Soil sample conservation from field to lab for heterotrophic respiration assessment

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Method Article

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A B S T R A C T

We evaluated (1) whether the sample transport time could lead to a significant loss of carbon through microbial respiration and to a change of measured respiration rates, which can be a problem in areas difficult to access, with a long travel time from field to laboratory; (2) whether the method used to quantify heterotrophic respiration for agricultural soils is adequate for horizons that remain always water-saturated or close to saturation. Surface horizons and deep Bh of Amazonian podzols were sampled and kept under refrigeration to maintain moisture of sampling time. Incubations of aliquot of the same sample were initiated on the sampling day and 3, 6, 9 and 12 days after sampling. Other aliquots were conducted on a tension table to given water potential (60 cm $_{H2O}$) prior to incubation.

- Soil samples, whether disturbed or not, should not be dried but kept at sampling moisture in semi-open plastic bags under refrigeration at 4 °C, respiration monitoring must be conducted without prior water potential adjustment.
- In such conditions,12 days between sampling and beginning of measurement did not affected respiration results.
- The method used for agricultural soils gave different results and does not make sense for soils under perudic moisture regime.

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Introduction

Soil organic carbon mineralization rates are usually evaluated using soil respiration, positively correlated to soil carbon and to microbial biomass and consisting of the sum of radicular and microbial respiration [1]. The part not derived from root respiration, called soil heterotrophic respiration, is an indicator of microbial activity which regulates the turnover of organic matter (OM) in the soil. The soil heterotrophic respiration is an extremely variable parameter, difficult to quantify [2]. Amongst the main factors that may affect the respiration rate are soil structure, substrate quality and availability, temperature, soil moisture and porosity. For each condition, there is an optimum moisture that maximizes respiration: a too high moisture can limit O_2 diffusion and, if below ideal, the access of heterotrophic organisms to soil organic carbon (SOC) becomes restricted [3]. The measurement of soil heterotrophic respiration is of great importance for many purposes and especially for determining the carbon balance of soils in the context of climate change [4].

Soil microbial respiration rate can be determined in situ, using respiration chambers placed on the topsoil [5], or in the laboratory by measuring the CO_2 production of a sample placed in incubation chamber. A classical method, widely used for agricultural soils, was described by Paul et al. [6]. Before incubated, the soil sample is initially dried, sieved and subsequently wetted until reaching the field capacity, or directly conducted to field capacity moisture without drying. Such a procedure is well-adapted to agricultural soils that frequently reach field capacity in place. Many soils having a high organic carbon content, however, remain usually all along the year waterlogged or at a moisture level closer to saturation than to field capacity [7,8]. Drying the samples or bringing them to field capacity will therefore be a radical ecological change for the existing microbial communities, which could alter the respiration rates. Recent studies have shown that ex-situ measurement of soil respiration with pre-dried and disturbed samples can give questionable results [9].

This consideration brings up another problem. Many large soil carbon pools around the world are in areas that are difficult to access, as tropical and boreal large forests [10]. Reaching such places requires several days of travel in difficult conditions which do not always allow the transport and installation of heavy instrumentation, or which are incompatible with an extended stay on site necessary for in situ measurements. The question raised is then that of the effect of transport time on the mineralization results, do we lose a significant part of very labile organic matter in a few days?

In this context, we evaluated a method that allows samples from topsoil or from deeper horizons to be brought back from places located several travel days away from the laboratory, without the need to dry them.

Field sampling

The samples are collected using a Ø 70 mm hand auger and, regarding deep samples, casing the borehole with 75 mm PVC pipes to avoid collapsing. If possible, undisturbed soil cores are sampled with stainless steel volumetric rings (bulk density rings), following the method proposed by Comeau et al. [9]. Many deep Bh, however, are too hard for a non-disturbing sampling. After careful root

removal if necessary, soil samples are stored in semi-open plastic bags to maintain a low air exchange between the soil and the environment and kept under refrigeration at 4 °C, to be maintained as close to field moisture as possible and to minimize SOC mineralization before experiments [11]. Refrigeration during fieldwork time and during the return trip must be maintained by different methods depending on the available infrastructure: cooler placed inside a larger fishing cooler with ice blocks, kerosene refrigerator or electric refrigerator with generator.

