PERGAMON

Soil Biology and Biochemistry 31 (1999) 395–405

Soil Biology & Biochemistry

Relationships of chloroform fumigation–incubation to soil organic matter pools

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Accepted 11 September 1998

Abstract

Microbial biomass is part of the active pool of soil organic matter that plays focal roles in decomposition of organic materials, nutrient cycling and biophysical manipulation of soil structure. We compared two commonly used variants of the chloroform fumigation–incubation method in their relationships with other active, passive and total soil C and N pools in soils from Texas, Georgia, Alberta and British Columbia. The relationship of potential C mineralization with chloroform fumigation–incubation without subtraction of a control was much stronger ($r^2 = 0.81 \pm 0.10$ among five data sets with a total of 844 observations) than with subtraction of a control ($r^2 = 0.30 \pm 0.22$). Similarly, the relationship of soil organic C with chloroform fumigation–incubation without subtraction of a control was better ($r^2 = 0.80 \pm 0.13$) than with subtraction of a control ($r^2 = 0.38 \pm 0.32$). Relationships of net N mineralization, flush of N following fumigation–incubation, flush of CO_2-C during the first day following rewetting of dried soil, particulate organic C and N, mean weight diameter of water-stable aggregation and total porosity with chloroform fumigation–incubation were also better without subtraction of a control than with subtraction of a control. In analyses of data taken from published reports, chloroform fumigation–incubation without subtraction of a control was better related with active soil C pools than with subtraction of a control. Chloroform fumigation–incubation without subtraction of a control, unlike that with subtraction of a control, should be considered a more robust method to determine microbial biomass under a wide range of environmental conditions.

1. Introduction

Soil microbial biomass is an important component of soil quality assessment because of its important roles in nutrient dynamics, decomposition of natural and synthetic organic amendments and physical stabilization of aggregates. Numerous methods are now available to estimate this active pool of soil organic matter. Exhaustive reviews are available to describe these methods (Jenkinson and Ladd, 1981; Smith and Paul, 1990; Alef, 1993; Horwath and Paul, 1994; Martens, 1995). However, there is growing concern that some of these methods may not be as reliable as others (Vance et al., 1987b; Couteaux et al., 1990; Wardle and Ghani, 1995; Horwath et al., 1996; Wu et al., 1996), despite the intention of measuring the same pool. The most common methods utilized are variants of chloroform fumigation–incubation (CFI) (Jenkinson and Powlson, 1976b), chloroform fumigation–extraction (Vance et al., 1987a), substrate-induced respiration (Anderson and Domsch, 1978) and adenosine triphosphate (Webster et al., 1984).

Chloroform fumigation–incubation has become the most widely used method for determining microbial biomass C, becoming a citation classic (Martens, 1995). It is the standard by which most other newer methods are compared. However, CFI is not recommended when soils are extremely acidic (i.e. pH < 4.5) (Vance et al., 1987b) or when soils have received recent organic amendments (Martens, 1985). Under these conditions, negative microbial biomass estimates have been calculated as the control evolves more CO_2 than the fumigated sample. Due to this potential aberration, debate has been ongoing as

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0038-0717/99/$19.00
PII: S0038-0717(98)00142-4

to whether a control should be subtracted (Voroney and Paul, 1984) or which control should be subtracted (Jenkinson and Powlson, 1976b; Chaussod and Nicolardot, 1982; Smith et al., 1995) when using CFI. Shan-Min et al. (1987) compared microbial biomass estimates using CFI with four different controls and concluded that all variants were highly related if soil were given a 7-d conditioning incubation. They recommended that a control be subtracted because the magnitude of microbial biomass was similar among estimates using controls derived from unfumigated soil (0–10 d or 10–20 d) or fumigated soil (10–20 d), but 50 to 90% greater using no control. Similarly, Wu et al. (1996) argued that the reason for subtracting a control was that estimates of microbial biomass were larger, often by a factor of 2 or more if no control were subtracted compared with subtraction of a control. This argument implies that CFI with subtraction of a control (CFI/F-C) is accurately proportioned to microbial biomass.

Microbial biomass estimates using CFI/F-C have been calibrated to those using direct microscopic counting (Jenkinson et al., 1976; Vance et al., 1987b). Other workers however, have found that CFI/F-C underestimates microbial biomass compared with direct microscopic counting (Schnürer et al., 1985; Ingham et al., 1991). Compared with direct counting, CFI/F-C resulted in underestimation of microbial biomass, but CFI without subtraction of a control (CFI/F) resulted in overestimation of microbial biomass (Horwath et al., 1996). Martens (1995) stated that direct microscopic counting of microorganisms is fraught with uncertainties, including inability to accurately distinguish between dead and living cells, selection of factors used to convert cell shapes into mass and skills of the investigator. Validating microbial biomass estimates with direct counting, therefore should be used only in combination with other techniques.

Despite the numerous uncertainties about the accuracy of CFI/F-C to estimate microbial biomass, chloroform fumigation–extraction, substrate-induced respiration and adenosine triphosphate methods have been developed and calibrated against it. We have been measuring microbial biomass using CFI in several short- and long-term studies. Our objectives were to compare CFI/F and CFI/F-C to a suite of other active and total soil C and N pools that, according to our current understanding of soil organic matter dynamics, should be intrinsically related to soil microbial biomass. For example, in general soil microbial biomass should be related to steady-state C and N mineralization under standard environmental conditions, because of balances among substrate availability and utilization and microbial population dynamics. Also, in general soil microbial biomass constitutes a portion of total organic C within a fairly narrow range, based on resource allocation and environmental controls. We realize that on a finer scale of examination, there may be small divergences among microbial biomass, C and N mineralization and soil organic C. This may occur due to differences in substrate availability and utilization as a result of various chemical or physical conditions interacting with the soil biological community structure. However in the long-term, steady-state condition of most soils, the relationships among active organic matter pools should become well defined.

