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AN EXTRACTION METHOD FOR MEASURING SOIL MICROBIAL BIOMASS C

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Summary—The effects of fumigation on organic C extractable by 0.5 M K_2SO_4 were examined in a contrasting range of soils. E_C (the difference between organic C extracted by 0.5 M K_2SO_4 from fumigated and non-fumigated soil) was about 70% of F_C (the flush of CO_2-C caused by fumigation during a 10 day incubation), meaned for ten soils. There was a close relationship between microbial biomass C, measured by fumigation–incubation (from the relationship Biomass $C = F_C/0.45$) and E_C, given by the equation:

$$\text{Biomass } C = (2.64 \pm 0.060)\, E_C$$

that accounted for 99.2% of the variance in the data. This relationship held over a wide range of soil pH (3.9–8.0).

ATP and microbial biomass N concentrations were measured in four of the soils. The (ATP)/(E_C) ratios were very similar in the four soils, suggesting that both ATP and the organic C rendered decomposable by $CHCl_3$ came from the soil microbial biomass. The C:N ratio of the biomass in a strongly acid (pH 4.2) soil was greater (9.4) than in the three less-acid soils (mean C:N ratio 5.1).

We propose that the organic C rendered extractable to 0.5 M K_2SO_4 after a 24 h $CHCl_3$-fumigation (E_C) comes from the cells of the microbial biomass and can be used to estimate soil microbial biomass C in both neutral and acid soils.

INTRODUCTION

The increase in extractable N, P and S following fumigation of soil has been used to estimate the amounts of N (Brookes *et al.*, 1985), P (Brookes *et al.*, 1982; Hedley and Stewart, 1982) and S (Saggar *et al.*, 1981; Strick and Nakas, 1984) held in the soil microbial biomass. Chloroform has been used as the fumigant with these methods and with the fumigation–incubation method for measuring microbial biomass C (Jenkinson and Powlson, 1976b) and N (Shen *et al.*, 1984; Voroney and Paul, 1984) because it is an effective biocide, and does not solubilize non-microbial soil organic matter or render it decomposable (Jenkinson, 1976).

Powlson and Jenkinson (1976) measured the C rendered extractable by fumigation (E_C), defined as [(organic C extracted by 0.5 M K_2SO_4 from a fumigated soil) − (organic C extracted from a non-fumigated soil)] and also the CO_2-C flush (F_C), defined as [(CO_2-C evolved from fumigated soil, incubated aerobically at 25°C for 10 days) − (CO_2-C evolved from non-fumigated soil, incubated under the same conditions)]. In this paper we re-examine Powlson and Jenkinson's (1976) results and also include recent analyses of soils taken from two of their original sites and from two other sites.

The main aim was to see if the amounts of C which could be directly extracted from soils following fumigation were related to microbial biomass C as measured by the fumigation–incubation method (Jenkinson and Powlson, 1976b) or by a modified fumigation–incubation method for use in acid soils (Vance *et al.*, 1987b). The relationship between organic C thus extracted and biomass N and ATP concentrations was also examined. There have been other reports (Voroney, 1985) of good agreement between total extractable C released by chloroform and the CO_2-C flush, which suggest that direct extraction may be a valid procedure for measuring soil biomass C (R. P. Voroney, Pers. Comm.).

MATERIALS AND METHODS

Soils

The data for soils 1–6 (Table 1) are taken from Powlson and Jenkinson (1976) who gave details of the sampling and properties of these soils. The other four (soils 7–10) were sampled in 1985. For each of these four soils, two composite samples, each consisting of 10 soil cores of 5 cm dia. were taken to a depth of 23 cm (including the litter layer, if present) and kept at 5°C until use. Before biological analyses, the soils were sieved (< 6.35 mm) and then incubated [40% water holding capacity (WHC), 25°C for 10 days]. Soil samples were air-dried and ground for measurement of total soil organic C (Kalembasa and Jenkinson, 1973) and total soil N (Bremner, 1965). Soil pH was measured on air-dried samples using a 1:2 soil–water paste.

