Characterization of the heavy, hydrolysable and non-hydrolysable fractions of soil organic carbon in conventional and no-tillage soils

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ABSTRACT

Quantification of soil organic carbon (SOC) pool fractions under different tillage systems is important in understanding SOC dynamics and storage. Two major pools of SOC that can be isolated are the light (LF) and heavy fractions (HF). Few studies have quantified the effect of tillage systems on the hydrolysable (HYF) and non-hydrolysable fractions (NHF) which comprise the HF. The objective of this study was to evaluate if there were significant changes in the quantity and δ13C of the HF, HYF, and NHF fractions of SOC after six years of no-tillage (NT), on arable soils that were previously under conventional tillage (CT). Our study used δ13C natural abundance (rotation of C3 and C4 crops) on a calcareous Typic Hapludalf soil in southern Ontario, Canada. The HF (> 1.7 g cm⁻²) was isolated using density fractionation and separated into its HYF and NHF using acid hydrolysis (6 M HCl) for three soil depths: 0–10, 10–20, and 20–30 cm. The HF pool (90–93% of SOC) was significantly greater (P < 0.05) in the NT (28.9) than the CT system (25.5 Mg C ha⁻¹) only for the 0–10 cm depth. The dominant SOC fraction from the HF pool was the NHF (62–65% of SOC) for both tillage systems. However, the HYF (25–30% of SOC) was significantly greater in the NT (9.3) than the CT system (7.0 Mg C ha⁻¹) only for the 0–10 cm depth. Additionally, there was a significantly higher proportion of C₄-derived C in the HF only at the 0–10 cm depth of NT soils. Differences in the δ13C of the whole soil and SOC fractions show a preservation of newly derived C in the HF, HYF and NHF of NT soils. We conclude that the adoption of NT systems on arable soils increases the quantity of HF and HYF in the 0–10 cm depth only, but there is no difference in carbon sequestration potential when treatments are compared over the 0–30 cm depth in the short-term.

1. Introduction

Soil organic carbon (SOC) pools, dynamics and storage are affected by conventional (CT) and no-tillage (NT) systems (Angers et al., 1995; Six et al., 1999; Lupwayi et al., 2004; Huggins et al., 2007; Schjønning et al., 2007; Alvarez and Steinbach, 2009; Beyaert and Voroney, 2011). SOC is the carbon stored in soil organic matter (SOM), which refers to the organic fraction of soil that consists of a mixture of plant and animal residues in various stages of decomposition, microbial biomass, and substances made microbiologically or chemically from by-products (Schnitzer, 2000; Wel and Brady, 2016). Lehmann and Kleber (2015) provides empirical evidence that SOM is a continuum of progressively decomposing organic carbon-containing substances but argues against the formation of stable humic substances. According to Schnitzer (2000), there will be decomposition and synthesis of products during SOM formation.

Decomposition of crop residues by the soil microbial biomass results in the residue organic C being oxidised to CO₂ by soil organisms and microbial products becoming stabilized by sorption to mineral surfaces and forming humus (Jenkinson and Rayner, 1977; Voroney et al., 1989). During this continuum of decomposition, two major pools of SOM can be identified. Firstly, there is a transitory intermediate pool known as the light fraction (LF) consisting of plant and microbial residues in various states of decomposition (Gregorich and Beare et al., 2008), and secondly a heavy fraction (HF) pool that consists of more stable, low molecular weight organic compounds and high molecular weight humified organic matter (Christensen, 1996). These pools are normally recovered by density fractionation, which is the physical separation of SOM using a liquid of known specific gravity, into a low- and high-density fraction, known as the LF and HF, respectively (Strickland and Sollins, 1987; Angers et al., 1997; Sohi et al., 2001; Tan et al., 2007; Cerli et al., 2012).

There have been many studies on the physical separation of the SOC pool into the LF and HF (Janzen et al., 1992; Cambardella and Elliot, 2008); however, few studies have quantified the effect of tillage systems on the hydrolysable (HYF) and non-hydrolysable fractions (NHF) which comprise the HF. The objective of this study was to evaluate if there were significant changes in the quantity and δ13C of the HF, HYF, and NHF fractions of SOC after six years of no-tillage (NT), on arable soils that were previously under conventional tillage (CT). Our study used δ13C natural abundance (rotation of C3 and C4 crops) on a calcareous Typic Hapludalf soil in southern Ontario, Canada. The HF (> 1.7 g cm⁻²) was isolated using density fractionation and separated into its HYF and NHF using acid hydrolysis (6 M HCl) for three soil depths: 0–10, 10–20, and 20–30 cm. The HF pool (90–93% of SOC) was significantly greater (P < 0.05) in the NT (28.9) than the CT system (25.5 Mg C ha⁻¹) only for the 0–10 cm depth. The dominant SOC fraction from the HF pool was the NHF (62–65% of SOC) for both tillage systems. However, the HYF (25–30% of SOC) was significantly greater in the NT (9.3) than the CT system (7.0 Mg C ha⁻¹) only for the 0–10 cm depth. Additionally, there was a significantly higher proportion of C₄-derived C in the HF only at the 0–10 cm depth of NT soils. Differences in the δ13C of the whole soil and SOC fractions show a preservation of newly derived C in the HF, HYF and NHF of NT soils. We conclude that the adoption of NT systems on arable soils increases the quantity of HF and HYF in the 0–10 cm depth only, but there is no difference in carbon sequestration potential when treatments are compared over the 0–30 cm depth in the short-term.

