PERGAMON

Soil Biology & Biochemistry 33 (2001) 1501–1507

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Molar concentration of K_2SO_4 and soil pH affect estimation of extractable C with chloroform fumigation–extraction

R.L. Haney[a,*], A.J. Franzluebbers[b], F.M. Hons[a], L.R. Hossner[a], D.A. Zuberer[a]

[a]*Department of Soil and Crop Sciences, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843-2474, USA*
[b]*U.S. Department of Agriculture—Agricultural Research Service, J. Phil Campbell Sr. Natural Resource Conservation Center, 1420 Experiment Station Road, Watkinsville, GA 30677-2373, USA*

Received 8 November 1999; received in revised form 17 January 2001; accepted 2 March 2001

Abstract

Methods of determining soil microbial biomass need to be reliable and produce consistent results across soils with a wide range of properties. We investigated the effect of extractant molarity (distilled water and 0.001, 0.01, 0.1, and 0.5 M K_2SO_4) on the flush of C (i.e. the difference between fumigated and unfumigated subsamples) with the chloroform fumigation–extraction method in soils of different pH. Extraction efficiency of 0.5 M K_2SO_4 relative to water was dependent upon soil pH. The ratio of extractable C in water to that in 0.5 M K_2SO_4 for five acidic soils was 1.5 ± 0.3 in unfumigated controls, 1.4 ± 0.2 in fumigated samples, and 1.8 ± 0.7 in fumigated minus control flushes, respectively. Ratios in six alkaline soils were 1.0 ± 0.2, 0.9 ± 0.2, and 0.8 ± 0.2, respectively. Flocculation/dispersion of organic colloids and changes in the diffuse double layer surrounding clay particles are possible reasons for differences in extractable C with changes in extractant molarity and soil pH. Chloroform fumigation–extraction with any of the extractants was less related to soil organic C and potential C and N mineralization during 50 days of incubation ($r^2 = 0.51 \pm 0.11$) than was chloroform fumigation–incubation without subtraction of a control ($r^2 = 0.74 \pm 0.08$). Changes in microbial biomass estimates with changes in extractant molarity and soil pH suggest that chloroform fumigation–extraction may not be reliable in a wide range of soils.

Keywords: Extractable carbon; Chloroform fumigation–extraction; Microbial biomass; Soil pH

1. Introduction

Microbial biomass is the key biological component of soil that affects decomposition, nutrient cycling, and aggregation. In general, soil microbial biomass C (SMBC) is highly related with, but constitutes only a small fraction of the soil organic C (SOC) content (Smith and Paul, 1990). Relatively rapid turnover of the SMBC pool compared with other components of SOC, however, suggests that we need to know more about how this pool liberates or sequesters nutrients based on its pool size under various situations.

Development of the chloroform fumigation–incubation (CFI) method (Jenkinson and Powlson, 1976) allowed soil scientists to become increasingly proficient at quantifying SMBC compared with older techniques of plate counting and microscopic enumeration. Underestimation of SMBC in recently amended, low pH, or waterlogged soils with the CFI method with subtraction of a control (CFI/F − C) prompted the development of the chloroform fumigation–extraction (CFE) method to try to overcome these difficulties (Vance et al., 1987; Tate et al., 1988). A common argument for selecting CFE over CFI has been the rapidity with which results are obtained. However, unless an estimation of SMBC is needed in a soil testing laboratory where rapid turnaround time of analyses is needed, the CFE method offers little labor savings in a research laboratory, because often times more labor is needed to extract and operate analytical equipment with CFE than is needed for setting up incubation jars and titrating with CFI. Nonetheless, CFE has become a more popular method in recent years. However, evidence has been mounting that suggests poor correlation of CFE-labile C with SOC and potential C mineralization under steady-state conditions (Franzluebbers et al., 1999) and with double-stranded DNA under exponential growth conditions (Marstorp and Witter, 1999).

The typical extractant in the CFE method has been 0.5 M K_2SO_4 (Vance et al., 1987; Tate et al., 1988). Potassium sulfate was selected for extracting soluble organic C in many laboratories after the discovery that Cl^-, such as in KCl, interfered with colorimetric determination of soluble organic C when oxidized with potassium dichromate (Quinn

* Corresponding author. Tel.: +1-409-845-8738; fax: +1-409-845-0456.
E-mail address: rhaney@acs.tamu.edu (R.L. Haney).

