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DO PHYSICAL PROPERTIES OF SOIL AFFECT CHLOROFORM EFFICIENCY IN LYSING MICROBIAL BIOMASS?

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Summary—Evolution of CO_2 was measured *during* a 24 h $CHCl_3$ fumigation at 25°C in eight different soils (*CO_2-F*) The cumulative production of such CO_2 was always lower than that evolved under the same conditions by unfumigated soils (*CO_2-NF*). However, for five of the eight soils, *CO_2-F* accounted for more than 50% of *CO_2-NF*. Neither *CO_2-F* nor *CO_2-NF* were singly or multiply correlated to soil pH, water holding capacity, Hg porosity and carbonate content, i.e. chemico-physical variables that could influence the release of CO_2 from soil. Also other considerations suggest that abiological evolution of CO_2 was likely to be negligible; thus, both *CO_2-F* and *CO_2-NF* were mostly of biological origin and, probably, the biomass surviving chloroform exposure, or partially lysed, was substantial. The ratio *CO_2-F* to *CO_2-NF* was taken as an indirect physiological assessment of the efficiency of $CHCl_3$ in lysing microbial cells as, in principle, the lower *CO_2-F* compared to *CO_2-NF* the more efficient the chloroform fumigation. This ratio was significantly multiply correlated with several combinations of independent variables, including a wide range of soil physico-chemical properties (Hg porosity, storage porosity, water holding capacity, sand, silt, organic C and carbonate contents) related to soil structure. These significant correlations were functionally confirmed because the dynamics of the *CO_2-F* evolution *during* fumigation was related to the relative structural stability of the soils. Other correlations seemed to indicate that the positive relationships reported earlier between clay content and organic C made extractable by $CHCl_3$ (E_C: from which biomass C is calculated in the FE method for estimating soil microbial biomass) could be largely dependent on the relative ability of chloroform to permeate pores in different soils. It is highly probable that the efficiency of $CHCl_3$ in lysing microbial cells is strongly influenced by the soil structural properties. This could be one of the reasons why the calibration procedures for calculating K_C and K_{EC} generate quite dissimilar values. © 1997 Elsevier Science Ltd

INTRODUCTION

The development of physiological and indirect-extraction techniques for estimating the carbon immobilized in the soil microbial biomass (B_C) has overcome some of the problems inherent in direct microscopical methods, such as conversion of observed biovolume to biomass carbon, objective reproducibility of results and tediousness (Jenkinson and Ladd, 1981). The two most used indirect methods employ chloroform vapour to selectively lyse the living part of the organic matter, i.e. the microbial biomass, without altering the decomposability of the non-living organic components of the soil (Jenkinson, 1966). The C released by $CHCl_3$ fumigation is then measured either as the C mineralized during 10 days of incubation at 25°C or as the C extracted by saline solution. These approaches are known as the fumigation–incubation (FI) and the fumigation–extraction (FE) methods, respectively (Jenkinson and Powlson, 1976a; Vance *et al.*, 1987).

A soil moisture of from 50 to 60% of the water holding capacity has been proposed as a standard condition for fumigation (Jenkinson, 1988), but this does not prevent large differences in the fraction of the $CHCl_3$-killed biomass subsequently mineralized (K_C) or extracted by K_2SO_4 (K_{EC}) in particular soils (Voroney *et al.*, 1993; Powlson, 1994; Wardle and Ghani, 1995; Smith *et al.*, 1995). It is not strictly necessary that $CHCl_3$ lyses completely all the living soil microbial biomass when comparing the effects of agricultural practices, chemical or climatic factors on B_C of a given soil (Smith and Paul, 1990), since this implies a comparison within the same type of soil and thus an absolutely exact estimation of B_C is not required. The efficiency of $CHCl_3$ in lysing soil microbial biomass has not yet been adequately investigated and only problems arising from the assessment of K_C and K_{EC} have been acceptably solved (Martens, 1995; Joergensen, 1996). Jenkinson (1966) found that prolonged exposure to $CHCl_3$ of soil did not significantly

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increase the evolution of CO_2 and concluded that effectively all organisms are killed under fumigation. Lynch and Panting (1980) showed that the efficiency of $CHCl_3$ in killing bacteria increased from 81.9% in undisturbed soils to 89.1% in sieved soils. Ingham and Horton (1987) found that, while protozoa were undetectable after fumigation, bacteria and fungi were only reduced to 37–79% of their original populations. Martin and Foster (1985) and Foster (1988) showed, using transmission electron microscopy, that some microbes which were either embedded in the mucigel (2–3 μm inside) or deep in micropores were not lysed by chloroform. However, Arnebrant and Schnürer (1990) suggested that $CHCl_3$ fumigation kills all the soil organisms within 12 h because almost all the ATP disappeared within this period; after 2.5 h the soil contained less than 18% of the original ATP. Survival of bacteria inoculated into soil decreased with increasing exposure time to chloroform and depended on whether the cells were introduced into larger or smaller pores (White *et al.*, 1994). Greenfield (1995) by using five species of bacteria and four species of fungi found that considerably less N is extractable by K_2SO_4 from bacterial than from fungal cells after chloroform fumigation.

