 1000\ \mu L\ L^{-1}\ of\ CO_2 = 100{,}000\ \mu L = 100\ mL\ of\ CO_2$$

Where V_{add} the volume of pure CO_2 gas that should be added to the 10 L reference gas tank (filled to 100 L at 10 bar); in this example 100 mL of pure CO_2 gas should be added to a 10L reference gas tank filled with 100 L at 10 bar of (CO_2 free) synthetic air in order to bring the total reference gas CO_2 concentration to 1000 ppm.

2.7.3 Calculating the volume of CO_2 with high $\delta^{13}C$ and the volume of CO_2 at natural abundance levels to mix to create a CO_2 gas with a desired $\delta^{13}C$ value.

Use the mixing model equations:

$$C_{AB} = C_A \times f_A + C_B \times f_B \tag{2.4}$$

$$f_A + f_B = 1 \tag{2.5}$$

$$C_{AB} = C_A \times f_A + C_B \times (1 - f_A) \tag{2.6}$$

$$f_A = \frac{C_{AB} - C_B}{C_A - C_B} \tag{2.7}$$

Where: C_{AB} = atom% ^{13}C of desired CO_2 gas; C_A = atom% of CO_2 gas_A for mixing (such as anatural abundance CO_2 gas source); C_B = atom% ^{13}C of CO_2 gas_B for mixing (such as 99 atom% ^{13}C); f_A is the fraction of CO_2 gas_A that should be mixed into gas_{AB}; and f_B is the fraction of CO_2 gas_B that should be mixed into gas_{AB}

Note: use the following equation to convert δ^{13}C values to atom% ^{13}C (as there is no linear relationship between δ^{13}C and atom%, all isotopic calculations must be done in atom%).

$$^{13}C\ atom\% = \frac{100\times 0.01118\times(\frac{\delta^{13}C}{1000}+1)}{1+0.01118\times\left(\frac{\delta^{13}C}{1000}+1\right)} \qquad (2.8)$$

EXAMPLE to determine how much labelled $^{13}CO_2$ gas (99 atom% ^{13}C) to add to an unenriched CO_2 gas at natural abundance to create a CO_2 gas with a desired δ^{13}C value of 200‰:

> If the desired CO_2 gas (gas$_{AB}$) should have a 1.324 atom% ^{13}C (or 200‰ δ^{13}C) and a 99 atom% $^{13}CO_2$ gas (gas$_B$) and a natural abundance CO_2 gas (gas$_A$) with 1.097 atom% ^{13}C (or -8‰ δ^{13}C) are available for mixing, then the fraction of gas$_A$ (f_A) and gas$_B$ (f_B) that should be mixed together for gas$_{AB}$ can be calculated with the following:
>
> $$f_A = \frac{C_{AB} - C_B}{C_A - C_B} = \frac{1.324 - 99}{1.097 - 99} = 0.9977$$
>
> $$f_B = 1 - f_A = 0.0023$$
>
> with 0.9977 as the fraction of gas$_A$ and 0.0023 as the fraction of gas$_B$ to mix into gas$_{AB}$.
>
> Using the fractions to calculate the required volumes, for a CO_2 gas tank with a volume of 0.5 L at 10 bar that holds 5 L gas at STP (as calculated using 2.7.1.):
>
> $f_A \times 5\,L = 0.9977 \times 5\,L = 4.989\,L$ of natural abundance CO_2 gas (gas$_A$)
>
> $f_B \times 5\,L = 0.0023 \times 5\,L = 0.012\,L$ of 99 atom% ^{13}C CO_2 gas (gas$_B$)
>
> Use these calculated volumes to prepare your reference gas, following the procedure in 2.6. replacing “CO_2 gas source” with gas$_A$ and “synthetic air” with gas$_B$.

3. MEASUREMENTS USING THE LASER CO_2 CARBON ISOTOPE ANALYSER IN CONTINUOUS FREE FLOW MODE

3.1. SCOPE AND FIELD OF APPLICATION

Changes in CO_2 levels and isotope composition are often dynamic and are due to both biotic and abiotic processes. To understand these dynamics, CO_2 fluxes must be monitored regularly. Laser CO_2 carbon isotope analysers that can continuously monitor the concentration and $\delta^{13}C$ of CO_2 are useful for providing high resolution data that are necessary for understanding CO_2 dynamics. With a simple inlet that can be set to sample and flow continuously, one can measure second to second changes in CO_2 concentration and $\delta^{13}C$ of whatever area the inlet is directed at [2]. With an accompanying outlet, one can measure second to second changes in CO_2 concentration and $\delta^{13}C$ of a closed-loop system (a closed system in which gas is sampled and returned to the sampled area without exchange with gas outside the sealed system, see Figure 3.). This closed-loop system can be used, for example, to measure rates of CO_2 build up and the $\delta^{13}C$ of CO_2 gas sources [3].

This section describes how to use the laser CO_2 carbon isotope analyser to measure CO_2 in continuous free flow mode and acquire accumulated data. The same methods would be used whether measuring in an open system (only measuring CO_2 concentration at point of inlet) or closed-loop system (measuring the buildup or loss of CO_2 in a closed system with the inlet sampling CO_2 and the outlet returning CO_2 to avoid artificial changes to CO_2 concentration in the system). Only calculations of data output will differ.

3.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Laser isotope analysers frequently measure gas samples that contain moisture. A nafion drier should be placed upstream the analyser, on the sampling line in between where a sample is taken in and where the sample enters the analyser, in order to remove water from sampled gas before measurement. This is done because removal of water vapor is essential to ensure high precision of measurements.

Depending on the resolution, amount of data desired and measurement time, data can be recorded in the instrument regularly over a range of temporal intervals, such as every second to 100 seconds. This tradeoff is dependent upon the user's needs.

Also depending on the type of data desired, the inlet can continuously measure an open area to measure general changes in CO_2 composition over time in an open air system, or the inlet and outlet can be connected to a closed-loop area to measure build up of CO_2 and changes in CO_2 composition over time (Figure 3.). However, caution should be applied if using an open air system, as factors within the proximity, such as wind, the number of people present, and the presence of exhaust from machinery, can cause minor to significant changes in CO_2 that interfere with sample measurements.

Data correction should always be applied after data is collected. Additionally, attention should be given if CO_2 concentration or $\delta^{13}CO_2$ is close to the limits of the guaranteed specification range of the spectroscope. This specification range differs for instrumentation. However, as an example based on testing maximum CO_2 concentrations on a Los Gatos laser CO_2 carbon isotope analyser, in a serial dilution of CO_2 concentrations ranging from 1500 to 400 ppm, the laser spectroscope correctly kept a constant $\delta^{13}CO_2$ value of 470 ‰. Appropriate CO_2 reference gases should be used to capture the range of CO_2 in which samples are measured at. Creating reference gases and performing data correction are discussed in Chapter 1 of this TECDOC.

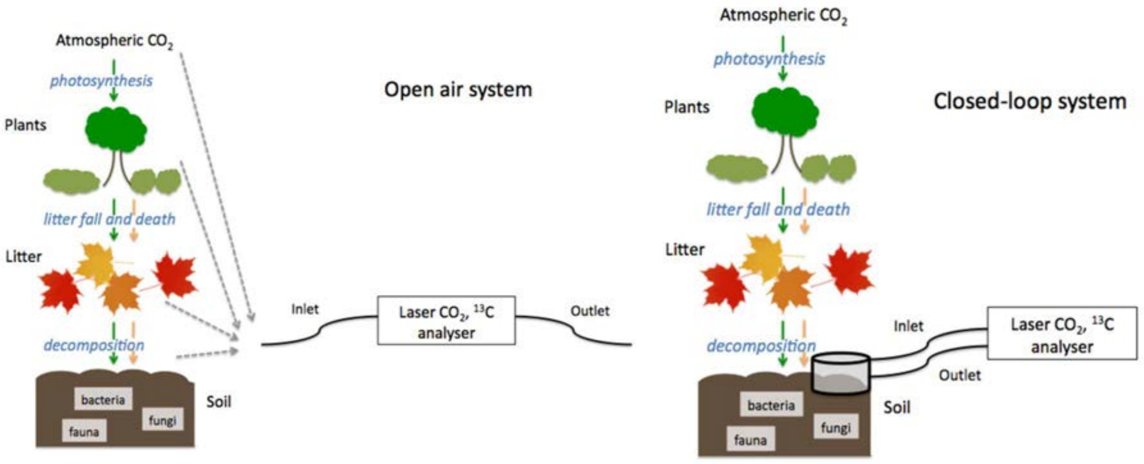


Figure 3. Diagram of an open (left) versus closed-loop (right) measurement system.

3.3. PRINCIPLE

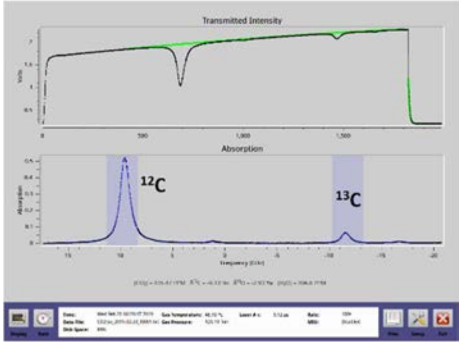
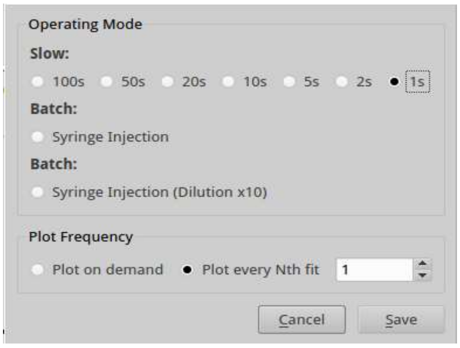
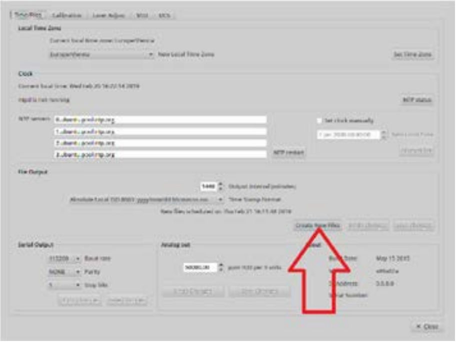
Measurements of CO_2 in continuous free flow mode by a laser CO_2 carbon isotope analyser can provide uninterrupted high resolution data, recorded up to every second. Depending on the set up of the analyser inlet and outlet, the analyser can take measurements in an open or closed-loop system. Only calculations of data output will differ. To ensure accurate and precise measurements that can be compared between times of measurement and between measurements on different instrumentation, it is important to calibrate the instrument with a CO_2 reference gas before taking measurements and to re-measure a reference gas every hour to check for and correct drift (further discussed in data management SOP).

