Soil organic carbon pools across paired no-till and plowed Alfisols of central Ohio

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Abstract

No-till (NT) farming can restore the soil organic carbon (SOC) pool of agricultural soils, but the SOC pool size and retention rate can vary with soil type and duration of NT. Therefore, the objectives of the present study were to determine the effects of NT and soil drainage characteristics on SOC accumulation across a series of NT fields on Alfisols in Ohio, USA. Sites under NT for 9 (NT9), 13 (NT13), 36 (NT36), 48 (NT48) and 49 (NT49) years were selected for the study. Soil was somewhat poorly-drained at the NT48 site but moderately-well drained at the other locations. The NT48 and NT49 on-station sites were under continuous corn (Zea mays) while the other sites were farmers' fields in a corn-soybean (Glycine max) rotation. At each location, the SOC pool (0-30 cm) in the NT field was compared to that of an adjacent plow-till (PT) and woodlot (WL). At the NT36, NT48 and NT49 sites, the retention rate of cornderived C was determined using stable C isotope (¹³C) techniques. In the 0-10 cm soil layer, SOC concentration was significantly higher under NT than PT, but a tillage effect was rarely detected below that depth. Across sites, the SOC pool in that layer averaged 36.4, 20, and 40.8 Mg C ha⁻¹ at the NT, PT and WL sites, respectively. For the 0-30 cm layer, the SOC pool in NT (83.4 Mg C ha⁻¹) was still 57 % higher compared to PT. However, there was no consistent trend in the SOC pool with NT duration probably due to the legacy of past management practices and SOC content differences that may have existed among the study sites prior to their conversion to NT. The retention rate of corn-derived C was 524, 263 and 203 kg C ha⁻¹ yr⁻¹ at the NT36, NT48 and NT49 sites. In contrast, the retention rate of corn-C under PT averaged 25 and 153 kg C ha⁻¹ yr⁻¹ at the NT49 (moderately-well drained) and NT48

(somewhat poorly drained) sites, respectively. The conversion from PT to NT resulted in greater retention of corn-derived C. Thus, adoption of NT would be beneficial to SOC sequestration in agricultural soils of the region.

Keywords: Tillage, conventional tillage, carbon sequestration, alfisols, stable C isotope

Introduction

No-till (NT) farming eliminates pre-plant tillage operations with the current year's crop planted directly into residues left on the surface from the previous crop (Triplett and Dick, 2008). No-till has been promoted as a soil management practice that improves long-term agricultural productivity and profitability through reduced input costs, improved water use efficiency, and increased soil organic matter (SOM). Since annual plowing leads to soil organic carbon (SOC) oxidation, elimination of plowing with NT adoption of NT management practice can lead to progressive restoration of SOC in agricultural soils (West & Post, 2002; Jacinthe *et al.*, 2009; Olson, 2013). However, the extent of C sequestration by NT has recently been challenged as possibly being overly optimistic (Powlson *et al.*, 2015). Other factors that contribute to SOC accrual in NT systems include better water retention, moderation of soil temperature by crop residues left on the land surface, protection against soil erosion, improved soil structure, physical protection of organic C within soil aggregates, and evolution of fungal-dominated microbial communities (Frey *et al.*, 1999).

While changes in land management often elicit a rapid response in biochemical properties linked to C cycling (Frey *et al.*, 1999; Jacinthe & Lal, 2005), their impacts on the SOC pool generally exhibit a significant lag phase; therefore, both short-term and long-term studies are often required to properly document temporal trends in SOC pool. Reported estimates of C sequestration rates in agricultural soils vary greatly, ranging from 0 - 1000 kg C ha⁻¹ yr⁻¹ (West & Post, 2002; Armstrong *et al.*, 2003).

Rates of SOC sequestration depend on several factors including climate, soil mineralogy, profile characteristics, and land management practices (West & Post, 2002). Tan & Lal (2005) concluded the conversion of Ohio's Alfisols from conservation tillage

to NT could result in an average sequestration of 620 kg C ha⁻¹ yr⁻¹. However, similar to numerous other regional assessments, Tan and Lal (2005) did not specify the time over which this estimated C benefit was observed.

A steady increase in the SOC pool, with the maximum rate of SOC accrual observed during the first decade (an annual gain of 570 kg C ha⁻¹), is reported with NT duration reaching a new C equilibrium within the second to third decade (Follett, 2001; West & Post, 2002; Alvaro-Fuentes et al., 2014). Similarly in reclaimed southern Indiana farmlands, sequestration rates averaged 800 kg C ha⁻¹ yr⁻¹ during the first decade of restoration but declined to 250 kg C ha⁻¹ yr⁻¹ during the second decade (Jacinthe & Lal, 2009).