In principle, the efficacy of $CHCl_3$ in lysing soil microorganisms may depend on a number of soil physico-chemical and biological properties, such as the porosity, the texture, the specific surface, the water holding capacity (the solubility of $CHCl_3$ in water is 8 g l^{-1} at 20°C), the swelling clay content, the microbial community composition and the growth phase of the microorganisms. Microbial debris released by $CHCl_3$ fumigation may be mineralized by surviving microorganisms, or by enzymes which either remain active after cell death or, possibly, are stabilized by interactions with soil colloids (Burns, 1986). Differences among soils in the amount of microbial C mineralized *during* the 24 h chloroform fumigation could lead to different amounts of microbial C being either mineralized to CO_2 during the following 10 d (FI method) or extracted by K_2SO_4 (FE method). A measurement of the CO_2 evolved *during* fumigation could be helpful for assessing the efficiency of $CHCl_3$ vapour in killing microbial biomass.

The aims of this work were: (1) to determine the extent and kinetics of CO_2 evolution *during* fumigation of soil; (2) to study the influence of soil physico-chemical properties on the amount of CO_2 thus evolved; (3) to relate the quantity of organic C made extractable to K_2SO_4 by $CHCl_3$ fumigation (E_C) to soil physico-chemical properties.

MATERIALS AND METHODS

Eight soils with diverse physico-chemical properties were collected from surface horizons (5–20 cm) at different sites in Italy and England (Table 1). The soils were sieved (< 2 mm), stored at 5°C for 1 month and then kept for 1 week in covered, but not sealed, plastic bags at room temperature with the moisture adjusted to 60% of the water holding capacity (WHC). After this period the soil moisture content was between 51 and 55% of WHC.

In order to measure CO_2 evolution from unfumigated soils, an aliquot of fresh soil equivalent to 250 g oven-dry weight soil was introduced into a desiccator (21 cm dia) containing 30 ml of distilled water on the bottom and a glass beaker with 4 ml of 1 M NaOH. Determination of CO_2 evolved *during* fumigation was carried out as above, but with a glass beaker containing 50 ml of $CHCl_3$ stabilized with 2-methyl-2-butene (Merck, Darmstadt, Germany, Kaiser *et al.*, 1992) also placed in the desiccator. Seven separate desiccators were prepared in this way and CO_2 evolution *during* fumigation was determined after 1, 2, 4, 7, 10.5, 16 or 24 h. In addition, there was one control desiccator for each time, containing water, NaOH solution and 50 ml of $CHCl_3$ but no soil, so that the acidifying effect of chloroform could be taken into account. In the case of unfumigated soils, the control desiccator contained only NaOH solution and water. Then all the desiccators were evacuated by a rotary vacuum pump for 110 s, until the $CHCl_3$ had bubbled for 1 min, and sealed before being incubated at 25°C in the dark. The NaOH solutions were removed from the unfumigated soils after 24 h and from the fumigated soils after 1, 2, 4, 7, 10.5, 16 or 24 h. The trapped CO_2 was determined by titration with HCl as described by Badalucco *et al.* (1992a). The desiccator which had been used for the 24 h fumigation was evacuated eight times, each for 1 min, to remove $CHCl_3$; an identical vacuum treament was applied to the unfumigated soils. Both fumigated and unfumigated soil samples were then extracted by 0.5 M K_2SO_4 in order to determine B_C according to the method of Vance *et al.* (1987), except that the excess dichromate was potentiometrically titrated using a platinum electrode and an inflection point procedure (Skoog and West, 1976). Unlike the original FE method (Vance *et al.*, 1987), in which unfumigated soils (control) were immediately extracted by K_2SO_4, in our work they were submitted to the same vacuum and incubation conditions as the fumigated soils before extraction. This was done so that the only difference between fumigated and unfumigated soils was exposure to chloroform.