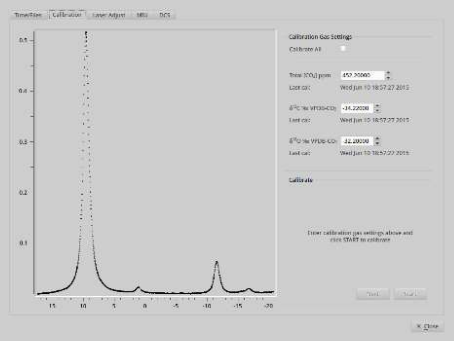
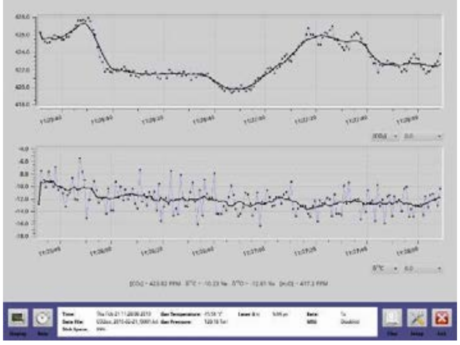
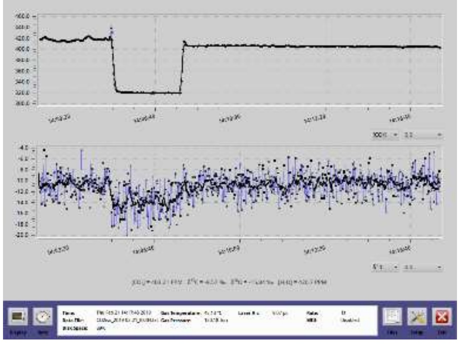
3.4. EQUIPMENT

- Carbon dioxide carbon isotope analyser
- Nafion drier
- Teflon tubing (1/8” × 1/4”)
- CO_2 reference gas(es)
- Gas flow meter

3.5. PROCEDURE

The following procedure is to measure CO_2 concentration and $\delta^{13}CO_2$ with a laser CO_2 carbon isotope analyser in continuous free flow mode. This will continually measure air pulled into the inlet until the instrument is stopped. *A Los Gatos laser CO_2 carbon isotope analyser has been selected as an example for illustration, though the procedure is relevant to additional models of any provider.*

Step	Procedure	
Step 1: **Instrumentation warm-up**	Turn on and warm-up instrumentation (*for the Los Gatos analyser, allow for at least 2 hours*). Drift in CO_2 measurements will occur if instrument is calibrated before warm-up.	
Step 2: **Confirm instrumentation warm-up**	Check the laser adjustment (*for the Los Gatos analyser, click the **Display icon** on the main screen until the reading for Absorption vs. Frequency is available for confirmation. In the bottom plot, the black dots (measured) and the blue line (fitted) should align*). Also confirm that **Gas Temp** has warmed to company recommendations (*for the Los Gatos Analyser*, 45°C).	
Step 3: **Switch instrumentation to continuous flow mode**	Select the flow mode (continuous free flow mode) and the rate at which data should be stored, such as 1, 2, 5, 10, 20, 50 or 100 seconds (*for the Los Gatos Analyser, click the **Rate icon** on the main screen to make the selection*).	Operating Mode Slow: ○ 100s ○ 50s ○ 20s ○ 10s ○ 5s ○ 2s ● 1s Batch: ○ Syringe Injection Batch: ○ Syringe Injection (Dilution x10) Plot Frequency ○ Plot on demand ● Plot every Nth fit 1
Step 4: **Create new file**	To ensure that measurements recorded for the day are on the same file, create a new file that will be used for the entire day (for the Los Gatos Analyser, click the **Setup icon** on the main screen, then the **Time/Files tab** on the next screen and the **"Create New File" button)**.	

Step 5: **Instrument calibration**	Supply CO_2 reference gas to instrument by a tube attachment regulated at the same flow rate as the analyser (*for the Los Gatos Analyser, use 1-2 L min^{-1}*). Ensure correct flow of gas before attaching to instrument inlet using a flow meter.	
	Calibrate the analyser after entering the known CO_2 ppm and $\delta^{13}C$ of the reference gas as measured on additional instrumentation (*for the Los Gatos Analyser, select* ***Setup icon*** *and then* ***Calibrate tab*** *and follow instrument instructions until calibration is complete*).	
Step 6: **Confirm instrument calibration**	Allow 1-2 minutes for instrument to begin to record output data after calibration. Confirm that CO_2 ppm and $\delta^{13}C$ measurements of the reference gas are similar to those entered in the calibration process. Record the start and end time of the reference gas measurement.	
Step 7: **Measure samples**	Disconnect the inlet from the CO_2 reference tank and direct the inlet to the area for desired CO_2 measurement and record time on instrument monitor of the start and end of measurement. If measuring gas with a high $\delta^{13}C$ or CO_2 concentration higher than 500 ppm, allow instrumentation to purge with unenriched atmospheric gas for 2 minutes in between each	

	sample to ensure no carry-over of CO_2. Repeat process for each sample.	
Step 8: **Confirm instrument calibration**	Confirm instrument calibration every hour and check for drift in measurements by re-measuring a CO_2 reference gas. Note the start and end of the CO_2 reference gas measurement. Purge the analyser by allowing the reference gas to flow 1 minute prior to measuring for another minute.	
Step 9: **Collecting recorded data**	Once all samples have been measured, copy the data from the analyser to a USB memory stick. Transfer the file that was created for measurements that day (*for the Los Gatos Analyser, click the **Files icon** and then the **Mount USB button** and drag the desired file to the right side screen containing contents of the USB stick. When complete, click the **Unmount USB button** then click the **Close button***).	
Step 10: **Turning off the instrument**	To turn off the instrument, ensure that the software is ready for shutdown first (for the Los Gatos Analyser, click the **Exit** icon on the main screen and follow analyser instructions).	

4. MEASUREMENTS USING THE LASER CO_2 CARBON ISOTOPE ANALYSER IN INJECTION MODE

4.1. SCOPE AND FIELD OF APPLICATION

Laser CO_2 carbon isotope analysers can also be placed in an injection mode, in which individual gas samples can be injected with an airtight gas syringe and measured. This method is particularly useful when analysing (low-volume) samples that have been collected and stored prior to analysis and are typically used for studies that require a relatively low number of measurements. Because continuous flow mode has a higher flow rate of, for example 1 L min^{-1} for the Los Gatos Analyser, low-volume samples are too rapidly consumed in this mode and should be measured in injection mode instead.

This section describes how to use the laser CO_2 carbon isotope analyser to measure CO_2 in injection mode and acquire data.

4.2. BACKGROUND INFORMATION AND CONSIDERATIONS

Because instrument calibration cannot be performed in injection mode, data correction using reference gas(es) will need to be performed after measurements (see protocol on Data Management). Furthermore, samples should be measured at least twice to confirm values.

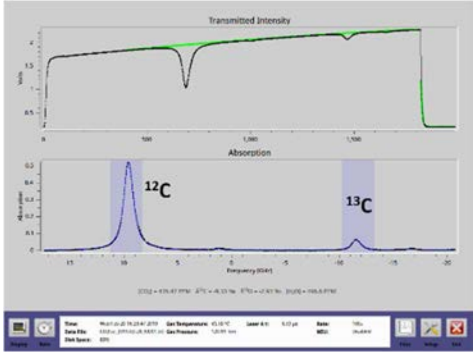
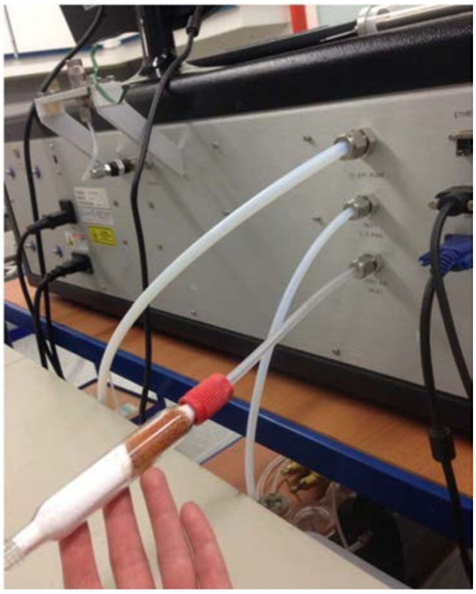
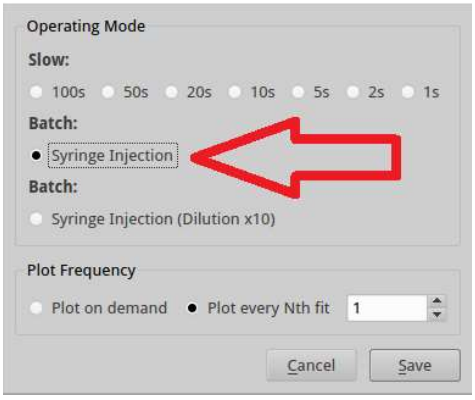
4.3. EQUIPMENT

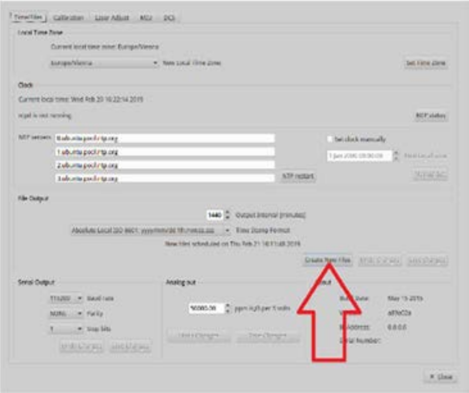
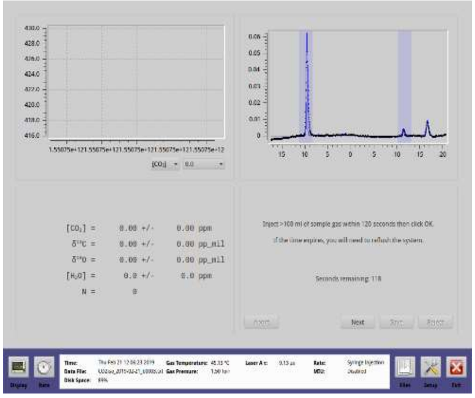
- Rotary vacuum pump (pumping speed > 1 m^3 h^{-1}, ultimate vacuum > 10^{-3} mbar)
- Gas tight syringe (100 mL)
- Gas bag or gas vial
- CO_2 reference gas(es)

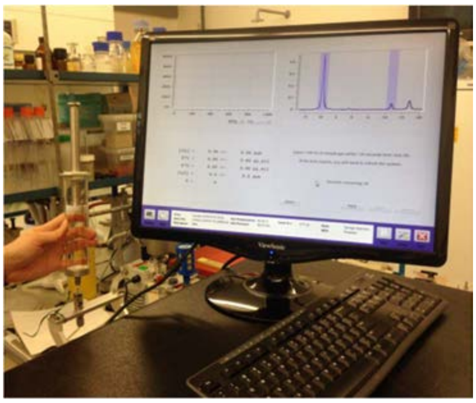
4.4. PROCEDURE

The following procedure is to measure CO_2 concentration and $\delta^{13}C$-CO_2 with a laser CO_2 carbon isotope analyser in injection mode. This will measure gas injected into the injection port. *A Los Gatos laser CO_2 carbon isotope analyser has been selected as an example for illustration, though the procedure is relevant to additional models of any provider.*

Step	Procedure	
Step 1: **Instrumentation warm-up**	Turn on and warm-up instrumentation (*for the Los Gatos analyser, allow for at least 2 hours*). Drift in CO_2 measurements will occur if instrument is calibrated before warm-up.	
Step 2: **Confirm instrumentation warm-up**	Check the laser adjustment (*for the Los Gatos analyser, click the **Display icon** on the main screen until the reading for Absorption vs. Frequency is available for confirmation. In the bottom plot, the black*	

	dots (measured) and the blue line (fitted) should align). Also confirm that **Gas Temp** has warmed to company recommendations (*for the Los Gatos Analyser*, 45°C).	
Step 3: **Attach CO_2 free air source to instrumentation**	Attach a CO_2 free air source to the "ZERO AIR INLET" on the back of the instrument. If only measuring a few number of samples, a synthetic air bag or a CO_2 scrubber (as seen on picture, containing a combination of Carbosieve and magnesium perchlorate ($Mg(ClO_4)_2$) can be attached to this inlet. If measuring a larger batch of samples, attach this directly to a synthetic air gas line supplied at a pressure of 5 to 10 psig (or 1.36 to 1.70 bar).	
Step 4: **Switch instrumentation to injection mode**	Switch the instrument to injection/batch mode (for the Los Gatos Analyser, click the **Rate icon** on the main screen, then select the Batch mode with "Syringe Injection" or, if samples contain very high concentrations of CO_2 (over 10 000 ppm), "Syringe Injection (Dilution X10)". Click Save. Allow time for the instrument to purge and flush with zero air.	Operating Mode Slow: ○ 100s ○ 50s ○ 20s ○ 10s ○ 5s ○ 2s ○ 1s Batch: ● Syringe Injection Batch: ○ Syringe Injection (Dilution x10) Plot Frequency ○ Plot on demand ● Plot every Nth fit 1