0038-0717/01/$ - see front matter
PII: S0038-0717(01)00065-7

and Salomon, 1964). However, the reason for using 0.5 M K_2SO_4 rather than a more dilute extractant or water is unclear, and the choice seems to have been arbitrarily selected despite the common use of water as an extractant for soluble organic C in many studies (Herbert and Bertsch, 1995). Powlson and Jenkinson (1976) used 0.5 M K_2SO_4 to extract C before and after chloroform fumigation. The findings of that study prompted further investigations into extracting chloroform-labile C, which resulted in reports of significant relationships between CFE-labile C using 0.5 M K_2SO_4 and the standard estimate of SMBC at the time, CFI/F − C [CFI/F − C = −82 + 1.71 CFE, $r^2 = 0.70$, $n = 9$, relationship from values reported in Table 1 of Vance et al. (1987) without an outlier having high SOC]; [CFI/F − C = −92 + 1.06 CFE, $r^2 = 0.76$, $n = 19$ (Tate et al., 1988)].

In some soils, soluble organic C can decrease dramatically with increasing ionic strength (Evans et al., 1988). There is growing evidence that extractant molarity may not only affect the quantity of soluble organic C, but also the estimation of SMBC with the CFE method. When soil was extracted with 0.05 M K_2SO_4, estimates of SMBC were five times greater than by direct counting (Guggenberger et al., 1999). Extractable soil C was 2.9 times greater in distilled water than in 0.5 M K_2SO_4 in a soil with pH of 6.5, but 17% less in a soil with pH of 8.3 (Haney et al., 1999). The effect of extractant molarity needs to be investigated further in order to understand the applicability of the CFE method for a wide range of soils.

Our objectives were to (1) investigate the impact of decreasing extractant molarity on estimation of CFE-labile C in soils with a wide range in pH and (2) compare estimates of CFE-labile C with other biologically relevant soil C and N pools.

2. Materials and methods

Twelve soil samples were collected from eight locations in Georgia and Texas to achieve a range in soil pH and SOC (Table 1). Using a glass electrode, soil pH in water (1:2 soil:water, w/v) ranged from 4.65 to 8.3 and in 0.5 M K_2SO_4 (1:2, w/v) ranged from 4.6 to 7.9. Soil organic C ranged from 4 to 52 mg g^{-1} and was dissimilarly distributed with soil pH.

Dried soil was sieved to pass a 2-mm screen to remove coarse organic residues and stones. Thirty-six subsamples (25-g portions) of each soil (12 subsamples × three replications × 12 soils = 432 total) were placed into glass beakers, wetted to near 50% water-filled pore space (gravimetric water content in Table 1), and incubated in the dark at 25°C for 7 days in 1-l canning jars along with a vial of 10 ml of 1 M KOH to capture evolved CO_2 and a vial of water to maintain humidity.

At the end of 7 days of incubation, 15 subsamples of each soil were fumigated with $CHCl_3$ for 24 h, then extracted with 100 ml of either (1) distilled water, (2) 0.001 M K_2SO_4, (3) 0.01 M K_2SO_4, (4) 0.1 M K_2SO_4, or (5) 0.5 M K_2SO_4 by shaking for 1 h, filtering, and analyzing the extract immediately for soluble organic C with an OI Corporation Model 700 carbon analyzer using glucose standards for calibration. The pH of all extractants was 6.5. At the end of 7 days of incubation, an additional 15 subsamples of each soil were extracted and analyzed in the same manner as above, except without prior fumigation. The flush of extractable C due to fumigation was calculated as the difference between fumigated and unfumigated samples (Vance et al., 1987). No k value was assumed for either CFE or CFI methods, since we wanted only to compare relative differences among methodologies. Absolute estimates of SMBC would have required estimation of a separate k value for each soil and extractant.

Three additional subsamples of each soil were removed at 7 days, fumigated with $CHCl_3$ for 24 h, then incubated for a further 10 days to determine the flush of CO_2 evolved. The flush of CO_2–C following fumigation was calculated without subtraction of a control (CFI/F) and with subtraction of the 7–17 days unfumigated CO_2–C (CFI/F − C) (Jenkinson and Powlson, 1976; Voroney and Paul, 1984).

Three subsamples of each soil were incubated in the presence of alkali for up to 50 days and the alkali trap changed at 7, 17, 28 and 35 days. Net N mineralization was calculated from the difference in inorganic N between samples incubated for 0 and 50 days. Seven-g portions of dried soil were shaken with 28 ml of 2 M KCl for 30 min, filtered, and analyzed for NO_3-N + NO_2-N by Cd reduction and NH_4-N by salicylate-nitroprusside autoanalyzer techniques (Bundy and Meisinger, 1994). Soil organic C was determined using the modified Mebius method with heating to 150°C for 30 min (Nelson and Sommers, 1982).

