Evaluation of structural chemistry and isotopic signatures of refractory soil organic carbon fraction isolated by wet oxidation methods

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Abstract Accurate quantification of different soil organic carbon (SOC) fractions is needed to understand their relative importance in the global C cycle. Among the chemical methods of SOC fractionation, oxidative degradation is considered more promising because of its ability to mimic the natural microbial oxidative processes in soil. This study focuses on detailed understanding of changes in structural chemistry and isotopic signatures of SOC upon different oxidative treatments for assessing the ability of these chemicals to selectively isolate a refractory fraction of SOC. Replicated sampling (to ~ 1 m depth) of pedons classified as Typic Fragiudalf was conducted under four land uses (woodlot, grassland, no-till and conventional-till continuous corn [Zea mays L.]) at Wooster, OH. Soil samples (<2 mm) were treated with three oxidizing agents (hydrogen peroxide (H_2O_2), disodium peroxodisulfate ($Na_2S_2O_8$) and sodium hypochlorite (NaOCl)). Oxidation resistant residues and the bulk soil from A1/Ap1 horizons of each land use were further analyzed by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy and accelerator mass spectrometry to determine structural chemistry and ^{14}C activity, respectively. Results indicated that, oxidation with NaOCl removed significantly less SOC compared to $Na_2S_2O_8$ and H_2O_2. The NMR spectra revealed that NaOCl oxidation preferentially removed lignin-derived compounds at 56 ppm and at 110–160 ppm. On the other hand, the SOC resistant to $Na_2S_2O_8$ and H_2O_2 oxidation were enriched with alkyl C groups, which dominate in recalcitrant macromolecules. This finding was corroborated by the ^{14}C activity of residual material, which ranged from −542 to −259‰ for $Na_2S_2O_8$ resistant SOC and −475 to −182‰ for H_2O_2 resistant SOC as compared to relatively greater ^{14}C activity of NaOCl resistant residues (−47 to 61‰). Additionally, H_2O_2 treatment on soils after light fraction removal was more effective in isolating the oldest (^{14}C activity of −725 to −469‰) SOC fraction. The $\Delta^{14}C$ signature of SOC removed by different oxidizing agents, calculated by mass balance, was more or less similar irrespective of the difference in labile SOC removal efficiency. This suggests that SOC isolated by many fractionation methods is still a mixture of much younger and older material and therefore it is very important that the labile SOC should be completely removed before measuring the turnover time of stable and refractory pools of SOC.

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Abbreviations

ANOVA	Analysis of variance
CT	Conventional-till
CPMAS	Cross polarization magic angle spinning
$Na_2S_2O_8$	Disodium peroxodisulfate
HCl	Hydrochloric acid
HF	Hydrofluoric acid
H_2O_2	Hydrogen peroxide
LSD	Least significant difference
NT	No-till
NMR	Nuclear magnetic resonance
OM	Organic matter
SPIDER	Saturation pulse induced dipolar exchange with recoupling
$NaHCO_3$	Sodium bicarbonate
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
SOC	Soil organic carbon

Introduction

Soil organic carbon (SOC) is the third largest global C pool comprising 1,550 PgC in the top 1 m of soil (Batjes 1996; Lal 2007). Transferring atmospheric CO_2 into the SOC pool is an important option to help mitigate global warming. The SOC exists as a heterogeneous mixture of different fractions with turnover times ranging from minutes to millennia. The biological stability of SOC is influenced by several factors including chemical structure of SOC, soil environmental factors including temperature, moisture and aeration, and soil physical and chemical properties (Krull et al. 2003). Mechanisms of SOC stabilization can be summarized as chemical recalcitrance of the organic matter (OM), physical protection as a result of inaccessibility to microbes and/or enzymes due to encapsulation of OM into aggregates, and increased stability due to interaction of SOC with mineral matrices (Sollins et al. 1996; Baldock and Skjemstad 2000). These mechanisms, combined with the input-loss ratio of C, determine the turnover rates and subsequent pools of SOC in soils. The total SOC

pool can be compartmentalized into different fractions or pools of varying stability. Unfortunately, different authors use different terminology to describe these SOC pools (Krull et al. 2003). In this study, the term *labile pool* refers to easily mineralizable fraction with rapid turnover time ranging from months to years (Krull et al. 2003). The term *stable pool* is used to describe an intermediate fraction with turnover rates in the range of decadal to centennial time scale (Bruun et al. 2007; Baisden and Amundson 2003). The *refractory pool* refers to a fraction that persists in soil for long time and has turnover time of thousands of years (Krull et al. 2003). Global estimates of rapidly cycling SOC pools range from 250 to 530 Pg C (Trumbore 1997), suggesting that major part of the global SOC pool is assigned to the stable and refractory fractions. Quantification of different SOC pools is important for understanding C dynamics and for increasing the accuracy of model prediction. The turnover rates of different SOC fractions have been successfully calculated by radiocarbon technique, if samples at various times before and after the peak atmospheric nuclear weapon testing are available (Trumbore 1997; Baisden et al. 2002). The δ^{13}C values of soil organic matter (SOM) can also be used as a tracer to study the SOC dynamics in areas where C_3 vegetation was replaced by C_4 plants or vice versa (e.g., Balesdent et al., 1987). However, there is no consensus in the literature on the appropriate chemical treatment for the experimental isolation of SOC fractions with distinct turnover rates.

Over the years, an array of physical and chemical fractionation protocols have been used for separating the SOC fractions with varying stability. Among the chemical methods, acid hydrolysis (Trumbore and Zheng 1996; Leavitt et al. 1996; Paul et al. 1997; Silveira et al. 2008), and chemical oxidation by various oxidizing agents including hydrogen peroxide (H_2O_2) (Theng et al. 1999; Eusterhues et al. 2005), disodium peroxodisulphate ($Na_2S_2O_8$) (Eusterhues et al. 2003; Lorenz et al. 2006), and sodium hypochlorite (NaOCl) (Kleber et al. 2005; Siregar et al. 2005; Zimmermann et al. 2007) have been used frequently. Both acid hydrolysis and chemical oxidation preferentially remove labile organic compounds such as proteins, nucleic acids, and polysaccharides and retain relatively stable SOC fraction that is enriched in alkyl and aromatic C components (Leavitt

et al. [1996]; Paul et al. [2001]; Mikutta et al. [2006]). Ultimately, the residue remained after both acid hydrolysis and chemical oxidation is a heterogeneous mixture of different C inputs. However, chemical oxidation causes changes in SOC composition similar to that observed during natural microbial oxidative processes (Cuypers et al. [2002]; Eusterhues et al. [2003]) and is more efficient at preferentially removing young SOC inputs than acid hydrolysis (Bruun et al. [2007]).

Though chemical oxidation procedures are promising methods of separating SOC fractions with distinct stability, the relative efficiency of different oxidizing agents in separating stabilized SOC is not known. Most studies determine the efficiency of oxidizing agents in separating SOC fractions based on the content of residual OC remaining after oxidation treatment. A few studies, which determined mean ^{14}C ages of oxidation-resistant OC showed a wide variation of SOC age, ranging from modern to several thousand years (e.g. Theng et al. [1992]; Balesdent [1996]; Eusterhues et al. [2005]; Kleber et al. [2005]). In addition, several studies (e.g. Bruun et al. [2007]; Helfrich et al. [2007]; Favilli et al. [2008]) which compared the oxidation efficiency of different chemicals in similar soil types and land uses have produced contradictory results. Bruun et al. ([2007]) oxidized soil samples (0–7.5 cm) from a similar soil incubated with ^{14}C-labelled barley *(Hordeum vulgare* L.) for 40 years (1964–2004) and 40 days (1 June to 11 July, 2005) and found that both $Na_2S_2O_8$ and NaOCl are unlikely to isolate a stable OC fraction as they consistently removed more 40-years-old C than 40-days-old C. Helfrich et al. ([2007]) indicated that, among the several chemical methods tested, $Na_2S_2O_8$ and H_2O_2 were the most efficient in isolating a refractory fraction of SOC. According to Favilli et al. ([2008]), H_2O_2 was superior to NaOCl in isolating the oldest and most refractory SOC.

Since SOM contains multiple, and simple to complex biomolecules with varying chemical nature and function, a detailed understanding of the changes in structural chemistry of SOM with different oxidative treatments would be valuable for assessing the ability of these oxidizing agents in selective removal of labile SOC. The ^{13}C nuclear magnetic resonance (NMR) spectroscopy offers a unique opportunity to examine the structural chemistry of SOC by

distributing the C among classes of compounds, such as alkyl C, O/N-alkyl C, aromatic C, and carboxyl C (Ussiri and Johnson [2007]). During the initial stages of SOC decomposition, O/N-alkyl C (mostly derived from carbohydrates and proteins) is selectively utilized by microbes followed by the decomposition of aromatic C (derived from lignin and phenols) (Baldock et al. [1997]). As the degree of decomposition progresses, the relative proportion of alkyl C, which is dominantly present in aliphatic macromolecules such as fats, waxes, resins, cutin and suberin, increases (Baldock et al. [1992]; Preston [1996]). Thus, the alkyl C:O/N-alkyl C ratio, calculated from ^{13}C NMR data, has been used as an index for estimating the biodegradability of SOC (Baldock and Preston [1995]). Another useful index is the % aromaticity, which is the ratio of aromatic C (110–160 ppm) to the sum of aromatic and total aliphatic C (0–110 ppm) (Fründ et al. [1994]).

