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Evaluation of Three Incubation Designs for Mineralization Kinetics of Organic Materials in Soil

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ABSTRACT

Carbon (C) mineralization was assessed during incubations of a Mediterranean sandy soil amended with various organic by-products covering a wide range of C and nitrogen (N) contents. The laboratory incubation systems consist in measuring continuously the soil respiration (as CO_2-C) in closed chambers, or less current, in pre-storing soil containers in semi-open chambers until transferred and measured for CO_2-C evolved in closed 'measuring-jars'. The latest were improved, the new designs permitting to test a much greater number of by-products with a minimum handling. No significant differences were found between the results obtained by the different incubation systems. The storage systems using pre-storage of soils gave reproducible cumulative CO_2-C curves. Results obtained with the pre-storage systems could be compared confidently to C mineralization data from studies using permanent closed chambers. One of them was specially reliable and can thus be recommended for long-term incubation experiments.

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INTRODUCTION

Carbon and/or N mineralization studies are still widely undergone in order to better understand the organic matter dynamics in soils (Smith and Hadley, 1989; Kaboneka et al., 1997). They are also very helpful to evaluate the quality of various organic materials (Ajwa and Tabatabai, 1994; Sorensen and Jensen, 1995) or composts applied to soils (Levi-Minzi et al., 1990; Hébert et al., 1991; Paré et al., 1998). Estimations of carbon mineralized as CO_2 from such organic material-treated soils were improved by the use of infrared methods (e.g., on airflow or in static chambers). But acidimetry on CO_2-C alkali traps still provides powerful and cost-effective measurements of soil respiration.

Laboratory incubation experiments on soil organic matter and organic material decomposition are supposed to be easier to manage than field experiments (Nakadai et al., 1993; Mueller et al., 1998). However, one can be faced with some constraints, for example, material, laboratory space, and complexity of handling represent several obstacles when many organic materials are to be tested. Furthermore, additional difficulties arise when studying C and N dynamics concomitantly. This implies indeed, (i) either to work on the same soil sample throughout the experiment, (ii) or to destroy the sample periodically. In the first case [(i)], the experiment deals with a minimum set of soil samples; periodical inorganic N percolations with saline solutions (Stanford and Smith, 1972) are used (e.g., Quemada and Cabrera, 1995), but are not representative of natural conditions. On the opposite, in the other case [(ii)], the destruction for inorganic N extraction imposes multiplication of soil samples. The conception of incubation designs fitted to such studies would probably overcome these difficulties.

The aim of our study was thus to design an incubation system, which allowed: 1) suitable experimental conditions with the simplest laboratory material and equipment, 2) a maximum number of organic materials to be tested in a minimum space, and 3) CO_2-C measurement on alkali trap solutions by acidimetry, from a soil sample periodically destroyed (removed from the incubation system) for inorganic N analysis.

MATERIALS AND METHODS

Soil and Organic By-Product Materials

We used a sandy soil (11.5% clay, 69.3% sand), classified as fluvisol (FAO-UNESCO-ISRIC, 1988) or Udifluvent (Soil Survey Staff, 1975). It was collected in the 0-20 cm layer of a field experimental design in Théza (Plain of Réart, Perpignan, France), and previously described by Servat and Callot (1966). It had a pH_{H2O} of 6.6, and contained 4.44 g C kg^{-1}, 0.49 g N kg^{-1}, and 0.55 g P kg^{-1}. It had a bulk density of 1.5 Mg m^{-3}, a cation-exchange capacity of 56 mmol c^{+} kg^{-1}, and a water-holding capacity (WHC) of 220 g kg^{-1}. The sampled plot (greenhouse soil) had not received any organic input (crop residues or compost) for the last seven years. The soil was partially air dried at room temperature (20°C) until it could be crushed and passed through a 2-mm sieve, then air dried to constant weight.

The experiment was fitted to test seventeen agricultural and food industry by-products (designated as Added Organic Materials or *AOM*). The *AOM* were air dried (at 25°C to prevent inorganic N losses), then finely ground (0.2 mm) before use. We present here the results for two sets of *AOM*, in order to compare the incubation designs and check the reproducibility of CO_2-C measurements:

Set 1: Grape cake (Grap; 494 g C kg^{-1}; 22.5 g N kg^{-1}), a winery by-product; feather meal (Feath; 471 g C kg^{-1}; 152 g N kg^{-1}), an animal feed obtained by hydrolysis of poultry feather.

