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Assessment of soil organic carbon and total nitrogen under conservation management practices in the Central Claypan Region, Missouri, USA

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ABSTRACT

Conservation management practices including upland vegetative filter strips (VFS) and no-till cultivation have the potential to enhance soil carbon sequestration and other ecosystem services in agroecosystems. A modified two-factor analysis of variance (ANOVA) with subsamples was used to compare SOC and TN on a concentration, soil volume and soil mass basis in claypan soils planted to different conservation management practices and as a function of landscape position. The three conservation management practices (no-till cultivation, grass VFS and agroforestry VFS) and four landscape positions (summit, shoulder, backslope and footslope) investigated were compared 10 years after VFS establishment in a no-till system planted to maize (*Zea mays.* L.)–soybean (*Glycine max* (L.) Merr.) rotation. Two soil depth increments (0–5 cm and 5–13 cm) were modeled separately to test for treatment effects. In the surface layer, mean SOC concentration was significantly greater in the VFS soils compared to no-till. On a soil volume or mass basis, no significant differences in SOC stocks were found among treatment means. Concentration and mass based TN values were significantly greater in the grass VFS relative to no-till in the surface layer. A rapid slaking stability test, developed to separate particulate, adsorbed and occluded organic carbon (PAO-C) and nitrogen (PAO-N), showed that VFS soils had significantly greater mean PAO-C and PAO-N concentrations, soil volume and soil mass based stocks than no-till. In addition, comparison of SOC:TN and PAO-C:PAO-N ratios suggest reduced decomposition and mineralization of SOC in the PAO fraction. No significant treatment effects were detected in total or PAO soil fractions in the subsurface layer or among landscape position in either depth increment. Study results emphasize the need to compare soil carbon and nitrogen stocks on a soil volume and/or soil mass basis using bulk density measurements. Additionally, the rapid PAO separation technique was found to be a good indicator of early changes in SOC and TN in the systems studied. Overall, this research indicates that grass VFS may sequester TN more rapidly than agroforestry VFS and that a greater proportion of SOC and TN may be stabilized in VFS soils compared to no-till.

1. Introduction

Soil organic matter, typically measured as soil organic carbon (SOC), has many different critical biogeochemical functions in the global carbon cycle. For example, SOC is an important factor in soil fertility and agricultural productivity (Doran et al., 1998). Additionally, SOC affects the degradation, sorption and mobility of agricultural pollutants (Chu et al., 2010; Li et al., 2005) and heavy metals (Li and Shuman, 1997) and it is a potential source or sink of atmospheric carbon dioxide (Wander and Nissen, 2004). Many environmental and anthropogenic factors interact with geomorphic processes to affect the retention, distribution and transformation of SOC and total nitrogen (TN) in soil. Agricultural practices (e.g., conventional tillage) have been implicated in SOC and TN losses (Fu et al., 2010; Helfrich et al., 2006; Mann, 1986; Yimer et al., 2007). In contrast, conservation management practices, such as no-till cultivation and vegetative filter strips (VFS), have the potential to mitigate the effects of conventional cultivation. Conservation management practices also provide several ecosystem services including carbon sequestration in soil and biomass (Chen et al., 2004; Puget and Lal, 2005; Tufekcioglu et al., 2003), reduced runoff and sediment loss (Beke, 1989; Veum et al., 2009), pollutant reduction or sorption (Barling and Moore, 1994; Chu et al., 2010) and increased wildlife diversity (Semlitsch and Jensen, 2001). From 1982 to 1997, agricultural soil erosion in the USA declined by over a billion tons per year, and 25% of this decrease has been attributed to conservation management efforts (Wiebe and Gollehon, 2006).

In addition to affecting total SOC and TN concentration, agricultural cultivation may impact the distribution of SOC and TN vertically in the soil profile. In particular, reduced tillage systems allow for accumulation of organic matter at the surface while

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conventional tillage incorporates organic matter below the surface and homogenizes soil in the plow layer (Angers and Eriksen-Hamel, 2008; Dou et al., 2008; Lal et al., 2007; Tatzber et al., 2008; Wander et al., 1998), especially in comparison to soils under natural vegetation (Paz-González et al., 2000). In agricultural systems, spatial variation in SOC is usually attributed to differences in vegetation, management and terrain (Bricklemyer et al., 2005), which subsequently influence other factors such as infiltration, water content, porosity, aggregation and temperature.

Many studies have compared the effects of conventional tillage to conservation management practices on SOC (Angers and Eriksen-Hamel, 2008; Blanco-Canqui et al., 2009b; Christopher et al., 2009); however, few studies have been devoted to comparison of carbon stocks under 'best management practices' such as no-till or agroforestry in cropping systems (Morgan et al., 2010). Riparian filter strips, the focus of nearly all published studies of filter strips, separate aquatic and terrestrial habitats with the primary goal improving water quality (Barling and Moore, 1994; Liu et al., 2008; Parsons et al., 1994; Tate et al., 2004), while upland filter strips are located in the terrestrial landscape and are designed to improve water quality and protect soil resources at the same time. Upland filter strips are also usually maintained (e.g. mowing, fertilization and herbicide application) in contrast to riparian filter strips, where the vegetation is often allowed to undergo natural succession. Elucidating the effects of upland filter strips on carbon stocks is necessary to fill a current gap in our knowledge base regarding conservation management practices.

Evaluation of nutrient stocks and the ability to detect treatment effects is often hampered by large spatial variability coupled with limited experimental design (Ellert et al., 2001; VandenBygaart et al., 2007). Even though some studies find differences after one decade, (Lal, 2004; Lal et al., 1998), up to 200 years may be required before differences in total SOC become detectable after land-use changes are implemented (Knops and Tilman, 2000). Detecting change in nutrient storage is generally accomplished by taking measurements over time, which may not account for exogenous factors, or by comparing treatments after a period of time to a conventionally managed control (Ellert et al., 2001). Sampling depth is also a consideration; at a minimum, soil should be sampled to a depth that captures the effects of the management practice or horizonation, whereas a sampling depth of 30–50 cm is generally recommended for evaluation of total stocks (Ellert et al., 2001; VandenBygaart et al., 2007; VandenBygaart et al., 2011).