There are a few studies that have used NMR spectroscopy to understand the structural composition of SOC resistant to chemical oxidation (Eusterhues et al. [2005]; Mikutta et al. [2006]). Moreover, evaluation on how different oxidizing agents affect the structural chemistry of oxidation resistant SOC in similar soil types or land use regimes is completely lacking. Therefore, the focus of this study was to isolate SOC resistant to different oxidizing agents (H_2O_2, $Na_2S_2O_8$ and NaOCl) and evaluate the changes in structural chemistry and isotopic signatures (Δ^{14}C, δ^{13}C) of the SOC resistant to different oxidizing agents and their relative implications as a refractory pool of SOC. The specific objectives were to: (1) quantify the loss of SOC upon oxidation in four land uses of a similar soil type, (2) compare the structural chemistry of SOC before and after oxidation using solid-state ^{13}C-cross polarization magic angle spinning (CPMAS) NMR spectroscopy, and (3) evaluate the isotopic signatures as influenced by the oxidative treatments.

Materials and methods

Site description

This study was conducted at the Ohio Agricultural Research and Development Center in Wooster, Ohio,

USA (40°48′N, 82°00′W). The soils of this site belong to Wooster series (mixed, mesic, Typic Fragiudalf) with silt-loam texture (USDA-SCS 1984). The parent material is low-lime glacial till with a discontinuous loess mantle of up to 51 cm in thickness. These soils are well drained and contain a fragipan at a depth of 50–90 cm (USDA-SCS, 1984). The native vegetation was a continuous cover of mixed, deciduous hardwood forest. Relic forest remnants occur along creeks and as small woodlots (USDA-SCS 1984). The mean annual temperature of the site is 9.1°C with 173 frost-free days occurring each year and the mean annual precipitation is 905 mm (Dick et al. 1998). The study included woodlot, grassland and agricultural land uses. The woodlot is part of the remnant hardwood forest, which dominated the area prior to land clearing for agriculture in the mid 1830s. The agricultural site is part of a long-term tillage experiment (Triplett–van Doren plots) initiated in 1962 to study the effects of tillage and crop sequences (van Doren et al. 1976). This site was maintained under grass meadow for 6 years before starting the experiment. For the present study, no-till (NT) and conventional-till (CT) management plots under continuous corn (*Zea mays* L.) were sampled. Typically, NT is defined as complete elimination of pre-plant tillage. The seeds in the NT plots were sown using a planter with single coulter to cut through the crop residues and loosen the soil ahead of standard planter units. The CT consisted of one moldboard plowing to a depth of 20–25 cm in spring of each season followed by two 10-cm deep secondary operations (disking, field cultivator) prior to planting. The CT treatment resulted in a complete soil surface inversion and nearly 100% incorporation of crop residues. Fertilizer was applied in the spring of every year based on soil test recommendations, with the same rates of N, P and K applied to both the tillage treatments. The fertilizer materials were broadcasted on soil surface for NT and incorporated for CT plots. Lime was broadcasted in the fall as required to maintain pH close to 6.0 in the Ap horizon. Herbicide types and rates were slightly varied over the years. The cumulative amount of herbicide application was reported as 119 kg a.i. ha^{-1} for NT and 111 kg a.i. ha^{-1} for CT soils (Dick 1984). The woodlot and grassland areas are located very close to the Triplett–van Doren plots on the similar soil type.

Soil sampling and processing

Three replicates of horizonwise samples were collected from the pits dug down to the glacial till in each of the four land uses during summer 2005. For this study, five horizons (Ap1, Ap2, Ap3, Bt1 and Btx1) were selected for all the land uses except woodlot, where horizonation was slightly different and samples of A1, A2, E and Bt1 horizons were selected. No Btx horizon was present in the soil profile of woodlot. In addition, woodlot profile contained an E horizon, instead of Ap3 horizon in other land uses (Table 1). The samples were air dried, gently ground with a wooden roller, and sieved through a 2-mm sieve. Triplicate samples per treatment for initial C and N analyses and chemical oxidation were roller ground to pass through a 250-μm sieve.

Soil analyses

Soil pH was determined in 1:2 soil weight to water volume ratio suspension using pH meter (Thomas 1996) and proportion of clay was calculated from the textural analysis conducted by the hydrometer method (Gee and Or 2002). The procedure for H_2O_2 oxidation was slightly modified from Helfrich et al. (2007). Briefly, 1 g of soil was wetted with 10 ml distilled water for 10 min. Then, 30 ml of 10% H_2O_2 was added and at the end of sometimes intense initial reaction at room temperature, the oxidation was performed at 50°C in a water bath. After 2–3 days when the frothing subsided completely, the suspension was centrifuged at 2500×g for 15 min and the supernatant was decanted. Oxidation with H_2O_2 was repeated two more times. The samples were then washed three times with 40 ml deionised water and freeze-dried.

For $Na_2S_2O_8$ oxidation, 0.5 g of soil was first dispersed in 250 ml distilled water. Then, 20 g $Na_2S_2O_8$ buffered with 22 g sodium bicarbonate ($NaHCO_3$) was added and allowed to react for 2 days at 80°C in a water bath. After oxidation, the suspension was centrifuged at 2500×g for 15 min and the supernatant was decanted. The samples were washed three times with 40 ml deionised water and freeze dried (Helfrich et al. 2007). The $Na_2S_2O_8$ oxidized samples were then acid washed to remove the traces of carbonate remaining from the $NaHCO_3$

Table 1 Basic parameters of soils used in the experiment

Land use	Horizon	Depth (cm)	pH 1:1 soil water	Clay (g kg^{-1})	SOC (g kg^{-1})	C:N ratio
Woodlot	A1	0–4	5.9 (0.3)[a]	140 (25)	37 (0.8)	13.6 (0.03)
	A2	4–13	5.3 (0.1)	129 (16)	28 (0.3)	13.3 (0.1)
	E	13–28	4.2 (0.1)	89 (8)	11 (0.1)	10.2 (0.2)
	Bt1	41–62	4.0 (0.2)	208 (25)	2.7 (0.2)	4.7 (0.2)
Grassland	Ap1	0–4	5.4 (0.2)	158 (18)	33 (0.5)	10.1 (0.2)
	Ap2	4–10	5.4 (0.2)	159 (30)	25 (0.1)	9.8 (0.1)
	Ap3	10–20	5.6 (0.1)	160 (12)	15 (0.1)	8.7 (0.04)
	Bt1	20–33	6.0 (0.04)	196 (18)	7.1 (0.3)	7.4 (0.5)
	Btx1	76–109	4.8 (0.1)	221 (21)	1.8 (0.1)	3.5 (0.1)
No-till corn	Ap1	0–3	5.4 (0.4)	138 (17)	40 (1.2)	11.2 (0.5)
	Ap2	3–10	5.4 (0.2)	158 (25)	26 (1.6)	9.5 (0.7)
	Ap3	10–22	5.8 (0.2)	150 (20)	14 (0.3)	8.9 (0.2)
	Bt1	33–48	6.2 (0.1)	218 (9)	2.8 (0.1)	4.4 (0.3)
	Btx1	77–88	4.5 (0.2)	210 (24)	1.9 (0.1)	3.5 (0.1)
Conventional-till corn	Ap1	0–3	5.8 (0.3)	135 (16)	10 (0.5)	10.6 (0.4)
	Ap2	3–17	6.2 (0.1)	138 (9)	10 (0.4)	10.3 (0.6)
	Ap3	17–31	6.4 (0.1)	130 (13)	10 (0.2)	8.3 (0.04)
	Bt1	31–44	6.6 (0.2)	183 (9)	3.6 (0.2)	5.4 (0.3)
	Btx1	70–92	4.6 (0.03)	224 (17)	1.9 (0.1)	3.5 (0.1)

[a] Mean values ($N = 3$) followed by standard deviation in parentheses

added during the oxidation process. Briefly, 3 g of finely ground (<250 μm), $Na_2S_2O_8$ resistant residue were treated with 30 ml of 1 mol l^{-1} HCl for 24 h with occasional stirring (Ussiri and Lal 2008). After removing the supernatant by centrifugation, the residue was washed with deionized water six times and freeze dried.

Oxidation with NaOCl was achieved by treating 5 g of soil with 50 ml of 6% NaOCl, adjusted to pH 8 with concentrated hydrochloric acid (HCl). After 6 h at 25°C, the suspension was centrifuged and the supernatant was decanted. The extraction with NaOCl was repeated two more times. Afterwards, the samples were washed twice with 50 ml of 1 M sodium chloride (NaCl), three times with deionised water, and freeze-dried (Helfrich et al. 2007).