Abbreviations: δ13C, isotopic signature of carbon; CT, conventional tillage; HF, heavy fraction; HYF, hydrolysable fraction; LF, light fraction; NHF, non-hydrolysable fraction; NT, no-tillage; SOC, soil organic carbon; SOM, soil organic matter

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Soil organic matter stability is considered not only as a function of molecular structure but also as an ecosystem property, arising from complex interactions between biotic and abiotic factors (Schmidt et al., 2011). SOM dynamics and modeling have conceptually divided the SOM fractions into compartments or pools of different turnover rates and lower C concentration than the LF (Christensen, 1996). The HF can be separated into its HYF and NHF when subjected to chemical fractionation methods such as acid hydrolysis (Leavitt et al., 1996).

The HYF consists mainly of sugars, polysaccharides, amino acids, and peptides that are formed during the breakdown of cellulose, hemicellulose and proteins from the crop residues (Schmitzer and Preston, 1983; Rovira and Vallejo, 2002). The NHF (recalcitrant) is comprised of stable aromatic and long chain compounds synthesized during microbial decomposition (Baldock et al., 1997; Paul et al., 2001) and inherent plant litter components which are highly resistant to degradation (e.g. lipids, cutin, suberin, tannin, and lignin), and charcoal (Rovira and Vallejo, 2002). Acid hydrolysis thus removes labile components and is useful in isolating a stable older soil C fraction (Leavitt et al., 1996; Paul, 2016). Variations in the acid hydrolysis procedure are mainly due to temperature and reflux time. Conditions range from 6M HCl at 96 °C for 18 h (Plante et al., 2006) to 6M HCl at 116 °C for 16 h (Collins et al., 2000; Paul et al., 2001; 2006).

Researchers have attributed three mechanisms responsible for the stabilization of organic matter in temperate soils (Voroney et al., 1989; Christensen, 1996; Six et al., 2002; Krull et al., 2003; von Lützow et al., 2006; Conceição et al., 2013). These include:

(i) Biochemical recalcitrance of SOM to microbial (enzymatic) activity and non-enzymatic chemical reactions (Krull et al., 2003; von Lützow et al., 2006).

(ii) Physical protection or occlusion of SOM to microbes by micro- and macroaggregates (Hassink et al., 1997). A recent study suggests that SOM stability is governed by accessibility to microbial degradation rather than recalcitrance (Dungait et al., 2012).

(iii) Chemical stabilization due to interaction of SOM with mineral (silt and clay) surfaces (organo-mineral complexes) and formation of organic-metal complexes (Schrumpf et al., 2013). Stabilization processes determine the decomposition rates, mean residence time, and the quantity and quality of the substrate in soil carbon pools (Sollins et al., 1996).

Soil organic matter stability is considered not only as a function of molecular structure but also as an ecosystem property, arising from complex interactions between biotic and abiotic factors (Schmidt et al., 2011). SOM dynamics and modeling have conceptually divided the SOM fractions into compartments or pools of different C mineralisation rates, stability and mean residence time (Jenkinson and Rayner, 1977; Parton et al., 1987). As an example, the CENTURY model (Parton et al., 1987) shows SOM as part of three kinetic pools with varying turnover times. There is an ‘active’ SOM pool conceived as consisting mainly of the soil microbial biomass, microbial products, and root exudates (turnover time 1–5 y). There is a ‘slow’ pool consisting of physically protected or SOM more resistant to microbial degradation, with an intermediate turnover time of 20–40 y. There is also a ‘passive’ pool consisting of chemically recalcitrant SOM with a turnover time of 200–1500 y. The LF is included in the conceptually defined ‘active’ pool, the HYF is representative of the ‘slow’ pool, and the NHF is analogous to the ‘passive’ pool (Collins et al., 2000).

Determining the nature of individual soil carbon pools as a function of molecular structure but also as an ecosystem property, arising from complex interactions between biotic and abiotic factors (Schmidt et al., 2011). SOM dynamics and modeling have conceptually divided the SOM fractions into compartments or pools of different C mineralisation rates, stability and mean residence time (Jenkinson and Rayner, 1977; Parton et al., 1987). As an example, the CENTURY model (Parton et al., 1987) shows SOM as part of three kinetic pools with varying turnover times. There is an ‘active’ SOM pool conceived as consisting mainly of the soil microbial biomass, microbial products, and root exudates (turnover time 1–5 y). There is a ‘slow’ pool consisting of physically protected or SOM more resistant to microbial degradation, with an intermediate turnover time of 20–40 y. There is also a ‘passive’ pool consisting of chemically recalcitrant SOM with a turnover time of 200–1500 y. The LF is included in the conceptually defined ‘active’ pool, the HYF is representative of the ‘slow’ pool, and the NHF is analogous to the ‘passive’ pool (Collins et al., 2000).