The temporal variation in SOC sequestration rate should be considered when making future projections of C pool capacity of agricultural soils (Follett, 2001). This aspect has not been systematically accounted for in previous studies investigating the C sequestration benefits of NT adoption. As illustrated by Alvaro-Fuentes et al. (2014), the data needed to address this information gap should be derived from repeated measurements of SOC pool at specific experimental sites using the same sampling protocol. Since such data are not widely available, a chronosequence of short-term and long-term NT sites can be used to quantitatively estimate the temporal variation in C sequestration rates.

In light of these considerations, a study was conducted to assess trends in the SOC pool for central Ohio (USA) Alfisols managed under NT for 49 years. These unique study sites provide an opportunity to trace the conversion of C from crop residues into a stable soil C pool, and thus determine the effect of management practices and soil drainage characteristics. Therefore, the objectives of the present study were to: (1) assess the effects of tillage on SOC pool in cultivated Alfisols of central Ohio, (2) monitor the effect of soil drainage characteristic on SOC pool, and (3) estimate the SOC sequestration benefits of NT through paired comparison with PT (plow-till) fields using stable C isotope (¹³C) techniques. It was hypothesized that the SOC pool will be greater under NT than in adjacent PT fields, and that the differential rate of SOC sequestration will decrease with NT duration.

Material and methods

Description of the study sites

The study sites included three farmer-managed NT fields (9-36 years) under a corn-soybean (*Gycine max*, L.) rotation and two longterm experimental plot sites under continuous corn (48 and 49 years) in Ohio, U.S.A (Table 1 and Fig. 1). At the time of soil sampling in 2010, the selected sites were under NT for 9 (Bucyrus, NT9), 13 (Mount Gilead, NT13), 36 (Centerburg, NT36), 48 (South Charleston, NT48), and 49 years (Wooster, NT49). The study sites at South Charleston and Wooster represent some of the world's longest continuous NT experiments. The NT48 site is somewhat poorly drained and has higher clay content than the NT49 site which is well drained (Table 1). Each NT site was paired to a nearby PT field under the same cropping sequence, and was compared to an adjacent secondary growth forest (woodlot). The forest sites were included to measure SOC pool potential of undisturbed soils, and represent steady state endpoints under the climatic conditions of the region. Additional information regarding the location and general soil characteristics at the study sites can be found in Jacinthe *et al.*, (2014).

At the NT sites, there was no soil disturbance except for the narrow slots for seed and fertilizer injection. The PT treatment consisted of fall moldboard plowing (20-25 cm) followed by surface disking (7.5-10 cm) in the spring. Occasionally, an additional

finishing tillage operation was applied. These experimental sites received N fertilizer at an annual rate of 134-180 kg N ha⁻¹ either as urea or urea-ammonium nitrate. During the corn year, farmer-managed fields received 160-190 kg N ha⁻¹ (starter and side-dress) primarily as anhydrous NH₃; no N fertilizer was applied to soybean. Soils at the study sites are Alfisols developed from glacial till and were classified as Canfield (*Aquic Fragiudalfs*), Cardington (*Aquic Hapludalfs*), Centerburg (*Aquic Hapludalfs*) and Wooster (*Oxyaquic Fragiudalfs*) soil series in the upslope position, and as Bennington (*Aeric Epiaqualfs*), Crosby (*Aeric Ochraqualf*) and Condit (*Typic Epiaqualfs*) in down slope landscape positions. While four of the locations (Bucyrus, Mount Gilead, Centerburg, and Wooster) were established on moderately well-drained soils, the South Charleston site was established on a somewhat poorly-drained Crosby silt loam soil (Table 1). Climate was temperate with mean annual temperature of 9.9 °C and precipitation of 1023 mm.

Soil sampling and analysis

This study was not a replicated field plot experiment and therefore, because of the lack of true replication, on-farm sites with similar slope, elevation and soil type were selected to serve as pseudo-replications. Four sampling areas (approximately 0.3 ha) were identified in each farmer's field to capture spatial variability. Within each sampling area , soil samples were randomly collected at 0-5, 5-10, 10-20 and 20-30 cm depths from three sampling points (within a 40-50 m radius) using hand augers after removing plant residue from surface. For each sampling area, a composite sample (~ 500 g) was prepared for each depth by pooling and mixing the soil material retrieved from the three sampling points. At the Wooster and South Charleston locations, soil samples were collected at 0-5, 5-10, 10-20 and 20-30 cm depths from four plots for each tillage treatment. Within each sampling area, intact soil cores were also

extracted for the determination of soil bulk density (ρ_b) (Grossman & Reinsch, 2002). Composite soil and core samples were placed in a labeled and sealed plastic bags, transported to the laboratory and stored at 4°C pending further processing and analysis.