Concentrations of total C and N in soil samples before and after oxidation were measured by the dry combustion method (Vario Max CN Analyzer, Elementar GmbH, Hanau, Germany). The SOC was assumed to be equal to the total C since inorganic C concentrations were negligible and soil pH was <6.6.

The relative change in C:N ratios before and after oxidative treatments was calculated as a percentage (Schmidt and Gleixner 2005):

$$\text{Relative change in C:N ratio} = 100 \times (\text{C:N}_{\text{after oxidation}} - \text{C:N}_{\text{before oxidation}})/(\text{C:N}_{\text{before oxidation}}).$$

Solid-state ^{13}C-CPMAS-NMR analysis

A composite sample from the three replications of surface soil (A1/Ap1 horizon) before and after the oxidative degradation was analyzed for chemical composition using solid-state ^{13}C-CPMAS-NMR spectroscopy. Prior to the analysis, the oxidized samples were treated with 10% hydrofluoric acid (HF) to remove paramagnetic materials and to concentrate OC through dissolution of the mineral matrix (Schmidt et al. 1997; Kiem et al. 2000). This was achieved by treating 10 g of oxidized soil with 50 ml of 10% HF in a polyethylene bottle. The suspension was shaken horizontally for approximately 12 h. After

centrifugation, the supernatant was removed. The HF treatment was repeated four times. Finally, the residue was washed three times with deionised water and freeze dried.

Solid-state ^{13}C-CPMAS-NMR analyses were conducted at the Department of Chemistry, Ohio State University with a Bruker DSX 300 spectrometer operating at a ^{13}C resonance frequency of 75.48 MHz. Samples were packed into a 4 mm (outside diameter) zirconium rotor with a Kel-F$^®$ cap and spun at a frequency of 10 kHz using a contact time of 1.5 ms and a recycle delay time of 1 s. In order to obtain the NMR spectra with an acceptable signal to noise ratio, the unoxidized samples and NaOCl resistant residues required approximately 65,000 scans for a period of 18 h, and the H_2O_2 and $Na_2S_2O_8$ resistant residues required approximately 1,46,600 scans for 42 h. The spectra were divided into four major chemical shift regions, representing alkyl C (0–45 ppm), O/N-alkyl C (45–110 ppm), aromatic C (110–160 ppm) and carboxyl C (160–220 ppm). The relative intensities of the chemical shift regions were determined by the integration of ^{13}C-CPMAS-NMR spectra over given chemical shift ranges after phase and baseline corrections. Chemical shift assignments were externally referenced to the adamantine resonance at 29.5 ppm.

Isotopic measurements

The ^{14}C activity of a composite sample from three replicates of surface soil (A1/Ap1 horizon) before and after oxidation was measured at W.M. Keck Carbon Cycle Accelerator Mass Spectrometer facility at University of California-Irvine. The untreated and oxidized samples containing approximately 1 mg of C were combusted at 900°C to produce CO_2 and the evolved CO_2 was cryogenically purified, reduced to graphite using sealed zinc tube reduction and analyzed for radiocarbon content (Xu et al. 2007; Nowinski et al. 2008). Radiocarbon data are reported as Δ^{14}C, which is the fractional deviation, in parts per thousand (‰), of the ^{14}C/^{12}C ratio of sample from that of a standard of fixed isotopic composition (0.95 times the activity of an oxalic acid standard (OX1) in 1950, which is normalized to a δ^{13}C of −19‰ (Stuiver and Polach 1977):

$$\Delta^{14}C = (F^{14}C \times \exp(-k^{14}C \times (y - 1950)) - 1) \times 1000$$

where,

$$F^{14}C = \frac{\left[\frac{^{14}C}{^{12}C + ^{13}C}\right]_{sample}}{\left[\frac{^{14}C}{^{12}C + ^{13}C}\right]_{OXI}}$$

k^{14}C, radioactive decay constant for ^{14}C, equal to 1/8,267 year^{-1}; y = year of ^{14}C measurement.

The Δ^{14}C values are corrected to a δ^{13}C value of −25‰ to account for the assumption that plants discriminate twice as much against ^{14}C as they do against ^{13}C (Stuiver and Polach 1977; Castanha et al. 2008). Negative Δ^{14}C values indicate the predominance of SOC old enough for radioactive decay of ^{14}C (half-life = 5,730 years) to have occurred and positive Δ^{14}C values indicate the presence of bomb ^{14}C produced by atomic weapons testing during 1960s.

The radiocarbon age of the sample was calculated from the F^{14}C and Libby mean life of radiocarbon (8,033 years) by the expression,

$$\text{Radiocarbon age (years BP)} = -8,033 \ln(F^{14}C)$$

BP, means years before 1950, as the activity of OX1 standard is defined for 1950.

An aliquot of the purified CO_2 obtained from combustion samples used for the ^{14}C analyses was placed in a He-filled vial and analyzed for ^{13}C using a Gas Bench II interface coupled to a Delta Plus continuous flow isotope ratio mass spectrometer (Xu et al. 2007). The ^{13}C data are expressed as δ^{13}C (‰):

$$\delta^{13}C = \left(^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard}\right)/ \left(^{13}C/^{12}C_{standard}\right) \times 10^3$$

Mass balance calculations

The percent of the original SOC that is retained after oxidation was calculated based on the following equation:

$$\% \text{ SOC in residue} = \frac{(\text{Residue wt.})(\% \text{ SOC}_{residue})}{(\text{Original soil wt.})(\% \text{ SOC}_{soil})}$$

The radiocarbon signature of SOC removed by oxidation is derived from the mass balance of SOC and ^{14}C:

$$\Delta^{14}C_{\text{oxidized}} = \frac{100\% \times \Delta^{14}C_{\text{original SOC}} - \% \text{SOC in residue} \times \Delta^{14}C_{\text{residueSOC}}}{(100\% - \% \text{SOC in residue})}$$

A similar equation was used to calculate the $\delta^{13}C$ of SOC removed by oxidation.

Density fractionation

The bulk soil samples from the surface horizon (A1/Ap1) of four land uses were subjected to a density fractionation to separate the light fraction (LF) from heavy fraction (HF) (Golchin et al. 1994; John et al. 2005). Ten grams of soil was placed in a centrifugation tube and 40 ml of sodium polytungstate (SPT) solution with a density of 1.6 g cm^{-3} (Sometu, Berlin, Germany) was added. The tubes containing the soil-SPT mixture were shaken in a reciprocating shaker at 300 rpm for 15 min. After sedimentation for 30 min, the solution was centrifuged at 5100×g for 30 min. The supernatant with floating LF was filtered using Millipore AP20 glass-fiber filter under vacuum. Both LF and the HF were washed thoroughly with deionised water and oven dried at 50°C. Subsamples of LF and HF from each land use were separately subjected to chemical oxidation process.

Statistical analysis

The analysis of variance (ANOVA) for testing the difference among the chemical oxidation methods for SOC concentration within a horizon of each land use was computed using PROC GLM (fixed effects model) of SAS (SAS Institute Inc. 2002). The statistical significance was evaluated at the $P \leq 0.05$ level and the mean effects were separated using the F protected least significant difference (LSD) test.

Results and discussion

Depth distribution of soil organic carbon and C:N ratio

The SOC concentration within the selected horizons ranged from 2.7 to 37 g kg^{-1} in woodlot, 1.8 to 33 g kg^{-1} in grassland, 1.9 to 40 g kg^{-1} in NT corn and 1.9 to 10 g kg^{-1} in CT corn (Table 1). Among the horizons, the SOC concentration was the highest in A1/Ap1 horizon for all land uses, where C addition from plant litter was the highest (Jobbágy and Jackson 2000). A sharp decrease in SOC concentration with depth was observed in all land uses except CT corn. The uniform SOC concentration from Ap1 to Ap3 horizons of CT corn is probably due to the incorporation of crop residues by tillage operations. Soil C:N ratios were also varied among different horizons, ranging from 4.7 to 13.6 in woodlot, 3.5 to 10.1 in grassland, 3.5 to 11.2 in NT corn and 3.5 to 10.6 in CT corn (Table 1). Narrowing of C:N ratios with depth, consistent among land uses, provided an indication that SOM in subsoil is probably more stabilized than that in surface soil (Kramer et al. 2003).

