Differential soil organic carbon storage at forb- and grass-dominated plant communities, 33 years after tallgrass prairie restoration

Matt D. Ampleman, Kerri M. Crawford & David A. Fike

🕮 Springer

Springer

REGULAR ARTICLE

Differential soil organic carbon storage at forb- and grass-dominated plant communities, 33 years after tallgrass prairie restoration

Matt D. Ampleman · Kerri M. Crawford · David A. Fike

Abstract

Background and aims Dominance of C_4 grasses has been proposed as a means of increasing soil organic carbon (SOC) sequestration in restored tallgrass prairies. However, this hypothesis has not been tested on long time scales and under realistic (e.g. N-limited) environmental conditions. We sampled a restoration in southern Illinois 33 years after establishment to determine the effects of varying plant communities on SOC sequestration in the top 50 cm of soil.

Methods SOC, total nitrogen (TN), and the stable isotopic composition of SOC ($\delta^{13}C$) were used to calculate SOC sequestration rates, N storage, and the relative contributions of C_3 vs. C_4 plant communities as a function of soil depth.

Results While both a forb-dominated and a mixed forb-grass plant community showed positive sequestration rates (0.56 ± 0.13 and 0.27 ± 0.10 Mg C ha^{-1} yr^{-1}, respectively), a C_4 grass-dominated community showed SOC losses after 33 years of restoration (-0.31 ± 0.08 Mg C ha^{-1} yr^{-1}). Soil $\delta^{13}C$ values were significantly more negative for forb-dominated plant communities, increasing the confidence that plant communities were stable over time and an important contributor to differences in SOC stocks among transects.

Conclusion These results suggest that functional diversity may be necessary to sustain sequestration rates on the scale of decades, and that dominance of C_4 grasses, favored by frequent burning, may lead to SOC losses over time.

Keywords Prairie restoration · Soil organic carbon sequestration · C_4 grasses · Forbs · Stable carbon isotopes

M. D. Ampleman (✉) · D. A. Fike
Department of Earth & Planetary Sciences, Washington University, 1 Brookings Dr., CB 1169,
St. Louis, MO 63105, USA
e-mail: mdamplem@wustl.edu

K. M. Crawford
Tyson Research Center, Washington University,
1 Brookings Dr., St. Louis, MO 63130, USA

Introduction

Grassland restorations have been shown to sequester soil organic carbon (SOC) at rates of ~0.5 Mg C Ha^{-1} yr^{-1} averaged over several decades through increased belowground primary productivity and stabilization of soil organic matter (Burke et al. 1995; Potter et al. 1999; Conant et al. 2001; McLauchlan et al. 2006; Kucharik 2007; Matamala et al. 2008). These values are comparable to SOC sequestration rates obtained by reforestation (Post and Kwon 2000), where sequestration is defined as the net accumulation (or loss) of SOC compared to a control land use. However, not all restored grasslands have shown significant sequestration, and questions remain about whether measured SOC accumulation rates

(e.g. 0.5 Mg C Ha^{-1} yr^{-1}) can persist through time (Baer et al. 2000; Brye and Kucharik 2003). Furthermore, strategies for enhancing SOC accumulation provide conflicting views on the role of plant diversity and specific functional groups (e.g. Wedin and Tilman 1996; Tilman et al. 2001; Matamala et al. 2008). In general, dependence of soil properties on plant functional groups has been difficult to evaluate, due to poor recruitment, statistical challenges, and plant community changes over time (Kucharik et al. 2006; McLauchlan et al. 2006; Cahill et al. 2009). Overcoming these challenges is critical to determining effective management strategies for SOC sequestration and other ecosystem services.

Stable isotope measurements provide an important and powerful tool to help address these challenges. In prairies, two primary plant groups – C_3 and C_4 plants – fix atmospheric carbon by differing photosynthetic pathways with distinct isotopic signatures (Hatch and Slack 1966; Hayes 2001). The C_3 prairie plants include some grasses and the vast majority of forb species (Sage and Monson 1999), and produce biomass with isotopic values ($\delta^{13}C$) between -35‰ and −21‰ (Deines 1980; O'Leary 1988). This category includes leguminous forb species, which host nitrogen-fixing bacteria in their root nodules and are important regulators of N availability in prairie ecosystems. In contrast, C_4 prairie plants consist almost exclusively of grasses and sedges, and produce biomass with $\delta^{13}C$ values between −20‰ and −9‰ (Deines 1980; O'Leary 1988). C_4 grasses also have greater N-use efficiencies and may have a competitive advantage over C_3 species under sufficient sunlight; in low-N, warm, and water-limited environments; and where regular burning is applied (Seastedt et al. 1991; Knops and Tilman 2000; Wedin 1995). Due to their differing isotopic values, the relative contributions of C_3 vs. C_4 plants to SOC can be evaluated as a function of depth by examining carbon isotope profiles. However, this method has rarely been used in grassland restoration studies (Mahaney et al. 2008).

On account of their high N-use efficiencies, the establishment of C_4 grasses has been promoted as a means of increasing prairie biomass and organic matter deposition (Knops and Tilman 2000; Matamala et al. 2008). These warm-season grasses typically have greater belowground net primary productivity and root turnover than other prairie plants and may provide greater contributions to SOC pools (Craine et al. 2002; Matamala

et al. 2008). This latter possibility has not been tested over long time scales, however, and may be difficult to verify (McLauchlan et al. 2006). Experimental plots have demonstrated that the presence of C_4 grasses together with leguminous forbs (mostly C_3 plants) may account for the greatest productivity gains in restored grassland plots (Tilman et al. 1997). In non-experimental plots, however, C_4-dominated restorations have not significantly out-performed other restorations in terms of productivity and SOC accumulation (e.g. McLauchlan et al. 2006), and it is not clear if C_4 grasses can sustain sequestration rates under non-ideal (e.g. N-limited) conditions (Mahaney et al. 2008). Dominance of C_4 grasses can also change litter quality and decrease N availability in restored prairies with consequences for carbon sequestration (Baer et al. 2002; Camill et al. 2004). Accordingly, restoration practices that contribute to the dominance of C_4 grasses, such as frequent burning (Gibson 1988; Seastedt et al. 1991; Collins et al. 1995) and intentional selection of C_4 grasses, should be considered in light of these associated changes to the N cycle (Baer et al. 2002). Special consideration should be afforded to annual burn regimes, which tend to favor the transition to C_4-dominated plant communities (Gibson 1988; Collins et al. 1995). This preference may be driven by N-volatilization through fire leading to N-limitation, a condition in which C_4 grasses are competitively advantaged (Seastedt et al. 1991). Other studies have found altered light availability to be a major determinant of C_4 competitiveness following burning (Turner and Knapp 1996). C_4 grasses may further this progression by offering easily flammable material, and by decreasing N-mineralization rates in prairie ecosystems (Wedin and Tilman 1990; Baer et al. 2002; Camill et al. 2004). All of these factors tend to drive a prairie ecosystem towards C_4 dominance where fire is frequent and early (i.e.. in the spring; Hulbert 1988). However, C_4 grasses may dominate without the effects of fire as well, and the resulting impacts on N and C cycling may have unintended consequences for SOC sequestration (Knops and Tilman 2000; Baer et al. 2002; Camill et al. 2004; Cahill et al. 2009).

For determining sequestration, it is important to measure SOC changes at depth, which may arise due to historical and recent land use (Kravchenko and Robertson 2011). Many restoration studies have only sampled to 20 cm depth, and have found significant SOC gains only in the top 5–15 cm of the soil (Conant et al. 2001; e.g. Kucharik et al. 2003; McLauchlan et al.

2006; Kucharik 2007). Furthermore, the majority of recent prairie studies have provided sequestration rates by depth increments, a method which is vulnerable to disparities in soil density (Ellert and Bettany 1995; VandenBygaart and Angers 2006; Lee et al. 2009). By using an equivalent soil mass (ESM) for all samples, SOC stocks can be corrected for changes in soil density caused by restoration, a factor which can greatly affect measured SOC stocks (Ellert et al. 2002). For these reasons, appropriate density corrections and sampling depths should be applied in sequestration studies.

