Soil organic carbon dynamics 75 years after land-use change in perennial grassland and annual wheat agricultural systems

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Abstract The dynamics of roots and soil organic carbon (SOC) in deeper soil layers are amongst the least well understood components of the global C cycle, but essential if soil C is to be managed effectively. This study utilized a unique set of land-use pairings of harvested tallgrass prairie grasslands (C_4) and annual wheat croplands (C_3) that were under continuous management for 75 years to investigate and compare the storage, turnover and allocation of SOC in the two systems to 1 m depth. Cropland soils contained 25 % less SOC than grassland soils (115 and 153 Mg C ha^{-1}, respectively) to 1 m depth, and had lower SOC contents in all particle size fractions (2000–250, 250–53, 53–2 and <2 μm), which nominally correspond to SOC pools with different stability. Soil bulk $\delta^{13}C$ values also indicated the significant turnover of grassland-derived SOC up to 80 cm depth in cropland soils in all fractions, including deeper (>40 cm) layers and mineral-associated (<53 μm) SOC. Grassland soils had significantly more visible root biomass C than cropland soils (3.2 and 0.6 Mg ha^{-1}, respectively) and microbial biomass C (3.7 and 1.3 Mg ha^{-1}, respectively) up to 1 m depth. The outcomes of this study demonstrated that: (i) SOC pools that are perceived to be stable, i.e. subsoil and mineral-associated SOC, are affected by land-use change; and, (ii) managed perennial grasslands contained larger SOC stocks and exhibited much larger C allocations to root and microbial pools than annual croplands throughout the soil profile.

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Introduction

Developing agricultural management strategies that increase soil organic carbon (SOC) stocks to mitigate climate change and sustain ecosystem processes for food production and environmental quality is a global priority (Lal 2010; Powlson et al. 2011). The majority of SOC is derived from root C which has a longer mean residence time than shoot inputs (Rasse et al. 2005); therefore, increasing root biomass in soil appears an obvious mechanism to increase SOC stocks. Furthermore, decomposing root biomass and rhizoexudates are spatially discrete sources of organic matter in the soil that drive the activity of the soil biological community. Root and microbial (bacterial and fungal) byproducts and the castings of invertebrates encourage the formation of aggregates, which physically protect SOC (Or et al. 2007; Rawlins et al. 2007). Root-associated fungi have been observed to play a key role in increasing stable SOC pools (Clemmensen et al. 2013), but their establishment is disrupted in soil under regular tillage. The reduction in SOC after land-use change from perennial plant communities to annual agriculture may be associated with a reduction in root C inputs (Culman et al. 2010; DuPont et al. 2010).

Temperate grasslands allocate large proportions of C belowground and have average root:shoot ratios and root biomass stocks that are an order of magnitude larger than those observed in annual croplands (Jackson et al. 1996). This large belowground allocation of primary productivity has resulted in large SOC pools in grasslands, which confer soil fertility for highly productive agriculture after land-use change. Thus, the majority of the world's temperate grasslands have been converted to cropland (Hoekstra et al. 2005). It is estimated that temperate grasslands lose up to 40 % of their SOC within 20 years after the onset of cultivation (Davidson and Ackerman 1993; Guo and Gifford 2002; Miller et al. 2004). Few studies, however, have documented the turnover of SOC in deeper (>40 cm) soil layers following the conversion of grasslands to annual agriculture.

Deeper soil horizons (subsoils) are perceived to be physically, chemically and biologically stable compared to topsoils and may provide increased capacity for additional SOC storage (Lorenz and Lal 2005). Consequently, the development of perennial cropping systems with large-rooted cultivars has been recommended to address issues of food security, global climate change and soil degradation by increasing SOC stocks in deeper soil horizons (Glover et al. 2007; Kell 2011). In the USA, recognition of the potential ecological benefits of deep rooted perennial grasses has led to suggestions that managed temperate grasslands should be developed to simulate tallgrass prairie ecosystems (Tilman et al. 2006; Glover et al. 2007; DeLuca and Zabinski 2011).

A small number of sites have been identified in north-central Kansas that serve as benchmarks of the long-term ecological effects of harvesting perennial grasslands for biomass production (Glover et al. 2010). These sites feature a unique pairing of never-cultivated tallgrass prairies that have been harvested for hay annually for approximately 75 years and adjacent wheat (*Triticum aestivum*) fields that have been under intensive cultivation for a similar time period. Improved soil quality (physical, chemical and biological), greater plant and insect diversity, reduced nitrate leaching, and larger annual biomass production at much lower rates of input compared to the wheat system, have been observed in the perennial grassland plots (Culman et al. 2010; Glover et al. 2010). A detailed examination of the storage and turnover of SOC, however, has not been conducted at these sites.