Soil textural analysis was performed by using the pipet method (Gee and Bauder, 1986). Soil structural stability was assessed on the basis of the residual weight of aggregates with dia > 0.25 mm, measured after wet-sieving by a procedure and an equipment similar to those suggested by Kemper and Rosenau (1986). Soil stability was expressed as

Table 1. Some physical and chemical properties of soils. Organic C, total N, cation exchange capacity (CEC), pH (water) were determined as reported by Badalucco *et al.* (1992b)

Soil	Site (Country)	USDA classification	Vegetation cover	Sand	Silt	Clay	Organic C	Total N	CEC*	pH	Carbonates	WHC†	Hg porosity‡	Storage porosity‡	SSA§	RSS¶
				%			%				%					
1	Vico (I)	Typic Udivitrand	*Quercus cerris* L.	85.6	10.9	3.5	4.31	0.32	34.7	5.5	2.8	91.5	234.4	145.6	10.3	1.55
2	S. Matteo (I)	Typic Melanudand	*Quercus cerris* L.	78.9	17.8	3.3	9.18	0.71	51.9	6.5	4.0	105.7	75.5	47.8	8.0	1.00
3	Faggeta (I)	Typic Hapludand	*Fagus sylvatica* L.	46.7	31.6	21.7	2.40	0.17	21.4	5.2	3.0	83.6	184.4	90.0	16.4	2.73
4	S. Martino (I)	Andic Dystrochrept	*Castanea sativa* L.	73.0	14.1	12.9	2.63	0.19	23.1	6.8	3.8	75.2	174.4	85.6	16.6	1.50
5	Romola (I)	—	Fallow	81.9	6.7	11.4	0.71	0.07	25.3	7.2	4.2	45.0	68.4	51.7	8.3	1.23
6	Pisa (I)	—	Winter wheat	55.0	21.3	23.7	0.69	0.11	18.8	7.7	13.9	43.8	63.0	24.4	10.4	1.95
7	Bologna (I)	—	Maize	31.9	34.3	33.8	0.58	0.15	26.9	8.0	8.5	45.5	72.9	20.8	21.4	3.73
8	Wye (U.K.)	—	Maize	20.6	56.9	22.5	2.61	0.16	26.9	8.1	59.3	49.6	124.1	72.4	8.1	3.41

*cmol (+) kg^{-1} dry soil.
†Water holding capacity, ml H_2O 100 g^{-1} dry soil.
‡mm^3 g^{-1} dry soil.
§Specific surface area, m^2 g^{-1} dry soil.
¶Relative structure stability.

relative structural stability (RSS) by assigning the value 1 to the least structural stability measured among the eight soils analysed. Pore-size distribution was determined in the range 7.4 nm to 50 μm of equivalent pore diameter (E.P.D.) by the mercury intrusion method (Lawrence, 1977) using a Carlo Erba Porosimiter 2000. Total volume of pores (Hg Porosity) and the volume of pores between 0.5 and 50 μm of E.P.D. were also measured (Table 1). These, according to Greenland and Hayes (1981), correspond to a class of storage pores (Storage Porosity). Water holding capacity (WHC) consisted of the total water content of soil equilibrated for 24 h with a body of free water through capillarity and under atmospheric pressure. Specific surface area was measured by N_2 adsorption at 770K with a Carlo Erba Sorptomatic 1900 apparatus (Brunauer *et al.*, 1938). Total carbonate content was determined by using the Dietrich–Fruehling calcimeter which measures the CO_2 evolved after treating soil with concentrated HCl.

Each experiment was carried out in triplicate. Correlation and multiple regression analyses were performed by the computer program Excel 4.0 (Microsoft, U.S.A.).

RESULTS AND DISCUSSION

The cumulative evolution of CO_2 *during* the 24 h $CHCl_3$ fumigation (CO_2-*F*) was always lower than that produced, under the same conditions, by unfumigated soils (CO_2-*NF*); moreover, for five of the eight soils used CO_2-*F* was more than 50% of CO_2-*NF*. The lowest percentage (20.2) was observed in S. Matteo soil and the highest (85.2) in Wye soil (Table 2). It is possible that some of the CO_2 evolved by both fumigated and unfumigated soils came partly from dissolved CO_2 that was present in soil air or solution (as CO_2, HCO_3^- and CO_3^-) at the beginning of the 24 h incubation. However, such CO_2 cannot be dominant for three reasons: (a) most of the gaseous CO_2 in the soil air and solution was certainly removed from the desiccators during their evacuation for 110 s by the rotary vacuum pump before starting the incubation; (b) neither CO_2-*F* nor CO_2-*NF* were significantly correlated with pH, WHC, Hg porosity or carbonate content, i.e. physico-chemical variables influencing the release of CO_2 from soil (Table 3); (c) neither $CO_2 - F$ nor $CO_2 - NF$, considered as dependent variables, were multiply significantly correlated with all above four independent variables [Table 4, eqns (1) and (2)]. The release of CO_2-*F* from carbonates reacting with $CHCl_3$ was excluded because no CO_2 was detected by g.c. (thermal conductivity detector) in a suspension consisting of 1 ml of water, 0.2 g of pulverized $CaCO_3$ and 1 ml of $CHCl_3$ during a 24 h incubation at room temperature and under agitation. Thus, since abiological evolution of CO_2

