Published March, 2012

Effects of Profile Depth and Management on the Composition of Labile and Total Soil Organic Matter

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Carbon in deeper soil layers may be more stable than that in surface soil due to differences in source, composition, and environmental conditions. We studied the effects of profile depth on soil organic matter (SOM) composition and stability by comparing soils which had received organic amendments since 1991 to non-amended soils from the same study. We used thermometric analysis and excitation-emission fluorescence spectroscopy to characterize total SOM as well as a highly labile fraction of soil organic carbon (SOC) extracted with water (about 1% of total SOC), a moderately labile fraction extracted by pyrophosphate (5–18% of total SOC), and the resistant fraction remaining in the soil after pyrophosphate extraction. The concentrations of both total and water-extractable C decreased with depth. Amended soils had more total SOC than non-amended soils at the 0- to 15- and 15- to 30-cm depths, but not at the 30- to 45-cm depth, and greater amounts of water-extractable C at all depths. The most thermally labile fraction of SOM in the bulk samples was not affected by either depth or amendment, while the thermally resistant fraction decreased slightly with depth, but was unaffected by amendment. The thermally refractory fraction was unaffected by amendment but increased with depth. Thermal analysis of the residue after sequential extraction confirmed that the sequential extraction procedure removed relatively labile C and left behind more stable organic matter. Both thermal analysis, of soil pre- and post-pyrophosphate extraction, and fluorescence of pyrophosphate-extractable C suggested that C deeper in the profile was more microbially decomposed than surface C.

Abbreviations: DI, deionized; DSC, differential scanning calorimetry; EEM, excitation emission matrix fluorescence spectroscopy; OM, organic matter; PARAFAC, parallel factor analysis; SOC, soil organic carbon; SOM, soil organic matter; TG, thermogravimetric; WEOC, water-extractable organic carbon.

Enriching soil C through the recycling of organic wastes provides beneficial ecosystem services such as nutrient recycling (Schröder, 2005), improvement of soil quality (Clapp et al., 2007), and C sequestration (Freibauer et al., 2004; Smith and Powlson, 2000). Repeated applications of organic amendments enrich labile fractions of C (Griffin and Porter, 2004; Mallory and Griffin, 2007), enhance soil microbial biomass and activity (Fraser et al., 1988; Witter et al., 1993), increase soil aggregation (Grandy et al., 2002; Mallory and Porter, 2007), and increase water-holding capacity (Weil and Magdoff, 2004).

The accumulation of organic matter in soils is controlled by the balance between C inputs and losses. Numerous studies comparing soil management systems under similar tillage regimes have demonstrated that the change in soil C over time is linearly related to the level of C inputs (Griffin and Porter, 2004; Paustian et al.,

Soil Sci. Soc. Am. J. 76:408–419
Posted online 6 Feb. 2012
doi:10.2136/sssaj2011.0273
Received 28 July 2011.
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1992). However, most investigators have focused on C in surface soils. Carbon retained in deeper soil layers may be more stable than that in surface soil due to differences in source, composition, and environmental conditions (Rumpel and Kögel-Knabner, 2011). Subsurface soil receives a large proportion of inputs from root residues (including exudates) which appear to be more effective than shoot residues in building SOM (Gregorich et al., 2001; Rasse et al., 2006). Additional C inputs are introduced to subsurface soil from upper soil layers via water transport of dissolved and particulate organic C and bioturbation (Rasse et al., 2006). Turnover of these C inputs is influenced by environmental conditions in these lower strata, which appear to discourage decomposition (Gill and Burke, 2002; Collins et al., 1999). Temperature, moisture, and soil texture, key factors controlling SOM decomposition rates on a regional scale, also vary with depth in a soil profile (Gill and Burke, 2002) as do microbial and fungal activities (Taylor et al., 2002). Fontaine et al. (2007) suggested that C in deep soil layers is protected from decomposition due to the lack of fresh plant residues to stimulate microbial activity, while Salome et al. (2010) suggested that physical separation between substrate and decomposers may limit decomposition in the subsoil. Taken together, the environmental conditions in subsurface soil may discourage decomposition such that much of the C in deeper soil layers can be considered spatially or physically protected.

There is surprisingly little information on the chemistry and stability of OM in subsurface horizons (Rumpel and Kögel-Knaber, 2011) or on how subsurface OM is affected by soil management. The majority of long-term studies have focused on changes in the top 15 or 20 cm of soil, yet subsurface soil contains half or more of total soil C stocks, and, notably, these stocks have markedly different turnover kinetics than surface soil C (Fontaine et al., 2007; Rasse et al., 2006). A better understanding of the characteristics of SOM in subsurface horizons is needed to improve modeling of organic matter turnover and storage on a landscape scale (Viaud et al., 2010). Studies of the effects of manure-amended systems on deeper soil C storage are scant and inconclusive, with gains in deeper soil C storage detected by some (Grant et al., 2001; Sommerfeldt et al., 1988) but not by others (Poudel et al., 2001).

Characterizing SOM presents numerous challenges because of the intermixture of organic and mineral components in soils. Historically researchers have employed alkaline solutions to extract a C-rich fraction from soils, with subsequent acidification and fractionation of the extracts before chemical characterization. There is a large body of literature describing the chemistry of such humic fractions, but recently researchers have questioned the relevance of such fractions as models of SOM (Kleber and Johnson, 2010). We combined two approaches to quantify and characterize SOM: (i) using water and pyrosphosphate to sequentially extract relatively chemically unaltered, labile fractions of soil C and characterizing them by excitation emission matrix (EEM) fluorescence spectroscopy combined with parallel factor analysis (PARAFAC), and (ii) using thermal analysis to examine whole SOM before and after the extraction sequence. Pyrophosphate has been used to extract soil C due to its ability to remove iron- and aluminum-bound organic matter by complexing with di- and trivalent cations (Schnitzer and Schuppli, 1989; Wattel-Koekkoek et al., 2001). Fluorescence spectroscopy combined with PARAFAC has been shown to be a sensitive method to provide quantitative, chemically meaningful information on the composition of SOM (Ohno and Bro, 2006).Thermal methods can be applied to bulk soil with no pretreatment and offer a rapid and inexpensive means of characterizing SOM (Plante et al., 2009). Our specific objectives were to characterize the relative stability and composition of SOM at three different depths within the soil profile in soils that have contrasting management histories and significantly different quantities of organic matter.

MATERIALS AND METHODS

Soil Sampling and Sample Processing

Soil samples were collected from a subset of plots of the Maine Potato Ecosystem Project, which was initiated at the Aroostook Research Farm in Presque Isle, Maine in 1991 to examine the effects of different management systems on the productivity and economic viability of potato (*Solaum tuberosum* L.) production (Gallandt et al., 1998). The site (46.65°N, 68.02° E) has a mean annual temperature of 4.6°C and mean annual precipitation of 895 mm. In total, the research plots cover 5.8 ha (14.6 × 41 m each), with the dominant soil type being a gravelly, well-drained Caribou loam (fine-loamy, isotic, frigid, Typic Haplorthod). Clay mineral species present in the soil include quartz, kaolinite, illite, chlorite/vermiculite/smectite (Soil Geomorphology Laboratory, University of Nebraska, Lincoln, NE).

