Differences in Soluble Organic Matter After 23 Years of Contrasting Soil Management

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Soluble organic matter (OM) is a small but very mobile and reactive fraction of soil organic matter (SOM). We characterized the properties of soluble OM from 0- to 125-cm soil depth in maize (*Zea mays* L.)-based agricultural systems that were managed with conventional tillage (CT) and no-tillage (NT) for 23 yr. Water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM) were obtained in a sequential extraction procedure using dilute ($0.01M$ $CaCl_2$) and concentrated (0.5 M K_2SO_4) salt solutions. Subsequently, WEOM and SEOM were characterized using resin fractionation, specific ultraviolet absorption at 254 nm ($SUVA_{254}$) and humification indices (HI). Concentrations of C and N of SOM and SEOM and C of WEOM declined with increasing soil depth. In contrast to less pronounced differences in C and N of SEOM in CT and NT soils, those of WEOM were greater (52–116% for C and 26–86% for N) in NT than CT soils throughout the soil profile. The proportion of C and N in SOM recovered as WEOM and SEOM increased with soil depth (three to nine times for C and two to five times for N). The proportion of hydrophilic-C increased with soil depth, but the proportion of humified OM decreased with soil depth. Consistent with reports that indicate CT enhances OM decomposition, our data suggest WEOM and particularly SEOM fractions in CT soils are more decomposed than NT soils down to 125-cm depth. The relative pool size and properties of WEOM and SEOM demonstrated a higher in situ contribution of SOM to soluble OM pool with increasing soil depth.

Abbreviations: CT, conventional tillage; HI, humification index; HPI, hydrophilic; NT, no-tillage; SEOC, salt-extractable organic carbon; SEOM, salt-extractable organic matter; SEON, salt-extractable organic nitrogen; SOC, soil organic carbon; SOM, soil organic matter; SUVA, specific ultraviolet absorption; TN, total nitrogen; WEOC, water-extractable organic carbon; WEOM, water-extractable organic matter; WEON, water-extractable organic nitrogen.

Soluble OM is a small fraction of SOM that has gained increasing interest in studies of terrestrial ecosystem C and N dynamics. Soluble OM contains a relatively high proportion of labile compounds (Haynes, 2005) and serves as the medium for soil microbial activity (Metting, 1993). The downward movement of soluble OM originating from topsoil plays an important role in sustaining subsoil microbial activity (Buscot, 2005). Microbially processed OM and other less decomposable compounds of soluble OM have been suggested to contribute substantially to long-term C storage, particularly at soil depth (Kalbitz and Kaiser, 2008). At the watershed scale, soluble OM flux through leaching and runoff may comprise a substantial portion of N losses from forests and agro-ecosystems (Perakis and Hedin, 2001; Sollins and McCorison, 1981; Van Kessel et al., 2009).

Soluble OM is operationally defined as organic compounds smaller than 0.45 µm (Herbert and Bertsch, 1995). In cropping systems, soil disturbance typically

limits in situ monitoring of soil solution using lysimeters; thus, soil extraction with aqueous solutions is commonly used to obtain soluble OM (Chantigny, 2003). Although there is no standard extraction method for obtaining soluble OM, it has been suggested dilute salt solutions obtain OM present in soil solution, while concentrated salt solutions release OM that is bound to minerals (exchangeable OM) (Jones and Willett, 2006; Zsolnay, 2003). These two fractions have been referred to as water- and salt-extractable organic matter (WEOM and SEOM, respectively). The size and properties of these fractions may serve as early indicators of changes in SOM dynamics in response to land-use and management (Burton et al., 2007; Haynes, 2000).

Dynamics of soluble OM have been extensively studied in forest soils, but similar information is less available in agroecosystems (Chantigny, 2003). Agricultural management affects soluble OM dynamics by affecting SOM inputs and decomposition. However, the depth at which SOM is affected by land management practices, including tillage, is not clear (e.g., Baker et al., 2007).

Conventional agricultural management practices including annual tillage have led to substantial OM losses in the highly productive soils of the Midwest. (Allmaras et al., 2000). As a result, reduced tillage has been adopted in part to mitigate and reverse the consequences of intensive agriculture on SOM contents. Although reduced tillage increases SOM contents in surface soils, the effect of tillage on subsoil OM content has been of recent debate (Baker et al., 2007; West and Post, 2002). In fact, conventional tillage systems may store more OM in subsoils due to deeper crop rooting (Baker et al., 2007; Powlson and Jenkinson, 1981).

Soluble OM concentrations and properties can be used to indicate differences in OM inputs and decomposition among different tillage strategies with the same crops. Our objective was to determine the effect of tillage (conventional vs. no-till) on the concentration and properties of WEOM and SEOM from the topsoil to 125-cm soil depth. We hypothesized that (i) the concentration and properties of soluble OM differ between conventional and no-till systems; and (ii) the influence of tillage on soluble OM properties is attenuated from topsoil to subsoil.

Table 1. Physical and chemical properties of soil at the experimental site.

Soil depth	Sand†	Silt†	Clay	CaCO₃	pH‡
cm			g kg⁻¹		
0–25	221	503	276	<1	7.3
25–50	312	324	364	17	7.7
50–75	342	329	329	26	8.1
75–100	588	229	183	63	8.2
100–125	711	171	118	102	8.3

† Particle distribution determined based on Kettler et al. (2001).