Step 5: **Create new file**	To ensure that measurements recorded for the day are on the same file, create a new file that will be used for the entire day (for the Los Gatos Analyser click the **Setup icon** on the main screen, then the **Time/Files tab** on the next screen and the **Create New File button**).	
Step 6: **Measuring a CO_2 reference gas**	Connect a gas bag valve to a vacuum pump. Open valve and evacuate bag. Close the valve before removing the evacuated bag.	
	Connect the gas bag to the CO_2 reference gas. Open valve and fill bag with roughly 500 mL of reference gas. Close the valve before removing the filled bag.	
	When the instrument indicates it is ready for a sample gas to be injected, measure the reference CO_2 gas first as a sample.	 [CO₂] = 0.00 +/- 0.00 ppm δ¹³C = 0.00 +/- 0.00 pp_mil δ¹⁸O = 0.00 +/- 0.00 pp_mil [H₂O] = 0.0 +/- 0.0 ppm N = 0 Inject >100 ml of sample gas within 120 seconds then click OK. If the time expires, you will need to reflush the system. Seconds remaining: 118

	Place empty gas syringe into septum of filled gas bag and flush 2-3 times with CO_2 reference gas by pulling CO_2 from bag and releasing it into the open air. After flushing, fill syringe with instrument specified injection volume (*for the Los Gatos Analyser, 100 mL*) of reference gas.	
	Place the filled syringe into the injection port and initiate sampling. Let the vacuum empty the syringe. Remove the syringe. (for the Los Gatos Analyser, click the **Next button** on the main screen and follow instrument instructions until sampling is complete). Allow time for the instrument to deliver the sample into cell, equilibrate the gas and measure.	
	Save the measurement (for the Los Gatos Analyser, Click the **Save button** on the main screen and note the text document the sample is saved as, example “CO2iso_2018-01-23_b003.txt”).	 $[CO_2]$ = 504.56 +/- 0.02 ppm $\delta^{13}C$ = -18.70 +/- 0.11 pp_mil $\delta^{18}O$ = -21.47 +/- 0.58 pp_mil $[H_2O]$ = 5326.9 +/- 9.8 ppm N = 320
Step 7: **Measure samples**	Measure same volume of sample gas in same way as the CO_2 reference gas.	

Step 8: **Instrument calibration check**	After every hour, confirm instrument calibration and check for drift by re-measuring a CO_2 reference gas.	
Step 9: **Collecting recorded data**	Once all samples have been measured, copy the data from the analyser to a USB memory stick. Transfer the file that was created for measurements that day (*for the Los Gatos Analyser, click the **Files icon** and then the **Mount USB button** and drag the desired file to the right side screen containing contents of the USB stick. When complete, click the **Unmount USB button** then click the **Close button***).	
Step 10: **Turning off the instrument**	To turn off the instrument, ensure that the software is ready for shutdown first (for the Los Gatos Analyser, click the **Exit** icon on the main screen and follow analyser instructions).	

5. DATA MANAGEMENT OF LASER CO_2 CARBON ISOTOPE ANALYSER COLLECTED MEASUREMENTS

5.1. BACKGROUND

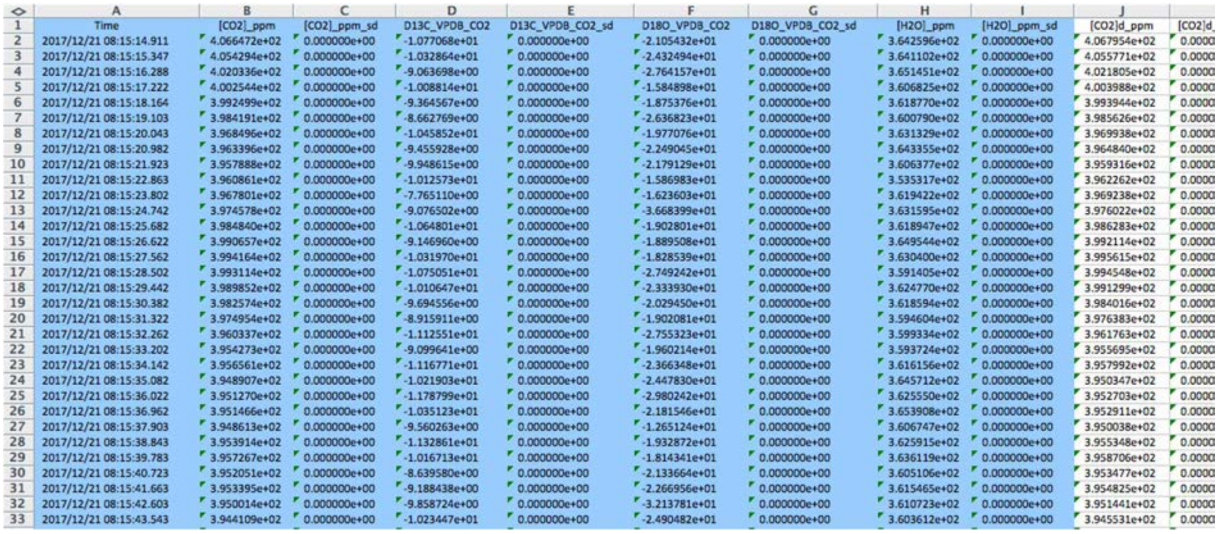
Data collected on a laser CO_2 carbon isotope analyser is stored in text format and must be transferred onto appropriate data processing software prior to data analysis. Data output can be large, especially when data is collected in continuous free flow mode. It is therefore necessary to use software that can easily convert data stored as text into a spreadsheet. Microsoft Office Excel is an accessible software program that can do this task. Described below is a protocol on how to manage and correct data in Excel.

5.2. ADVANTAGES AND POTENTIAL LIMITATIONS

Data management and correction can be performed on Excel, but to perform advanced statistical analysis, further software and instruction is necessary. As the type of statistical analysis will vary greatly depending on the research question being asked, we focus strictly on how to manage and correct data collected on the laser CO_2 carbon isotope analyser. Methods for statistical analysis are outside the scope of this publication.

5.3. PRINCIPLE

The laser CO_2 carbon isotope analyser stores over 35 columns of data, but the first 9 columns hold the most relevant data for users (Figure 4.). Specifically, these columns contain the time of collected data, CO_2 ppm, standard deviation of CO_2 ppm, $\delta^{13}C$ of CO_2, standard deviation of $\delta^{13}C$ of CO_2, $\delta^{18}O$ of CO_2 (and H_2O), standard deviation of $\delta^{18}O$ of CO_2 (and H_2O) and concentration of water and its respective standard deviation. As the standard deviation each data output is likely minimal, there are only 4 relevant columns (time, CO_2 ppm, $\delta^{13}C$ and H_2O ppm). The following protocol describes how to obtain this data and calculate CO_2 fluxes and sources in Excel.



	A	B	C	D	E	F	G	H	I	J	
1	Time	[CO2]_ppm	[CO2]_ppm_sd	D13C_VPDB_CO2	D13C_VPDB_CO2_sd	D18O_VPDB_CO2	D18O_VPDB_CO2_sd	[H2O]_ppm	[H2O]_ppm_sd	[CO2]d_ppm	[CO2]d_
2	2017/12/21 08:15:14.911	4.066472e+02	0.000000e+00	-1.077068e+01	0.000000e+00	-2.105432e+01	0.000000e+00	3.642596e+02	0.000000e+00	4.067954e+02	0.0000
3	2017/12/21 08:15:15.347	4.054294e+02	0.000000e+00	-1.032864e+01	0.000000e+00	-2.432494e+01	0.000000e+00	3.641102e+02	0.000000e+00	4.055771e+02	0.0000
4	2017/12/21 08:15:16.288	4.020336e+02	0.000000e+00	-9.063698e+00	0.000000e+00	-2.764157e+01	0.000000e+00	3.651451e+02	0.000000e+00	4.021805e+02	0.0000
5	2017/12/21 08:15:17.222	4.002544e+02	0.000000e+00	-1.008814e+01	0.000000e+00	-1.584898e+01	0.000000e+00	3.606825e+02	0.000000e+00	4.003988e+02	0.0000
6	2017/12/21 08:15:18.164	3.992499e+02	0.000000e+00	-9.364567e+00	0.000000e+00	-1.875376e+01	0.000000e+00	3.618770e+02	0.000000e+00	3.993944e+02	0.0000
7	2017/12/21 08:15:19.103	3.984191e+02	0.000000e+00	-8.662769e+00	0.000000e+00	-2.636823e+01	0.000000e+00	3.600790e+02	0.000000e+00	3.985626e+02	0.0000
8	2017/12/21 08:15:20.043	3.968496e+02	0.000000e+00	-1.045852e+01	0.000000e+00	-1.977076e+01	0.000000e+00	3.631329e+02	0.000000e+00	3.969938e+02	0.0000
9	2017/12/21 08:15:20.982	3.963396e+02	0.000000e+00	-9.455928e+00	0.000000e+00	-2.249045e+01	0.000000e+00	3.643355e+02	0.000000e+00	3.964840e+02	0.0000
10	2017/12/21 08:15:21.923	3.957888e+02	0.000000e+00	-9.948615e+00	0.000000e+00	-2.179129e+01	0.000000e+00	3.606377e+02	0.000000e+00	3.959316e+02	0.0000
11	2017/12/21 08:15:22.863	3.960861e+02	0.000000e+00	-1.012573e+01	0.000000e+00	-1.586983e+01	0.000000e+00	3.535317e+02	0.000000e+00	3.962262e+02	0.0000
12	2017/12/21 08:15:23.802	3.967801e+02	0.000000e+00	-7.765110e+00	0.000000e+00	-1.623603e+01	0.000000e+00	3.619422e+02	0.000000e+00	3.969238e+02	0.0000
13	2017/12/21 08:15:24.742	3.974578e+02	0.000000e+00	-9.076502e+00	0.000000e+00	-3.668399e+01	0.000000e+00	3.631595e+02	0.000000e+00	3.976022e+02	0.0000
14	2017/12/21 08:15:25.682	3.984840e+02	0.000000e+00	-1.064801e+01	0.000000e+00	-1.902801e+01	0.000000e+00	3.618947e+02	0.000000e+00	3.986283e+02	0.0000
15	2017/12/21 08:15:26.622	3.990657e+02	0.000000e+00	-9.146960e+00	0.000000e+00	-1.889508e+01	0.000000e+00	3.649544e+02	0.000000e+00	3.992114e+02	0.0000
16	2017/12/21 08:15:27.562	3.994164e+02	0.000000e+00	-1.031970e+01	0.000000e+00	-1.828539e+01	0.000000e+00	3.630400e+02	0.000000e+00	3.995615e+02	0.0000
17	2017/12/21 08:15:28.502	3.993114e+02	0.000000e+00	-1.075051e+01	0.000000e+00	-2.749242e+01	0.000000e+00	3.591405e+02	0.000000e+00	3.994548e+02	0.0000
18	2017/12/21 08:15:29.442	3.989852e+02	0.000000e+00	-1.010647e+01	0.000000e+00	-2.333930e+01	0.000000e+00	3.624770e+02	0.000000e+00	3.991299e+02	0.0000
19	2017/12/21 08:15:30.382	3.982574e+02	0.000000e+00	-9.694556e+00	0.000000e+00	-2.029450e+01	0.000000e+00	3.618594e+02	0.000000e+00	3.984016e+02	0.0000
20	2017/12/21 08:15:31.322	3.974954e+02	0.000000e+00	-8.915911e+00	0.000000e+00	-1.902081e+01	0.000000e+00	3.594604e+02	0.000000e+00	3.976383e+02	0.0000
21	2017/12/21 08:15:32.262	3.960337e+02	0.000000e+00	-1.112551e+01	0.000000e+00	-2.755323e+01	0.000000e+00	3.599334e+02	0.000000e+00	3.961763e+02	0.0000
22	2017/12/21 08:15:33.202	3.954273e+02	0.000000e+00	-9.099641e+00	0.000000e+00	-1.960214e+01	0.000000e+00	3.593724e+02	0.000000e+00	3.955695e+02	0.0000
23	2017/12/21 08:15:34.142	3.956561e+02	0.000000e+00	-1.116771e+01	0.000000e+00	-2.366348e+01	0.000000e+00	3.616156e+02	0.000000e+00	3.957992e+02	0.0000
24	2017/12/21 08:15:35.082	3.948907e+02	0.000000e+00	-1.021903e+01	0.000000e+00	-2.447830e+01	0.000000e+00	3.645712e+02	0.000000e+00	3.950347e+02	0.0000
25	2017/12/21 08:15:36.022	3.951270e+02	0.000000e+00	-1.178799e+01	0.000000e+00	-2.980242e+01	0.000000e+00	3.625550e+02	0.000000e+00	3.952703e+02	0.0000
26	2017/12/21 08:15:36.962	3.951466e+02	0.000000e+00	-1.035123e+01	0.000000e+00	-2.181546e+01	0.000000e+00	3.653908e+02	0.000000e+00	3.952911e+02	0.0000
27	2017/12/21 08:15:37.903	3.948613e+02	0.000000e+00	-9.560263e+00	0.000000e+00	-1.265124e+01	0.000000e+00	3.606747e+02	0.000000e+00	3.950038e+02	0.0000
28	2017/12/21 08:15:38.843	3.953914e+02	0.000000e+00	-1.132861e+01	0.000000e+00	-1.932872e+01	0.000000e+00	3.625915e+02	0.000000e+00	3.955348e+02	0.0000
29	2017/12/21 08:15:39.783	3.957267e+02	0.000000e+00	-1.016713e+01	0.000000e+00	-1.814341e+01	0.000000e+00	3.636119e+02	0.000000e+00	3.958706e+02	0.0000
30	2017/12/21 08:15:40.723	3.952051e+02	0.000000e+00	-8.639580e+00	0.000000e+00	-2.133664e+01	0.000000e+00	3.605106e+02	0.000000e+00	3.953477e+02	0.0000
31	2017/12/21 08:15:41.663	3.953395e+02	0.000000e+00	-9.188438e+00	0.000000e+00	-2.266956e+01	0.000000e+00	3.615465e+02	0.000000e+00	3.954825e+02	0.0000
32	2017/12/21 08:15:42.603	3.950014e+02	0.000000e+00	-9.858724e+00	0.000000e+00	-3.213781e+01	0.000000e+00	3.610723e+02	0.000000e+00	3.951441e+02	0.0000
33	2017/12/21 08:15:43.543	3.944109e+02	0.000000e+00	-1.023447e+01	0.000000e+00	-2.490482e+01	0.000000e+00	3.603612e+02	0.000000e+00	3.945531e+02	0.0000