An additional factor making it difficult to compare research outcomes of studies examining the effects of agricultural management practices is that different methods are used to calculate SOC and TN stocks. For example, SOC data is commonly reported as concentration measurements (kg SOC per kg soil), by equivalent soil volume (kg SOC per m^2 to a fixed soil depth) or by equivalent soil mass (kg SOC per m^2 to a fixed soil mass). Both the equivalent soil volume and equivalent soil mass methods account for differences in soil bulk density (Ellert and Bettany, 1995; Ellert et al., 2001; Gregorich et al., 1994; VandenBygaart and Angers, 2006). Although the equivalent soil mass based method is generally accepted as the most reliable, it may not be appropriate in areas with perennial woody vegetation such as an agroforestry system (Ellert et al., 2001). Long-term or large-scale studies designed to capture these changes are expensive and not always feasible.

Soil bulk density, structure and aggregate stability are soil properties that may be indicative of resistance to erosion and improved stability and have been related to cultivation practices and soil organic matter content (Blanco-Canqui et al., 2009a; Cambardella and Elliott, 1993; Ruehlmann and Korschens, 2009; Schlesinger, 1986; Watts and Dexter, 1997). Lack of tillage combined with planting and harvesting traffic may lead to compaction and increased soil bulk density under no-till management and a strong negative correlation between SOC and bulk density is well documented in the literature (Blanco-Canqui et al., 2009b; Ruehlmann and Korschens, 2009). A variety of laboratory aggregate stability methods are commonly used to empirically relate the structural cohesion of soil to various aspects of soil function. These methods differ primarily in the technique of soil disruption including sieving (wet and dry), slaking, shaking, sonication and use of chemical dispersants (Cambardella and Elliott, 1994; Kemper and Rosenau, 1986; Pikul et al., 2009; Six et al., 1999). The distribution of SOC and TN among soil fractions is positively correlated with aggregate stability and has implications for nutrient losses through erosion and leaching (Barthes and Roose, 2002). Therefore, subsequent analysis of stable aggregates or other fractions for SOC or TN content provides a measure of the degree of structural isolation and protection in soil (Eynard et al., 2005; Swanston et al., 2005; Virto et al., 2009) and serves as an early indicator of SOC change that is not always evident with total carbon measurements (Gregorich et al., 1994).

Another gage of soil organic matter quality is the SOC:TN ratio. Soil nitrogen is inextricably linked to the carbon cycle through biotic processes associated with vegetative productivity and organic matter decomposition (Post et al., 1985). Differences in SOC:TN ratio may be indicative of the composition of the organic material being added to the soil or of the decomposition process. As organic matter is decomposed, SOC is mineralized to carbon dioxide while much of the nitrogen is recycled in the soil, resulting in a lower SOC:TN ratio relative to the initial organic substrate (Gilmour, 1961; Jenkinson, 1988; Parsons and Tinsley, 1975). Therefore, a larger SOC:TN ratio indicates a retardation of the decay process while a lower SOC:TN ratio is considered indicative of more advanced stages of organic matter decomposition (e.g. mineralization).

Soil formation in a landscape is the result of pedogeomorphic processes including physical weathering, chemical weathering and losses/gains associated with solute and soil transport; thus, the occurrence and distribution of soil types and their properties are influenced by landscape position (Wilson and Gallant, 2000). Over time, soil is transported down-slope, accumulating in depressions (Minasny and McBratney, 2001) and landscape position has been shown to be a factor in the spatial distribution of SOC and TN in soils with accelerated erosion and leaching (Chamran et al., 2002; Gessler et al., 2000; Hancock et al., 2010; Yoo et al., 2006). Landscape position is also a factor affecting the rate of carbon sequestration in reclaimed prairie soils (Guzman and Al-Kaisi, 2010) and depth to argillic horizon and associated hydraulic properties in claypan soils (Jiang et al., 2007).

A better understanding of the complex interactions among agronomic practices, conservation practices and the environment is necessary to describe and predict the impacts of land use change on soil SOC and TN. Therefore, the objectives of this study were to evaluate the effects of three conservation management practices (i.e., grass VFS, agroforestry VFS and no-till) and four landscape positions (i.e., summit, shoulder, backslope and footslope) on the concentration, soil volume based stocks and soil mass based stocks of SOC, TN, particulate, adsorbed and occluded organic carbon (PAO-C) and nitrogen (PAO-N) in an agroecosystem with claypan soils planted to a maize (*Zea mays.* L.)–soybean (*Glycine max* (L.) Merr.) rotation.

2. Materials and methods

2.1. Study site

The Greenley Memorial Research Center experimental site, originally designed as a paired watershed study to investigate soil solution and runoff, is located in Knox County, Missouri, USA (40° 01′N, 92° 11′W) in Major Land Resource Area 113, the Central Claypan Area (USDA-NRCS, 2006). Claypan soils are characterized by an abrupt, argillic sub-soil horizon imparting vertic properties to the soil such as shrink-swell and cracking behavior (Baer and Anderson, 1997). These soils experience complex runoff and infiltration phenomena; they are poorly drained soils with perched water tables dominated by lateral, subsurface flow and restricted upward movement of water when surface evaporation is high (Arnold et al., 2005; Blanco-Canqui et al., 2002; Ross et al., 1944). Even though claypan soils are not extensive globally, over 16,000 km^2 are classified as Epiaqualfs (Stagnic Luvisols) in the Central Claypan Area. Worldwide, soils with restrictive sub-horizons cover approximately 2.9 million km^2, potentially having a large impact on global biogeochemical cycles (Kitchen et al., 2010; USDA-NRCS, 1998; USDA-NRCS, 2006).

The study site is a small catchment (9.25 ha) facing north with gentle slopes (0 to 3%) that have been cultivated for over 60 years and consistently managed since 1991 in a no-till, maize and soybean rotation. In 1997, two types of upland contour VFS were planted; grass VFS were installed in the west subcatchment (3.16 ha), agroforestry VFS were installed in the center subcatchment (4.44 ha) and the east subcatchment (1.65 ha) was left under no-till management to serve as the control (Fig. 1). The filter strip design exceeds recommendations for upland filter strips in cropped land (USDA-NRCS, 2010); each filter strip is approximately 4.5 m wide and planted on the contour with a VFS to crop width ratio ranging from 1:8 to 1:5. Overall, the filter strips account for approximately 8 to 10% of the land area and have a combined VFS width of 22.5 to 27 m in each subcatchment. The grass VFS consist of redtop (*Agrostis gigantea* Roth), brome (*Bromus* spp.), and birdsfoot trefoil (*Lotus corniculatus* L.). The agroforestry VFS consist of pin oak (*Quercus palustris* Muenchh.), swamp white oak (*Q. bicolor* Willd.) and bur oak (*Q. macrocarpa* Michx.) planted approximately 3 m apart within the grass species. The filter strips are mowed periodically and treated with herbicides before planting and after harvest to inhibit vegetative succession.