Plots sampled for this study were in either 4-yr (potato-soybean [*Glycine max* (L.) Merr.]-potato-barley [*Hordeum vulgare* L.]) or 2-yr (potato-barley) rotation cycles in a randomized complete block design with two factors (crop type and amendment) and four replicates. Although the specific rotations used changed in 1999 (see Alyokhin et al. (2005) and Gallandt et al. (1998) for a description of previous rotations), a comparison has been maintained between two contrasting soil treatments: Amended, which has received manure, compost, and supplemental fertilizer, and Non-amended, which has received only inorganic fertilizer. Solid-bedded beef manure and cull potato compost were applied annually from 1991 to 1993 and semi-annually (potato year only) from 1994 to 1998. After 1998 compost applications were discontinued and only beef manure was applied to the potato and grain crops at rates of 67 and 45 Mg ha^{-1}, respectively (Mallory et al., 2010). Amendments were applied and incorporated just before planting. Four amended and four non-amended plots were sampled on 4 June 2009 just before amendment application and crop planting. All sampled plots were in barley in 2008 and were planted with potato in 2009.

Using the core method described by Blake and Hartge (1986) four separate samples were taken per plot for bulk density at depth increments of 0 to 15, 15 to 30, and 30 to 45 cm. After oven drying and weighing, bulk density samples were sieved (2 mm) and bulk densities corrected for the presence of rocks. Six separate soil samples were taken per plot for each depth incre-

ment and thoroughly mixed to form one composite sample per plot for each depth increment. The 24 composite samples were air-dried and sieved through a 2-mm sieve before sequential extraction and further analysis.

Soil Analysis

Total soil organic C was determined using a Leco CN-2000 Analyzer. Soil pH was measured using a 1:1 deionized/distilled water (DI-H_2O): dry soil ratio. Water-extractable organic carbon (WEOC) was determined twice, sequentially, using a method similar to that of Piper et al. (2006).The initial water extract represents the most labile fraction of SOM which is composed of a complex, heterogeneous mixture of molecules most likely to be directly involved in ecosystem processes such as nutrient and metal mobilization and C cycling (Zsolnay, 1996; Chantigny, 2003). Briefly, 15 g of air-dry soil was added to a 50-mL plastic centrifuge tube with 30 mL of deionized water. Tubes were shaken on a table shaker for 1 h at room temperature, centrifuged for 30 min at 900 × *g*, filtered through Whatman GF/C glass fiber filters and then through Millipore nylon 47-mm diam. 0.45-μm filters. The second water-extract was designed to sample a less mobile water-soluble fraction, less influenced by the contents of lysed microbial cells. The second water extraction was conducted by adding enough deionized water to the tubes to return the water content to 30 mL and again shaking, centrifuging, and filtering as described above. After removal of the second water supernatant, a third extraction was performed by adding 25 mL of 0.125 mol L^{-1} sodium pyrophosphate preadjusted to pH 5.0 to the tubes and again repeating the previously described steps. The pyrophosphate fraction consists of molecules solubilized through a ligand exchange reaction, which removes Fe and Al cations (Schnitzer and Schuppli, 1989; Wattel-Koekkoek et al., 2001). This fraction likely represents material that was chemically sorbed to soil surfaces and protected from decomposition due to this sorption. The pyrophosphate extracts were filtered only through the Millipore nylon 0.45-μm filters with no pre-filtration with GF/C. All extract solutions were analyzed for dissolved organic C using a Shimadzu TOC-5000 Analyzer.

Thermometric Analysis

Thermometric analyses were used to characterize SOM in the solid phase before and after extraction of the labile components. Analyses of bulk soils and soil residues after sequential extraction were performed using a Netzsch STA 409PC Luxx simultaneous thermal analyzer equipped with a type-S (Pt/PtRh) TG-DSC sample carrier (Netzsch-Gerätebau GmbH, Selb, Germany). Before analysis, samples were lightly ground in a mortar and pestle to pass a 500-mm sieve. Samples (30 mg) were placed in a Pt/Rh crucible with an identical and empty crucible used as the reference, and heated from ambient to 105°C, held at this temperature for 15 min to remove moisture, then heated to 700°C at 10°C min^{-1} under an oxidizing atmosphere of 30 mL min^{-1} of synthetic air (20% O_2 and N_2 balance) and 10 mL min^{-1} of N_2 as a protective gas. The instrument was previously calibrated for temperature and enthalpy sensitivity using the melting points of five salts, and calcium oxalate was periodically used as a check. Differential scanning calorimetry (DSC) data were corrected for baseline drift using instrumental correction runs, as well as a posteriori corrections. Net energy content (in mJ) of the combination of organic matter combustion and mineral reactions was determined by integrating the DSC heat flux (in mW) over the exothermic region 190 to 600°C, and thermogravimetric (TG) mass loss was determined for the same range. Energy density (J mg^{-1} OM) was thus determined by dividing DSC net energy content by TG mass loss. The stability of SOM was characterized by determining the proportion of TG mass loss in the thermally labile (150–380°C, Exo_1), thermally resistant (380–500°C, Exo_2), and thermally refractory (500–600°C, Exo_3) components (sensu Lopez-Capel et al., 2005). We also calculated two additional thermal indices of SOM stability suggested by Rovira et al. (2008), the temperature at which half of the exothermic mass loss has occurred (TG-T_{50}) and the temperature at which half of the net exothermic energy has been released (DSC-T_{50}).

Excitation Emission Matrix Fluorescence and Parallel Factor Analysis

The soil water and pyrophosphate extract solutions were diluted to 15 mg C L^{-1} to minimize inner-filtration effects. Fluorescence measurements were obtained using a Hitachi F-4500 spectrofluorometer (Hitachi; San Jose, CA) with the excitation range set from 240 to 400 nm and the emission range set from 300 to 500 nm in 3-nm increments. Instrumental parameters were: excitation and emission slits, 5 nm; response time, 8 s; and scan speed, 240 nm min^{-1}. The EEM fluorescence landscape was obtained by setting the EX range from 240 to 400 nm and EM range from 300 to 500 nm in 3 nm increments. Pre-processing steps were used to minimize the influence of scatter lines and other attributes of the EEM landscape before PARAFAC modeling. An EEM that was collected from a control DI-H_2O solution was subtracted from each sample EEM to remove the lower intensity Raman scatter effects (Christensen et al., 2005). The blank subtraction will not adequately remove the higher intensity Rayleigh scatter lines. Thus, the Rayleigh scatter lines and the region immediately adjacent to the region where the emission and excitation wavelengths are equal were removed by setting the fluorescence intensity values of these data points as missing. In addition, EEM data have a triangular-shaped region where the emission wavelength is less than the excitation wavelength (upper left-hand corner), which is physically impossible, and these data pairs were set to zero. The PARAFAC modeling approach has been described in detail elsewhere (Ohno and Bro, 2006). The PARAFAC modeling was conducted with MATLAB, Release 14 (Mathworks, Natick, MA) using PLS_Toolbox version 4.0 (Eigenvector Research, Manson, WA). A non-negativity constraint was applied to the parameters to allow only chemically relevant results because negative concentrations and fluorescence intensities are chemically impossible assuming that quenching and inner filter effects are negligible. Parallel factor analysis models with two to eight components were computed. The correct

number of components in the data set was determined by the core consistency diagnostic score, which should be close to 100% for appropriate models. The core consistency provides an estimate of how well the model captures trilinear information, and if the consistency turns low, that is, toward zero, it is a strong indication that the model is invalid (Bro and Kiers, 2003).