In this study, the unique long-term land-use pairings of grassland and cropland described above were exploited to address the objectives of comparing the storage, turnover, and allocation of organic C in these soils. We quantified SOC stocks up to 1 m depth in multiple SOC pools: bulk SOC, visible root biomass C, primary particle size fractions (2000–250, 250–53, 53–2 and <2 μm) and microbial biomass C (MBC). Separating soil into primary particle sizes and analyzing the associated SOC is a widely used method for comparing the storage and cycling of SOC in distinct pools (Christensen 2001; Bol et al. 2009). In general, sand-sized particles of SOC, or particulate organic matter (POM), constitute recently deposited, undecomposed plant residues that tend to reflect the molecular composition of their source materials (Wander 2004; Grandy and Neff 2008; Von Lutzow

Table 1 Site locations and soil descriptions

Site (Location)	Soil series	Soil description	Texture class Surface (0–10 cm)	% Clay
Niles (N 38°58′145″, W 97°28′616″)	Hord	Fine-silty, mixed, mesic, Cumulic Haplustoll	Silt loam	26
Buckeye (N 39°2′344″, W 97°7′798″)	Hobbs	Fine-silty, mixed, mesic, Mollic Ustifluvent	Silty clay loam	34
New Cambria (N 38°53′54″, W 97°32′615″)	Detroit	Fine, smectitic, mesic, Pachic Argiustoll	Silt loam	23
Goessel (N 38°15′333″, W 97° 22′307″)	Goessel	Fine, smectitic, mesic, Typic Haplustert	Silty clay loam	37
Five Creek (N 38°22′665″, W 97°18′788″)	Muir	Fine-silty, mixed, mesic, Cumulic Haplustoll	Silt loam	22

et al. 2007). Conversely, SOC associated with silt and clay is composed of simpler, microbially-processed C compounds and more stable organo-mineral associations (Von Lutzow et al. 2007; Grandy and Neff 2008). Therefore, in this study, the use of stable ^{13}C isotope analysis in tandem with soil particle size fractionation allowed the observation of SOC pools with relatively distinct turnover times that could be related to a land-use change event 75 years previously. We estimated SOC turnover in each of the pools using natural abundance stable ^{13}C isotope analysis to determine the mean residence times of C_4 tallgrass prairie-derived SOC under C_3 wheat production.

Materials and methods

Study site

The study was conducted on five farms in north-central Kansas that had long term (~75 years) paired land uses of grasslands, i.e. remnant tallgrass prairie meadows used for hay production, and croplands, i.e. conventionally farmed winter wheat cultivation as described by Glover et al. (2010). The site names and locations of the farms are listed in Table 1. All sites were on deep alluvial soils in footslope or stream terrace landscape positions (Table 1). The grasslands were mown annually to ~10 cm height for hay in June and had not received fertilizer applications. The croplands received annual applications of 70 kg N ha^{-1}. Mean annual deposition of inorganic

N in precipitation in the region is 4.6 kg N ha^{-1} year^{-1} (Blair et al. 1998). The mean annual temperature in the region is 13.2 °C and the mean annual precipitation is 730 mm. The soil physical properties (pH, total C, total N, total P, total K, water stable aggregates) for the soil profile to 1 m depth for samples taken in 2006/2007 are given in Culman et al. (2010). The plant community composition of the grasslands was dominated by C_4 perennial grasses and was similar among sites (Glover et al. 2010).

Soil sampling

Soil sampling was carried out during May and June 2008. Both land uses at all five sites were sampled during each of the two dates. May corresponds to peak biomass for the cropland while June is the peak of biomass for the grassland. Five 4 cm diameter soil cores were taken at each site to 1 m depth using an 'X' design to accommodate in-field variation. The cores were divided into 0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm depth increments. The mass of the cores was recorded for each depth for bulk density (ρ_b) measurements. The five cores were bulked according to depth and mixed to achieve a representative homogenous field sample for each site, giving experimental replication of $n = 5$. A field moist sub-sample (~ 50 g) was removed for extraction of microbial biomass carbon (MBC). Gravimetric water content was determined for 100 g sub-samples that were oven dried at 105 °C. The remainder of the soil was air-dried.

Root sampling

Three 6 cm diameter soil cores up to 1 m depth were collected from both land uses at four of the sampling locations (roots were not sampled at Five Creek) and separated into 0–10, 10–20, 20–40, 40–60, 60–80 and 80–100 cm depth increments. Visible roots were separated from soil using a hydropneumatic root elutriator (Smucker et al. 1982) and oven dried at 50 °C for 48 h and weighed to determine root mass per unit area.

Microbial biomass carbon

MBC was determined in sieved (6.75 mm) field moist soil from all sites collected in May and June using chloroform-fumigation extraction (Vance et al. 1987). Soil samples (10 g) were fumigated with chloroform (50 ml) for 24 h then extracted with 80 ml 0.5 M K_2SO_4. The extract was filtered using 0.45 μm pore diameter glass fiber syringe filters. Control samples were extracted using the same process, but were not fumigated. The samples were frozen prior to analysis.