Set 2: Compost (449 g C kg^{-1}; 25 g N kg^{-1}), an industrial organic amendment; cocoa cake (437 g C kg^{-1}; 45 g N kg^{-1}), a food-industry by-product; olive pulp (469 g C kg^{-1}; 20 g N kg^{-1}), an olive-mill by-product.

Incubation Designs

Five hundred mg *AOM* (equivalent to 28 t *AOM* ha^{-1} applied into the top 20 cm of soil) were incorporated with 25 g air-dried soil in a 100-mL plastic container, and homogeneously mixed by ten radial and ten lateral rotations of the temporarily capped container. Periodically, deionized water was cautiously sprayed to bring samples at 75% WHC, with particular attention to minimize the breakdown of soil aggregates (slaking). This adjustment was done on a top loading analytical balance. Amended soil was then placed in a 1.2-L air-tight measuring-jar (wide-mouthed glass canning jar) with 10 mL water to maintain a moisture-saturated atmosphere, and a 50-mL vial (polypropylene screw-capped vial) containing 20 mL aqueous NaOH solution 0.25 mol L^{-1} (Titrisol) to trap respired CO_2. A control (a measuring-jar containing soil but no *AOM*) was used to assess the basal soil respiration. Three blank replicates (measuring-jars containing no soil, but water and the NaOH vial) were made to account for carbonatation of NaOH when opening the jars and replacing the NaOH vials before the next measurement period.

Samples (soil amended, control) were incubated in triplicate at 28°C (±1°C) in a ventilated incubator for 180 days. We decided to adopt 28°C, which is close to the usual temperature of incubation experiments as revealed by the literature (Table 1). The temperature effect on C mineralization, as expressed by the Q_{10} coefficient, was found indeed to be far weaker at moderate to high (20-30°C and above) than at low temperatures(<10°C; Kirshbaum, 1995; Kätterer et al., 1998). Respired CO_2 trapped in alkali solutions was measured at day 1, 2, 3, 5, 7, 10, 14, 20, 28, 41, 61, 90, 120, 152, and 180. Inorganic-N was determined by extraction of each sample at 8 sampling occasions (day 1, 2, 5, 10, 20, 41, 90, and 180). Three scenarios were then considered.

TABLE 1. Some conditions used to study C and N mineralization during laboratory incubations.

Study	Soil sample (g)	Temperature (°C)	Soil moisture (%WHC[a] or % d.w.[b])	Days or frequency of measures	Chamber volume (mL)	Method used for trapping CO_2-C	Method used for CO_2-C determination	References
short-term N dynamics	40 for C	25	field capacity	3 times/1st week 2 times/week (2nd and 3rd) 1 time/ week (4th to 9th)	250	air-tight during 3 to 24h	gas chromatography	Paul and Beauchamp (1994)
	40 for N	25	field capacity	0, 0.5, 1, 3, 6 and 12 weeks				
C mineralization (0, 0.5, 1 and 2% d.w.)	100	8, 22, 30	60% WHC 20% dry weight	13 measures between day 2 to 21	300	0.5 mol NaOH L^{-1}	$BaCl_2$ 0.5 mol HCl L^{-1}	Levi-Minzi et al. (1990)
N mineralization index	300 for N	20	80% WHC	1, 3, 6, 9, 12 weeks				Groot and Houba (1995)
	20 for C	25	60% WHC	10 days	1500	1 mol NaOH L^{-1}	$BaCl_2$ 0.05 mol HCl L^{-1}	
C and N mineralizations, inorganic-N availability	25	15		0, 2, 3, 6, 11, 15, 18, 29, 40, 69, 124 days	1000	0.25 mol NaOH L^{-1}	$BaCl_2$ 0.1 mol HCl L^{-1}	Recous et al. (1995)
C, S and N mineralizations	80 + 80 SiO_2 for N	21	field capacity	14, 28, 56, 84 days				Janzen and Kucey (1988)
	80 + 80 SiO_2 for C	21	field capacity	1, 3, 7, 10, 14, 21, 28, 42, 56, 84 days	150	1 mol NaOH L^{-1}	direct titration	
humification and mineralization modelling	250	28	80% WHC	0, 15, 30, 90, 180, 270 days				Pansu and Sidi (1987)
C and N mineralizations /soil texture, microbes	250 for C	20	60% WHC	0, 1, 2, 5, 8, 12 weeks	1500	0.5 mol NaOH L^{-1}	$BaCl_2$ 0.5 mol HCl L^{-1}	Hassink (1994a, 1994b, 1995)
Q_{10} temperature and SOM[c] decomposition		25, 33, 35						Kirschbaum (1995)
C and N cycles from root mucilages	100	25	20% dry weight	0, 2, 3, 4, 7, 10, 14, 21, 52, 70, 126, 185 days	500	0.25 mol NaOH L^{-1}	$BaCl_2$ 0.5 mol HCl L^{-1}	Mary et al. (1993)
manure C and N mineralizations	10	25	60% WHC	70 days	1000	0.1 mol NaOH L^{-1}	$BaCl_2$ 0.1 mol HCl L^{-1}	Kirchmann (1991)
sheep manure C and N mineralizations	30 for C	22		0, 3, 5, 7, 10.5, 14, 28 days		1 mol NaOH L^{-1}		Hébert et al. (1991)
	2700 for N	22		0, 4, 8, 16 weeks				
sheep manure C and N mineralizations	50	20	55% WHC	0, 7, 14, 28, 84 days	2000	1 mol KOH L^{-1}	$BaCl_2$ 0.25 mol HCl L^{-1}	Sørensen and Jensen (1995)
sheep and chicken manures N availability	70	25	75% WHC	2, 4, 6, 8, 12, 16 weeks	150			Serna and Pomares (1993)