Laboratory procedure

For disturbed samples, a 100 g aliquot of soil is packed in a volumetric ring. Volumetric ring with disturbed or undisturbed sample is closed at the bottom by a porous nylon canvas, weighted and immediately placed in 800 mL glass vial sealed with a metal cap with a rubber septum for sampling the atmosphere of the vial with a syringe. The vial is weighed at the beginning of the incubation and after each gas collection to monitor the sample moisture. The vial atmosphere is sampled at a frequency adapted to the objective of the study. After each collection, a flash-out is performed for 5 min.

Method validation

Sampling

Samples were taken from representative podzol soils in two remote areas of the Upper Rio Negro basin, state of Amazonas, Brazil, near the banks of the Cubate and Uaupés rivers at 00° 35′ 48.6′'N, 67° 53′ 37.2′' W and 00° 10′ 11.2′' N, 67° 48′ 56.3′' W, respectively. In both areas, the soils developed on sandy continental sediments. In the Cubate area, the soil was situated in a slightly depressed area subject to frequent surface waterlogging; the vegetation was an evergreen forest of Campinarana type [12]. It had a surficial 50-cm thick organo-mineral P horizon overlying a 10-cm thick organo-mineral A horizon; the E and Bh horizons were 80- and 520-cm thick, respectively. In the Uaupés area, the soil was situated on a gentle slope inclined toward the river, the vegetation was an evergreen forest of Campinarana Florestada type [12]. It had a surficial 20-cm thick A horizon overlying a 30-cm thick transitional A-E horizon; the E and Bh horizons were 660- and more than 310-cm thick, respectively. All samples were disturbed samples. For the Cubate P and the Uaupés A horizons, composite samples were made over the entire horizon thickness. For the Bh horizons, composite samples were made over the Bh upper 50 cm for the Cubate soil and the Bh upper 200 cm for the Uaupés soils.

Incubation

To evaluate a possible influence of the time between sampling and incubation, incubations of aliquot of the same sample were initiated on the sampling day ($t = 0$) and 3, 6, 9 and 12 days after sampling ($t = 3$, 6, 9 and 12, respectively). The vial atmosphere was sampled 1 day after the beginning of incubation (day 0), then every 3 days until day 13, then every 7 days until day 27, on day 42 then every month until day 520. During incubation, no vial showed a variation in sample weight greater than 1%. CO_2 was measured by gas chromatography on a Shimadzu GC-17A equipment.

To evaluate possible differences with the method described by Paul et al. [6], 100 g of soil packed without previous drying in a volumetric ring according to the procedure described above was taken into the tension table, slowly saturated then conducted to a water potential corresponding to 60 cm of water column. The ring was then placed in a 800 ml glass vial held open for 7 days for stabilization, then closed for gas collection as described above. This procedure was only realized for the Uaupés samples.

Mineralization kinetics

The dynamics of OM mineralization were fitted considering various hypothesis: 1 to 4 OM pools, each pool having a first order kinetics; temporal succession of microbial populations. The respiration curves were fitted by the Excel Solver using the Evolutionary algorithm to find the parameters values that minimized the normalized root mean square deviation (NRMSD).

Table 1
Main characteristics of the studied horizons. Size fractions are 0–2 µm (clay), 2–50 µm (silt), 0.05–0.2 mm (fine sand) and 0.2–2 mm (coarse sand).

	Munsell colour	SOC	Clay	Silt	Fine sand	Coarse sand	Moisture at sampling
		%	% of the mineral fraction				% w/w
P Cubate	7,5YR2,5/1	12.4	1.5	45.2	35.2	18.1	113
A Uaupés	10YR2/2	1.9	0.0	3.0	32.7	63.5	18
Bh Cubate	7,5YR2,5/1	3.5	0.0	11.1	68.3	21.1	27
Bh Uaupés	10YR3/3 & 10YR2/1	2.1	7.8	3.6	32.2	56.3	19