2. Materials and methods

Soils were collected from various depth increments to 0.2 m from several long-term management sites (Table 1) in Texas, Georgia, Alberta and British Columbia during April through June of 1992 to 1997, prior to planting of row crops or summer forage growth.

Chloroform fumigation–incubation to estimate soil microbial biomass C was determined with some modifications to the original method proposed by Jenkinson and Powlson (1976b). Smaller quantities of soil (30 to 60 g) were moistened to either −33 kPa or 50% water-filled pore space and placed into 1-l canning jars in the presence of 10 ml of either 0.5 or 1.0 M KOH or NaOH. Conditions other than fumigation were the same between fumigated and unfumigated soil within a data set. Soil was incubated at $25 \pm 1^{\circ}C$. In data sets using dried soil, a conditioning period of 7 to 10 d allowed the soil to establish a steady-state level of microbial activity (Franzluebbers et al., 1996). In data sets using field-moist soil, no conditioning period was used. CO_2-C evolved during 10 d following fumigation was determined by titration of alkali with 0.5 or 1.0 M HCl. The flush of CO_2-C evolved following fumigation was calculated with subtraction of a control (10 to 20 d period if available or during the 0 to 10 d period) as proposed in the original method by Jenkinson and Powlson (1976b) and without subtraction of a control as suggested by Voroney and Paul (1984).

Steady-state C mineralization from the unfumigated soil was determined during a 0 to 10 d period in data sets using undried soil and during either a 7 to 17 d or 10 to 24 d period using dried soil. Carbon mineralization following rewetting of dried soil has been shown to stabilize after $\approx$3 d of incubation at 25°C (Franzluebbers et al., 1996). Soil organic C and N were determined either by dry combustion in soils from Georgia with pH less than 6.5 or by dichromate oxidation with heating to 100°C for 1 h. Particulate organic C and N were determined from material >0.05 mm, similar to the method described by Cambardella and Elliott (1992). The mean weight di-

Table 1
Soil and environmental characteristics of samples

Location	USDA soil classification	pH	Mean annual temperature (°C)	Mean annual precipitation (mm)	Land management
Weslaco TX (26°N, 97°W)	Hidalgo sandy clay loam (fine-loamy, mixed, hyperthermic Typic Calciustoll)	7.6	25	500	Irrigated maize under conventional, reduced and no tillage
Corpus Christi TX (27°N, 97°W)	Victoria clay (fine, montmorillonitic, hyperthermic Udic Pellustert)	8.0	22	765	Sorghum, maize under conventional and reduced tillage with 0 to 60 kg N ha^{-1}
College Station TX (30°N, 96°W)	Weswood silty clay loam (fine, mixed, thermic Fluventic Ustochrept)	8.2	20	978	Sorghum, wheat, soybean under conventional and no tillage
Overton TX (32°N, 94°W)	Bowie fine sandy loam (fine-loamy, siliceous, thermic Plinthic Paleudult)	5.9	20	1050	Bermudagrass hay receiving 0 to 450 kg N ha^{-1} as poultry manure
Stephenville TX (32°N, 98°W)	Windthorst fine sandy loam (fine, mixed, thermic Udic Paleustalf)	6.5	18	750	Bermudagrass hay receiving 0 to 450 kg N ha^{-1} as dairy manure
Farmington GA (33°N, 83°W)	Cecil, Madison and Pacolet sandy loam, loam, and sandy clay loam (clayey, kaolinitic, thermic Typic Kanhapludults)	6.0	17	1250	Bermudagrass pasture receiving N via inorganic, clover, poultry litter sources under low and high grazing pressure
Lubbock TX (33°N, 101°W)	Acuff loam (fine-loamy, mixed, thermic Aridic Paleustoll)	7.4	16	457	Sorghum receiving 0 to 200 kg N ha^{-1}
Watkinsville GA (34°N, 83°W)	Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludult)	6.0	17	1250	Cotton, rye under no tillage with various frequencies and intensities of tillage
Vernon TX (34°N, 99°W)	Hollister clay loam (fine, mixed, thermic Pachic Paleustoll)	6.9	15	525	Cotton with wheat and cowpea cover crop
Bushland TX (35°N, 101°W)	Pullman clay loam (fine, mixed, thermic Vertic Paleustoll)	6.0	17	425	Wheat, sorghum under conventional and no tillage with 0 to 50 kg N ha^{-1}
Rycroft AB (55°N, 118°W)	Falher clay (fine, montmorillonitic, frigid, Typic Natriboralf)	5.7	2	449	Barley, wheat, pea under conventional and no tillage
Beaverlodge AB (55°N, 119°W)	Hythe clay loam (fine, montmorillonitic, frigid Mollic Cryoboralf)	6.7	2	452	Barley, canola, pea under conventional and no tillage
Dawson Creek BC (55°N, 120°W)	Donnelly silt loam (fine-loamy, mixed, frigid Typic Cryoboralf)	5.5	1	504	Barley under conventional and no tillage
Rolla BC (55°N, 120°W)	Donnelly sandy loam (coarse-loamy, mixed, frigid Typic Cryoboralf)	6.6	1	504	Wheat, canola, barley under conventional and no tillage

ameter of water-stable aggregation was determined from whole soil retained on 1, 0.25 and 0.05 mm screens, as described in Franzluebbers and Arshad (1996c). Net N mineralization was determined from the inorganic N (NO_3-N + NO_2-N + NH_4-N) concentration at 0 and 24 d of incubation following rewetting of dried soil using Cd reduction and salicylate autoanalyzer techniques from 2 M KCl extracts (Bundy and Meisinger, 1994). The flush of N due to chloroform fumigation was calculated from the difference in inorganic N at 7 d following rewetting of dried soil, when soil was fumigated and that 10 d later. Total porosity was calculated from determination of bulk density and assuming a particle density of 2.65 Mg m^{-3}.