The effect of extractant molarity on extractable C was compared with an LSD at $P < 0.05$ within each soil. Regression was used to test the strength of relationships among soil properties.

3. Results and discussion

Extractable C with 0.5 M K_2SO_4 from the unfumigated controls was 11 ± 5 mg g^{-1} SOC (mean ± standard deviation among the 12 soils). Extractable C from fumigated soil nearly doubled (18 ± 6 mg g^{-1} SOC). These results are on the higher end of those obtained in other studies, where extractable C has ranged from 1 to 8 mg g^{-1} SOC in unfumigated controls and from 5 to 16 mg g^{-1} SOC in fumigated soil (Powlson and Jenkinson, 1976; Sparling and West, 1988b; Anderson and Joergensen, 1997).

Variation in extractable C for both unfumigated and fumigated samples with changes in extractant molarity was large for most soils (Fig. 1). Across the 12 unfumigated soils, water extracted an average of 243 mg C kg^{-1} soil, 0.001 M K_2SO_4 extracted an average of 208 mg C kg^{-1}

Table 1
Characteristics of soils

Location	Series	Texture[a]	USDA soil classification	Soil pH in		Organic C (mg g^{-1})	Depth of sampling (cm)	Water content (g g^{-1})	Land management[b]
				water	0.5 M K_2SO_4				
Waynesboro GA	Lakeland	S	Thermic, coated Typic Quartzipsamments	4.65	4.6	5.9	30–60	0.11	Cropped (cotton, soybean)
Watkinsville GA	Cecil	SL	Fine, kaolinitic, thermic Typic Kanhapludults	4.8	4.7	15.0	0–15	0.16	Pasture (bermudagrass)
Amarillo TX	Pullman	SCL	Fine, mixed, superactive, thermic Torrertic Paleustolls	5.7	4.6	11.6	0–7.5	0.31	Cropped (sorghum, wheat)
Overton TX	Bowie	fSL	Fineloamy, siliceous, semiactive, thermic Plinthic Paleudults	6.3	4.75	4.1	0–7.5	0.08	Pasture (bermudagrass)
Stephenville TX	Windthorst	fSL	Fine, mixed, thermic Udic Paleustalfs	6.4	5.55	18.3	0–7.5	0.25	Pasture (bermudagrass)
Watkinsville GA	Pacolet	SCL	Fine, kaolinitic, thermic Typic Kanhapludults	6.6	4.8	52.3	2–5	0.42	Pasture (tall fescue)
Watkinsville GA	Cecil	SL	Fine, kaolinitic, thermic Typic Kanhapludults	7.3	4.9	47.9	2–5	0.38	Pasture (tall fescue)
Malone TX	Houston Black	C	Veryfine, smectitic, thermic Oxyaquic Hapluderts	7.75	7.2	13.7	0–10	0.35	Cropped (sorghum, wheat)
College Station TX	Weswood	SiCL	Finesilty, mixed, superactive, thermic Udifluventic Ustochrepts	8.0	7.4	23.7	0–7.5	0.33	Pasture (bermudagrass)
Granger TX	Krum	C	Fine, smectitic, thermic Udertic Haplustolls	8.1	7.9	16.4	0–10	0.30	Cropped (cotton, sorghum)
Weslaco TX	Hidalgo	SCL	Fineloamy, mixed, hyperthermic Typic Calciustolls	8.2	7.6	9.8	0–7.5	0.25	Cropped (cotton, corn)
College Station TX	Weswood	SiCL	Finesilty, mixed, superactive, thermic Udifluventic Ustochrepts	8.3	7.3	15.1	0–7.5	0.28	Cropped (sorghum, wheat)

[a] S (sand), SL (sandy loam), SCL (sandy clay loam), fSL (fine sandy loam), C (clay), SiCL (silty clay loam).
[b] Cotton (Gossypium hirsutum), soybean (Glycine max), bermudagrass (Cynodon dactylon), sorghum (Sorghum bicolor), wheat (Triticum aestivum), tall fescue (Festuca arundinacea), corn (Zea mays).