[a]WHC=waterholding capacity.
[b]d.w.=dry weight.
[c]SOM=soil organic matter.

Scenario 1

Each sample was located in its proper air-tight measuring-jar, and CO_2-C could be measured until the sample had to be removed from the system, for a new inorganic N extraction (Figure 1a). When adopting Scenario 1, the number of required jars at a specific sampling occasion can be formulated according to Equation [1]:

$$J_i = b + r(n \times p_i) \qquad [1]$$

Where J_i is the number of jars at the sampling occasion I, b=3 (blanks), r=3 (sample replicates), n=17 (number of AOM tested), and p_i the remaining sampling occasions (8-i).

Scenario 2

This system was adapted from Paul and Beauchamp (1994), Quemada and Cabrera (1995), and Hadas and Portnoy (1994, 1997). It consisted in storing soil samples, until they were CO_2-C measured. The three sample replicates (for each AOM tested) were stocked together in one 'stocking-jar', and left in contact with fresh air by means of a needle-perforated plastic film covering the stocking-jar; this limited the anaerobiosis risk during such a long incubation time. The three stocked samples were maintained in the same temperature and air moisture than the measuring-jars, until being transferred and kept in three measuring-jars during the 'measurement-period' (Figure 1b). For example, the stocking-jar referenced 'Feath 1-2' contained the three replicates corresponding to the Feath treatment from day 0 to day 1. At day 1, each replicate was then transferred and maintained in one air-tight measuring-jar from day 1 to day 2 (which was the next sampling occasion). When adopting Scenario 2, the number of jars required at a specific sampling occasion can be formulated according to Equation (2):

$$J_i = b + n\left[m + r\left(\frac{p_i - 1}{k}\right)\right] \qquad [2]$$

where J_i is the number of jars at the sampling occasion i, b=3 (blanks), r=3 (sample replicates), n=17 (number of AOM tested), p_i remaining sampling occasions (8-i), m='measuring-jars', and k='stocking-jars'.

Scenario 3

The replicates were stocked in 'stocking-plates' (60*30*10 cm polyvinylchloride plates) filled with 1-cm deionized water and covered with a needle-perforated plastic film to provide aerobic conditions. Then, following Scenario 2, each replicate was transferred and kept in one air-tight measuring-jar during the measurement period (Figure 1c).