Statistical Analysis

Concentrations of extracted C and thermal and fluorescence parameters were analyzed using the GLM procedure of SYSTAT 12 (San Jose, CA). Where depth effects were significant ($p \leq 0.05$), Fisher's least significant differences was calculated. The paired *t* test procedure of SYSTAT was used to evaluate whether there were differences in soil thermal parameters before versus after sequential water and pyrophosphate extraction. When differences were significant, the GLM procedure of SYSTAT was used to evaluate treatment effects. The classical discriminant analysis function of SYSTAT was used to generate grouping by treatment and depth based on fluorescence parameters (concentrations of each of four components determined by PARFAC modeling of the data) and thermometric parameters (%TG mass loss in Exo_1, Exo_2, and Exo_3, energy density, TG-T_{50} and DSC-T_{50}). Ellipses representing 95% confidence limits for the depth groupings were calculated using a MATLAB script.

RESULTS AND DISCUSSION

Total and Extractable Carbon Quantities

Amended soils contained greater amounts of C on both a weight and area basis than non-amended soils in the top two depths (Table 1). Amendment addition approximately doubled C content in the surface 15 cm and also significantly increased C in the 15- to 30-cm layer. Differences in C between amended and non-amended were not significant at the 30- to 45-cm depth. Carbon content decreased significantly with depth in both amended and non-amended soils. In this experiment, tillage depth was between 15 and 30 cm, and therefore in both amended and non-amended soils there was some mixing of material from the 0- to 15-cm depth with material from the 15- to 30-cm depth. In the amended soils, there was a difference in C content between these two horizons suggesting partial, rather than complete, mixing of the two due to tillage. Tillage likely did not extend below 30 cm suggesting that the source of C at this depth, for both amended and non-amended plots, was root growth, leaching of dissolved C, and natural mixing of plant residue due to activity of soil organisms. In the non-amended soils, about 23% of the C in the top 45 cm was located below 30 cm, and in the amended soil about 17% was located below 30 cm. Taken together, the results indicate that amended-derived C was concentrated at the surface with minimal translocation to deeper layers.

Water-extractable organic C in the initial extraction ranged from about 100 mg kg^{-1} soil in the deepest layer of the non-amended soils to almost 300 mg kg^{-1} in the surface layer of the amended soils (Table 1). Concentrations of C in the second water extracts were slightly less than half of the concentrations in the initial water extracts (Table 1). For both the initial and second water extracts, the concentrations of WEOC were greater in amended than non-amended plots for all three depths. In general WEOC concentrations declined with depth (Table 1). The WEOC concentrations were different among depths for every depth except between 0 to 15 and 15 to 30 cm in non-amended plots for the second extract.

Initial WEOC values expressed as a percentage of total C, were around 1 to 1.25% and were not affected by depth or amendment. The second water extract removed a smaller percentage of total C, 0.4 to 0.6%; these values were greater in the 15- to 30- and 30- to 45-cm depths than in the 0- to 15-cm depth for the amended soils (Table 1). These values were in the range, or slightly greater than, those of other investigators. Fierer and Schimel (2003) found 0.2 to 0.5% WEOC in air-dry surface soils, and Provenzano et al. (2010) found 0.1 to 0.4% WEOC in oven-

Table 1. Total and extractable organic carbon for the amended and non-amended soils at three sampling depths.

Soil Treatment	Depth	Total organic C		Water-extractable organic C (WEOC)				Pyrophosphate-extractable organic C	
				Initial		Second			
	cm	g kg^{-1}	kg ha^{-1}	mg kg^{-1}	% of TOC	mg kg^{-1}	% of TOC	mg kg^{-1}	% of TOC
Amended	0–15	29.8	50,400	289	0.98	127	0.43	1626	5.48
	15–30	16.7	32,700	199	1.20	94	0.57	1630	9.82
	30–45	9.8	17,500	125	1.24	61	0.62	1809	16.81
Non-amended	0–15	14.4	26,700	166	1.16	67	0.47	1397	9.72
	15–30	12.2	23,300	139	1.17	59	0.49	1495	12.46
	30–45	8.6	14,700	101	1.21	45	0.54	1572	18.15
					ANOVA				
Source of variation									
Soil treatment		***	***	***	NS†	***	NS	NS	**
Depth		***	***	***	NS	***	*	NS	***
Soil Tmt x Depth		***	**	***	NS	***	NS	NS	NS
Fisher's $LSD_{0.05}$		3.8	6800	21	NS	11	0.12	NS	2.48

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NS = not significant at the 0.05 probability level.

dry A, B, and C horizons. Using field-moist soils generally yields somewhat less WEOC (e.g., 0.01–0.3%, Gregorich et al., 2000). The amount of WEOC varies from soil to soil depending primarily on soil C content. The percentage of total SOC as WEOC can be expected to vary also due to differences in extraction methodology among investigators. There is uncertainty in the literature about how much of the increase in WEOC observed with soil drying is due to the lysing of microbial cells. Fierer and Schimel (2003), using ^{14}C labeled glucose additions to soil, found drying increased WEOC by more than 200% and that the increase was not due to increased solubility of microbial biomass C. They suggested that drying and subsequent rewetting may disrupt microaggregates and increase the solubility of soil organic C.

Pyrophosphate extracted much greater amounts of C than water, as expected (Table 1). There were no significant effects of depth or amendment on the amount of C extracted by pyrophosphate, suggesting that mineral surface area or surface functional groups determine the amount of pyrophosphate-extractable C. Clay content did not differ by depth or amendment (data not shown). However, expressed as a percentage of total C, the C extracted by pyrophosphate was significantly affected by both amendment and depth. Although pyrophosphate extracted only 5 to 10% of the C in the 0- to 15-cm layer, it extracted 17 to 18% of the C in the 30- to 45-cm layer. Pyrophosphate extracted more C as a percentage of the total C present in non-amended soil than in amended soil in both the 0- to 15- and 15- to 30-cm depths. Taken together, the extraction results suggest a greater relative presence of unstabilized C, not bound to soil surfaces, in the amended soils compared with the non-amended, as well as in the surface horizons compared with the deeper horizons.

