Pergamon

Soil Biol. Biochem. Vol. 30, No. 8/9, pp. 1221–1224, 1998

Printed in Great Britain
PII: S0038-0717(97)00224-1
0038-0717/98 $19.00 + 0.00

SHORT COMMUNICATION

MEASUREMENT OF BIOMASS C, N AND ^{14}C OF A SOIL AT DIFFERENT WATER CONTENTS USING A FUMIGATION-EXTRACTION ASSAY

J. L. CHOTTE,[1]* J. N. LADD[2] and M. AMATO[2]

[1]Centre ORSTOM, BP 1386, Dakar, Senegal and [2]CSIRO, Division of Soils, PO Box No. 2, Glen Osmond, SA 5064, Australia

(Accepted 9 September 1997)

INTRODUCTION

Soil microbial biomass plays a key role in soil organic matter turnover. Microbial biomass C and N have been assayed by a variety of methods, such as fumigation-incubation (Jenkinson and Powlson, 1976; Chaussod and Nicolardot, 1982; Ross, 1990) substrate-induced respiration (Anderson and Domsch, 1978), and fumigation-extraction (Vance *et al.*, 1987; Amato and Ladd, 1988; Ladd and Amato, 1988; van Gestel *et al.*, 1993; Kabir *et al.*, 1994). Recent reviews have listed and compared the performance of these methods (Sparling and Ross, 1993; Martens, 1995). Moisture contents of assayed soils have ranged from 40% to 60% of soil water-holding capacity. In the fumigation-extraction method described by Amato and Ladd (1988) and Ladd and Amato (1988), biomass C, N and ^{14}C were assayed on soil samples incubated at 40% WHC. Biomass C and N estimations were based on the gain in ninhydrin-reactive N after fumigation, multiplied by 21 and 3.1, respectively; biomass ^{14}C was calculated from the gain of extractable ^{14}C after fumigation, multiplied by 3.25. These factors were obtained by calibration against the fumigation-incubation assay (Jenkinson and Powlson, 1976), where soils were also incubated at 40% WHC. The reliability of the procedure is dependent on the efficiency of chloroform in killing soil microorganisms and on the activity of proteases to release ninhydrin-reactive N, and of hydrolases to release soluble organic ^{14}C. Amato and Ladd (1994) have indicated that both soil moisture content and soil pH modify the conversion factor required for calculating biomass C. Studies on the location of biota in soil aggregates have involved measurements of microbial biomass in soil fractions of ranging particle size (Jocteur Monrozier *et al.*, 1991; Richaume *et al.*, 1993; Ladd *et al.*, 1996; Sorensen *et al.*, 1996; Chotte *et al.*, 1998). Despite consistent results, several potential sources of error have been pointed out, such as the failure to accommodate the effect of differences in moisture content of fractions on biomass ^{14}C assay (Sorensen *et al.*, 1996). We report the effect of soil moisture on the performance of a fumigation-extraction method for measuring biomass C, N and ^{14}C. We compared the quantities of ninhydrin-reactive N, soluble C (organic and inorganic) and soluble ^{14}C extracted before and after fumigation of a Vertisol when assayed at different moisture contents.

The soil, a Vertisol (Black Earth), was collected (0–10 cm depth) at a field site near Northfield, South Australia, in late Spring 1993. The soil was stored at 4°C at its field moisture content (20% of WHC) pending further use. Incubation and treatments: The soil was thoroughly mixed, sieved (8 mm), and conditioned at 28°C for 4 d. Soil subsamples (equivalent to 10 g oven dried-soil) were incubated with ^{14}C-glucose (1 mg C g^{-1} soil; 287 Bq g^{-1} soil) for 3 d. Soil moisture content was adjusted to 40% WHC by the addition of the glucose solution and soil subsamples were compacted to the bulk density 1.0. After 3 d of incubation, distilled water was added to soil samples to bring moisture contents in triplicate to 60%, 80%, 100% and 200% WHC, respectively. A set of three subsamples was left at 40% WHC as a reference soil. Fumigated soil subsamples were exposed to $CHCl_3$ vapor for 10 d. Extraction: Fumigated and unfumigated soil samples were suspended in KCl solution (1:3 dry soil/solution, w/v; 2 M final concentration), shaken at 25°C for 1 h and then centrifuged for about 5 min (2,000 *g*). Extracts were filtered (0.45 μm) and stored frozen pending further analysis.