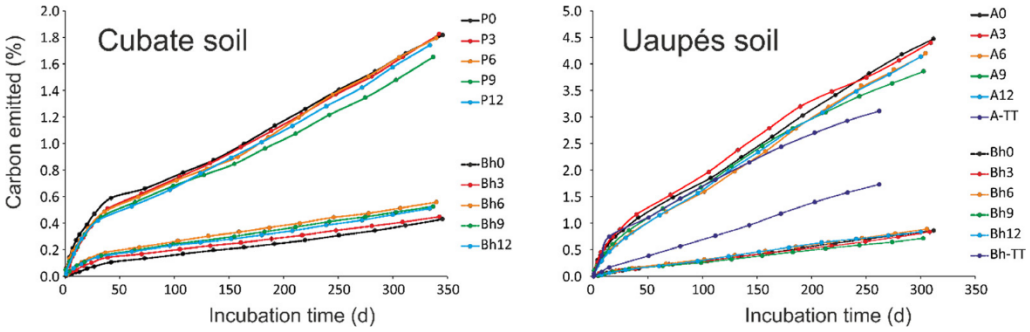

Fig. 1. Carbon emitted during incubation. Experiment names: the letter (P, A or Bh) refers to the horizon type; the number (0 to 12) refers to the time elapsed between sampling and beginning of incubation; TT refers to samples previously placed on tension table.

Soil samples analyses

An aliquot of each soil sample was used to determine soil granulometry and carbon content. In the laboratory, the samples were submitted to drying at 35 °C in an air circulation oven. Particle size distribution was obtained by the Robinson pipette method. Part of the samples was manually grinded in agate gravel in particles smaller than 0.106 mm to ensure good carbon analysis. The total organic carbon content (TOC) was obtained by dry combustion using a Shimadzu TOC-L coupled with SSM-5000A solid sample module; there is no carbonate minerals in these acid soils. Moisture content before soil incubation was determined by drying at 105 °C for 24 h.

Results and discussion

Organic carbon, granulometry and soil moisture at sampling are shown in Table 1. The hydromorphic P horizon of the Cubate soil had a loamy texture with a very high SOC (12.4%) and moisture, all other samples were sandy with a SOC ranging from 1.9 to 3.5%. The two Bhs had similar characteristics.

Soil moisture remained unchanged during the 12 days between successive incubations. It changed, however, for the Uaupés A and Bh aliquot placed on the tension table, for which moisture at the beginning of incubation was 10% and 18%, respectively. Results of the respirometry measurements are given on Fig. 1. For each curve, a two-pool model (fast pool and slow pool having a fast and slow OM turn-over, respectively) was enough to describe the experimental data; an example is given on Fig. 2 and the corresponding equation describing C mineralization with time is:

$$Cem = C_f\left(1 - e^{-k_f t}\right) + C_s\left(1 - e^{-k_s t}\right)$$

where Cem is the C mineralization (g^{-1}), C_f and C_s are the relative size of the fast and slow pool, respectively, k_f and k_s are the mineralization rate (y^{-1}) in the fast and slow pool, respectively. Note that the carbon pools sizes Cf and Cs are relative size, their sum being equal to 1.

The Normalized Root Mean Square Error (NRMSD) ranged between and 5.0 10^{-3} and 1.7 10^{-2}, which is satisfactory: NRMSD has not been improved using more pools or temporal succession of

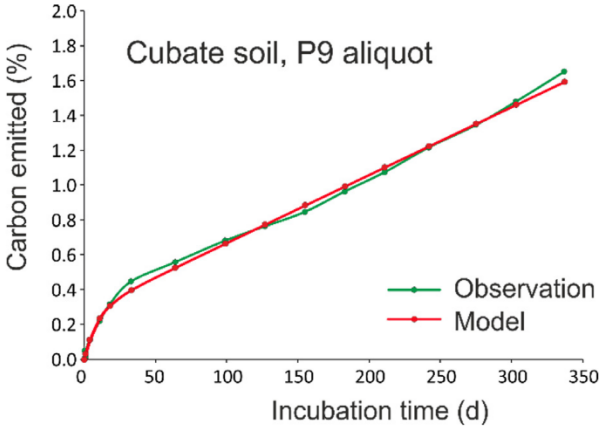

Fig. 2. Example of modelling: respirometry measurement of the Cubate P9 aliquot.