The soils sampled in 1985 from Broadbalk Wilderness (soil no. 7) (deciduous woodland, 3.2% C, 0.29% N, pH 7.7, 26% clay); Meathop Wood (soil no. 8) (deciduous woodland, 3.4% C, 0.27% N, pH 5.4, 32% clay); Park Grass (soil no. 9) (unmanured grassland, 3.1% C, 0.26% N, pH 5.1, 22% clay) and Geescroft Wilderness (soil no. 10) (deciduous woodland, 2.8% C, 0.19% N, pH 4.2, 24%

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Table 1. CO_2-C evolved and total C extracted from 10 soils with and without $CHCl_3$-fumigation

Soil No.[a]	Soil	Symbol used in Fig. 1	pH	Fumigation–incubation: CO_2-C evolved non-fumigated	Fumigation–incubation: CO_2-C evolved fumigated	Fumigation–incubation: F_C[b]	Direct extraction: K_2SO_4-C extracted non-fumigated	Direct extraction: K_2SO_4-C extracted fumigated	Direct extraction: E_C[c]	Ratio E_C/F_C
				(μg C g^{-1} soil)						
1	Broadbalk Continuous Wheat Experiment. Farmyard manure plot (022)	△	7.6	122	370	248	120	270	150	0.61
2	Broadbalk Continuous Wheat Experiment. Unmanured plot (03)	▽	8.0	64	164	100	40	100	60	0.60
3	Broadbalk Wilderness	□	7.5	270	824	554	350	650	300	0.54
4	Whitehorse Field	○	6.4	89	251	162	120	220	100	0.62
5	Flint Field	+	6.3	318	1994	1676	500	1720	1220	0.73
6	Geescroft Wilderness	■	3.9	148	152	152	240	350	110	0.72
7	Broadbalk Wilderness	◀ ▶	7.7	287	582	295	112	322	210	0.71
8	Meathop Wood	✱	5.4	150	411	261	118	383	265	1.01
9	Park grass	X	5.1	182	484	302	82	330	248	0.82
10	Geescroft Wilderness		4.2	125	130	130	123	242	119	0.91

[a]Soils 1–6, data from Powlson and Jenkinson (1976); Soils 7–10, this paper.
[b]F_C = [(CO_2-C evolved from fumigated soil) − (CO_2-C evolved from non-fumigated soil)] except for Geescroft Wilderness (soils 6 and 10), where F_C = (CO_2-C evolved from fumigated soil) (Vance *et al.*, 1987b).
[c]E_C = [(organic C extracted from fumigated soil)—(organic C extracted from non-fumigated soil)].

clay). Soils 7 and 10 came from sites that had been used in the earlier work (Powlson and Jenkinson, 1976). Details of the Meathop Wood site are given by Vance *et al.* (1987a) and the Park Grass Experiment by Warren and Johnston (1964).

Measurement of biomass C, N and ATP

Biomass C (B_C) was measured by the fumigation–incubation method (Jenkinson and Powlson, 1976b), from the relationship $B_C = F_C/k_C$ where F_C = [(CO_2-C evolved from fumigated soil, 0–10 days) − (CO_2-C evolved from non-fumigated soil)] and k_C, the proportion of microbial C evolved as $CO_2 = 0.45$ for 10-day incubations at 25°C (Jenkinson and Ladd, 1981). Each soil received the conventional inoculum (*ca* 10 mg) of the corresponding non-fumigated soil. For soils 7–9, which received a conditioning incubation, the 0–10 day period was used as the control. For soils 1–5, the 10–20 day period was used as control because the soils had been stored at −15°C and not given a conditioning incubation before fumigation (Powlson and Jenkinson, 1976). For the strongly acidic Geescroft Wilderness soil (soils 6 and 10), biomass C was calculated from B_C = (CO_2-C evolved from fumigated soil, 0–10 days)/0.45) (Vance *et al.*, 1987b). Biomass was measured on three replicate portions of moist soil, each containing 25 g oven dry soil.

