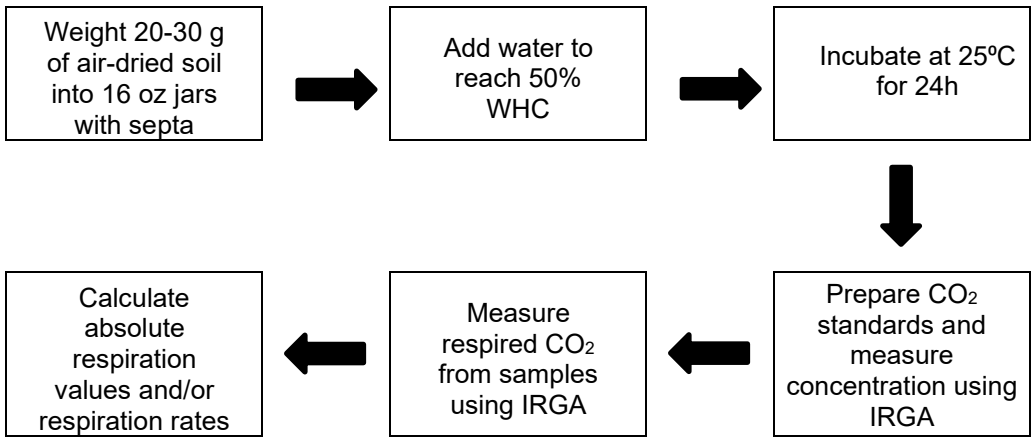
SOP: **Quantification of Soil Respiration by Infrared Gas Analyzer (IRGA)**

Overview:

This standard operating procedure (SOP) describes a protocol for estimating soil respiration by quantifying the concentration of CO_2 evolved from air-dried soil after rewetting. The method was originally reported by Franzluebbers et al. (1996) and expanded by Franzluebbers et al. (2000). This method uses an Infrared Gas Analyzer (IRGA) unit to measure the concentration of CO_2 released from soil. Soils that are ground to pass a <2 mm sieve are typically used.



Safety:

All standard safety protocols and online safety training via UIUC Division of Research Safety (DRS) are required.
Personal protection (PPE) for this procedure include:
Hand Protection: Disposable nitrile gloves
Body Protection: Laboratory coat is recommended

Instrumentation & Consumables:

Sample preparation and incubation

- Analytical balance capable of weighing to two decimal places
- 16 oz wide-mouth glass jars (also known as Mason jars)
- Mason jar caps with rubber septa installed and sealed with silicone caulk
- 5 or 10 mL pipette and tips
- 18.2MΩ-cm water

- Incubator capable of maintaining 25ºC

CO_2 measurement

- Q-S151 CO_2 Analyzer (0-2000 ppm), i.e. Infrared Gas Analyzer (IRGA)
- Carrier gas tank (100% N_2 gas)
- CO_2 standard gas tank (1% CO_2, 99% N_2)
- Gas regulators
- 3 mL syringe + needle
- Glass exetainers for standards

Detailed Procedure:

I. Sample preparation and incubation

1. Label 16 oz mason jars with their corresponding sample ID's.
2. Weight 20-30 g (± 0.05) f air-dried soil into jars. 2-3 analytical replicates are recommended. Record exact weight of soil to at least two-decimal places (e.g. 20.00 g).
3. Place uncapped samples in a fume hood to ensure the initial level of CO_2 in each jar is uniform.
4. While keeping the samples under the fume hood, using a pipette add the corresponding amount of 18.2MΩ-cm water to reach 50% of each sample's water holding capacity (WHC). Water should be dispensed in a circular motion making sure to uniformly rewet all the surface of the soil (do not mix or disturb the soil afterwards). Record exact time when water was dispensed into each sample.
5. Careful not to breathe into the samples and introduce excess CO_2, cap the jars while still under the fume hood.
6. Place samples in an incubator at 25ºC for 24h-96h, or your determined length of incubation.