Regardless of the method of comparison, however, most restored prairies still represent C sinks relative to the atmosphere; whereas agricultural activities remain a C source to the atmosphere, especially when factoring in emissions associated with fertilizer production, tractor fuel, pesticides, and other inputs (Lal et al. 1998). A number of important questions remain, however, regarding sequestration strategies. Namely, what factors can best sustain sequestration on the scale of decades, and what can explain the low sequestration rates of some restoration projects (e.g. Baer et al. 2000; Kucharik 2007; Stockmann et al. 2013). It is possible that succession and a loss of diversity with prairie age, have contributed to the poor success of some restoration projects (Matamala et al. 2008); however, few data have been reported on the effects of directional plant community changes on SOC accumulation (Kucharik et al. 2006; Mahaney et al. 2008; Matamala et al. 2008).

In order to address these questions, four transects were assessed at a 33-year tallgrass prairie restoration in southern Illinois. Sections of the prairie had been restored in stages and exhibit varying assemblages of grasses and forbs, providing the opportunity for paired-site comparison with adjacent cropland under pre-existing management. SOC stocks were measured by plant community and depth, as a means of determining the factors responsible for the success – or lack thereof – of SOC accrual at a tallgrass prairie restoration. The study was designed to consider (1) if the use of stable C isotopes could help evaluate the effect of plant community on SOC storage after restoration; (2) whether transects dominated by C_3 or C_4 plants resulted in differential SOC sequestration as a function of depth; and (3) if significant SOC sequestration could be demonstrated through 50 cm of soil on an annually burned, 33-year prairie restoration.

Materials and methods

Site description

The Heartland Prairie is located at Gordon Moore Park (GMP) in southern Illinois, on a formerly glaciated upland plain near the Mississippi River (Fig. 1). This site was specifically chosen as a long-running restoration (33 years) where previous land use had been maintained on adjacent plots. Specifically, GMP had been restored from cropland that had been cultivated for more than 100 years, growing a rotation of corn (C_4) and soybeans (C_3). Adjacent plots, separated by a gravel access road, are still cultivated yearly under the same corn and soybean rotation. Restoration at GMP progressed in stages, beginning in the spring of 1977 and continuing in 1-to-8 acre sections through the early 1990s. The prairie was planted to be a high-diversity assemblage of local remnant species, including over
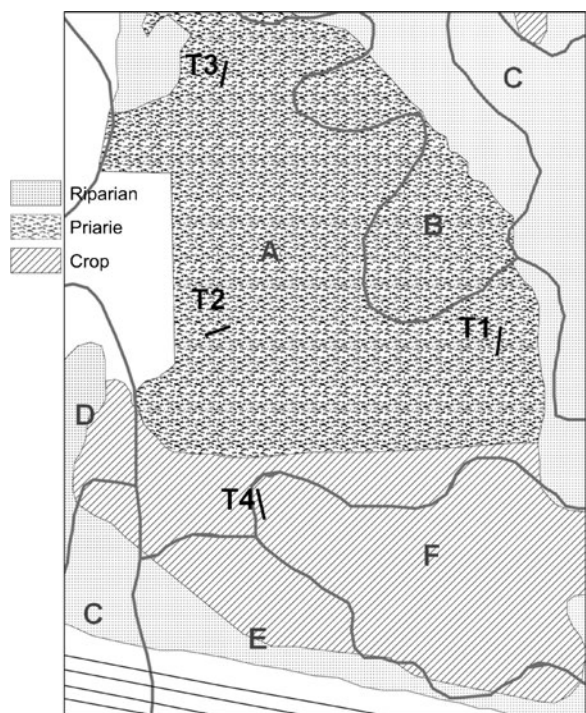
Fig 1 Location of study site: Gordon Moore Park in Alton, Illinois. Transect T1 is the Grass Transect, T2 the Mixed Transect, T3 the Forb Transect, and T4 the Crop Transect. MUSYM (map unit symbol) soil units are outlined with gray polygons: **A**, Winfield silt loam, 2–5 % slopes. **B**, Winfield silt loam, 2–5 % slopes, eroded. **C**, Menfro silt loam, 10–18 % slopes, eroded. **D**, Menfro silt loam, 5–10 % slopes, eroded. **E**, Menfro silt loam, 2–5 % slopes. **F** Winfield silty clay loam, 5–10 % slopes, severely eroded (USDA 2009)

100 forbs and 9 grasses over 28 acres (Newman 1998). No precise records of yearly sown seed were kept, but each planting contained a mixture of both grasses and forbs, with the proportion of planted forbs vs. grasses increasing with later plantings. Through the first 23 years of restoration, the prairie has been burned annually in its entirety every spring to control weeds. No burns were conducted 3 of the 10 years preceding the study, but regular burning of the entire prairie resumed in 2008 (Online Resource 1). The intensity of burns has varied with the location in the prairie, with weak burns occurring in the most forb-dominated sections, a factor that may affect both long-term community structure and carbon sequestration. Charcoal occurrence was not commonly observed in soil samples or at the surface.

Soils at the site are silty hapludalfs of the Winfield series. The prairie soils are mostly silt loams with 2–5 % and 5–10 % slopes, while the crop soils are silty clay loams on 2–5 % and 5–10 % slopes. The steepest portions of both the cropland and the prairie are designated as eroded, with a small amount of the cropland sections designated as severely eroded in the USGS soil survey (Fig. 1; USDA 2009).

Sampling

To compare how SOC accumulated under communities of C_3 vs C_4 plant groups, we established four 21-mtransects at GMP, one at the oldest prairie plot, planted in 1977 containing >66 % C_4 grasses (Grass Transect, 15 cores); two from younger portions of the prairie, planted in 1983 (Mixed Transect, 15 cores) and in 1987 (Forb Transect, 5 cores), containing <50 % and <10 % C_4 grasses, respectively; and a fourth taken from the cropland to serve as an approximate baseline (Crop Transect, 7 cores) (Fig. 1). All the prairie transects were taken from the same soil unit, Winfield silt loam, with designated slopes of 2–5 %. Thus, soil texture was constant, and variation in slope was minimal across transects, as transects were deliberately taken from flat locations, undissected by swales. Although the Grass Transect is closer to the riparian zone, it remains 100 m from the ephemeral creek on the property and more than 10 m higher than the creek itself (Fig. 1). Accordingly, we do not expect soil moisture content to vary appreciably among the transects.

Soils were sampled in the fall and winter of 2009 using a 1.9-cm inner diameter, single gauge corer. Two cores were retrieved successively from each sampling location, one from 0 to approximately 30 cm depth, and a second from approximately 30 cm depth to 50 cm depth. These cores were then cut into depth increments of 10 cm, before applying a correction for ESM based on bulk soil density. Bulk soil density was calculated as the dry weight of the soil increment, measured after drying at 70 °C for 24 h, divided by the volume of the soil core (i.e. 1.13×10^{-4} m^3 for each 10 cm increment). Fewer cores were taken from the Forb and Crop transect (5 and 7 as opposed to 15), because they were sampled later in the study, after it was determined that a smaller sample size was sufficient for statistical comparison. Accordingly, each successive sampling location was 1.52 m from the previous location, for the Crop and Forb Transects, and 6.10 m from the previous location for the Grass and Mixed Transects, with two additional cores taken from off-transect locations on the cropland (see Fig. 1).

The composition of these plant communities reflects both initial species' distribution and potentially a shift towards C_4 grasses over time. While the oldest transect was planted with a higher percentage of grasses than the other two transects, we also expect that the older two transects may have shifted towards greater C_4 representation over time, as annually burned prairies often trend towards C_4 dominance (Gibson 1988; Collins et al. 1995). The forb-dominated transect was likely unaffected by this shift in vegetation, as it was poorly burnt in most years, possibly due to low fuel potential of some forb species (Nan Adams, personal communication). Accordingly, restoration age and plant community type are confounded in this study. However, prairie managers indicate that plant community composition was relatively stable over time (Nan Adams, personal communication), and the length of restoration indicates that these transects have surpassed what is usually the period of greatest change for both plant communities and soil properties, i.e. the first 10 years of restoration (Knops and Tilman 2000; Baer et al. 2002).