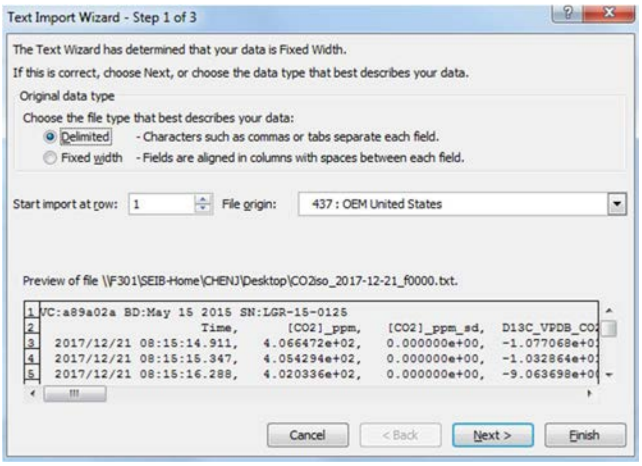
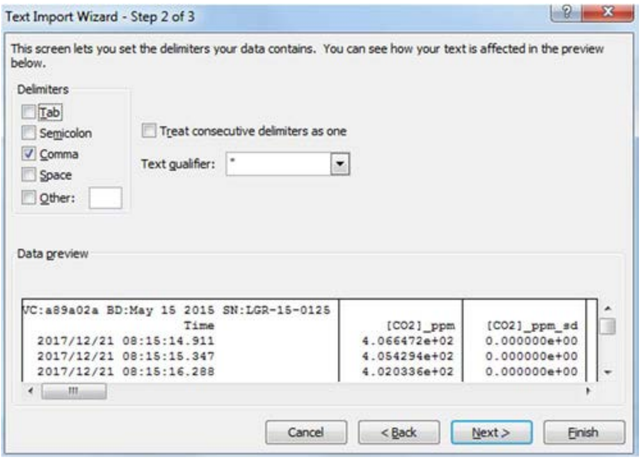
Figure 4. An example of data transferred onto Microsoft Office Excel from a CO_2 Carbon Isotope Analyser (in this case, Los Gatos). The 9 columns mentioned in 5.3. are highlighted.

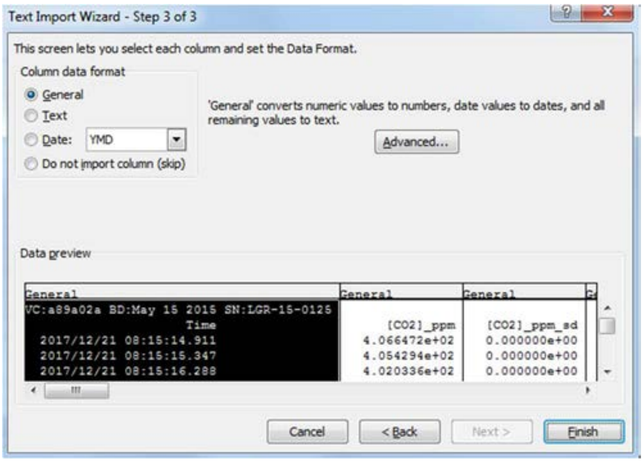
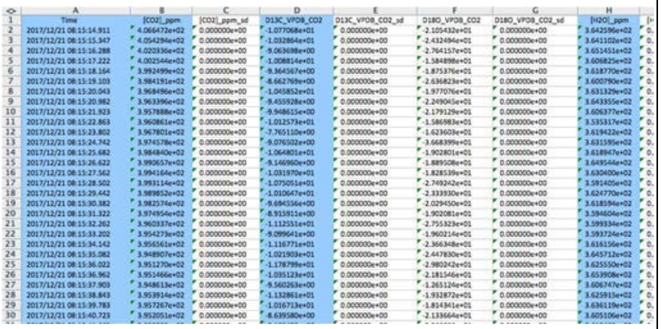
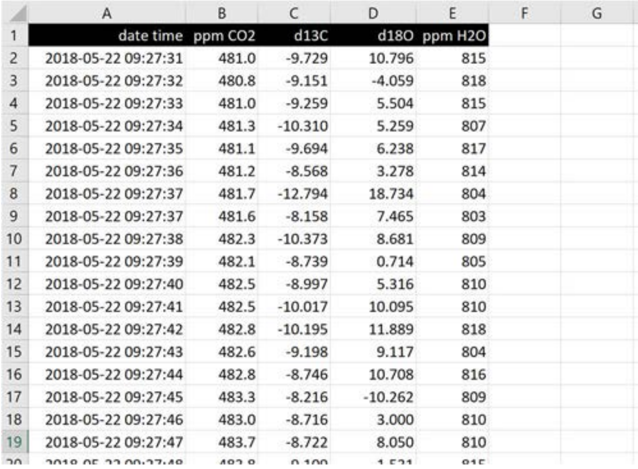
5.4. SOFTWARE

Microsoft Office Excel

5.5. PROCEDURE

The following procedure is to process data collected by the laser CO_2 carbon isotope analyser. *A Los Gatos laser CO_2 carbon isotope analyser has been selected as an example for illustration, though the procedure is relevant to additional models.*

Step	Procedure	
Step 1: **Importing data in Excel**	In Excel, open the analyser text document. You will be queued to note how the original type is separated. Click Delimited (with characters) then click next.	 Text Import Wizard - Step 1 of 3 The Text Wizard has determined that your data is Fixed Width. If this is correct, choose Next, or choose the data type that best describes your data. Original data type Choose the file type that best describes your data: ● Delimited - Characters such as commas or tabs separate each field. ○ Fixed width - Fields are aligned in columns with spaces between each field. Start import at row: 1 File origin: 437 : OEM United States Preview of file \\F301\SEIB-Home\CHENJ\Desktop\CO2iso_2017-12-21_f0000.txt. 1 VC:a89a02a BD:May 15 2015 SN:LGR-15-0125 2 Time, [CO2]_ppm, [CO2]_ppm_sd, D13C_VPDB_CO2 3 2017/12/21 08:15:14.911, 4.066472e+02, 0.000000e+00, -1.077068e+0 4 2017/12/21 08:15:15.347, 4.054294e+02, 0.000000e+00, -1.032864e+0 5 2017/12/21 08:15:16.288, 4.020336e+02, 0.000000e+00, -9.063698e+0
	Next select “comma” as the type of delimiter. Click next.	 Text Import Wizard - Step 2 of 3 This screen lets you set the delimiters your data contains. You can see how your text is affected in the preview below. Delimiters ☐ Tab ☐ Semicolon ☑ Comma ☐ Space ☐ Other: ☐ Treat consecutive delimiters as one Text qualifier: " Data preview VC:a89a02a BD:May 15 2015 SN:LGR-15-0125 Time | [CO2]_ppm | [CO2]_ppm_sd 2017/12/21 08:15:14.911 | 4.066472e+02 | 0.000000e+00 2017/12/21 08:15:15.347 | 4.054294e+02 | 0.000000e+00 2017/12/21 08:15:16.288 | 4.020336e+02 | 0.000000e+00

	Finally select a “general” format and click finish.	
	Save the imported text data as an original file. Continue by copying columns with data on time, CO_2 ppm, $\delta^{13}C$ CO_2, and H_2O ppm.	
	Create a new worksheet within this workbook, example “1”. Copy the rows of data from “LGR” that are associated with the time the sample was run. In this case, standard 1 was run from 9:27:31 to 9:37:281 on 2018- 05- 22.	

Text Import Wizard - Step 3 of 3

This screen lets you select each column and set the Data Format.