A portion of each composite sample was air-dried and passed through a 2-mm mesh screen, then a fraction of each sample (<2 mm) was crushed to pass through a 250 μ m mesh sieve. This finely ground soil was used for the determination of total C (TC) and soil inorganic C (SIC) concentration. Soil pH was measured in 1:2 soil to water suspensions using an Orion Star pH-meter (Thermo Fisher, Beverly, MA), and electrical conductivity (EC) was measured in 1:5 soil to water extracts (Rhoades, 1996). Total C and N (TN) concentrations were determined by the dry combustion (960 °C) using a Vario TOC analyzer (Elementar Inc., Hanau, Germany). The SIC concentrations were determined by decomposing carbonates in a sealed serum bottle (0.2 g of soil, 0.5 mL of 2 M HCl) and measuring evolved CO₂ by gas chromatography (Jacinthe *et al.*, 2009). Most samples contained trace amounts of SIC (mean: 0.14 g kg⁻¹), the highest was detected in those from the Mount Gilead site (mean: 1.4 ± 0.2 g kg⁻¹; Table 1). The SOC concentration was calculated as the difference between total C and SIC. Concentrations of SOC and TN (g kg⁻¹) were multiplied by bulk density (ρ_b) value (Mg m⁻³) and soil depth (m) to convert concentration to mass per area (Mg ha⁻¹) for comparing SOC and TN pools from different tillage treatments and sites. The SOC pool (Mg ha⁻¹) in each layer was computed using Eq. 1:

SOC or
$$N_{pool} = SOC \text{ or } N/10^3 \rho_b d \times 10^4 (m^2 h a^{-1})$$
 (1)

where SOC is soil organic C and N is total N concentration (g kg⁻¹), ρ_b is bulk density (Mg m⁻³), and *d* is soil layer thickness (m). The SOC and N pools for whole soil profiles were estimated on an equal mass basis referenced to 0-30 cm depth (Ellert *et al.*, 2002). On this basis, total SOC and N pools were estimated up to 30 cm depth for all treatments.

Soil samples from the Centerburg, Wooster, and South Charleston sites were analyzed for ¹³C/¹²C using isotope ratio mass spectrometry (IRMS) at the Stable Isotope Facility of the University of California, Davis (Model 2020 Europa mass spectrometer, PDZ Europa, Crewe, UK). For each soil layer, the SOC_c pool (SOC pool attributable to corn vegetation) was determined using the isotope mixing equation (Bernoux *et al.*, 1998):

$$SOC_{c} = SOC_{stock} \frac{\delta_{t} - \delta_{r}}{\delta_{c} - \delta_{r}}$$
 (2)

where δ_t is ¹³C enrichment (‰) at a given soil depth, δ_r is ¹³C enrichment (‰) of the reference woodlot (WL) soil at that depth, and δ_c is mean ¹³C enrichment of corn residue. Corn residues were collected at the time of harvest. This material was air-dried at room temperature, mixed, finely crushed, sieved (150 µm) and analyzed for ¹³C concentration. Average composition for corn residue was: C = 45.58±0.11 %; N = 0.54 ± 0.04 %; and δ^{13} C = -12.43 ± 0.01‰. Corn carbon sequestration rate was calculated from the corn-derived C (C4-C) pool divided by duration of tillage. The C4-C was estimated assuming that corn was grown continuously at South Charleston (48 years) and Wooster (49 years), and 18 years (out of a total 36 years because of the corn-soybean crop rotation) at the

Centerburg site. The C sequestration benefit from NT was calculated by subtracting the corn C sequestration rate under PT from that under NT.

Statistical analysis

Analysis of variance (ANOVA) was conducted separately for each soil depth with tillage management (NT vs. PT) as the treatment factor. Analysis was conducted using the general linear model (GLM) procedure of SAS (SAS, 2007). Statistical significance was determined at $p \le 0.05$, unless otherwise stated. Tillage treatment means and interactions were separated by the least-significant-difference (LSD) multiple comparison.