We did not pursue replication of transects due to the lack of restorations of comparable age and management within a 100-mile radius of our facilities. GMP was chosen for to its accessibility, documented management history, length of restoration, and the presence of multiple plant communities. Recognizing the limitations of a single-site approach, we deemed that these unique benefits warranted study despite a lack of

replication. We also note that the Crop Transect appears to be at the edge of the eroded soils unit (Fig. 1). Any SOC losses over time, or initial disparity in SOC stocks, due to erosion will hinder our ability to accurately assess SOC sequestration rates in these sites. To minimize this possibility, the Crop Transect was located at the summit of a small rolling hill, where the slope was minimal, and the erosion assumed to be negligible. Furthermore, current cropping practices tend to minimize erosion: the disk till method used on the crop fields during the last several decades left crop residues for protection from wind and water erosion (Ray Sinclair, personal communication, September 2009). To capture the spatial variation of SOC in cultivated rows, the cropland soil was sampled across the ridge and furrow topography: two cropland cores were taken from ridge-top locations, two were taken from ridge-slope locations, and one was taken from the middle of the furrow. These measures should allow the Crop Transect to capture the spatial variation of the soils, while providing a conservative estimate of sequestration based on marginally or un-eroded soils at flat or gently sloping sites.

Preparation and analysis of soils

Soil cores were broken and dried at 70 °C; passed through a 1.5 mm sieve, at which point rocks, roots, and other vegetation were removed; and ground by mortar and pestle, reducing grains to a powder for combustion. Soils of the Winfield series are designated as containing no carbonates (USDA 2009), but samples were tested to ensure pH values less than 7 before proceeding with analysis.

Total organic carbon (TOC), total nitrogen (TN), and carbon isotopes ($\delta^{13}C$) were measured by catalytic combustion and mass spectrometry, using a Costech ECS 4010 elemental analyzer coupled to a Thermo Fischer Delta V Plus isotope ratio mass spectrometer. We used TN and TOC measurements to determine if N and C have accumulated or been lost over time, and $\delta^{13}C$ data to evaluate long-term plant community composition and expected SOC contributions from C_3 vs. C_4 species at depth. Each depth increment was measured in duplicate for SOC. To determine SOC stocks, we multiplied TOC values by the density of each soil core fraction and its thickness to obtain SOC in g C m^{-2} before correcting for ESM. Here, we define sequestration strictly as the net formation (or loss) of

SOC, compared to a control, following land use change (Post and Kwon 2000). Sequestration rates were determined by taking the difference of SOC in restored prairies and cropland and finally dividing by restoration age to achieve the rate in Mg C ha^{-1} yr^{-1}. For this study, three ESMs were chosen to represent the 0–30 cm, 0–50 cm, and 30–50 cm depth increments. ESMs were chosen as the lowest average core mass measured for that depth increment. At GMP, the average soil mass cored to a depth of 50 cm ranged from 162.0 g (at the Mixed Transect) to 186.3 g (at the cropland). Therefore, if these transects were compared by depth, an average 24.3 g of extra mass would be measured at the cropland site, artificially inflating its SOC stock relative to prairie transects.

Plant cover survey

Percent plant cover was estimated for each prairie transect by visual inspection on 19-April-2010, several weeks after the prairie had been burned. Surveys included fifteen 1-m^2 plots along each transect, representing the maximum number of soil cores taken for any one transect. Ground cover was classified as one of three types: grass, forb, or bare ground. At the Forb Transect, % cover was reported as a single value for the whole transect (95 % forbs and 5 % grasses), due to the extreme dominance of forbs and homogeneity in cover. To present plant community composition as a single variable, plant cover is reported as the ratio of grass cover to forb cover as an areal percentage.

C_3 vs C_4 contributions to SOC

A simple end-member mixing model was applied to evaluate C_3 and C_4 plants' ultimate contributions to the existing SOC pool, using the ratio

$$\frac{C_4 \, SOC}{C_3 \, SOC} = \frac{\left(\delta^{13}C_{C3, \, reported} - \delta^{13}C_{SOC,observed}\right)}{\left(\delta^{13}C_{SOC,observed} - \delta^{13}C_{C4,reported}\right)} \quad (1)$$

where $\delta^{13}C_{SOC,observed}$ was evaluated at each transect for the upper and lower ESM; $\delta^{13}C_{C3, \, reported} = -28‰$; and $\delta^{13}C_{C4, \, reported} = -13‰$, representing the median of commonly reported values for C_3 and C_4 biomass in grassland ecosystems (Fernandez et al. 2003; Mahaney et al. 2008). In general, $\delta^{13}C$ of C_3- and C_4-derived SOC will vary slightly from these averages and precise measurements of on-site vegetation's $\delta^{13}C$ should be

used where possible. Additionally, $\delta^{13}C$ of deeper, more decomposed SOC can be shifted to slightly heavier or lighter ratios by a number of processes (Boström et al. 2007). We do not account for these depth effects here, but note that many of them would produce a systematic shift in $\delta^{13}C$ and would not affect comparisons among the transects studied here.

Statistical analysis

First, we tested whether transects did, in fact, significantly differ from one another in plant community composition. To achieve this, we employed an ANOVA model with the fixed effect of transect (Proc GLM, SAS Institute 2009). Grass/forb ratios were log transformed prior to analysis to meet assumptions of normality of residuals and homogeneity of variances. Next, using the same ANOVA model, we tested whether transects differed in SOC. SOC was tested for both the "upper" ESM (~0 cm–30 cm), and "lower" ESM (~30 cm–50 cm), as well as the "total" ESM (~0–50 cm). These depths were chosen to evaluate sequestration generally above and below the plow horizon. Then, we tested how four soil properties, as averaged over the top 30 cm of soil, differed among transects using M/ANOVA models with the fixed effect of transect (Proc GLM, SAS Institute 2009). The following response variables were examined: $\delta^{13}C$, TOC, TN, and C:N. The MANOVA analysis found a significant effect of transect on all response variables considered together ($F_{12,45}$=13.97, P<0.0001); we subsequently performed protected ANOVA on each response. We chose to run an analysis on the top 30 cm of soil as that is where we expected soil properties to most strongly reflect recent plant community composition. Replicates within a transect were excluded if they did not include all values for the 0–10 cm, 10–20 cm, and 20–30 cm depths. All responses met assumptions of normality of residuals and homogeneity of variances.

Finally, we examined how those four soil properties responded to transect and depth over the entire soil profile using repeated measured mixed models (Proc MIXED, KR-corrected, SAS Institute 2009), where depth within a replicate was the repeated measure. The full model included the fixed effects of transect and depth, the transect by depth interaction, and the random effect of replicate nested within transect. As before, if a replicate for one of the responses was missing values for a depth class, it was excluded in

the analysis of that response to avoid skewing results. Covariance models were chosen for each response based on AIC. Following all statistical tests, Tukey's HSD was used to determine what aspects of a treatment were significantly different from one another when a significant treatment effect was detected.

For SOC, an outlier core from the Cropland Transect was removed to normalize that data set. This core was taken from the furrow and had an SOC value more than 3 standard deviations lower than the mean. Removing this outlier provides a more conservative estimate of sequestration for the Forb and Mixed Transects, and therefore does not invalidate the trends discussed here.

Results

Plant cover survey

The three transects differed in their grass/forb ratios, which were used to categorize the transects by plant community (Table 1). The Grass Transect had a significantly higher grass/forb ratio (1.95±0.49) than the Mixed Transect (0.86±0.15; $F_{1,28}$=6.79, P=0.01). In these transects, the following plants were most common: the C_4 grasses *Andropogon gerardii* and *Sorghastrum nutans*; the forbs *Helianthus grosseserratus*, *Eryngium yuccifolium*, *Silphium laciniatum*, and *Silphium terebinthinaceum*; and the legume *Senna hebecarpa*. C_3 grasses were not observed at these transects. The third transect, the Forb Transect, was dominated by forbs, and in particular was typified by *Helianthus grosseserratus*. There was no observed variation in plant cover among the replicates within the Forb Transect. Thus, this transect could not be included in the statistical models; however, the grass/forb ratio of this transect, 0.06, falls well outside the range of values for the Grass Transect and the Mixed Transect.