Determining the nature of individual soil carbon pools as affected by tillage practices, allows for a better understanding of soil organic matter dynamics, and its contribution to greenhouse gas emissions from soil. Due to the complex and heterogeneous nature of SOM, many techniques have been developed to study the characteristics of the different SOM pools (Stevenson, 1994; von Lützow et al., 2006). This study however, focused on measurements of the HF and its sub-fractions (HYF and NHF). The effects of tillage practices on the LF organic matter was previously reported in Ramnarine et al. (2015).

The objective of this study was to investigate the effects of conventional (CT) and no-tillage (NT) systems on the quantity and δ13C signature of the heavy (HF), hydrolysable (HYF), and non-hydrolysable (NHF) fractions of SOM in arable soils. It also aims to evaluate if there were significant changes in the above SOC fractions after six years of no-tillage, on soils that were previously under conventional tillage.

2. Material and methods

2.1. Study site

This study was carried out at the Elora Research Station (43°38′ N, 80°25′ W), southern Ontario, Canada. The soil is a Typic Haplustalf derived from calcareous glacial till parent material. The soil is a silt loam (sand–270 g kg⁻¹, silt–560 g kg⁻¹, clay–170 g kg⁻¹) with an average pH of 7.3, total N of 1.8 g kg⁻¹ and TOC of 21.2 g kg⁻¹ in the top 0–20 cm soil depth. The site receives a mean annual precipitation of 900 mm and average monthly temperature ranges from −7.1 °C in January to 19.8 °C in July.

The research site consisted of four large-scale plots (2 CT and 2 NT), each 100 m × 150 m (1.5 ha). All plots were initially managed under conventional tillage, but in October 1999, two of the plots were converted to no-tillage. In this study, conventional tillage refers to primary tillage in the fall with a moldboard plow to a depth of 15–20 cm, followed by secondary tillage in the spring with a cultivator. From 2000, the site was planted with either a C3 crop (soybean [Glycine max (L.) Merr.] or winter wheat – Triticum aestivum L.) in annual rotation with a C4 crop (corn – Zea mays L.). Corn was grown in 2000, 2003 and 2005, winter wheat in 2002, and soybean in 2001. Historical data show that corn was first grown in 1969 in rotation with winter wheat, soybean, alfalfa (Medicago sativa L.), and barley (Hordeum vulgare L.). The corn-soybean-wheat rotation is typical and predominant for southern and eastern Canada.

The “C4-derived” carbon from corn is an important tool that can be used to analyse soil tillage effects. Due to isotopic fractionation in photosynthesis, the tissues of C3 and C4 plants have specific isotopic signatures. C3 and C4 plants have average δ13C values of −27‰ and −13‰, respectively (Smith and Epstein, 1971). Thus, soils developed under C3 or C4 vegetation contain SOM with a δ13C signature reflecting the source of their plant inputs. The 14C natural abundance technique utilizes the natural differences in δ13C signature of C3 and C4 plants as a tracer during the decomposition of plant residues in the soil (Balesdent et al., 1987, 1990; Ehleringer, 1991; Angers et al., 1995). Because of this difference in isotopic signature, the fate of these different C substrates can be traced into the various SOC pools affected by tillage systems. Corn is also one of the major C4 crops grown in the world and have been used in other tillage-related studies as a tracer for understanding SOC dynamics (Balesdent et al., 1990; Gregorich et al., 1995; Wanniarachchi et al., 1999; Allmaras et al., 2004; Murage et al., 2007; Mishra et al., 2010; Karlen et al., 2013; Rutkowska et al., 2018).

The control 13C site was a native northern hardwood forest soil located adjacent to our research site. With the exception of tillage management, agronomic practices were identical for all the plots.

2.2. Soil sampling and preparation

Soil sampling was carried out in October 2005 (after corn harvest) using a hydraulic core sampler (38 mm i.d.) to obtain the top 0–30 cm soil depth. Studies on the vertical distribution of SOC is normally
focused on the 0–30 cm of the soil profile (Lorenz and Lal, 2005), since this represents the zone most affected by tillage practices. For this reason, we focused our research on changes occurring in SOC fractions in the 0–30 cm depth.

Each of the four large-scale plots was divided into six subplots (50 m × 50 m). Of the six subplots, four were randomly selected and the centre of each was chosen as a sampling site. Within the sampling sites, four soil cores were collected at four positions relative to the harvested corn plant (spacing 75 cm × 25 cm) – on the plant, midway between plant within the rows, quarter-way (¼) and half-way (½) between plant rows. Each soil core was divided into three, to obtain the 0–10, 10–20, and 20–30 cm depths. In summary, 192 samples (4 plots × 4 sampling sites × 4 sampling positions × 3 depths) were collected. The number of replicates analysed from the CT and NT treatments was 32 for each soil depth.

Soil samples at field moisture content were gently hand-crushed and visible crop residues were removed by handpicking. The samples were then air-dried and passed through a 2-mm sieve to obtain the fine-earth fraction used for soil analyses. Soil moisture content was obtained by drying a 10 g subsample of the soil at 105 °C for 24 h.