*Author for correspondence.

Table 1. Relationships between components extracted from fumigated and unfumigated soil (y) and water content (%WHC) of the soil (x)

		$y = a + bx$			
y	x	a	b	r	significance
		Ninhydrin-reactive N ($\mu g\ N\ g^{-1}$ soil)			
unfumigated soil	%WHC	0.6	−0.0017	0.52	0.05
fumigated soil	%WHC	44.2	0.029	0.63	0.01
gain = fumigated − unfumigated	%WHC	43.6	0.031	0.66	<0.01
		Soluble organic C ($\mu g\ C\ g^{-1}$ soil)			
unfumigated soil	%WHC	16.1	−0.027	0.41	0.12
fumigated soil	%WHC	260.8	0.961	0.89	<0.01
gain = fumigated − unfumigated	%WHC	244.6	0.989	0.91	<0.01
		Soluble organic ^{14}C (i) (dis $s^{-1}\ g^{-1}$ soil)			
unfumigated soil	%WHC	0.9	−0.0011	0.19	0.50
fumigated soil	%WHC	43.3	0.210	0.97	<0.01
gain = fumigated − unfumigated	%WHC	42.5	0.211	0.97	<0.01
		Soluble organic ^{14}C (ii) S.A. (dis $s^{-1}\ g^{-1}$ C)			
unfumigated soil	%WHC	62.2	−0.006	0.01	0.97
fumigated soil	%WHC	170.0	0.103	0.37	0.17
gain = fumigated − unfumigated	%WHC	176.7	0.084	0.32	0.25

Ninhydrin-reactive nitrogen was determined from a 0.5 ml of extract from each of the fumigated and unfumigated soils. Aliquots were mixed with 1.5 ml 2 M KCl and 2.0 ml of freshly-prepared ninhydrin reagent. Tubes were placed in a boiling waterbath for 15 min, cooled, and 5 ml of 50% ethanol were added to each. Absorbances at 570 nm were calibrated against standard solutions of L-leucine and ammonium sulfate (Moore and Stein, 1954). Total and inorganic soluble C in the extracts were determined using a Dohrman DC-180 organic carbon analyser. CO_2, released by acidification and by UV-promoted persulfate oxidation of organic material, was measured by infrared absorbance. Organic soluble C in the extracts was calculated by difference. Extractable ^{14}C of fumigated and unfumigated soils was measured using 0.5 ml aliquots mixed with 10 ml of LKB Hiphase III scintillation cocktail. Because inorganic carbonate ^{14}C was found to be very low (<0.03% of input ^{14}C) (Chotte *et al.*, 1998), extractable ^{14}C was considered to be organic.

Statistical analysis indicated that ninhydrin-reactive N, extracted from fumigated and unfumigated soils, and the gain in ninhydrin-reactive N were positively and significantly ($P < 0.05$) correlated with soil water content (Table 1), and were in agreement with data obtained by Amato and Ladd (1994). The conversion factors K_C (21) and K_N (3.1) to convert the gains in ninhydrin-reactive N to biomass C and N, and calculated from a range of soils assayed at 40% WHC, can be adjusted for soils incubated at higher moisture content. Because the reliability of these factors has clearly been demonstrated for soils incubated at 40% WHC (Amato and Ladd, 1988), we can assume that the true value of biomass N corresponded to those obtained at this WHC%. Then, this allows the calculation of the adjusted factors to be made for each soil moisture content. Nevertheless, the relationships between the factors K_C and K_N and WHC% (Table 2) demonstrate that the extent of adjustment due to soil moisture content was slight. For example, for soil assayed at saturation (100% WHC) the adjusted K_C and K_N values were 20.2 and 2.98 respectively, being little different from those obtained under standard (40% WHC) conditions. The use of the standard conversion factors for the calculation of the biomass C and N of soils assayed at water contents between 40% and 100% WHC would at worst lead to an overestimation of 3%. We currently advocate the use of 21 and 3.1, as proposed originally by Amato and Ladd (1988), for converting ninhydrin-reactive N extracted from soil in the pH range 6.0–8.6 to biomass C and N, provided that water contents of soils or particle size fractions of soils do not exceed saturation, free water being eliminated by a mild centrifugation.