Particle size fractionation

Soil collected in June only was divided into four particle size fractions: coarse sand (2,000–250 μm), fine sand (250–53 μm), silt (53–2 μm) and clay (<2 μm). Briefly, air-dried soil was sieved (2 mm) and 20 g was dispersed in 60 ml of 5 g l^{-1} sodium hexametaphosphate shaken for 16 h (Cambardella and Elliott 1992). The soil slurry was passed through nested 250 and 53 μm sieves using de-ionized water. The residual finer fractions were separated into silt and clay by sequential decantation and siphoning and the clay suspension was floccu-lated using 0.5 M $MgCl_2$ (Rutledge et al. 1967). All fractions were washed into pre-weighed glass beak-ers and oven dried (45 °C).

Total organic carbon and stable ^{13}C isotope analysis

Dried and ground samples of root biomass, whole soil and particle size fractions from each soil depth increment were analyzed for total C using a PDZ Europa ANCA-GSL elemental analyzer (Sercon Ltd., Cheshire, UK). Liquid extracts from the MBC samples were analyzed for total organic C (TOC) using a O.I. Analytical Model 1010 TOC Analyzer (OI Analytical, College Station, TX, USA). Both of these instruments were coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Chesire, UK) to determine the δ^{13}C values of the samples. All C analysis was conducted at the stable isotope facility at the University of California, Davis.

Total SOC pools were calculated according to Lal et al. (1999) based on the depth interval and ρ_b. The C concentration in each particle size fraction was calculated (g C kg soil^{-1}) according to Sollins et al. (1999):

$$C_{(fr)} = C_{fr}W_{fr}10, \tag{1}$$

where, $C_{(fr)}$ is the C (g kg soil^{-1}) in each fraction, C_{fr} is the % concentration of C in the fraction, and W_{fr} is the dry mass of the fraction (g fraction^{-1} g soil^{-1}).

All δ^{13}C values were reported relative to the international reference standard Pee Dee Belemnite (PDB):

$$\delta^{13}C(\permil) = \left[(^{13}C/^{12}C_{sample}{}^{13}C/^{12}C_{reference}) - 1 \right]1000 \tag{2}$$

where, $^{13}C/^{12}C_{sample}$ refers to the individual sample, and $^{13}C/^{12}C_{reference}$ refers to PDB. The standard deviation of the δ^{13}C values for reference standards was 0.2 ‰ for solid samples and 0.4 ‰ for liquid samples.

The MBC concentration was calculated using the method of Voroney et al. (1993). The δ^{13}C values of the MBC were calculated according to an equation from Ryan and Aravena (1994):

$$\delta^{13}MBC = \left(\delta^{13}C_{FUM}C_{FUM} - \delta^{13}C_{CONT}C_{CONT} \right)/ \\ (C_{FUM} - C_{CONT}) \tag{3}$$

where, C_{FUM} and C_{CONT} are the mass of C in the fumigated and control samples, and $\delta^{13}C_{FUM}$ and $\delta^{13}C_{CONT}$ are the δ^{13}C values of the fumigated and control samples, respectively.

The relative proportions of SOC derived from the C_3 wheat and the C_4 prairie grasses were calculated for the whole soil and all particle size fractions in the samples from the cropland sites according to a two end

member mixing model, which assumes that plant C decomposes to SOC without isotopic fractionation (Balesdent and Mariotti, 1996):

$$\% \, C_3 = (\delta_s - \delta_4)/(\delta_3 - \delta_4) \qquad (4)$$

where, $\% \, C_3$ is the proportion of C derived from wheat, δ_s is the $\delta^{13}C$ (‰) of the cropland soil sample, δ_4 is the $\delta^{13}C$ value (‰) of the prairie soil, and δ_3 is the $\delta^{13}C$ value (‰) of the wheat roots for each depth. We used the natural abundance $\delta^{13}C$ values of the visible root biomass extracted from the soils at each depth to calculate the turnover rates of bulk SOC under different land use because the intensive aboveground harvesting activities suggest that roots are the primary belowground C input in both systems, especially at depth; and, Skjemstad et al. (1990) reported that the $\delta^{13}C$ values of roots varied by up to ±2 ‰ with depth up to 80 cm.

The mean residence time (MRT) of SOC was derived by estimating a first order decay rate constant (k) (after Jastrow et al. 1996), assuming that both C_3 and C_4 carbon undergo exponential decay, and that there is no interaction between C_3 and C_4 carbon during decomposition in order to compare relative decay rates (after Skjemstad et al. 1990), according to the equation

$$k = -(x_t/x_0)/t, \qquad (5)$$

where, x_t is the concentration of C derived from C_4 plant biomass remaining in the soil and each particle size fraction of the cropland soil; x_0 is the concentration of C derived from C_4 plant biomass in the corresponding sample from the prairie grassland soil, which represents the baseline (time zero) condition; and, t is the length of time since the conversion from grassland to cropland cultivation (75 years). MRT is then equal to $1/k$ for each sample

Data analysis

All data were analyzed using a mixed model analysis of variance (ANOVA) with the PROC MIXED procedure in SAS software (Cary, NC, USA). Depth and treatment were treated as fixed effects, and site as a random effect. Values presented reflect the analysis of the effect of treatment by depth and were considered significant at $p < 0.05$.