Soil organic carbon resistant to chemical oxidation

The amount of SOC resistant to oxidation significantly differed among oxidizing agents in all horizons of the four land uses (Table 2). Among the land uses, SOC resistant to NaOCl ranged from 38 to 50% of total SOC in A1/Ap1 horizon, 42 to 54% in A2/Ap2 horizon, 41 to 49% in E/Ap3 horizon, 48 to 66% in Bt1 horizon and 64 to 79% in Btx1 horizon; SOC resistant to Na$_2$S$_2$O$_8$ ranged from 4 to 10% in A1/Ap1 horizon, 5 to 11% in A2/Ap2 horizon, 8 to 12% in E/Ap3 horizon, 14 to 38% in Bt1 horizon and 37 to 43% in Btx1 horizon; and SOC resistant to H$_2$O$_2$ ranged from 4 to 8% in A1/Ap1 horizon, 6 to 10% in A2/Ap2 horizon, 10 to 11% in E/Ap3 horizon, 16 to 26% in Bt1 horizon and 36 to 46% in Btx1 horizon. These results indicate that, irrespective of soil horizons and land uses, oxidation with NaOCl removed significantly less SOC compared to Na$_2$S$_2$O$_8$ and H$_2$O$_2$. The SOC removal efficiency of Na$_2$S$_2$O$_8$ and H$_2$O$_2$ were generally similar except in Bt1 horizons of woodlot soils, where H$_2$O$_2$ removed significantly higher amount of SOC than Na$_2$S$_2$O$_8$. In addition, the

Table 2 Effect of wet oxidation methods on soil organic carbon removal in different horizons

Horizon	Oxidants	Woodlot		Grassland		No-till corn		Conventional-till corn	
		SOC resistant to oxidation (g kg^{-1})	% of total SOC	SOC resistant to oxidation (g kg^{-1})	% of total SOC	SOC resistant to oxidation (g kg^{-1})	% of total SOC	SOC resistant to oxidation (g kg^{-1})	% of total SOC
A1/Ap1	NaOCl	14 (1.7) a	38	15 (0.6) a	45	20 (0.7) a	50	4.0 (0.05) a	40
	$Na_2S_2O_8$	1.6 (0.05) b	4	1.4 (0.3) b	4	1.6 (0.1) b	4	1.0 (0.1) b	10
	H_2O_2	2.5 (0.1) b	7	1.3 (0.2) b	4	1.5 (0.1) b	4	0.8 (0.1) b	8
A2/Ap2	NaOCl	12.8 (0.2) a	45	13.2 (0.8) a	54	12.6 (0.5) a	49	4.9 (0.2) a	42
	$Na_2S_2O_8$	1.6 (0.05) b	6	1.3 (0.2) b	5	1.4 (0.1) b	5	1.3 (0.1) b	11
	H_2O_2	2.0 (0.1) b	7	1.4 (0.1) b	6	1.4 (0.1) b	6	1.2 (0.07) b	10
E/Ap3	NaOCl	5.2 (0.1) a	49	7.4 (0.3) a	49	5.9 (0.3) a	42	4.8 (0.4) a	41
	$Na_2S_2O_8$	1.3 (0.03) b	12	1.4 (0.0) b	9	1.1 (0.06) b	8	1.4 (0.2) b	11
	H_2O_2	1.0 (0.2) b	10	1.6 (0.2) b	11	1.3 (0.05) b	10	1.3 (0.01) b	11
Bt1	NaOCl	1.8 (0.1) a	66	3.4 (0.2) a	48	1.7 (0.1) a	58	2.0 (0.2) a	55
	$Na_2S_2O_8$	1.0 (0.01) b	38	1.0 (0.1) b	14	0.93 (0.02) b	33	0.97 (0.05) b	27
	H_2O_2	0.70 (0.01) c	26	1.2 (0.1) b	16	0.72 (0.05) c	25	0.87 (0.1) b	24
Btx1	NaOCl	NA	NA	1.4 (0.3) a	79	1.4 (0.2) a	72	1.3 (0.06) a	64
	$Na_2S_2O_8$			0.78 (0.03) b	43	0.76 (0.02) b	40	0.72 (0.04) b	37
	H_2O_2			0.82 (0.2) b	46	0.76 (0.08) b	40	0.70 (0.09) b	36

$N = 3$; Standard deviation in brackets; Values in a column within a horizon followed by different letters are significantly different at $P \leq 0.05$ using Fisher's protected LSD

proportion of total SOC resistant to $Na_2S_2O_8$ and H_2O_2 increased progressively with increase in soil depth in all land uses, in contrast to no clear patterns between SOC resistant to NaOCl and soil depth. In general, the SOC protective or loading capacity of mineral particles increases with decreasing total SOC concentration from surface to subsurface soils. At the higher SOC loading, where most mineral bonding sites are already occupied with organic compounds, the SOC may be associated relatively weakly to the mineral surfaces and therefore may be more susceptible to chemical oxidation. On the other hand, at lower SOC loading, organic compounds occupy a relatively larger portion of mineral surfaces with more direct bondings with minerals (Kaiser and Guggenberger 2003; Mikutta et al. 2005). Therefore, the increase in proportion of $Na_2S_2O_8$ and H_2O_2 resistant SOC with depth may partly be attributed to a more intimate association of SOC with mineral surfaces at lower SOC loadings (Kaiser and Guggenberger 2003; Eusterhues et al. 2003, 2005). Helfrich et al. (2007) reported that, contrary to the treatment with H_2O_2 or $Na_2S_2O_8$, the proportion of NaOCl-resistant SOC in the Rotthalmünster soils of Germany

did not increase with progressive decrease in SOC loading. These results suggest that, as compared to NaOCl, the treatment with $Na_2S_2O_8$ and H_2O_2 were able to isolate a SOC fraction which is more refractory in nature. Bruun et al. (2007), who compared several methods for the isolation of a stable SOC on two soils incubated with ^{14}C-labelled barley straw for either 40 days or 40 years, found that the SOC resistant to both NaOCl and $Na_2S_2O_8$ contained a larger proportion of 40-days-old C than of 40-years old C and therefore they are unlikely to isolate a stable fraction of SOC.

C:N ratio of oxidation resistant residues

The C:N ratios of residues remaining after oxidative treatments differed from that of the untreated soil in all the land uses (Fig. 1). Oxidation using NaOCl resulted in increased C:N ratios in all horizons, as revealed by the positive relative change (7–53%, Fig. 1). Increased C:N ratio suggests a preferential oxidation of N-rich OM fractions by NaOCl. Mikutta et al. (2005) also reported higher C:N ratios for NaOCl oxidized residues as compared to untreated
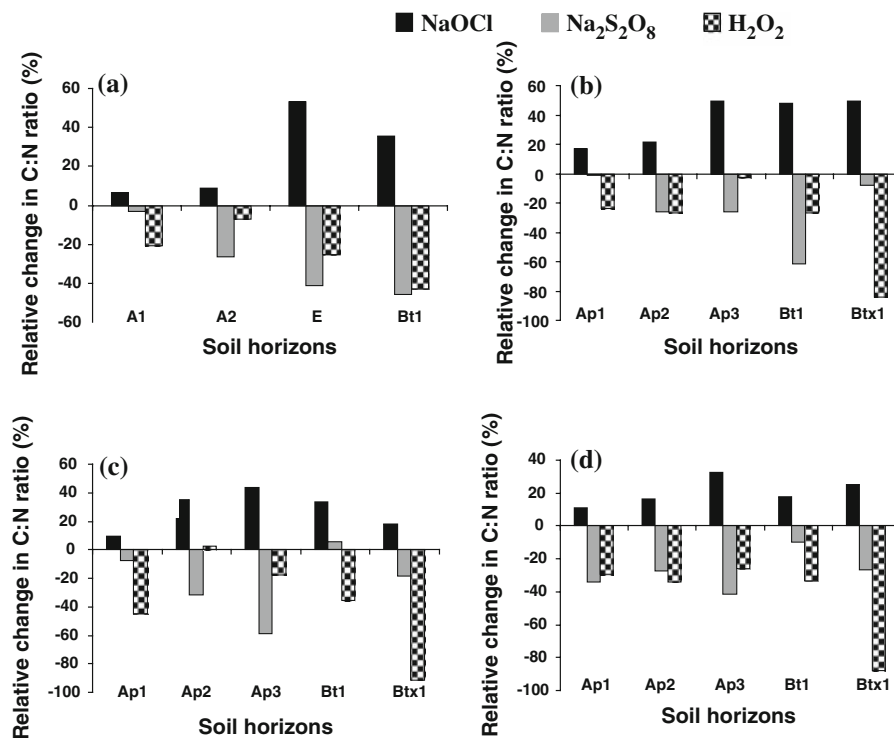
Fig. 1 Changes in C:N ratios of oxidation resistant residues relative to untreated soils: **a** woodlot, **b** grassland, **c** no-till corn, **d** conventional-till corn

soils, and attributed this to lesser stability of N-containing compounds of SOM to NaOCl oxidation than C-containing compounds.

Oxidation using $Na_2S_2O_8$ and H_2O_2 in general decreased the C:N ratios (Fig. 1). This result was in accordance with Leifeld and Kögel-Knabner (2001) and Plante et al. (2004), who also observed lower C:N ratios for H_2O_2 oxidized residues compared to untreated soils. The lower removal of N than C and consequent decreases in the C:N ratio of residues after H_2O_2 oxidation was suggested to result from interaction of N with mineral surfaces, protection within microaggregates, or fixation of NH_4^+ to minerals (Helfrich et al. 2007). Cheshire et al. (2000) conducted an analysis of the nature and proportion of SOM functional groups resistant to H_2O_2 oxidation and identified significant amounts of amino acids in the residual clay fraction after oxidation in a Scottish Podsol. These findings also supported the stability of N-compounds to H_2O_2 oxidation. According to Lorenz et al. (2006), the relative change in C:N ratios of $Na_2S_2O_8$ oxidized

residues were variable, but mostly negative. The C:N ratio data was supplemented with data from NMR and radiocarbon analyses for evaluating the efficacy of oxidizing agents to isolate a refractory SOC fraction.