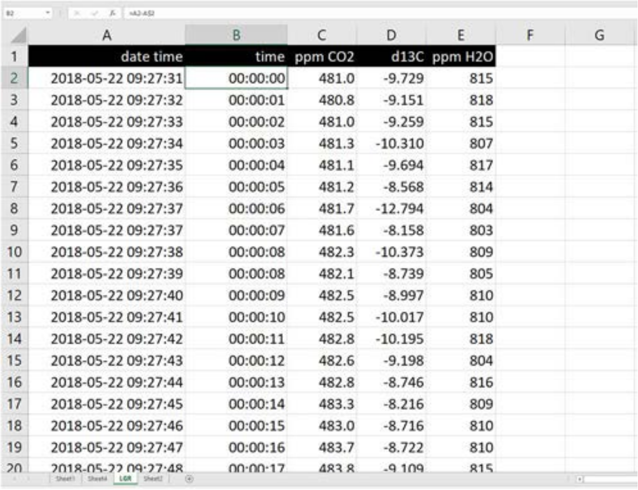
Column data format

- ● General
- ○ Text
- ○ Date: YMD
- ○ Do not import column (skip)

'General' converts numeric values to numbers, date values to dates, and all remaining values to text.

```
General                                          General          General
VC:a89a02a BD:May 15 2015 SN:LGR-15-0125
                     Time                        [CO2]_ppm        [CO2]_ppm_sd
  2017/12/21 08:15:14.911                        4.066472e+02     0.000000e+00
  2017/12/21 08:15:15.347                        4.054294e+02     0.000000e+00
  2017/12/21 08:15:16.288                        4.020336e+02     0.000000e+00
```

	A	B	C	D	E	F	G
1	date time	ppm CO2	d13C	d18O	ppm H2O		
2	2018-05-22 09:27:31	481.0	-9.729	10.796	815		
3	2018-05-22 09:27:32	480.8	-9.151	-4.059	818		
4	2018-05-22 09:27:33	481.0	-9.259	5.504	815		
5	2018-05-22 09:27:34	481.3	-10.310	5.259	807		
6	2018-05-22 09:27:35	481.1	-9.694	6.238	817		
7	2018-05-22 09:27:36	481.2	-8.568	3.278	814		
8	2018-05-22 09:27:37	481.7	-12.794	18.734	804		
9	2018-05-22 09:27:37	481.6	-8.158	7.465	803		
10	2018-05-22 09:27:38	482.3	-10.373	8.681	809		
11	2018-05-22 09:27:39	482.1	-8.739	0.714	805		
12	2018-05-22 09:27:40	482.5	-8.997	5.316	810		
13	2018-05-22 09:27:41	482.5	-10.017	10.095	810		
14	2018-05-22 09:27:42	482.8	-10.195	11.889	818		
15	2018-05-22 09:27:43	482.6	-9.198	9.117	804		
16	2018-05-22 09:27:44	482.8	-8.746	10.708	816		
17	2018-05-22 09:27:45	483.3	-8.216	-10.262	809		
18	2018-05-22 09:27:46	483.0	-8.716	3.000	810		
19	2018-05-22 09:27:47	483.7	-8.722	8.050	810		

<table>
<tr>
<td></td>
<td>Paste the relevant data in a new workbook on a new worksheet, example “LGR” and insert “time” using the formula: “B=A2-A$2”, copy this formula to all rows.</td>
<td>

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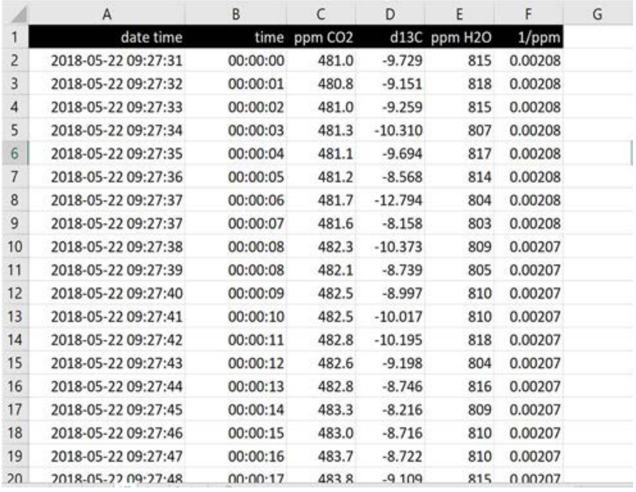
<tr><th></th><th>A</th><th>B</th><th>C</th><th>D</th><th>E</th><th>F</th><th>G</th></tr>
<tr><td>1</td><td>date time</td><td>time</td><td>ppm CO2</td><td>d13C</td><td>ppm H2O</td><td></td><td></td></tr>
<tr><td>2</td><td>2018-05-22 09:27:31</td><td>00:00:00</td><td>481.0</td><td>-9.729</td><td>815</td><td></td><td></td></tr>
<tr><td>3</td><td>2018-05-22 09:27:32</td><td>00:00:01</td><td>480.8</td><td>-9.151</td><td>818</td><td></td><td></td></tr>
<tr><td>4</td><td>2018-05-22 09:27:33</td><td>00:00:02</td><td>481.0</td><td>-9.259</td><td>815</td><td></td><td></td></tr>
<tr><td>5</td><td>2018-05-22 09:27:34</td><td>00:00:03</td><td>481.3</td><td>-10.310</td><td>807</td><td></td><td></td></tr>
<tr><td>6</td><td>2018-05-22 09:27:35</td><td>00:00:04</td><td>481.1</td><td>-9.694</td><td>817</td><td></td><td></td></tr>
<tr><td>7</td><td>2018-05-22 09:27:36</td><td>00:00:05</td><td>481.2</td><td>-8.568</td><td>814</td><td></td><td></td></tr>
<tr><td>8</td><td>2018-05-22 09:27:37</td><td>00:00:06</td><td>481.7</td><td>-12.794</td><td>804</td><td></td><td></td></tr>
<tr><td>9</td><td>2018-05-22 09:27:37</td><td>00:00:07</td><td>481.6</td><td>-8.158</td><td>803</td><td></td><td></td></tr>
<tr><td>10</td><td>2018-05-22 09:27:38</td><td>00:00:08</td><td>482.3</td><td>-10.373</td><td>809</td><td></td><td></td></tr>
<tr><td>11</td><td>2018-05-22 09:27:39</td><td>00:00:08</td><td>482.1</td><td>-8.739</td><td>805</td><td></td><td></td></tr>
<tr><td>12</td><td>2018-05-22 09:27:40</td><td>00:00:09</td><td>482.5</td><td>-8.997</td><td>810</td><td></td><td></td></tr>
<tr><td>13</td><td>2018-05-22 09:27:41</td><td>00:00:10</td><td>482.5</td><td>-10.017</td><td>810</td><td></td><td></td></tr>
<tr><td>14</td><td>2018-05-22 09:27:42</td><td>00:00:11</td><td>482.8</td><td>-10.195</td><td>818</td><td></td><td></td></tr>
<tr><td>15</td><td>2018-05-22 09:27:43</td><td>00:00:12</td><td>482.6</td><td>-9.198</td><td>804</td><td></td><td></td></tr>
<tr><td>16</td><td>2018-05-22 09:27:44</td><td>00:00:13</td><td>482.8</td><td>-8.746</td><td>816</td><td></td><td></td></tr>
<tr><td>17</td><td>2018-05-22 09:27:45</td><td>00:00:14</td><td>483.3</td><td>-8.216</td><td>809</td><td></td><td></td></tr>
<tr><td>18</td><td>2018-05-22 09:27:46</td><td>00:00:15</td><td>483.0</td><td>-8.716</td><td>810</td><td></td><td></td></tr>
<tr><td>19</td><td>2018-05-22 09:27:47</td><td>00:00:16</td><td>483.7</td><td>-8.722</td><td>810</td><td></td><td></td></tr>
<tr><td>20</td><td>2018-05-22 09:27:48</td><td>00:00:17</td><td>483.8</td><td>-9.109</td><td>815</td><td></td><td></td></tr>
</table>

</td>
</tr>
<tr>
<td>**Step 2:**

Source calculation</td>
<td>Make a new column on worksheet “1”, containing the inverse of the CO_2 concentration (in this case, “F2=1/C2).</td>
<td>

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<tr><th></th><th>A</th><th>B</th><th>C</th><th>D</th><th>E</th><th>F</th><th>G</th></tr>
<tr><td>1</td><td>date time</td><td>time</td><td>ppm CO2</td><td>d13C</td><td>ppm H2O</td><td>1/ppm</td><td></td></tr>
<tr><td>2</td><td>2018-05-22 09:27:31</td><td>00:00:00</td><td>481.0</td><td>-9.729</td><td>815</td><td>0.00208</td><td></td></tr>
<tr><td>3</td><td>2018-05-22 09:27:32</td><td>00:00:01</td><td>480.8</td><td>-9.151</td><td>818</td><td>0.00208</td><td></td></tr>
<tr><td>4</td><td>2018-05-22 09:27:33</td><td>00:00:02</td><td>481.0</td><td>-9.259</td><td>815</td><td>0.00208</td><td></td></tr>
<tr><td>5</td><td>2018-05-22 09:27:34</td><td>00:00:03</td><td>481.3</td><td>-10.310</td><td>807</td><td>0.00208</td><td></td></tr>
<tr><td>6</td><td>2018-05-22 09:27:35</td><td>00:00:04</td><td>481.1</td><td>-9.694</td><td>817</td><td>0.00208</td><td></td></tr>
<tr><td>7</td><td>2018-05-22 09:27:36</td><td>00:00:05</td><td>481.2</td><td>-8.568</td><td>814</td><td>0.00208</td><td></td></tr>
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<tr><td>11</td><td>2018-05-22 09:27:39</td><td>00:00:08</td><td>482.1</td><td>-8.739</td><td>805</td><td>0.00207</td><td></td></tr>
<tr><td>12</td><td>2018-05-22 09:27:40</td><td>00:00:09</td><td>482.5</td><td>-8.997</td><td>810</td><td>0.00207</td><td></td></tr>
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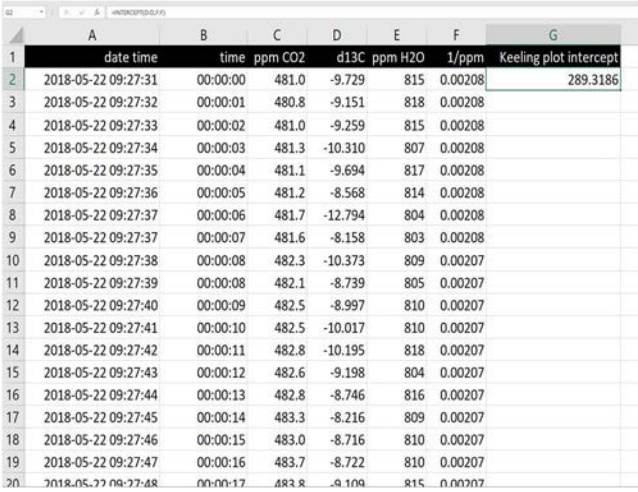
<tr><td>15</td><td>2018-05-22 09:27:43</td><td>00:00:12</td><td>482.6</td><td>-9.198</td><td>804</td><td>0.00207</td><td></td></tr>
<tr><td>16</td><td>2018-05-22 09:27:44</td><td>00:00:13</td><td>482.8</td><td>-8.746</td><td>816</td><td>0.00207</td><td></td></tr>
<tr><td>17</td><td>2018-05-22 09:27:45</td><td>00:00:14</td><td>483.3</td><td>-8.216</td><td>809</td><td>0.00207</td><td></td></tr>
<tr><td>18</td><td>2018-05-22 09:27:46</td><td>00:00:15</td><td>483.0</td><td>-8.716</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>19</td><td>2018-05-22 09:27:47</td><td>00:00:16</td><td>483.7</td><td>-8.722</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>20</td><td>2018-05-22 09:27:48</td><td>00:00:17</td><td>483.8</td><td>-9.109</td><td>815</td><td>0.00207</td><td></td></tr>
</table>
</td>
</tr>
<tr>
<td></td>
<td>Calculate the intercept of the Keeling plot (the linear regression with $1/[CO_2]$ on the x-axis and ^{13}C on the y-axis) using “=INTERCEPT(D:D,F:F)”</td>
<td>