$\delta^{13}C$

Values for $\delta^{13}C$ corroborate the designations from the plant community survey (Fig. 1). Averaged $\delta^{13}C$ values for the top 30 cm were significantly different among the three prairie transects (Table 2; $F_{3,25}$=23.82, P<0.0001). The Forb Transect had the most negative, C_3-like, value (−23.3±0.3‰) and the Grass transect had the least negative, most C_4-like value (−18.2±0.3‰). Differences

Table 1 Transect plant cover and SOC stocks. SOC stocks reported as g C m^{-2} with standard errors

Transect plant cover and SOC stocks

Plant community	Grass/Forb	Estimated C$_4$/C$_3$ SOC		SOC Stock (g C kg^{-1} m^2 × ESM)			Apparent SOC Accum.	SOC Accum. Rate
		0–30 cm	30–50 cm	Upper ESM	Lower ESM	Total ESM	Total ESM	Total ESM
ESM/Depth	–			279 kg m^{-2}	289 kg m^{-2}	568 kg m^{-2}	(g C kg^{-1} m^2 × ESM)	(Mg C ha^{-1} yr^{-1})
ESM/(Units)	–							
Crop (n=6)	–	1.1±0.2	2.6±0.7	4,120±230 a	2,390±120 c	6,510±240 b	–	–
Forb (n=5)	0.06±0.00*	0.46±0.1	1.6±0.3	6,020±210 b	1,780±50 d	7,800±180 d	1,290±300	0.56±0.13
Mixed (n=9)	0.86±0.15 a	1.1±0.2	2.6±0.7	4,220±70 a	3,020±110 d	7,240±120 c	730±270	0.27±0.10
Grass (n=6)	1.95±0.49 b	1.9±0.4	2.2±0.5	4,170±70 a	1,300±90 a	5,470±80 a	−1,040±250	−0.31±0.08

Means in each column followed by the same letters are not significantly different at $p<0.05$

* The plant cover for the Forb transect was homogenous and is reported as a single log transformed grass-to-forb ratio. Log transformed grass-to-forb ratios for the other two transects are reported as means (n=15)

among transects held when the entire soil profile was examined (Table 2; $F_{3,24}$=18.01, $P<0.0001$), and across all transects $\delta^{13}C$ values became less negative with depth (Fig. 2), from −21.0±0.5‰ at 0–10 cm to −17.5±0.2‰ at 40–50 cm ($F_{4,56}$=76.32, $P<0.0001$). The ratio, $\frac{C_4\ SOC}{C_3\ SOC}$ increased with depth for all transects, and was highest for the Grass Transect and lowest for the Forb Transect (Table 1).

Soil organic carbon stocks and sequestration rates

The three prairie transects and the crop transect differed significantly in TOC averaged over the top 30 cm (Fig. 3, Table 3; $F_{3,23}$=92.21, $P<0.0001$) and over the entire 50 cm depth (Table 3; $F_{3,\ 32}$=55.66, $P<0.0001$). Over the top 30 cm, the Forb Transect had the highest value, 2.51±0.06 %, the Grass Transect had the lowest value, 1.26±0.02 % and the Mixed and Crop Transects had intermediate values for %TOC, 1.51±0.03 % and 1.61±0.11 %. Across all transects, %TOC significantly decreased with depth ($F_{4,48}$=211.37, $P<0.0001$), but the degree of decrease differed among the transects ($F_{12,64}$=18.53, $P<0.0001$). The decrease with depth was most pronounced for the Forb Transect (3.70 ±0.21 % at 0–10 cm; 0.57±0.03 % at 40–50 cm) and was least pronounced for the Mixed Transect (1.99 ±0.09 % at 0–10 cm; 1.04±0.05 % at 40–50 cm).

SOC stocks followed the patterns described above regarding TOC and were significantly different among the transects for the entire soil profile sampled (total ESM, $F_{3,23}$=35.26, $P<0.0001$; upper ESM, $F_{3,23}$=35.10, $P<0.0001$, lower ESM, $F_{3,23}$=52.10, $P<0.0001$). Values are reported for three equivalent soil masses (ESM), 279, 568, and 289 kg m^{-2}, representing approximately the top 30 cm, the top 50 cm and the 30–50 cm depth, respectively (Table 1). Results for the total ESM are as follows: both the Forb (23 years) and Mixed Transects (27 years) had significant gains in SOC of 1,290±300 and 730±270 g C m^{-2}, respectively, as compared to the proxy baseline of the crop SOC (Table 1). In contrast, the High Grass Transect (33 years) had a significant loss in SOC of 1,040±250 g C m^{-2}, as compared to the crop SOC, over 33 years. When divided by the age of each transect, the SOC differences yield sequestration rates of 0.56±0.13 and 0.27±0.10 Mg C ha^{-1} yr^{-1} for the Forb and Mixed Transects, respectively, while the Grass Transect lost carbon at a rate of −0.31±0.08 Mg C ha^{-1} yr^{-1}. There is substantial difference between sequestration rates calculated solely on the basis of depth

Table 2 Sequestration rates by depth and ESM for each transect. The disparity between these two methods is caused by the extra mass in a depth-based sampling that would not be included in ESM calculations. White cells represent transect-depth pairs where the ESM measurement provides a higher sequestration rate, or lower carbon loss rate, than the corresponding depth measurement

Sequestration rates compared by ESM and depth

Transect	Age	Method	Upper rate[a] (Mg C ha^{-1} yr^{-1})	Mass not sampled (g)	Lower rate[b] (Mg C ha^{-1} yr^{-1})	Mass not sampled (g)	Total rate[c] (Mg C ha^{-1} yr^{-1})	Mass not sampled (g)
Grass	33	Depth	−0.10±0.08	−5.6	−0.24±0.03	7.7	−0.34±0.08	2.1
		ESM	−0.13±0.06		−0.17±0.03		−0.31±0.08	
Mixed	27	Depth	−0.07±0.10	8.8	0.19±0.05	15.5	0.18±0.11	24.3
		ESM	−0.02±0.08		0.29±0.06		0.27±0.10	
Forb	23	Depth	0.51±0.14	20.6	−0.11±0.06	3.1	0.40±0.14	23.7
		ESM	0.73±0.11		−0.17±0.07		0.56±0.13	

[a] Upper ESM: 279 kg m^{-2}, 0–30 cm

[b] Lower ESM: 289 kg m^{-2}, 30–50 cm

[c] Total ESM: 568 kg m^{-2}, 0–50 cm

versus ESM-corrected rates (Table 2): at 0–50 cm depth, the Forb and Mixed Transects exhibit depth-based sequestration rates of 0.40±0.14 and 0.18±0.11 Mg C ha^{-1} yr^{-1}, respectively, while the Grass Transect exhibits a depth-based sequestration rate of −0.34±0.08 Mg C ha^{-1} yr^{-1}. For all but two depth-transect combinations, uncorrected measurements provided lower sequestration rates (or higher losses) than those measured by ESM (Table 2).

Sequestration trends by depth were also significantly different among the prairie transects, as indicated by the shallower ($F_{3,23}=35.10$, $P<0.0001$) and the deeper ($F_{3,28}=52.10$, $P<0.0001$) ESM results (Table 2). The

Fig 2 Averaged $\delta^{13}C$ values for each transect by depth. The *gray line* marks the division between $\delta^{13}C$ ranges reported for C_3 and C_4 plant communities (Deines 1980; O'Leary 1988). *Error bars* denote the standard error

Forb Transect accumulated the most SOC in the shallower ESM (1,800 g C m^{-2} more than any other transect), while the Mixed Transect accumulated the most SOC in the deeper ESM (almost 700 g C/m^2 more than any other transect) (Table 1). Finally, the Grass Transect had carbon stocks less than or equal to those at the Crop Transect for all soil depths that were sampled.

