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Measuring Soil C Mineralization Rates

Basic Idea:

A wide variety of experiments require the measurement of soil C mineralization rates (otherwise known as microbial respiration rates or microbial CO_2 production). The basic method is as follows: put a known weight of soil in a gas-tight vessel, seal the vessel, measure (or infer) initial headspace CO_2 concentrations, incubate the soil at constant temperature for a given period of time, measure headspace CO_2 concentrations at the end of the incubation. While that is the basic idea, choosing a specific protocol for your specific experiment can be a tad more difficult. Below I have outlined a basic static incubation protocol followed by some notes for customizing the protocol to fit your specific needs. C mineralization rates are generally reported in one of the following manners: μg C-CO_2/ g soil/ h, μg C-CO_2/g soil C/ h, or mg C-CO_2/ g soil / day.

Basic Protocol:

- Weigh 5 – 10g soil into a 50mL plastic centrifuge tube. Seal the tube with a gas tight lid equipped with a rubber O-ring and a rubber septum. Let the sample equilibrate at the chosen incubation temperature for 15 –30 minutes.
- Take an initial (T0) headspace gas sample. Pull 5 mL of CO_2-free air (air scrubbed through soda lime) into a glass syringe. Attach a hypodermic needle to the syringe and puncture the septum of the 50 mL centrifuge tube. Inject the CO_2-free air, pump the syringe 3 – 4 times, and withdraw 5mL of headspace air. Run the headspace air on an IRGA equipped for static CO_2 measurements (see diagram). Record time of sampling and CO_2 concentrations (in ppm).
- Take a second headspace gas sample 2 – 24 h (see below for a discussion of incubation times) after the initial sampling, using the exact same technique used to collect the T0 sample.
- Calculate C mineralization rates, change in headspace CO_2 concentrations (in μg C-CO_2 or mg C-CO_2) per gram soil (dry wt. equivalent) or per gram soil C, per unit incubation time (hour or day). Use the universal gas law to convert ppm CO_2 (μmol CO_2/ mol air) to μg C-CO_2, as follows:

$$n = \frac{PV}{RT}$$

where n = mol air in vessel
V = volume of vessel (mL)

P = 1 atm
R = 82.05 mL atm/ mol K
T = temp in K = 273 + ^{o}C

So, a 50mL vessel at 20°C contains 2.08 mmol air.

To calculate μg $C\text{-}CO_2$:

$$\mu g\ C\text{-}CO_2 = mmol\ air * ppm\ CO_2(\mu mol\ C/\ mol\ air) * 10^{-3}\ mol/mmol * 12\ \mu gC/\mu molC$$

So, a 50mL vessel at 20°C with 5000 ppm CO_2 contains 125 μg $C\text{-}CO_2$. Figure 1 shows the conversion factors to calculate μg $C\text{-}CO_2$ for a number of vessels of different volumes.

Notes/Warnings:

- *Headspace CO_2 concentration.* The headspace CO_2 concentration at the end of the incubation (T1) is an important factor to consider when choosing incubation times and headspace:soil ratios. The final headspace CO_2 concentration is going to be a function of 3 things: headspace volume, soil C mineralization rates, and amount of soil in the vessel. You want the concentration of CO_2 in the headspace to stay below 2% (20,000ppm), CO_2 concentrations above 2% are likely to inhibit microbial activity. Likewise, if the headspace CO_2 concentrations are too close to ambient, it is difficult to accurately measure C mineralization rates as the T0 and T1 concentrations may be very close. As a rough approximation, aim to have CO_2 headspace concentrations at the end of the incubation that are somewhere between 1000 and 10,000 ppm CO_2. For soils with high C mineralization rates use either a larger headspace: soil ratio or shorter incubation times.
- *Continuous accumulation vs. "snap-shots".* If trying to measure respiration rates over an extended period of time (e.g. a month long C mineralization potential assay) you can leave your containers sealed for long periods or just measure them briefly (2-24 h, as described above). If you don't have leakage or excessive accumulation, you get integrated CO_2 production with a long incubation. With short incubations you need to interpolate between incubations periods.
- *Pre-injection*. In order to keep the pressure in the vessel at equilibrium, it is necessary to inject air into the vessel that is equal to the volume of headspace gas removed. One can either use ambient (lab) air or air that has been scrubbed with soda lime to remove much of the CO_2. Generally, CO_2-free air is the better choice for pre-injection since lab air CO_2 concentrations can vary significantly (especially with heavy breathing by the researcher). On the other hand, with a large headspace volume, the pre-injection volume is only a small portion of total headspace volume, so lab variability may not be significant compared to rapid CO_2 buildup. With slow respirations, use scrubbed air. Calculate the dilution due to pre-injection when calculating headspace CO_2 concentrations.
- *Leakage.* This can be a big problem. Check it out by "incubating" 3 empty containers that you breathed into before sealing. Treat them just like samples and measure at the same time. Dramatic decreases in [CO_2] will reveal leakage.
- *T0 measurements – necessary or extraneous?* One way to save time is to not measure headspace CO_2 concentrations at the start of the incubation (T0) and assume that CO_2 concentration inside the vessel equals the concentration of CO_2 in

the lab air at the time the vessel was sealed. This time-saving technique will only work if your final (T1) headspace CO_2 concentrations are quite high (in which case any error in T0 CO_2 estimation will have a negligible effect on the final calculation of C mineralization rates) and if you make sure the tubes are well ventilated in front of a fan or in a hood before sealing (also make sure not to breath directly into the vessels). When in doubt, measure T0 headspace CO_2 concentrations

Figure 1: Multiply ppm CO_2 detected by the factors below to get μg C-CO_2 in jar.

Temperature	0	5	10	15	20	25	30
Vessel Volume (mL)							
50	0.0268	0.0263	0.0258	0.0254	0.0250	0.0245	0.0241
55.58	0.0298	0.0292	0.0287	0.0282	0.0277	0.0273	0.0268
72	0.0386	0.0379	0.0372	0.0366	0.0359	0.0353	0.0348
233	0.1248	0.1226	0.1204	0.1183	0.1163	0.1144	0.1125
487	0.2609	0.2562	0.2517	0.2473	0.2431	0.2390	0.2351
974	0.5218	0.5124	0.5034	0.4946	0.4862	0.4780	0.4701
1920	1.0286	1.0101	0.9922	0.9750	0.9584	0.9423	0.9267