Structural composition of SOC before and after oxidation as revealed by NMR analysis

In general, the NMR spectra of the oxidized and untreated surface soils (A1/Ap1 horizon) possessed peaks in the resonance areas of alkyl C (0–45 ppm), O/N-alkyl C (45–110 ppm), aromatic C (110–160 ppm), and carboxyl C (160–220 ppm) (Fig. 2). In the untreated whole soil samples, major peaks in the alkyl C region were found in the vicinity of 24, 30 and 33 ppm (Fig. 2a), which are ascribed to long chain methine and methylene type C structures present in fatty acids, waxes and resins (Malcolm 1989). The O/N-alkyl C region was characterized by O and N substituted aliphatic C and is dominated by signals near 56, 62, 72 and 104 ppm. The resonance

Fig. 2 ^{13}C NMR spectra of soil organic carbon before and after oxidation: **a** untreated, **b** NaOCl treated, **c** $Na_2S_2O_8$ treated, **d** H_2O_2 treated soils of Wooster, OH
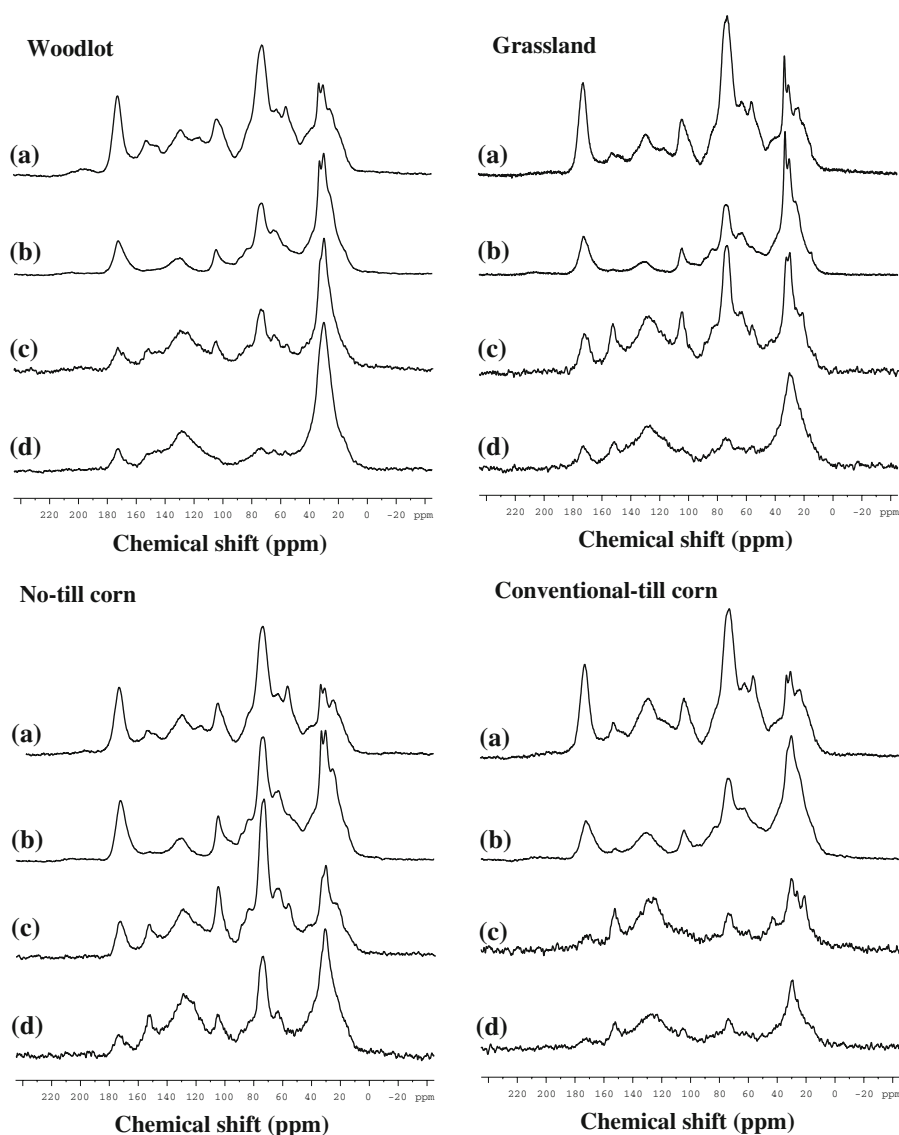
at 56 ppm is mostly due to methoxyl C (Hatcher 1987) associated with lignin. The peaks at 62, 72 and 104 ppm generally arise from carbohydrates and are dominated by O-substituted C, ring C and anomeric C, respectively. The aromatic C region was dominated by peaks near 129 ppm (C- or H-substituted aromatic C) and 153 ppm (O-substituted aromatic C such as in phenols), and the carboxylic C region was characterized by a single dominant signal near 173 ppm from carboxylic, amide or ester C (Malcolm 1989).

The relative signal intensities of different C groups showed that the untreated soils of all land uses were

dominated by O/N-alkyl C, with values ranging from 46% (CT) to 50% (grassland and NT) (Fig. 3). The untreated soils of different land uses also contained 20–24% alkyl C, 15 to 21% aromatic C and 11–12% carboxylic C. Among the land uses, the alkyl C:O/N-alkyl C ratio of untreated soils followed the order, 0.40 (NT corn) < 0.45 (CT corn) < 0.48 (grass-land) < 0.49 (woodlot) (Table 3). The higher alkyl C:O/N-alkyl C ratio for CT corn than NT corn and for woodlot than grassland were likely driven by differences in quality of plant residues. The grasses were enriched in carbohydrates, which are dominated by O/N-alkyl C, where as the forest litter had significant
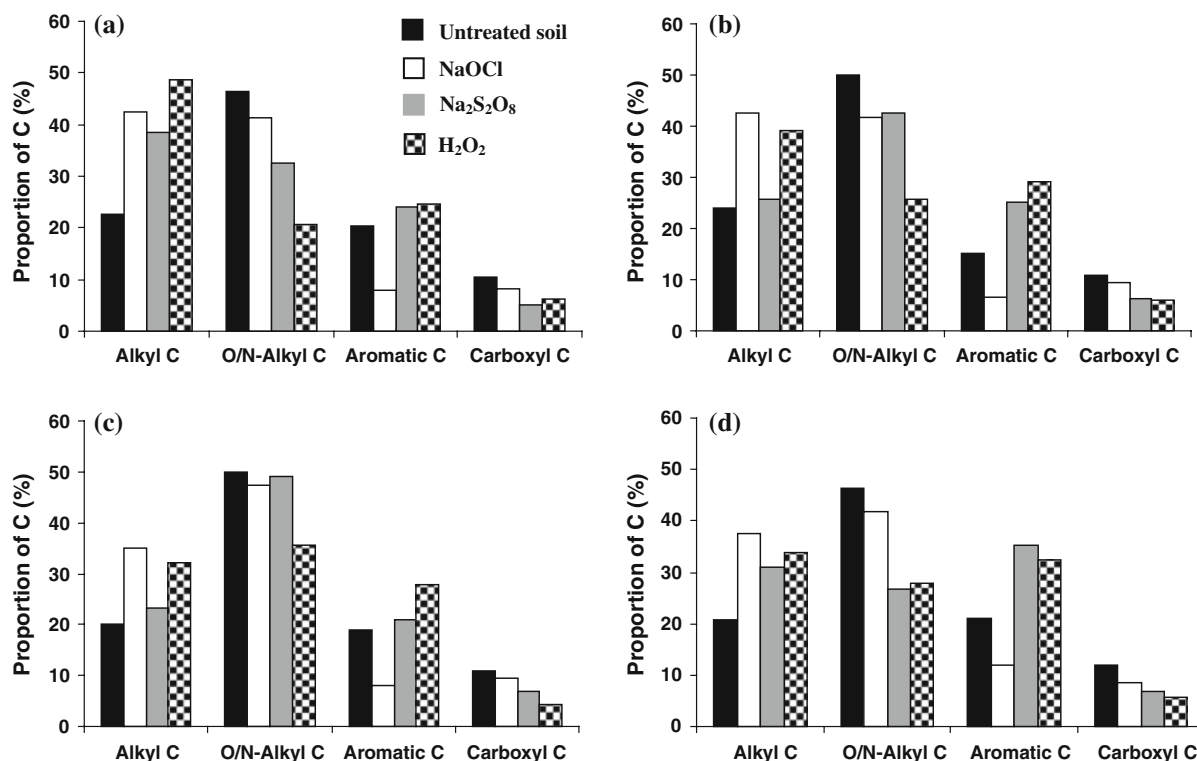
Fig. 3 Relative distributions of carbon functional groups based on solid-state ^{13}C NMR analysis: **a** woodlot, **b** grassland, **c** no-till corn, **d** conventional-till corn

Table 3 Comparison of alkyl C:O/N-alkyl C ratios and % aromaticity derived from solid-state ^{13}C NMR integration data

| Land uses | Alkyl C | | | | Aromaticity (%) | | | |
| | O/N-Alkyl C | | | | | | | |
	Untreated	NaOCl	Na$_2$S$_2$O$_8$	H$_2$O$_2$	Untreated	NaOCl	Na$_2$S$_2$O$_8$	H$_2$O$_2$
Woodlot	0.49	1.03	1.18	2.37	0.31	0.16	0.29	0.31
Grassland	0.48	1.02	0.6	1.51	0.26	0.16	0.32	0.35
No-till corn	0.40	0.74	0.47	0.91	0.30	0.18	0.28	0.32
Conventional-till corn	0.45	0.9	1.16	1.22	0.33	0.21	0.42	0.38

amounts of recalcitrant macromolecules such as tannins, waxes, fats and lignin, which are dominated by alkyl C (Marín-Spiotta et al. 2008).