Soils in the study site were formed in loess underlain by weathered glacial till (Watson, 1979) and are characterized by an abrupt, argillic, claypan horizon occurring from 20 to 62 cm below the surface (Udawatta et al., 2006). Soil series mapped in the study area follow the landscape and are classified by the USDA-NRCS as Putnam silt loam (fine, smectitic, mesic Vertic Albaqualfs) in the summit and shoulder positions and Kilwinning silt loam (fine, smectitic, mesic Vertic Epiaqualfs) in the backslope and footslope positions (Fig. 1). The 30-year, average annual precipitation for the Knox County (Novelty, MO) weather station is 95.9 cm. A summary description of the site and comprehensive details can be found in Veum et al. (2009) and the references therein.

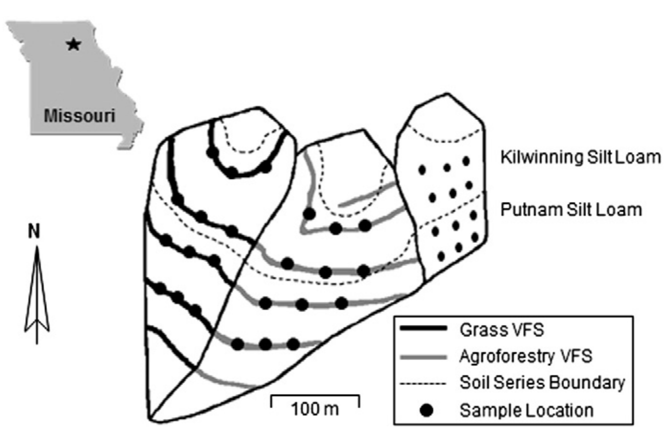

Fig. 1. Greenley Memorial Research Center study site in Knox County, Missouri (star). Black dots indicate sampling locations. Thick black lines are grass VFS, gray lines are agroforestry VFS and dotted lines represent soil series boundaries.

2.2. Soil sampling and laboratory analyses

An exploratory semivariogram analysis of cropped soil in the study area (36 samples collected under no-till with distances varying from 3 to 30 m apart) suggested a geostatistical range of 35 m for SOC_c, 40 m for TN_c and 25 m for bulk density. Subsequent samples were collected along the contour at an interval close to the estimated geostatistical range for SOC_c to avoid spatial correlation. Surface (0–5 cm) and subsurface (5–13 cm) soil samples were collected from four landscape positions (summit, shoulder, backslope and footslope) and three conservation management practices (grass VFS, agroforestry VFS and no-till). The shallow sampling depth was deliberately selected in order to isolate the dynamic surface layer most likely to exhibit treatment effects. Three subsamples from each combination of landscape position and conservation management practice were collected, totaling 36 sample locations in the study site (Fig. 1). Soil cores were collected using 7.62 cm diameter by 5.08 cm height aluminum rings for the surface (0–5 cm) and 7.62 cm diameter by 7.62 cm height aluminum rings for the subsurface (5–13 cm) following the core method of Grossman and Reinsch (2002). Surface residues and litter were removed prior to soil sample collection. Gravimetric water content of the soil in the rings was determined by placing samples in a forced-air oven set at 105 °C. Bulk density was measured using the soil core sample and adjusting for volumetric water content. For general characterization of soil properties, 1 kg of bulk soil from each depth at each location was also collected using a hand trowel.

Cation exchange capacity (CEC), base saturation and particle size were determined on air-dried, ground soil samples by the University of Missouri Soil Characterization Laboratory following USDA-NRCS procedures (Burt, 2004). All other laboratory analyses used in this study were performed in triplicate on each subsample. Samples were reanalyzed as appropriate for quality control purposes. General soil characteristics by conservation management practice are summarized in Table 1.

Soil was sieved (<2 mm), air-dried, ground and analyzed on a LECO® combustion analyzer for SOC and TN concentration measurements (SOC_c and TN_c). Soil volume (SOC_v and TN_v) and soil mass (SOC_m and TN_m) based stocks were calculated as the product of gravimetric concentration, bulk density and the appropriate thickness of the soil layer (Ellert and Bettany, 1995; Gregorich et al., 1994; Lal et al., 2001; VandenBygaart and Angers, 2006). In this study, all three calculations were used to evaluate treatment effects on SOC and TN in the surface (0–5 cm; 0–70 kg m^{-2}) and subsurface (5–13 cm; 70–130 kg m^{-2}) layers.

Table 1
Mean soil properties in the 0–5 cm and 5–13 cm layers of the study soils by conservation management practice. The standard error of the mean is stated in parentheses.

Soil property	Grass	Agroforestry	No-till
Surface (0–5 cm)			
Bulk density (g cm^{-3})	1.10 (0.027)	1.02 (0.023)	1.29 (0.020)
CEC_{NaOAc} ($cmol_c$ kg^{-1})[a]	23.3 (1.20)	23.6 (0.51)	21.0 (0.40)
Base $saturation_{naoac}$ (%)	92.8 (2.33)	87.8 (1.58)	90.0 (2.27)
Soil pH_w	6.6 (0.11)	6.0 (0.16)	6.3 (0.12)
Textural class	Silt loam	Silt loam	Silt loam
Clay content (g kg^{-1})	242.5 (17.59)	239.2 (9.42)	218.7 (6.22)
Subsurface (5–13 cm)			
Bulk density (g cm^{-3})	1.30 (0.011)	1.25 (0.024)	1.39 (0.024)
CEC_{NaOAc} ($cmol_c$ kg^{-1})[a]	20.8 (1.03)	19.9 (0.60)	19.62 (0.63)
Base $saturation_{naoac}$ (%)	96.2 (0.58)	96.2 (2.9)	92.2 (2.46)
Soil pH_w	7.0 (0.09)	7.0 (0.05)	6.9 (0.08)
Textural class	Silt loam	Silt loam	Silt loam
Clay content (g kg^{-1})	246.8 (17.49)	226.2 (9.53)	227.8 (0.47)

[a] Cation exchange capacity.

