Supplement of:

Decomposability of soil organic matter over time: The Soil Incubation Database (SIDb, version 1.0) and guidance for incubation procedures

In the main manuscript, we focused on essential variables to measure and report for inclusion in the Soil Incubation Database (SIDb). Here, we focus on suggested variables to measure and methods to consider that go beyond what is required for inclusion in SIDb but are nevertheless important to consider prior to and during soil incubations, both to increase interpretability of results and to allow for inter-site comparions. Most of the recommendations made here are applicable for incubation studies using soils from any regions; however, some ecosystems may require reporting of additional variables.

Additional information on soil characteristics

As explained in the main manuscript Table 1), soil depth and soil carbon (C) are the two most important and necessary soil characteristics to report for incubated soil samples.

In addition, it can be useful to measure pH values in the soil solution on a regular basis during the incubation for the following reasons; first, if the soil pH is more neutral or basic, C mineralization might be confounded by high carbonate concentrations (Billings et al., 2004); second, pH values are known to change over the course of an incubation, thereby possibly affecting both the microbial community composition (Fierer et al., 2007) and the solubility of CO_2 in the soil water, which has to be considered when calculating CO_2 flux rates from incubation studies. Additionally, microbial growth and many microbial nutrient cycling processes (e.g. N mineralization, nitrification and denitrification), and mineral-organic associations are affected by pH (Kalbitz and Kaiser, 2008).

Another variable to consider, particularly for anaerobic incubations, are soil redox conditions as they can have an important effect on the oxidation state of mineralized soil C released (Knorr and Blodau, 2009; Peters and Conrad, 1996). Accurately measuring soil redox potential in an incubation can be difficult and costly due to large spatial heterogeneity of soil redox conditions, however obtaining this information is valuable when interpreting results in the context of the potential for a soil to produce methane. Anoxic processes can occur at redox potentials (Eh) between +300 to -300 mV (DeLaune and Reddy, 2005). At higher redox values, facultative anaerobic processes occur (e.g. NO_3^- reduction) followed by iron and sulfate reduction. Fermentation can occur over a wide range of redox values, and multi-step fermentation may be important in full mineralization under anoxic conditions (Megonigal et al., 2004). Before methanogenesis can occur, terminal electron acceptors higher on the redox ladder (e.g. NO_3^-, Fe(III), SO_4^{2-}) must be depleted (Peters and Conrad, 1996) and the redox potential must be sufficiently negative (DeLaune and Reddy, 2005). Observations of methanogenesis at higher redox potentials have been made (Peters and Conrad, 1996), which is likely due to micro-scale variability in soil redox potential (Sexstone et al., 1985). Thus, the activity of soil microorganisms in anaerobic incubations has a large effect on the redox potential, and therefore the gas flux, as microbes exhaust electron acceptors that yield more energy than methanogenesis, which then finally allows methanogenic conditions to occur (Peters and Conrad, 1996; Tveit et al., 2013).

Simple microbial parameters that are usually well correlated with heterotrophic respiration rates are microbial biomass and microbial stoichiometry (Čapek et al., 2015). Microbial biomass is often estimated by the chloroform-fumigation extraction method, which also allows measuring microbial C and/or nitrogen and thus allows stoichiometric analyses. Other measures of microbial biomass use biomarkers, such as DNA or phospholipid fatty acids to estimate microbial biomass or activity-based methods, such as the substrate-induced respiration method.

Incubation setup

Experimental incubation setups commonly consist of either incubation jars, such as mason jars fitted with Swagelok bulkhead fittings and gas-tight septa, or incubation vials with silicone septa, which contain the incubated soil and ensure an airtight headspace for analytical measurements. Incubation vessels should always be cleaned and sterilized prior to beginning the experiment. One consideration in selecting the size of the incubation jars or vials is the size of the sample and potential for CO_2 and/or CH_4 production; the headspace must be small enough to detect a change in gas concentrations but not so small that the headspace reaches levels of CO_2 beyond those expected in the field or under anoxic conditions.

Soil preparation includes the decision to homogenize soil via sieving or other methods, to remove roots, to maintain oxic or anoxic conditions, to optimize soil moisture, and whether to pre-incubate the soils. Drying and grinding of soils should be avoided as this leads to mineralization of C released from organisms killed by drying and rapid rehydration as well as potentially releasing C that was physically protected, e.g. via aggregation (Carter and Gregorich, 2007).