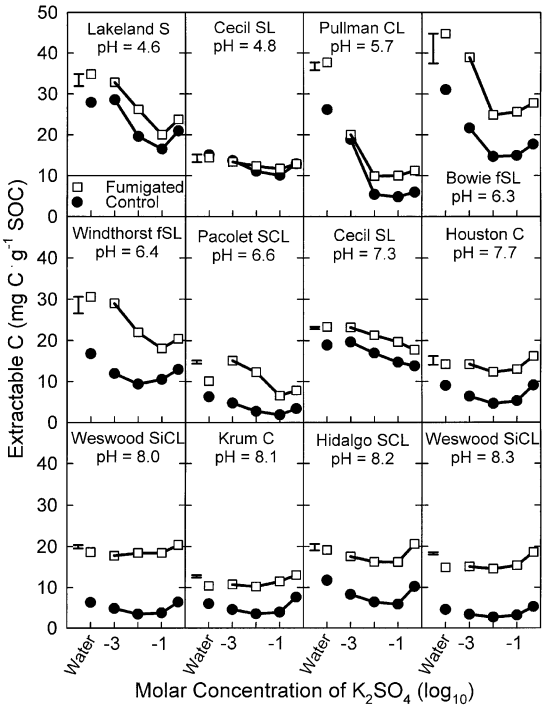

Fig. 1. Extractable C from fumigated and unfumigated soils as affected by extractant molarity (i.e. water and 0.001, 0.01, 0.1, and 0.5 M K_2SO_4). Error bar in each subpanel represents LSD among extractants at $P = 0.05$.

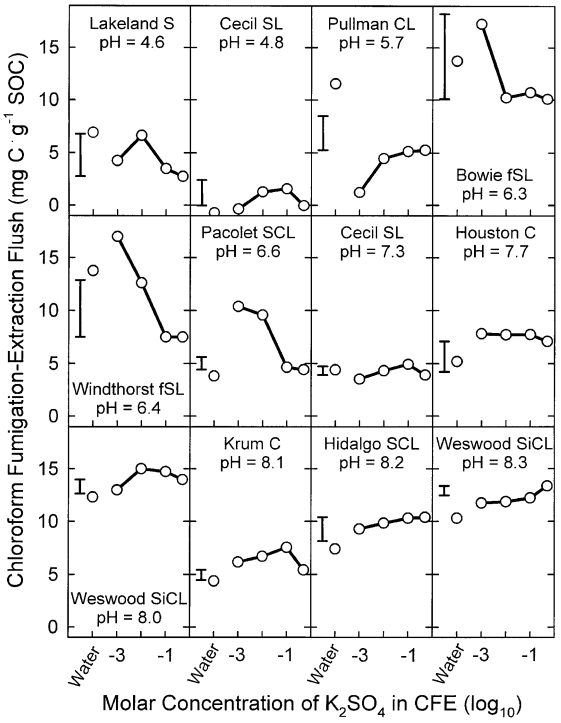

Fig. 2. Chloroform fumigation flush of extractable C (fumigated minus control, CFE/F − C) as affected by extractant molarity (i.e. water and 0.001, 0.01, 0.1, and 0.5 M K_2SO_4). Error bar in each subpanel represents LSD among extractants at $P = 0.05$.

soil, 0.01 M K_2SO_4 extracted an average of 153 mg C kg^{-1} soil, 0.1 M K_2SO_4 extracted an average of 141 mg C kg^{-1} soil, and 0.5 M K_2SO_4 extracted an average of 176 mg C kg^{-1} soil. The CFE flush (i.e. fumigated minus control) across the 12 soils was an average of 129 mg C kg^{-1} soil in water, 159 mg C kg^{-1} soil in 0.001 M K_2SO_4, 161 mg C kg^{-1} soil in 0.01 M K_2SO_4, 136 mg C kg^{-1} soil in 0.1 M K_2SO_4, and 125 mg C kg^{-1} soil in 0.5 M K_2SO_4. Compared with CFE flushes, CFI/F − C (average of 250 mg C kg^{-1} soil) and CFI/F (average of 470 mg C kg^{-1} soil) were both higher. None of the estimates included a k factor, which would be necessary to convert values to soil microbial biomass estimates. Obviously, k values would have to be widely different between CFI and CFE methods, but also among CFE methods with different extractants.