A rapid physical slaking test was used to isolate a physically stabilized fraction of SOC and TN and evaluate treatment effects. This fraction includes particulate, adsorbed and occluded organic carbon (PAO-C) and nitrogen (PAO-N). The separation of PAO-C and PAO-N was performed using a slaking stability test adapted from Kemper and Koch (1966) and Yoder (1936). Specifically, 100 g of air-dried sieved soil (<2 mm) was slaked in deionized water over a 23 cm diameter, 53 μm sieve and wet-sieved for 2 min at 30 s per cycle. The soil residue retained on the sieve was backwashed, dried at 50 °C until the mass stabilized, followed by grinding with a mortar and pestle. The proportion of residue to whole soil was calculated by mass difference on a sand-free basis with the sand content determined for each individual sample location. The SOC and TN content of the PAO fraction was determined on a LECO® combustion analyzer and the proportion of PAO-C and PAO-N in the soil was calculated by multiplying the proportion of occluded SOC or TN in the PAO residue by the proportion of PAO residue to whole soil on a sand-free basis. Stocks in this fraction were calculated and compared on a concentration (PAO-C_c and PAO-N_c), soil volume (PAO-C_v and PAO-N_v) and soil mass (PAO-C_m and PAO-N_m) basis.

2.3. Statistical analysis

Due to the original experimental design imposed on the study site, testing the main effects of conservation management practice and landscape position was accomplished using a two-factor ANOVA from a completely randomized design (Kuehl, 2000). However, similar to many environmental studies, a standard statistical analysis was not possible due to the inclusion of subsamples and the lack of replication among the different treatment factors. Specifically, each landscape by conservation management practice treatment combination has no "true" replication. Instead, each experimental unit has one replicate with three subsamples. Consequently, using a standard two-factor ANOVA with subsamples results in an error term with zero degrees of freedom and, thus, a proper hypothesis test for the landscape position by conservation management practice interaction term could not be formulated. Specifically, the interaction term served as the replication, or error term, in formulating tests for main effects.

In environmental studies, subsamples are often incorrectly treated as true replicates. This ignores the fact that the subsamples are correlated (i.e., they come from the same experimental unit) and may lead to incorrect statistical and scientific inference. Nevertheless, as is the case with many environmental studies involving designed experiments, it is still possible to conduct a proper statistical analysis capable of drawing meaningful scientific inference. In this direction, a modified two-factor ANOVA, appropriate for this study, was employed.

The statistical analysis is based on a two-factor ANOVA with sub-samples. Specifically, the following model is considered

$$y_{ijk} = \mu + \alpha_i + \beta_j + e_{ij} + d_{ijk},$$

where μ denotes the overall mean, α_i denotes the main effect due to conservation management practice ($i = 1,2,3$), β_j denotes the main effect due to landscape position ($j = 1,2,3,4$), e_{ij} is the random error term and d_{ijk} is the random effect due to subsamples ($k = 1,2,3$) with e_{ij}~iid $N(0,\sigma_e^2)$ and d_{ijk} ~iid $N(0,\sigma_d^2)$ and mutually independent. This model differs from the traditional two-factor ANOVA in that there is an additional random effect (d_{ijk}) due to subsampling. Additionally, due to the lack of replication, the two-way interaction between conservation management practice and landscape position was used as the random error term (i.e., the replication). A full justification for this model specification is provided in the Appendix. Table A1 provides a comparison of the two-factor ANOVA with subsamples employed in this study to a two-factor ANOVA where subsamples are treated as true replicates. This comparison illustrates how treating the subsamples as true replicates could lead to incorrect scientific inference about the soil properties investigated at the selected study site.

All two-factor ANOVA analyses were conducted using SAS Proc Mixed (SAS® software, Version 9.1.3, SAS Institute Inc., Cary, NC, USA.). In addition, Tukey's HSD procedure was implemented to test for pair-wise differences in treatment means. In doing so, the Tukey's HSD statistic uses the correct estimate for the variance by utilizing the interaction term as the random error term in the ANOVA while accounting for the subsamples (Littell et al., 2006). All analyses were conducted at the $\alpha = 0.05$ significance level.

To examine the relationship between bulk density and SOC, a simple linear regression was considered across all subsamples. As such, this regression does not explicitly account for possible correlation due to subsampling. In order to validate the regression assumptions a residual analysis was conducted and no departures were found. Finally, using the Shapiro–Wilk test for normality, there was insufficient evidence to reject the null hypothesis that the residuals are normally distributed ($p = 0.508$). To assess the paired differences between the mean total SOC:TN ratio and mean PAO-C:PAO-N ratio, subsamples were aggregated by group (mean of three subsamples within each conservation management practice by landscape position combination, n = 12). The normality of the distribution of the paired differences was validated (Shapiro–Wilk test for normality, $p = 0.323$) and a paired *t*-test was conducted, accounting for the fact that the ratios are related (i.e., matched pairs) rather than independent variables (Mason et al., 1989).

3. Results

3.1. Bulk density, total SOC and TN

In the surface layer, the mean bulk density of no-till soil was significantly greater than the grass ($p = 0.004$) and agroforestry ($p < 0.001$) VFS soils. In the subsurface layer, the mean bulk density of no-till soil was significantly greater than the agroforestry ($p = 0.017$) VFS soil only. Overall, the grass and agroforestry VFS soils had significantly greater mean total SOC_c in the surface layer than no till ($p = 0.032$ and 0.034), corresponding to an increase of 23% and 22%, respectively. When mean surface stocks of SOC_v or SOC_m were calculated, no detectable differences were found among conservation management practices. The grass VFS soil had significantly greater surface mean TN_c and TN_m than no till ($p = 0.031$ and $p = 0.049$) and no significant differences were detected in the subsurface layer or among landscape positions (either depth) for mean SOC_c, TN_c, SOC_v, TN_v, SOC_m or TN_m (Table 2).