Results

Whole soil SOC

The cropland soils contained significantly less SOC than the grassland soils from 0 to 40 cm depth, and at 80 to 100 cm depth (Table 2). The cropland soils contained 30 % less SOC in the top 60 cm, and 25 % less SOC up to 1 m depth, than the grassland soils (Table 2). SOC concentrations declined significantly with increasing depth in both systems. There was 15 % less SOC from the 40–80 cm depth in the croplands compared to the grassland (Table 2). The bulk $\delta^{13}C$ values of the soils under either land use were significantly different to 80 cm depth (Table 3); the bulk $\delta^{13}C$ values of the grassland soils ranged from −16.2 ‰ (0–10 cm) to −12.7 ‰ (80–100 cm), and the cropland soils ranged from −20.1 ‰ (0–10 cm) to −15.4 ‰ (80–100 cm). Using the $\delta^{13}C$ values, we calculated that approximately 50 % of the total SOC at the 0–10 cm depth, and 90 % of SOC at the 60–80 cm depth, was derived from C_4 plant biomass (Fig. 1a).

Sand-sized SOC fractions

SOC concentrations decreased significantly in both the coarse sand (2 mm–250 μm) and fine sand (250–53 μm) in the cropland soils up to 60 cm depth (Table 2) compared to whole soil. The coarse and fine sand fractions accounted for 3 and 7, and 2 and 5 %, of total SOC in the grassland and cropland soils, respectively. The $\delta^{13}C$ values of the coarse and fine sand fractions were distinct between the two land uses, to a depth of 60 cm in the coarse sand fraction and 80 cm in the fine sand fraction (Table 3). $\delta^{13}C$ values for the coarse sand fraction suggested a complete turnover to entirely C_3 wheat-derived C in the soil surface (0–10 cm), while 85 % of the coarse sand SOC was from the previous C_4 grassland biomass at 60–80 cm depth (Fig. 1b). The fine sand fraction also demonstrated extensive turnover in the 0–10 cm depth such that only 3 % of the SOC was derived from the C_4 grasses, while 85 % of the fine sand C was relic C_4-derived SOC at the 60–80 cm depth (Fig. 1c).

Table 2 Mean ($n = 5$) soil bulk density (Mg m^{-3}), soil C pools (Mg ha^{-1}) and organic carbon concentrations (g C kg^{-1} DW soil) in whole soil and particle size fractions at different depths (0–100 cm) under grassland or cropland

Depth (cm)	Land use	ρ_b (Mg m^3)	SOC Pools (Mg ha^{-1})	Organic carbon (g C kg^{-1} DW soil)				
				Whole soil	Coarse sand	Fine sand	Silt	Clay
0–10	Grassland	1.03***	26.63**	25.9***	1.58**	3.23***	8.12***	13.24***
	Cropland	1.27	17.75	14.0	0.99	1.72	3.37	9.22
10–20	Grassland	1.19***	21.74**	18.3***	0.57**	1.45***	5.42***	11.97***
	Cropland	1.39	14.84	10.7	0.13	0.47	2.24	8.28
20–40	Grassland	1.28***	38.46**	15.1***	0.30**	0.63***	3.69***	10.05***
	Cropland	1.34	26.36	9.9	0.06	0.28	2.24	8.01
40–60	Grassland	1.36 n.s.	28.09**	10.4***	0.21*	0.68*	2.07**	9.49**
	Cropland	1.34	22.80	8.6	0.08	0.21	1.82	7.24
60–80	Grassland	1.43 n.s.	20.82 n.s.	7.1 n.s.	0.12 n.s.	0.15 n.s.	1.21 n.s.	5.82 n.s.
	Cropland	1.37	19.03	7.2	0.13	0.28	1.30	5.49
80–100	Grassland	1.49**	17.04 n.s.	5.8 n.s.	0.21 n.s.	0.21 n.s.	1.03 n.s.	4.57 n.s.
	Cropland	1.40	14.24	5.2	0.11	0.20	1.18	4.41

n.s. indicates that the treatment effect was not significant ($p < 0.05$);

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

Table 3 Mean ($n = 5$) $\delta^{13}C$ values (‰) for carbon pools (roots, MBC, bulk soil and particle size fractions) at different depths (0–100 cm) under grassland or cropland

Depth (cm)	Land use	Carbon Pool $\delta^{13}C$ (‰)						
		Roots	Bulk soil	Coarse sand	Fine sand	Silt	Clay	MBC
0–10	Grassland	−15.3***	−16.2***	−18.3**	−18.1***	−16.9***	−15.6**	−15.1**
	Cropland	−24.0	−20.1	−24.7	−24.0	−21.8	−18.5	−19.5
10–20	Grassland	−14.8***	−14.2***	−15.6**	−16.5***	−15.2***	−14.0**	−13.5**
	Cropland	−25.6	−17.5	−24.6	−23.3	−19.8	−16.8	−18.7
20–40	Grassland	−14.2***	−13.4***	−15.2**	−15.9***	−14.5***	−13.5***	−13.1**
	Cropland	−25.7	−15.1	−22.1	−20.8	−17.2	−15.3	−15.8
40–60	Grassland	−14.4***	−13.2***	−14.9**	−15.7***	−14.9***	−13.6**	−12.0**
	Cropland	−25.3	−14.5	−16.1	−19.3	−17.2	−14.8	−15.8
60–80	Grassland	−16.4***	−13.5*	−16.1 n.s.	−16.9**	−16.3*	−14.1 n.s.	−11.5*
	Cropland	−25.7	−14.8	−14.6	−18.1	−17.9	−15.3	−14.5
80–100	Grassland	−13.7***	−12.7 n.s.	−12.7 n.s.	−15.2 n.s.	−16.8 n.s.	−14.1 n.s.	−10.5 n.s.
	Cropland	−27.7	−15.4	−15.2	−18.0	−18.3	−16.2	−13.1

n.s. indicates that the treatment effect was not significant ($p < 0.05$)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