Avoiding disturbance effects caused by soil homogenization can be accomplished by cutting soil cores into sections and incubating each intact section. However, with intact cores, recently cut live roots remain in the soil and may artificially increase substrate availability and result in artifacts in the initial rates of CO_2 production. For anaerobic incubations, minimizing soil disturbance and exposure to oxygen during sampling, preparation and incubation is critical as exposure to oxygen can disrupt fermentation and methanogenesis, which can decrease the potential rates of CH_4 production (Nilsson and Öquist, 2013), at least during the initial stages of incubation. However, keeping soil cores and soil aggregates intact can result in anaerobic microsites within the soil during aerobic incubations (Knorr and Blodau, 2009), which may affect interpretation. Homogenizing soils disturbs the structure of soil aggregates and may eliminate the spatial disconnect between microbes and organic matter, thus leading to increased mineralization rates.

When examining long-term changes in aerobic C dynamics over time, it can be favorable to start out the incubation with multiple subsets of the same soil core in one jar and then repeatedly taking out one subset for detailed soil and microbial analysis over the course of incubation (there needs to be enough soil left for reliable flux measurements after samples are taken out for analysis). In summary, keeping soils intact without homogenizing prior to incubation will keep soils closer to field conditions but it will require a higher number of replicates to incubate as heterogeneity will be higher without homogenizing.

Pre-incubating soils is generally recommended as it reduces a confounding effect of high initial respiration rates that are due to a short-lived flush of respiration caused by sample collection and preparation even if soils are kept intact. Pre-incubation duration varies widely, from 0 to 180 days (Whitman et al., 2014). For studies in which soils are dried and rewetted, 2 to 9 days is typical for soils to return to basal respiration rates of undried controls (Hamer et al., 2007) but may not return to basal rates even after more than 50 days depending on soil texture (Chowdhury et al., 2011). Based on the literature and our personal experience, we recommend at least 4 days and preferably 7 days of preincubation, but caution that specific study questions may dictate shorter or longer preincubation periods.

Initial experimental setup requires recording jar weights of empty jars, determining soil moisture, and adding the prepared soil for incubation, and weighing and recording the soil plus jar weight. Using this weight as a benchmark and checking weekly to adjust soil moisture can ensure that soils do not become either waterlogged or water-stressed, both of which affect C fluxes. If anoxic conditions are desired, this step can be conducted in a glove box to ensure anoxia.

Flux measurements

The length and frequency of flux measurements are important for characterizing C fluxes in soil incubations. Aerobic respiration rates follow an exponential decline which requires frequent measurements at the beginning of the experiment to capture the depletion of fast cycling C. Generally, at the beginning of an incubation, measurements are taken at short intervals for the first few weeks (every 2-3 days) and then extended to weekly measurements. Depending on the length of incubation, later measurements can be taken monthly or even yearly as there is little change in respiration rates once fast cycling C has been respired. Flux values should be closely monitored and the frequency of measurements adjusted if needed. Frequent flux measurements are especially important to characterize the shape of the time series curve when using inverse modeling to separate soil C into fast and slower cycling pools as discussed earlier. Frequent measurements even after the initial decline in respiration rate improve the detection limit of small differences in the slower cycling C pools (Schädel et al., 2013). Before beginning an incubation, jars should be flushed to ensure ambient CO_2 concentrations.

The duration of anaerobic incubations needs to be sufficiently long enough to achieve favorable conditions for CH_4 production, which can be inhibited by availability of alternate electron acceptors (Knorr and Blodau, 2009) and by methanogen communities (Tveit et al., 2013). During anaerobic incubations, CO_2 fluxes should be measured alongside CH_4 fluxes.

Isotope Incubations

The C isotope composition (^{13}C and ^{14}C) of the bulk soil C pool as well as that of respired CO_2 and CH_4 reveals information on C dynamics and the age of soil C pools and of respired C

(Trumbore, 2000). In most cases, multiple measurements of the isotopic composition of respired gases are not possible during an incubation, however, radiocarbon measurements of the respired CO_2 at the end, or multiple measurements during the course of an incubation can provide valuable information on the age of the C being respired (Dioumaeva et al., 2002; Nowinski et al., 2010), giving insights into the decomposition of different C pool fractions over the course of an incubation. The isotopic signature (^{13}C and ^{14}C values) of respired CO_2 in combination with different soil fractions (Biasi et al., 2005; Crow et al., 2006; Czimczik and Trumbore, 2007; Dioumaeva et al., 2002; Dutta et al., 2006) can be useful to identify the C sources that contribute to total C efflux.