The larger average CFE flushes with more dilute extractants than with 0.5 M K_2SO_4 would not appear to result in misleading estimates of SMBC at first glance since a different k_{EC} value could be employed to compensate for the difference. However, extraction efficiency was found to depend largely on soil pH (Fig. 2). In three of the six acidic soils (i.e. Lakeland, Pullman, and Windthorst), CFE flushes were significantly greater with water than with 0.5 M K_2SO_4. In addition, CFE flushes were significantly greater with 0.001 M K_2SO_4 in the Windthorst soil and with 0.001 and 0.01 M K_2SO_4 in the Pacolet soil compared with 0.5 M K_2SO_4. However, under alkaline soil pH, CFE flushes were significantly less with water than with 0.5 M K_2SO_4 in four of six soils (i.e. Krum, Hidalgo, and the two Weswood soils). The ratio of CFE flush with water compared with 0.5 M K_2SO_4 as extractant (excluding the acidic Cecil soil, which had negative CFE flushes in water and in 0.5 M K_2SO_4) was 1.8 ± 0.7 in the five acidic soils, 0.8 ± 0.2 in the six alkaline soils, and formed a strong linear relationship along the soil pH gradient (Fig. 3). These results suggest that k_{EC} values would have to vary by as much as 150% to obtain the same SMBC estimate using a different molar concentration of the extractant. The highest CFE flush occurred with water in two soils, with 0.001 M

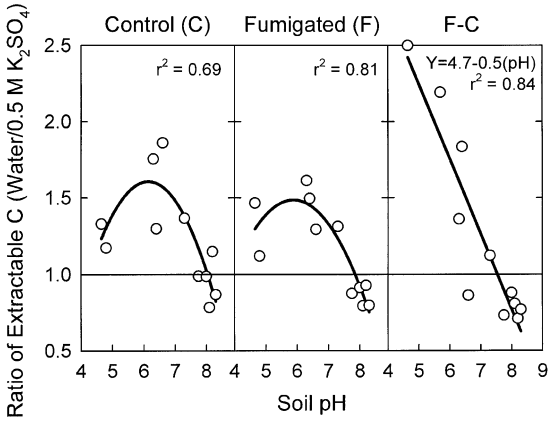

Fig. 3. Ratio of extractable C in water to that in 0.5 M K_2SO_4 as a function of soil pH in unfumigated controls (C), in fumigated soils (CFE/F), and in the difference between fumigated and control soils (CFE/F − C).

K_2SO_4 in four soils, with 0.01 M K_2SO_4 in one soil, with 0.1 M K_2SO_4 in three soils, and with 0.5 M K_2SO_4 in two soils (Fig. 2). A review of k_{EC} values reported in several studies by various techniques suggests that only 70% of values fell within the range of 0.35–0.55, meaning that 30% of soils might have k_{EC} values either <0.35 or >0.55 (Joergensen, 1996)

The large variation in extractable C as a function of extractant molarity suggests that CFE is greatly influenced by soil chemical properties, in which ionic strength of the extractant plays a key role in selecting the fraction of potentially extractable C. Ionic strength is related to molarity of the extractant according to the equation:

$$I = 0.5 \times \Sigma[c_i \times Z_i^2]$$

where, I is ionic strength, c_i is the molar concentration of the ith ionic species, and Z_i is the electrical charge of the ith ionic species. Extracting solutions of 0.001 and 0.5 M K_2SO_4 would have ionic strengths of 0.003 and 1.5, respectively. Varying extractant ionic strength has been useful in biochemical industries for separating different types of proteins. Proteins are generally very soluble and 'salted out' of solution by increasing ionic strength from near 0 to 2 (Voet and Voet, 1990). Increasing the ionic strength of solution results in two conditions, both of which cause large molecules like proteins and solubilized organic C to shrink, i.e. a decrease in intramolecular charge repulsion and a decrease in the diffuse double layer surrounding the organic colloid (Swift, 1996). Increasing ionic strength in non-calcareous soils reduces the diffuse double layer and causes soil colloids to flocculate, which could remove extractable C from solution by collapsing clay interlayers to which soluble organic C binds. In high-pH calcareous soils, increasing the molar concentration of K_2SO_4 replaces Ca^{2+} on the exchange complex causing an increase in the diffuse double layer, which disperses soil and allows solubilized C to stay in solution.

Similar to observations by Brookes et al. (1985); Tate et al. (1988), we found a white precipitate in some soils upon extraction with 0.5 M K_2SO_4, but not with lower ionic strengths. We analyzed the material with X-ray diffraction and identified it as polyhalite [$K_2Ca(SO_4)_2$]. We found this mineral formation mostly in soils with pH > 7.7 and in the Windthorst soil with a pH of 6.4, which had received repeated applications of Ca-enriched dairy cattle manure. Polyhalite formation may be an important factor for expressing the 'salting out' effect by altering ionic strength once the extractant is exposed to soil (i.e. by removing some of the salt from solution), thus differentially affecting extractable C based on Ca^{2+} availability in soil.