Thermometric Analysis

Analysis of the bulk samples showed that the most thermally labile fraction of SOM (i.e., Exo_1) was not affected by either depth or amendment (Table 2).The thermally resistant fraction of SOM (i.e., Exo_2) decreased slightly with depth with significant differences between each depth except 0 to 15 and 15 to 30 cm for non-amended, but was unaffected by amendment overall. The thermally refractory fraction of SOM (i.e., Exo_3) was unaffected by amendment but increased with depth, with significant differences between the surface and the deepest horizon for amended soils (Table 2). The net energy density of the SOM averaged 32 (±1.5) J mg^{-1} C and was unaffected by depth or amendment (Table 2). Assuming SOM contains about 50% C (Pribyl, 2010), this translates to a value of 16 J mg^{-1} OM, which is within the range reported by others for SOM and decomposing plant litter (Rovira et al., 2008; Duguy and Rovira, 2010). The DSC-T_{50} decreased with depth and was consistently less in non-amended than amended soils (Table 2). Conversely, TG-T_{50} increased with depth and was consistently greater in non-amended than amended soils, though the differences were not statistically significant. Taken together, the results suggest that SOM in the amended treatments and at the surface is more thermally labile. Rovira et al. (2008) previously found that as plant litter decomposes, its organic matter is lost at higher temperatures (i.e., increasing TG-T_{50}), even though its stored energy is released at lower temperatures (i.e., decreasing DSC-T_{50}), and thus our results would suggest that SOM at depth is more highly decomposed, which is also consistent with our fluorescence data.

The shapes of the DSC curves differed significantly before versus after the sequential extraction of labile SOM fractions (Fig. 1). Differences were most significant at lower temperatures (i.e., near the peak at 320°C), with smaller differences in energy flux at higher temperatures. Net energy density of the organic matter in the extraction residues was not significantly different ($p = 0.370$, by paired t test) from the bulk soil, averaging 33.1 ± 1.3 J mg^{-1} C for the residues versus 32.3 ± 1.5 J mg^{-1} C for the bulk soils, though the trend was for slightly higher energy density for each sample after sequential extraction. Similar to bulk soils, energy density of the extraction residues was unaffected by

Table 2.Thermochemical parameters for the bulk amended and non-amended soils.

Soil treatment	Depth	Thermogravimetric mass loss (%)			Energy density	TG-T_{50}	DSC-T_{50}
		EXO_1 (150–380°)	EXO_2 (380–500°)	EXO_3 (500–600°)			
	cm				mJ mg^{-1}C	°C	
Amended	0–15	58.1	29.1	12.8	31,900	357	355
	15–30	55.4	27.9	16.7	31,800	361	342
	30–45	51.6	26.6	21.8	35,200	377	332
Non-amended	0–15	53.2	28.3	18.5	32,100	368	341
	15–30	53.9	27.7	18.4	32,400	366	336
	30–45	50.8	25.8	22.7	30,900	382	327
				ANOVA			
Source of variation							
Soil treatment		NS†	*	NS	NS	NS	***
Depth		NS	***	*	NS	NS	***
Soil Tmt x Depth		NS	NS	NS	NS	NS	**
Fisher's $LSD_{0.05}$		NS	1.1	7	NS	NS	3

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NS = not significant at the 0.05 probability level.

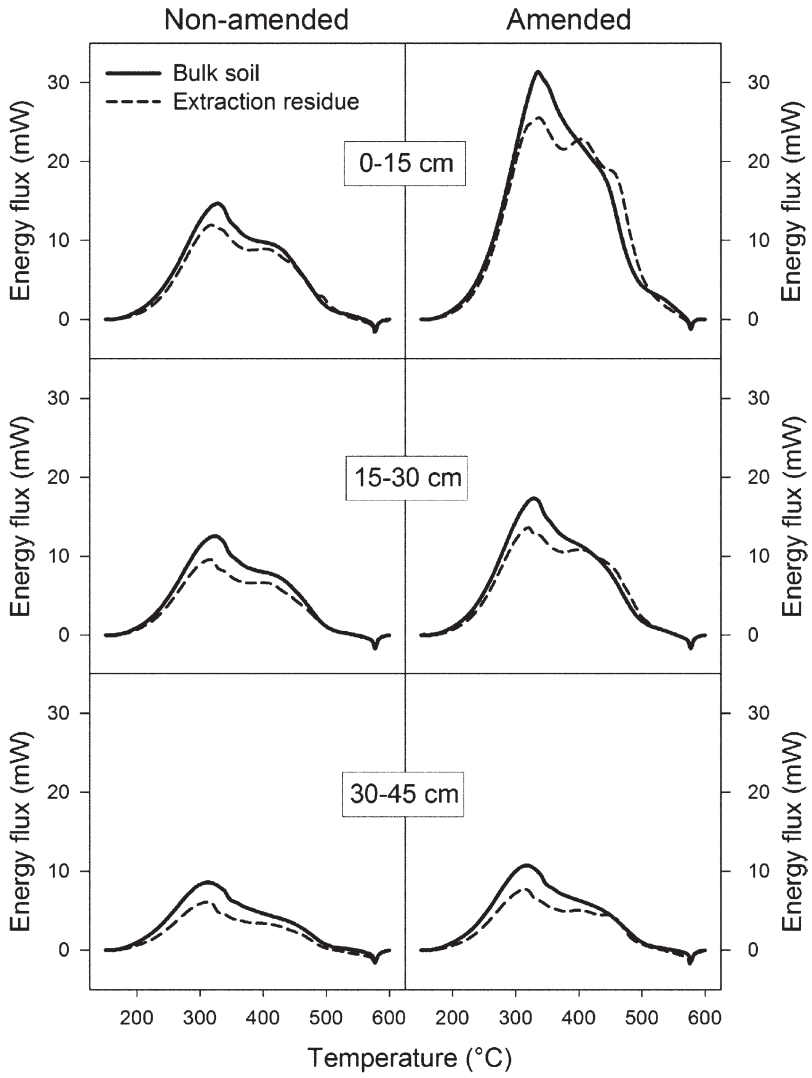

Fig. 1. Differential scanning calorimetry energy flux as a function of temperature for amended and non-amended soils, three different depths, before and after extraction with pyrophosphate.

depth or amendment (Table 3). The proportion of thermogravimetric mass loss at low temperatures (e.g., Exo_1) was significantly lower in the extraction residues compared with the bulk soils ($p < 0.001$, by paired t test), and this decrease was matched with significant increases in Exo_2 ($p < 0.001$, by paired t test) and smaller increases in Exo_3 ($p < 0.001$, by paired t test). Thermogravimetric- and DSC-T_{50} values were also greater in the extraction residues compared with the bulk soil ($p < 0.001$ for both, by paired t test). Differences in TG-T_{50} between bulk soils and extraction residues increased with depth ($p = 0.037$), while differences in DSC-T_{50} showed the opposite trend, decreasing with depth ($p = 0.014$), and were also lesser in non-amended samples ($p = 0.001$). Taken together, the trends in the differences among the thermometric analyses before and after sequential extraction confirm that the extraction procedure isolated relatively labile pools of SOM and left behind a more stable fraction.