Table 2. Carbon dioxide evolved during 24 h at 25°C in unfumigated (CO_2-*NF*) and chloroform fumigated (CO_2-*F*) soils; organic C extracted by K_2SO_4 from unfumigated (E_C-NF) and fumigated soils (E_C-*F*). E_C = (E_C-*F*) minus (E_C-*NF*)

Soil no.	CO_2-*NF*	NO_2-*F*	E_C-*NF*	E_C-*F*	E_C	CO_2-*F* to CO_2-*NF*	CO_2-*F* to E_C
	μCO_2-C g^{-1} dry soil		μg C g^{-1} dry soil				
1	29.3 ± 1.7*	19.0 ± 1.7	70.5 ± 3.4	134.7 ± 10.7	64.2	0.648	0.296
2	31.2 ± 2.8	6.3 ± 0.8	95.1 ± 8.3	128.4 ± 9.4	33.3	0.202	0.189
3	14.0 ± 1.6	8.7 ± 0.6	107.4 ± 11.5	192.1 ± 14.9	84.7	0.621	0.103
4	19.7 ± 2.0	10.8 ± 0.9	52.2 ± 5.6	125.6 ± 11.5	73.4	0.548	0.147
5	6.8 ± 1.0	3.0 ± 0.4	26.3 ± 3.6	44.6 ± 4.2	18.3	0.441	0.164
6	23.7 ± 2.2	14.8 ± 1.1	8.4 ± 1.1	51.9 ± 5.5	43.5	0.624	0.340
7	35.7 ± 3.1	14.8 ± 1.6	27.2 ± 2.9	122.4 ± 8.8	95.2	0.415	0.156
8	22.3 ± 2.5	19.0 ± 1.5	65.0 ± 6.4	121.9 ± 5.2	56.9	0.852	0.334

* Standard deviation.

seems negligible, the above considerations substantiate the hypothesis that both CO_2-*F* and CO_2-*NF* were mostly of biological origin and, consequently, the biomass surviving chloroform was substantial.

It is well known that the chloroform fumigation causes a marked decrease in the size of the living microbial biomass and that microorganisms surviving fumigation are usually located at sites where they are protected from $CHCl_3$ vapour (Jenkinson, 1988; Foster, 1988). Three hypotheses (not necessarily mutually exclusive) may be suggested to explain the CO_2-*F* evolution pattern: (1) it is the result of respiratory activity of those microorganisms which had not yet been killed by fumigation; (2) the enzymes from killed microorganisms are active during the fumigation period and metabolic processes, such as C mineralization, are still occurring; and (3) CO_2 is evolved abiotically as a result of the change in partial pressure of CO_2 in the soil atmosphere due to the incubation in the presence of NaOH. There should be no substantial difference between the CO_2-*F* produced through (1) and (2) above, as both are attributable to microbial biomass, although in different physiological states (still living, lysing or lysed). If we accept that the exposure to $CHCl_3$ alone does not induce an increase in the release of abiotic CO_2 from soil, for example by altering soil chemical and/or physical properties, it is reasonable to assume that the sum of the CO_2 present at the beginning of incubation *plus* that likely produced abiotically during the incubation in a fumigated sample, driven by the presence of NaOH, should be very similar to that of the respective unfumigated sample; so that the decrease in CO_2 production by fumigated soils compared to unfumigated soils is likely to be mainly caused by the effects of exposure to chloroform on microbial biomass survival and activity, *unless most of the* CO_2-*F is of abiotic origin*, but this is highly unlikely due to the above considerations.

In principle, the smaller CO_2-*F* is, relative to CO_2-*NF*, the more efficient is chloroform in lysing microbial cells or inhibiting enzymes involved in the respiratory processes. If we disregard hypotheses (2) and (3), which appears legitimate, the ratio CO_2-*F* to CO_2-*NF* may be taken as an indirect physiological assessment of the efficiency of chloroform in lysing microbial cells; consequently, the efficiency is maximal when that ratio is near 0 (that is when microorganisms are totally and sharply lysed) and minimal when near 1 (that is when microorganisms are lysed in low number and slowly).