The inverse relationship between soil pH and the ratio of CFE flush with water to that with 0.5 M K_2SO_4 (Fig. 3) suggests that SMBC estimates with the traditional CFE method (i.e. using 0.5 M K_2SO_4) might be underestimated at low pH or overestimated at high pH. The ratios of SOC-to-CFE with 0.5 M K_2SO_4, potential C mineralization-to-CFE with 0.5 M K_2SO_4, potential N mineralization-to-CFE with 0.5 M K_2SO_4, and CFI/F-to-CFE with 0.5 M K_2SO_4 all decreased with increasing pH, which further suggests that CFE with 0.5 M K_2SO_4 might be underestimating SMBC at low pH or overestimating SMBC at high pH. These observations are consistent with those of other studies having a wide range of soil pH, where the ratio of SOC-to-CFE with 0.5 M K_2SO_4 decreased significantly with increasing soil pH (Joergensen et al., 1995; Anderson and Joergensen, 1997). The ratio of potential C mineralization-to-CFE with 0.5 M K_2SO_4 decreased slightly, but the ratio of substrate induced respiration-to-CFE with 0.5 M K_2SO_4 increased with increasing soil pH (Anderson and Joergensen, 1997). Interestingly, of all soil properties measured, CFE with 0.1 M K_2SO_4 and with 0.5 M K_2SO_4 were the only soil properties related to soil pH (Table 2).

Although we employed a standard set of extracting conditions in our study, changes in extraction methodology can lead to major differences in SMBC estimates with the CFE technique. For example, varying extraction time from 0.5 to 4 h resulted in coefficients of variation in extractable C from unfumigated controls of 18 ± 8%, from fumigated soils of 9 ± 1%, and from CFE flushes of 7 ± 2% among four different soils (Tate et al., 1988). Varying shaking speed from 50 to 250 rev min^{-1} resulted in coefficients of variation in CFE flushes of 8 ± 4% among three soils (Tate et al., 1988). Varying fumigation time from 1 to 5 days resulted in coefficients of variation in extractable C of 20 ± 8% in two soils (Brookes et al., 1985). Varying the temperature during fumigation from 25 to 50°C resulted in coefficients of variation in extractable C of 97 ± 1% from unfumigated controls, of 37 ± 3% from fumigated soils, and of 19 ± 7% from CFE flushes in two soils (Brookes et al., 1985). These variations can all be attributed to the interaction among soil physical, chemical, and biological properties. Unfortunately, methodological differences are frequently found among different studies, such that comparison of SMBC estimates among studies may not be reliable. Further, soils varying in organic matter, texture, pH, and aggregation are not likely to respond similarly to even a specific set of conditions, as we have illustrated with variations in pH among soils. Different soil response to extraction due to chemical and physical differences among soils may be the reason for widely differing extraction efficiencies among soils within the same study (Vance et al., 1987; Sparling and West, 1988a; Ross, 1990; Martikainen and Palojärvi, 1990; Zagal, 1993).

We have shown that extractability of soluble C is complicated by ionic strength of the extractant. Soil microbial biomass N is also frequently determined with CFE using 0.5 M K_2SO_4 (Brookes et al., 1985). A high salt concentration for extracting soluble N has been justified to displace all available NH_4^+ on negatively charged soil colloids. However, there is little justification for using a high salt concentration to extract soluble C, especially since there

Table 2
Correlation matrix of properties from 12 soils. CFE flush is chloroform fumigation–extraction (fumigated minus control), CFI is chloroform fumigation–incubation with subtraction of a control (CFI/F − C) and without subtraction of a control (CFI/F), SOC is soil organic C, CMIN is potential C mineralization during 50 days incubation, and NMIN is potential N mineralization during 50 days incubation. *, **, *** denote significance at $P \leq 0.1$, $P \leq 0.01$, and $P \leq 0.001$, respectively. NS is not significant