Total nitrogen and C:N ratios

Transects differed significantly in total nitrogen (TN) averaged over the top 30 cm (Table 3; $F_{3,22}=8.30$, $P=0.0007$). The Grass Transect and Crop transect had the lowest TN, 0.12±0.00 % and 0.12±0.01 %, while the Mixed Transect and the Forb Transect had greater TN, 0.22±0.02 % and 0.20±0.01 %, respectively (Fig. 3). However, the difference between Forb and Grass was marginally significant ($P=0.0523$) at this depth. When the 50 cm soil depth was examined, results for differences among transects were similar (Fig. 3; Table 3; $F_{3,21}=7.47$, $P=0.0013$): mean TN of the transects preserved their rank order, but the difference between the Forb and Grass Transects became significant ($P<0.0001$). The difference between the Mixed and Grass Transects was significant for both 0–30 cm ($P=0.0035$) and 0–50 cm depths ($P=0.0005$). TN strongly decreased with depth for all transects ($F_{4,45}=54.79$, $P<0.0001$). Note that these values include both organic and inorganic N, though the latter typically represents a marginal amount of total soil N (Wedin and Tilman 1990).

Over the top 30 cm, the Forb Transect and the Crop Transect tended to have higher C:N ratios than the
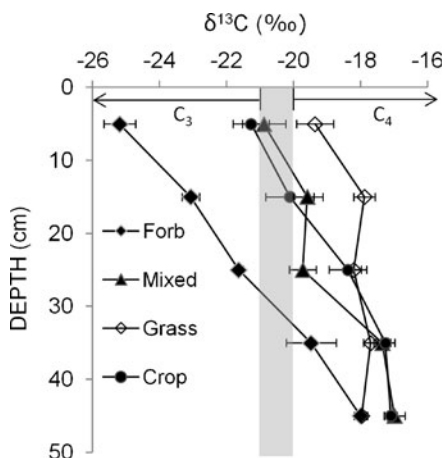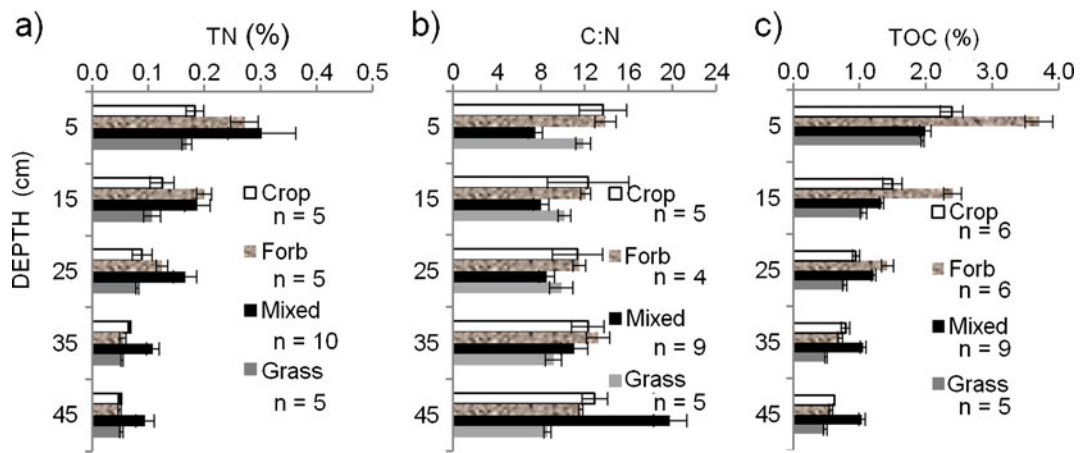
Fig 3 (**a**) TN, (**b**) C:N, and (**c**) TOC averaged across transects. *Error bars* represent standard errors calculated for *n* samples

Mixed Transect (Forb: 12±0.4; Crop: 14±2.1; Mixed: 8.0±0.7; $F_{3,20}$=5.80, *P*=0.0051), but none were significantly different from the intermediate C:N value for the Grass Transect (10.69±0.54). The effect of transect-dependence disappeared when the C:N ratio of the entire soil depth was examined (Table 3; $F_{3,18}$=0.46, *P*=0.71). The C:N ratio did not differ significantly with depth (Table 3; $F_{4,41}$=1.36, *P*=0.26).

Discussion

Understanding the impacts of different plant functional groups on SOC sequestration is an important step in evaluating management practices at restoration sites.

Here, we used stable C isotopes (δ^{13}C) to provide additional, time-averaged constraints on plant community composition and to investigate the corresponding effects on C and N storage. We report that a grass-dominated transect lost SOC over time, while two transects with more forbs gained SOC at rates within the higher range reported for grassland restorations (Burke et al. 1995; Potter et al. 1999; Baer et al. 2000; Conant et al. 2001; Brye and Kucharik 2003; McLauchlan et al. 2006; Kucharik 2007; Matamala et al. 2008;). Specifically, the transect with the highest abundance of C_4 grasses and a C_4-like δ^{13}C SOC signature had TOC and TN values lower than the cropland for all depths sampled, consistent with a frequent burn regime and successive loss of TN at the

Table 3 Results from general linear models and mixed models testing the effects of transect and depth on soil properties. General linear models tested the effects of transect over the top 30 cm of soil. Mixed models tested the effects of transect and depth

over the top 50 cm of soil using depth within a replicate as a repeated measure. Replicates within transects were excluded if values were not available for all depth increments (0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, and 40–50 cm)

Effect of transect and depth on soil properties

	δ^{13}C			TOC			TN			C:N		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
(0–30 cm)												
Transect	3, 25	23.82	**<0.0001**	3, 23	92.21	**<0.0001**	3, 22	8.30	**0.0007**	3, 20	5.80	**0.0051**
(0–50 cm)												
Transect	3, 24	18.01	**<0.0001**	3, 32	55.66	**<0.0001**	3, 21	7.47	**0.0013**	3, 18	0.46	0.7131
Depth	4, 56	76.32	**<0.0001**	4, 44	192.79	**<0.0001**	4, 45	54.79	**<0.0001**	4, 41	1.36	0.2646
Transect x depth	12, 70	10.26	**<0.0001**	12, 59	17.08	**<0.0001**	12, 57	2.29	**0.0186**	12, 53	1.31	0.2392

Bold *P*-values were significant at *P*<0.05

site (Seastedt et al. 1991; Ojima et al. 1994). However, a forb-dominated transect showed SOC stock gains in the top portion of the soil (~0–30 cm), while the Mixed Transect showed significant SOC stock gains concentrated in the deeper soil (~30–50 cm). The distribution of plants at these sites and the resulting SOC changes are consistent with initial species distributions of sown seed and a spatially variable burn regime; specifically weak burning in the all-forb transect may have increased N and C stores at this transect relative to the grass transect. In summary, these results suggest that dominance of C_4 grasses, and the management practices that encourage them, may counteract carbon sequestration on the scale of decades.

$\delta^{13}C$ Constrains plant community composition and C_3 vs C_4 contributions to SOC

The $\delta^{13}C$ profiles shown here verify the results of plant community surveys and their expected contributions to SOC as a function of soil depth, providing an important constraint that is underutilized in other studies. In general, soil $\delta^{13}C$ profiles for prairie plots were shifted towards C_3 (−35 to −21‰) and C_4 (−20 to −9‰) signatures for forb-dominated and grass-dominated plots, respectively, leading to isotopic profiles that were significantly different from that displayed by the crop transect (Fig. 2). Mean turnover of SOC at the depths sampled in this study (0–50 cm) typically occurs on the order of years to centuries (Balesdent et al. 1988; Trumbore 1997; Jones and Donnelly 2004). The isotopic signature at this site, therefore, is likely to reflect organic matter added in the last several decades, including material added throughout the time span of this restoration. Accordingly, these results provide an important constraint on the long-term composition of these plant communities and increase the confidence that differences in SOC totals are attributable to varying abundances of C_4 grasses vs. forbs.

Depth-dependent contributions from plant functional groups were apparent in the $\delta^{13}C$ signal and SOC trends. For all transects, we recorded a more C_4-like signal at depth and a more C_3-like signal at the surface. Additionally, results from the simple end-member mixing model indicate greater forb (C_3) contributions to surface SOC and greater C_4 grass contributions to deeper SOC for these transects (Table 1). This inference is supported by higher belowground primary productivity measurements for C_4 grasses compared to

forbs (Craine et al. 2002). However, the overall depth distribution of SOC will also be affected by erosion and translocation of SOC to greater depths, century-scale shifts in $\delta^{13}C$ of atmospheric CO_2, and isotopic fractionation during decomposition (Ehleringer et al. 2000). Together, these factors typically result in typical depth-based enrichments of 3‰ for C_3 forest ecosystems (Ehleringer et al. 2000; Boström et al. 2007). It is unclear if this trend extends to grasslands, and disagreement exists over the size and causes of this depth-based shift in $\delta^{13}C$ (Ehleringer et al. 2000).