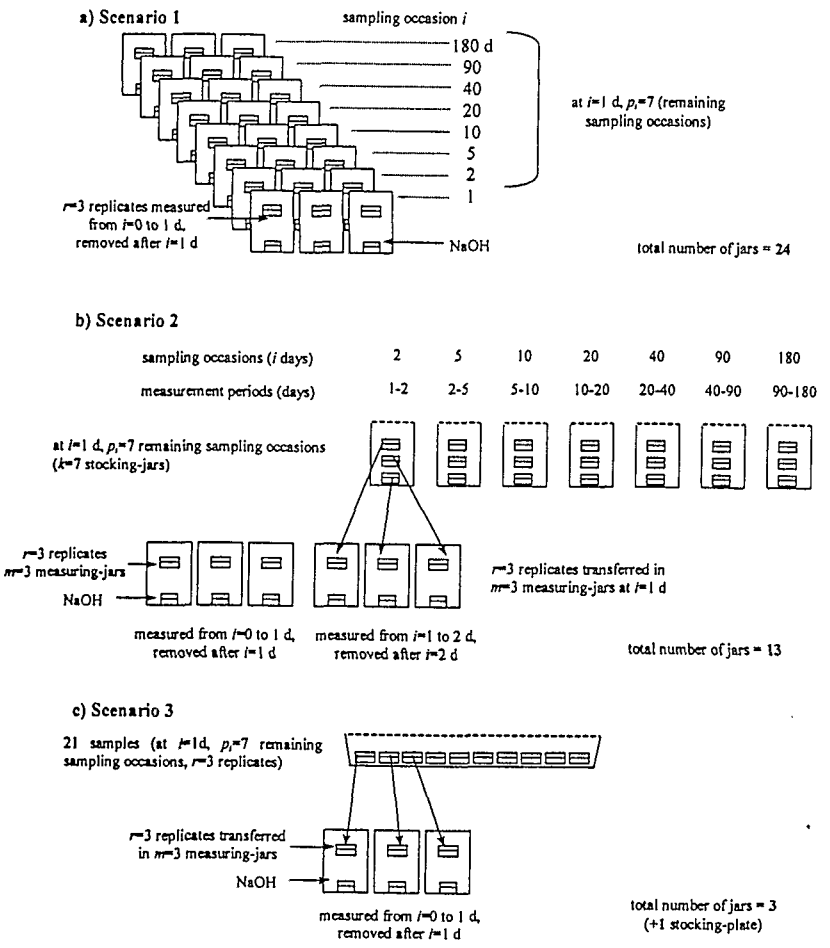

FIGURE 1. Number of jars required for n=1 *AOM* tested (add b=3 jars for blanks in each scenario), samples being removed at 1, 2, 5, 10, 20, 40, 90, and 180 d with: a) Scenario 1 (Equation 1, b=3, r=3 replicates per *AOM*, continuously CO_2-C measured until removed); b) Scenario 2 (Equation 2, b=3, r=3, samples stocked in 1 semi-open stocking-jar until transferred in their individual air-tight measuring-jars until removed); c) Scenario 3 (Equation 3, b=3, r=3, samples stocked in a semi-open stocking-plate then transferred in their individual air-tight measuring-jars until removed).

The *AOM* set 1 (grape cake, feather meal) was incubated with soil on a 10-day period according to the three scenarios and set 2 (compost, cocoa cake, olive pulp) incubated according to Scenario 2 or 3 on a 180-day period.

Measurements of Carbon Dioxide

The rates of CO_2-C (or inorganic N) released by soils amended with organic materials are generally high during the first stages of incubation, then become relatively low. The CO_2-C needs thus to be frequently measured within the first two weeks. The test *AOM* presented a wide range of OM contents, and were susceptible to differ greatly in the amounts of CO_2-C produced or O_2 absorbed. We estimated the CO_2-C respired during a six-month experiment, assuming that approximately 70% of the carbon input would be respired along this period.

Anaerobiosis, which may limit the oxidizing ability of microorganisms, and the limited CO_2-C absorption capacity of NaOH solutions, may lead to important errors in CO_2 measurements. It was therefore necessary to calculate the quantity of absorbed-O_2 and carbonated-NaOH in between two measurements: absorbed O_2 during this period, could indeed represent approximately 20 to 35% of the initial oxygen. With 20 mL of 0.5 mol NaOH L^{-1}, 20 to 33% of the initial soda should be carbonated, which corresponded to 1-1.5 mmol respired-CO_2 or absorbed-O_2, and about 10% of the initial oxygen. With the sampling occasions finally chosen, 20 mL of 0.25 mol NaOH L^{-1} were sufficient in most cases.

When adopting acidimetry for CO_2 determination on alkali trap solutions, two techniques are generally used (Table 1): direct HCl titration of NaOH/Na_2CO_3 solutions, or NaOH titration after $BaCO_3$ precipitation ($BaCl_2$ method). Preliminary tests (unpublished data) showed a better accuracy with the $BaCl_2$ method which was then adopted in this experiment.