Results

Soil organic C and N concentrations

Overall, SOC concentration was consistently higher under NT compared to PT at most depths (Fig. 2). In the 0-5 cm layer, SOC concentration was significantly (P<0.05) higher under NT than under PT. Across all study sites, mean SOC concentration in the 0-5 cm layer was 27.6 g kg⁻¹ under NT and 12.9 g kg⁻¹ under PT (Fig. 2). A significant (P<0.05) effect of NT on SOC concentration was also observed in the 5-10 cm layer at the NT13, NT36, and NT48 sites. With the exception of the NT36 site (Centerburg), NT management did not significantly affect SOC concentration in the 10-20 or 20-30 cm soil layer. Relative to PT, increases in SOC

concentration with NT adoption were 113, 63, 53, and 41% in the 0-5, 5-10, 10-20, and 20-30 cm layers, respectively. At the sites under NT for 48-49 years, the increase in SOC concentration was more pronounced at the somewhat poorly drained site (NT48) than at the moderately well drained site (NT49). In the 0-5 cm soil layer, SOC concentration was 235 and 90 % higher under NT compared to PT at the somewhat poorly drained and moderately-well drained sites, respectively. In the 5-10 cm soil layer, an increase in SOC concentration with NT adoption (115% relative to PT) was observed at the somewhat poorly drained but not at the moderately-well drained site.

Across study sites, total N concentrations were higher (24%) under NT (mean of all sites: 2.2 g N kg⁻¹) than under PT (mean of all sites: 1.8 g N kg⁻¹) (Fig. 2). In general, SOC and N concentrations tended to be higher in the top soil layer than deeper in the soil profile under NT. In contrast, concentrations of SOC and total N were more uniformly distributed at sites under PT.

Soil organic C and total N pools

Across sites, the SOC pool for 0-10 cm depth ranged from 31 to 39 Mg ha⁻¹ for NT, 13 to 25 Mg ha⁻¹ for PT, and 19 to 64 Mg ha⁻¹ for WL. Similarly, the SOC pool for 0-30 cm depth ranged from 59 to 113 Mg ha⁻¹ for NT, 36 to 70 Mg ha⁻¹ for PT, and 70 to 214 Mg ha⁻¹ for WL. At the surface layer of 0-10 cm depth, average SOC pools across all NT sites (36 Mg C ha⁻¹) was 82% higher compared to PT (20 Mg C ha⁻¹) (P < 0.05) (Table 2). Increase in SOC pools at NT9, NT13, NT36, NT48, and NT49 sites over PT were 56, 111, 65, 177, and 48% for 0-10 cm depth, 58, 17, 83, 127, and 38% for 0-20 cm depth, and 53, 40, 102, 106, and 3% for 0-30 cm depth, respectively.

Soil organic C pools at the South Charleston (NT48) and Wooster (NT49) sites were compared to assess the effect of soil drainage. Relative to PT, the SOC gain in 0-10, 0-20, and 0-30 cm depths of NT were 23, 33, and 38% at somewhat poorly drained soils (NT48) compared to only 10, 15, and 2% for moderately-well drained soils (NT49) (Table 2). The average SOC pool for 0-10 cm depth across all PT sites was 49% and NT sites 89% of the pool in WL. Similarly, the average SOC pool for 0-30 cm depth across all PT sites was 41% and NT sites 65% of the pool in WL. Thus, the PT fields may have lost nearly 50 to 60% of their SOC pool due to annual plowing, while a net gain in SOC was observed at the NT sites. Tillage did not affect total N pool. Cumulative N pool in the 0-10 cm depth ranged 2.65-3.55 Mg N ha⁻¹ under NT, 2.36-3.4 Mg N ha⁻¹ under PT, and 2.05-5.65 Mg N ha⁻¹ under woodlot (Table 2).

Carbon sequestration

The stable isotope technique was used to refine estimates of C sequestration with NT adoption. The δ^{13} C of SOM in NT ranged from - 14.9% to -23.6%, in PT the values ranged from -16.7% to -25.8%, and in woodlots from -27% to -23.2% (Table 3). The δ^{13} C signature decreased with increase in soil depth. The δ^{13} C signature was lower in well-drained NT soils (NT49) compared to poorly drained soils (NT48). The δ^{13} C signature of woodlots were much lower than the NT. Reflecting the isotopic influence of corn biomass, the δ^{13} C of SOM was always higher in the agricultural fields (range: -25.8 to -14.9 %) relative to reference woodlots.