‡ pH was measured with deionized water at a ratio of 1:2 (soil/water)

MATERIALS AND METHODS
Study Site and Soil History

Soil sampling was conducted at the Agricultural Engineering Research Farm of Iowa State University, IA (42°80′19″ N, 93°84′59″ W; elevation: 341m), on Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls; Table 1). The experimental site was in continuous corn production between 1987 and 1996. In 1988 three tillage treatments [CT, moderate, and NT] were established in four replicates. In 1997, the entire experimental area was planted to soybean (*Glycine max* L.). In 1998, a corn–soybean–wheat (*Triticum aestivum* L.)/clover (*Trifolium pratense* L.) rotation was initiated with all phases represented each year. The experimental design was a randomized complete block design with treatments arranged as a split-plot with four replicates. Main plots (22.8 by 27.4 m) were tillage system and subplots (7.6 by 13.1 m) consisted of the application of compost or no compost. Soil sampling for this research only occurred in CT and NT no compost treatment. Moldboard plow depth in CT was approximately 20 cm. Additional details about the research site and management can be found in Singer et al. (2010). The tillage main plots were maintained during the entire period from 1987 to 2010. However, after the 3-yr rotation commenced in 1998, moldboard plowing only occurred the fall before corn and soybean production years. After harvesting soybean in the fall and before planting winter wheat, CT plots had one pass with a tandem disk and one pass with a field cultivator.

Soil Sampling and Extraction Procedure

Soil samples (0–125 cm) were collected on 30 Aug. 2010 with a hydraulic soil sampler using 6-cm diam. polyethylene sleeves. Four 125-cm soil cores were collected from each of four replicate CT and NT subplots (total cores = 32) in the wheat/clover phase of the crop rotation. Soil cores were transferred to the laboratory within 2 h and were cut into 25-cm depth increments. Within depth increments, subsamples from each plot were pooled, mixed, and sieved (2 mm) after removal of visible litter. For purposes of discussion, 0 to 25 cm was considered topsoil. Soil total C and N were measured using a CHN2000 analyzer (LECO Corp., St. Joseph, MI) after removal of carbonate by acidification (Skjemstad and Baldock, 2007). Carbonate was determined based on the evolved CO_2 after acidifying soils with 5% HCl (Nelson, 1982). The high inorganic C/organic C ratios could create errors in SOC at depths >75 cm (Table 1). To avoid the influence of variable soil moisture content at different soil depths on the concentration and properties of extractable OM, soils were incubated at 40% of their water holding capacity (pre-determined) for 2 wk (Wilke, 2005). A sequential extraction procedure was used to obtain WEOM and SEOM fractions. The WEOM was obtained by adding 100 mL 0.01 M $CaCl_2$ to a centrifuge bottle containing 50 g soil. The suspension was shaken in an orbital shaker at 150 rpm for 10 min and centrifuged at 2200 rpm for 12 min. The supernatant was filtered (0.45 μm) through a pre-leached cellulose nitrate (Millipore) filter. The

soluble OM obtained through such a procedure most likely represents the properties of OM existing in soil solution in situ (Zsolnay, 2003). The centrifuged bottles were weighed and the remaining moisture in the soils was calculated. The SEOM was subsequently extracted from the same soils in a two-step process. First, 100 mL 0.5 M K_2SO_4 was added to the soils and the suspension was shaken in a reciprocal shaker at180 rpm for 30 min followed by 12 min centrifuging at 2200 rpm. The supernatant was decanted and stored. Subsequently, the tubes were weighed and the procedure was repeated by adding a second 100 mL 0.5 M K_2SO_4 solution. The two supernatants were pooled and filtered as described above. The OM obtained during described procedure is considered to reflect the exchangeable pool of OM (Jones and Willett, 2006; Perakis and Hedin, 2001). Although decomposition of low molecular organic compounds (comprising a small portion of soluble OM) may occur during soil extraction procedure (Rousk and Jones, 2010), we assumed that this did not affect our results under experimental conditions due to short extraction time conducted for WEOM and high ionic strength of SEOM. The subsamples of WEOM and SEOM were stored at 4°C and analyzed within <5 d. Before analysis, the aliquots of WEOM and SEOM samples were allowed to warm to room temperature and shaken manually. All SEOM samples were diluted at least five times before analysis.

Chemical and Spectroscopic Analysis

Carbon and N concentrations of WEOM and SEOM were measured in acidified extracts on a TOC/TN analyzer (LiquiTOC, Elementar; Hanau, Germany), using high temperature (820°C) catalytic oxidation method. Mineral N (NH_4^+ + NO_3^-) was determined on a microplate spectrophotometer (Sims et al., 1995; Hood-Nowotny et al., 2010). Soluble organic N was calculated as the difference between the concentration of the total and mineral N.

Soluble OM is often characterized based on the proportion of its hydrophilic (HPI) C and N to assess its interaction with soil mineral particles (Herbert and Bertsch, 1995) and its association with leaching/retention of nutrients and pollutants (e.g., Kaiser et al., 2001; Raber et al., 1998). The resin fractionation was performed based on the procedure defined by Leenheer (1981), modified by Amery et al. (2009). Before the fractionation, DAX-8 resin was cleaned according to the method of Lepane (1999). Briefly, 30 mL of aliquots of acidified (pH 2) extracts was injected with a peristaltic pump into a DAX-8 packed column (Cromaflex, 25 × 150 mm) at rate of 180 mL h^{-1}. The initial 10 mL of leachate was decanted and the remainder of the leachate was recirculated through the column. After each sample, the column was rinsed with 30 mL 0.05 M HCl (180 mL h^{-1}). The collected solutions were pooled and classified as HPI-OM fraction. After each run, the column was rinsed with successive cycles of two 30 mL 0.1 M HCl and two 30 mL of 0.1 M NaOH at 450 mL h^{-1} (first run) and 100 mL h^{-1} (second run). Finally, the column was rinsed with 0.01 M HCl until the pH and UV_{254}

absorbance was stable. The C and N concentrations of the HPI-OM were determined as above.