Calculations

The quantity of respired CO_2-C at a sampling occasion i (mineralized *AOM* as a fraction of added C), precipitated as $BaCO_3$, was calculated by difference between the remaining vs. initial NaOH concentrations according to:

$$Cm_{ia} = \frac{CO_2C_{ia}^a - CO_2C_{ia}^c}{TAC} \quad [5]$$

$$\overline{C}m_i = \frac{1}{r}\sum_{a=1}^{r} Cm_{ia} \quad [5']$$

$$\overline{SC}m_i = \overline{SC}m_{i-1} + \overline{C}m_i \quad [5'']$$

where Cm_{ia} = mineralized fraction of *AOM* sample a at sampling occasion i, $CO_2C_{ia}^a$, and $CO_2C_{ia}^c$ are the amounts of C evolved from the amended and control ia samples, respectively; TAC is total added C expressed in the $CO_2C_{ia}^a$ and $CO_2C_{ia}^c$

unit, $\overline{Cm}_i$ = mean respired fraction of *AOM* at sampling occasion *i* (*r*=3 replicates), $\overline{SCm}_i$ and $\overline{SCm}_{i-1}$ (no dimension) are mean cumulated values of *AOM* mineralized fractions (with SCm_0=0).

The $\overline{SCm}_i$ is very interesting for practical use since it does not depend on any unit. For example, a value $\overline{SCm}_i$ = 0.35 at day 120, means (under comparable mineralization conditions) that for a *AOM* application of 1 Mg (C) ha^{-1}, 350 kg of C will be mineralized within 4 months following application.

Careful data control must be done during the experiment, especially when working with cumulative values, since variances are added when summing the means. The pooled variance of Cm_i is:

$$s^2_{Cm_i} = \frac{1}{rp-p}\sum_{i=1}^{p}\sum_{\alpha=1}^{r}\left(Cm_{i\alpha} - \overline{C}m_i\right)^2 \qquad [6]$$

where *p* is the total number of sampling occasions with *r* samples. Equation 6 can be sufficient in studies with non-cumulative data (Hess and Schmidt, 1995); else the cumulative confidence intervals (at the 95% level of probability, Student $t_{0.975}$) can be calculated according to Pansu et al. (1998):

$$SCm_i = \overline{SC}m_i \pm t^{rp-p}_{0.975} \times s_{Cm_i}\sqrt{\frac{i}{r}} \qquad [6']$$

RESULTS AND DISCUSSION

Practicabilities of Incubation Designs

Among the three incubation designs presented, Scenario 1 can be chosen if the incubator (or thermostated room) capacity is not a limiting factor. In this case, thanks to the large pool of measuring-jars (and CO_2-C trapping vials) existing during the first stages of the incubation, many CO_2-C replicate measurements (J_i) could be done. This, in turn, provided a good reproducibility of the results during these early stages. The number of CO_2-C trapping vial replicates then decreased along the experiment, due to the removal of selected soil samples for inorganic N analysis to reach the lower limit *r*=3 at the last sampling occasion. A second advantage was the possibility to measure CO_2-C emissions on the same soil sample until its removal from the experimental system. Nevertheless, the incubator capacity being generally a limiting factor, Scenario 1 rapidly could be set aside.

Scenario 2 provided a less important pool of possible CO_2-C measuring vials (i.e., reduced to the number of replicates *r*=3) throughout the whole experiment. But it offered new advantages comparatively to Scenario 1: (i) the possibility to have a distinct treatment reference on the stocking-jar and on the measuring-jars, avoiding confusions especially when managing a great number of jars and (ii) a less important number of jars in the incubator which made the handling easier. For example with Scenario 1 (and 8 sampling occasions) and according to Equation (1), if the incubator had the capacity for 80 jars, only 3 *AOM* could be tested (75 jars at day 0). On the opposite with a similar capacity (80 jars), and according to Scenario 2 (Equation 2), 7 *AOM* could be incubated (73 jars at day 0). However, with this scenario, CO_2-C emissions were not measured on the same sample over long durations of time. Nevertheless, it was assumed that the sample replicates which were selected for measurement were representative of the whole pool of the *AOM* tested. But in this case, soil desiccation had to be checked very cautiously (depending on the upper or lower location of the stocked soil samples in the stocking-jars).

The stocked soil samples in Scenario 3, as in Scenario 2, were in contact with the atmosphere renewed naturally. The material used in Scenario 3 provided additional advantages as compared to Scenario 1 and Scenario 2. It permitted (i) to save more space (capacity: 30 samples per 'stocking-plate'), (ii) to reduce or eliminate the differences in desiccation among replicates. In this scenario, indeed, all soil samples were on the same level in the stocking-plate, i.e., were equally exposed to air-drying/water-saturated air. But this system required to be careful in managing the soil sample: the *r* replicates of a same treatment were not isolated and referenced as compared to Scenario 2.