Tillage significantly affected corn-derived C (C4-C) (Table 4). The SOC pool associated with C4-C under NT was 1.7 times greater in NT48 site and 8.1 times greater in NT49 site than PT. Whereas SOC pool associated with C3-C was 2.2 times greater in NT48 and 0.9 times of NT49 than PT. Corn-derived C pools were highest in the top soil layers (0-10 cm) of NT fields. Conversely, C

pools attributable to C₃-vegetation (old C) tended to increase with soil depth. The amount of C₄-C at the whole 0-30 cm soil layer at the NT36, NT49 and NT48 sites was 9.4, 9.7 and 12.6 Mg C ha⁻¹, respectively (Table 4). Between 27-69% of these amounts was located in the top 10 cm soil layer. Corresponding rates of corn C sequestration were 524, 203, and 263 kg C ha⁻¹ yr⁻¹ at the NT36, NT49 and NT48 sites, respectively.

Discussion

Soil organic C and total N pools

A primary objective of this study was to compare the SOC pools under different tillage practices, and determine whether NT adoption positively impacts SOC accumulation. Fields under NT for varying length of time were sampled, with the expectation that this chronosequence approach would show a gradual increase in SOC pool with NT duration. While the study results demonstrate the benefits of NT on SOC, no temporal trend in SOC pool with NT duration was detected.

Overall, our results have consistently shown higher SOC pool in NT compared to PT (Fig. 2). This finding supports earlier studies that long-term adoption of NT increases SOC content, enhances soil quality, and improves soil resilience (Lal *et al.*, 1998; Olson, 2013). In a global analysis of long-term agricultural management practices, West & Post (2002) reported that conversion of PT to NT can potentially increase the rate of SOC accumulation, thereby fostering the transfer of CO₂ from the atmosphere to soils.

As observed in previous studies (Puget & Lal, 2005; Álvaro-Fuentes *et al.*, 2014), the depth distribution of SOC and total N was more stratified in NT than PT, especially for NT13, NT48, and NT49 (Fig. 2). The stratification of chemical elements under NT has

been ascribed to the accumulation of crop residues on the land surface. The SOC accrual in NT soil has been attributed to the incorporation of dead roots and faunal transport of residues (Balesdent *et al.*, 2000). Furthermore, NT fields maintain significantly greater residue on the soil surface compared to chisel tillage and moldboard plowing (Olson, 2013). When NT is practiced continuously, crop residues accumulate on the soil surface and enhance aggregation and formation of macro-pores increasing SOC retention (Blanco-Canqui & Lal, 2004). The lack of stratification under PT is likely due to mixing of the different soil layers and incorporation of crop residues into the plow layer by tillage operations.

Assuming that the woodlots provide a reasonable measure of C pool capacity for soils in the region, the ratio of NT SOC pool to woodlot SOC pool may be an approximate indicator of soil C saturation. At sites under NT for 9 and 13 years, the SOC pool (0-20 cm) was an average equivalent to 55% of the woodlot, whereas the older NT sites (NT36 and NT49) had SOC pools nearly equal (97%) to that in the woodlot. The most notable exception to this trend was the NT48 site where the SOC pool was only 52% of the woodlot. The SOC pool in woodlots was quite variable, ranging between 55 and 122 Mg C ha⁻¹ in the 0-20 cm layer. It is important to note that, in this agriculture-intensive landscape, woodlots are often former farmlands (wet spot, flood prone areas) that had been abandoned over the years, and thus may have different development histories. The woodlot at Mount Gilead was near an agricultural drainage ditch and may have experienced periods of subsurface soil wetness. These site-specific conditions may have contributed to the large variation in SOC pool for the woodlots.

Since clay content is one of the determining factors of soil C pool capacity (Hassink, 1997), it is not surprising that higher SOC pool (0-10 cm) was measured in the WL at the fine-textured somewhat poorly drained site in South Charleston than at the other

WL(Table 1 and 2). Thus, it is reasonable to expect future increases in SOC pool at the South Charleston NT plots. While the forest stands at Bucyrus, Centerburg and South Charleston were visibly mature (>80 years), the Wooster woodlot was an aggrading stand without a developed litter layer (Mestelan, 2008). Also the presence of a fragipan (Mestelan, 2008) at the Wooster site may have restricted root penetration deeper into the soil profile and subsurface SOC accumulation in the woodlot. Reduction in root development and crop yield has been observed at several US Midwest sites where fragipans occur at intermediate to shallow depths (Graveel *et al.*, 2002). Such a subsurface feature could have restricted root penetration and negatively affected C deposition with depths at our Wooster site.