Published data reporting soil organic C and C mineralization from fumigated and unfumigated soil were selected to further assess the relationships of CFI/F and CFI/F-C with soil organic C, steady-state C mineralization, microbial biomass by direct counting, and adenosine triphosphate. Steady-state C mineralization was taken as the CO_2-C evolved during the 10 to 20 d period if available or during the 0 to 10 d period.

Regression analyses were performed using SAS (SAS Institute Inc., 1990). Relationships of CFI/F and CFI/F-C with various pools of soil organic matter were evaluated by comparing coefficients of determination (r^2).

3. Results and discussion

Relationships of CFI/F with C mineralization ($r^2 = 0.74$, $n = 90$) and CFI/F with soil organic C ($r^2 = 0.65$, $n = 90$) were strong in a set of four soils varying in clay content from northern Alberta and British Columbia (data from Franzluebbers and Arshad, 1996a,b; Fig. 1). However, there was no relationship of CFI/F-C with C mineralization and only

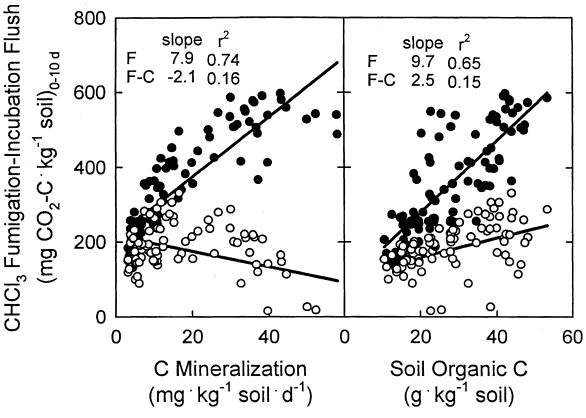

Fig. 1. Relationships of C mineralization and soil organic C with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from four Boralfs in northern Alberta and British Columbia.

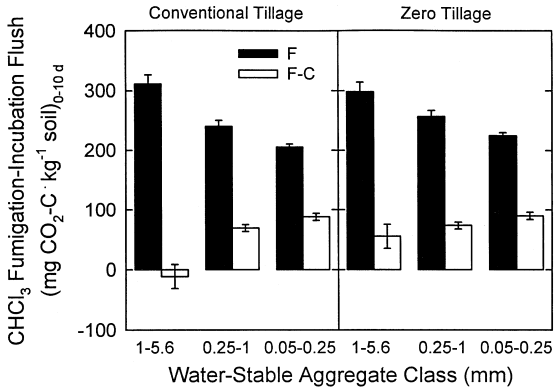

Fig. 2. Effect of water-stable aggregate class and tillage on chloroform fumigation–incubation flush without subtraction of a control (F) and with subtraction of a control (F-C) from four Boralfs in northern Alberta and British Columbia sampled to a depth of 0–50 mm.

a weak relationship of CFI/F-C with soil organic C. Carbon mineralization was only weakly related to soil organic C ($r^2 = 0.26$), suggesting that CFI/F represented an intermediate pool between these active and total pools of organic matter. Interpretation of tillage management and environmental characteristics on the standing stock of microbial biomass and its specific respiratory activity using CFI/F was consistent with that of C mineralization and soil organic C (Franzluebbers and Arshad, 1996a,b), but not using CFI/F-C. As an example of the potential discrepancy in interpretation, estimates of microbial biomass using CFI/F decreased with decreasing size of water-stable aggregates in these soils from Alberta and British Columbia (Fig. 2). Macroaggregates (>0.25 mm) also contained greater quantities of soil organic C (Franzluebbers and Arshad, 1996c) and potentially mineralizable C (Franzluebbers and Arshad, 1997) than microaggregates. However using CFI/F-C, microbial biomass estimates increased with decreasing water-stable aggregate class, even producing negative values for large macroaggregates that were highly enriched in total and mineralizable C. Using CFI/F-C, interpretation of microbial biomass data would have been disconnected with other soil C pools.

Relationships of CFI/F with C mineralization ($r^2 = 0.88$, $n = 96$) and CFI/F with soil organic C ($r^2 = 0.95$, $n = 96$) were very strong in a silty clay loam from Texas (data from Franzluebbers et al., 1995; Fig. 3). Although the relationships of CFI/F-C with C mineralization and CFI/F-C with soil organic C were also strong ($r^2 = 0.57$ and 0.84), they were weaker than those of CFI/F with these properties. Carbon mineralization was highly related to soil organic C ($r^2 = 0.82$). It appears that using CFI/F-C would not have greatly affected interpretation of management effects on microbial biomass in this study.

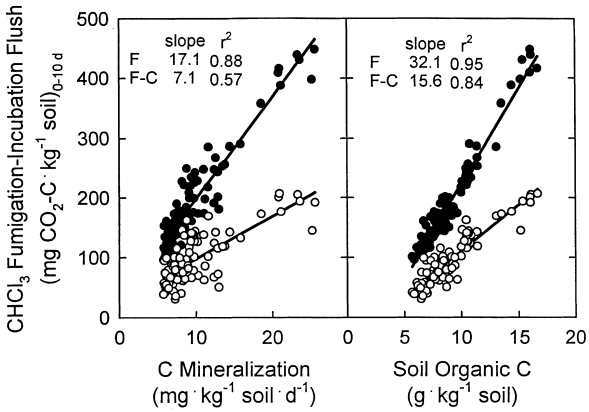

Fig. 3. Relationships of C mineralization and soil organic C with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from an Ustochrept in southcentral Texas.