The $SUVA_{254}$ has been shown to be strongly correlated with the proportion of aromatic compounds in dissolved organic matter (DOM) (Weishaar et al., 2003). The UV absorbance property of extracts was determined with a PerkinElmer 552 spectrophotometer in a 1-cm path-length quartz cell (SPECROCELL, Oreland, PA). The samples with UV absorbance >0.3 cm^{-1} were diluted before the analysis. The $SUVA_{254}$ of the samples was calculated after normalizing UV absorbance data as follows:

$$SUVA_{254} = 100 \times UV_{abs}/b \times C \qquad [1]$$

where SUVA is specific ultraviolet absorption (L mg^{-1} C m^{-1}); UV_{abs} is ultraviolet absorption at 254 nm; b is the path length of the UV (cm); and C is C concentration of the samples (mg C L^{-1}).

We used the fluorescence emission property of soluble OM to determine the humification index (HI) of WEOM and SEOM. The HI concept suggests that during the humification process, the emission spectra of fluorescing molecules shifts toward longer wavelengths as a result of a decrease in the H/C ratio of fluorescing compounds. Accordingly, the proportion of fluorescence region at longer to shorter wavelengths can be used to quantify the relative degree of humification (Zsolnay et al., 1999). The HI was determined by methods suggested by Zsolnay (HI_Z) and Ohno (HI_{O-Z}):

$$HI_Z = (\Sigma I_{435-480})/(\Sigma I_{300-345}) \qquad [2]$$

$$HI_{O-Z} = (\Sigma I_{435-480})/(\Sigma I_{300-345} + \Sigma I_{435-480}) \qquad [3]$$

where I is the fluorescence intensity at the given wavelengths (Ohno, 2002; Zsolnay et al., 1999). HI_{O-Z} varies from 0 to 1 and has been suggested to be more sensitive than HI_Z when comparing samples with a broad range of C concentration (Ohno, 2002). Higher HI values indicate a larger proportion of humified compounds or an increased degree of humified OM (Zsolnay et al., 1999).The excitation-emission fluorescence spectra were recorded using an excitation wavelength of 254 nm and emission range of 300 to 480 nm at 5 nm with a spectrofluorometer (Jobin-Yvon Horiba Fluoromax, Kyoto, Japan).

Statistics

Data were analyzed with a two-way ANOVA and linear regression using SPSS (version 18) to compare the effect of tillage and soil depth on the size of C and N pools and properties of soluble OM fractions. Log transformation of data was performed to improve data normality and eliminate heteroscedasticity. Tukey's test was also used to compare the effect of different soil depths on the properties of SOM and its soluble fractions. Since soil depth cannot be randomized, this has to be considered when interpreting the effect of soil depth. All data are represented as mean ± standard error (SE) of four replicates unless otherwise has been mentioned.

RESULTS

Total Soil Organic Matter

The concentration of soil organic carbon (SOC) decreased from topsoil to 125-cm soil depth by 94% in CT soils and 92% in NT soils ($P < 0.01$; Table 2). Across all depths, the concentration of SOC was greater in NT than CT soils ($P < 0.01$). However, the magnitude of difference in SOC concentration between tillage treatments varied at each soil depth with the largest difference at 50 to 75 cm (121%).

Similar to SOC, total nitrogen (TN) concentration of soils declined with depth in both NT and CT soils ($P < 0.01$; Table 2). The decrease in TN concentration from topsoil to 125-cm soil depth was similar (85%) in both tillage systems but less than that of SOC. Across all depths, the overall concentration of TN was greater in NT than CT soils ($P = 0.01$). Similar to SOC, the magnitude of differences in TN concentrations between tillage treatments varied at each depth with the largest difference at depth 50 to 75 cm (85%).

We observed a decline in the C/N ratio of SOM from topsoil (C/N = 12.1) to 125-cm soil depth (C/N < 6.5), indicating proportional N enrichment of SOM with increasing soil depth ($P < 0.01$; Table 2). In contrast to C and N concentrations in SOM, tillage did not affect the C/N ratio of SOM.

Extractable Organic Matter
Carbon and Nitrogen Concentration

Carbon concentration of WEOM and SEOM decreased ($P < 0.01$) along the soil profile (Table 2, Fig. 1a and 1b). However, the decline in C concentration with soil depth was more rapid in SEOM (78 and 77% in NT and CT soils, respectively) than WEOM (60 and 49% in NT and CT soils, respectively). In comparison to water-extractable organic carbon (WEOC), the concentration of salt-extractable organic carbon (SEOC) was substantially larger (Table 2), averaging 9.5 times greater in topsoil and 4.8 times greater at soil depth (100–125 cm).

In contrast to WEOC, water-extractable organic nitrogen (WEON) did not decline with depth, but WEON concentrations were greater in NT than CT soils ($P < 0.01$, Table 2, Fig. 1c and 1d). However, salt-extractable organic nitrogen (SEON) concentrations declined with depth (71% in NT and 67% in CT soils). The concentration of SEON was considerably larger than WEON, ranging from 4.1 to 10.4 times higher in CT soils and from 4.1 to 7.8 times higher in NT soils. The concentration of SEON was not affected by tillage system.