Sequestration rates of corn-derived carbon

This chronosequence study was designed with the implicit hypothesis that SOC sequestration would increase with NT duration. This hypothesis is supported by the results of Alvaro-Fuentes et al. (2014) from a plot study in the Mediterranean region, as well as those of Jacinthe and Lal (2009) from an investigation of reclaimed Indiana farmlands. However, the data presented in this study (Table 2) did not support this expectation. Regardless of soil depth, no consistent trend in SOC pool with NT duration was observed. This observation contrasts with our earlier finding of a linear increase in the methane oxidation capacity of soils with NT duration (Jacinthe *et al.*, 2014). Unlike controlled experimental plots, on-farm investigations can present unique interpretation challenges due to the difficulty of ascertaining if the baseline SOC pool was similar between each pair of PT and NT fields. As suggested by the wide range of SOC pools in the PT fields (range: 36.1- 69.5 Mg C ha⁻¹; Table 2), it is likely that the study sites had vastly different SOC contents at the time of conversion to NT, although the differences between paired fields at a location is assumed to be much less than the differences between the location sites. Although study sites were established on similar soil types, prior land management legacy and farming practices may have led to different SOC accumulation trajectories following conversion to NT. Furthermore, the wide variation in SOC pool measured at the woodlots (Table 2) indicates that the C storage capacity of these Alfisols varies with location.

The rate of retention of corn-derived C (C₄ vegetation) was determined at three of the study sites using stable C isotope techniques. The rate of corn-C sequestration in NT was 524, 203, and 263 kg C ha⁻¹ yr⁻¹ at the NT36, NT48 and NT49 sites, respectively (Table 4). This study hypothesized that the rate of SOC sequestration would decrease with NT duration. Although the trend in corn-C retention rates between the NT36, NT48, and NT49 sites may be taken as evidence supporting this hypothesis, one needs to exercise caution given the limited number of study sites. For the NT36 site, for example, the land-use history prior to NT conversion in 1978 is not well known; therefore, it is possible that our estimated corn-derived C pool also includes the contribution of corn crops predating the conversion of the site to NT. As recognized in previous studies (Leifeld and Fuhrer, 2010; Jacinthe et al, 2011), the interpretation of uncontrolled on-farm investigations often presents unique challenges due in part to the difficulty to ascertain the similarity of baseline soil characteristics among study sites. These considerations aside, this study results provide several lines of evidence demonstrating the positive impact of NT adoption on C sequestration in cultivated Alfisols of the US Midwest. First, the SOC pool was consistently higher under NT than PT (Table 2), although the effect of tillage was generally significant in the surface soil layers (0-10 and 10-20 cm) probably due to difference in soil hydrology, land-use history, among other factors. Second, and perhaps a more powerful illustration of the C sequestration benefits of NT, the retention of corn-derived C was found to be significantly greater under NT than

PT, regardless of the soil depth considered (Table 4). Our results showed that, by switching from PT to NT, 1.6 times more cornderived C can be sequestered (For the 0-30 cm soil layer: 110 and 177 kg C ha⁻¹ y⁻¹, at the somewhat poorly drained and moderatelywell drained sites, respectively).

Corn, as a C₄ plant, produces residue with a distinctly heavy ¹³C isotopic signature (δ^{13} C: -19 to - 9 ‰) compared to C₃ (δ^{13} C: -35 to -20 ‰) (Farquhar *et al.*, 1989). Therefore, at sites where shifts in C₃ to C₄ vegetation occurred, the difference in ¹³C signature has been extensively used as an *in-situ* marker of old and recent SOC pools to assess C pool and turnover rate (Bernoux *et al.*, 1998; Puget *et al.*, 2005). At the three long-term sites, the SOC is a mixture of C from C₃ vegetation and C₄ (corn residue) deposited to the soil over the past 36-49 years.

In addition to the retention of corn-derived C, the SOC pool associated with C₃ vegetation was also greater under NT than PT, especially in the surface soil layers and at the somewhat poorly drained site (Table 4). These results suggest that NT practice may contribute to the protection of existing SOC pool from mineralization. This preservation likely involves physical protection of C within soil aggregates due to limited soil disturbance (Dungait *et al.*, 2012), and moderation of soil temperature by crop residues on the land surface. Since the effect of tillage on C3-derived C was most pronounced at the somewhat poorly drained site, this preservation mechanism is likely linked to soil texture and hydrology.

Summary and conclusions

In the upper 0-10 cm soil layer, the SOC pool was significantly (P<0.04) greater under NT than PT. Contrary to expectations, a temporal trend in the SOC pool with NT duration was not observed, probably due to differences in the SOC pool that may have existed among the study sites at the time of their conversion to NT. This factor must be taken into account when designing future studies. Nonetheless, through application of stable ¹³C isotope techniques, sequestration rates of corn-derived C ranging between 203 and 524 kg C ha⁻¹ yr⁻¹ were recorded at the older (36 to 49 years) NT sites in the chronosequence. In contrast, the average retention rate of corn-C was only 89 kg C ha⁻¹ yr⁻¹ under PT. These results demonstrate the significance of NT as a management option to restore depleted SOC pool in agricultural soils.