Unlike ninhydrin-reactive N, soluble organic C and soluble organic ^{14}C extracted from unfumigated soils were significantly not correlated with %WHC. However, like ninhydrin-reactive N, amounts of organic C and ^{14}C extracted from fumigated soils and

Table 2. Relationships between the conversion factors (K_N, K_C and $K_{^{14}C}$) (y) and water content (%WHC) of the soil (x)

		$y = a + bx$			
y	x	a	b	r	significance
K_N	%WHC	3.18	−0.002	0.66	<0.01
K_C	%WHC	21.5	−0.013	0.66	<0.01
$K_{^{14}C}$	%WHC	3.65	−0.010	0.94	<0.01

the gains after correction for unfumigated control soils were positively correlated with %WHC. Moreover, this correlation revealed sharp increases in extractable C and ^{14}C with increasing moisture content of assayed soil. For example, the gain of soluble organic ^{14}C (dps g^{-1} soil) after fumigation for soil assayed at 100% WHC was 30% higher than that for soil assayed at 40% WHC. Badalucco *et al.* (1992) demonstrated that, for soils assayed at 50% WHC, amounts of phenol-reactive C and anthrone-reactive C represented higher proportions of total extractable C after fumigation than before. At the same time, N-compounds were found to account for a lower proportion of total extractable N after than before fumigation. They argued that non-biomass sugars are solubilized after fumigation, while non-biomass N was not. In our experiment, soluble organic ^{14}C extracted from fumigated soil would not have included any added ^{14}C-glucose, which is entirely metabolized during the 3 d of incubation (van Veen *et al.*, 1985; Ladd *et al.*, 1992). Nevertheless, sugar ^{14}C from non-biomass sources may be released in increasing amounts from fumigated soils of increasing water contents and thus may account for increasing proportions of total, KCl-extractable organic ^{14}C. However, it can also be argued that the origin of extractable organic ^{14}C was exclusively from biomass killed by fumigation and that carbohydrases were more affected by soil moisture content than proteases (Ladd *et al.*, 1996). Whatever their sources, these organic compounds were likely to be derived from the same pool, since the specific activity (S.A.) of organic C (dps g^{-1} C) extracted at different moisture contents was not significantly correlated with %WHC and similar (Table 1).

We can conclude that the performance of the fumigation assay in measuring biomass C, N and ^{14}C is affected by soil moisture content, but that the conversion factor can be readily adjusted for water content. Whilst adjustments of K_C and K_N based on extractable ninhydrin-reactive compounds are trivial, adjustments for these factors based on extractable organic C and ^{14}C are far greater (Table 2). For soils assayed at 100% WHC, the adjusted value was 2.65. This suggests that standardization of assay conditions regarding %WHC is less demanding in the former method, which therefore may be more readily applicable to a routine biomass assay of a range of soils or to soil sampled at different times of the year. Moreover, we recommend that in studies of soil particle sizes, free water be removed from soil fractions to maintain moisture content below saturation. Since K_C adjustments were already suggested for acidic soils assayed at 40% WHC (Amato and Ladd, 1994), corrections for $K_{^{14}C}$ are likely to be needed for these soils assayed at higher water content.

Acknowledgements—This work was conducted while J. L. Chotte was a visiting scientist at the Division of Soils, CSIRO. The financial support of the Division and of l'Institut Français de Recherche Scientifique pour le Développement en Coopération (Orstom) is gratefully acknowledged. We thank Mr. A. Beech of the Analytical Services Laboratory, Division of Soils, CSIRO, for technical assistance.