As a proportion of total SOC, WEOC and SEOC increased with increasing soil depth ($P < 0.01$, Table 3), but the magnitude of increase was larger in CT than NT soils. The proportion of WEOC to SOC was higher in NT compared to that of CT soils ($P = 0.02$). In contrast, the proportion of SEOC to SOC was higher in CT than NT soils ($P = 0.05$, Table 3). Similar to C, as a proportion of TN, WEON and SEON increased with increasing soil depth ($P < 0.01$, Table 3). As a proportion of total SOC and TN, C ($r = 0.59$–0.92) and N ($r = 0.76$–0.96) of WEOM and SEOM were negatively related (both $P \leq 0.05$) to clay percentage of soil. Similarly, C ($r = 0.58$–0.92) and N ($r = 0.65$–0.97) of the WEOM and SEOM were positively related (both $P \leq 0.05$) to percentage of soil $CaCO_3$ at soil depth 25 to 125 cm (data not shown).

Table 2. Concentrations of organic C and total N of soil organic matter (SOM) and C and N of salt- extractable organic matter (SEOM) and water-extractable organic matter (WEOM).†

Soil depth	Conventional-till						
	C	N	C/N	SEOC	SEON	WEOC	WEON
cm	—— g kg^{-1} ——			———— µg g^{-1} ————			
0–25	32.6 ± 4[a]§	2.7 ± 0.2[a]	12.1 ± 0.3[a]	92.2 ± 9.8[a]	8.3 ±1.2[a]	7.1 ± 0.4[a]	0.75 ± 0.1
25–50	17.0 ± 4[b]	1.7 ± 0.3[b]	9.9 ± 0.7[b]	73.3 ± 13.9[b]‡	7.3 ±1.7[a]	6.6 ± 0.3[a]	0.74 ± 0.2
50–75	6.6 ± 0.9[c]	0.8 ± 0.0[c]	8.5 ± 0.9[c]	58.4 ± 7.6[b]	4.6 ±0.4[b]	6.3 ± 0.5[a]	0.77 ± 0.2
75–100	3.5 ± 0.3[c]	0.5 ± 0.0[c]	7.8 ± 1.2[c]	31.7 ± 5.9[c]	3.7 ±0.7[c]	4.3 ± 0.3[b]	0.58 ± 0.1
100–125	2.0 ± 0.1[c]	0.4 ± 0.0[c]	6.0 ± 0.9[c]	21.2 ± 1.1[c]	2.8 ± 0.5[c]	3.6 ± 0.4[b]	0.56 ± 0.2
	No-till						
0–25	34.2 ± 4[a]	2.8 ± 0.3[a]	12.1 ± 0.2[a]	104 ± 12.9[a]	10.1 ± 1.4[a]	13.7 ± 1.5[a]	1.32 ± 0.2
25–50	22.3 ± 4[b]	2.0 ± 0.4[b]	11.1 ± 0.4[b]	89.6 ± 14.6[b]	7.6 ±1.6[b]	11.1 ± 1.5[b]	1.16 ± 0.1
50–75	14.6 ± 4[b]	1.4 ± 0.3[b]	10.1 ± 0.5[b]	77.6 ± 15.5[b]	7.7 ±1.5[b]	11.0 ± 1.0[b]	1.21 ± 0.1
75–100	3.8 ± 0.6[c]	0.6 ± 0.1[c]	6.7 ± 0.3[c]	31.8 ± 4.5[c]	3.9 ±0.5[c]	9.3 ± 1.2[b]	1.08 ± 0.1
100–125	2.6 ± 0.2[c]	0.4 ± 0.0[c]	6.4 ± 0.3[c]	22.7 ±2.7[c]	2.9 ±0.3[c]	5.5 ± 0.7[c]	0.71 ± 0.2
	Analysis of variance¶						
Factor	SOM			SEOM		WEOM	
	C	N	C/N	C	N	C	N
Tillage	<0.01	0.01	0.33	0.14	0.11	<0.01	<0.01
Depth	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.19
Tillage × depth	0.18	0.41	0.32	0.90	0.78	0.64	0.79

† Data are mean ± SE ($n = 4$).

‡ SEOC, salt-extractable organic carbon; SEON, salt-extractable organic nitrogen; WEOC, water-extractable organic carbon; WEON, water-extractable organic nitrogen.

§ Small letters in a column indicate differences across soil depth based on Tukey's test.

¶ The values represent the probability value (two-way ANOVA) of the impact of tillage, soil depth, and their interactions on the given parameters.

Properties of Water-extractable Organic Matter and Salt-extractable Organic Matter

In both WEOM and SEOM, the proportion of HPI–C generally increased with depth ($P < 0.01$, Fig. 2a and 2b, Table 4). Across depths, the proportion of HPI–C varied from 54 to 76% (mean 69% $\pm$ 10) in WEOM, and from 36 to 67% (mean 50% $\pm$ 12) in SEOM. The proportion of HPI–N of both WEOM and SEOM varied widely, but similar to HPI–C it was larger in the WEOM than SEOM fraction (Fig. 2c and 2d) and there was no effect of soil depth (Table 4). We observed an overall greater proportion of HPI–C (53 $\pm$ 13% vs. 46 $\pm$ 10%; $P = 0.02$) and HPI–N (72 $\pm$ 17% vs. 59 $\pm$ 21%; $P = 0.05$) in SEOM of NT than CT soils