Organic C rendered extractable to 0.5 M K_2SO_4 by fumigation was measured as described by Jenkinson and Powlson (1976a). Briefly, moist soil was exposed to $CHCl_3$ for 24 h, the fumigant removed and the soil then extracted with 0.5 M K_2SO_4; a non-fumigated control was extracted under the same conditions at the time fumigation commenced. Organic C in the extracts was determined by dichromate digestion. A full description of the procedure used to measure C rendered extractable by $CHCl_3$ is given below.

Total N rendered extractable by fumigation (soils 7–10 only) was measured (Brookes *et al.*, 1985) on the same 0.5 M K_2SO_4 soil extracts used for measurements of extractable C. At the same time, soil ATP (again soils 7–10 only) was extracted with the trichloroacetic acid-phosphate-paraquat reagent (Jenkinson and Oades, 1979) and measured according to Tate and Jenkinson (1982).

RESULTS AND DISCUSSION

Relationship between biomass C and organic C rendered extractable by $CHCl_3$

The amounts of CO_2-C evolved and C extracted by 0.5 M K_2SO_4 from fumigated and non-fumigated soils are shown in Table 1. Meaned over the ten soils, E_C was about 70% of F_C, so that rather less C was rendered extractable by 24 h $CHCl_3$-fumigation than was mineralized during the 10 day incubation. Presumably some non-extractable portions of the killed biomass also contribute to F_C. Similarly, Brookes *et al.* (1985) found that E_N; defined as [(total N extracted by 0.5 M K_2SO_4 from soil fumigated for 24 h) − (total N extracted from non-fumigated soil)] was about 80% of F_N; defined as [(N mineralized by fumigated soil incubated for 10 days) − (N mineralized by non-fumigated soil incubated under the same conditions)].

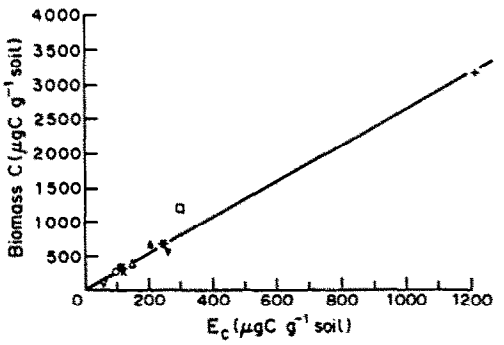

Fig. 1. Relationship between microbial biomass C, as measured by fumigation–incubation and organic C released by $CHCl_3$, as extracted by 0.5 M K_2SO_4 (E_C). For symbols see Table 1.

The relationship between biomass C, measured by fumigation–incubation, and E_C is shown in Fig. 1. There was a close linear relationship between the two variables, with the regression accounting for 98.6% of the variance in the data and the intercept not significantly different from zero. The regression equation (Fig. 1), calculated with the regression fitted through zero was:

$$\text{Biomass C} = (2.64 \pm 0.060)\, E_C. \qquad (1)$$

This regression contained data from one soil (5; a grassland soil with nearly 10% organic C) that contained more than three times as much biomass C as any of the others (Powlson and Jenkinson, 1976). If this soil is omitted, the regression equation becomes: Biomass $C = (2.78 \pm 0.140)\, E_C$, accounting for 90% of the variance in the data. The two regression equations are clearly so similar that there is little justification for considering soil 5 atypical and it is therefore included in equation (1).

Relationship between ATP and C rendered mineralizable or extractable by $CHCl_3$

ATP was measured in soils 7–10 only. In these soils (Table 2) there was the customary close relationship between ATP (as measured following extraction with TCA) and microbial biomass C, as measured by the fumigation method, with a mean value for the four soils of 10.1 μmol ATP g^{-1} biomass C. This value is comparable to the ATP concentration of 10.6 found for arable and grassland soils (Jenkinson *et al.*, 1979; Tate and Jenkinson, 1982) and to that (9.1) in a range of neutral and acid forest soils (Vance *et al.*, 1987b).