Relationships of CFI/F with C mineralization ($r^2=0.68$, $n=92$) and CFI/F with soil organic C ($r^2=0.68$, $n=92$) were significant, but relationships between CFI/F-C and these properties were non-existent in 10 different soils varying in texture from eight sites separated by as much as 7° of latitude and 7° of longitude in Texas (unpublished data, 1998; Fig. 4). Carbon mineralization was weakly related ($r^2=0.54$) to soil organic C in these soils. Interpretation of management and environmental effects from CFI/F-C amidst information concerning steady-state C mineralization and soil organic C would have been difficult to justify. For example, using CFI/F-C resulted in very similar estimates of microbial biomass regardless of the large variation in C mineralization and soil organic C.

Relationships of CFI/F with C mineralization ($r^2=0.91$, $n=90$) and CFI/F with soil organic C ($r^2=0.88$, $n=90$) were very strong in a Cecil sandy loam (clayey, kaolinitic, thermic Typic Kanhapludult) from Georgia (Franzleubbers et al., 1999; Fig. 5). Similar to previous results, the relationships of CFI/F-C with C mineralization and CFI/F-C with soil organic C were weaker than of CFI/F with these properties. Carbon mineralization was highly related to soil organic C ($r^2=0.82$). In addition, stronger relationships of CFI/F with soil organic N ($r^2=0.88$) were observed than of CFI/F-C with soil organic N ($r^2=0.61$). Using CFI/F, microbial biomass would have been greater with various non-inversion tillage systems with a cover crop compared with conventional inversion tillage without cover crop (Table 2). Additional biochemical data indicated similar differences between non-inversion and inversion tillage methods (Franzleubbers et al., 1999). However using CFI/F-C, microbial biomass was greater only with the in-row chisel tillage system compared with conventional tillage, resulting in a very different interpretation of the effect of alternative tillage systems on soil biogeochemical properties.

Relationships of CFI/F with mean weight diameter of water-stable aggregation ($r^2=0.56$, $n=10$) and CFI/F with particulate organic N ($r^2=0.86$, $n=10$) were stronger than relationships of CFI/F-C with these properties (Franzleubbers et al., 1999; Fig. 6). Significant relationships of CFI/F with these physical and chemical soil properties indicate the important linkages of microbial biomass with soil structural manipulation and the detrital food web at the soil surface.

Relationships of CFI/F with net N mineralization ($r^2=0.57$, $n=24$) and CFI/F with total porosity ($r^2=0.91$, $n=24$) were strong, but relationships between CFI/F-C and these properties were non-existent (data from Franzluebbers and Arshad, 1996a,b; Fig. 7). A significant relationship of CFI/F with net N mineralization indicates the linkage between microbial biomass and potential nutrient turnover that has been

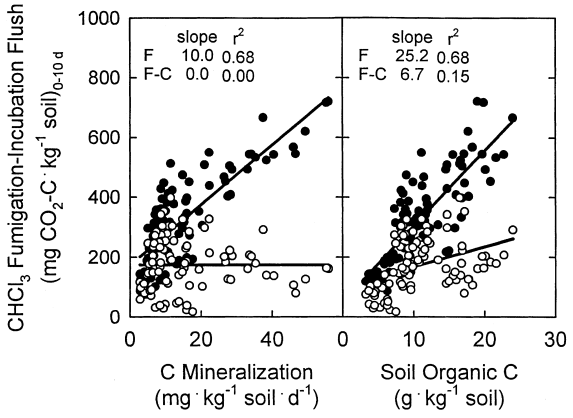

Fig. 4. Relationships of C mineralization and soil organic C with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from 10 soils separated by as much as 900 km in Texas.

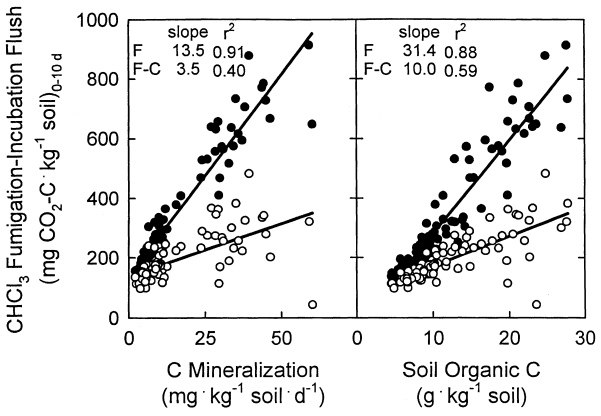

Fig. 5. Relationships of C mineralization and soil organic C with chloroform fumigation-incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from a Kanhapludult in Georgia.