Silt and clay SOC fractions

The cropland soils had 50 % less SOC in the silt fraction and 27 % less in the clay fraction than the grassland soils to 60 cm depth (Table 2). The clay fraction (<2 μm) contained the highest proportions of the SOC in grassland and cropland soils from 0 to 40 cm depth (63 and 71 %, respectively). The silt fraction (53–2 μm)

Fig. 1 Depth profile of the estimated proportions of SOC contributed by wheat (C_3) and prairie (C_4) carbon sources in the whole soil, particle size fractions, and MBC of the Cropland sites
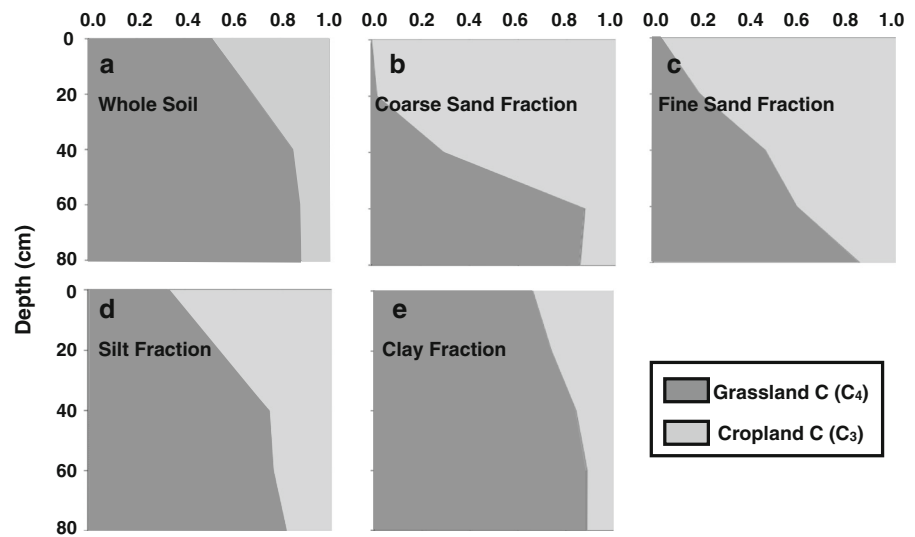

Table 4 Mean residence times of whole soil and particle-sized fractions of soil at up to 80 cm depth in croplands

Depth (cm)	% loss original C4 carbon	Mean residence time (years)				
		Whole soil	Coarse sand	Fine sand	Silt	Clay
0–10	57	74	7	23	46	108
0–20	52	83	25	35	52	122
20–40	45	102	42	51	65	150
40–60	38	135	58	77	90	195
60–80	29	268	90	–	277	357

accounted for 27 % of measured SOC in the grasslands and 21 % in the cropland soils (0–40 cm). The $\delta^{13}C$ values of the silt- and clay-sized fractions were significantly different through the 60–80 and 40–60 cm depths, respectively (Table 3). Estimated SOC turnover in the silt fraction was such that C_4-derived SOC was 33 % in the 0–10 cm depth and 80 % in the 60–80 cm depth (Fig. 1d). The clay fraction retained the highest percentages of C_4-derived SOC with 66 % at 0–10 cm depth and 89 % at the 60–80 cm depth (Fig. 1e).

Mean residence times (MRTs) of SOC

The MRTs of the SOC associated with the different pools were estimated using the difference in the $\delta^{13}C$ values of the SOC in the cropland versus the grassland soils (Table 4). The MRT for bulk soil increased in proportion to depth from 74 years (0–10 cm depth) to 268 years (60–80 cm depth), and increased dramatically

below 60 cm depth. There were distinct MRTs for each particle size fraction: those of the coarse and fine sand particle size fractions were consistent with annual to decadal turnover times (7–90 years), intermediate in the silt particle size fraction (46–277 years), and centennial for the clay fraction (108–357 years).

Root biomass C

Root biomass C was significantly greater in the grassland soils compared to the cropland soils at all depths. There were more visible plant roots throughout the 1 m profile of the grassland soils, equating to 400–600 % more root biomass C compared to the cropland soils (Fig. 2). The grassland soils had more than 600 % more root biomass C in the 0–10 cm depth than the cropland soil in June and the difference between the soils remained statistically significant at depths up to 60–80 cm where the grassland soils had 20 times more root biomass C than the cropland soils (Fig. 2). The $\delta^{13}C$ values of root biomass C were distinct across all depths and attested to the C_3 or C_4 composition of the plant communities in the respective systems (Table 3).