While decomposition, in particular, can result in small isotopic enrichments in residual SOC, we do not expect this effect to be the sole cause of the differential $\delta^{13}C$ depth trends reported here. The Mixed Transect, for example, demonstrates a ~3‰ enrichment with depth, despite the presence of apparently "new", less-decomposed SOC at 30–50 cm (as indicated by high C:N ratios and apparent gains in SOC). If decomposition were the major influence on isotopic enrichment at this transect, we would expect more negative $\delta^{13}C$ values than were recorded at this depth. Furthermore, the Forb Transect demonstrates an enrichment in $\delta^{13}C$ with depth (~7‰) that is much larger than can be accounted for by decomposition alone. Accordingly, we consider the $\delta^{13}C$ profiles at these transects to be a first-order indicator of past and present C_4 contributions at depth.

More broadly, the results presented here demonstrate that $\delta^{13}C$ measurements can help elucidate the impact of C_3 and C_4 plant communities on SOC storage. While $\delta^{13}C$ has been used elsewhere for determination of historic prairie-forest edges (Wang et al. 1993), response of plant functional groups to management alternatives (Sinton et al. 2000), and the effects of C_4 grass introduction in old fields (Mahaney et al. 2008), we are unaware of any study which uses $\delta^{13}C$ to evaluate C_3 vs C_4 impacts on SOC sequestration at depths greater than 20 cm (Obrien et al. 2009). This study demonstrates that, even without detailed biomass production estimates, $\delta^{13}C$ measurements can provide (1) an additional means of statistically distinguishing two or more plant communities, (2) verification that plant communities at a site are stable over time (years to decades), and (3) confirmation that the SOC in the soil is a product of those species observed at the surface. Accordingly, this method provides a clear link between the SOC stocks measured belowground and

the plant communities observed aboveground, and should be considered as a supplement to measurements of SOC stocks and fluxes.

Depth and rate of SOC sequestration dependent on plant community

The sequestration rates measured at the Forb and Mixed Transects (0.56±0.13 and 0.27±0.10 Mg C ha^{-1} yr^{-1}, respectively) fall at the high end of the range reported for restoration projects (Post and Kwon 2000; Matamala et al. 2008). These totals take into account SOC changes throughout the upper 568 kg m^{-2} of soil, and have been averaged over 23 and 27 growing seasons, respectively, on a small privately owned prairie restoration. We note that our methods (comparing by ESM), and the depth to which we sample, contribute to these relatively high rates of sequestration. However over the same depths, and using the same methods, we measured a loss of SOC at the grass-dominated transect, at an apparent rate of −0.31 (± 0.08) Mg C ha^{-1} yr^{-1} over 33 years. Losses at the Grass Transect were reported for both the shallow and deeper ESM's. In this study, prairie soils sampled to 50 cm, had on average 9.0 % less mass than crop soils sampled to the same depth (Table 2), a disparity that is typical for prairie restoration studies (Kucharik 2007; Lee et al. 2009). As a result, sequestration rates measured by depth for all three transects were lower than those measured by an equivalent soil mass.

These rates are also dependent on an accurate baseline as measured in the cropland soils. It is possible that SOC stocks in the adjacent cropland soils have changed since prairie establishment or that SOC stocks were different among transects prior to restoration. Soil designations made by the USGS Soil Survey indicate that portions of the cropland had been severely eroded (Fig. 1, USDA 2009). To avoid eroded sections of the cropland, the crop transect was taken from the summit of a small rolling hill, but it is still within the extrapolated boundaries of the eroded section, and we acknowledge that the crop transect exists as a proxy baseline, due to the lack of SOC measurements at inception of the prairie restoration project. However, we consider initial variation among prairie transect SOC to be minimal, as these transects are located within the same soil unit. Furthermore, the consistency of cropping practices throughout a century of continuous cultivation should minimize variability among

these locations (Davidson and Ackerman 1993; Bowman et al. 2002; Goidts et al. 2009; Zhang et al. 2011). These plots were restored after more than 100 years of cultivation and were thus likely to have SOC stocks with low spatial variability and minimal changes over time. It is also possible that plant communities may have shifted over time. However, observations suggest that plant communities were relatively stable after restoration (Nan Adams, personal communication). Furthermore, δ^{13}C data confirmed that the present plant communities are representative of SOC contributions on a decadal scale.

The difference in the sequestration rates at the three transects may be partly due to the difference in plant communities. It has largely been assumed that C$_4$ grasses are the key to successful sequestration in restored prairie, on account of their high belowground productivity (Knops and Tilman 2000; Kucharik et al. 2001; Baer et al. 2002; Camill et al. 2004). However, the opposite was observed at Gordon Moore Park: the transect with the greatest percentage grass cover (the Grass Transect) and the heaviest (most C$_4$-like) isotopic profile exhibited large SOC losses, while the transect with the fewest C$_4$ grasses (the Forb Transect) demonstrated the highest sequestration rates. Hence C$_4$ grasses alone could not account for the positive sequestration seen at the successful transects, and in this particular case, dominance of C$_4$ grasses was associated with a loss in SOC. However, this trend may be related to fire and N-limitation as discussed below, rather than strictly the presence of the grasses themselves.

The presence of C$_4$ grasses, C$_3$ forbs, and other plant functional groups (e.g. legumes and C$_3$ grasses) has been identified as a major control on the variation of soil N and productivity in prairie ecosystems (Wedin 1995; Tilman et al. 1997; Hooper and Vitousek 1998). Here we report higher N levels in two forb-containing transects, and lower N levels in a third transect dominated by grasses. The Grass Transect also had higher C:N ratios in the top 20 cm than the Mixed Transect, consistent with C$_4$ grasses' typical stoichiometry (biomass C:N>80; Wedin and Tilman 1990; Wedin 1995; Craine et al. 2002), but this trend was just short of significance at a 95 % confidence interval. We also measured a higher C:N ratio in the Forb Transect than in the Mixed Transect, contrary to expectations based on the typically lower C:N ratios of forbs (biomass C:N<60; Craine et al. 2002). In this situation, it is important to consider that younger, less decomposed litter typically has higher C:N

ratios, which decrease over time (Wedin 1995). Notably, the Forb Transect appeared to be contributing large amounts of new SOC to the soil, based on the sequestration rates provided here and the observable presence of large amounts of standing litter. We expect that the presence of new, presumably less decomposed SOC inflated the soil C:N ratio of this transect relative to the other prairie transects, but we do not have conclusive evidence for how functional groups and C:N ratios influenced nitrogen availability at this site.

Loss of SOC at the grass transect

In contrast to the SOC gains at the other two transects, the losses recorded at the Grass Transect (0.31 ± 0.08 Mg C ha^{-1} yr^{-1}) are exceptional, but not unprecedented, given their magnitude and duration (Kucharik 2007). As the oldest transect, the grass transect may have been expected to accumulate the most SOC, but the opposite was observed. Losses of SOC in grassland ecosystems may be due to two major causes: a decrease in productivity and associated organic matter inputs and/or an increase in decomposition. Don et al. (2009) hypothesized that decreases in C inputs at depth explain SOC losses of some newly restored grazing lands. In grassland systems, it is also possible for root exudates to stimulate decomposition of pre-existing organic matter, i.e. priming (Bottner et al. 1999), and thereby increase the rate of decomposition. It is unclear which or both of these are at work at GMP. The pattern of losses described here is consistent with a decrease in productivity, due to annual burning and N-limitation, as discussed in the next section, but increased decomposition rates cannot be ruled out. Neither is it clear when the losses were accrued. In this study, only the oldest transect (33 years) displayed losses, whereas the youngest transect (23 years) displayed the highest sequestration rates. The possible time-dependence of C losses, as observed here and in other studies (Kucharik 2007), merits further investigation, especially in light of possible plant community shifts over time as observed in other restoration plots (e.g. Knops and Tilman 2000; Baer et al. 2002; Camill et al. 2004).