Table 2
Effect of 4 yr of different tillage systems on chloroform fumigation–incubation flush with subtraction of a control (CFI/F-C), chloroform fumigation–incubation without subtraction of a control (CFI/F), soil organic C (SOC), particulate organic C (POC), basal soil respiration (BSR), C mineralization (CMIN) and net N mineralization (NMIN) from a depth of 0–25 mm of a Typic Kanhapludult in Georgia (Franzleubbers et al., 1999)

Tillage system[a]	CFI/F-C (mg kg^{-1})	CFI/F (mg kg^{-1})	SOC (g kg^{-1})	POC (g kg^{-1})	BSR (mg kg^{-1} d^{-1})	CMIN (mg kg^{-1} 24 d^{-1})	NMIN (mg kg^{-1} 24 d^{-1})
CT	209	294	11	3	9	458	40
PP	255	544	17	7	29	1503	66
ST	223	605	19	9	38	1795	70
IC	334	694	23	12	36	1730	89
Contrast							
CT vs PP	NS	*	*	*	**	**	*
CT vs ST	NS	**	*	**	***	**	**
CT vs IC	*	**	**	**	***	**	***

[a] CT is conventional inversion tillage using chisel and disk, PP is non-inversion tillage using paraplow to loosen subsoil in autumn with no tillage planting, ST is non-inversion tillage using secondary cultivation to control weeds during summer with no tillage planting, and IC is non-inversion tillage using in-row chisel to loosen seed furrow at planting.
*, ** and *** are significant at $P \leq 0.1$, $P \leq 0.01$ and $P \leq 0.001$, respectively.
NS is not significant.

commonly observed in forest (Myrold, 1987) and in cropland ecosystems (Franzluebbers et al., 1994, 1996). A strong relationship of CFI/F with total porosity indicates important interactions among microbial biomass, substrate availability, decomposition, and biophysical manipulation of soil structure. Plant roots and residues provide substrates for fauna and microorganisms, which process this material into stable organo-mineral complexes that alter soil structure and porosity. These important biogeochemical processes would not be readily explainable using CFI/F-C as a method to estimate microbial biomass.

Relationships of CFI/F with the flush of N mineralization following fumigation ($r^2 = 0.70$, $n = 92$) and CFI/F with the flush of C mineralization during the first day after rewetting of dried soil ($r^2 = 0.58$, $n = 92$) were strong, but relationships between CFI/F-C and these properties were non-existent (1998, unpublished data; Fig. 8). Soils were from Texas as described for Fig. 3. The C-to-N ratio of the flush following fumigation as taken from the slope of these relationships was more realistic of microbial communities with CFI/F (slope = 6.6) than with CFI/F-C (slope = 1.2). As described by Franzluebbers et al. (1996) from a different set of soils from Texas, the initial flush of C mineralization following rewetting of dried soil appears to be primarily related to a fraction of the microbial biomass that has been killed by drying.

Relationships of CFI/F with soil organic C, particulate organic C and C mineralization during 24 d

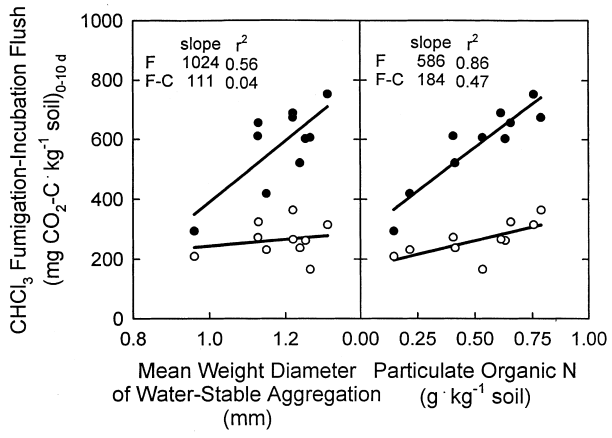

Fig. 6. Relationships of mean weight diameter of water-stable aggregation and particulate organic N with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from the surface 0 to 25 mm of a Kanhapludult in Georgia.

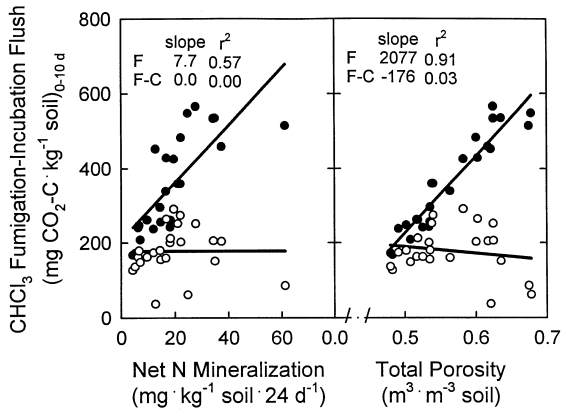

Fig. 7. Relationships of net N mineralization and total porosity with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from four Boralfs in northern Alberta and British Columbia.

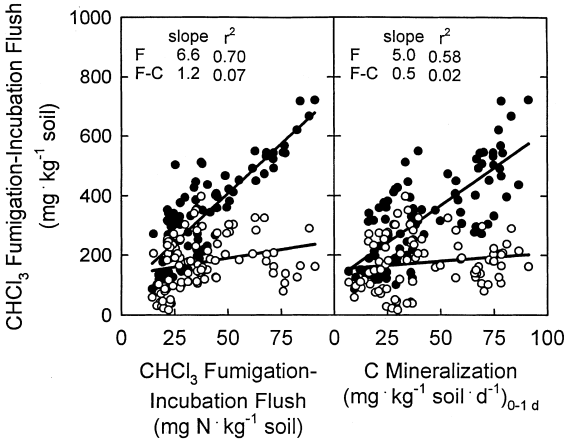

Fig. 8. Relationships of the flush of N following fumigation and the flush of C during the first day following rewetting of dried soil with chloroform fumigation–incubation flush without subtraction of a control (F, •) and with subtraction of a control (F-C, ○) from 10 soils separated by as much as 900 km in Texas.

were very strong in a set of 476 samples representing seven soil series (clayey, kaolinitic, thermic Typic Kanhapludults) under coastal bermudagrass pasture in Georgia (1998, unpublished data; Fig. 9). There were weak, negative relationships between CFI/F-C and these soil properties. Relationships between particulate organic C and soil organic C ($r^2 = 0.90$), between C mineralization and soil organic C ($r^2 = 0.86$) and between C mineralization and particulate organic C ($r^2 = 0.89$) were all highly significant. Clearly, use of CFI/F-C in this study would have been very problematic when interpreting management effects on soil quality, especially in the light of these other active and passive pools of organic matter.