REFERENCES

Amato M. and Ladd J. N. (1988) Assay for microbial biomass based on ninhydrin-reactive nitrogen extracts of fumigated soils. *Soil Biology & Biochemistry* **20,** 107–114.

Amato M. and Ladd J. N. (1994) Application of the ninhydrin-reactive N assay for microbial biomass in acid soils. *Soil Biology & Biochemistry* **26**(9), 1109–1115.

Anderson J. P. E. and Domsch K. H. (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology & Biochemistry* **10,** 215–221.

Badalucco L., Gelsomino A., Dell'Orco S., Grego S. and Nannipieri P. (1992) Biochemical chracterization of soil organic compounds extracted by 0.5 M K_2SO_4 before and after chloroform fumigation. *Soil Biology & Biochemistry* **24,** 569–578.

Chaussod R. and Nicolardot B. (1982) Mesure de la biomasse microbienne dans les sols cultivés. I. Approche cinétique et estimation simplifiée du carbone facilement minéralisable. *Revue d'Ecologie et de Biologie du Sol* **19,** 201–512.

Chotte J. L., Ladd J. N. and Amato M. (1998) Sites of microbial assimilation and turnover of ^{14}C soluble and particulate substrates decomposing in a clay soil. *Soil Biology & Biochemistry* **30,** 205–218.

Jenkinson D. S. and Powlson (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biology & Biochemistry* **8,** 209–213.

Jocteur Monrozier L., Ladd J. N., Fitzpatrick R. W., Foster R. C. and Raupach M. (1991) Components and microbial biomass content of size fraction in soils of contrasting aggregation. *Geoderma* **49,** 37–62.

Kabir M. D., Chotte J. L., Rahman M., Bally R. and Jocteur Monrozier L. (1994) Distribution of soil fractions and location of soil bacteria in a Vertisol under cultivation and perennial grass. *Plant and Soil* **163,** 243–255.

Ladd J. N. and Amato M. (1988) Relationships between biomass ^{14}C and soluble organic ^{14}C of a range of fumigated soils. *Soil Biology & Biochemistry* **20,** 115–116.

Ladd J. N., Jocteur Monrozier L. and Amato M. (1992) Carbon turnover and nitrogen transformations in an Alfisol and Vertisol amended with [U-^{14}C] glucose and [^{15}N] ammonium sulfate. *Soil Biology & Biochemistry* **24,** 359–371.

Ladd J. N., van Gestel M., Jocteur Monrozier L. and Amato M. (1996) Distribution of organic ^{14}C and ^{15}N in particle-size fractions of soils incubated with ^{14}C, ^{15}N-labelled glucose/NH_4 and legume and wheat straw residues. *Soil Biology & Biochemistry* **28,** 893–905.

Martens R. (1995) Current methods for measuring microbial biomass C in soil: Potential and limitations. *Biology and Fertility of Soils* **19,** 87–99.

Moore S. and Stein W. H. (1954) A modified ninhydrin-reagent for the photomoetric determination of aminoacids and related compounds. *Journal of Biological Chemistry* **211,** 907–913.

Richaume A., Steinberg C., Jocteur Monrozier L. and Faurie G. (1993) Differences between direct and indirect enumeration of soil bacteria: the influence of soil struc-

ture and cell location. *Soil Biology & Biochemistry* **25**, 641–643.

Ross D. J. (1990) Measurements of microbial biomass C and N in grassland soils by fumigation-incubation procedures: influence of inoculum size and the control. *Soil Biology & Biochemistry* **22**, 289–294.

Sorensen P., Ladd J. N. and Amato M. (1996) Microbial assimilation of ^{14}C of ground and unground plant materials decomposing in a loamy sand and a clay soil. *Soil Biology & Biochemistry* **28**, 1425–1434.

Sparling G. P. and Ross D. J. (1993) Biochemical methods to estimate soil microbial biomass: current developments and applications. In *Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture*, eds K. Mulongoy and R. Merckx, pp. 21–37. Wiley, New York.