The C/N ratios of WEOM and SEOM were generally comparable between CT and NT soils (Fig. 2e and 2f). Only the C/N ratio of SEOM declined with soil depth from 10.7 to 7.7 in NT and 11.2 to 9.2 in CT soils ($P = 0.05$; Table 4). The SUVA values of both WEOM and SEOM decreased from topsoil to 125-cm soil depth ($P < 0.01$, Fig. 3a and 3b, Table 4). The decrease of SUVA value for WEOM was 47% in NT and 62% in CT whereas in SEOM it was 54% in NT and 77% in CT. Across all depths and tillage, the SUVA value of SEOM (mean 2.0 $\pm$ 1 L mg C^{-1} m^{-1}) was greater than WEOM (mean 1.4 $\pm$ 0.7 L mg C^{-1} m^{-1}). The SUVA value of WEOM was substantially higher in topsoils in CT than NT, but there was not a difference at other soil depths. Similar to SUVA, both HI$_Z$ and HI$_{O–Z}$ values of WEOM and SEOM declined with increasing soil depth ($P < 0.01$, Fig. 3c–3f, Table 4). The average HI$_{O–Z}$ and HI$_Z$ values of SEOM (0.95 $\pm$ 0.02 and 26 $\pm$ 13, respectively) were considerably larger than that of the WEOM (0.88 $\pm$ 0.05 and 9 $\pm$ 5, respectively). The HI$_{O–Z}$ values of WEOM and SEOM were larger in CT than NT soils ($P < 0.01$ and $P = 0.05$,

respectively; Table 4). In contrast, only the HI$_Z$ value of WEOM was affected by tillage ($P = 0.01$, Fig. 3).

DISCUSSION

We could not reject our hypotheses: tillage affected soluble OM concentrations and properties, not only in topsoil but also far below the tillage depth (20 cm). Averaged across the 0- to 125-cm depth, concentrations of WEOC and WEON were 44 and 38% greater, respectively, in NT soils than CT soils. In contrast, concentrations of SOC and TN were only 23 and 18% greater, respectively, in NT than CT soils. This is consistent with the literature, indicating that plant residue and tillage have a greater effect on the amount of readily soluble OM (WEOM) than SOM or SEOM (Cookson et al., 2008; Haynes, 2005; Jensen et al., 1997).

Soluble Organic Matter Fractions

The higher concentrations of SEOM than WEOM in this research are consistent with operational definitions of soluble OM. The WEOM has been suggested to closely resemble the small pool of in situ, readily soluble OM (Reemtsma et al., 1999; Ros et al., 2010). In contrast, SEOM reflects the OM pool that is potentially soluble, but associated with mineral surfaces (Matlou and Haynes, 2006; Qualls, 2000).Compared to total SOM C/N ratios, extractable OM C/N ratios were lower in topsoil, but greater at soil depth below 50 cm (Table 2, Fig. 2d and 2f), suggesting dilute and concentrated salt solutions
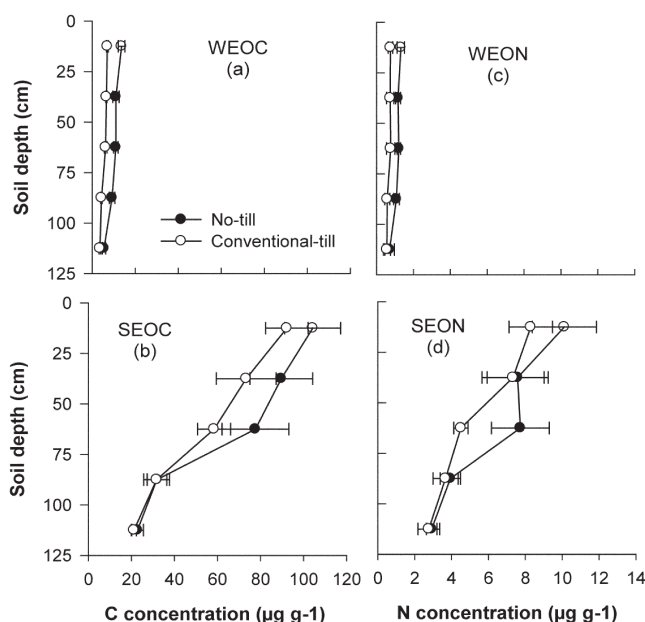
Table 3. Concentration of C and N of water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM) as a percentage of soil organic carbon (SOC) and total nitrogen (TN).

| Soil depth	Percent of SOC		Percent of TN	
	WEOC†	SEOC	WEON	SEON
cm				
	Conventional-till			
0–25	0.02^{c}‡	0.29^{b}	0.03^{c}	0.31^{c}
25–50	0.05^{c}	0.46^{b}	0.04^{c}	0.43^{c}
50–75	0.10^{b}	0.92^{a}	0.10^{b}	0.58^{b}
75–100	0.13^{b}	0.89^{a}	0.12^{b}	0.79^{a}
100–125	0.18^{a}	1.08^{a}	0.16^{a}	0.82^{a}
	No-till			
0–25	0.04^{c}	0.31^{c}	0.05^{c}	0.37^{c}
25–50	0.06^{c}	0.43^{c}	0.07^{c}	0.38^{c}
50–75	0.08^{b}	0.57^{b}	0.10^{b}	0.60^{b}
75–100	0.26^{a}	0.89^{a}	0.14^{a}	0.72^{a}
100–125	0.21^{a}	0.87^{a}	0.18^{a}	0.72^{a}
	Analysis of variance§			
Factor	WEOC/SOC	SEOC/SOC	WEON/TN	SEON/TN
Tillage	0.02	0.05	0.07	0.78
Depth	<0.01	<0.01	<0.01	<0.01
Tillage × depth	0.09	0.17	0.78	0.95

† WEOC, water-extractable organic carbon; SEOC, salt-extractable organic carbon; WEON, water-extractable organic nitrogen; SEON, salt-extractable organic nitrogen.

‡ Small letters in a column indicate differences across soil depth based on Tukey's test.

§ The values represent the probability value (two-way ANOVA) of the impact of tillage, soil depth, and their interactions on the given parameters.