Excitation Emission Matrix Fluorescence/Parallel Factor Analysis Fluorescence Spectroscopy

The EEM spectra of the three sequential extracts of the amended and non-amended 0- to 15-cm soils are shown as representative spectra, with characteristics typical for the entire soil set (Fig. 2). Visual inspection of the water extract spectra suggests the presence of four fluorophores at EX and EM wavelength pairs of <240/440, 250/<300, 270/355, and 315/440 nm. The pyrophosphate spectra indicate the presence of two of the fluorophores visible in the water extract spectra, the 240/440 and 315/440-nm wavelength pairs. These

Table 3. Thermochemical parameters for the sequential extraction (water+pyrophosphate) residues from amended and non-amended soils.

Soil treatment	Depth	Thermogravimetric mass loss, %			Energy density	TG-T_{50}	DSC-T_{50}
		EXO_1 (150–380°)	EXO_2 (380–500°)	EXO_3 (500–600°)			
	cm				mJ $mg^{-1}C$	°C	
Amended	0–15	53.6	33.8	12.6	34,400	369	369
	15–30	50.8	31.9	17.3	33,600	378	353
	30–45	46.0	29.7	24.3	33,900	400	338
Non-amended	0–15	50.0	30.7	19.3	33,400	381	348
	15–30	48.8	29.4	21.9	32,500	385	338
	30–45	44.4	27.8	27.8	30,800	410	324
				ANOVA			
Source of variation							
Soil treatment		NS†	***	*	NS	NS	***
Depth		**	***	**	NS	*	***
Soil Tmt x Depth		NS	NS	NS	NS	NS	NS
Fisher's $LSD_{0.05}$		5.7	1.9	6.2	NS	28	9

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NS = not significant at the 0.05 probability level.

fluorophores have been previously observed in organic matter extracted from different sources and have been characterized in the following way. The <240/440 nm and 315/440 peaks have been designated as A and C, respectively, and attributed to humic-like materials (Coble, 1996). The two other peaks are from protein-like materials containing tyrosine (250/<300 nm, 'B') or tryptophan (270/355 nm, 'T') (Kowalczuk et al., 2009).

The EX and EM locations of the four component model (the most appropriate model based on the core consistency diagnostic test) are shown in Fig. 3. We compared these modeled EEM components with components determined in other studies involving

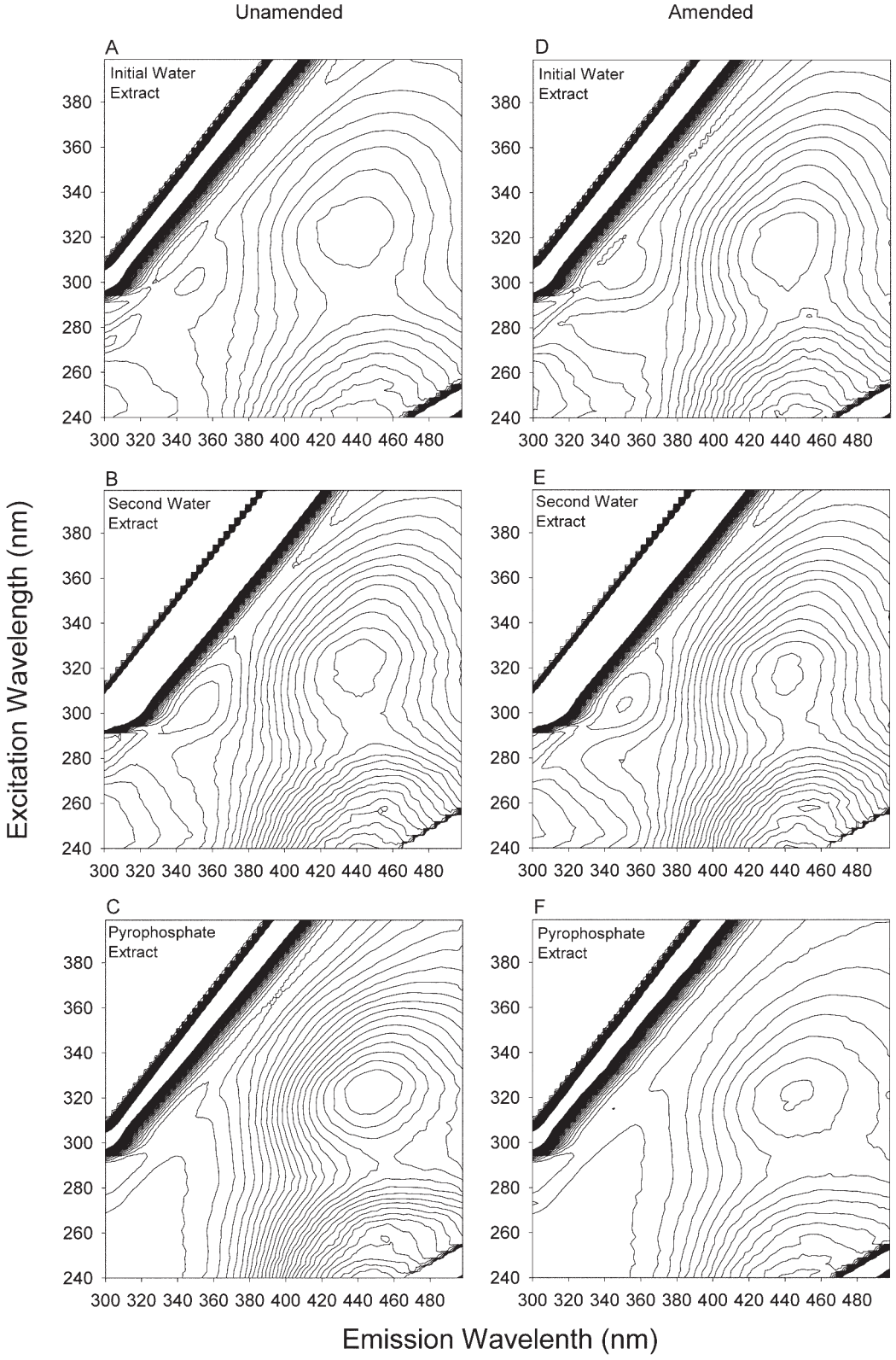

Fig. 2. Representative excitation emission matrix (EEM) spectra of the three sequential extracts of the amended and non-amended soils, 0- to 15-cm depth.

terrestrial organic matter. Component 1 is very similar to the fulvic-like component assigned by Santín et al. (2009). Component 2 is comparable with Component 2 in Santín et al. (2009) and Component 1 in Chen et al. (2010), which were designated as humic-like. Component 3 is similar to Component 6 reported by Chen et al. (2010), suggested to be a product of microbial degradation of humic-like precursors. In addition, Component 3 matches the component which increased 199% in concentration following the decomposition of plant biomass in a laboratory incubation study which supports the assignment of this component to microbial decomposition products (Hunt and Ohno, 2007). Component 4 is protein-like, incorporating both tyrosine- and tryptophan-containing materials (Chen et al., 2010).