Note: Exact sample mass and incubation time may change depending on your individual incubation experiment approach. For instance, experiments aiming to measure other indicators post-incubation may require an increased soil mass. However, a minimum of 20.0 g should be used since it is crucial to cover at least 1.0 cm up from the bottom of the jar. Incubation times may range from 24h (e.g. short-term C mineralization) up to 28 days or more (e.g. long-term C mineralization). For more details on recommended time points for CO_2 measurement, refer to the table below.

Table 1. Potential incubation lengths and recommended time points for CO_2 measurement.

Length of incubation	Recommended time points for CO_2 measurement	Comments
24 h	24h	Higher variability amongst replicates than 4-day incubation. CO_2 may also be measured at 4h, 6h, 8h and/or 12h by discretion of the researcher. Measurements at 6h have been found to be sensitive to treatment effects (Wade et al., 2018).
4 days	24h, 2d, 3d, 4d	Recommended method for short-term C mineralization. CO_2 may also be measured at 4h, 6h, 8h and/or 12h by discretion of the researcher. Measurements at 6h have been found to be sensitive to treatment effects (Wade et al., 2018).
7 days	24h, 2d, 3d, 7d	CO_2 may be measured at either 3d or 4d depending on method. Measurements at 3d (recommended) have been found to be sensitive to treatment effects (Wade et al., 2018). However, other commonly used methods propose measurements of CO_2 to be made at 4d (Schindelbeck et al., 2016, CASH).
14 days	24h, 2d, 3d, 7d, 14d	
28 days	24h, 2d, 3d, 7d, 14d, 21d, 28d	

II. CO_2 measurement

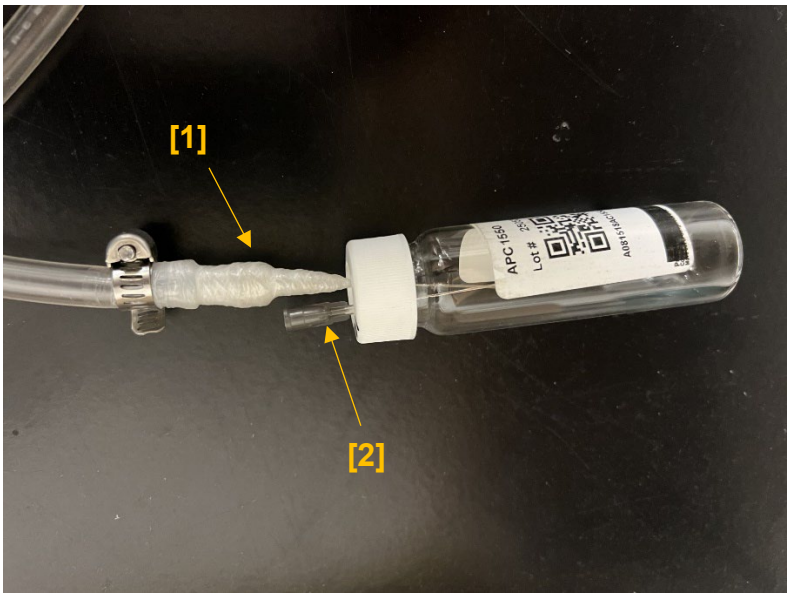
1. **Preparing the IRGA**
   - 1.1. Turn on the Q-S151 CO_2 analyzer by pressing the power button (upper left). A maximum number of 1 will appear on the screen.
   - 1.2. Turn on the gas tank containing the N_2 carrier gas and set the gas flow to 300 mL/min.
   - 1.3. Wait until the reading on the display of the analyzer goes down to zero and stabilizes (e.g. warm up) before continuing with the next steps.

Note: The analyzer may take at least 1 hour to warm up, so make sure you turn the analyzer on at about 1.5 hours before the scheduled time to measure your samples. This will allow enough room for instrument warm up and standard preparation.