Carbon Mineralization Data

Basal soil respiration from unamended soil was followed according to the three scenarios on the same 10-day period (Figure 2). As was observed for *AOM* mineralization, the respiration pattern of the control soil seemed to be almost the same for the three scenarios. Over 10 days, basal soil respiration was 76 (±24), 80 (±17.9) and 84 (±17.9) mg CO_2-C kg^{-1} soil according to Scenario 1, 2, and 3, respectively (Figure 2).

In the case of Grap amended soils (Figure 3), the three different scenarios gave almost the same patterns for CO_2-C mineralization. The mean cumulated value of mineralized *AOM* fraction in 10 days ($\overline{SCm}_{10d}$) for Grap represented 7.4 (±0.41), 8.9 (±0.83), and 8.0 (±0.36)% of the initial added organic carbon according to Scenario 1, 2, and 3, respectively (Figure 3). These data were relatively low, realistic and consistent with what is expected for a plant material characterized by a C: N ratio equal to 22 (Rubins and Bear, 1942). The low N amount (in comparison with the large pool of C) is a limiting factor for microbial growth, hence for respiration.

The CO_2-C mineralization of Feath followed also the same pattern whatever the scenario used. However, this mineralization was much higher than that obtained for Grap. The $\overline{SCm}_{10d}$ fraction for Feath was 43.2 (±8.5), 42.5 (±2.6), and 48.3 (±2.2)% of the initial added organic carbon according to Scenario 1, 2, and 3, respectively (Figure 3). During this short period (first 10 days), the soil microorganisms were able to respire a large part (up to 50%) of the initial carbon. These figures were not unusual for an animal by-product as Feath (Rubins and Bear, 1942). Nitrogen, here, was not a limiting factor and was available like

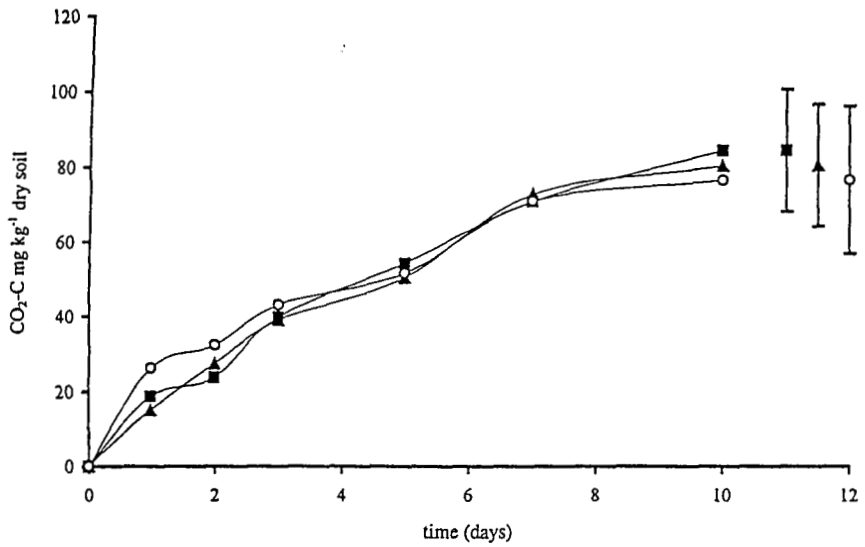

FIGURE 2. Respiration patterns of control soil according to: Scenario 1 = only measuring-jars (○); Scenario 2 = stocking-jars and measuring-jars (▲); Scenario 3 = stocking-plate and measuring jars (■). Vertical bars are the greatest 95% cumulative confidence intervals at sampling occasion i=10 days (Equation 6').