Soil property	CFE (water)	CFE (0.001 M)	CFE (0.01 M)	CFE (0.1 M)	CFE (0.5 M)	CFI (F − C)	CFI (F)	pH (water)	pH (K_2SO_4)	SOC	CMIN (50 days)	NMIN (50 days)
CFE flush (water)	–	**	**	***	***	NS	**	NS	NS	*	**	**
CFE flush (0.001 M K_2SO_4)	0.75	–	***	**	**	*	**	NS	NS	**	**	**
CFE flush (0.01 M K_2SO_4)	0.78	0.97	–	***	***	**	***	NS	NS	**	**	**
CFE flush (0.1 M K_2SO_4)	0.85	0.75	0.85	–	***	*	**	*	NS	**	**	*
CFE flush (0.5 M K_2SO_4)	0.87	0.78	0.86	0.98	–	*	**	*	NS	*	*	*
CFI/F − C	0.38	0.63	0.73	0.67	0.62	–	***	NS	NS	**	*	*
CFI/F	0.69	0.78	0.83	0.80	0.75	0.85	–	NS	NS	***	***	***
pH (water)	0.38	0.30	0.34	0.60	0.61	0.29	0.41	–	***	NS	NS	NS
pH (0.5 M K_2SO_4)	0.10	0.05	0.08	0.34	0.37	0.20	0.22	0.85	–	NS	NS	NS
Soil organic C	0.58	0.73	0.79	0.69	0.62	0.77	0.81	0.17	− 0.19	–	***	**
CMIN (0–50 days)	0.76	0.79	0.79	0.69	0.64	0.61	0.90	0.29	− 0.01	0.82	–	***
NMIN (0–50 days)	0.73	0.80	0.78	0.61	0.60	0.62	0.86	0.25	− 0.04	0.79	0.93	–

are pH-dependent and possibly mineralogical-dependent effects on extractability of C because of variations in mineral surface charge. Solubilized C is diverse in molecular weight and charge (Merckx and Martin, 1987; Badalucco et al., 1992), and therefore, its extraction is fundamentally more complex than extraction of simple inorganic compounds.

Soil microbial biomass should be highly related with potential C and N mineralization because substrate availability in agricultural soils often limits microbial growth and activity. Potential C and N mineralization were highly related to CFI/F, but only weakly related to CFE with 0.5 M K_2SO_4 as extractant (Table 2). The CFE flushes with 0.001 and 0.01 M K_2SO_4 as extractants were better related to potential C and N mineralization than those with 0.5 M K_2SO_4, but still not as well related as CFI/F. Soil organic C was also most closely related to CFI/F and much less related to the CFE flush with 0.5 M K_2SO_4. In a review of eight previously published studies with soils from England, New Zealand, and Germany ($n = 108$), potential C mineralization was also much better related with CFI/F ($r^2 = 0.73$) than with CFE ($r^2 = 0.18$) (Franzluebbers et al., 1999). Disparate relationships between SOC with CFI/F ($r^2 = 0.68$) and SOC with CFE ($r^2 = 0.25$) were also found in this same review. The chemical complications associated with the CFE method may make it unreliable as a technique to estimate this important, temporally sensitive pool of soil organic matter, the microbial biomass. The CFI/F method remains a more robust method of estimating SMBC.

4. Summary and conclusions

Decreasing extractant molarity from the traditional 0.5 M K_2SO_4 in the CFE method generally led to higher estimates of SMBC at low pH and lower estimates of SMBC at high pH. Apart from the microbial control of C availability in solution, our results suggest that extractant molarity and soil pH are important factors regulating the quantity of extractable C. We have shown that extractant molarity and soil pH are important variables that interact to yield vastly different estimates of SMBC using the CFE method. Chloroform fumigation–extraction estimates with 0.5 M K_2SO_4 were only weakly related with SOC and potential C and N mineralization. Chloroform fumigation–extraction is unreliable to estimate SMBC if soils have different pH, which often occurs due to management effects on soil. Our results suggest that CFE (i.e. a chemical extraction procedure) may not be suitable to accurately estimate the biologically active pool of soil organic matter, the microbial biomass.

Acknowledgements

We thank Dr Paul Lindahl, a bioinorganic chemist from the Department of Chemistry at Texas A&M University, for

his suggestions and insight, which greatly influenced the course of this research. We appreciate the comments of Dr Joe Dixon and Dr Lindahl during review of this manuscript. We thank Dr Norman White for X-ray diffraction determinations.

References

Anderson, T.H., Joergensen, R.G., 1997. Relationship between SIR and FE estimates of microbial biomass C in deciduous forest soils at different pH. Soil Biology and Biochemisty 29, 1033–1042.

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

Brookes, P.C., Kragt, J.F., Powlson, D.S., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biology and Biochemistry 17, 831–835.

Bundy, L.G., Meisinger, J.J., 1994. Nitrogen availability indices. In: Weaver, R.W., Angle, J.S., Bottomley, P.S. (Eds.). Methods of Soil Analysis, Part 2. Book Series 5—Soil Science Society of America, Madison, WI, pp. 951–984.