The structural chemistry of SOC resistant to different oxidizing agents as revealed by solid-state ^{13}C-CPMAS-NMR analysis varied considerably (Figs. 2, 3). Major changes observed in NaOCl oxidation residues include complete removal of signals near 56 ppm which arises from lignin (Fig. 2b), 36–48% reduction in signals at 110–160 ppm among land uses (Fig. 2b), and 36–48%

reduction in aromaticity (Table 3) compared to the untreated whole soils. These findings indicated that NaOCl oxidation selectively removed lignin-derived compounds. In order to remove the interference of lignin and other aromatic structures from the black C determination, Simpson and Hatcher (2004) employed NaOCl oxidation of soils and subsequent NMR analysis. Similar to our results, they also observed that lignin moieties were successfully removed by NaOCl oxidation, which facilitated accurate estimation of black C by NMR analysis.

Mikutta et al. (2006) also reported that lignin compounds of SOM were preferentially removed by NaOCl when compared with the losses of bulk OC.

Loss of lignin moieties in this study corroborates the observed increase in C:N ratios of NaOCl oxidized residues compared to unoxidized soils (Fig. 1), indicating that N-containing compounds are more vulnerable to NaOCl oxidation than C-containing compounds (Fig. 1). These N compounds could be directly bonded to aromatic rings in lignin residues. Although aromatic binding of N has been demonstrated under laboratory conditions, the field detection attempts using NMR techniques were unsuccessful until when Schmidt-Rohr et al. (2004) developed saturation-pulse-induced dipolar exchange with recoupling (SPIDER), an NMR technique involving MAS for selective detection of C that are bonded to N. With this advanced technique, significant amounts of N-bonded aromatic C around 134 ppm (N–C_{arom}) and N-bonded aliphatic C (N–CH) around 56 ppm were detected (Schmidt-Rohr et al. 2004; Olk et al. 2006). From the observed signal removal at 56 ppm and signal intensity reduction at 110–160 ppm upon NaOCl oxidation (Figs. 2, 3), it can be deduced that the widening of relative C:N ratio after NaOCl oxidation could be due to the preferential losses of N bonded to the aromatic (134 ppm) and aliphatic C (56 ppm). Thus, the NMR data showed that oxidation with NaOCl resulted changes in specific functional groups of SOM and these changes do not support evidence for an increasing degree of SOC decomposition.

The NMR spectra of the residues resistant to $Na_2S_2O_8$ and H_2O_2 showed a relative increase in alkyl and aromatic C, and a relative decrease in O/N-alkyl and carboxyl C than the control spectra (Figs. 2, 3). These trends indicate that changes in structural chemistry of SOC as a result of $Na_2S_2O_8$ and H_2O_2 oxidation were in agreement with the selective preservation of alkyl compounds present in recalcitrant macromolecules (e.g. fatty acids, waxes and resins) with increasing degree of humification (Kögel-Knabner et al. 1988; Baldock et al. 1997; Cuypers et al. 2002). These results further support the notion that $Na_2S_2O_8$ and H_2O_2 oxidation preferentially removed mostly labile SOC (Bruun et al. 2007). A comparison between $Na_2S_2O_8$ and H_2O_2 oxidized spectra across the land uses revealed that the proportion of alkyl C showed an increasing trend

for H_2O_2 resistant SOC (32–49%) than for $Na_2S_2O_8$ resistant SOC (23–39%). Concomitantly, the proportion of O/N-alkyl C was generally lower for H_2O_2 (21–36%) than for $Na_2S_2O_8$ (27–49%) resistant SOC (Figs. 2c, d, 3). The proportion of aromatic and carboxyl C were comparable between these two treatments. The relative enrichment of alkyl C upon oxidation with H_2O_2 was also evident from the alkyl C:O/N-alkyl C ratio, which was 1.1 to 2.5 times higher for SOC resistant to H_2O_2 than that resistant to $Na_2S_2O_8$ (Table 3). The observed increase in resonance at alkyl region of H_2O_2 resistant C, however, cannot be directly related to the chemical stability of SOC because some of the fresh aliphatic plant residues dominated by labile compounds can be resistant to H_2O_2 oxidation (von Lützow et al. 2007; Helfrich et al. 2007). It was proved when the LF was exposed to chemical oxidation. Among the land uses, the H_2O_2 oxidation left behind a fraction of bleached LF with 30–56% of total OC. However, the LF remaining after $Na_2S_2O_8$ oxidation was too low for any reliable measurement of C. A similar test conducted by Eusterhues et al. (2005) on forest litter layers reported that 26–79% of total OC in L and Of layers were resistant to H_2O_2 treatment. For the Oh horizons, the chemically resistant fraction was smaller, but still in the range of 8–19% of total OC. This result, which has been confirmed elsewhere (von Lützow et al. 2007), suggests that horizons with large amounts of relatively fresh plant material contain considerable amounts of H_2O_2-resistant organic matter.

Isotopic analyses of SOC before and after oxidation

The untreated A1/Ap1 soils of all the land uses were characterized by a higher ^{14}C activity (39–103‰) (Table 4), indicating that these soils contain ^{14}C produced due to atmospheric thermonuclear weapon testing during 1960s (bomb ^{14}C), and a significant portions of the SOM are exchanging C with atmospheric CO_2 on decadal or shorter time scales (Trumbore 1997). Among the oxidizing agents, the ^{14}C activity of SOC resistant to NaOCl was distinctly different from that resistant to $Na_2S_2O_8$ and H_2O_2 (Table 4). The ^{14}C activity of NaOCl resistant SOC was only slightly lower than the untreated whole soils. These results, along with the lower C removal

Table 4 The ^{14}C activity of soil organic carbon susceptible and resistant to oxidation in the surface soil

Land uses	Untreated soils	NaOCl oxidation		Na$_2$S$_2$O$_8$ oxidation		H$_2$O$_2$ oxidation		H$_2$O$_2$ oxidation after LF removal	
	Δ^{14}C of SOC (‰)	Δ^{14}C of retained SOC (‰)	Δ^{14}C of oxidized SOC (‰)	Δ^{14}C of retained SOC (‰)	Δ^{14}C of oxidized SOC (‰)	Δ^{14}C of retained SOC (‰)	Δ^{14}C of oxidized SOC (‰)	Δ^{14}C of retained SOC (‰)	Δ^{14}C of oxidized SOC (‰)
Woodlot	103	61	129	−259	116	−182	124	−469	119
Grassland	75	49	96	−378	91	−288	90	−550	88
No-till corn	65	34	96	−366	87	−326	80	−614	79
Conventional-till corn	39	−47	97	−542	80	−475	84	−725	97

efficiency (Table 2) and preferential removal of aromatic compounds (Fig. 2) by NaOCl oxidation indicate that NaOCl oxidation is not effective in removing all young SOC from soils. Based on SOC recovery efficiency and ^{14}C ages, Favilli et al. (2008) reported that NaOCl is a weak reagent to oxidize all labile OM in soil. On the contrary, the Na$_2$S$_2$O$_8$ and H$_2$O$_2$ resistant residues were depleted of ^{14}C activity as revealed by the negative values of Δ^{14}C (Table 4), indicating that these chemicals are more efficient in isolating a refractory SOC fraction enriched with aliphatic C functional groups. Since a portion of LF was resistant to H$_2$O$_2$ oxidation, the ^{14}C activity of the heavy fraction resistant to H$_2$O$_2$ was measured. It showed remarkably lower ^{14}C activity than the activity of H$_2$O$_2$ resistant SOC containing plant residues (Table 4). These results indicate that removing LF, which consists of mainly labile SOC, increases the turnover time of the H$_2$O$_2$ resistant SOC fraction. Among the four land uses, the relative decrease in activity of H$_2$O$_2$ resistant SOC upon LF

removal was slightly greater for soils from woodlot and NT corn, where the proportion of LF was relatively higher.