The ratio (ATP)/(E_C) was remarkably constant in all four soils, with a mean value of 0.026. This is strong independent evidence that both ATP and the organic C rendered extractable by $CHCl_3$ come from the same fraction of the soil organic matter—the soil microbial biomass.

Biomass C in strongly acid soils

The constancy of the (ATP)/E_C ratio in soils 7–10 suggests that a similar fraction of the soil microbial biomass is rendered extractable by $CHCl_3$ in soils that differ widely in pH. The close relationship between biomass C and E_C in Fig. 1 was only obtained when biomass C was calculated by the *modified* fumigation–incubation procedure for the two strongly acidic soils (Nos 6 and 10) (Vance *et al.*, 1987b). In this modification, biomass C in strongly acid soils is calculated from the CO_2-C released by fumigated soil, without subtraction of a control. If a control is subtracted in the usual way, the calculated biomasses in soils 6 and 10 are very small and both the ATP concentration of this biomass and the ratio E_C/F_C becomes unreasonably large. These results are in accord with the conclusions of Powlson and Jenkinson (1976) and Vance *et al.* (1987a) that the original fumigation–incubation method for measuring microbial biomass gives low results in strongly acid soils.

The relationship between F_C and E_N

The organic material rendered extractable by $CHCl_3$ is relatively rich in N (C:N ratios 3.2–6.6; Table 3), compared to either the soil organic matter as a whole or the calculated C:N ratios of the soil microbial biomass (Table 3). The biomass C:N ratios in Table 3 are calculated from E_C and E_N, assuming that biomass $C = 2.64\ E_C$ [equation (1)] and that biomass $N = 1.85\ E_N$ (Brookes *et al.*, 1985, where this relationship is given as $B_N = E_N/0.54$), so that their correctness depends on the validity of these relationships. The mean C:N ratio of the soil microbial biomass for the four soils in Table 3 is 6.2 but this mean conceals considerable variability and the biomass in the strongly acid soil 10 had a markedly wider C:N ratio than the others. Whether this wider C:N ratio is general in strongly acid soils or is a feature of this particular soil remains to be seen.

Proposed method for measuring microbial biomass C in soil by extraction

Six portions of moist soil, each containing 50 g oven-dry soil, are weighed into 100 ml glass beakers. Three of these serve as controls and are extracted immediately with 200 ml 0.5 M K_2SO_4, as below. The other three are fumigated in a desiccator (lined with wet filter paper to maintain humidity) containing about 25 ml ethanol-free $CHCl_3$ in a small beaker

Table 2. Biomass C, ATP and E_C in soils 7–10

Soil No.	Biomass C (μg C g^{-1} soil[a])	ATP (nmol g^{-1} soil[a])	ATP content of soil biomass, (μmol ATP g^{-1} biomass C)	E_C (μg C g^{-1} soil[a])	ATP/E_C
7	656[b] ± 70.2	6.64 ± 0.854	10.1	210 ± 24.9	0.032
8	580[b] ± 90.1	6.42 ± 0.511	11.1	265 ± 41.8	0.024
9	671[b] ± 79.6	6.03 ± 0.524	9.0	248 ± 28.5	0.025
10	289[c] ± 34.8	2.96 ± 0.097	10.2	119 ± 18.1	0.025

[a]Mean ± SD.
[b]Biomass C measured by fumigation–incubation: control subtracted.
[c]Biomass C measured by fumigation–incubation: control not subtracted—see text.