The concentrations (in arbitrary units) of the four components as estimated in the first loading of the PARAFAC model are shown in Table 4. It is important to recognize that the concentrations presented in Table 4 are based on their fluorescence signal contribution rather than their true chemical concentration contribution. Expression on a chemical concentration basis would require knowledge of the quantum fluorescence efficiencies of the individual components, which are unknown.

In both water extracts, the amended treatment led to higher concentrations of all four components in all three depths, except for Component 2 from the 30- to 45-cm soil depth (Table 4). The addition of C-rich manure amendments affected the composition, as well as the amount, of the WEOC from all soil depths by approximately doubling the fulvic-like Component 1 and more than tripling the humic-like Component 2. The microbial degradation Component 3 increased, on average, by a factor of 1.7. Animal manures have been reported to contain high proportions of these humic- and fulvic-like components (Ohno and Bro, 2006).

Concentrations of all four components in both water extracts declined with depth in all amended treatments. For non-amended treatments, concentrations of all components except Component 3, associated with microbial decomposition, declined with depth. Relative component concentrations are shown in Fig. 4. Comparison of Fig. 4A and 4D clearly shows the increasing contribution of Component 3, associated with microbial degradation, and decreasing contribution of the humic-like Component 2 with depth. This suggests that the humic-like component is preferentially being microbially degraded com-

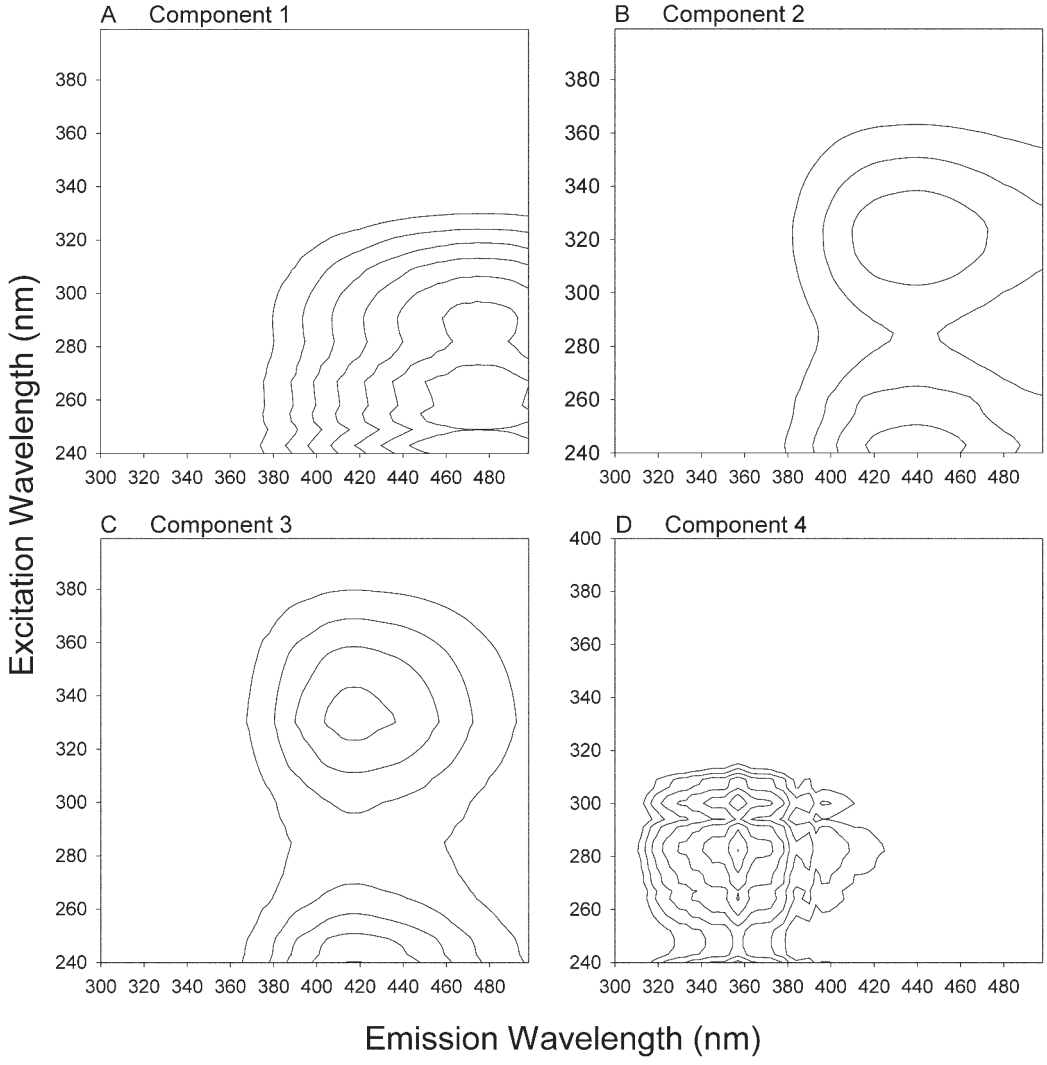

Fig. 3. The EX and EM locations of fluorophores determined by the four component model.

Table 4. Concentration of PARAFAC components (CMP1–4) in arbitrary units for the water and pyrophosphate extracts of the amended and non-amended soils at three sampling depths.

Soil Treatment	Depth (cm)	Water-extractable organic C (WEOC)								Pyrophosphate-extractable organic C			
		Initial				Second							
		CMP1	CMP2	CMP3	CMP4	CMP1	CMP2	CMP3	CMP4	CMP1	CMP2	CMP3	CMP4
Amended	0–15	40.1	23.9	26.8	14.8	25.1	12.3	12.8	6.6	188	309	6.6	3.2
	15–30	34.2	12.0	22.4	6.6	22.9	7.8	13.8	3.5	265	407	24.8	2.0
	30–45	15.4	2.7	15.1	3.6	11.7	2.7	10.1	2.4	307	506	32.0	7.6
Non-amended	0–15	15.4	7.8	12.6	6.3	12.0	5.6	7.8	3.6	205	381	3.5	4.1
	15–30	16.7	4.3	15.1	4.3	11.9	3.5	8.9	2.5	245	425	14.8	3.9
	30–45	8.9	0.7	10.8	2.7	7.0	1.0	7.2	1.7	267	456	35.1	4.5
						ANOVA							
Source of variation													
Soil treatment		***	***	***	***	***	***	***	***	NS	NS	NS	NS
Depth		**	***	**	***	***	***	*	***	*	NS	*	NS
Soil Tmt x Depth		***	***	NS†	***	*	**	NS	***	NS	NS	NS	NS
Fisher's $LSD_{0.05}$		5.3	2.2	3.9	0.4	3.8	1.5	2.4	0.6	96.7	NS	23.7	NS

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† NS = not significant at the 0.05 probability level.