As shown in Table 3, the CO_2-*F*-to-CO_2-*NF* ratio was not significantly singly correlated to any of the measured soil properties, whereas it was significantly multiply correlated to several combinations of independent variables including a wide range of soil physical properties related to soil structure (Table 4). In each of the four significant multiple correlations found, a measure of either Hg or storage porosity is involved, thus suggesting that the lysing efficiency of chloroform could be influenced by its diffusion path through soil pores. Probably soil organic matter played a major role in retaining water as the WHC was positively correlated with both total organic C and total N (Table 3). The negative correlation between WHC and total clay content suggests that swelling clays were unimportant in our soils. The significant involvement of the WHC in the multiple correlation with the CO_2-*F*-to-CO_2-*NF* ratio [Table 4, eqn (3)] is likely caused by the appreciable solubility of $CHCl_3$ in water (8 g l^{-1} at 20°C), since soils were incubated at 51–54% WHC and usually both microbial biomass and activity are positively related to intermediate soil moisture contents (Lund and Goksoyr, 1980). Besides porosity and WHC, also sand, silt, total organic C and carbonate contents play a significant role in the multiple correlation with the CO_2-*F*-to-CO_2-*NF* ratio [Table 4, eqns (4)–(6)]. The last four variables are crucial for establishing the soil texture, aggregation and structure, and thus they may regulate, even indirectly, the diffusion path of both chloroform and CO_2 during soil fumigation.

In order to investigate the statistically significant correlations found, the dynamics of CO_2 evolution *during* fumigation was followed in soils 1, 2 and 3. As the respective values of the ratio CO_2-*F* to CO_2-*NF* were 0.648, 0.202 and 0.621 (Table 2), we could expect the efficiency of chloroform in lysing microbes to be greater in soil 2 than in soils 1 and 3. The relative structural stabilities (RSS) of these

Table 3. Matrix of linear correlation coefficients (r) and significance levels (P) between the various physiological, chemical and physical properties determined in the eight soils used

	CO_2-NF	CO_2-F	CO_2-F to CO_2-NF	E_C-NF	E_C-F	E_C	CO_2-F to E_C	pH	Sand%	Silt%	Clay%	Org C	Tot N	CEC	$CaCO_3$%	WHC	SSA	Hp Por	Stor Por	(Hg-Stor) Por
CO_2-F	0.542																			
CO_2F to CO_2-NF	−0.233	0.677[d]																		
E_C-NF	0.017	−0.120	−0.046																	
E_C	0.221	0.165	0.106	0.841[b]																
CO_2-F to E_C	0.379	0.459	0.255	0.193	0.693[d]															
pH	0.263	0.696[d]	0.540	−0.311	−0.391	−0.296														
Sand%	0.170	0.206	0.081	−0.716[c]	−0.607	−0.147	0.361													
Silt%	−0.183	−0.464	−0.480	0.071	−0.226	−0.505	−0.162	−0.521												
Clay%	0.202	0.506	0.529	0.161	0.339	0.401	0.262	0.419	−0.942[a]											
Org C	0.111	0.293	0.295	−0.408	0.001	0.546	−0.028	0.556	−0.857	0.634[d]										
Tot N	0.374	−0.162	−0.461	0.686[d]	0.365	−0.251	−0.020	−0.409	0.404	−0.148	−0.702[d]									
CEC	0.496	−0.175	−0.590	0.586	0.308	−0.223	−0.047	−0.335	0.392	−0.182	0.622	0.978[a]								
carbonates%	0.487	−0.172	−0.607	0.488	0.185	−0.314	−0.013	−0.221	0.395	−0.179	−0.634[d]	0.921[b]	0.949[a]							
WHC	0.028	0.528	0.651[d]	−0.045	−0.053	−0.036	0.598	0.570	0.700[d]	0.832[c]	0.336	−0.128	−0.210	−0.414						
SSA	0.231	−0.162	−0.347	0.821[c]	0.627[d]	0.043	−0.252	−0.788[c]	0.515	−0.309	−0.711[c]	0.860[b]	0.813[c]	0.692[d]	−0.414					
Hg Por	0.250	0.090	−0.090	−0.077	0.422	0.867[b]	−0.572	−0.016	−0.346	0.124	0.605	−0.397	−0.309	−0.383	−0.327	−0.119				
Stor Por	−0.047	0.363	0.454	0.541	0.649[d]	0.455	−0.041	−0.724[c]	0.203	−0.078	−0.347	0.139	0.014	−0.062	−0.122	0.529	0.140			
(Hg − Stor) Por	−0.109	0.295	0.405	0.537	0.493	0.180	0.074	−0.720[c]	0.367	−0.182	−0.565	0.256	0.114	0.103	−0.085	0.561	−0.143	0.946[a]		
RSS	0.046	0.393	0.443	0.454	0.747[c]	0.748[c]	−0.190	−0.604	−0.054	0.077	0.007	−0.042	−0.124	−0.274	−0.152	0.396	0.498	0.900[b]	0.710[c]	
	0.260	0.527	0.456	−0.075	0.318	0.676[d]	0.059	0.405	−0.944[a]	0.852[b]	0.866[b]	−0.475	−0.445	−0.408	0.545	−0.485	0.520	−0.054	−0.232	0.196

See Tables 1 and 2 for abbreviations.
[a]Significant at $P < 0.001$.
[b]Significant at $P < 0.01$.
[c]Significant at $P < 0.05$.
[d] Significant at $P < 0.1$.