Vance E. D., Brookes P. C. and Jenkinson D. S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* **19**, 703–707.

van Gestel M., Merckx R. and Vlassak K. (1993) Microbial biomass and activity in soils with fluctuating water contents. *Geoderma* **56**, 617–626.

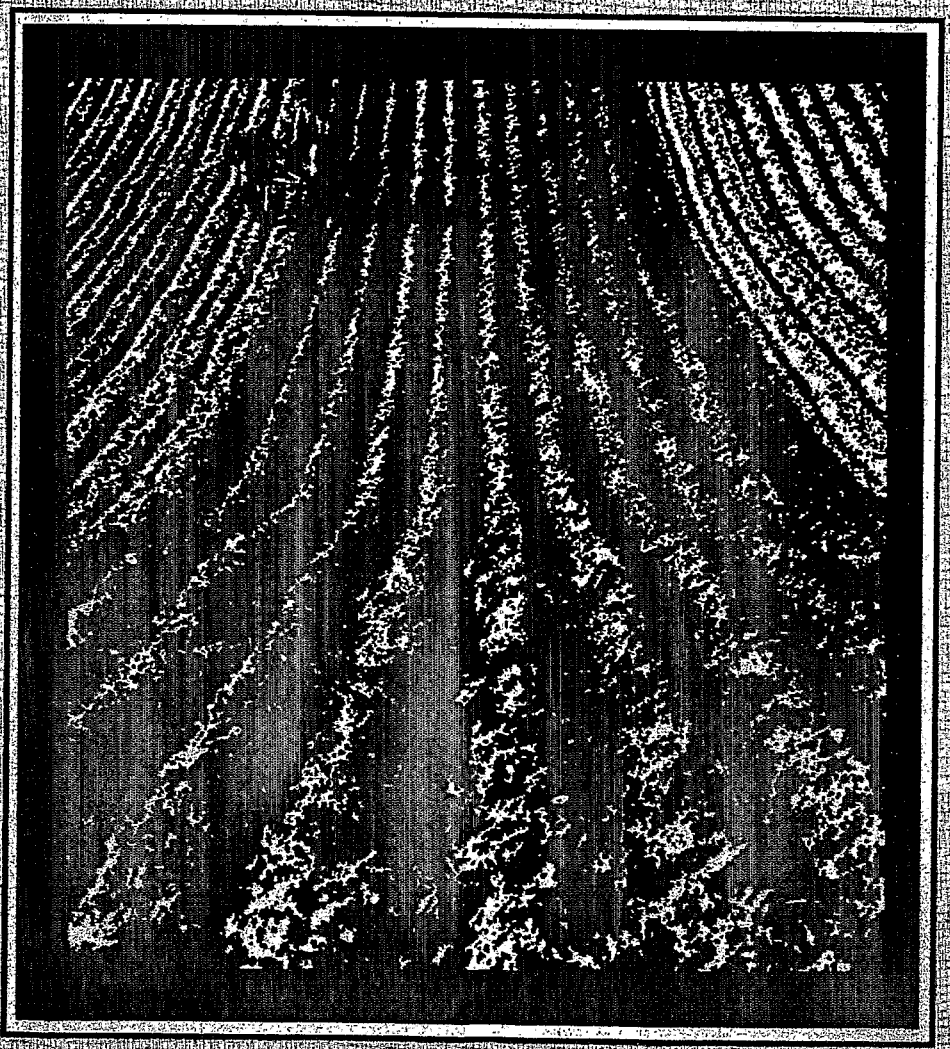
van Veen J. A., Ladd J. N. and Amato M. (1985) Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [U-^{14}C] glucose and [^{15}N] $(NH_4)_2SO_4$ under different moisture regimes. *Soil Biology & Biochemistry* **17**, 747–756.

Soil Biology & Biochemistry

ISSN 0038-0717

ISSS-AISS-IBG

Vol. 30 No. 8/9
August 1998
Editor-in-Chief J S WAID



PERGAMON