To more closely examine the general relationship between bulk density and SOC_c in the surface layer, the following simple linear regression relationship ($R^2 = 0.50$) was determined using data from all subsamples (n = 36) in the study (Fig. 2):

$$BD = 1.73 - 0.02\ SOC_c,$$

where BD is bulk density (g cm^{-3}) and SOC_c is soil organic carbon (g OC kg soil^{-1}) and the slope parameter was significant ($p < 0.001$). In this study, the range of SOC_c was relatively narrow in comparison to the work of Ruehlmann and Korschens (2009), allowing for the use of a linear regression model instead of a non-linear model, as was used in their meta-analysis of a wide range of soils.

Table 2
Means of soil total organic carbon (SOC), total nitrogen (TN) and sand-free, particulate, adsorbed and occluded soil organic carbon (PAO-C) and total nitrogen (PAO-N) expressed on a concentration, soil volume and soil mass (represented by the subscripts c, v and m, respectively) basis by conservation management practice. Values followed by a different lowercase letter within columns and for a given layer are significantly different (using Tukey's HSD) at $\alpha = 0.05$. The standard error of the mean is stated in parentheses.

	Surface layer (0–5 cm depth)								Surface layer (0–70 kg soil m^{-2})			
	Concentration means (g kg^{-1})				Volume based means (kg m^{-2})				Mass based means (kg m^{-2})			
	SOC_c	PAO-C_c	TN_c	PAO-N_c	SOC_v	PAO-C_v	TN_v	PAO-N_v	SOC_m	PAO-C_m	TN_m	PAO-N_m
Grass	33.0a	33.9a	3.10a	2.76a	1.84a	1.74a	0.174a	0.142a	2.14a	2.39a	0.200a	0.182a
	(1.23)	(1.32)	(0.159)	(0.100)	(0.035)	(0.066)	(0.0081)	(0.0050)	(0.060)	(0.099)	(0.0094)	(0.0071)
Agroforestry	32.9a	35.5a	2.78ab	2.80a	1.71a	1.69a	0.145a	0.133ab	2.08a	2.57a	0.176ab	0.189a
	(1.49)	(2.02)	(0.108)	(0.132)	(0.063)	(0.070)	(0.0051)	(0.0046)	(0.076)	(0.150)	(0.0058)	(0.0098)
No-till	26.9b	22.6b	2.29b	1.87b	1.77a	1.37b	0.151a	0.114b	1.85a	1.52b	0.157b	0.118b
	(1.11)	(0.93)	(0.055)	(0.064)	(0.070)	(0.056)	(0.0027)	(0.0039)	(0.076)	(0.063)	(0.0034)	(0.0042)
	Subsurface layer (5–13 cm depth)								Subsurface layer (70–130 kg soil m^{-2})			
	Concentration means (g kg^{-1})				Volume based means (kg m^{-2})				Mass based means (kg m^{-2})			
	SOC_c	PAO-C_c	TN_c	PAO-N_c	SOC_v	PAO-C_v	TN_v	PAO-N_v	SOC_m	PAO-C_m	TN_m	PAO-N_m
Grass	20.9a	17.8a	3.10a	1.56a	2.06a	1.63a	0.174a	0.142a	1.26a	1.00a	0.106a	0.081a
	(0.85)	(0.60)	(0.083)	(0.053)	(0.072)	(0.051)	(0.0073)	(0.0043)	(0.051)	(0.035)	(0.0050)	(0.0029)
Agroforestry	20.6a	17.8a	2.78a	1.59a	1.95a	1.55a	0.165a	0.139a	1.24a	0.98a	0.105a	0.081a
	(0.74)	(0.53)	(0.085)	(0.046)	(0.047)	(0.031)	(0.0060)	(0.0028)	(0.044)	(0.032)	(0.0051)	(0.0029)
No-till	18.9a	15.6a	2.29a	1.34a	1.98a	1.52a	0.157a	0.131a	1.13a	0.87a	0.089a	0.069a
	(1.13)	(1.00)	(0.066)	(0.056)	(0.091)	(0.083)	(0.0056)	(0.0053)	(0.068)	(0.059)	(0.0040)	(0.0033)

3.2. Particulate, adsorbed and occluded organic carbon (PAO-C) and nitrogen (PAO-N)

A simple, rapid slaking test was used to isolate a soil fraction more sensitive to land use effects than whole soil. The residues from this method include particulate, adsorbed and occluded fractions of organic carbon (PAO-C) and nitrogen (PAO-N). The proportion of soil residue, calculated by mass difference, ranged from 60 to 80%, similar to the results reported in Kemper and Rosenau (1986). The remaining residue was analyzed for total organic carbon and total nitrogen on a concentration (PAO-C_c and PAO-TN_c), soil volume (PAO-C_v and PAO-N_v) and soil mass (PAO-C_m and PAO-N_m) basis and corrected for sand content (cf. Section 2.2). The mean sand content was not significantly different among the VFS and no-till soils, ranging from 75 g kg^{-1} to 85 g kg^{-1} in the surface layer and from 71 g kg^{-1} to 81 g kg^{-1} in the subsurface layer.

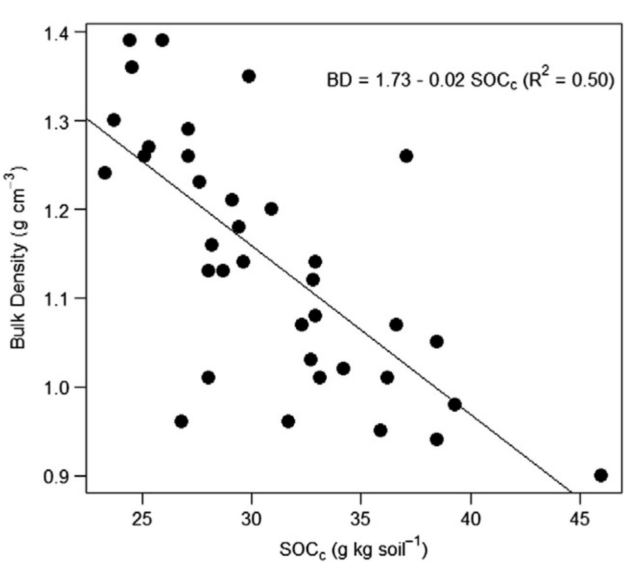

Fig. 2. Scatter plot and regression relationship for regression of soil organic carbon (SOC) concentration on bulk density (BD) using all subsamples (n = 36) in the 0–5 cm surface layer. Note the slope parameter (SOC_c) was statistically significant ($p < 0.001$).