Table 3. The relationship between C rendered extractable by fumigation (E_C) and N rendered extractable by fumigation (E_N) in soils 7–10

Soil No.	E_C (μg C g^{-1} soil[b])	E_N (μg N g^{-1} soil[b])	E_C/E_N	C:N ratio of biomass[a]	C:N ratio of soil
7	210 ± 24.9	66 ± 7.7	3.2	4.5	11.0
8	265 ± 41.8	69 ± 7.7	3.8	5.5	12.6
9	248 ± 28.5	67 ± 7.2	3.7	5.3	11.9
10	119 ± 18.1	18 ± 4.8	6.6	9.4	14.7

[a]Assuming that biomass C = 2.64 E_C [equation (1)] and that biomass N = 1.85 E_N (Brookes *et al.*, 1985).
[b]Mean ± S.D.

with a few boiling chips. The desiccator is evacuated until the $CHCl_3$ has boiled for 2 min and then placed in the dark at 25°C. After 24 h, the beaker of $CHCl_3$ is removed and residual $CHCl_3$ vapour in the soil removed by repeated evacuation before extraction. For extraction, soil is transferred to a 350 ml plastic bottle, 200 ml 0.5 M K_2SO_4 added, the bottles shaken on an oscillating shaker for 30 min and the suspensions filtered (Whatman No. 42). Organic C in the extracts is determined by digesting the filtered extract (8 ml) with 66.7 mM $K_2Cr_2O_7$ (2 ml), HgO (70 mg) and a mixture (15 ml) of two parts H_2SO_4 (98% acid) and one part H_3PO_4 (88% acid). The mixture is boiled gently under reflux for 30 min, allowed to cool and diluted with 20–25 ml water, added through the condenser as a rinse. The excess dichromate is determined by back-titration with ferrous ammonium sulphate (33.3 mM is convenient) in 0.4 M H_2SO_4, using 25 mM 1,10-phenanthroline-ferrous sulphate-complex solution (supplied by BDH Ltd) as indicator. The acidified ferrous ammonium sulphate solution is standardised against the 66.7 mM $K_2Cr_2O_7$ ('cold blank'). The amount of dichromate consumed is that remaining in a blank digestion with 8 ml 0.5 M K_2SO_4 ("hot blank"), less that remaining in the digest of the extract. Extractable C is calculated assuming that 1 ml 66.7 mM $K_2Cr_2O_7$ (i.e. 1 ml of 0.4 N $K_2Cr_2O_7$) is equivalent to 1200 μg C and biomass C from the relationship Biomass C = 2.64 E_C, where E_C is the difference between C extracted from the fumigated and non-fumigated treatments, both expressed as μg C g^{-1} oven dry soil.

It is essential to remove ethanol from the $CHCl_3$ before use (see Jenkinson and Powlson, 1976b) as commercially-available $CHCl_3$ always contains ethanol as a stabilizer. All operations with $CHCl_3$ should be performed in an efficient fume cupboard. CH_3Br can be used in place of $CHCl_3$, giving almost identical results (Powlson and Jenkinson, 1976), but is less convenient and more toxic. The HgO is added to eliminate interference from halides (Quinn and Salomon, 1964) and is necessary because pure $CHCl_3$ decomposes rapidly to phosgene and HCl. If more than 75% of the dichromate is consumed by 8 ml of extract, the determination must be repeated with less extract, a corresponding volume of 0.5 M K_2SO_4 being added to give a total of 8 ml. The 0.5 M K_2SO_4 extracts should preferably be analyzed immediately after extraction, although they can be stored at 1–2°C for 1–2 wk if necessary. A white precipitate (presumably hydrated $CaSO_4$) forms on storage of extracts from some soils. This precipitate can be dispersed by ultrasonification and does not interfere with the subsequent analysis.

CONCLUSIONS

A new method is proposed for estimating microbial biomass C in soil from the C rendered extractable by exposure to $CHCl_3$. Because this method has only been tested on 10 soil samples, of which two were taken at different times from the same sites, it should be treated with reserve until its validity has been established on a wider range of soils, particularly on soils of high clay content. The new method may prove useful in acid soils, in freshly sampled soils and in soils recently amended with substrates, where the fumigation–incubation technique breaks down.

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