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		Mount Gilead		Bucyrus		Centerburg		South Charleston		Wooster						
Site ID		NT9		NT13		NT36		NT48		NT49						
Location	Latitude	40°33″08″N		40°48″22″N		40°18″16″N		39°49″31″N		40°48″33″N						
	Longitude	82	°49″54′	″W	82	°58″23′	Ψ	82°	41″44	″W	83	38″12	″W	81°	656″14	Ψ
	Elevation (m)		329			303			372			343			310	
Climate	$MAT^{*}(^{\circ}C)^{\dagger}$		10.4			10.3			10.5			10.8			10.3	
	MAP ^{**} (mm)		990			1024			1086			1045			988	
Initiation of no-till		2001		1997		1974		1962		1962						
No-till duration (yr)		9		13		36		48		49						
Crop rotation		Cor	Corn-soybean		Corn-soybean		Corn-soybean		Corn-Corn		Corn-Corn					
Soil type			Alfisol	S	Alfisols		Alfisols		Alfisols		Alfisols					
Soil drainage characteristics		MWD		MWD		MWD		SPD		WD						
Land use		NT	PT	WL	NT	РТ	WL	NT	PT	WL	NT	PT	WL	NT	PT	WL
Soil properties [‡]																
Clay content (g 100 g ⁻¹ soil) §		NA^\dagger	NA	NA	NA	NA	NA	22	29	22	23	30	20	22	20	18
Silt cont	tent (g 100 g ⁻¹ soil)	NA	NA	NA	NA	NA	NA	40	39	47	53	49	45	59	56	59
pH §		7.5	7.4	5.7	6.0	5.1	4.8	6.2	5.7	5.9	6.3	6.9	6.3	6.9	5.9	5.2
EC $(\mu S \text{ cm}^{-1})^{\dagger}$ §		59	63	54	NA	NA	NA	114	64	50	121	74	343	57	44	47

Table 1 Location and general description of the study sites in Ohio

[†]Abbreviations: well-drained (WD), moderately well-drained soil (MWD), somewhat poorly drained soil (SPD), mean annual temperature (MAT), mean annual precipitation (MAP), no-till (NT), plow-tillage (PT), woodlot (WL), electrical conductivity (EC), Not available (NA) [‡]Measured at 0-10 cm depth

		SOC pool (Mg C ha ⁻¹)								
Depth	Treatment	Mt. Gilead (NT9)	Bucyrus (NT13)	Centerburg (NT36)	South Charleston (NT48)	Wooster (NT49)				
0-10 cm	NT	39 a†	38 a	38 a	36 a	31 a				
	РТ	25 b	18 b	23 b	13 b	21 b				
	WL	19	48	40	63	34				
0-20 cm	NT	71 a	49 a	75 a	59 a	54 a				
	РТ	45 b	42 a	41 b	26 a	39 b				
	WL	122	92	77	108	55				
0-30 cm	NT	107 a	59 a	113a	74 a	64 a				
	РТ	70 a	42 a	56 b	36 a	62 a				
	WL	214	118	93	147	70				
			<u>A</u> 1	nalysis of variance P	> <u>F</u>					
0-10 cm		0.04	0.03	0.01	0.04	0.01				
0-20 cm	Tillage	0.04	0.07	0.02	0.10	< 0.01				
0-30 cm		0.12	0.08	0.03	0.12	0.60				
				TN pool (Mg ha ⁻¹))					
0-10 cm	NT	3.33 a	2.81 a	2.97 a	, 3.55 a	2.65 a				
• • • • • •	РТ	2.79 a	3.40 a	2.36 a	3.27 a	2.57 a				
	WL	2.05	2.62	2.69	5.65	3.04				
0-20 cm	NT	6.41 a	4.85 b	5.73 a	5.92 a	5.01 a				
	РТ	5.02 a	6.57 a	4.93 a	5.43 a	5.35 a				
	WL	6.46	6.35	5.94	9.69	5.43				
0-30 cm	NT	9.67 a	6.94 a	8.81 a	8.31 a	6.96 a				
	РТ	9.15 a	9.38 a	7.27 a	7.50 a	8.73 a				
	WL	10.73	9.59	8.60	12.85	7.64				
		Analysis of variance $P > F$								
0-10 cm		0.11	0.23	0.11	0.67	0.69				
0-20 cm	Tillage	0.34	0.04	0.20	0.53	0.55				
0-30 cm	~	0.37	0.06	0.16	0.49	0.26				

Table 2 Soil organic carbon (SOC) and total nitrogen (TN) pool in soil under no-till (NT), plow-tillage (PT), and woodlot (WL)

[†]For a given soil depth and location, means followed by different letters indicate significant difference between tillage

treatment at P < 0.05. Woodlot SOC and TN pools were not included in the analysis of variance and are reported for comparison.