Similar to total SOC, the mean surface PAO-C_c was significantly greater in the grass ($p = 0.006$) and agroforestry ($p = 0.003$) VFS soils than in no-till. In contrast to total SOC, the grass VFS and agroforestry VFS had significantly greater mean PAO-C_v stocks than no-till ($p = 0.017$ and 0.031) and significantly greater mean PAO-C_m stocks than no-till ($p = 0.005$ and 0.002). These results represent a 57% increase in the grass VFS and a 69% increase in the agroforestry VFS over no-till in organic carbon in the PAO fraction on a mass basis. With respect to nitrogen, the grass and agroforestry VFS exhibited significantly greater mean PAO-N_c ($p = 0.005$ and 0.004), PAO-N_v ($p = 0.012$ and 0.049) and PAO-N_m ($p = 0.008$ and 0.005) stocks than no-till in the surface layer. These results correspond to a 54% increase in PAO-N_m in the grass VFS and a 60% increase in PAO-N_m in the agroforestry VFS over no-till. No significant differences were detected in the subsurface layer or among landscape positions (either depth) for mean PAO-C_c, PAO-N_c, PAO-C_v, PAO-N_v, PAO-C_m or PAO-N_m (Table 2).

3.3. SOC and TN ratios

No statistically significant differences in the total SOC:TN or PAO-C:PAO-N ratios were found among conservation management practices or landscape positions in surface or subsurface layers. An overall comparison of the total SOC:TN ratio with the PAO-C:PAO-N ratio was conducted using a paired *t*-test. Subsamples were aggregated by group (mean of three subsamples within each conservation management practice by landscape position combination, n = 12), and the difference between the ratios was significant ($p = 0.002$). This suggests that the mean PAO-C:PAO-N ratio (12.3 to 1) was greater than the mean total SOC:TN ratio (11.5 to 1) since the mean difference between PAO-C:PAO-N and total SOC:TN ratios is greater than zero. Additionally, this is an indication that the organic matter sequestered in the PAO fraction of the soil is less decomposed and more protected than the whole soil organic matter.

4. Discussion

Vegetated filter strips are known to effectively increase SOC_c in VFS soils compared to bare soils (Staddon et al., 2001), and research on grass and agroforestry riparian buffers by Tufekcioglu et al. (2003) found increased fine root biomass and fine root carbon in the buffers relative to cropped soil. In general, studies find that no-till cultivation is also a highly effective conservation management practice, often acting as a carbon sink from the atmosphere as opposed to conventional tillage (Dou et al., 2008; Izaurralde et al., 2007; Lal et al., 2007). Few studies, however, compare SOC among conservation practices and studies of agroecosystems with restrictive subsurface soil horizons are rare. Although significant differences in mean total SOC_c were found between the VFS and no-till soils in this study, no significant differences were evident when mean stocks were calculated on a soil volume or soil mass basis. No-till soils are subject to compaction and often have greater bulk density (Ruehlmann and Korschens, 2009; Schlesinger, 1986) and this study illustrates the need to account for bulk density differences when evaluating nutrient stocks in soil.

The results of this study suggest that VFS soils either do not sequester more total SOC stocks than no-till or that the VFS soils in this study may not have reached equilibrium levels of SOC. Hansen (1993) found soil under poplar plantations on previously cultivated land required 12–18 years to make significant gains in near surface SOC and that most gains occurred at greater depths. Therefore, the ten year duration of this study may not be sufficient to detect differences when VFS are compared to another conservation management practice such as no-till. In fact, small changes in SOC generally require an unrealistic sampling intensity (hundreds to thousands) in order to achieve the statistical power to detect differences (Garten and Wullschleger, 1999; Kravchenko and Robertson, 2011; Yang et al., 2008). Moreover, this degree of sampling intensity may potentially lead to an artificial reduction in variance that comes from ignoring positive spatial correlation (VandenBygaart and Angers, 2006). Several studies indicate that more than one decade is needed to observe changes in the SOC pool without intensive sampling, even though the treatments may be acting as a carbon-sink upon establishment (Conant et al., 2011; Oelbermann et al., 2004).

In addition to the short study duration, the modest changes in SOC may be partially due to a system where crop growth and hydrology are dominated by a restrictive claypan layer. Prairie and forest soils in the claypan region are generally low in productivity, highly leached and acid (Ross et al., 1944). Cultivated soils in this region have experienced extensive erosion and degraded soil quality over the past several decades, leading to reduced crop productivity (Kitchen et al., 1999; Kitchen et al., 2010). Therefore, the establishment and growth of the grass and agroforestry VFS in this study may have been adversely affected by the presence of the claypan layer, protracting the time needed for changes in total SOC and TN to be detectable at this site.

The PAO fraction is therefore of particular interest in a study where small changes in SOC are coupled with the limitations of a small sampling area. In contrast to SOC stocks, the PAO fraction exhibited significantly greater mean concentration, soil volume and soil mass based stocks in VFS soils compared to no-till after only ten years. The PAO fraction indicates that changes in the distribution and quality of SOC and TN have occurred and demonstrates that the PAO separation technique is an effective early indicator of management effects in the soils studied. The physicochemical protection afforded by aggregation inhibits the biochemical attack of otherwise decomposable organic matter, sequestering carbon in the soil (Jastrow et al., 2007). A strong feedback loop exists between organic matter and aggregate stability and the extent of protection in soil aggregates is dependent on soil properties, vegetation (in particular roots), soil microorganisms, management practices and environmental conditions (Amezketa, 1999; Jastrow et al., 2007; Six et al., 2000; Sollins et al., 1996).

Many soil separation methods, differing primarily in the technique of soil disruption, are currently in use as a measure of the degree of structural isolation and protection of carbon in the soil. Each of these techniques separates a fraction of soil carbon representing different biological, chemical and/or physical aspects of soil and method selection is based on time constraints and study objectives. These include sieving (wet and dry), slaking, shaking, sonication and use of chemical dispersants (Cambardella and Elliott, 1994; Kemper and Rosenau, 1986; Pikul et al., 2009; Six et al., 1999), all of which are more time consuming methods than the method used in this study. In contrast, the PAO fractionation method was rapid, simple and an effective early indicator of SOC protection in VFS that was not evident with total SOC measurements. Additionally, the mean difference between PAO-C:PAO-N and total SOC:TN ratios in the surface layer ($n=36$) was significantly different from zero. Together, these results suggest increased protection and reduced mineralization of SOC in VFS soil compared to soil under no-till management.