Fig. 1. Changes in concentration of C and N of water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM) from topsoil to 125 cm of conventional and no-tillage soils. (Data are mean ± SE; $n = 4$).
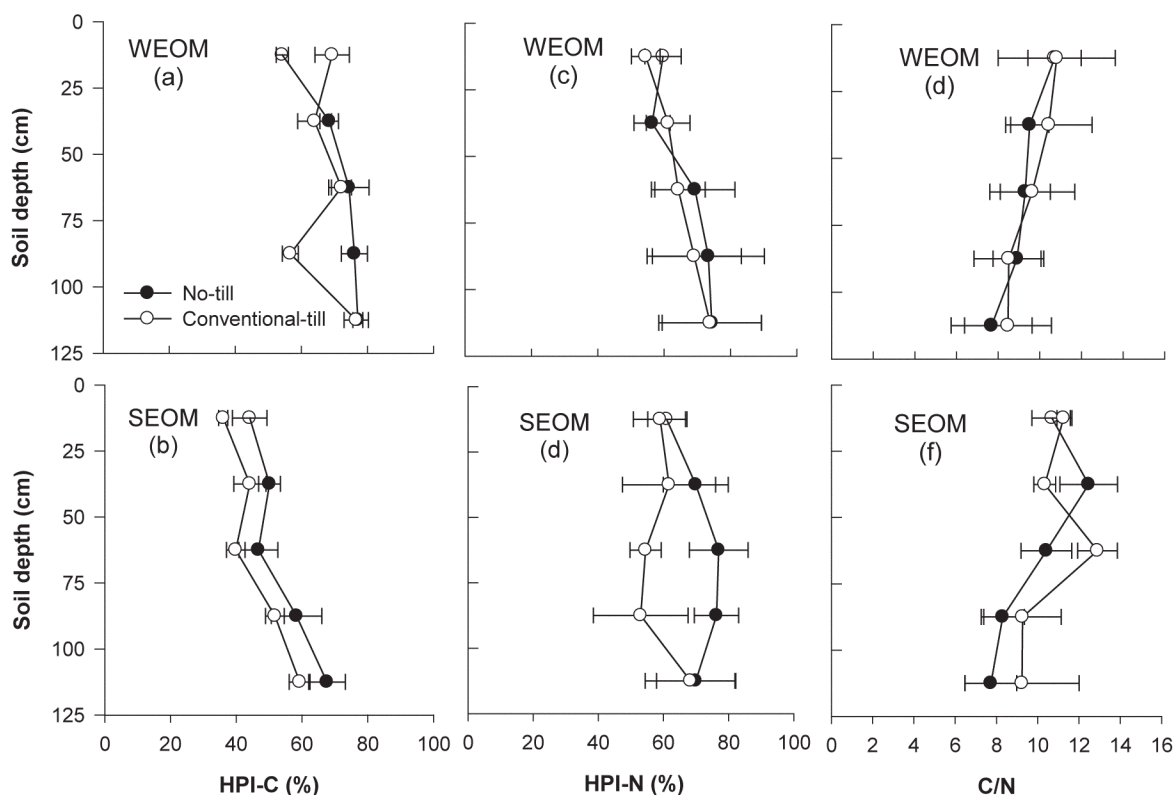
Fig. 2. Changes in proportion of hydrophilic-carbon (HPI–C) and nitrogen (HPI–N) and C/N ratios of water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM) from topsoil to 125 cm of conventional and no-tillage soils (Data are mean ± SE; n = 4).

selectively released relatively N-rich OM in topsoil and relatively N-depleted OM at soil depth.

The considerably higher proportion of HPI-C in WEOM than SEOM (Fig. 2a and 2b) demonstrates that a higher proportion of HPI-C was initially obtained during the first extraction step (WEOM), while less HPI–C was released during the subsequent extraction (SEOM). Because the proportion of hydrophilic compounds of the soluble OM reflects the labile fraction and nutrient-rich portion of soluble OM (Kaiser, 2001; Qualls and Haines, 1992), different proportions of HPI–C in WEOM and SEOM implies that the two fractions are comprised of biochemically different compounds. This is consistent with Toosi et al. (unpublished data, 2012) who observed a greater biodegradability of WEOM than SEOM

obtained from soils under different vegetations. Compared to HPI-C, the relatively high proportions of HPI–N (Fig. 2c and 2d) indicate that HPI–OM is comprised of relatively N-rich compounds compared to total SOM (Kaiser, 2001; Qualls and Haines, 1991).