2.3. Measurements of TOC, N and δ13C in whole soil and fractions

Before measuring total organic carbon (TOC) concentrations and δ13C in subsamples, inorganic carbonates were removed by acid fumigation (Harris et al., 2001; Ramnarine et al., 2011). TOC is a measure of the concentration of organic carbon (ppm, %, or g kg−1) in a soil sample. Soil C and N stocks (Mg ha−1) were calculated on the fine earth fraction (< 2 mm) of the soil, using soil C and N concentrations (g kg−1), thickness of layer (10 cm), and soil bulk density (Mg m−3). Soil bulk density was calculated by dividing the mass of oven-dried soil by the volume of the soil core for each depth.

Soil samples for TOC, N and δ13C analysis were placed in 8 by 5 mm tin capsules (sealed and compressed), with soil sample weights ranging from 20 to 60 mg depending on organic C concentration (modified from Harris et al., 2001). These sample weights provided about 420 μg of organic C required for mass spectrometer analysis. Samples were analyzed at the Stable Isotope Laboratory, University of Saskatchewan, Saskatoon, Canada. TOC, N, and δ13C was simultaneously determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) using an ANCA-GSL elemental analyzer (EA) coupled to a Tracermass spectrometer (Europa Scientific, Crewe, UK). The stable carbon isotope ratio was expressed as δ13C in per mil (%δ) units and calculated as δ13C (%δ) = [(Rsample/Rstandard − 1) × 1000, where Rsample and Rstandard is the 13C/12C ratio of the sample and standard, respectively (Harris et al., 2001; Ramnarine et al., 2015). The international standard PeeDee Belemnite with an isotope ratio (R) of 0.0112372 was used as the reference and the precision of δ13C measurements was < 0.1‰.

2.4. Recovery of the heavy fraction by density fractionation

Density fractionation (Gregorich and Beare et al., 2008) was used to remove the light fraction (LF) in order to recover the heavy fraction (HF). A 30-g sample of soil was placed in a 250-mL centrifuge bottle (polypropylene, wide-mouth) and 100 mL of sodium iodide (NaI) solution was added. Based on studies on similar agricultural soils in Canada (Janzen et al., 1992; Wanniarachchi et al., 1999; Murage et al., 2007; Soon et al., 2007), the specific gravity of the NaI solution used was 1.7 g cm−3, as determined with a Precision® hydrometer (Thomas Scientific, Swedesboro, NJ). The bottles were capped, placed upright, and shaken on an end-to-end platform shaker for 1 h at 160 rpm. The suspension was allowed to settle for 48 h at room temperature.

The LF was isolated using a modification of the “suction method” as described by Strickland and Solins (1987). The particulate LF floating on the surface of the NaI solution was isolated using a water-jet vacuum filtration system to allow for recovery of the HF. The vacuum filtration system (aspiration unit) consisted of a 300 mL, 47-mm magnetic filter funnel (Pall Corporation, East Hills, NY) fitted with a 0.45 μm Whatman® nylon membrane filter (Whatman International Ltd, Maidstone, England) and attached to a 1-L side-arm conical flask using 8-mm (i.d.) Tygon® tubing and a 2-mL pipette tip cut at an angle of 45°.

The residual organo-mineral HF, was washed by adding 100 mL of 0.05 M CaCl2 (to flocculate mineral particles), shaking for 1 h, followed by further washing with deionized water (centrifuging at 8000 × g for 20 min) three times (modified from Sohi et al., 2001). This fraction was retained for acid hydrolysis.

2.5. Acid hydrolysis of heavy fraction

Hydrolysable (HYF) and non-hydrolysable (NHF) fractions were obtained by acid hydrolysis (Leavitt et al., 1996) of the heavy fraction (HF). The HF samples were treated with 0.5 M HCl (150 mL) for 24 h to remove inorganic carbonates (modified from Collins et al., 1999). The samples were washed three times with deionized water (centrifuging for 20 min at 8000 × g) to remove chlorides, followed by drying at 60 °C for 48 h. The HF samples were ground (< 250 μm) and 2 g was taken for acid hydrolysis (Leavitt et al., 1996). Also, a subsample of the HF was analyzed for C, N and δ13C isotope ratio mass spectrometry.

The 2-g samples were refluxed with 50 mL of 6 M HCl in 250-mL digestion tubes at 115 °C for 16 h (Leavitt et al., 1996). After cooling, the supernatant (HYF) and the residue (NHF) were transferred to a vacuum filtration system fitted with Whatman® nylon membrane filter (47 mm, 0.22 μm) and repeatedly washed with deionized water until free of chlorides. The NHF was oven-dried at 60 °C (Plante et al., 2006), weighed, finely ground and analyzed for C, N and δ13C.