In the subsurface layer, no significant differences were found among conservation management practices or landscape positions for mean SOC_c, SOC_v or SOC_m. This is consistent with studies of no-till cultivation showing that treatment effects are limited to the surface layer where organic matter is concentrated (Angers and Eriksen-Hamel, 2008; Blanco-Canqui and Lal, 2008; Pikul et al., 2009). Whereas strong landscape effects are found in the steeply sloping (0–66%, average 22%) study site of Gessler et al.(2000) and Chamran et al. (2002), the lack of significant effects from landscape position in this study may be due to the gentle (0–3%) slope of the study site, the small catchment area, and the similarity of the soil series found at each landscape position. Further, a complication in many environmental studies is that landscape position is fixed in space and thus randomization is not possible (Essington, 2004). Therefore, significant effects due to this factor become difficult to interpret; however, this study only found significant treatment effects due to conservation management practices and, since this factor was randomized within the study design, the results are interpretable. Moreover, this study was concerned with soil stocks only, and the sequestration potential of other carbon and nitrogen pools (i.e. above- and below-ground biomass) was not evaluated. Under perennial vegetation, upwards of 60% of total net primary productivity occurs below ground as roots (Jones and Donnelly, 2004; Krug and Hollinger, 2003) and it is estimated that 10–40% of biomass in forested sites is contained in root biomass (MacDicken, 1997; Oelbermann et al., 2004; Young et al., 1998). Therefore, a large pool of organic carbon is contained in the perennial biomass of the grass and agroforestry VFS in this study. In addition, estimates of crop yield, surface residues, gaseous efflux, redistribution and leaching are important considerations for future studies at this site seeking a more comprehensive analysis of nutrient cycling at the ecosystem level.

The lack of significant differences in mean SOC and TN stocks between VFS and no-till confirms that no-till is a highly effective conservation management practice in agroecosystems. As the study continues, differences in total SOC and TN stocks between VFS and no-till soils may emerge if carbon and nitrogen are accumulating at different rates. However, the PAO fraction has demonstrated sensitivity to management effects in the short-term. The dissimilarity in PAO stocks between VFS and no-till soils indicate that differences in carbon and nitrogen distribution have occurred after ten years. Additional investigations into soil microbiological quality and SOC characteristics of the soils studied may also provide greater insight into the role of conservation management practices over relatively short time periods (e.g. 10 years).

5. Conclusions

As demonstrated in this study, ignoring the underlying experimental design by treating subsamples as true replicates (pseudoreplication) can lead to incorrect scientific inference. Critical to a proper statistical analysis was the use of a modified two-factor

ANOVA accounting for the lack of replication in the study design. Additionally, this study illustrates how the choice of SOC quantification method (concentration, soil volume or soil mass) influences the scientific inference and conclusions, emphasizing the need for bulk density measurements for valid assessment of soil nutrient stocks.

Overall, this study finds that VFS soils have not yet sequestered significantly greater stocks of total SOC than no-till soils and indicates that grass VFS may sequester TN more rapidly than agroforestry VFS. Future research is necessary to determine if the ten year duration of the study was sufficient for the soils to reach equilibrium levels of SOC or TN. In contrast to total SOC and TN, a rapid, inexpensive slaking-stability test separated a physically stable soil fraction (PAO) exhibiting significantly greater mean concentration, soil volume and soil mass based stocks in VFS soils compared to no-till. Thus, a higher proportion of SOC and TN are contained in the physically stable fraction of soils planted to VFS. This stable fraction also exhibited a greater organic carbon to nitrogen ratio than the whole soil, suggesting increased SOC protection in this fraction. Together, these results indicate differences in the distribution and quality of SOC and TN stocks between VFS and no-till soils. Overall, this study contributes to a richer understanding of agronomic effects on soil carbon and provides information for improved management of agricultural resources for long-term sustainability and profitability.

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Appendix

Two-factor ANOVA with subsamples.

1. Statistical analysis

The study consisted of two factors, landscape position and conservation management practice. In order to test for the effects of these factors, an analysis of variance (ANOVA) was used. In particular, due to the original study design, the analysis should be treated as a two-factor ANOVA with subsamples, from a completely randomized design (CRD). However, due to lack of replication, there are zero error degrees of freedom under this model specification and thus proper hypothesis tests cannot be formulated for the landscape by conservation management practice interaction. For a comprehensive discussion see Kuehl (2000).

Fortunately, it is still possible to conduct a proper test for the main effect due to conservation management practice and, in principal, for the main effect due to landscape position. In this case, due to the lack of replication, the interaction term becomes the error term (i.e. the replication). As a result, the model becomes

$$y_{ijk} = \mu + \alpha_i + \beta_j + e_{ij} + d_{ijk}$$

where $i=1,\ldots,a$, $j=1,\ldots,b$, $k=1,\ldots,n$, with $e_{ij}\sim$ iid $N(0,\sigma_e^2)$ and $d_{ijk}\sim$ iid $N(0,\sigma_d^2)$ mutually independent. For our specific case $a=3$, $b=4$, and $n=3$; therefore, the correct ANOVA table is given by

Source	Degrees of freedom	Mean square	Expected mean square
α	2	$MS_\alpha = \mathbf{Y}'\mathbf{M}_\alpha\mathbf{Y}/2$	$12Q_\alpha + 3\sigma_e^2 + \sigma_d^2$
β	3	$MS_\beta = \mathbf{Y}'\mathbf{M}_\beta\mathbf{Y}/3$	$9Q_\beta + 3\sigma_e^2 + \sigma_d^2$
error($\alpha\beta$)	6	$MS_e = \mathbf{Y}'\mathbf{M}_e\mathbf{Y}/6$	$3\sigma_e^2 + \sigma_d^2$
subsampling	24	$MS_{sub} = \mathbf{Y}'\mathbf{M}_{sub}\mathbf{Y}/24$	σ_d^2
Total	35		

where $Q_\alpha = \sum_{i=1}^{a}(\alpha_i - \overline{\alpha})2/(a-1)$ and $Q_\beta = \sum_{j=1}^{b}\left(\beta_j - \overline{\beta}\right)2/(b-1)$ respectively.