Evans, A., Zelazny, L.W., Zipper, C.E., 1988. Solution parameters influencing dissolved organic carbon levels in three forest soils. Soil Science Society of America Journal 52, 1789–1792.

Franzluebbers, A.J., Haney, R.L., Hons, F.M., Zuberer, D.A., 1999. Assessing biological soil quality with chloroform fumigation–incubation: why subtract a control? Canadian Journal of Soil Science 79, 521–528.

Guggenberger, G., Elliott, E.T., Frey, S.D., Six, J., Paustian, K., 1999. Microbial contributions to the aggregation of a cultivated grassland soil amended with starch. Soil Biology and Biochemistry 31, 407–419.

Haney, R.L., Franzluebbers, A.J., Hons, F.M., Zuberer, D.A., 1999. Soil C extracted with water or K_2SO_4: pH effect on determination of microbial biomass. Canadian Journal of Soil Science 79, 529–533.

Herbert, B.E., Bertsch, P.M., 1995. Characterization of dissolved and colloidal organic matter in soil solution: a review. In: McFee, W.W., Kelly, J.M. (Eds.). Carbon Forms and Functions in Forest Soils. Soil Science Society of America, Madison, WI, pp. 63–88.

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

Joergensen, R.G., Anderson, T.-H., Wolters, V., 1995. Carbon and nitrogen relationships in the microbial biomass of soils in beech (*Fagus sylvatica* L.) forests. Biology and Fertility of Soils 19, 141–147.

Joergensen, R.G., 1996. The fumigation–extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. Soil Biology and Biochemistry 28, 25–31.

Marstorp, H., Witter, E., 1999. Extractable dsDNA and product formation as measures of microbial growth in soil upon substrate addition. Soil Biology and Biochemistry 31, 1443–1453.

Martikainen, P.J., Palojärvi, A., 1990. Evaluation of the fumigation–extraction method for the determination of microbial C and N in a range of forest soils. Soil Biology and Biochemistry 22, 792–802.

Merckx, R., Martin, J.K., 1987. Extraction of microbial biomass components from rhizosphere soils. Soil Biology and Biochemistry 19, 371–376.

Nelson, D.W., Sommers, L.E., 1982. Total carbon, organic carbon, and organic matter. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.). Methods of Soil Analysis, Part 2. Agronomy Monograph 9—American Society of Agronomy and Soil Science Society of America, Madison, WI, pp. 539–594.

Powlson, D.S., Jenkinson, D.S., 1976. The effects of biocidal treatments on metabolism in soil. II. Gamma radiation, autoclaving, air-drying and fumigation. Soil Biology and Biochemistry 8, 179–188.

Quinn, J.G., Salomon, M., 1964. Chloride interference in the dichromate oxidation of soil hydrolysates. Soil Science Society of America Proceedings 28, 456.

Ross, D.J., 1990. Estimation of soil microbial C by a fumigation–extraction method: Influence of seasons, soils and calibration with the fumigation–incubation procedure. Soil Biology and Biochemistry 22, 295–300.

Smith, J.L., Paul, E.A., 1990. The significance of soil microbial biomass estimations. In: Bollag, J.M., Stotzky, G. (Eds.). Soil Biochemistry, vol. 6. Marcel Dekker, New York, pp. 357–396.

Sparling, G.P., West, A.W., 1988a. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and ^{14}C labelled cells. Soil Biology and Biochemistry 20, 337–343.

Sparling, G.P., West, A.W., 1988b. Modifications to the fumigation–extraction technique to permit simultaneous extraction and estimation of soil microbial C and N. Communications in Soil Science and Plant Analysis 19, 327–344.

Swift, R.S., 1996. Organic matter characterization. In: Sparks, D.L. (Ed.). Methods of Soil Analysis, Part 3. Book Series 5—Soil Science Society of America, Madison, WI, pp. 1011–1026.

Tate, K.R., Ross, D.J., Feltham, C.W., 1988. A direct extraction method to estimate soil microbial C: effects of experimental variables and some different calibration procedures. Soil Biology and Biochemistry 20, 325–329.

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

Voet, D., Voet, J.G., 1990. Biochemistry. John Wiley and Sons, New York.

Voroney, R.P., Paul, E.A., 1984. Determination of k_C and k_N in situ for calibration of the chloroform fumigation–incubation method. Soil Biology and Biochemistry 16, 9–14.

Zagal, E., 1993. Measurement of microbial biomass in rewetted air-dried soil by fumigation–incubation and fumigation–extraction techniques. Soil Biology and Biochemistry 25, 553–559.