		δ ¹³ C (‰)								
Depth	Treatmen t	Centerburg (NT36)	South Charleston (NT48)	Wooster (NT49)						
	NT	-20.7	-14.9a	-17.2a†						
0-5 cm	РТ	-	-16.8b	-24.8b						
	WL	-26.5	-26.3	-27.1						
	NT	-21.2	-17.8b	-19.3a						
5-10 cm	РТ	-	-16.7a	-24.8b						
	WL	-26.2	-25.3	-26.1						
	NT	-23.6	-20.1b	-21.8a						
10-20cm	РТ	-	-16.7a	-25.2b						
	WL	-26.0	-16.7a -24.8b -25.3 -26.1 -20.1b -21.8a -16.7a -25.2b -24.3 -25.4 -21.2b -22.1a	-25.4						
	NT	-23.3	-21.2b	-22.1a						
20-30cm	РТ	-	-17.6a	-25.8b						
	WL	-25.1	-23.2	-25.4						
		<u>Analysis of variance P > F</u>								
0-5cm		n/a	<0.01	< 0.01						
5-10cm	NT vg DT	n/a	0.04	< 0.01						
10-20cm	1 N I VS. F I	n/a	0.04	< 0.01						
20-30cm		n/a	0.02	0.02						

Table 3 Isotopic composition (¹³C) of organic carbon in soils under no-till (NT), plow tillage (PT), and forest landuse (WL) at the Centerburg, Wooster, and South Charleston sites

[†]For a given soil depth and location, means followed by different letters indicate significant difference between tillage treatments at P < 0.05. Woodlot data were not included in the analysis of variance and are reported for comparison.

		Location and soil organic carbon fraction (Mg C ha ⁻¹)										
Depth	Treatment	Centerbur	rg (NT36)	South Charle	eston (NT48)	Wooster (NT49)						
	-	C4-C	С3-С	C4-C	С3-С	C4-C	С3-С					
0-5cm	NT	3.01	17.3	6.06a	14.6a	4.21a	12.6a					
	PT	- †	-	1.74b	4.48b	0.67b	9.63a					
5-10cm	NT	2.22	15.1	3.09a	12.4a	2.47a	11.6a					
	РТ	-	-	1.67a	5.09b	0.37b	9.87a					
10-20cm	NT	2.31	34.9	2.61a	20.2a	2.20a	20.7a					
	PT	-	-	2.53a	10.4a	0.15b	18.7a					
20-30cm	NT	1.90	36.4	0.87a	14.6a	0.86a	11.7a					
	PT	-	-	1.42a	8.75a	0.02b	22.7a					
0-30cm	NT	9.44	104	12.6a	61.8a	9.74a	54.0a					
	PT	-	-	7.36a	28.8b	1.21b	60.9a					
		Corn carbon sequestration rate (kg C ha ⁻¹ yr ⁻¹)‡										
	NT	524	4.3	26	3a	203a						
	PT	-		15	3b	2:	25b					
		Carbon benefit of NT (kg C ha ⁻¹ yr ⁻¹)										
				1	177							

Table 4 Corn-derived carbon (C₄-C) and old carbon (C₃-C) under no-till (NT) and plow tillage (PT) at Centerburg, Wooster, and South Charleston

 \dagger : samples from PT fields were not analyzed for ¹³C.

For a given soil depth, means with different letters within a column, tillage treatment are significantly different at P < 0.05. ‡ Rates were computed by assuming 18 corn crops at Centerburg and 48 and 49 corn years at the other sites.



Figure 1 Locations of the study sites in Ohio, USA.



Figure 2 Total N and soil organic carbon (SOC) concentration in soils under plow tillage (PT) and no-till (NT). Crop fields at the study sites were under no-till for 9 (NT9), 13 (NT13), 36 (NT36), 48 (NT48) and 49 (NT49) years. Soil was somewhat poorly-drained at the S. Charleston sites, but moderately well-drained to well-drained at the other sites. For a given soil depth, data points followed by different letters are significantly different at P < 0.05.