In matrix notation the model can be expressed as

$$Y = X\delta + Zu + D$$

where $Y=(Y_{111},Y_{112},Y_{113},\ldots,Y_{341},Y_{342},Y_{343})'$, $\delta=(\alpha_1,\alpha_2,\ldots,\beta_3,\beta_4)'$, $X=[1_{36}|\mathbf{I}_3\otimes 1_4\otimes 1_3|1_3\otimes\mathbf{I}_4\otimes 1_3]$, $Z=[\mathbf{I}_3\otimes\mathbf{I}_4\otimes 1_3]$, $u=(e_{11},e_{12},e_{13},\ldots,e_{32},e_{33},e_{34})'$, and $D=(d_{111},d_{112},d_{113},\ldots,d_{341},d_{342},d_{343})'$. Note, $\mathbf{I}_k$ is the $k\times k$ identity matrix, $\otimes$ denotes the Kronecker product and 1_k denotes the vector of ones of length k; e.g.,

$$\mathbf{I}_3\otimes 1_4 = \begin{pmatrix} 1_4 & 0 & 0 \\ 0 & 1_4 & 0 \\ 0 & 0 & 1_4 \end{pmatrix}.$$

Further, the defining matrix, $\mathbf{M}_\cdot$, associated with each (quadratic form) sum of squares $Y'M_\cdot Y$ can be expressed as

$$\mathbf{M}_\alpha = (\mathbf{I}_3 - 1/3\mathbf{J}_3)\otimes 1/4\mathbf{J}_4\otimes 1/3\mathbf{J}_3,$$
$$\mathbf{M}_\beta = 1/3\mathbf{J}_3\otimes(\mathbf{I}_4 - 1/4\mathbf{J}_4)\otimes 1/3\mathbf{J}_3,$$
$$\mathbf{M}_e = \mathbf{M}_{\alpha\beta} = (\mathbf{I}_3 - 1/3\mathbf{J}_3)\otimes(\mathbf{I}_4 - 1/4\mathbf{J}_4)\otimes 1/3\mathbf{J}_3,$$

where $\mathbf{J}_k$ is equal to the $k\times k$ matrix of ones. Finally, the covariance matrix of $\mathbf{Y}$ is given by $\Sigma=\sigma_e^2(\mathbf{I}_{12}\otimes\mathbf{J}_3)+\sigma_d^2\mathbf{I}_{36}$.

In the above model it is important to point out that we are not asserting that the interaction of two fixed effects is being accounted for by a random variable. The issue is that if one includes the interaction term in this model, there are no error degrees of freedom due to lack of replication. However, if one assumes that the model has no significant interaction term, then a constrained model can be imposed (i.e., a model with no interaction term). In this case, the sum-of-squares for the error term becomes the sum-of-squares for the interaction (which is no longer in the model) plus the sum-of-squares for the error. Due to the lack of true replication, this reduces to the sum-of-squares for the interaction. In other words, the interaction serves as the replication and is thus a random effect in the constrained model. Under the constrained model, proper tests for main effects can be formulated.

The effects model, as stated above, is necessary for deriving the relevant test statistics and their associated distributions. However, as currently stated, this model is over-parameterized and would lead to estimation problems without imposing some constraints. The estimation constraints, imposed by SAS Proc Mixed (SAS® software, Version 9.1.3, SAS Institute Inc., Cary, NC, USA.), consist of setting the last factor level to zero (e.g., the corresponding row in the design matrix **X** is set to zero). It is important to note that this constraint is not unique; instead one could use a sum-to-zero constraint (i.e., the sum over all of the levels of a given factor equals zero). However, the sum-to-zero constraint becomes intractable with unbalanced designs and is thus less general and not imposed in SAS (Littell et al., 2006).

Let $\mathbf{E}[MS_\alpha]$ and $\mathbf{E}[MS_e]$ denote the expected mean square for conservation management practice and expected mean square error respectively. Under the null hypothesis, H_0, of no main effect due to conservation management practice, it follows that $Q_\alpha = 0$. Thus, under the null hypothesis, $\mathbf{E}[MS_\alpha] = \mathbf{E}[MS_e]$ and the appropriate test statistic for testing no main effect due to conservation management practice is

$$F = \frac{MS_\alpha}{MS_e}$$

Now, under H_0, it is straightforward to show that $\mathbf{M}_\alpha \Sigma / (3\sigma_e^2 + \sigma_d^2)$ is idempotent and symmetric with rank equal to two and that the noncentrality parameter is equal to zero. Therefore, it follows that (Theorem 2, Page 57, Searle, 1997), $\mathbf{Y}'\mathbf{M}_\alpha\mathbf{Y} / (3\sigma_e^2 + \sigma_d^2)$ follows a central chi-squared distribution with two degrees of freedom. Analogously, $\mathbf{Y}'\mathbf{M}_e\mathbf{Y} / (3\sigma_e^2 + \sigma_d^2)$ follows a central chi-square distribution with six degrees of freedom. With the distributions of the sum of squares for conservation management practice and sum of squares for error established, we can combine the two to form an *F*-statistic. However, the quadratic forms need to be independent. Now, since $\mathbf{M}_\alpha \Sigma \mathbf{M}_e = 0$, $\mathbf{Y}'\mathbf{M}_\alpha\mathbf{Y} / (3\sigma_e^2 + \sigma_d^2)$ and $\mathbf{Y}'\mathbf{M}_e\mathbf{Y} / (3\sigma_e^2 + \sigma_d^2)$ are independent (Theorem 3, Page 59, Searle, 1997). Additionally, note that

$$F = \frac{MS_\alpha}{MS_e} = \frac{\frac{\mathbf{Y}'\mathbf{M}_\alpha\mathbf{Y}}{(3\sigma_e^2+\sigma_d^2)2}}{\frac{\mathbf{Y}'\mathbf{M}_e\mathbf{Y}}{(3\sigma_e^2+\sigma_d^2)6}} \sim \frac{\chi_2^2/2}{\chi_6^2/6}.$$

Hence, *F* follows a central *F* distribution with two numerator degrees and six denominator degrees of freedom (Searle, 1997; Page 101). The test statistic for landscape position can be derived analogously. It is important to emphasize that treating the subsamples as true replication would result in an incorrect test statistic. In fact, the test statistic derived in this case would have the same numerator degrees of freedom. However, the denominator degrees of freedom would now be 24 and could thus result in incorrect scientific inference (Table A1).

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