<table>
<tr><th></th><th>A</th><th>B</th><th>C</th><th>D</th><th>E</th><th>F</th><th>G</th></tr>
<tr><td>1</td><td>date time</td><td>time</td><td>ppm CO2</td><td>d13C</td><td>ppm H2O</td><td>1/ppm</td><td>Keeling plot intercept</td></tr>
<tr><td>2</td><td>2018-05-22 09:27:31</td><td>00:00:00</td><td>481.0</td><td>-9.729</td><td>815</td><td>0.00208</td><td>289.3186</td></tr>
<tr><td>3</td><td>2018-05-22 09:27:32</td><td>00:00:01</td><td>480.8</td><td>-9.151</td><td>818</td><td>0.00208</td><td></td></tr>
<tr><td>4</td><td>2018-05-22 09:27:33</td><td>00:00:02</td><td>481.0</td><td>-9.259</td><td>815</td><td>0.00208</td><td></td></tr>
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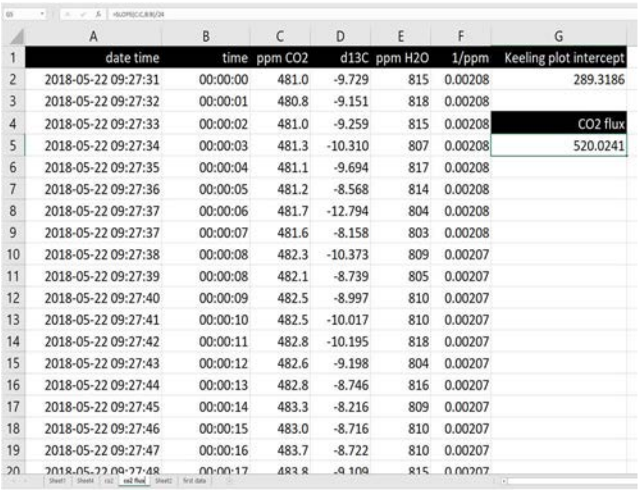
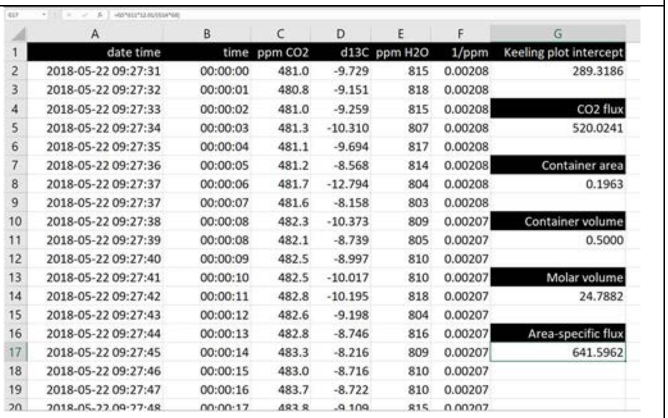
<tr><td>10</td><td>2018-05-22 09:27:38</td><td>00:00:08</td><td>482.3</td><td>-10.373</td><td>809</td><td>0.00207</td><td></td></tr>
<tr><td>11</td><td>2018-05-22 09:27:39</td><td>00:00:08</td><td>482.1</td><td>-8.739</td><td>805</td><td>0.00207</td><td></td></tr>
<tr><td>12</td><td>2018-05-22 09:27:40</td><td>00:00:09</td><td>482.5</td><td>-8.997</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>13</td><td>2018-05-22 09:27:41</td><td>00:00:10</td><td>482.5</td><td>-10.017</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>14</td><td>2018-05-22 09:27:42</td><td>00:00:11</td><td>482.8</td><td>-10.195</td><td>818</td><td>0.00207</td><td></td></tr>
<tr><td>15</td><td>2018-05-22 09:27:43</td><td>00:00:12</td><td>482.6</td><td>-9.198</td><td>804</td><td>0.00207</td><td></td></tr>
<tr><td>16</td><td>2018-05-22 09:27:44</td><td>00:00:13</td><td>482.8</td><td>-8.746</td><td>816</td><td>0.00207</td><td></td></tr>
<tr><td>17</td><td>2018-05-22 09:27:45</td><td>00:00:14</td><td>483.3</td><td>-8.216</td><td>809</td><td>0.00207</td><td></td></tr>
<tr><td>18</td><td>2018-05-22 09:27:46</td><td>00:00:15</td><td>483.0</td><td>-8.716</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>19</td><td>2018-05-22 09:27:47</td><td>00:00:16</td><td>483.7</td><td>-8.722</td><td>810</td><td>0.00207</td><td></td></tr>
<tr><td>20</td><td>2018-05-22 09:27:48</td><td>00:00:17</td><td>483.8</td><td>-9.109</td><td>815</td><td>0.00207</td><td></td></tr>
</table>
</td>
</tr>
</table>

Step 3: **Flux calculation**	Calculate the CO_2 flux (ppm h^{-1}) as the slope of the CO_2 ppm (column B in this example) by the time (column A in this example) and then divide by 24 as Excels' time format is in days. Example "=SLOPE(C:C,B:B)/24" (see calculation in 5.6.2).	
	To calculate the area-specific flux of carbon emitted (µg m^{-2} h^{-1}) see calculation in section 5.6.3. Example "=G5*G11*12.01/(G14*G8)".	

	A	B	C	D	E	F	G
1	date time	time	ppm CO2	d13C	ppm H2O	1/ppm	Keeling plot intercept
2	2018-05-22 09:27:31	00:00:00	481.0	-9.729	815	0.00208	289.3186
3	2018-05-22 09:27:32	00:00:01	480.8	-9.151	818	0.00208	
4	2018-05-22 09:27:33	00:00:02	481.0	-9.259	815	0.00208	CO2 flux
5	2018-05-22 09:27:34	00:00:03	481.3	-10.310	807	0.00208	520.0241
6	2018-05-22 09:27:35	00:00:04	481.1	-9.694	817	0.00208	
7	2018-05-22 09:27:36	00:00:05	481.2	-8.568	814	0.00208	
8	2018-05-22 09:27:37	00:00:06	481.7	-12.794	804	0.00208	
9	2018-05-22 09:27:37	00:00:07	481.6	-8.158	803	0.00208	
10	2018-05-22 09:27:38	00:00:08	482.3	-10.373	809	0.00207	
11	2018-05-22 09:27:39	00:00:08	482.1	-8.739	805	0.00207	
12	2018-05-22 09:27:40	00:00:09	482.5	-8.997	810	0.00207	
13	2018-05-22 09:27:41	00:00:10	482.5	-10.017	810	0.00207	
14	2018-05-22 09:27:42	00:00:11	482.8	-10.195	818	0.00207	
15	2018-05-22 09:27:43	00:00:12	482.6	-9.198	804	0.00207	
16	2018-05-22 09:27:44	00:00:13	482.8	-8.746	816	0.00207	
17	2018-05-22 09:27:45	00:00:14	483.3	-8.216	809	0.00207	
18	2018-05-22 09:27:46	00:00:15	483.0	-8.716	810	0.00207	
19	2018-05-22 09:27:47	00:00:16	483.7	-8.722	810	0.00207	
20	2018-05-22 09:27:48	00:00:17	483.8	-9.109	815	0.00207	

	A	B	C	D	E	F	G
1	date time	time	ppm CO2	d13C	ppm H2O	1/ppm	Keeling plot intercept
2	2018-05-22 09:27:31	00:00:00	481.0	-9.729	815	0.00208	289.3186
3	2018-05-22 09:27:32	00:00:01	480.8	-9.151	818	0.00208	
4	2018-05-22 09:27:33	00:00:02	481.0	-9.259	815	0.00208	CO2 flux
5	2018-05-22 09:27:34	00:00:03	481.3	-10.310	807	0.00208	520.0241
6	2018-05-22 09:27:35	00:00:04	481.1	-9.694	817	0.00208	
7	2018-05-22 09:27:36	00:00:05	481.2	-8.568	814	0.00208	Container area
8	2018-05-22 09:27:37	00:00:06	481.7	-12.794	804	0.00208	0.1963
9	2018-05-22 09:27:37	00:00:07	481.6	-8.158	803	0.00208	
10	2018-05-22 09:27:38	00:00:08	482.3	-10.373	809	0.00207	Container volume
11	2018-05-22 09:27:39	00:00:08	482.1	-8.739	805	0.00207	0.5000
12	2018-05-22 09:27:40	00:00:09	482.5	-8.997	810	0.00207	
13	2018-05-22 09:27:41	00:00:10	482.5	-10.017	810	0.00207	Molar volume
14	2018-05-22 09:27:42	00:00:11	482.8	-10.195	818	0.00207	24.7882
15	2018-05-22 09:27:43	00:00:12	482.6	-9.198	804	0.00207	
16	2018-05-22 09:27:44	00:00:13	482.8	-8.746	816	0.00207	Area-specific flux
17	2018-05-22 09:27:45	00:00:14	483.3	-8.216	809	0.00207	641.5962
18	2018-05-22 09:27:46	00:00:15	483.0	-8.716	810	0.00207	
19	2018-05-22 09:27:47	00:00:16	483.7	-8.722	810	0.00207	
20	2018-05-22 09:27:48	00:00:17	483.8	-9.109	815	0.00207	

A Macro for the flux and keeling plot calculations in Excel is available upon request.

5.6. CALCULATIONS

5.6.1. Calculating the soil surface area and volume of measurement chambers.

$$A = \pi \times r^2 \qquad (5.1)$$

Where A = area; r = radius of container

Use the equation to calculate the volume:

$$volume = A \times h \qquad (5.2)$$

Where A = area of container; h = height of container

In the subsequent flux calculations, the container volume is in litres. Therefore to obtain the container volume in *L*, convert for example from m^3 using:

$$1\,L = 0.001\,m^3$$

In addition to the volume of chamber the "dead volume" has to be considered:

The “dead volume” results from the volume of the tubing between analyser and measurement chamber, and of the volume of the measurement cell of the analyser. In general, the volume of the measurement chamber should be much larger than the “dead volume”. If this is the case, the dead volume is negligible. On the other hand, if this is not the case, the dead volume should be added to the measurement chamber volume.

5.6.2. Calculating the flux of CO_2-C.

Calculate the rate of change of CO_2 concentration per hour (ppm h^{-1}) as:

$$CO_{2ra} = \frac{\sum_{i=1}^{n}(t_i - t_{av})(CO_{2i} - CO_{2av})}{\sum_{i=1}^{n}(t_i - t_{av})^2} \tag{5.3}$$

Where CO_{2rate} = slope of the linear regression line of CO_2 concentration versus time over the whole measurement time, CO_{2i} = the CO2 concentration at time *i*, CO_{2av} = the average CO_2 ppm over the whole measurement time; t_i = the time point i; t_{av} = the average time calculated as the sum of all t_i divided by the number of time points

Excel will calculate this slope very easily, as explained below in the procedure.