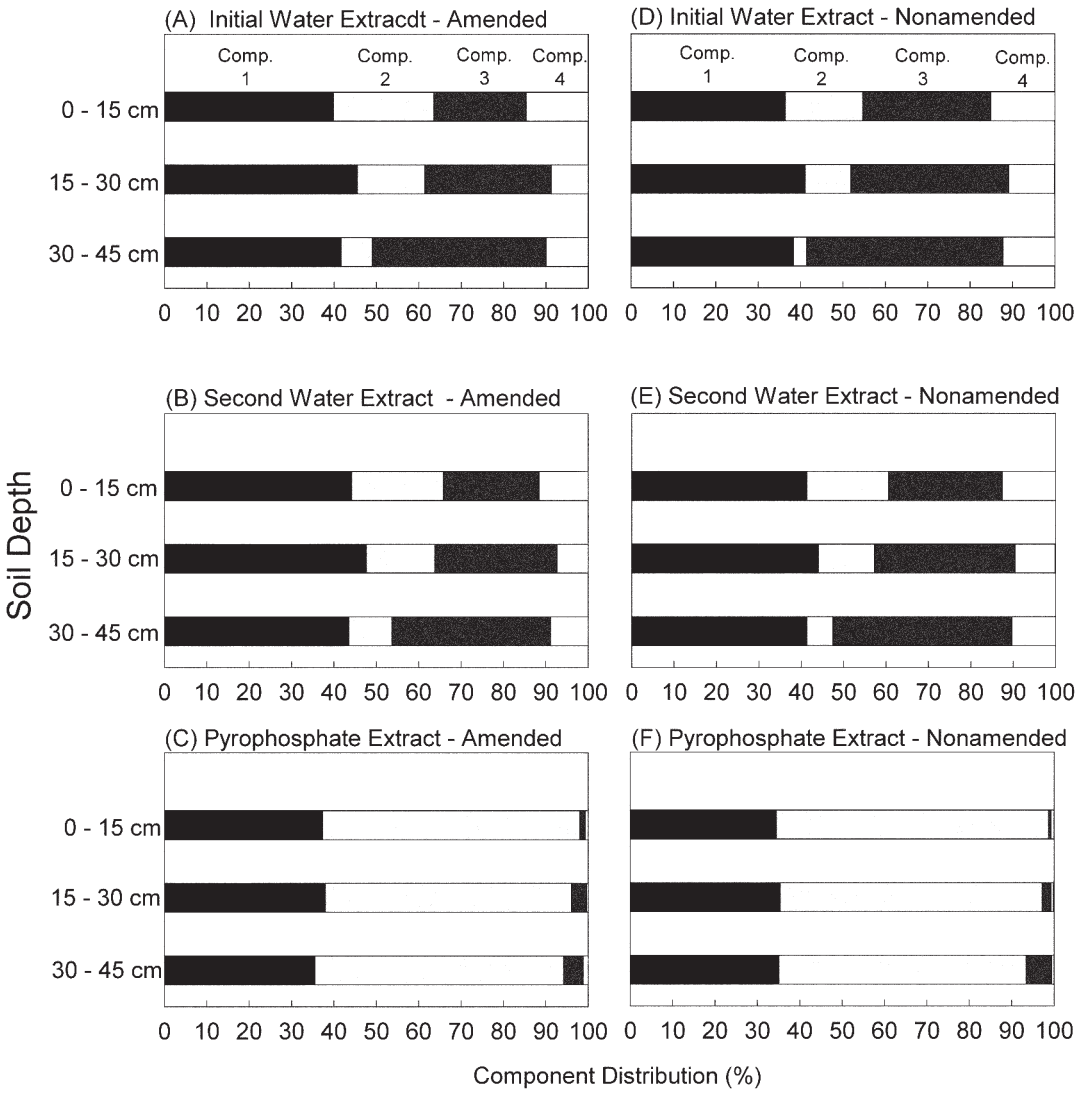

Fig. 4. Relative component concentrations for the three sequential extracts of the amended and non-amended soils at three different depths.

pared with the fulvic-like Component 1, which showed little change with depth as a proportion of total. The relative concentrations of the protein-like Component 4 were similar between the amended and non-amended treatments and declined somewhat with depth. The second water extracts mirrored the results seen with the initial extracts. The concentrations in the second water extracts as compared with the initial extract for the humic- and fulvic-like components were 77% for the non-amended soils and 63% for the amended soils indicating that the non-amended soils had a greater relative ability to release humic- and fulvic-like material to the second sequential water extract than the amended soils. This is likely due to the annual amendment of the manure which maintains non-stabilized organic matter in the soil.

In contrast to the water extracts, there were no effects of manure amendment on the concentration of any of the four PARAFAC-derived components of the pyrophosphate extractable fraction (Table 4). Despite no effect of depth on concentration of pyrophosphate extractable C (Table 1), concentrations of Component 1, in the amended soils, and Component 3, in both amended and non-amended soils, increased with depth. The consistent increase in Component 3 with depth suggested relatively more microbially decomposed material at depth compared with the surface (Table 4). The humic- and fulvic-like components represented >90% of the extracted organic matter for both treatments and all depths (Fig. 4C and 4F). It is surprising that after 18 yr of organic amendment both the amount of pyrophosphate-extractable C and its PARAFAC signature remain the same as in non-amended soil (Tables 1 and 4). This clearly suggests that amendments with C-rich materials are not likely to affect the moderately labile fraction of the SOM pool that is bound to soil surfaces by complexation with Fe and Al (i.e., the pyrophosphate-extractable fraction).