2. **Standard preparation and measurement**

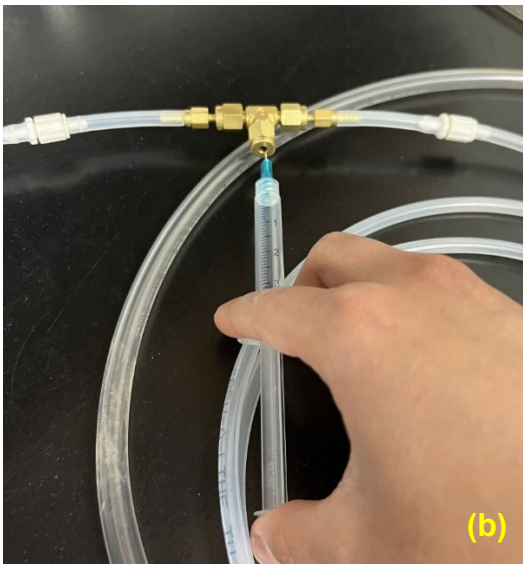
2.1. Turn on the CO_2 tank (1000 ppm) and set outflow pressure to 10-20 PSI using the regulator.

2.2. Insert the outflow needle+tube [1] from the CO_2 tank into a glass vial, then insert a secondary needle [2] to allow gas flow. Let the CO_2 gas flow for at least 5-10 minutes.



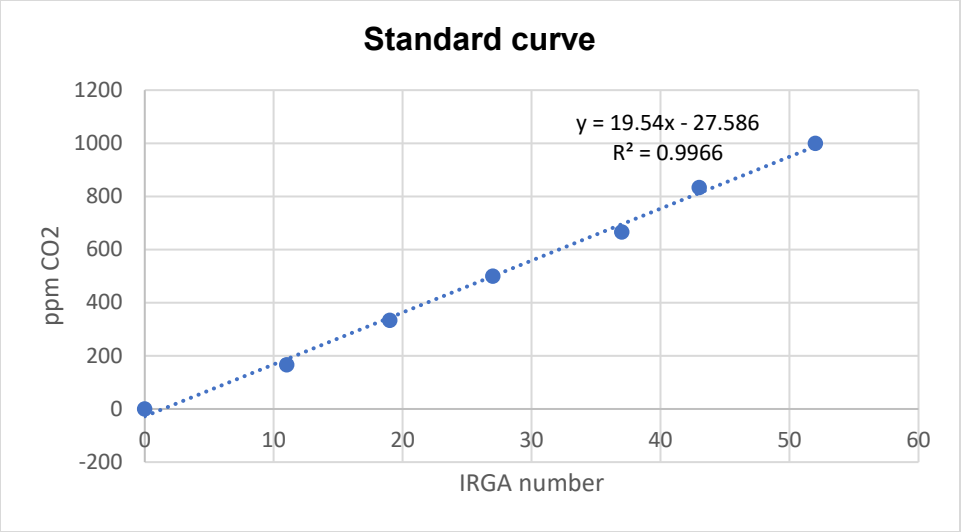
2.3. Prepare the CO_2 standards based on the table below. While CO_2 is still flowing, insert the 3 mL syringe into the glass vial septa and draw the appropriate volume of CO_2 gas (a). Then quickly, insert the syringe into the main injection port and quickly inject the gas (b). Record the maximum number shown in the IRGA screen (e.g. IRGA reading).

Standard (ppm)	**Volume of CO_2 gas (mL)**
166.7	0.5
333.3	1.0
500.0	1.5
666.7	2.0
833.3	2.5
1000.0	3.0



2.5. Fit a standard curve using the IRGA readings and known CO_2 standard concentrations in ppm. An acceptable R^2 is ≥ 0.98, but R^2 ≥ 0.99 is preferred.

IRGA reading	**ppm CO_2**
0	0
11	166.7
19	333.3
27	500.0
37	666.7
43	833.3
52	1000.0



Note: As N_2 is used as a carrier gas it has a dilution effect on the reading of CO_2 shown in the display of the analyzer (e.g. 1000 ppm = ~52 read), relative to the reading when performing a calibration of the instrument (e.g. 1000 ppm = ~1000 read) in which there is a continuous flow of pure CO_2. Hence, a standard curve (e.g. correction curve) of varying concentrations is needed to estimate the concentration of CO_2 in samples.