Effects of land-use change on the soil microbial biomass

The MBC pool to 1 m depth was 2.5 and 1.1 Mg ha^{-1} in May; 3.7 and 1.3 Mg ha^{-1} in June, for grassland and cropland soils, respectively (Fig. 3). The MBC pools were larger in June than in May. The $\delta^{13}C$

Fig. 2 Depth profile of total root C (kg C ha^{-1}). Error bars indicate standard error of the means for each depth. Asterisks indicate a significant treatment effect between land uses for a given depth: $*p < 0.05$, $**p < 0.01$, and $***p < 0.0001$
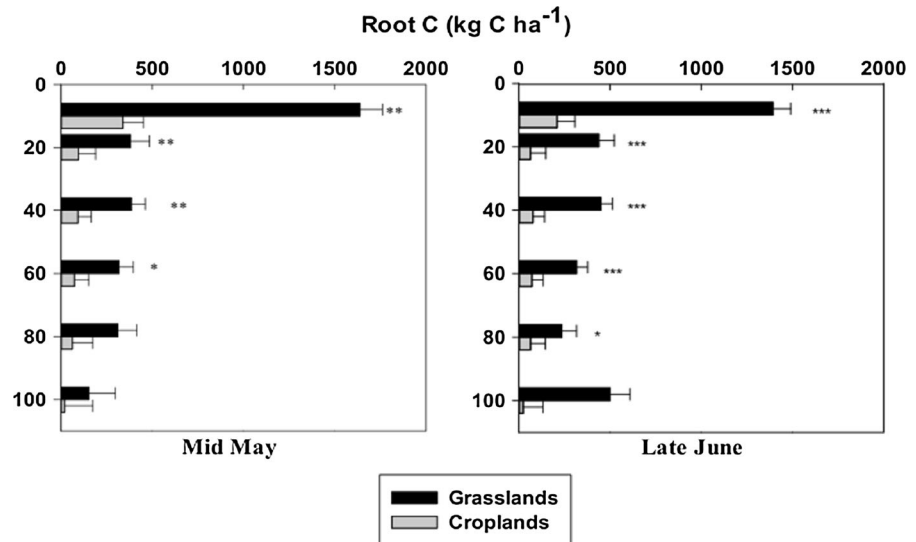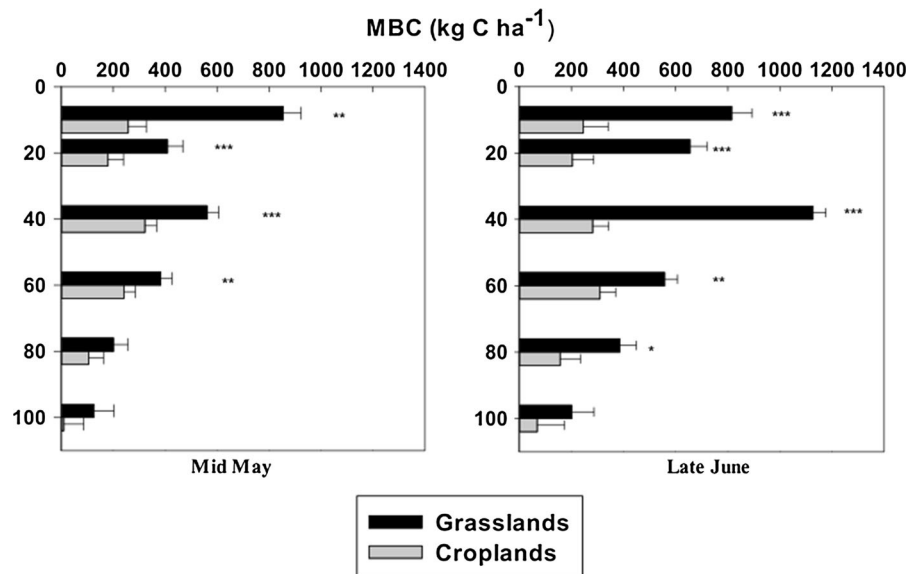

Fig. 3 Depth profile of MBC (kg C ha^{-1}). Error bars indicate standard error of the means for each depth. Asterisks indicate a significant treatment effect between land uses for a given depth: $*p < 0.05$, $**p < 0.01$, and $***p < 0.0001$

values of the MBC in the grassland in June were similar to those of the root biomass in the surface horizons (~ -14 ‰ in the 0–20 cm depth), but these became relatively ^{13}C-enriched with increasing depth (-10 ‰ at 80–100 cm; Table 2).