Radiocarbon incubations, where the ^{14}C of respired CO_2 is measured, are a common type of incubation designed to use the radiocarbon signature to trace the transit time of C in the soil (e.g. Nagy et al., 2018; Schuur and Trumbore, 2006) or to partition heterotrophic and autotrophic respiration, which have different signatures (Carbone et al., 2011; Hicks Pries et al., 2013; Schuur and Trumbore, 2006). They are commonly short-term, involving one single radiocarbon measurement made at the end of the incubation. In contrast, standard non-radiocarbon incubations are often longer. In the design of incubation experiments, there are opportunities to combine radiocarbon and time series incubations as complementary methods to address related questions of C pool structures and transit times. Studies using ^{14}C should report time series of CO_2 in addition to the total duration in supplemental information or a data repository. Long-term incubation studies should be aware of the benefit of adding multiple ^{14}C measurements to further constrain the source or dynamics of the C pool/s respired. Since long-term incubations should initially release C predominantly from faster pools and then from slower pools, this behavior could be confirmed with measurements of the ^{14}C content of the respired CO_2 at different times during incubation.

Laboratory setup of radiocarbon incubations is similar to common setups used for time series measurements, with some additional considerations. First and foremost, sufficient CO_2 must be accumulated in the headspace to make a radiocarbon measurement (typically ~0.5mg C), which leads to the build-up of relatively high CO_2 concentrations. When deciding how to standardize the duration of the incubation across samples, three criteria are commonly used: (1) total amount of time elapsed (days), (2) total amount of C respired (mg C), or (3) fraction of initial soil C respired. In all cases, the criterion used should be reported, and the % of C respired should be calculated and reported. Finally, although temperature and moisture should be highly controlled for time series incubations, the ^{14}C signature of the respired C is much less sensitive to temperature, as the same C is consumed by microbes independent of incubation temperature, merely at a faster rate under warmer conditions (Hicks Pries et al., 2017) .

References:

Biasi, C., Rusalimova, O., Meyer, H., Kaiser, C., Wanek, W., Barsukov, P., Junger, H. and Richter, A.: Temperature-dependent shift from labile to recalcitrant carbon sources of arctic heterotrophs, Rapid Communications in Mass Spectrometry, 19(11), 1401–1408, doi:10.1002/rcm.1911, 2005.

Billings, S. A., Schaeffer, S. M. and Evans, R. D.: Soil microbial activity and N availability with elevated CO2 in Mojave desert soils, Global Biogeochemical Cycles, 18(1), doi:10.1029/2003gb002137, 2004.

Čapek, P., Diáková, K., Dickopp, J.-E., Bárta, J., Wild, B., Schnecker, J., Alves, R. J. E., Aiglsdorfer, S., Guggenberger, G., Gentsch, N., Hugelius, G., Lashchinsky, N., Gittel, A., Schleper, C., Mikutta, R., Palmtag, J., Shibistova, O., Urich, T., Richter, A. and Šantrůčková, H.: The effect of warming on the vulnerability of subducted organic carbon in arctic soils, Soil Biology and Biochemistry, 90, 19–29, doi:10.1016/j.soilbio.2015.07.013, 2015.

Carbone, M. S., Still, C. J., Ambrose, A. R., Dawson, T. E., Williams, A. P., Boot, C. M., Schaeffer, S. M. and Schimel, J. P.: Seasonal and episodic moisture controls on plant and microbial contributions to soil respiration, Oecologia, 167(1), 265–278, doi:10.1007/s00442-011-1975-3, 2011.

Carter, M. R. and Gregorich, E. G.: Soil Sampling and Methods of Analysis, 2nd ed., CRC Press., 2007.

Chowdhury, N., Burns, R. G. and Marschner, P.: Recovery of soil respiration after drying, Plant Soil, 348(1), 269, doi:10.1007/s11104-011-0871-2, 2011.

Crow, S. E., Sulzman, E. W., Rugh, W. D., Bowden, R. D. and Lajtha, K.: Isotopic analysis of respired CO2 during decomposition of separated soil organic matter pools, Soil Biology & Biochemistry, 38(11), 3279–3291, doi:10.1016/j.soilbio.2006.04.007, 2006.

Czimczik, C. I. and Trumbore, S. E.: Short-term controls on the age of microbial carbon sources in boreal forest soils, Journal of Geophysical Research-Biogeosciences, 112(G3), doi:10.1029/2006jg000389, 2007.

DeLaune, R. D. and Reddy, K. R.: Redox Potential, in Encyclopedia of Soils in the Environment, edited by D. Hillel, pp. 366–371, Elsevier, Oxford. [online] Available from: http://www.sciencedirect.com/science/article/pii/B0123485304002125, 2005.

Dioumaeva, I., Trumbore, S., Schuur, E. A. G., Goulden, M. L., Litvak, M. and Hirsch, A. I.: Decomposition of peat from upland boreal forest: Temperature dependence and sources of respired carbon, Journal of Geophysical Research-Atmospheres, 108(D3), doi:10.1029/2001jd000848, 2002.

Dutta, K., Schuur, E. A. G., Neff, J. C. and Zimov, S. A.: Potential carbon release from permafrost soils of Northeastern Siberia, Global Change Biology, 12(12), 2336–2351, doi:10.1111/j.1365-2486.2006.01259.x, 2006.