Table 4. Multiple regression equations, r^2 adjusted for freedom degrees and significance (P) of coefficients by considering CO_2-NF, CO_2-F, $(CO_2-F$ to $CO_2-NF)\times 100$ and E_C as dependent variables, and pH (water), $CaCO_3$ (%), WHC, Hg Por, Stor Por, Sand (%), Silt (%), Clay (%), Org C as independent variables

No.	Equation	Adjusted r^2	P				
			a	b	c	d	e
1	$CO_2\text{-}NF = (13.321 \pm 9.368)\times(\text{pH}) + (-0.177 \pm 0.293)\times(CaCO_3) + (0.403 \pm 0.265)\times(\text{WHC}) + (0.068 \pm 0.103)\times(\text{Hg Por}) - 102.211 \pm 83.538$	−0.227	0.1980	0.5652	0.1723	0.5299	0.2607
2	$CO_2\text{-}F = (4.676 \pm 4.954)\times(\text{pH}) + (0.056 \pm 0.155)\times(CaCO_3) + (0.021 \pm 0.140)\times(\text{WHC}) + (0.087 \pm 0.054)\times(\text{Hg Por}) - 33.035 \pm 44.177$	0.1064	0.3767	0.7284	0.8836	0.1553	0.4790
3	$(CO_2\text{-}F \text{ to } CO_2\text{-}NF)\times 100 = (0.261 \pm 0.087)\times(\text{Hg Por}) + (-0.643 \pm 0.233)\times(\text{WHC}) + 65.201 \pm 14.302$	0.5604	0.0199	0.0280	0.0026		
4	$(CO_2\text{-}F \text{ to } CO_2\text{-}NF)\times 100 = (-0.574 \pm 0.234)\times(\text{Sand}) + (0.319 \pm 0.141)\times(\text{Stor Por}) + 66.940 \pm 14.487$	0.4692	0.0437	0.0576	0.0024		
5	$(CO_2\text{-}F \text{ to } CO_2\text{-}NF)\times 100 = (0.308 \pm 0.108)\times(\text{Stor Por}) + (0.672 \pm 0.264)\times(\text{Silt}) + (-3.685 \pm 1.535)\times(\text{Org C}) + 28.118 \pm 11.559$	0.6694	0.0294	0.0383	0.0474	0.0452	
6	$(CO_2\text{-}F \text{ to } CO_2\text{-}NF)\times 100 = (0.667 \pm 0.146)\times(CaCO_3) + (-3.122 \pm 0.988)\times(\text{Org C}) + (0.177 \pm 0.043)\times(\text{Hg Por}) + 33.060 \pm 6.790$	0.8565	0.0025	0.0159	0.0044	0.0018	
7	$E_C = (0.292 \pm 0.091)\times(\text{Hg Por}) + (1.959 \pm 0.558)\times(\text{Clay}) - 10.172 \pm 10.720$	0.6793	0.0148	0.0099	0.5839		
8	$E_C = (0.297 \pm 0.103)\times(\text{Hg Por}) + (-2.241 \pm 1.200)\times(\text{Silt}) + (-2.103 \pm 0.813)\times(\text{Sand}) + 200.397 \pm 72.644$	0.6065	0.1041	0.0230	0.0361	0.0282	

See Table 1 and Table 2 for abbreviations.

three soils were 1.6, 1 and 2.7, respectively (Table 1). This determination was reliable since, as expected, RSS was significantly negatively correlated to sand content and positively correlated to both silt and clay content (Table 3). During the first 4 h of fumigation, the CO_2 evolution rates were similar if the CO_2 production was expressed as a percentage of that evolved by the end of the fumigation (24 h). For example, the percent CO_2 evolved during the first 4 h of fumigation was 31.6, 30.9 and 33.5 of the total evolved in 24 h in soils 1, 2 and 3, respectively. The CO_2 evolved between 0 and 10.5 h from soil 2 was 73.8% of that evolved during the entire 24 h fumigation, whereas from soil 1 it was 42.2% and from soil 3 it was 47.9%; between 10.5 and 24 h, the rate of CO_2 evolution was markedly lower in the soil 2, which shows the poorest structural stability (Fig. 1). These results suggest that CO_2 evolution is related to the spatial location of microorganisms in soil, thus probably depending on different physical and structural properties. Exposure to $CHCl_3$ for 4 h at comparable concentrations were probably sufficient to kill microbes at accessible sites, such as on the surface of organo-mineral particles and in the larger pores (White *et al.*, 1994). Once these less-protected microorganisms were killed, the porosity and consequently the tortuosity of the $CHCl_3$ diffusion path became a discriminating factor in determining the rate at which the