Discussion

Estimating SOC mean residence times

The most basic assumption of using natural abundance $\delta^{13}C$ values of soils to estimate the MRTs of SOC

following a land-use shift from C$_4$ to C$_3$ vegetation (or vice versa), is that the $\delta^{13}C$ signatures of plant biomass inputs are retained in SOC during decomposition (Balesdent et al. 1987; Balesdent and Mariotti 1996; Amundson and Baisden 2000). However, several factors may add variability to estimating source contributions using this method, including: changes in the $\delta^{13}C$ values of atmospheric CO_2; isotopic discrimination during decomposition; and, interspecific and intraspecific variation in $\delta^{13}C$ signatures in vegetation inputs caused by physiological stresses (Randerson et al. 1999; Amundson and Baisden 2000; Dungait et al. 2008, 2010). Furthermore,

[13]C-enrichment of SOC with depth occurs due to a number of processes; our results indicated ^{13}C-enrichment in all soil fractions with increasing depth in both systems, with the δ^{13}C values of prairie soils around 4 ‰ greater at 80–100 cm than the surface soil horizon (Table 3). Kinetic fractionation against the heavier isotope during microbial metabolism ('Rayleigh distillation') is assumed to lead to the ^{13}C-enrichment of 'older' SOC deposits observed with depth in most soils (Wynn et al. 2005; Bostrom et al. 2007). The downward leaching of dissolved organic carbon (DOC), including the preferential desorption of the heavier isotope in soil surface horizons and desorption of previously leached and adsorbed DOC associated with changes in soil processes following land-use changes may also contribute to altered δ^{13}C values with depth in soils (Sanderman et al. 2008; Kaiser and Kalbitz 2012).

The first-order decomposition equation used to calculate the MRTs of soil fractions assumes that: (1) all of the SOC from the initial C source (C_4 grasses herein) in a given sample or fraction decomposes exponentially; and, (2) that each soil fraction eventually reaches an equilibrium state composed entirely of newer C inputs (Skjemstad et al. 1990). Both the mixing model and the exponential decay process (k) assumed that each soil fraction is a single, well-mixed pool; however, all soil and soil fractions are heterogeneous and contain components that are more or less resistant to decomposition (Torn et al. 2009). Furthermore, whole SOC is likely to be more heterogeneous in its composition than more stabilized fractions (Rumpel et al. 2010), and therefore, MRT estimates derived from δ^{13}C values may be skewed compared to radiocarbon dating by an over-representation of actively cycling 'labile' SOC with relatively ^{13}C-enriched values (Six and Jastrow 2002; Marin-Spiotta et al. 2009; Denef et al. 2009; Derrien and Amelung 2011). Nonetheless, we observed wide differences in MRT estimates between sand-sized and mineral-associated SOC fractions (Table 4), based on the δ^{13}C values of the different particle sizes, suggesting faster and slower cycling SOC pools were associated with different particle size fractions as generally described. We considered that the δ^{13}C values of root samples taken from each sampling depth to be the most accurate inputs for the turnover models because, like Skjemstad et al. (1990), we observed differences in the δ^{13}C value of root biomass with

depth (Table 3), and Rasse et al. (2005) indicated that the majority of SOC is derived from root C. Despite all of the complexity and caveats regarding the precise estimation of MRTs using the methods described herein, the bulk soil MRTs in this study were similar to those calculated using δ^{13}C for cultivated and pasture soils (reviewed by Six and Jastrow 2002), and suggested the profound effect of land-use change on all SOC pools.

Increased turnover of SOC after land-use change

The SOC in all pools was significantly reduced in the cropland *versus* the grassland, but more so in the sand-sized fractions, similar to previous studies (Tiessen and Stewart 1983; Cambardella and Elliott 1992; Russell et al. 2005; Purakayastha et al. 2008). The decline in root inputs in cropland soils after 75 years is a likely mechanism for the reduction in sand-sized or POM SOC fractions, although the observed mean δ^{13}C values of these fractions were enriched in all horizons compared to a soil under a long-term C_3 plant community (e.g. −27 ‰; e.g. Bol et al. 2009). A previous study observed significantly higher root and litter decomposition rates in wheat systems compared with prairies attributed to disturbances such as tillage, more rapid decomposition of wheat roots during the warmest months of the year, and high transpiration rates of prairie grasses which limit soil moisture available for decomposition (Buyanovsky et al. 1987).

The relative proportion of C_4-derived C increased with depth in the soil profile suggesting preservation of subsoil C derived from the legacy grassland. Other processes may also have contributed to these observed changes in δ^{13}C values with depth, including the downward transport of ^{13}C in DOC, as described above (Sanderman et al. 2008). Subsoil C is increasingly recognized as an important contributor to total C stocks within a soil profile (Rumpel et al. 2012; Harper and Tibbett 2013). Deeper SOC is assumed to be very stable, with radiocarbon ages of more than 4,000 years reported (Jenkinson and Coleman 2008). However, the SOC turnover in deeper (>40 cm) soil horizons in croplands described herein is consistent with previous reports of a substantial proportion of subsoil C turning over on a decadal time scale (e.g. Baisden and Parfitt 2007). Agricultural tillage operations generally do not exceed 30 cm depth (Angers and Eriksen-Hamel 2008); thus, the observed SOC turnover in deeper

soil layers is consistent with mechanisms other than the direct disturbance of tillage. Possible factors include altered root C dynamics (Chabbi et al. 2009) and changes in the quantity and composition of DOC leaching downward in the soil profile (Kaiser and Kalbitz 2012). This study suggests that land-use change from natural vegetation (prairie) to annual cropland drives SOC loss in deeper soil horizons.