2.6. Estimation of corn-derived carbon in heavy fraction

The proportion of carbon derived from corn (fC4) inputs was estimated in the HF using a two-end member mixing model (Balesdent et al., 1987; Murage et al., 2007).

\[ \delta^{13}C_{HF} = f_{C4} \times \delta^{13}C_{corn} + (1 - f_{C4}) \times \delta^{13}C_{C3-HF} \]

Rearranging the equation gives:

\[ f_{C4} = \frac{\delta^{13}C_{HF} - \delta^{13}C_{C3-HF}}{\delta^{13}C_{corn} - \delta^{13}C_{C3-HF}} \]

where \( f_{C4} \) is the proportion of corn-derived carbon in the HF, \( \delta^{13}C_{HF} \) is the \( \delta^{13}C \) of the HF, \( \delta^{13}C_{corn} \) is the mean \( \delta^{13}C \) of corn residues (−12.2‰ for CT and NT soils), and \( \delta^{13}C_{C3-HF} \) is the average \( \delta^{13}C \) of the corresponding HF from uncultivated soil. The \( \delta^{13}C \) of the heavy fractions for a native forest soil located adjacent to the research site was obtained from a comparable study reported by Wanniarachchi et al. (1999). The \( \delta^{13}C \) values of the HF in the native soil were −26.5‰ for the 0–10 cm soil depth and −26.6‰ for the 10–20 and 20–30 cm soil depths.

2.7. Statistical analyses

The experiment was conducted as a split-split plot design, with tillage as the main plot, sampling location relative to the corn plant as the split-plot, and depth as the split-split plot. The statistical software used was SAS 9.1 (SAS Institute Inc., Cary, NC). Data were tested for normality using the Shapiro-Wilk test and then evaluated by analysis of variance (ANOVA) using proc GLM. Proc Mixed was used to obtain the least square means. Least significant differences (LSD) at P < 0.05 were used to determine significant differences between treatment means.
with values decreasing in depth from 18.5 g C kg⁻¹ soils, resulting in a C:N ratio of 9.5 to 12. Tan et al. (2007) also reported an average C:N ratio for the HF as 8.8, slightly lower.

22.0 g kg⁻¹ in many studies (Lal et al., 1999; West and Post, 2002; Murage et al., 2015). Increases in C concentrations in surface soils of NT have been reported.

Results and discussion

3.1. Total organic carbon and nitrogen concentrations in the SOC fractions

Total organic carbon (TOC) concentrations (whole soil) in the top 10 cm of the NT soils (24.1 g C kg⁻¹) were significantly higher than that of the CT soils (21.0 g C kg⁻¹) (Table 1). For the 10–30 cm depth, there were no significant differences in TOC for both tillage treatments. C:N ratios ranged from 12.7 for surface soils to 11.9 for the 20–30 cm depth. Increases in C concentrations in surface soils of NT have been reported in many studies (Lal et al., 1999; West and Post, 2002; Murage et al., 2017; Yang et al., 2008).

The HF carbon concentration in the 0–10 cm depth of the NT soils was significantly higher than that of the CT soils (Table 2). The HF carbon concentration for the 0–10 cm depth was 19.5 g kg⁻¹ and 22.0 g kg⁻¹ for CT and NT soils, respectively. There were no significant differences in HF carbon concentration for the lower depths between tillage treatments. However, both tillage treatments showed a decreasing trend in HF carbon concentration with depth (Table 2). Nitrogen concentrations in the HF ranged from 1 to 2 g kg⁻¹ in CT and NT soils, resulting in a C:N ratio of 9.5 to 12. Tan et al. (2007) also observed higher C concentrations in the 0–5 cm of NT soils versus CT soils, with values decreasing in depth from 18.5 g C kg⁻¹ to 10.0 g C kg⁻¹. They also reported an average C:N ratio for the HF as 8.8, slightly lower than the values found in this study. Pinheiro et al. (2015) reported HF carbon concentration of 12.3 g kg⁻¹ for soils under six years of NT system.

The carbon concentration of the NHF was not significantly different between tillage treatments for all sampling depths (Table 3). However, both tillage treatments showed a decreasing trend in NHF carbon concentration with depth. The N concentrations in the NHF were similar between tillage treatments, ranging from 0.5 to 1 g kg⁻¹ in the surface soils to 0.3 g kg⁻¹ in the sub-surface soils. The C:N ratio for the NHF ranged from 24 to 27 for the CT soils and 25 to 29 for the NT soils.

The carbon concentration of the HF in the NT soils was significantly higher for the 0–10 cm depth but significantly lower for the 10–20 and 20–30 cm depths when compared to the CT soils (Table 3). The HF carbon concentration for the CT soils was 5.3, 5.7, and 4.1 g kg⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. The NT soils also showed a decreasing trend in HF carbon concentration with values of 7.1, 4.9, and 3.1 g kg⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. HFN concentrations in the NT soils (for all depths) were significantly higher than that of the CT soils. The C:N ratio of the HF was 4.8 to 5.7 for the CT soils and 3.6 to 4.4 for the NT soils. The low C:N ratios in the HF may be due to contributions from soil microbial constituents (proteins, nucleic acids, polysaccharides) (Schnitzer and Preston, 1983; Silveira et al., 2008; Lehmann and Kleber, 2015).

3.2. Contributions of SOC fractions to total SOC stock

Soil bulk density was not significantly different between tillage treatments (1.33, 1.40, and 1.51 Mg m⁻³) for the 0–10, 10–20, and 20–30 cm depths, respectively. Total organic C and N stocks of the whole soil in the 0–30 cm depth in CT and NT soils were not significantly different after six years (Table 4). However, the 0–10 cm depth of the NT soils had about 10% more SOC than the CT soils (2.96 Mg ha⁻¹). It is important to note that the sequestration potential of no-tillage disappear when considered over the 0–30 cm depth. Pinheiro et al. (2015) reported that after six years of no-tillage, soil C stock in the 0–10 cm depth was higher in NT than in CT systems by 5.3 Mg ha⁻¹. Their values are higher, probably because of the input of crop-residues from short-term vegetable crops.