5.6.3. Calculating the flux of CO_2-C per area.

Calculate the amount of carbon flux emitted per area per hour (µg m^{-2} h^{-1}) as:

$$C_{flux} = \frac{(CO_{2rate} \times Vol \times 12.01)}{MV \times A} \tag{5.4}$$

Where C_{flux} = the amount of carbon flux emitted per area per hour (µg m^{-2} h^{-1}); CO_{2rate} = the rate of CO_2 flux (ppm h^{-1}); Vol held by container (and dead volume if non-negligible) (L); 12.01 g mol^{-1}= molar mass of C; MV = molar volume (L mol^{-1} of C = 24.79); A = area of CO_2 exchange (m^2)

5.6.4. Calculating the Keeling Plot intercept.

A Keeling plot is a plot of the $\delta^{13}C$ (on the Y axis) against the inverse of the CO_2 concentration (on the X axis). The intercept of the regression line of these two represents the $\delta^{13}C$ signature of the source (eg. respiration) in absence of dilution from atmospheric CO_2. The Keeling plot intercept ($\delta^{13}C_{source}$) is calculated based on the equations:

$$\partial^{13}C_s = \frac{\sum_{i=1}^{n}\left(\frac{1}{C_i} - \left(\frac{1}{C}\right)_{av}\right)\left(\partial^{13}C_i - \partial^{13}C_{av}\right)}{\sum_{i=1}^{n}\left(\frac{1}{C_i} - \left(\frac{1}{C}\right)_{av}\right)^2} \tag{5.5}$$

and

$$C_a = C_b + C_s \tag{5.6}$$

Where C_i = measured CO_2 ppm at timepoint i; $(1/C)_{av}$ = the average value of $1/CO_2$ ppm over the measurement time; C_s = CO_2 ppm produced by source, $\delta^{13}C_i$ the isotopic signature at time *i*, and $\delta^{13}C_{av}$ the average isotopic signature [4 and 5].

6. CASE STUDY: MEASURING CARBON LOSS OF MAIZE CROP RESIDUES APPLIED IN INCUBATED FRESH, REVIVED AND STORED SOILS

6.1. BACKGROUND

Carbon-13 (^{13}C) labelled plant material can be used to trace cycling of plant-derived carbon in numerous systems. For example, in agricultural systems, ^{13}C-labelled plant material can be used to measure storage of crop residue-derived carbon to soils in the form of soil organic carbon as well as to measure loss of crop residue carbon to the atmosphere in the form of CO_2. Often, incubation experiments are used to determine such soil carbon losses. Estimates of soil carbon losses derived from incubation experiments are scaled up to estimate ecosystem level amounts of carbon loss. As a demonstration, we use ^{13}C-labelled maize plant material (leaf and stem material) to estimate carbon loss as CO_2 from plant material and soil over 70 days in a soil incubation study.

Here, a soil incubation study has been conducted to estimate carbon loss from maize residues (mulch) applied to soils. Mulch loss is different on freshly collected soils, revived soils and stored soils. Although freshly collected soils should be used incubation studies in order to more closely represent field soils, this is often not feasible. Therefore, many studies need to revive stored soils by pre-incubating them after wetting prior to the actual experiment to avoid the flush of microbial activity that is stimulated by re-wetting dried stored soils [6 and 7]. Omitting this pre-incubation step leads to an overestimation of carbon loss due to the artificial flush of microbial activity that occurs when rewetting stored soils. In this experiment we revived an Austrian agricultural soil with water to verify whether soil incubation studies using revived soils can accurately estimate the amount of carbon lost from maize residues added to soils. To this end, CO_2 flux from revived as well as fresh and stored soils with added maize residues were compared in this experimental set up.

6.2. MEASURING SOIL AND MULCH CARBON LOSS

Because the laser CO_2 carbon isotope analyser measures both the concentration of CO_2 and its δ^{13}C value in sampled gas, it can be used to measure the amount of carbon respired from soil and determine its source when carbon sources have distinct isotopic signatures. We used soil from an agricultural site that has a δ^{13}C value of -27 to -28.5‰ and a ^{13}C-labelled maize material with a δ^{13}C of 400‰ as mulch (labelled maize was acquired from the Soil and Water Management & Crop Nutrition Laboratory of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture). If the δ^{13}C values of the sources are known, measuring the concentration and δ^{13}C value of CO_2 produced in incubated soils can be used to calculate the contribution of both carbon sources to the flux of CO_2.

All soils were collected from an Austrian Cambisol (from 0-15 cm soil depth) and sieved to 2 mm. Stored soils were dried and stored for 5 months. A portion of the stored soils was first revived for the soil incubation study by restoring soils to 20% gravimetric water content (with a spray bottle to ensure even water distribution) and placing it in jars (5.6 cm diameter, 8 cm height) to a height of 5 cm and a bulk density of 1.31 g cm^{-3}. These soils were loosely covered to allow for air-flow and were kept in a dark area at room temperature for 15 days prior to beginning the incubation study. Fresh soils were collected within 48 hours before beginning the study and were transported on ice, back to the laboratory and stored at 4°C prior to being placed in incubation jars. In the laboratory fresh soils were confirmed to also be at 20% gravimetric water content and stored soils were also raised to the same gravimetric water content as fresh and revived soils. Stored and fresh soils were then also filled in jars using the same method previously discussed. In total, three replicates each of fresh, revived and stored soil jars were prepared.

The day after all soil jars were prepared, bare soil CO_2 flux was measured in a closed-loop for each jar for five minutes by placing each jar in a 1 L container (9 cm diameter, 17 cm high) with an airtight lid and two ports connected to the inlet and outlet tubes of the CO_2 carbon isotope analyser. The CO_2 carbon isotope analyser was set to continuous flow mode. Over 5 cm of headspace was present between the top of the 1 L container and the top of the soil incubation jar, thus ensuring proper mixing of air within the container for CO_2 sampling (Figure 5.). To avoid sample carry over, the analyser was purged with ambient air for two minutes in between each sample measurement.

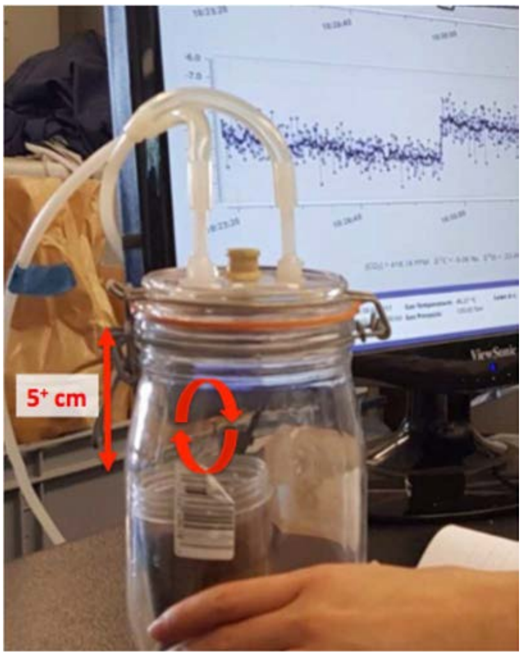


Figure 5. *A soil incubation jar within a sealed 1 L container connected to the inlet and outlet tubing of the laser CO_2 Carbon Isotope Analyser.*

Once CO_2 measurements of bare soils were completed, 0.21 g of ^{13}C-labelled maize mulch was added to each jar (a mulch application equivalent to 0.87 t ha^{-1}). Soil moisture was monitored based on the mass loss of each soil jar and restored with a spray water bottle three times a week. CO_2 measurements were always taken a day after water was applied to avoid measuring the pulse of CO_2 that occurs when water is applied to soil. Soil CO_2 measurements were again taken 1, 4, 7, 13, 15, 20, 30, 40, 54, 70 and 85 days after mulch application using the laser CO_2 carbon isotope analyser.

Data collected from measurements on the CO_2 carbon isotope analyser was processed in Excel and the flux of CO_2 ($\mu g\ m^{-2}\ h^{-1}$) and the Keeling plot intercept ($\delta^{13}C_{source}$) for each sampled jar was calculated. Graphical representation of analysed data and statistical analysis was generated using JMP 13 software (SAS, Cary, North Carolina, USA). A two-source mixing model was applied to determine the proportion of $\delta^{13}C_{source}$ that was derived from mulch as:

$$f_{13C_{mulch}} = \frac{\partial^{13}C_{source} - \partial^{13}C_{soil}}{\partial^{13}C_{mulch} - \partial^{13}C_{soil}} \times 100\% \qquad (6.1)$$

where $f_{13\mathrm{Cmulch}}$ is the ^{13}C proportion of $\delta^{13}C_{source}$ that was derived from mulch, $\delta^{13}C_{source}$ is the Keeling plot intercept and estimated δ^{13}C of the source of sampled CO_2, $\delta^{13}C_{soil}$ is the δ^{13}C of soil and $\delta^{13}C_{mulch}$ is the δ^{13}C of mulch. This model assumes that the source of CO_2 produced by sampled soil jars is exclusively from soil and mulch.

Because the proportion of ^{13}C derived from mulch in the $\delta^{13}C_{source}$ is equal to the proportion of carbon derived from mulch, $f_{13\mathrm{Cmulch}}$ can then be used to calculate both the proportion of C from mulch and soil in the measured CO_2 since:

$$f_{13C_{mulch}} = f_{C_{mulch}} \qquad (6.2)$$

and

$$f_{C_{mulch}} + f_{C_{soil}} = 1 \qquad (6.3)$$

where $f_{13\mathrm{Cmulch}}$ is the ^{13}C proportion of $\delta^{13}C_{source}$ that was derived from mulch, f_{Cmulch} is the proportion of C from mulch that contributed to measured CO_2, and f_{Csoil} is the proportion of C from soil that contributed to measured CO_2. This model likewise assumes that the source of CO_2 produced by sampled soil jars is exclusively from soil and mulch.

6.3. RESULTS AND DISCUSSION

Peak CO_2 loss for fresh and revived soils occurred between 4 to 7 days after mulch application and carbon loss ranged from 74.5 to 99.0 mg of C m^{-2} h^{-1} (Figure 6.). Stored soils had the largest rate of CO_2 loss on the day they were rewetted (163.6 mg of C m^{-2} h^{-1}) and respiration rates continually declined, even after mulch application. Thus, soil response to mulch was dwarfed by the large response of stored soil to rewetting. Although reviving stored soils with water did restore CO_2 trends (in response to mulch) similar to fresh soils, 9 to 47 mg of C m^{-2} h^{-1} more carbon was consistently lost from fresh than from revived soils. However, this difference was not statistically significant and only stored soils differed from fresh ($p = 0.04$) and revived soils ($p < 0.01$) in across time. For all soil types, rates of CO_2 loss plateau between 70 to 85 days after mulch application and carbon loss ranged from 11 to 23 mg of C m^{-2} h^{-1}.