carbon in large quantities (C:N=3). Feath is classified as an organic fertilizer according to the French norm NFU#42001 (1981), and its biochemical characteristics are typical of the organic fertilizer group defined by Robin (1997). With Scenario 1 even if fresh air was periodically introduced in the measuring-jar, continuous measurement in closed jars with alkali acting as a strong CO_2 sink might induce overestimated results (Sierra and Renault, 1995). Under low CO_2 concentrations, such as those measured in alkali-trap chambers (20-250 μL L^{-1}), the CO_2 emission rate has been reported to be enhanced by 20-70% as compared with that under the ambient CO_2 of 400 μL L^{-1} (Bekku et al., 1997; Sierra and Renault, 1995). On the other hand, Santruckova and Simek (1994) reported that elevated CO_2 air concentration could rapidly (1 day) affect CO_2 production from soil microorganisms without affecting O_2 consumption. According to the calculated pooled confidence intervals, there was no significant difference between Grap $\overline{SCm}_{10d}$ from Scenario 1 and 3 on one hand, and from Scenario 3 and 2 on another hand. According to the same calculations, there was no significant difference between Feath $\overline{SCm}_{10d}$ from Scenario 2 and 1 on one hand and from Scenario 1 and 3 on the other hand.

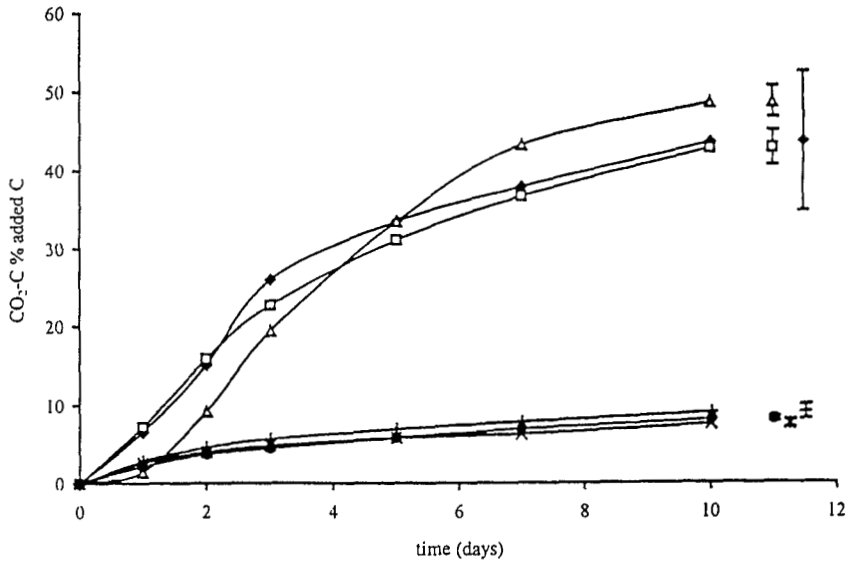

FIGURE 3. The CO_2-C mineralization patterns of Feath: Scenario 1 (◆); Scenario 2 (□); Scenario 3 (△) and Grap: Scenario 1 (×); Scenario 2 (+); Scenario 3 (●). Vertical bars are the greatest 95% cumulative confidence intervals at sampling occasion i=10 days (Equation 6').

Even for *AOM* with potentially high respiration rates (e.g., feather meal), the levels of carbonated NaOH were not too high. Between two measurements, most levels of the NaOH carbonatation were under 30%. The risk of NaOH saturation was thus effectively low. The levels reaching or exceeding 30-60%, were observed for *AOM* of animal origin: in this case, 20 mL NaOH solution 0.5 mol L^{-1} should be recommended.

Repeatability of Carbon Mineralization Data

Relative standard deviations (RSD) for basal soil respiration were almost similar but relatively high (RSD=31, 21, and 22% for Scenario 1, 2, and 3, respectively).

For Grap, the precision of the results decreased in the order Scenario 3>1>2 with RSD=4.5, 5.5, and 9.3%, respectively. For Feath, the precision of the results decreased in the order Scenario 3>2>1 with RSD=4.6, 6.1, and 19.7%, respectively. The higher value of the confidence interval of $\overline{SCm}_{10d}$ was twice that of the lower one in the case of Grap, and 4-fold in the case of Feath. There was no direct relationship between the $\overline{SCm}_{10d}$ values (differences due to the origin of the *AOM*)