Carbon stock in the HF (HFC) in the 0–10 cm depth was significantly different in ANOVA.
Table 4
Organic C and N stocks in whole soil (SOC) and heavy fraction (HF) for conventional tillage and no-tillage treatments.

<table>
<thead>
<tr>
<th>Soil depth cm</th>
<th>Element</th>
<th>Conventional tillage SOC or N (Mg ha⁻¹)</th>
<th>No-tillage SOC or N (Mg ha⁻¹)</th>
<th>LSDa</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>C</td>
<td>28.38 (1.38)</td>
<td>31.34 (0.60)</td>
<td>3.01</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>2.37 (0.11)</td>
<td>2.47 (0.05)</td>
<td>0.24</td>
<td>0.999</td>
</tr>
<tr>
<td>0–20</td>
<td>C</td>
<td>56.26 (2.52)</td>
<td>57.36 (0.68)</td>
<td>5.21</td>
<td>0.673</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>4.51 (0.20)</td>
<td>4.56 (0.05)</td>
<td>0.41</td>
<td>0.818</td>
</tr>
<tr>
<td>0–30</td>
<td>C</td>
<td>75.44 (2.97)</td>
<td>75.26 (0.79)</td>
<td>0.15</td>
<td>0.954</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>6.13 (0.23)</td>
<td>6.06 (0.07)</td>
<td>0.48</td>
<td>0.770</td>
</tr>
</tbody>
</table>

Higher in the NT soils, but there were no significant differences in HFC between tillage treatments in the lower depths (Table 4). HFC in the 0–10 cm depth was 25.5 Mg ha⁻¹ and 28.9 Mg ha⁻¹, for the CT and NT soils, respectively. These results indicate that the HF pool is a larger reservoir of C in the 0–10 cm depth of NT soils. The HF pool accounted for 90–93% of the total SOC for both tillage systems at all three soil depths (Table 4). The HFN stock was significantly higher in the NT soils compared to the CT soils for all sampling depths. Both tillage treatments showed a decreasing trend in HFC and N with depth. For the CT soils, HFC was 7.0, 7.8, and 6.1 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. NHF stock was significantly higher in the NT soils compared to the CT soils. Both CT and NT soils showed a decreasing trend in TOC with depth (Table 5).

3.3. δ13C of whole soil and SOC fractions

The δ13C of the total organic C (TOC–δ13C) in the 0–10 cm depth of NT soils was significantly higher (13C-enriched) than that of the CT soils (Table 6). There were no significant differences in TOC–δ13C for the lower soil depths between tillage treatments. Both CT and NT soils showed a decreasing trend in TOC–δ13C with depth (Table 6).

Table 5
Organic C and N stocks for non-hydrolysable (NH) and hydrolysable (HY) fractions in conventional tillage and no-tillage soils.

<table>
<thead>
<tr>
<th>Soil depth cm</th>
<th>Element</th>
<th>Conventional tillage NH or N (Mg ha⁻¹)</th>
<th>No-tillage NH or N (Mg ha⁻¹)</th>
<th>LSDa</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>C</td>
<td>18.55 (0.81)</td>
<td>19.60 (0.28)</td>
<td>1.71</td>
<td>0.221</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.68 (0.03)</td>
<td>0.69 (0.01)</td>
<td>0.06</td>
<td>0.827</td>
</tr>
<tr>
<td>10–20</td>
<td>C</td>
<td>17.67 (0.18)</td>
<td>17.36 (0.21)</td>
<td>1.49</td>
<td>0.679</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.70 (0.03)</td>
<td>0.63 (0.01)</td>
<td>0.06</td>
<td>0.015</td>
</tr>
<tr>
<td>20–30</td>
<td>C</td>
<td>11.62 (0.66)</td>
<td>12.21 (0.25)</td>
<td>1.40</td>
<td>0.398</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>0.48 (0.03)</td>
<td>0.49 (0.01)</td>
<td>0.06</td>
<td>0.651</td>
</tr>
<tr>
<td>0–10</td>
<td>C</td>
<td>47.83 (0.52)</td>
<td>49.17 (0.52)</td>
<td>3.34</td>
<td>0.426</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>1.85 (0.06)</td>
<td>1.80 (0.02)</td>
<td>0.13</td>
<td>0.402</td>
</tr>
</tbody>
</table>

Higher in the NT soils, but there were no significant differences in HFC between tillage treatments in the lower depths (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils (Table 5). However, for the 10–20 and 20–30 cm depths, HYF was significantly higher in the CT soils. The HYFC accounted for 25–30% of SOC for both tillage treatments (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils. Both tillage treatments showed a decreasing trend in HYFC and N with depth. For the CT soils, HYFC was 7.0, 7.8, and 6.1 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. For NT soils, HYF was 9.3, 6.9, and 4.6 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. A comparison of the total 0–30 cm depth showed no significant differences in HYFC but significantly higher hydrolysable N in the NT soils compared to the CT soils. A possible reason for the latter is that no-tillage also promotes a preservation of nitrogen-containing substances in the HF that are more resistant to microbial degradation and have a longer turnover time (Stevenson, 1994).