Annual burning, species distribution, and SOC sequestration

At GMP, the distribution of C_4 grasses, N stores, and SOC sequestration rates are consistent with the frequency and spatial patterns of burning at the site. In particular, the Forb Transect is located upwind on the prairie (to the NW) and has not burned thoroughly in recent years, despite the application of fire. In the study year, the entirety of the Forb Transect remained unburned, while the majority of the prairie, including the other two transects, was completely burned through the same treatment. After 33 years of annual burning, the Grass Transect, which is located downwind on the Prairie, had the lowest N concentration, possibly reflecting the cumulative loss of N through successive volatilization. In the present study we are unable to statistically assess the impact of fire on plant community composition, but the effects of frequent burns are documented elsewhere (e.g. Seastedt et al. 1991; Ojima et al. 1994) and may help explain the trends of plant community composition and SOC stocks provided here.

Frequency and intensity of fire may also directly affect SOC sequestration by reducing aboveground inputs of organic matter or by increasing recalcitrant charcoal inputs to SOC. However, we do not expect that either of these processes were important to the sequestration trends noted here. Despite reducing the amount of aboveground litter added to the soil, biomass burning in most circumstances does not decrease overall inputs to SOC, as fire increases above- and belowground productivity (Ojima et al. 1994; Turner et al. 1997; Johnson and Matchett 2001) and the turnover of root materials (Johnson and Matchett 2001), with roots representing a larger component of total organic matter inputs than aboveground litter (Wedin and Tilman 1990; Seastedt 1988; Brye et al. 2002). Furthermore, while charcoal inputs can increase SOC storage as pyrogenic carbon, charcoal was not commonly observed in these soil samples. Additionally, controls on the rate of pyrogenic carbon formation are poorly understood (Glaser and Amelung 2003), and it is unlikely that pyrogenic carbon drove the differences documented here, as the transects with higher apparent SOC accumulation were burnt less frequently and thoroughly.

Conclusion

Despite suggestions that dominance of C_4 grasses should be used as a means of increasing SOC sequestration rates, we report apparent SOC losses for a transect with the highest C_4 plant cover at a 33 year

prairie restoration A less frequently burned transect, with a dominance of forbs showed the highest sequestration rate (0.56 ± 0.13 Mg C ha^{-1} yr^{-1} over 23 years). Finally, a third transect with a mix of C_4 grasses and forbs showed a significant, positive sequestration rate (0.27 ± 0.10 Mg C ha^{-1} yr^{-1}) over 27 years. Dominance of C_4 grasses has been proposed as a means of increasing sequestration rates in the absence of diversity, due to the low-N needs and high productivity of these grasses (Knops and Tilman 2000; Matamala et al. 2008). However, empirical results differ as to the success of this strategy and the efficacy of restoration in general (Baer et al. 2002). This study supports the benefits of including forbs and other C_3 plants in prairie restorations, suggesting that a predominance of C_4 grasses, especially in low-N conditions and when maintained by burning, may compromise the aim of SOC sequestration. Accordingly, managing prairies for diversity and SOC sequestration do not have to be in opposition.

For these and for other restoration practices, it is critical to report the most accurate representation of SOC sequestration rates. As described here, depth-based measurements may yield sequestration measurements considerably less than those derived from the ESM method (Table 2; Ellert and Bettany 1995; Ellert et al. 2002; Lee et al. 2009). We recommend widespread use of the ESM method to allow for greater comparison between grassland sequestration studies, and to avoid systematic underestimation of sequestration rates, due to decompaction of restored soils. Finally, we support the use of stable C isotopes for greater clarification of below-ground processes, and for verifying expected contributions of C_3 vs. C_4 species and their effects on nutrient cycling, two factors which should be considered in sequestration and restoration strategies.

Author contributions Matt D. Ampleman: MDA and DAF conceived and designed the experiments. MDA performed the experiments and analyzed the data. KMC performed essential statistics. MDA, DAF, and KMC wrote the manuscript.

References

Baer SG, Rice CW, Blair JM (2000) Assessment of soil quality in fields with short and long term enrollment in the CRP. J Soil Water Conserv 55:142–146

Baer SG, Kitchen DJ, Blair JM, Rice CW (2002) Changes in ecosystem structure and function along a chronosequence of restored grasslands. Ecol Appl 12:1688–1701. doi:10.1890/1051-0761(2002) 012

Balesdent J, Wagner GH, Mariotti A (1988) Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci Soc Am J 52:118–124

Boström B, Comstedt D, Ekblad A (2007) Isotope fractionation and 13C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia 153(1):89–98. doi:10.1007/s00442-007-0700-8

Bottner P, Pansu M, Sallih Z (1999) Modelling the effect of active roots on soil organic matter turnover. Plant Soil 216:15–25. doi:10.1023/A:1004769317657

Bowman RA, Reeder JD, Wienhold BJ (2002) Quantifying laboratory and field variability to assess potential for carbon sequestration. Commun Soil Sci Plant Anal 33:1629–1642. doi:10.1081/CSS-120004304

Brye KR, Kucharik CJ (2003) Carbon and nitrogen sequestration in two prairie topochronosequences on contrasting soils in southern Wisconsin. Am Midland Nat 149:90–103. doi:10.1674/0003-0031

Brye KR, Gower ST, Norman JM, Bundy LG (2002) Carbon budget components for a prairie and managed agroecosystems: toward an improved understanding of the effects of land use. Ecol Appl 12(4):962–979

Burke IC, Laurenroth WK, Coffin DP (1995) Soil organic matter recovery in semiarid grasslands: implications for the Conservation Reserve Program. Ecol Appl 5:793–801. doi:10.2307/1941987

Cahill KJ, Kucharik CJ, Foley JA (2009) Prairie restoration and carbon sequestration: difficulties quantifying C sources and sinks using a biometric approach. Ecol Appl 19:2185–2201. doi:10.1890/08-0069.1

Camill P, McKone MJ, Sturges ST, Severud WJ, Ellis E, Limmer J, Martin CB, Navratil RT, Purdie AJ, Sandel BS, Talukder S, Trout A (2004) Community and ecosystem-level changes in species-rich tallgrass prairie restoration. Ecol Appl 14:1680–1694. doi:10.1890/03-5273

Collins SL, Glenn SM, Gibson DJ (1995) Experimental analysis of intermediate disturbance and initial floristic composition: decoupling cause and effect. Ecology 76:486. doi:10.2307/1941207

Conant RT, Paustian K, Elliott ET (2001) Grassland management and conversion into grassland: effects on soil carbon. Ecol Appl 11:343–355. doi:10.1890/1051-0761(2001) 011

Craine JM, Tilman D, Wedin D, Reich P, Tjoelker M, Knops J (2002) Functional traits, productivity and effects on nitrogen cycling of 33 grassland species. Funct Ecol 16(5):563–574. doi:10.1046/j.1365-2435.2002.00660.x

Davidson EA, Ackerman IL (1993) Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochem 20:161–193

Deines P (1980) The isotopic composition of reduced organic carbon. In: Fritz P, Fontes JC (eds) Handbook of environmental isotope geochemistry (the terrestrial environment), vol 1. Elsevier, Amsterdam, pp 329–406

Don A, Scholten T, Schulze ED (2009) Conversion of cropland into grassland: implications for soil organic-carbon stocks in two soils with different texture. J Plant Nutr Soil Sci 172:53–62. doi:10.1002/jpln. 200700158

Ehleringer JR, Buchmann N, Flanagan LB (2000) Carbon isotope ratios in belowground carbon cycle processes. Ecol Appl 10(2):412–422. doi:10.1890/1051-0761(2000) 010

Ellert BH, Bettany JR (1995) Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can J Soil Sci 75:529–538

Ellert BH, Janzen HH, Entz T (2002) Assessment of a method to measure temporal changes in soil carbon storage. Soil Sci Soc Am J66:1687–1695

Fernandez I, Mahieu N, Cadisch G (2003) Carbon isotopic fractionation during decomposition of plant materials of different quality. Glob Biogeochem Cycles 17(3):1075

Gibson DJ (1988) Regeneration and fluctuation of tallgrass prairie vegetation in response to burning frequency. Bull Tor Botan Club 115:1–12