Averaged between soil depths and tillage systems, SUVA values (Fig. 3a and b) suggest the proportion of aromatic compounds was greater in SEOM than WEOM (mean 2.0 ± 1 vs. 1.4 ± 0.7 L mg C^{-1} m^{-1}, respectively). Consistent with SUVA values, the greater HI of SEOM than WEOM (Fig. 3c–f) implies a greater proportion of more humified OM (Zsolnay et al., 1999). Together, HPI–C, SUVA, and HI properties indicate that SEOM is more decomposed than WEOM.
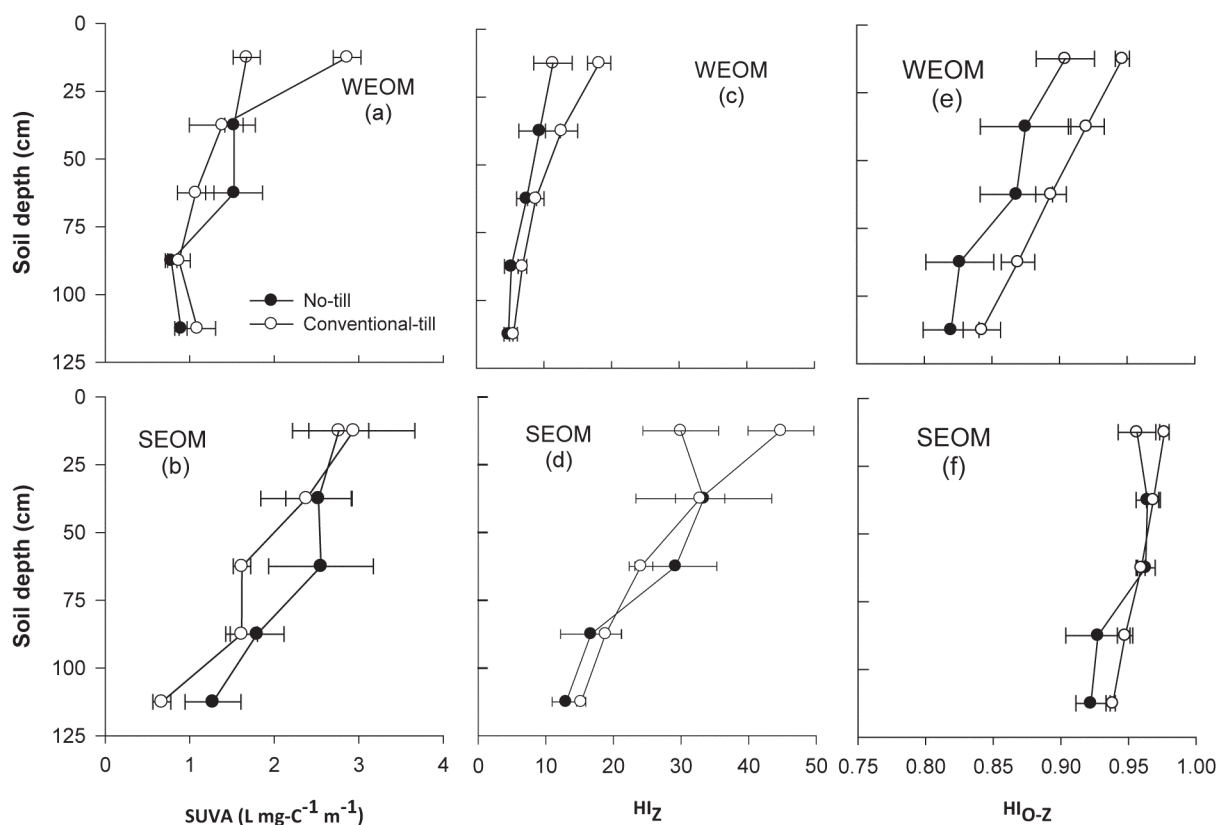
Tillage

In agreement with previous research, we observed higher concentrations of SOC and TN in NT compared to CT (Devine et al., 2011; Kumar and Goh, 1999) (Table 2). Additionally, these data show that differences in SOC and TN concentrations between tillage treatments were highest at soil depth 50 to 75 cm. The observed greater SOC and TN concentrations at soil depth (>30 cm) in NT than CT (e.g., Dou et al., 2007) is inconsistent with Baker et al. (2007) who suggested that greater SOC concentration in deeper layers of CT may offset the often reported higher SOC concentration in topsoils under NT. Unlike the C and N concentrations of SOM, its C/N ratio was not affected by tillage (Black and Tanaka, 1997; Devine et al., 2011).

Table 4. Probability values (two-way ANOVA) of the impact of tillage, soil depth, and their interactions on properties of water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM).

Factor	C/N	HPI–C†	HPI–N	SUVA	HI$_z$	HI$_{o–z}$
			WEOM			
Tillage	0.77	0.43	0.78	0.68	0.01	<0.01
Depth	0.63	<0.01	0.49	<0.01	<0.01	<0.01
Tillage × depth	0.99	<0.01	0.99	0.12	0.84	0.96
			SEOM			
Tillage	0.44	0.02	0.05	0.12	0.16	0.05
Depth	0.05	<0.01	0.93	<0.01	<0.01	<0.01
Tillage × depth	0.51	0.99	0.76	0.76	0.58	0.73

† HPI–C: hydrophilic carbon (%), HPI–N: hydrophilic nitrogen (%), SUVA: specific ultraviolet absorption at 254 nm (L mg^{-1} C m^{-1}), HI$_z$: humification index Zsolnay, HI$_{o–z}$: humification index Ohno–Zsolnay.

Fig. 3. Changes in specific ultraviolet absorption (SUVA$_{254}$) and humification indices (HI) of water-extractable organic matter (WEOM) and salt-extractable organic matter (SEOM) from topsoil to 125 cm of conventional and no-tillage soils (Data are mean ± SE; n = 4).

The properties of WEOM and SEOM indicated that a higher decomposition rate contributed to the lower OM concentration in CT soils. Differences in net primary productivity could not explain higher SEOM and WEOM concentrations because crop yields at our site were lower in NT soils (Singer et al., 2010; J.W. Singer, unpublished data, 2011). Instead, a greater downward movement of dissolved OM from topsoil may have resulted in higher SOM and WEOM in NT soils at depth below 25 cm (Devine et al., 2011; Dou et al., 2007).

Based on our findings for soluble OM, a higher proportion of fresh, relatively nondecomposed plant residues exist in NT soil. The HI data indicated a lower proportion of humified OM in both WEOM and SEOM from NT soils (Fig. 3c–3f, Table 4), consistent with higher proportions of HPI–C and HPI–N in SEOM in NT soils (Fig. 3a–3d). Furthermore, the SUVA value was considerably larger in WEOM of topsoil CT than NT. Previous studies (e.g., Gregorich et al., 2000) have shown that water-soluble OM is largely derived from native OM (vs. fresh OM input) and thus, closely resembles the properties of SOM. This suggests that the substantially higher SUVA value of the topsoil under CT is associated with the further decomposed SOM in CT soils. The influence of tillage on the properties of soluble OM is attributed to the impact of tillage on shifts in composition of microbial community, OM decomposition rate, and changing OM-aggregates dynamics (Halgason et al., 2010; Jacobs et al., 2011).