The ^{13}C isotopic signatures of oxidation resistant SOC are useful tracers for evaluating the effectiveness of different oxidizing agents for the separation of SOC fractions with distinct stability. In woodlot and grassland, where the native plant species are C$_3$ type and there was no shift to C$_4$ vegetation occurred over time, an enrichment of ^{13}C (less negative δ^{13}C values) was observed after oxidative treatments (Table 5). Compared to the δ^{13}C values of untreated samples, the δ^{13}C values of residues after oxidation in both the woodlot and grassland increased by 0.9 and 0.33‰, respectively, upon NaOCl oxidation, 0.66 and 1.63‰ upon Na$_2$S$_2$O$_8$ oxidation, 3.5 and 5.3‰ upon H$_2$O$_2$ oxidation and 5.4 and 6.8‰ upon H$_2$O$_2$ oxidation after LF removal. The largest enrichment of ^{13}C was therefore associated with the SOC present in the H$_2$O$_2$ resistant residue after LF removal. These results are in accord with other studies that

Table 5 The ^{13}C signatures of soil organic carbon susceptible and resistant to oxidation in the surface soil

Land uses	Untreated soils	NaOCl oxidation		Na$_2$S$_2$O$_8$ oxidation		H$_2$O$_2$ oxidation		H$_2$O$_2$ oxidation after LF removal	
	δ^{13}C of SOC (‰)	δ^{13}C of retained SOC (‰)	δ^{13}C of oxidized SOC (‰)	δ^{13}C of retained SOC (‰)	δ^{13}C of oxidizedSOC (‰)	δ^{13}C of retained SOC (‰)	δ^{13}C of oxidized SOC (‰)	δ^{13}C of retained SOC (‰)	δ^{13}C of oxidized SOC (‰)
Woodlot	−27.3	−26.4	−27.9	−26.7	−27.4	−23.8	−27.7	−21.9	−27.4
Grassland	−27.8	−27.5	−28.1	−26.2	−27.9	−22.5	−28.1	−21.0	−27.9
No-till corn	−18.2	−18.5	−17.8	−22.6	−18.0	−22.4	−18.3	−25.0	−18.1
Conventional-till corn	−19.4	−19.7	−19.2	−23.7	−18.9	−23.6	−19.2	−26.0	−19.3

documented enrichment of heavier ^{13}C compared to lighter ^{12}C with increasing degree of SOM decomposition (Nadelhoffer and Fry 1988; Balesdent et al. 1993; Bernoux et al. 1998). Such a trend in $\delta^{13}C$ signatures was not observed in soils from cultivated sites, where the $\delta^{13}C$ signatures of contemporary plant inputs are a mixture of both C_3 native species (average $\delta^{13}C$ of −27‰) and C_4 corn (average $\delta^{13}C$ of −12‰) (Table 5). The mean $\delta^{13}C$ signatures in two cultivated sites are as follows: −18.8‰ for unoxidized soils, −19.1‰ for SOC resistant to NaOCl oxidation, −23.2‰ for SOC resistant to $Na_2S_2O_8$ oxidation, −23.0‰ for SOC resistant to H_2O_2 oxidation and −25.5‰ for SOC resistant to H_2O_2 oxidation after LF removal. These data showed that the significant amount of young, corn-derived SOC that entered the soil over decadal time scales were most susceptible to oxidation with H_2O_2 (after LF removal) than with $Na_2S_2O_8$ and NaOCl.

Mass balance calculations

The $\Delta^{14}C$ and $\delta^{13}C$ values of the SOC removed by oxidation were estimated by mass balance calculations (Tables 4, 5). The data revealed that, irrespective of the differences in labile SOC removal efficiency, the isotopic signatures of the SOC removed by different oxidizing agents remained more or less similar. The $\Delta^{14}C$ activity of removed SOC ranged from 116 to 129‰ for woodlots, 88 to 96‰ for grassland, 79 to 96‰ for NT corn and 80 to 97‰ for CT corn (Table 4), suggesting that many of the millennial ages reported for organic matter indeed represent mixtures of much younger and much older material. Incomplete removal of labile SOC can thus lead to an underestimation of the turnover time of more stable fraction of SOC. Similarly the $\delta^{13}C$ data of removed SOC, estimated by mass balance, ranged from −27.4 to −27.9‰ for woodlots, −27.9 to −28.1‰ for grassland, −17.8 to −18.3‰ for NT corn and −18.9 to −19.3‰ for CT corn (Table 5). Lower than expected $\delta^{13}C$ values (−12‰) of removed SOC from NT and PT corn suggests that some relic C (C_3 SOC) was also removed by the oxidizing agents. Nonetheless, these results reiterate that the isotopic signature of the rapidly cycling labile SOC pool is influenced by the plant species from which it was derived.

Conclusions

The data presented support the conclusion that different chemicals commonly used for wet oxidation of SOC ($Na_2S_2O_8$, H_2O_2, NaOCl) react differently on total SOC. The NaOCl oxidation resulted in a lower SOC removal efficiency and left behind a residue with wider C:N ratios than the untreated soils. On the other hand, $Na_2S_2O_8$ and H_2O_2 oxidized substantial amounts of SOC and the residue remained had narrower relative C:N ratios. The solid-state ^{13}C NMR analysis was useful in evaluating the chemical transformations of SOC during the wet oxidation processes. The NaOCl oxidation preferentially removed the aromatic functional groups of SOM. However, the SOC resistant to $Na_2S_2O_8$ and H_2O_2 oxidation were enriched with alkyl C groups, which dominate in recalcitrant macromolecules. The isotopic analyses not only confirmed that $Na_2S_2O_8$ and H_2O_2 were more efficient than NaOCl in isolating a refractory fraction of SOC, but also provided evidence that H_2O_2 treatment after LF removal is more effective in isolating the oldest SOC.

Results obtained from this study will strengthen the current knowledge on the usefulness of different oxidizing agents on identifying the SOC fractions with homogeneous reactivity. Such information is important for accurate quantification of the effects of land use or management changes on longer term sequestration of SOC. The experimental isolation and quantification of SOC pool with homogeneous stability is also useful for validating the conceptual pools of the SOM prediction models for improving their process-based simulation accuracy.

References

Baisden WT, Amundson R (2003) An analytical approach to ecosystem biogeochemistry modeling. Ecol Appl 13:649–663

Baisden WT, Amundson R, Cook AC, Brenner DL (2002) Turnover and storage of C and N in five density fractions

from California annual grassland surface soils. Global Biogeochem Cycles 16(4):1117

Baldock JA, Preston CM (1995) Chemistry of carbon decomposition processes in forests as revealed by solid-state carbon-13 nuclear magnetic resonance. In: McFee WW, Kelly JM (eds) Carbon forms and functions in forest soils. Soil Sci Soc Am, Madison, WI

Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Org Geochem 31:697–710

Baldock JA, Oades JM, Waters AG, Peng X, Vassallo AM, Wilson MA (1992) Aspects of the chemical structure of soil organic materials as revealed by solid-state ^{13}C NMR spectroscopy. Biogeochem 16:1–42

Baldock JA, Oades JM, Nelson PN, Skene TM, Golchin A, Clarke P (1997) Assessing the extent of decomposition of natural organic materials using solid-state ^{13}C NMR spectroscopy. Aust J Soil Res 35:1061–1083

Balesdent J (1996) The significance of organic separates to carbon dynamics and its modeling in some cultivated soils. Eur J Soil Sci 47:485–493

Balesdent J, Mariotti A, Guillet B (1987) Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biol Biochem 19:25–30

Balesdent J, Girardin C, Mariotti A (1993) Site-related δ^{13}C of tree leaves and soil organic matter in a temperate forest. Ecology 74:1713–1721

Batjes NH (1996) Total carbon and nitrogen in the soils of the world. Eur J Soil Sci 47:151–163

Bernoux M, Cerri CC, Neill C, de Moraes JFL (1998) The use of stable carbon isotopes for estimating soil organic matter turnover rates. Geoderma 82:43–58

Bruun S, Thomsen IK, Christensen BT, Jensen LS (2007) In search of stable soil organic carbon fractions: a comparison of methods applied to soils labeled with ^{14}C for 40 days or 40 years. Eur J Soil Sci 59:247–256

Castanha C, Trumbore S, Amundson R (2008) Methods of separating soil carbon pools affect the chemistry and turnover time of isolated fractions. Radiocarbon 50:83–97

Cheshire MV, Dumat C, Fraser AR, Hiller S, Staunton S (2000) The interaction between soil organic matter and soil clay minerals by selective removal and controlled addition of organic matter. Eur J Soil Sci 51:497–509

Cuypers C, Grotenhuis T, Nierop KGJ, Franco EM, de Jager A, Rulkens W (2002) Amorphous and condensed organic matter domains: the effect of persulfate oxidation on the composition of soil/sediment organic matter. Chemosphere 48:919–931

Dick WA (1984) Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Sci Soc Am J 48:569–574

Dick WA, Blevins RL, Flye WW, Peters SE, Christenson DR, Pierce FJ, Vitosh ML (1998) Impacts of agricultural management practices on C sequestration in forest-derived soils of the eastern Corn Belt. Soil Till Res 47:235–244

Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner I (2003) Stabilization of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Org Geochem 34:1591–1600

Eusterhues K, Rumpel C, Kögel-Knabner I (2005) Stabilization of soil organic matter isolated via oxidative degradation. Org Geochem 36:1567–1575