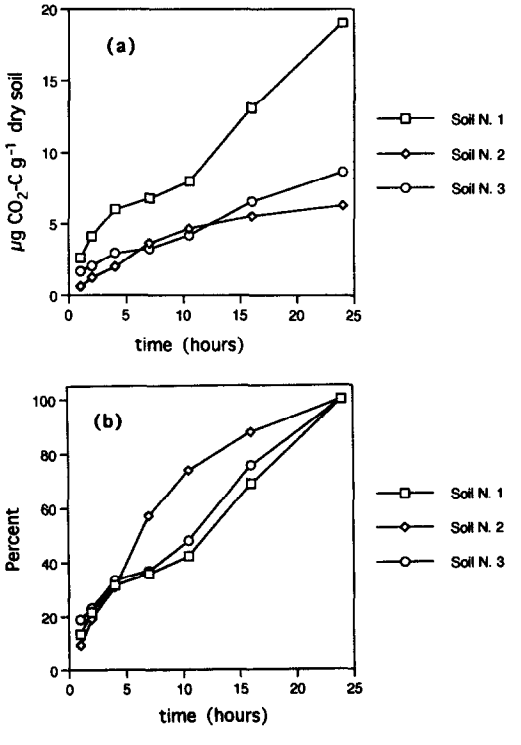

Fig. 1. Cumulative (a) and percent of total (b) CO_2 evolved *during* 24 h $CHCl_3$ fumigation at 25°C in soils 1, 2 and 3 (see Table 1 for details).

surviving microorganisms were progressively lysed. Thus, the efficiency of chloroform in lysing microbes was comparable in all three soils during the first 4 h of fumigation, but greater for soil 2 than for soils 1 and 3 between 4 and 24 h.

The ratio CO_2-F to E_C, where E_C is the organic C rendered extractable by $CHCl_3$, that is the basis for estimating B_C by the FE method (Vance *et al.*, 1987), it is a measure of the proportion of E_C which is potentially lost as CO_2-C *during* fumigation. This proportion is not constant, ranging from 10.3 to 34.0% (Table 2), further indicating that exposure to chloroform of soils with different structural, physico-chemical and biological properties (Table 1) may constitutively introduce a factor of variability relating measurements of biomass by FE to those by FI. The extent of such an uncertainty is very similar to that from an accelerated degradation of non-biomass organic C released by $CHCl_3$ exposure, estimated by Jenkinson (1976b) to be, at most, 20%. Such a release, if it occurs, would lead to an overestimation of B_C as measured by the FI method. This assumption was indirectly confirmed by Badalucco *et al.* (1990; 1992b) who argued that some non-biomass sugars were released during $CHCl_3$ fumigation of soil.

Soil pH significantly and negatively influenced the amount of K_2SO_4 extracted organic C in unfumigated soils, E_C-NF (r = -0.716, Table 3). Probably, under more acidic conditions, organic molecules tend to be more protonated, i.e. they are more strongly retained by soil negative colloids and thus less leachable. It was not surprising that the correlation between soil pH and E_C was not significant, since it is widely accepted that K_2SO_4 is able to extract from soil, as an average, only the 38% of the microbial C (Vance *et al.*, 1987).

Extractable organic C released by $CHCl_3$ (E_C) was linearly and positively correlated to (a) the specific surface area (SSA); and to (b) the difference between Hg porosity and storage porosity [(Hg-Stor) Por], that is the volume of pores with dia 0.5 μm (Table 3). It is difficult to explain the significance of the first correlation since several physical, chemical and biological soil factors can influence both microbial biomass and SSA, but it could not be a coincidence that techniques for estimating such variates resort to gas permeation (either $CHCl_3$ vapour or N_2). The correlation (b) seems to confirm that most soil microorganisms are less than 0.5 μm in diameter (Foster, 1988). Moreover, a positive correlation was found between relative structure stability (RSS) and E_C (Table 3) as previously reported by Jocteur-Monrozier *et al.* (1991), although significant only at $P < 0.1$. In addition, the total organic C made extractable by $CHCl_3$ fumigation (E_C-F) was positively and significantly correlated to the difference between Hg porosity and storage porosity (Table 3).

Soil microbial biomass C has been often, but not always, positively correlated to clay content, as in our work (Table 3). That correlation is explained in terms of the high ability of clay to retain soil organic matter and microorganisms (Wardle, 1992). In this work E_C was multiply significantly correlated to Hg porosity, silt and sand contents and (excluding the intercept) to Hg porosity and clay content [Table 4, eqns (7) and (8), respectively]. Thus, our results raise the possibility that the frequent positive correlation found between B_C and clay content could be, to a large extent, caused by the ability of chloroform to permeate soil pores.