Fierer, N., Morse, J. L., Berthrong, S. T., Bernhardt, E. S. and Jackson, R. B.: Environmental controls on the landscape-scale biogeography of stream bacterial communities, Ecology, 88(9), 2162–2173, doi:10.1890/06-1746.1, 2007.

Hamer, U., Unger, M. and Makeschin, F.: Impact of air-drying and rewetting on PLFA profiles of soil microbial communities, Journal of Plant Nutrition and Soil Science, 170(2), 259–264, doi:10.1002/jpln.200625001, 2007.

Hicks Pries, C. E., Schuur, E. A. G. and Crummer, K. G.: Thawing permafrost increases old soil and autotrophic respiration in tundra: Partitioning ecosystem respiration using δ13C and Δ14C, Global Change Biology, 19(2), 649–661, doi:10.1111/gcb.12058, 2013.

Hicks Pries, C. E., Castanha, C., Porras, R. C. and Torn, M. S.: The whole-soil carbon flux in response to warming, Science, 355(6332), 1420–1423, doi:10.1126/science.aal1319, 2017.

Kalbitz, K. and Kaiser, K.: Contribution of dissolved organic matter to carbon storage in forest mineral soils, Journal of Plant Nutrition and Soil Science, 171(1), 52–60, doi:10.1002/jpln.200700043, 2008.

Knorr, K.-H. and Blodau, C.: Impact of experimental drought and rewetting on redox transformations and methanogenesis in mesocosms of a northern fen soil, Soil Biology & Biochemistry, 41(6), 1187–1198, doi:10.1016/j.soilbio.2009.02.030, 2009.

Megonigal, J. P., Hines, M. E. and Visscher, P. T.: Anaerobic metabolism: linkages to trace gases and aerobic processes, [online] Available from: http://repository.si.edu//handle/10088/15579 (Accessed 8 August 2019), 2004.

Nagy, R. C., Porder, S., Brando, P., Davidson, E. A., Figueira, A. M. e S., Neill, C., Riskin, S. and Trumbore, S.: Soil Carbon Dynamics in Soybean Cropland and Forests in Mato Grosso, Brazil, Journal of Geophysical Research: Biogeosciences, 123(1), 18–31, doi:10.1002/2017JG004269, 2018.

Nilsson, M. and Öquist, M.: Partitioning Litter Mass Loss into Carbon Dioxide and Methane in Peatland Ecosystems, in Carbon Cycling in Northern Peatlands, pp. 131–144, American Geophysical Union. [online] Available from: http://dx.doi.org/10.1029/2008GM000819, 2013.

Nowinski, N. S., Taneva, L., Trumbore, S. E. and Welker, J. M.: Decomposition of old organic matter as a result of deeper active layers in a snow depth manipulation experiment, Oecologia, 163(3), 785–792, doi:10.1007/s00442-009-1556-x, 2010.

Peters, V. and Conrad, R.: Sequential reduction processes and initiation of CH4 production upon flooding of oxic upland soils, Soil Biology & Biochemistry, 28(3), 371–382, doi:10.1016/0038-0717(95)00146-8, 1996.

Schädel, C., Luo, Y., Evans, D. R., Fei, S. and Schaeffer, S. M.: Separating soil CO2 efflux into C-pool-specific decay rates via inverse analysis of soil incubation data, Oecologia, 171(3), 721–732, doi:10.1007/s00442-012-2577-4, 2013.

Schuur, E. A. G. and Trumbore, S. E.: Partitioning sources of soil respiration in boreal black spruce forest using radiocarbon, Global Change Biology, 12(2), 165–176, doi:10.1111/j.1365-2486.2005.01066.x, 2006.

Sexstone, A. J., Revsbech, N. P., Parkin, T. B. and Tiedje, J. M.: Direct measurement of oxygen profiles and denitrification rates in soil aggregates, Soil Science Society of America Journal, 49(3), 645–651, 1985.

Trumbore, S. E.: Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics, Ecological Applications, 10(2), 399–411, doi:10.2307/2641102, 2000.

Tveit, A., Schwacke, R., Svenning, M. M. and Urich, T.: Organic carbon transformations in high-Arctic peat soils: key functions and microorganisms, Isme Journal, 7(2), 299–311, doi:10.1038/ismej.2012.99, 2013.

Whitman, T., Zhu, Z. and Lehmann, J.: Carbon Mineralizability Determines Interactive Effects on Mineralization of Pyrogenic Organic Matter and Soil Organic Carbon, Environ. Sci. Technol., 48(23), 13727–13734, doi:10.1021/es503331y, 2014.