The estimated MRTs of prairie-derived SOC 75 years after land-use change to cropland demonstrated markedly longer turnover times in fractions associated with smaller particle sizes especially those in deeper soil horizons, which is consistent with the current understanding of SOC dynamics (Von Lutzow et al. 2007; Torn et al. 2009; Rumpel et al. 2012). The development of very stable complexes between organic carbon and specific clay minerals has been highlighted as a likely mechanism that confers resistance to decomposition (Six et al. 2002; Kögel-Knabner et al. 2008). Organo-mineral associations may further combine to form soil aggregates, thus enhancing SOC protection (Martens et al. 2003). In this study, the relative contribution of SOC in the clay fraction to total SOC increased in proportion to depth under both land uses, supporting the proposition that the majority of C in deeper soil layers tends to occur in organo-mineral associations (Rumpel et al. 2012). $\delta^{13}C$ values of the silt and clay fractions in the cropland soils also indicated a significant incorporation of wheat-derived C in these fractions (Fig. 1d, e). Sorption of DOC (Kaiser and Kalbitz 2012) and interactions of minerals with fine roots (Swanston et al. 2005; Rasse et al. 2005) are potential mechanisms by which recent C inputs are incorporated into mineral associations. Although physical protection may be the strongest SOC preservation mechanism in soils (Dungait et al. 2012), the observed significant difference in the $\delta^{13}C$ values of the clay fraction down the soil profile of the cropland soil compared to the prairie soil after 75 years strongly supports the observation by O'Brien et al. (2013) that a portion of organo-mineral associated SOC turns over on a decadal time scale and that these fractions are not uniformly stable.

An obvious mechanism for reduced SOC stocks is decreased inputs from roots in the soil profile. The root-to-shoot biomass ratio of the grasslands was 2.75 compared to 0.4 for the croplands (based on a 1.5:1 residue to grain ratio for wheat; Lal 1995), which are in the range of global averages for similar ecosystems (Jackson et al. 1996). Our observation of significant SOC decline in arable croplands is similar to that reported for other North American sites where native grasslands have been converted to agriculture and cultivated for several decades (Tiessen and Stewart 1983; Purakayastha et al. 2008; David et al. 2009) and are consistent with previous measurements of SOC pools at the study sites in 2007 (Culman et al. 2010). If the SOC pools of the grassland sites (135.7 Mg C ha^{-1} in 0–80 cm) are considered as a baseline of total SOC content, which is supported by observations of stable SOC pools in long-term hayfields at Rothamsted UK (Hopkins et al. 2009), then our estimates suggest that 42 % or 56.8 Mg C ha^{-1} of the original prairie SOC had been lost following land-use change, which is equivalent to total emissions of 210 Mg CO_2 ha^{-1}. This figure is consistent with the range of previous SOC loss estimates in tallgrass prairie-derived agricultural soils (Huggins et al. 1998; DeLuca and Zabinski 2011; Follett et al. 2009).

Soil microorganisms, which are also a pool of SOC (MBC), largely drive the decomposition of all other SOC pools. A range of factors that affect the activity of the soil microbial biomass may be precipitated by land-use change including changes in the supply and quality of C and nutrients, and changes to the soil environment including pH, oxygen and moisture status. The initial decomposition of the prairie root system, particularly immediately following tillage, may have triggered positive priming of SOC (Kuzyakov et al. 2000; Fontaine et al. 2007). The trend towards C_4 values of the MBC with depth in the cropland soils indicates that the soil microorganisms in the deeper soil horizons were using a mixture of novel and relic SOC from grasslands (Table 3). However, based on the $\delta^{13}C$ values, approximately three-quarters of the SOC in the cropland soils was derived from the previous grassland vegetation (Fig. 1a). This 'old' SOC preservation, i.e. at least 75 years old, throughout the soil profiles suggests that the potential of the soil microorganisms to access and mineralize a large proportion of the old grassland-derived C had been restricted after land-use change to wheat cultivation.

Conclusions

Land-use change from a prairie grassland where deep-rooting vegetation types predominate to wheat, which has a much smaller root system, caused a significant loss of SOC over 75 years. The natural abundance $\delta^{13}C$ values of the native grasses compared to the crop allowed the mean residence times for the different pools to be estimated. The SOC content of all soil C pools was reduced and was less in the sand-sized fractions, which had the most rapid MRT of the soil pools. However, there was also turnover in the clay-sized pool suggesting that physical protection of SOC in organo-mineral associations, even in deeper soil horizons, is also affected by perturbations to the surface soil horizons. The change in root biomass appeared to be key in the dynamics of the microbial biomass, which was larger in the grassland soils. Overall, this new work supports the premise that a portion of all SOC fractions are heavily influenced by land-use change and that production systems based on perennial grasses transfer greater quantities of C to SOC pools than annual crops, through larger C allocations to root and microbial pools.

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