Note: Check the background air for a quick validation of the standard curves. Draw air at the hallway outside of S-23; it should range 405-440 ppm. Do not use the air inside of the lab as it is inconsistently higher in CO_2.

3. **CO_2 measurement of samples**
   - 3.1. Remove the samples from the incubator shortly before measurement and record the exact time when samples were removed. Samples should be read as close to the time points pre-defined by your experiment. For example, when performing a 24h incubation, samples should be read as close to the 24h mark after adding water into the sample (hence the recording of time when water was added).
   - 3.2. Insert 3 mL syringe into septa in lid of soil sample. Pump syringe 5 times to thoroughly mix air inside the jar.
   - 3.3. Draw 3 mL of air from the sample and inject it into the IRGA at the minute mark of the predetermined time of measurement (e.g. 24h, 48h, 3d, etc.). Record the maximum reading and the exact time (hours:minutes, AM/PM) of measurement per sample.

Note: If the reading exceeds the maximum reading of the standard curve (e.g. sample exceeds 2000 ppm CO_2), the sample must be diluted until its reading fits within the standard curve. To dilute the sample, less volume should be injected into the analyzer. For example, a 3x dilution can be done by injecting 1 mL of sample only. Or if needed, a 6x dilution can be done by injecting 0.5 mL of

sample. Make sure to record the dilution fold used, to correct for the original CO_2 concentration.

3.4. Wait for the reading on the screen to return to zero and repeat steps above with the next sample. Make sure to flush the syringe with ambient air between each sample (by drawing and releasing ambient air 3-5 times).

Note: If the incubation runs longer than 24h or the CO_2 respired will be measured at several points in time, after finishing the first measurement:

Set up samples to continue incubation (as needed):

[1] de-gas the samples by opening the jars and placing them under a fume hood for several minutes. Then cap them and place them back in the incubator, and

[2] repeat **ALL** steps from the CO_2 measurement section (II) above when performing a new measurement (including making a new standard curve)

III. Clean up

1. Turn off the carrier (N_2) and standard (CO_2) tanks, making sure to release the pressure.
2. Turn off the IRGA analyzer (use power button).
3. If recording sample and standard readings and times using Excel, make sure to manually save the file or use the autosave option (this is safer).

IV. Calculations

Measurement of C mineralization are usually expressed in units of C respired per kilogram of dry soil (mg C kg soil^{-1}) or as rate per day (mg C kg soil^{-1} day^{-1}). To calculate, see corresponding Excel calculation template.

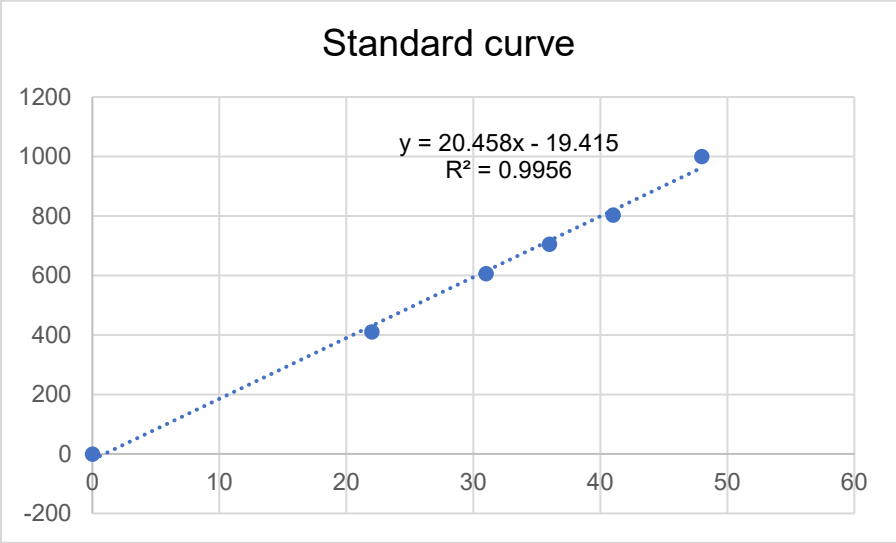
1. Convert the IRGA number measurements to CO_2 concentrations in ppm using the equation of the standard curve and the proper dilution folds.
2. Convert the adjusted ppm CO_2 (which is on a volume basis) to µg CO_2-C per L headspace with the below equation (ideal gas law).