Stronger relationships of CFI/F with C mineralization and soil organic C than of CFI/F-C with these soil properties were also found in published data (Table 3). Of 15 studies that contained data from more than four soils or long-term management systems, nine indicated significantly stronger relationships (as indicated by differences in levels at $P \leq 0.1$, $P \leq 0.01$ and $P \leq 0.001$) between CFI/F and C mineralization than between CFI/F-C and C mineralization. In all 15 studies, coefficients of determination between CFI/F and C mineralization were higher than between CFI/F-C and C mineralization. Five of the studies showed a stronger relationship between CFI/F and soil organic C than CFI/F-C and soil organic C. Our results from soils in widely different regions of North America with many combinations of management and depth distribution effects within soils corroborate results from these published studies, where steady-state C mineralization forms a closer relationship with CFI/F than with CFI/F-C.

Soil microbial biomass determination using CFI/F-C with recent residue addition has been previously shown to yield negative estimations (Martens, 1985). Under pasture conditions particularly (Fig. 9), we also observed this phenomenon. However, under these same difficult conditions, we obtained excellent relationships between CFI/F and potential C mineralization, particulate organic C and soil organic C (Fig. 9). This suggests that CFI can produce results that are meaningful within the context of other soil C pools, if a control is not subtracted from the flush following fumigation. Other investigators have lamented the problem of negative microbial biomass estimations and attempted to make methodological modifications to adjust for these intuitively erroneous occurrences (Sparling and Williams, 1986; Vance et al., 1987b; Cochran et al., 1989). However, CFI/F has consistently yielded significant relationships with other active and total soil C pools, suggesting its validity in measuring microbial biomass even under conditions of recent residue addition.

Subtraction of a control in CFI assumes that the quantity of non-living soil organic C mineralized to CO_2 in the fumigated soil is the same as that in the unfumigated soil. If this were true, the relationship of steady-state C mineralization with CFI/F would be expected to be somewhat stronger than with CFI/F-C,

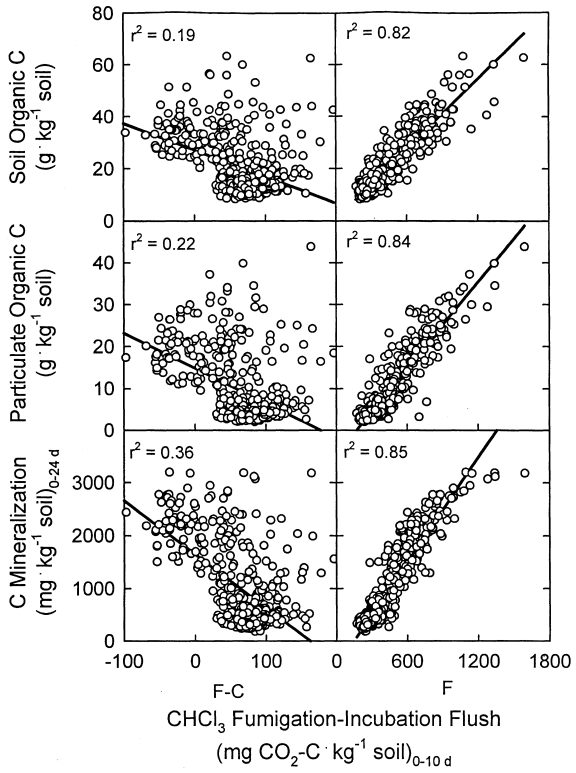

Fig. 9. Relationships of soil organic C, particulate organic C, and cumulative C mineralization during 24 d with chloroform fumigation–incubation flush without subtraction of a control (F) and with subtraction of a control (F-C) from seven Kanhapludults in Georgia.

Table 3
The flush of CO_2-C evolved during 10 d of incubation following chloroform fumigation without subtraction of a control (F) and with subtraction of a control (F-C) in relationship with C mineralization (i.e. the control; CMIN, mg kg^{-1} d^{-1}) and soil organic C (SOC, g kg^{-1}) from published data