Table 6
δ13C values of total organic carbon (TOC), heavy (HF), non-hydrolysable (NHF) and hydrolysable (HYF) fractions in conventional tillage and no-tillage soils.

<table>
<thead>
<tr>
<th>Soil depth cm</th>
<th>Conventional tillage TOC-δ13C (‰)</th>
<th>No-tillage TOC-δ13C (‰)</th>
<th>LSDa</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>–23.41 (0.11)</td>
<td>–20.41 (0.07)</td>
<td>0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>–23.50 (0.09)</td>
<td>–23.64 (0.05)</td>
<td>0.21</td>
<td>0.219</td>
</tr>
<tr>
<td>20–30</td>
<td>–25.41 (0.14)</td>
<td>–25.18 (0.07)</td>
<td>0.32</td>
<td>0.153</td>
</tr>
</tbody>
</table>

Higher in the NT soils, but there were no significant differences in HFC between tillage treatments in the lower depths (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils (Table 5). However, for the 10–20 and 20–30 cm depths, HYF was significantly higher in the CT soils. The HYFC accounted for 25–30% of SOC for both tillage treatments (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils. Both tillage treatments showed a decreasing trend in HYFC and N with depth. For the CT soils, HYFC was 7.0, 7.8, and 6.1 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. For NT soils, HYF was 9.3, 6.9, and 4.6 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. A comparison of the total 0–30 cm depth showed no significant differences in HYFC but significantly higher hydrolysable N in the NT soils compared to the CT soils. A possible reason for the latter is that no-tillage also promotes a preservation of nitrogen-containing substances in the HF that are more resistant to microbial degradation and have a longer turnover time (Stevenson, 1994).

3.3. δ13C of whole soil and SOC fractions

The δ13C of the total organic C (TOC–δ13C) in the 0–10 cm depth of NT soils was significantly higher (13C-enriched) than that of the CT soils (Table 6). There were no significant differences in TOC–δ13C for the lower soil depths between tillage treatments. Both CT and NT soils showed a decreasing trend in TOC–δ13C with depth (Table 6).

δ13C values of total organic carbon (TOC), heavy (HF), non-hydrolysable (NHF) and hydrolysable (HYF) fractions in conventional tillage and no-tillage soils.

<table>
<thead>
<tr>
<th>Soil depth cm</th>
<th>Conventional tillage TOC-δ13C (‰)</th>
<th>No-tillage TOC-δ13C (‰)</th>
<th>LSDa</th>
<th>P valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>–23.50 (0.13)</td>
<td>–21.61 (0.08)</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>–23.55 (0.10)</td>
<td>–23.69 (0.05)</td>
<td>0.22</td>
<td>0.227</td>
</tr>
<tr>
<td>20–30</td>
<td>–25.54 (0.13)</td>
<td>–25.30 (0.06)</td>
<td>0.29</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Higher in the NT soils, but there were no significant differences in HFC between tillage treatments in the lower depths (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils (Table 5). Hydrolysable N for all depths sampled in the NT soils was significantly higher than that of the CT soils. Both tillage treatments showed a decreasing trend in HYFC and N with depth. For the CT soils, HYFC was 7.0, 7.8, and 6.1 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. For NT soils, HYF was 9.3, 6.9, and 4.6 Mg ha⁻¹ for the 0–10, 10–20, and 20–30 cm depths, respectively. A comparison of the total 0–30 cm depth showed no significant differences in HYFC but significantly higher hydrolysable N in the NT soils compared to the CT soils. A possible reason for the latter is that no-tillage also promotes a preservation of nitrogen-containing substances in the HF that are more resistant to microbial degradation and have a longer turnover time (Stevenson, 1994).
The $\delta^{13}C$ of the HF carbon (HF–$\delta^{13}C$) in the 0–10 cm depth of NT soils was also significantly higher than that of the CT soils (Table 6). This enrichment implies that there was a significant increase in storage of C$_4$-derived carbon (from corn residues) in the NT system. However, the enrichment is only apparent on the 0–10 cm soil depth and there are no significant differences over the 0–30 cm depth. There were no significant differences in HF–$\delta^{13}C$ for the 10–20 and 20–30 cm depths between tillage treatments. The HF–$\delta^{13}C$ for the CT soils was $-23.5\%$, $-23.6\%$, and $-25.5\%$ for the 0–10, 10–20, and 20–30 cm depths, respectively. The HF–$\delta^{13}C$ for NT soils was $-21.6\%$, $-23.7\%$, and $-25.3\%$ for the 0–10, 10–20, and 20–30 cm depths, respectively. The $\delta^{13}C$ values of the HF in the native hardwood forest soil were $-26.5\%$ for the 0–10 cm soil depth.