In conclusion, it is highly probable that the efficiency of $CHCl_3$ in lysing microbial cells cannot be the same in different soils, since the function CO_2-F to CO_2-NF is strongly influenced by soil structural properties. This could be one of the main reasons why the calibration procedures for calculating K_C and K_{EC} generate quite dissimilar values (Martens, 1995; Wardle and Ghani, 1995; Smith *et al.*, 1995). However, to unambiguously relate chloroform efficiency in lysing microbial cells to soil physical properties, it is essential to follow the release of $^{14}CO_2$ during $CHCl_3$ fumigation from soils amended with ^{14}C-labelled microorganisms occupying either accessible or inaccessible sites. This should lead to $^{14}CO_2$ release at different rates. Such an experiment would also eliminate an alternative explanation of the results we present in this paper. In our experiments, the soils were conditioned for 1 week at room temperature in partially-closed plastic bags. The soils were then incubated for 24 h in the presence of 1 M NaOH, with or without $CHCl_3$. The partial pressure of CO_2 in the soil atmosphere would decrease in the presence of NaOH, leading to decomposition of a certain amount of the HCO_3^- held in the soil solution. The rate at which this abiotic CO_2 was released would also be related to porosity.

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Update

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ERRATUM

Do physical properties of soil affect chloroform efficiency in lysing microbial biomass? by L. Badalucco, F. de Cesare, S. Grego, L. Landi and P. Nannipieri. *Soil Biology & Biochemistry* **29,** 1135–1142 (1997).

The above article contained an error in column 1 of Table 3. The correct table now appears overleaf. The publishers wish to apologise to the authors for any inconvenience or embarrassment caused.

Table 3. Matrix of linear correlation coefficients (r) and significance levels (P) between the various physiological, chemical

	CO_2-NF	CO_2-F	CO_2-F to CO_2-NF	E_C-NF	E_C-F	E_C	CO_2-F to E_C	pH	Sand%	Silt%	Clay%	Org C
CO_2-F	0.542											
CO_2F to CO_2-NF	−0.233	0.677[d]										
E_C-NF	0.017	−0.120	−0.046									
$E_C - F$	0.221	0.165	0.106	0.841[b]								
E_C	0.379	0.459	0.255	0.193	0.693[d]							
CO_2-F to E_C	0.263	0.696[d]	0.540	−0.311	−0.391	−0.296						
pH	0.170	0.206	0.081	−0.716[c]	−0.607	−0.147	0.361					
Sand%	−0.183	−0.464	−0.480	0.071	−0.226	−0.505	−0.162	−0.521				
Silt%	0.202	0.506	0.529	0.161	0.339	0.401	0.262	0.419	−0.942[a]			
Clay%	0.111	0.293	0.295	−0.408	0.001	0.546	−0.028	0.556	−0.857	0.634[d]		
Org C	0.374	−0.162	−0.461	0.686[d]	0.365	−0.251	−0.020	−0.409	0.404	−0.148	−0.702[d]	
Tot N	0.496	−0.175	−0.590	0.586	0.308	−0.223	−0.047	−0.335	0.392	−0.182	0.622	0.978[a]
CEC	0.487	−0.172	−0.607	0.488	0.185	−0.314	−0.013	−0.221	0.395	−0.179	−0.634[d]	0.921[b]
carbonates%	0.028	0.528	0.651[d]	−0.045	−0.053	−0.036	0.598	0.570	0.700[d]	0.832[c]	0.336	−0.128
WHC	0.231	−0.162	−0.347	0.821[c]	0.627[d]	0.043	−0.252	−0.788[c]	0.515	−0.309	−0.711[c]	0.860[b]
SSA	0.250	0.090	−0.090	−0.077	0.422	0.867[b]	−0.572	−0.016	−0.346	0.124	0.605	−0.397
Hg Por	−0.047	0.363	0.454	0.541	0.649[d]	0.455	−0.041	−0.724[c]	0.203	−0.078	−0.347	0.139
Stor Por	−0.109	0.295	0.405	0.537	0.493	0.180	0.074	−0.720[c]	0.367	−0.182	−0.565	0.256
(Hg − Stor) Por	0.046	0.393	0.443	0.454	0.747[c]	0.748[c]	−0.190	−0.604	−0.054	0.077	0.007	−0.042
RSS	0.260	0.527	0.456	−0.075	0.318	0.676[d]	0.059	0.405	−0.944[a]	0.852[b]	0.866[b]	−0.475

See Tables 1 and 2 for abbreviations.
[a]Significant at $P < 0.001$.
[b]Significant at $P < 0.01$.
[c]Significant at $P < 0.05$.
[d] Significant at $P < 0.1$.