$$Cm\left(\frac{\mu g\ CO_2 - C}{L\ headspace}\right) = \frac{Cv \times M \times P}{R \times T}$$

Where:
Cv = Adjusted CO_2 ppm resulting from subtracting 410 (air ppm) from sample concentration obtained from curve
M = molecular weight of C (12 µg/µmol)
P = barometric pressure (1 atm)
R = universal gas constant (0.0820575 L × atm / K × mole)
T = incubation temperature in ºK (273.15 + ºC)

3. Convert μg CO_2-C L headspace^{-1} to μg CO_2-C g soil^{-1}
   3.1. Multiply μg CO_2-C L headspace^{-1} by the volume of headspace in the sample (L) and divide by the mass of the sample used in the incubation. This is equivalent to μg CO_2-C g soil^{-1}, or the more commonly reported mg CO_2-C kg soil^{-1}.
4. To convert to a rate (e.g. mg CO_2-C kg soil^{-1} day^{-1}), divide by the number of days incubated after the last CO_2 measurement event (e.g. number of days in incubation where the samples were completely sealed and CO_2 was accumulating after the last de-gasing). For example, if CO_2 was measured at 24h and 96h, at 96h there are 2 days after the last de-gasing event. Therefore, the absolute respiration values at 96h have to be divided by 2 to determine the respiration rate in mg/kg per day.

Example calculation:



IRGA number = 48
Dilution = 1
[CO_2 ppm] = (20.458(48) – 19.415) × 1 = 963 ppm
CO_2 from air = 410 ppm
Adjusted CO_2 concentration (Cv) = 963 – 410 = 553 ppm
M = 12 μg/μmol
R = 0.0820575
P = 1 atm
T = 298.15 ºK

$$Cm\left(\frac{\mu g\ CO_2 - C}{L\ headspace}\right) = \frac{553 \times 12\ \times 1}{0.0820575\ \times\ 298.15} = \mathbf{271.24}$$

Headspace volume = 0.4545 L

Soil mass = 30.0 g

$$\text{Respiration } (\text{mg } CO_2 - \text{C kg soil}^{-1}) = \frac{Cm \times headspace\ vol}{soil\ mass} = \frac{271.24 \times 0.4545}{30} = \mathbf{4.11}$$

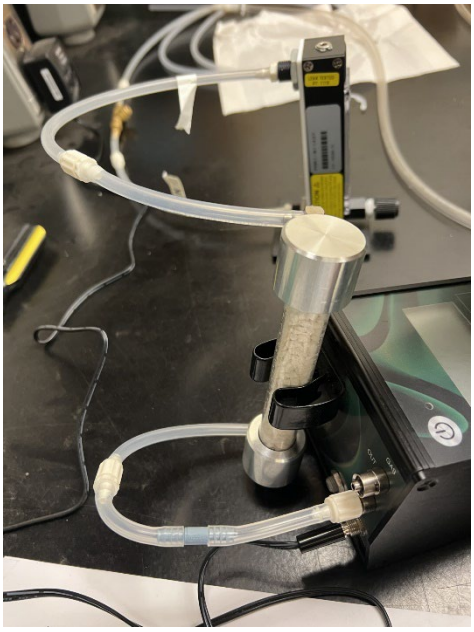
At 24h:

$$\text{Respiration rate } (\text{mg } CO_2 - \text{C kg soil}^{-1}\ day^{-1}) = \frac{Respiration}{Days\ incubated} = \frac{4.10}{1} = \mathbf{4.11}$$