Source	Observations	Dependent variable		Independent variable			
		type	mean ± S.D.	CFI/F		CFI/F-C	
				b_1	r^2	b_1	r^2
Jenkinson and Powlson (1976b)	9	CMIN	21 ± 16	19.0	0.98***	9.0	0.92***
		SOC	21 ± 11	25.8	0.88***	12.8	0.91***
Powlson and Jenkinson (1976)	13	CMIN	19 ± 17	28.6	0.85***	18.6	0.71***
		SOC	29 ± 23	21.5	0.92***	15.1	0.89***
Schnürer et al. (1985)	5	CMIN	7 ± 4	17.3	0.95**	7.3	0.78*
		SOC	29 ± 4	17.4	0.93**	8.4	0.99***
Sparling et al. (1986)	19	CMIN	86 ± 37	11.8	0.91***	1.8	0.20*
		SOC	45 ± 19	22.0	0.80***	5.0	0.38**
Shan-Min et al. (1987)	5	CMIN	14 ± 13	14.9	0.73*	4.9	0.23
		SOC	23 ± 13	16.9	0.93**	7.5	0.53
Vance et al. (1987a)	10	CMIN	18 ± 9	51.0	0.64**	41.0	0.53*
		SOC	36 ± 24	22.6	0.92***	19.8	0.92***
Vance et al. (1987b,c)	11	CMIN	35 ± 20	19.6	0.72***	9.6	0.38*
		SOC	84 ± 86	0.9	0.03	−0.4	0.01
Diaz-Raviña et al. (1989)	6	CMIN	22 ± 3	22.5	0.12	12.5	0.04
		SOC	80 ± 41	4.3	0.74*	3.8	0.63*
Srivastava and Singh (1989)	12	CMIN	24 ± 5	14.8	0.47**	3.9	0.06
		SOC	16 ± 6	13.3	0.66***	7.4	0.36*
Kaiser et al. (1992)	27	CMIN	9 ± 6	26.1	0.81***	16.1	0.62***
		SOC	28 ± 47	1.3	0.12*	0.6	0.05
Díaz-Raviña et al. (1993)	15	CMIN	22 ± 11	10.3	0.30*	0.3	0.00
		SOC	76 ± 45	2.5	0.27*	1.1	0.08
Wardle and Ghani (1995)	12	CMIN	43 ± 3	19.2	0.47**	12.2	0.26*
		SOC	83 ± 14	4.0	0.55**	3.2	0.50**
Groffman et al. (1996)	12	CMIN	65 ± 58	8.8	0.84***	7.8	0.81***
		SOC	142 ± 150	3.4	0.82***	3.0	0.80***
Horwath et al. (1996)	7	CMIN	51 ± 31	10.1	0.93***	0.1	0.00
		SOC	30 ± 21	−4.3	0.08	1.0	0.05
Beck et al. (1997)	20	CMIN	24 ± 29	13.6	0.96***	3.6	0.65***
		SOC	39 ± 34	11.3	0.91***	3.4	0.78***
Mean	207	CMIN	31 ± 32	11.8	0.80***	4.9	0.57***
		SOC	48 ± 57	3.8	0.59***	2.5	0.59***

*, **, and *** indicate significance at $P \leq 0.1$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

since part of the estimation of microbial biomass would be derived from steady-state C mineralization. In fact in pioneering work describing CFI, Jenkinson and Powlson (1976a) observed that after the first 4 d of incubation following fumigation, the rate of C mineralization from fumigated and unfumigated samples was similar up to 60 d of incubation. However, this observation was from a single soil receiving long-term application of farmyard manure. Evidence from several subsequent studies have indicated a much different response. From four soils sampled in summer and in winter in New Zealand, the rate of C mineralization at 10 d in fumigated soil was 80 ± 16% of that in unfumigated soil (Ross, 1987). Further, the rate of C mineralization in fumigated soil at 10 d was 74 ± 18% of that in unfumigated soil and 53 ± 24% at 20 d in similar soils (Ross, 1990). Similar trends of reduced C mineralization in fumigated relative to unfumigated soil at or after 10 d of incubation following fumigation have been observed in other soils (Ross et al., 1980; Martens, 1985; Smith et al., 1995; Wu et al., 1996). These results indicate that the microbial community that developed following fumigation had a significantly different basal soil respiration rate than in unfumigated soil.

The time when soil CO_2 evolution from fumigated soil subsides and begins to become less than the control soil varies with different soils (e.g. 4, 5, 6, 9 and 15 d for several soils from New Zealand (Ross et al., 1980; Ross, 1987); 0 to 2 d for a soil in Germany following wheat root addition (Martens, 1985); 8 d for a soil in England (Wu et al., 1996)). The time of maxi-

mum rate of soil CO_2 evolution following fumigation also appears to vary with different soils (e.g. 1 d for a soil in England (Jenkinson and Powlson, 1976a); 1, 3 and 5 d for soils in New Zealand (Ross et al., 1980; Ross, 1987); 6 d for a soil in Alaska (Cochran et al., 1989)). This variability among soils in response to fumigation, suggests that although some soils could be incubated for less than 10 d following fumigation, the standard 10-d incubation provides a reasonable period for most soils to express the flush of activity following fumigation. Further research into the duration of incubation among various soils could improve the accuracy of microbial biomass estimates.

The fact that negative microbial biomass estimates can be obtained using CFI/F-C suggests that the microbial community surviving fumigation is able to decompose primarily simple substrates of microbial origin and is less capable of decomposing more complex substrates such as plant residues. The ability of microorganisms surviving fumigation to decompose maize straw was severely reduced compared with unfumigated soil (Smith et al., 1995; Horwath et al., 1996). Further evidence to reject the hypothesis of similar capabilities of surviving microorganisms has been given by Zelles et al. (1997), in which phospholipid fatty acids in fumigated soil at 10 d were reduced to 50%, arginine deaminase activity was reduced to 40% and dehydrogenase activity was reduced to 4% of those in unfumigated soil.

From relative decomposition of ^{14}C-labelled straw in fumigated and unfumigated soil, a method for subtracting a partial control has been suggested (Smith et al., 1995; Horwath et al., 1996). In these studies, release of $^{14}CO_2$-C from fumigated soil during 10 d of incubation compared with that from unfumigated soil was $21 \pm 3\%$ (Smith et al., 1995) and $22 \pm 8\%$ (Horwath et al., 1996). Recalculating the data presented in Fig. 9 with subtraction of 32% of the control as suggested by Horwath et al. (1996) resulted in a significant improvement in relationships between CFI and soil organic C ($r^2 = 0.68$), particulate organic C ($r^2 = 0.69$) and C mineralization ($r^2 = 0.64$) compared with full subtraction of a control. However, CFI with subtraction of a partial control was not as strongly related to these active and total C pools as CFI/F. Despite adding a source of intermediately-available ^{14}C that attempted to mimic a fraction of soil organic C between humus and microbial by-products, some degradation of carbohydrates present within the straw probably occurred at the drying temperature of 70°C (van Soest, 1982). The percentage of total ^{14}C added in straw that was released as CO_2 during CFI was only 3 to 4% (Smith et al., 1995), which could have been rendered readily-available through drying and heating of the straw. Therefore, the evidence for partial subtraction of a control has not been adequate to promote its use.