Soil Depth

The pool size of WEOM and SEOM declined from topsoil to 125 cm (Table 2). The rapid decline in pool size of WEOM with soil depth is due to its decomposition and particularly, sorption to mineral components (Kaiser and Guggenberger, 2000). The less pronounced change in WEON or SEON relative to WEOC or SEOC with increasing soil depth suggests the microbial community at lower soil depth was primarily C limited (Kemmitt et al., 2008). Although dynamics of WEOM in relation to soil depth has been of recent focus (e.g., Hassouna et al., 2010), to our knowledge this is the first study addressing changes in the size and properties of SEOM from topsoil to depth below 30 cm. At this research site, the proportional decrease in C and N concentrations of SEOM to depth were greater than those of WEOM. We observed a stronger relationship between SEOC and SOC (R^2 = 0.75), and SEON and TN (R^2 = 0.67) than between WEOC and SOC (R^2 = 0.33), and WEON and TN (R^2 = 0.14) (data not shown). This suggests similarities in the dynamics of SEOM with SOM, regardless of soil depth and tillage.

The proportion of total OM extracted as WEOM and SEOM increased with soil depth (Table 3), demonstrating a higher proportion of readily soluble OM (WEOM) and exchangeable OM (SEOM) at soil depth. When topsoil was not included, the proportion of total OM that was extracted in WEOM and SEOM exhibited a negative relationship with clay content and a positive relationship with CaCO$_3$ content of the soil. Similarly, Corvasce et al. (2006) reported that the proportion

of WEOC to total SOC increased four times with depth (0–195 cm) and was negatively correlated with clay content ($R^2 = 0.90$). The higher proportion of soluble OM fractions within the soil profile appears to be due to weaker association of the released OM (potentially soluble) with the mineral phase in subsoil. The subsoil SOM is known for its proportionally higher microbial origin (Rumpel and Kögel-Knabner, 2011) and thus, is more soluble in aqueous solutions. In addition, given the considerable increase in soil $CaCO_3$ from topsoil down to 125 cm (Table 1), the increased proportion of total SOM that was SEOM with increasing soil depth may be due to the partial release of OM protected via Ca^{+2} complexation (Muneer and Oades, 1989) as a result of Ca^{+2}–K^+ exchange during SEOM extraction. Nevertheless, the strong correlation between extractable OM and clay or $CaCO_3$ may have also been affected by trends in clay and $CaCO_3$ with soil depth.

The proportional distribution of HPI–C and HPI–N fractions of WEOM and SEOM increased with depth (Fig. 2a and 2d), but the increase was only significant for HPI–C (Table 4). Previous studies have shown an increasing proportion of HPI–C (Hassouna et al., 2010) and HPI–N (Kaiser et al., 2001) with soil depth as a result of higher mobility of HPI–OM in soil (Jardine et al., 1989). Soluble OM contributes to nutrient losses in agricultural systems (e.g., Van Kessel et al., 2009). Given that HPI-OM comprises the major portion of N, P and S in soluble OM (Kaiser et al., 2001), the observed tillage-induced changes in proportion of HPI–OM seems to be of importance for understanding nutrient dynamics in agroecosystems and a possible mechanism of nutrient losses under NT management. Consistent with HPI–OM, the proportion of aromatic and humified compounds (assessed by $SUVA_{254}$ and HI) in WEOM and SEOM decreased with depth (Table 4). The decrease in HI and $SUVA_{254}$ of soluble OM with soil depth (e.g., Corvasce et al., 2006; Don and Schulze, 2008) has been attributed largely to the preferential retention of more aromatic and lignin-derived organic compounds of soluble OM during downward transport in soil (Kaiser et al., 2004). Alternatively, changes in properties of soluble OM with soil depth may be due to selective release of SOM components during exchange reactions (Fröberg et al., 2007; Sanderman et al., 2008) and/or in situ formation of soluble OM from native SOM. The latter can be supported by the observed decline in the C/N ratio of SEOM and WEOM with depth which was consistent with that of SOM (Tables 2) and in agreement with Kemmitt et al. (2008). Such a decline clearly indicates a larger proportion of microbially-driven OM (low C/N ratio) in the subsoil (Rumpel and Kögel-Knabner, 2011). Thus, given the lack of (or very limited) mobility of SEOM under field conditions, the decrease in SUVA and HI and increase in HPI–C proportion of SEOM with increasing soil depth seem to reflect the preferential release of less aromatic and humified components of SOM during extraction. Of the measures used to characterize soluble OM properties in this study, HI (particularly $HI_{O–Z}$) most consistently indicated effects of soil depth and tillage.

CONCLUSIONS

Both WEOM and SEOM ($<0.45\mu m$) are soluble forms of SOM, with SEOM representing a larger and more decomposed OM pool. Due to high spatial and temporal variability, the C concentration and properties of WEOM do not appear to be consistent indicators of agricultural management effects on SOM. Instead, SEOM has been suggested to more consistently reflect the impact of management on SOM (e.g., Graham et al., 2002). In this research, in contrast to WEOM, the pool size of OM extractable with a concentrated salt solution was not significantly affected by long-term tillage. However, our results showed that the composition of WEOM, and particularly SEOM, appeared to be more decomposed in CT compared to NT soils. The strong relationships between C and N concentrations as well as C/N ratios of SEOM and SOM suggest that properties of SEOM reflect SOM dynamics and are affected by soil management and depth. Additional long-term studies are required to assess the suitability of SEOM as an early indicator of land-use and management effects on SOM.

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