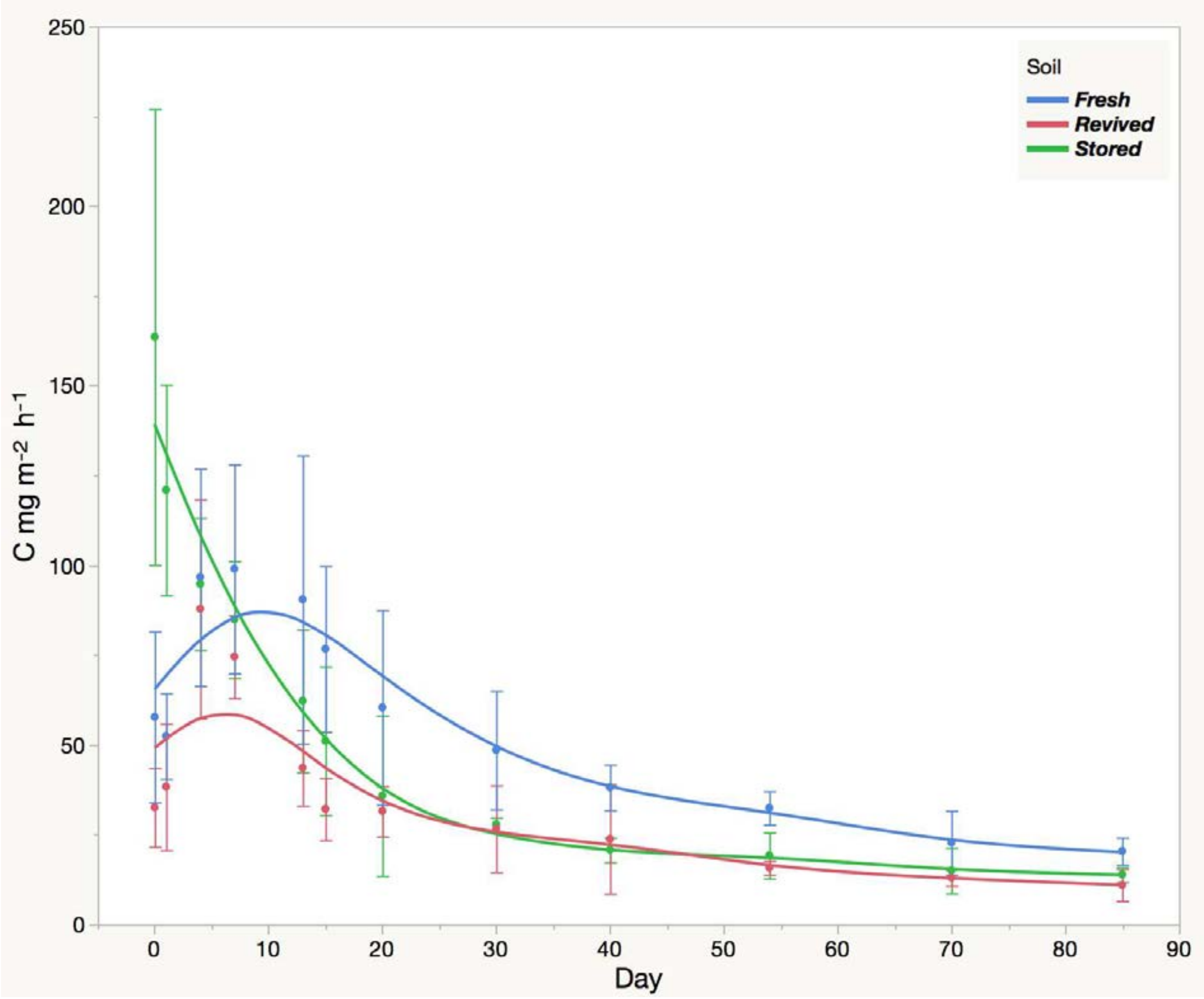


Figure 6. *Rate of carbon loss from soil incubation jars (in the form of CO_2) before mulch application (0 d) and after mulch application (1-85 d) for fresh, revived and stored soils. Each line represents the mean trend (and standard error with a 95% confidence interval) of three replicates per soil type and each point represents an individual jar measurement.*

The strength of the $\delta^{13}C$ signal in sampled CO_2 that was derived from ^{13}C-labelled mulch (Figure 7.) was similar to the trend in the rate of CO_2 loss over time (Figure 6.), with the highest $\delta^{13}C_{source}$ occurring 4 to 7 days after mulch application. This indicates that the peak in soil CO_2 flux observed on 4 to 7 days is due to the mulch application and loss of mulch-derived carbon. Unlike the CO_2 flux that varied between stored soils and the fresh and revived soils, all three soil types displayed a similar trend in $\delta^{13}C_{source}$ and there was no significant difference by soil type over time. Four days after mulch application $\delta^{13}C_{source}$ ranged from 172.3 to 294.5 and on day 7 it ranged from 169.7 to 238.3. No $\delta^{13}C_{source}$ data for day 85 is available as the 5 minutes closed-loop measurement was too short to generate enough CO_2 build up, thus causing standard deviation of measured $\delta^{13}CO_2$ to be too high and the accuracy of measurement to be too low.

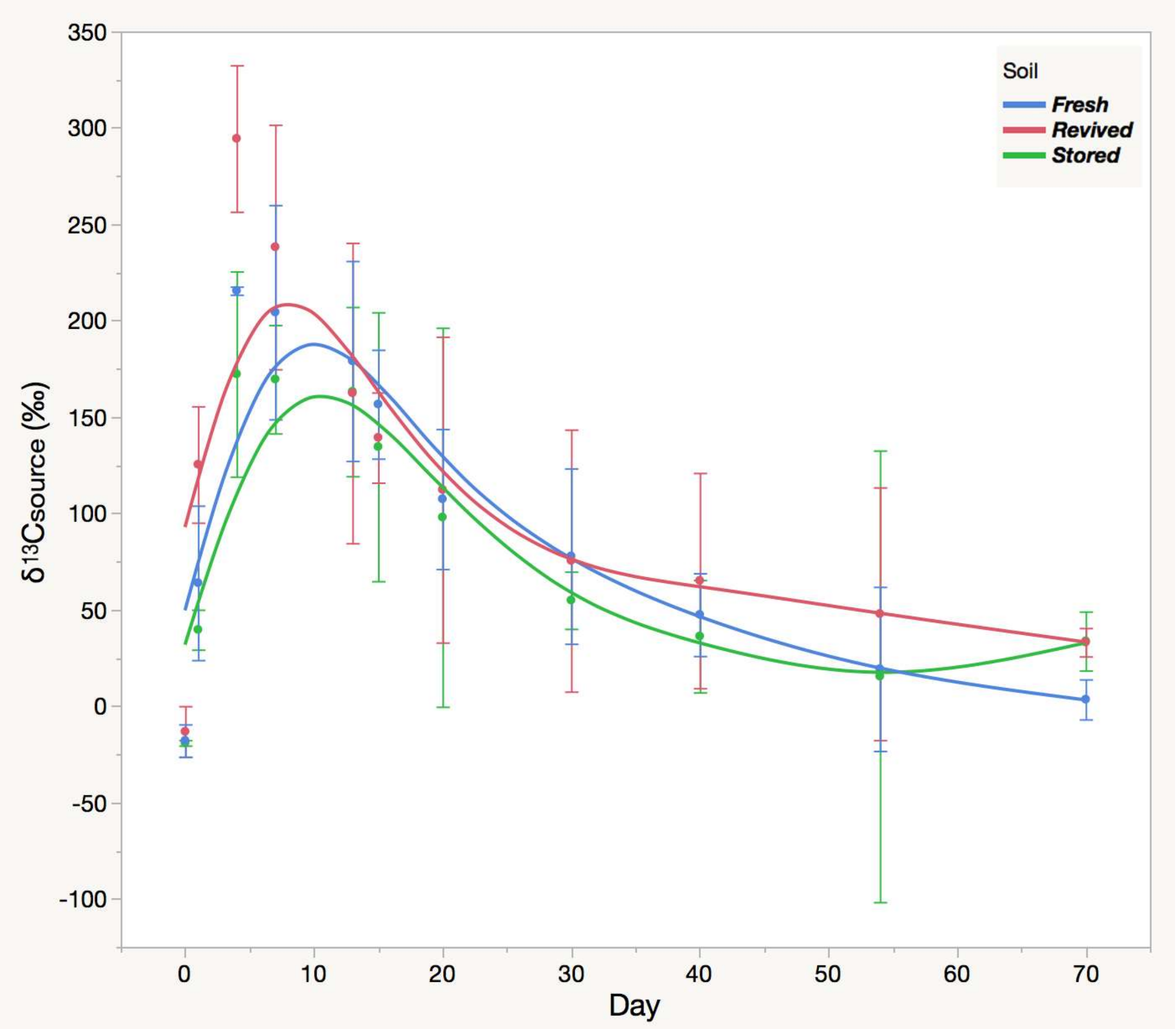


Figure 7. *Keeling plot intercept ($\delta^{13}C_{source}$) of CO_2 from soil incubation jars before mulch application (0 d) and after mulch application (1-85 d) for fresh, revived and stored soils. Each line represents the mean trend (and standard error with a 95% confidence interval) of three replicates per soil type and each point represents an individual jar measurement.*

A two-source mixing model was applied to determine the proportion of carbon derived from mulch. Four days after mulch application, on average 60% of the carbon was determined to be produced by mulch, while 7 days after application this proportion was on average 54%. As no significant difference in the mulch contributions could be observed between the soil pre-treatments, results were combined to illustrate varying contributions to the CO_2 losses by mulch and soil (Figure 8.) over time. From this, it can be concluded that between days 4 to 7 after mulch application mulch accounts for just as much CO_2 production as soil but that the days before and after that period most of the CO_2 is derived from soil (Figure 8.). For the stored soils that did not display a peak in CO_2 flux on days 4 to 7 (Figure. 6.), $\delta^{13}C_{source}$ indicates that the decrease in CO_2 flux on days 4 to 7 relative to days 0 and 1 (Figure 7.) is not as large as it would be if mulch did not contribute to CO_2 emissions.

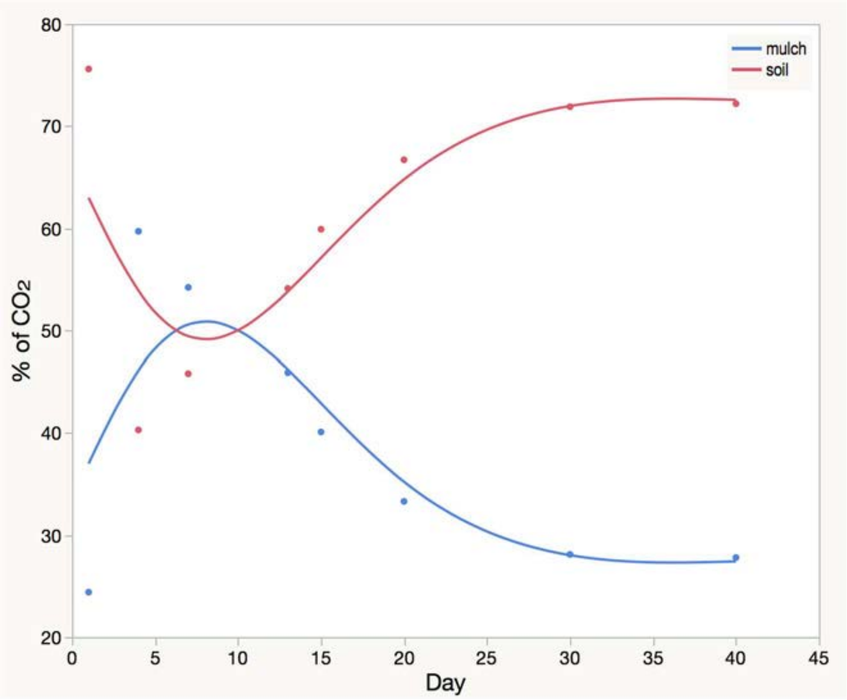


Figure 8. *Average percent of CO_2 derived from soil and mulch from soil incubation jars after mulch application (1-85 d) for fresh, revived and stored soils. Each line represents the mean trend of three replicates per soil type and each point represents an individual jar measurement.*

In conclusion, it is demonstrated that (i) the laser isotope CO_2 analyser can be used effectively to monitor CO_2 emissions and their source from incubated, amended soils and that (ii) a stored Austrian agricultural soil can be revived for an incubation study with the aim to measure soil CO_2 fluxes in response to maize mulch application. Further, it is confirmed that the soil CO_2 flux in response to mulch amendment is relatively fast and occurs within the first month of application in these incubated Austrian agricultural soils.

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