The $\delta^{13}C$ of the NHF carbon (NHF–$\delta^{13}C$) was not significantly different between tillage treatments for all soil depths (Table 6). The NHF–$\delta^{13}C$ for the CT soils was $-26.0\%$, $-26.1\%$, and $-26.3\%$ for the 0–10, 10–20, and 20–30 cm depths, respectively. The NHF–$\delta^{13}C$ for NT soils was $-25.9\%$ for the 0–10 and 10–20 cm depths and $-26.1\%$ for 20–30 cm depth. The NHF was less enriched in $^{13}C$ than the other SOC fractions and was nearest to the $\delta^{13}C$ of the native hardwood forest soil ($-26.5\%)$. Therefore, after six years of N, there was no significant input of C$_4$-derived C into the most recalcitrant SOC fraction. This indicates that most of the organic matter in this fraction originated from C$_3$ plants and there was no significant input of C$_4$-derived C. A possible reason is that even if corn has been grown on the site since 1969, the previous agricultural crops and the native vegetation of the land were C$_3$ plants. However, the slightly more enriched values in NHF–$\delta^{13}C$ for NT soils suggest that C$_4$-derived C is entering this pool but the length of time for any significant change to be observed is uncertain. There is also the likelihood that some of the corn residue materials (e.g. waxes) are not hydrolysable. The slightly lower NHF–$\delta^{13}C$ with depth for both tillage treatments can be related to the recalcitrance of SOM fractions with depth as reported in previous studies (Kögel-Knabner et al., 2005; Lorenz and Lal, 2005).

The $\delta^{13}C$ of the HYF carbon (HYF–$\delta^{13}C$) was significantly higher for the NT treatment in the 0–10 and 20–30 cm soil depths (Table 6). There were no significant differences in HYF–$\delta^{13}C$ in the 10–20 cm depth between tillage treatments. HYF–$\delta^{13}C$ shows greater enrichment in the 0–10 cm depth of the NT soils, since C$_4$-derived C would be more abundant on the surface. The HYF consists of the newly formed decomposition products of the crop residues (Schnitzer and Preston, 1983; Rovira and Vallejo, 2002). Paul et al. (2006) found that non-hydrolysable C also contains recent plant-derived lignin that is not soluble in acid. During microbial decomposition, “new” humus would likely be found on the surface of aggregates, rather than protected inside microaggregates or form organomineral complexes (Schnitzer, 2000) and possibly be resistant to acid hydrolysis.

4. Conclusions

This study showed that after six years of no-tillage (NT) on previously conventionally tilled (CT) soils there is evidence of soil organic carbon (SOC) changes occurring in the heavy fraction (HF), hydrolysable (HYF) and non-hydrolysable (NHF) fractions. Total organic carbon (TOC) concentrations of the whole soil, HF and HYF were significantly higher in the 0–10 cm depth of NT soils. After six years, TOC stocks for soils in both tillage systems were not significantly different at any of the soil depths, but the NT soils had $\sim 3$ Mg C ha$^{-1}$ more than CT soils. NT also significantly increased HF and HYF carbon stocks but had no effect on the NHF carbon stocks. The HF, NHF and HYF, accounted for 90–93%, 62–65%, and 25–30% of the total SOC, respectively.

Using $\delta^{13}C$ measurements allowed for discrimination between NT and CT systems. The $\delta^{13}C$ signature of the TOC, HF, and HYF suggest that there is significant $^{13}C$ enrichment at the 0–10 cm depth of NT soils. There was also a higher proportion of C$_4$-derived C in the HF at the 0–10 cm depth of NT soils. Differences in the $\delta^{13}C$ signature of the whole soil and SOC fractions show a preservation of newly-derived C in the HF and HYF of NT soils compared to the CT soils. NT effects are more apparent in the 0–10 cm depth with HF, HYF and NHF being similar at lower depths. In conclusion, our results suggest that the adoption of NT systems on arable soils increases the quantity of HF and HYF in the 0–10 cm depth only in the short-term but does not contribute to carbon sequestration when compared over the 0–30 cm depth.

Acknowledgements

The authors would like to thank Mr. Myles Stocki who conducted

Table 7
Propportion ($f_{C_4-HF}$) and quantity of C$_4$-derived carbon (C) in the heavy fraction (HF) of conventional tillage and no-tillage soils.

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>Fraction</th>
<th>Conventional tillage ($f_{C4-HF}$ (%))</th>
<th>No-tillage</th>
<th>LSD$^b$</th>
<th>P value$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10</td>
<td>HF</td>
<td>20.9 (0.9)</td>
<td>34.1 (0.5)</td>
<td>2.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>10–20</td>
<td>HF</td>
<td>21.1 (0.7)</td>
<td>20.2 (0.4)</td>
<td>1.5</td>
<td>0.209</td>
</tr>
<tr>
<td>20–30</td>
<td>HF</td>
<td>7.4 (0.9)</td>
<td>9.0 (0.6)</td>
<td>2.0</td>
<td>0.105</td>
</tr>
</tbody>
</table>

$^a$ Least significant difference calculated between two means at $P = 0.05$.

$^b$ Probability level for the F value for tillage effect in ANOVA.

$^c$ Numbers in parentheses are the standard errors of the means ($n = 32$).
the isotopic analyses. The funding for this research was provided by the Natural Sciences and Engineering Research Council (NSERC) Strategic and Discovery grants program.

References


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