Favilli F, Egli M, Cherubini P, Sartori G, Haeberli W, Delbos E (2008) Comparison of different methods of obtaining a resilient organic matter fraction in Alpine soils. Geoderma 145:355–369

Fründ R, Haider K, Lüdemann HD (1994) Impact of soil management practices on the organic matter structure—investigations by CPMAS ^{13}C NMR-spectroscopy. Zeitschrift für Pflanzenernährung und Bodenkunde 157:29–35

Gee GW, Or D (2002) Particle-size analysis. In: Dane JH, Topp GC (eds) Methods of soil analysis. Part 4: physical methods. SSSA Book Series No. 5. SSSA, Madison, WI, pp 255–289

Golchin A, Oades JM, Skjemstad JO, Clarke P (1994) Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy. Aust J Soil Res 32:285–309

Hatcher PG (1987) Chemical structural studies of natural lignin by dipolar dephasing solid-state ^{13}C nuclear magnetic resonance. Org Geochem 11:31–39

Helfrich M, Flessa H, Mikutta R, Dreves A, Ludwig B (2007) Comparison of chemical fractionations methods for isolating stable soil organic carbon pools. Eur J Soil Sci 58:1316–1329

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436

John B, Yamashita T, Ludwig B, Flessa H (2005) Storage of organic carbon in aggregate and density fractions of silty soils under different types of land use. Geoderma 128:63–79

Kaiser K, Guggenberger G (2003) Mineral surfaces and soil organic matter. Eur J Soil Sci 54:219–236

Kiem R, Knicker H, Körschens M, Kögel-Knabner I (2000) Refractory organic carbon in C-depleted arable soils, as studied by ^{13}C NMR spectroscopy and carbohydrate analysis. Org Geochem 31:655–668

Kleber M, Mikutta R, Torn MS, Jahn R (2005) Poorly crystalline mineral phases protect organic matter in acid subsoil horizons. Eur J Soil Sci 56:717–725

Kögel-Knabner I, Zech W, Hatcher PG (1988) Chemical composition of the organic matter in forest soils: the humus layer. Zeitschrift für Pflanzenernährung und Bodenkunde 151:331–340

Kramer MG, Sollins P, Sletten RS, Swart PK (2003) N isotope fractionation and measures of organic matter alteration during decomposition. Ecology 84:2021–2025

Krull ES, Baldock JA, Skjemstad JO (2003) Importance of mechanisms and processes of the stabilization of soil organic matter for modeling carbon turnover. Funct Plant Biol 30:207–222

Lal R (2007) Carbon sequestration. Phil Trans R Soc B 363:815–830

Leavitt SW, Follett RF, Paul EA (1996) Estimation of the slow and fast cycling soil organic carbon pools from 6 N HCl hydrolysis. Radiocarbon 38:230–231

Leifeld J, Kögel-Knabner I (2001) Organic carbon and nitrogen in fine soil fractions after treatment with hydrogen peroxide. Soil Biol Biochem 33:2155–2158

Lorenz K, Lal R, Shipitalo MJ (2006) Stabilization of organic carbon in chemically separated pools in no-till and meadow soils in northern Appalachia. Geoderma 137:205–211

Malcolm RL (1989) Applications of solid-state ^{13}C NMR spectroscopy to geochemical studies of humic substances. In: Hayes et al (eds) Humic substances II: in search of structure. Wiley, New York

Marín-Spiotta E, Swanston CW, Torn MS, Silver WL, Burton SD (2008) Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. Geoderma 143:49–62

Mikutta R, Kleber M, Jahn R (2005) Poorly crystalline minerals protect organic carbon in clay subfractions from acid subsoil horizons. Geoderma 128:106–115

Mikutta R, Kleber M, Torn MS, Jahn R (2006) Stabilization of organic matter: association with minerals or chemical recalcitrance? Biogeochem 77:25–56

Nadelhoffer KJ, Fry B (1988) Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci Soc Am J 52:1633–1640

Nowinski NS, Trumbore SE, Schuur EAG, Mack MC, Shaver GR (2008) Nutrient addition prompts rapid destabilization of organic matter in an arctic tundra ecosystem. Ecosystems 11:16–25

Olk DC, Cassman KG, Schmidt-Rohr K, Anders MM, Mao JD, Deenik JL (2006) Chemical stabilization of soil organic nitrogen by phenolic lignin residues in anaerobic agroecosystems. Soil Biol Biochem 38:3303–3312

Paul EA, Follett RF, Leavitt SW, Halvorson A, Peterson GA, Lyon DJ (1997) Radiocarbon dating for determination of soil organic matter pool sizes and dynamics. Soil Sci Soc Am J 61:1058–1067

Paul EA, Collins HP, Leavitt SW (2001) Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring ^{14}C abundance. Geoderma 104:239–256

Plante AF, Chenu C, Balabane M, Mariotti A, Righi D (2004) Peroxide oxidation of clay-associated organic matter in a cultivation chronosequence. Eur J Soil Sci 55:471–478

Preston CM (1996) Applications of NMR to soil organic matter analysis: history and prospects. Soil Sci 161:144–166

SAS Institute (2002) The SAS system for microsoft windows release 8.2. SAS Institute, Cary, NC

Schmidt MWI, Gleixner G (2005) Carbon and nitrogen isotope composition of bulk soils, particle-size fractions and organic material after treatment with hydrofluoric acid. Eur J Soil Sci 56:407–419

Schmidt MWI, Knicker H, Hatcher PG, Kögel-Knabner I (1997) Improvement of ^{13}C CPMAS NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10% hydrofluoric acid. Eur J Soil Sci 48:319–328

Schmidt-Rohr K, Mao JD, Olk DC (2004) Nitrogen-bonded aromatics in soil organic matter and their implications for a yield decline in intensive rice cropping. Proc Nat Acad Sci USA 101:6351–6354

Silveira ML, Comerford NB, Reddy KR, Cooper WT, El-Rifai H (2008) Characterization of soil organic carbon pools by acid hydrolysis. Geoderma 144:405–414

Simpson MJ, Hatcher PG (2004) Determination of black carbon in natural organic matter by chemical and solid-state ^{13}C nuclear magnetic resonance spectroscopy. Org Geochem 35:923–935

Siregar A, Kleber M, Mikutta R, Jahn R (2005) Sodium hypochlorite oxidation reduces soil organic matter concentrations without affecting inorganic soil constituents. Eur J Soil Sci 56:481–490

Sollins P, Homann P, Caldwell BA (1996) Stabilisation and destabilisation of soil organic matter: mechanisms and controls. Geoderma 74:65–105

Stuiver M, Polach HA (1977) Reporting of C-14 data—discussion. Radiocarbon 19:355–363

Theng BKG, Tate KR, Becker-Heidemann P (1992) Towards establishing the age, location, and identity of the inert soil organic matter of a Spodosol. Zeitschrift für Pflanzenernährung und Bodenkunde 155:181–184

Theng BKG, Ristori GG, Santi CA, Percival HJ (1999) An improved method for determining the specific surface areas of topsoils with varied organic matter content, texture and clay mineral composition. Eur J Soil Sci 50:309–316

Thomas GW (1996) Soil pH and soil acidity. In: Sparks DL (ed) Methods of soil analysis. Part 3: chemical methods. SSSA Book Series No. 5. SSSA, Madison, WI, pp 475–490

Trumbore SE (1997) Potential responses of soil organic carbon to global environmental change. Proc Nat Acad Sci USA 94:8284–8291

Trumbore SE, Zheng S (1996) Comparison of fractionation methods for soil organic matter ^{14}C analysis. Radiocarbon 38:219–229

USDA-SCS (United States Department of Agriculture-Soil Conservation Service) (1984) Soil survey of Wayne County, Ohio. US Government Printing Office, USDA, Washington DC p 94

Ussiri DAN, Johnson CE (2007) Organic matter composition and dynamics in a northern hardwood forest ecosystem 15 years after clear-cutting. Forest Ecol Manag 240:131–142

Ussiri DAN, Lal R (2008) Method for determining coal carbon in the reclaimed minesoils contaminated with coal. Soil Sci Soc Am J 72:231–237

van Doren DM, Triplett GB, Henry JE (1976) Influence of long-term tillage, crop-rotation, and soil type combinations on corn yield. Soil Sci Soc Am J 40:100–105

von Lützow M, Kögel-Knabner I, Ekschmitt K, Flessa H, Guggenberger G, Matzner E, Marschner B (2007) SOM fractionation methods: relevance to functional pools and to stabilization mechanisms. Soil Biol Biochem 39:2183–2207

Xu XM, Trumbore SE, Zheng SH, Southon JR, McDuffee KE, Luttgen M, Liu JC (2007) Modifying a sealed tube zinc reduction method for preparation of AMS graphite targets: reducing background and attaining high precision. Nucl Instrum Methods Phys Res B Beam Interact Mater Atoms 259:320–329

Zimmermann M, Leifeld J, Abiven S, Schmidt MWI, Fuhrer J (2007) Sodium hypochlorite separates an older soil organic matter fraction than acid hydrolysis. Geoderma 139:171–179