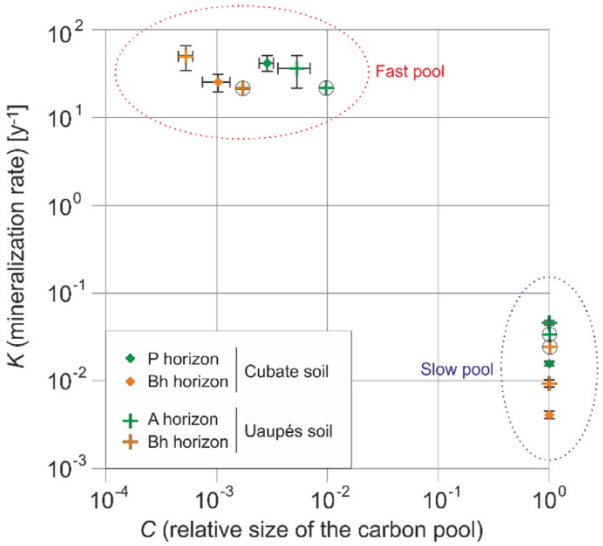

Fig. 3. Mean and standard deviation of relative carbon pools size and mineralization rates. Symbols within a circle indicate samples submitted to tension table before mineralization experiment.

microbial populations. The results of modelling are summarized on Fig. 3. For all models, less than 1% of the fast pool remained after 90 days.

Considering the mineralization experiments conducted at sampling moisture, no systematic effect was observed due to the time elapsed between sampling and incubation in the 0- to 12-days range. Differences between curves related to a given horizon can thus be attributed to variability between experiments. For both surface and Bh samples, the observed variability between soils was higher than experimental variability.

Results of mineralization experiments conducted after normalization of the water potential on tension table (Uaupés aliquot A-TT and Bh-TT) gave, however, results significantly different that mineralization experiments conducted at sampling moisture. For the surface A horizon, total mineralization of the A-TT aliquot at the end of the experiment was slightly lower than for aliquots kept at sampling moisture (−26%). Modelling showed that this was principally due to the slow pool,

with slightly lower size and lower mineralization rate. For the Bh horizon, total mineralization at of the Bh-TT aliquot at the end of the experiment was higher than for aliquots kept at sampling moisture (+107%). Modelling showed that this was principally due to a higher mineralization rate of the slow pool ($2.4\ 10^{-2}\ y^{-1}$ versus $9.3\ 10^{-3}\ y^{-1}$).

Difference in the handling of samples between A-TT and Bh-TT, on one side, and the other aliquots, on the other, was (1) more time at room temperature before beginning of mineralization, (2) sample conducted to saturation and then (3) to a water potential corresponding to 60 cm of water column (60 cm_{H2O}), corresponding to a potentially different moisture. Considering the A horizon, the lower mineralization rate of the A-TT aliquot can be related to a quite lower moisture (10%) than in aliquots kept at sampling moisture (18%). This is contradictory with the idea that field capacity is an optimum for microbial respiration [13,14], at least for such soils. Considering the Bh horizon, moisture in the Bh-TT aliquot (18%) was not significantly different than moisture in Bh aliquots kept at sampling moisture (19%). A longer time at room temperature does not appear to be a relevant factor because it would mainly impact the fast pool when the higher mineralization seems linked to the slow pool. The remaining factor is that the Bh-TT aliquot has been brought to saturation prior to desorption until 60 cm_{H2O}, which must have favoured the access of microorganisms to a mineralizable organic matter, or the replacement of a microbial population by another one [15].

Conclusion

When samples were kept cold in semi-open plastic bags to maintain their moisture at sampling time, the time elapsed between sampling and beginning of the experiment, up to 12 days, did not result in significant differences between mineralization results. Soil respiration studies under field moisture can therefore wait for samples to return to the laboratory, provided that refrigeration conditions are met during transport. Our method minimizes microbial ecological changes between sampling and laboratory incubations.

In comparison to these experiments, the aliquots conducted on tension table to a given water potential (60 cm_{H2O}) gave different results: a 26% lower mineralization for the A horizon and a 106% higher mineralization for the Bh horizon. The causes of these differences can be a different moisture, a favoured access of microorganisms to a mineralizable organic matter, or the replacement of a microbial population by another one; a more precise answer to these questions would require dedicated studies. It can be concluded, however, that if normalizing the water potential is justified for most of agricultural soils, it does not make sense for soils under perudic soil moisture regime. These results also show that evaluating changes in the rate of mineralization of organic matter as part of a change towards drier Amazonian climates would require specific mineralization studies under controlled soil moisture regime.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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