Chloroform fumigation–incubation with subtraction of a control was originally calibrated against direct counting of microorganisms (Jenkinson et al., 1976). Since then, several studies have attempted to corroborate this relationship with varying degrees of success. Although direct counting is fraught with uncertainties (Martens, 1995), it has been used to calibrate CFI because of its long established history. We plotted the results of six published reports where CFI/F and CFI/F-C could be regressed upon microbial biomass obtained with direct counting (Fig. 10). Although neither CFI method was strongly related to direct counting, CFI/F did relate better than CFI/F-C. Perhaps fortuitously, the slope of the regression of CFI/F on microbial biomass by direct counting was 0.40, which was very similar to the k_C factor of 0.41 suggested by Voroney and Paul (1984). There were large differences among studies in the relationship between CFI and microbial biomass by direct counting. Of the four studies with positive slopes between CFI and direct counting, slopes were 0.52 ± 0.31 between CFI/F and direct counting and 0.26 ± 0.21 between CFI/F-C and direct counting. Coefficients of determination regressing CFI on direct counting were always higher using CFI/F (0.85 ± 0.15) than CFI/F-C (0.62 ± 0.29) in these four studies. From these same four studies, coefficients of determination regressing C mineralization on microbial biomass by direct counting were 0.82 ± 0.12, although with widely differing slopes among studies. The strong relationship between C mineralization and direct counting further supports the use of C mineralization to validate CFI methods.

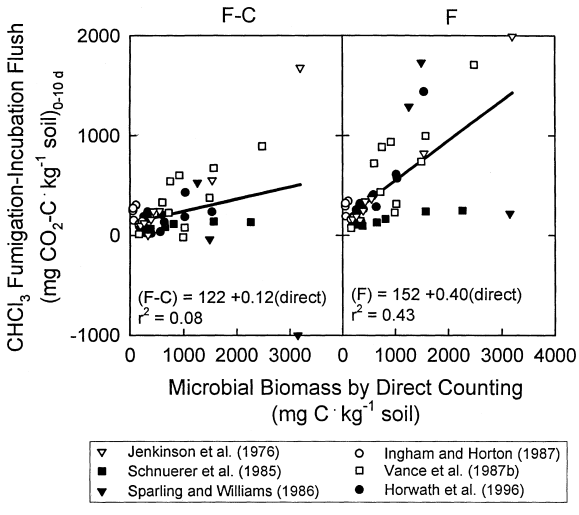

Fig. 10. Relationship of microbial biomass by direct counting with chloroform fumigation–incubation flush without subtraction of a control (F) and with subtraction of a control (F-C) from six published studies.

The relationship of CFI/F with adenosine triphosphate concentration in soil was somewhat stronger than the relationship of CFI/F-C with adenosine triphosphate from data taken from four published studies (Fig. 11). Relationship of C mineralization with adenosine triphosphate was also highly significant ($r^2 = 0.79$, $n = 36$). The concentration of adenosine triphosphate corroborates the use of CFI with or without subtraction of a control to estimate microbial biomass. Further, adenosine triphosphate concentration renders support for using steady-state C mineralization as a simple, but effective proxy for estimating an active soil C pool, which is related to microbial biomass.

In summary, soil microbial biomass C estimated using CFI/F was highly related to active pools of potential C and N mineralization, as well as to total organic C and N and to less active pools of particulate organic C and N, mean weight diameter of water-stable aggregation at the soil surface and total porosity. Relationships between CFI/F-C and these same pools of organic matter were always weaker or even non-existent. From published data, CFI/F was better related to several active soil C pools, including steady-state C mineralization, microbial biomass by direct counting and adenosine triphosphate concentration compared with CFI/F-C. Despite the inadequacy of the original CFI/F-C to obtain reasonable estimates of microbial biomass in recently amended soils, CFI/F displayed no complications in such situations. Differences in the microbial communities between fumigated and unfumigated soil in their abilities to decompose complex substrates make the principle of subtraction of a control unrealistic. Chloroform fumigation–incubation without subtraction of a control should be considered a more robust method to determine microbial biomass than CFI/F-C, especially since it is simple and reliable under a wide range of environmental conditions.

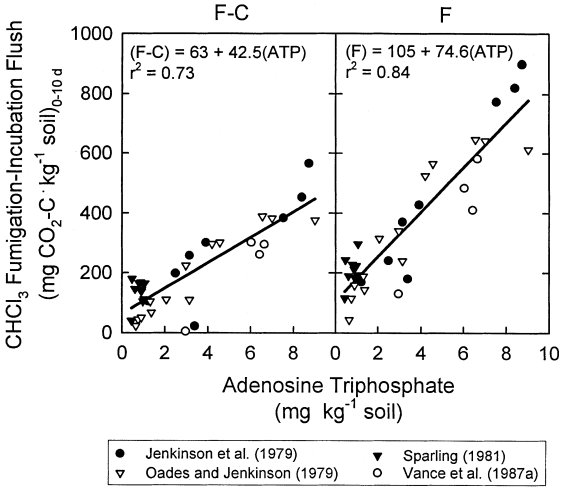

Fig. 11. Relationship of adenosine triphosphate with chloroform fumigation–incubation flush without subtraction of a control (F) and with subtraction of a control (F-C) from four published studies.

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