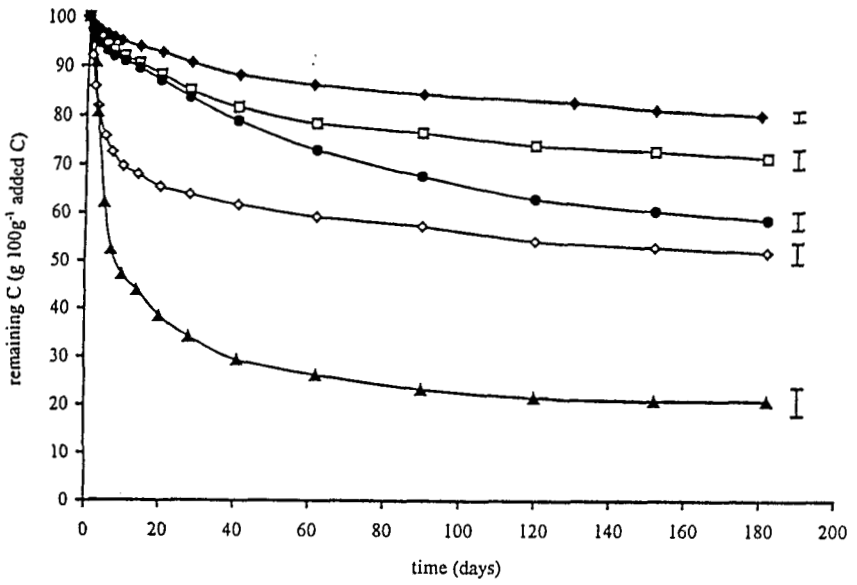

FIGURE 4. The CO_2-C mineralization patterns during a 6-month period of Feath (▲) and Grap (□) and some other *AOM*: compost (◆), cocoa cake (◇), olive pulp (●) according to Scenario 3. Vertical bars are the greatest cumulative confidence intervals at the end of the experiment (Equation 6').

and the confidence intervals, and between the $\overline{SCm}_{10d}$ and the RSD values. When Scenario 3 was utilized, the Grap RSD value was almost the same than that of Feath. It could indicate a limited risk of anaerobiosis with this scenario.

Poor repeatability was associated with low CO_2 emissions (basal soil respiration) whereas a good and constant one was obtained with both medium and high CO_2 emissions (Grap and Feath).

On a 6-month incubation period, a wide range of *AOM* gave reproducible mineralization patterns with Scenario 3 (Figure 4). Cumulated confidence intervals at the end of incubation (Equation 6') were not too high and should allow valuable comparisons between *AOM* mineralization kinetics.

CONCLUSIONS

The three incubation systems designed during this work allowed accurate and similar CO_2-C measurements. The results from permanent closed chambers with alkali traps along the whole experiment (Scenario 1) did not differ significantly from those obtained with punctual alkali trapping (Scenarios 2 and 3). Results obtained with Scenarios 2 and 3 can thus be compared confidently to C mineralization data using permanent closed chambers. Moreover, the risks of anaerobiosis or NaOH saturation were low with Scenarios 2 and 3. The main improvement with the stocking-jar (Scenario 2) and mostly the stocking-plate system (Scenario 3) as compared to Scenario 1, was to minimize the material needs (simplest equipment, minimum laboratory space), especially when dealing with a great number of treatments. The incubation design described as Scenario 3: (i) allows accurate CO_2-C measurements and slightest relative standard deviation on soil samples periodically removed from the system; (ii) requires the lowest laboratory space for a maximum range of *AOM*; (iii) provides the best experimental conditions (e.g., no difference in soil desiccation) with the simplest material and current laboratory equipment. Scenario 3 best fits the aim of our study and, therefore, could be reasonably recommended to carry out long-term carbon mineralization incubations.

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Distribution of Sludge-Borne Manganese in Field-Grown Maize

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ABSTRACT

The uptake and distribution of manganese (Mn) in field-grown maize (*Zea mays* L.) was studied in a long-term sewage sludge field trial on an acid sandy soil at Bordeaux. Since 1974, sewage sludge had been applied at levels of 10 t dry matter (DM) ha^{-1} $year^{-1}$ (SS 10) and 100 t DM ha^{-1} per 2 years (SS 100) on annually cropped maize plots. Treatment with farmyard manure (FYM) at a rate of 10 t DM ha^{-1} $year^{-1}$ served as unpolluted control. Five replicate plants per treatment were examined at six different growth stages. At each stage, the whole plant was separated into its different organs and the Mn distribution was determined in at least 12 different plant parts. Manganese concentrations were always higher in SS 100 plants compared to FYM and SS 10 treated plants. Significant treatment-dependent differences occurred almost all in the roots and in the different leaf levels while we found similar Mn concentrations in the stalk and in the reproductive organs. In the different stalk levels and in the ear composites we determined low Mn concentrations with critical deficiency values in FYM and SS 10 plants while Mn concentrations in SS 100 plants were in the normal range. Soil treatment also significantly influenced the initial absorption by the roots. Despite low