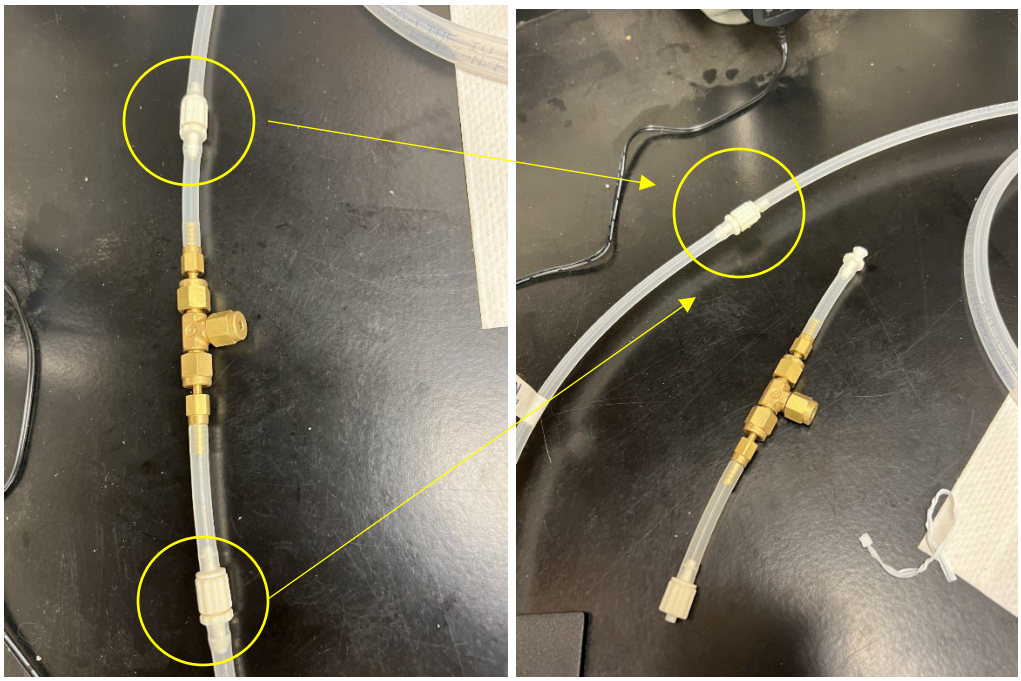
V. Calibrating the instrument

The CO_2 analyzer may drift as much as 100 ppm per year. Regular calibration checks and re-calibration are recommended, specially after long periods without use (e.g. >3-4 weeks). The analyzer output is linear, therefore 2 points are sufficient for calibration checks: zero CO_2 (0 ppm) and a standard CO_2 concentration (1000 ppm, from the CO_2 tank). To calibrate the instrument:

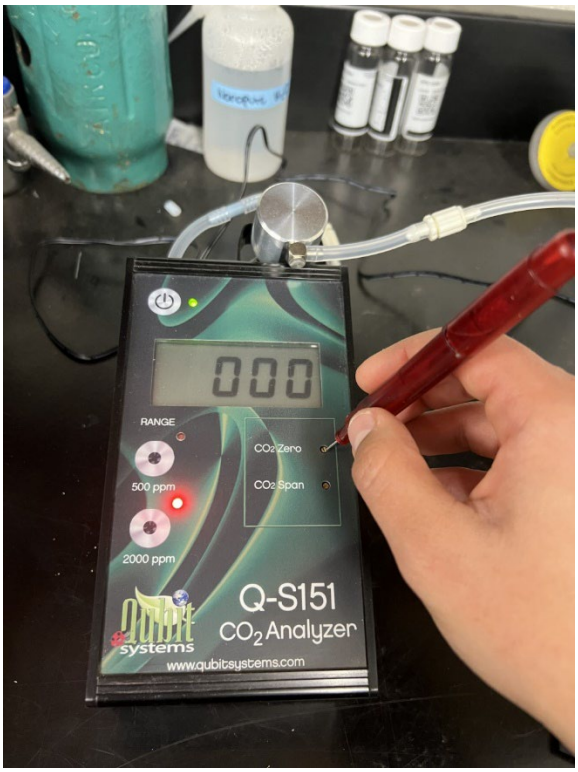
1. While the instrument is on and warmed up, turn off the N_2 gas inflow by closing the tank valve. Wait for the flow rate to go down to 0 mL/min.
2. Replace the drying tube on the back of the analyzer with the soda lime column (white). The column can be found at the supplies drawer underneath the analyzer.