CONCLUSIONS

A large percentage of total soil C resides in deeper soil layers, but little is known about its chemistry and stability (Rumpel

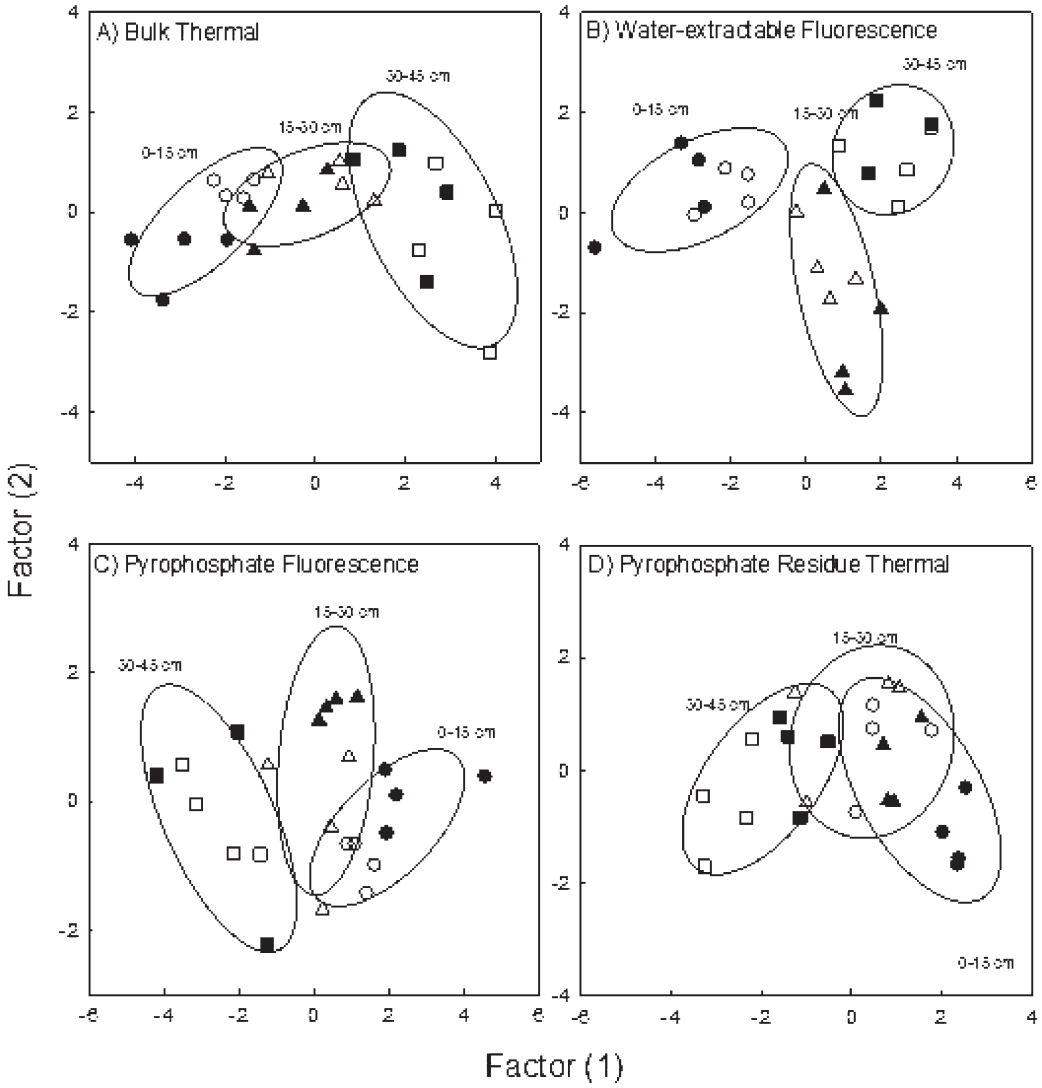

Fig. 5. Discriminant analysis of thermometric parameters (i.e., % Thermogravimetric [TG] mass loss in Exo1, Exo2, and Exo3, energy density, TG-T50 and DSC-T50) and fluorescence parameters (i.e., concentrations of each of four components determined by PARFAC modeling of the data). Open symbols represent non-amended treatment, and closed symbols represent amended treatment for samples from 0- to 15-cm depth (circles), 15- to 30-cm depth (triangles) and 30- to 45-cm depth (squares).

and Kögel-Knabner, 2011), and the few studies that exist suggest that its dynamics may be regulated by different mechanisms than surface soils (Salome et al., 2010). We studied both depth and soil treatment (an 18-yr history of applications of C-rich amendments) effects on the composition and stability of total SOM, as well as three fractions: a highly labile fraction extracted with water (about 1% of the total), a moderately labile fraction extracted by pyrophosphate (5–18% of total), and the resistant fraction remaining in the soil after pyrophosphate extraction. A synthesis of our findings can be illustrated by discriminant analysis of thermal and fluorescence parameters (Fig. 5). Results of the discriminant analysis showed that differences between depths were generally more pronounced than differences because of amendment treatment. The depths were most distinctly different for the more labile fractions of C, that is, fluorescence parameters from water extracts (Fig. 5b, no overlap between 95% confidence limits) and pyrophosphate extracts (Fig. 5c, a small amount of overlap between 0- to 15- and 15- to 30-cm depths based on 95% confidence limits). Thermal parameters for the bulk soils showed some overlap between depths (Fig. 5a). After extraction of the more labile and chemically distinct fractions, thermal parameters became more similar between depths, with significant overlap between 95% confidence limits for both 0- to 15- and 15- to 30-cm depths and 15- to 30- and 30- to 45-cm depths. Analysis of the soil residue after sequential extraction confirmed that the extractions removed a relatively labile pool and left behind a more stable pool of organic matter.

Eighteen years of C-rich amendments at our study site increased total soil C in the 0- to 15- and 15- to 30-cm depths, but not in the 30- to 45-cm depth, indicating that amendment-derived C was concentrated at the surface with minimal translocation to deeper soil layers. In addition, thermal analysis (increasing TG-T_{50}, and decreasing DSC-T_{50}, with depth) and the increasing concentration with depth of the fluorescent Component 3 (associated with microbial decomposition) in the pyrophosphate-extractable C fraction suggested that C deeper in the soil profile was more microbially decomposed than surface C. This finding supports some recent research (e.g., Liang and Balser, 2008), who found preferential accumulation of microbial amino sugars in subsurface soil, but contradicts other recent studies suggesting that C in deep soil layers is relatively protected from decomposition due to the lack of fresh plant residues to stimulate microbial activity (Fontaine et al. (2007) or environmental conditions unfavorable for microbial activity (Gill and Burke, 2002).

Our study did not, therefore, support the idea that C-rich amendments in agroecosystems are likely to significantly increase C sequestration deeper in soil profiles, because under the given climatic and soil conditions, relatively little added C was transported below surface layers and because deeper soil C showed signs of microbial decomposition. Carbon-rich amendments, while providing significant agronomic benefits, probably increase C storage only in surface horizons where much of the C remains susceptible to microbial decomposition if amendments cease.

ACKNOWLEDGMENTS

We thank Hannah Griffin, Kate Kirby, Andy Lannan, Katherine McPhee, Dan Ohno, and Stephanie Sosinski for help with sampling; Krzysztof Lesniewicz, Nate Severy, and Stephanie Sosinski for help with sample processing and analysis; and Paul Ohno for writing the algorithm to plot 95% confidence limits in Fig. 5. This project was supported by USDA-National Research Initiative Competitive Grant no. 2008-35107-04480 (Ohno) and 2010-65107-20351 (Plante) from the USDA National Institute of Food and Agriculture, and has also been supported by Hatch funds provided by the Maine Agricultural and Forest Experiment Station. This is Maine Agricultural and Forest Experiment Station Publication no. 3244.

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