3. Remove the main injection port and connect both ends to allow for continuous gas flow.



4. Turn on the N_2 gas tank and set the inflow rate at 300 mL/min. Wait 1-2 minutes. A reading = 000 or close should appear on the screen.
5. If the reading on the display of the analyzer does not read “000”, rotate the **CO_2 Zero** screw as needed until the reading = 000.



Note: If the zero reading is highly out of range (>40 ppm) or if rotating the CO_2 Zero does not work, the Coarse Zero adjustment on the back of the analyzer can be used to bring zero within range. Use the Coarse Zero with caution since very small adjustments result in large changes and there is delay in response to changes in Coarse Zero.

6. Once the zero CO_2 point has been calibrated, turn off again the N_2 gas inflow by closing the tank valve. Wait for the flow rate to go down to 0 mL/min.
7. Replace the soda lime column on the back of the analyzer with the drying tube. (Note: the soda lime column should be used to calibrate the Zero CO_2 point only. Not for standards and not for samples).
8. Disconnect the hose from the valve of the N_2 tank and connect it to the valve of the CO_2 tank.

9. Turn on the CO_2 gas tank and set the inflow rate at 300 mL/min. Wait 1-2 minutes. A reading = 1000 or close should appear on the screen.
10. If the reading on the display of the analyzer does not read “1000”, rotate the CO_2 Span screw as needed until the reading = 1000.
11. Once the standard 1000 ppm CO_2 point has been calibrated, turn off the CO_2 gas inflow by closing the tank valve. Wait for the flow rate to go down

to 0 mL/min. Then disconnect the hose from the valve of the CO_2 tank and connect it to the valve of the N_2 tank, as original.

12. Turn on the N_2 gas tank set the inflow rate at 300 mL/min. Wait 1-2 minutes. The reading should be = 000. If not zero, repeat calibration steps 1-5 until the zero CO_2 point reads = 000.
13. Once calibration is done, turn off any gas inflow and reconnect the main injection port.

References:

Franzluebbers, A.J., R.L. Haney, F.M. Hons, and D.A. Zuberer. 1996a. Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. Soil Sci. Soc. Am. J. 60:1133–1139.

Franzluebbers, A., R. Haney, C. Honeycutt, H. Schomberg, and F. Hons. 2000. Flush of carbon dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J. 64:613–623.

Wade, J., Culman, S.W., Hurisso, T.T., Miller, R.O., Baker, L., Horwath, W.R. 2018. Sources of Variability that Compromise Mineralizable Carbon as a Soil Health Indicator. Soil Sci. Soc. Am. J. 82:243-252.

Schindelbeck, R.R., B.N. Moebius-Clune, D.J. Moebius-Clune, K.S. Kurtz and H.M. van Es. 2016. Cornell University Comprehensive Assessment of Soil Health Laboratory Standard Operating Procedures, Available https://cpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/f/5772/files/2015/03/CASH-Standard-Operating-Procedures-030217final-u8hmwf.pdf (Verified 19 June 2018).

Suggested reading:

Wade, J., Culman, S.W., Hurisso, T.T., Miller, R.O., Baker, L., Horwath, W.R. 2018. Sources of Variability that Compromise Mineralizable Carbon as a Soil Health Indicator. Soil Sci. Soc. Am. J. 82:243-252.

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