Glaser B, Amelung W (2003) Pyrogenic carbon in native grassland soils along a climosequence in North America. Glob Biogeochem Cycles: 17(2). doi:10.1029/2002GB002019

Goidts E, Van Wesemael B, Crucifix M (2009) Magnitude and sources of uncertainties in soil organic carbon (SOC) stock assessments at various scales. Eur J Soil Sci 60:723–739. doi:10.1111/j.1365-2389.2009.01157.x

Hatch MD, Slack CR (1966) Photosynthesis in sugarcane leaves:a new carboxylation reaction and the pathway of sugar formation. Biochem J 101:103–111

Hayes JM (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic processes. Rev Mineral Geochem 43:225–277. doi:10.2138/gsrmg.43.1.225

Hooper DU, Vitousek PM (1998) Effects of plant composition and diversity on nutrient cycling. Ecol Monogr 68:121–149. doi:10.2307/2657146

Hulbert LC (1988) Causes of fire effects in tallgrass prairie. Ecology 69:46–58. doi:10.2111/07-047.1

Johnson LC, Matchett JR (2001) Fire and grazing regulate belowground processes in tallgrass prairie. Ecology, 82(12):3377–3389

Jones MB, Donnelly A (2004) Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO^2. New Phytol 164:423–439. doi:10.1111/j.1469-8137.2004.01201.x

Knops JMH, Tilman D (2000) Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. Ecology. doi:10.1890/0012-9658(2000) 081

Kravchenko AN, Robertson GP (2011) Whole-profile soil carbon stocks: the danger of assuming too much from analyses of too little. Soil Sci Soc Am J75(1):235–240. doi:10.2136/sssaj2010.0076

Kucharik CJ (2007) Impact of prairie age and soil order on carbon and nitrogen sequestration. Soil Sci Soc Am J 71(2):430–441. doi:10.2136/sssaj2006.0074

Kucharik CJ, Brye KR, Norman JR, Foley JA, Gower ST, Bundy LG (2001) Measurements and modeling of carbon and nitrogen cycling in agroecosystems of southern Wisconsin: potential for SOC sequestration during the next 50 years. Ecosyst 4:237–258. doi:10.1007/s10021

Kucharik CJ, Roth JA, Nabielski RT (2003) Statistical assessment of a paired-site approach for verification of carbon and nitrogen sequestration on Wisconsin Conservation Reserve Program land. J Soil Water Conserv 58:58–67

Kucharik CJ, Fayram NJ, Cahill KN (2006) A paired study of prairie carbon stocks, fluxes, and phenology: comparing the world's oldest prairie restoration with an adjacent remnant. Glob Chang Biol 12:122–139. doi:10.1890/08-0069.1

Lal R, Kimble JM, Follet RF, Cole CV (1998) The potential of U.S. cropland to sequester carbon and mitigate the greenhouse effect. Ann Arbor Press, Chelsea

Lee J, Hopmans JW, Rolston DE, Baer SG, Six J (2009) Determining soil carbon stock changes: simple bulk density corrections fail. Agric, Ecosyst Env 134:251–256. doi:10.1016/j.agee.2009.07.006

Mahaney WM, Smemo KM, Gross KL (2008) Impacts of C_4 grass introductions on soil carbon and nitrogen cycling in C_3-dominated successional systems. Oecologia 157:295–305. doi:10.1007/S00442-008-1063-5

Matamala R, Jastrow JD, Miller RM, Garten CT (2008) Temporal changes in C and N stocks of restored prairie: implications for C sequestration strategies. Ecol Appl 18:1470–1488. doi:10.1890/07-1609.1

McLauchlan KK, Hobbie SE, Post WM (2006) Conversion from agriculture to grassland builds soil organic matter on decadal timescales. Ecol Appl 16:143–153. doi:10.1890/04-1650

Newman DS (1998) A Proposal to Register The Nature Institute's Heartland Prairie Restoration in Madison County as an Illinois Land and Water Reserve. Prepared for The Nature Institute and the Alton Parks and Recreation Commission. Illinois Nature Preserves Commission, Coulterville, Illinois, USA

Obrien SL, Jastrow JD, Grimley DA, Gonzalez-Meler MA (2009) Moisture and vegetation controls on decadal–scale accrual of soil organic carbon and total nitrogen in restored grasslands. Glob Chang Biol 16(9):2573–2588

Ojima DS, Schimel DS, Parton WJ, Owensby CE (1994) Long- and short-term effects of fire on nitrogen cycling in tallgrass prairie. Biogeochem 24(2):67–84. doi:10.1007/BF02390180

O'Leary MH (1988) Carbon isotopes in photosynthesis. Biosci 38:328–335. doi:10.2307/1310735

Post WM, Kwon KC (2000) Soil carbon sequestration and land-use change: processes and potential. Glob Chang Biol 6:317–327. doi:10.1046/j.1365-2486.2000.00308.x

Potter KN, Torbert HA, Johnson HB, Tischler CR (1999) Carbon storage after long-term grass establishment on degraded soils. Soil Sci 164:718–725. doi:10.1111/j.1526-100X.2005.00060.x

Sage RF, Monson RK (1999) C4 plant biology. Academic Press, San Diego

SAS Institute (2009) The SAS system for windows, release 9.1. SAS Institute, Cary

Seastedt TR (1988) Mass, nitrogen, and phosphorus dynamics in foliage and root detritus of tallgrass prairie. Ecology 69:59–65

Seastedt TR, Briggs JM, Gibson DJ (1991) Controls of nitrogen limitation in tallgrass prairie. Oecologia 87:72–79

Sinton P, Seastedt T, Bennett B, Bock C, Bock J (2000) C_3 and C_4 species changes identified by $\delta^{13}C$ values of soil organic

matter in a Colorado prairie. West N Am Naturalist 60(4):445–450

Stockmann U, Adams MA, Crawford JW et al (2013) The knowns, known unknowns and unknowns of sequestration of soil organic carbon. Agric Ecosyst Environ 164:80–99. doi:10.1016/j.agee.2012.10.001

Tilman D, Knops J, Wedin D, Reich PB, Ritchie M, Seimann E (1997) The influence of functional diversity and composition on ecosystem processes. Science 277(5330):1300–1302. doi:10.1126/science.277.5330.1300

Tilman D, Reich PB, Knops J, Wedin D, Mielke E, Lehman C (2001) Diversity and productivity in a long-term grassland experiment. Science 294:843–845. doi:10.1126/science.1060391

Trumbore SE (1997) Potential responses of soil organic carbon to global environmental change. Proc Natl Acad Sci U S A 94:8284–8291. doi:10.1073/pnas.94.16.8284

Turner CL, Knapp AK (1996) Responses of a C_4 grass and three C_3 forbs to variation in nitrogen and light in tallgrass prairie. Ecology 77:1738–1749. doi:10.1111/j.0030-1299.2004.13110.x

Turner CL, Blair JM, Schartz RJ, Neel JC (1997) Soil N and plant responses to fire, topography, and supplemental N in tallgrass prairie. Ecology 78(6):1832–1843

USDA (2009) Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available online at http://websoilsurvey.nrcs.usda.gov/. Accessed 12 Dec. 2009

VandenBygaart AJ, Angers DA (2006) Towards accurate measurements of soil organic carbon stock change in agroecosystems. Canadian Journal of Soil Science 86(3):465–471. doi:10.4141/S05-106

Wang Y, Cerling TE, Effland WR (1993) Stable isotope ratios of soil carbonate and soil organic matter as indicators of forest invasion of prairie near Ames, Iowa. Oecologia 95:365–369. doi:10.1016/j.geoderma.2004.03.006

Wedin DA (1995) Species, nitrogen and grassland dynamics: the constraints of stuff. In: Jones CG, Lawton JH (eds) Linking species and ecosystems. Chapman and Hall, New York, pp 253–262

Wedin DA, Tilman D (1990) Species effects on nitrogen cycling: a test with perennial grasses. Oecologia 84:433–441. doi:10.1890/02-5005

Wedin DA, Tilman D (1996) Influence of nitrogen loading and species composition on the carbon balance of grasslands. Science 274(5293):1720–1723. doi:10.1126/science.274.5293.1720

Zhang W, Weindorf DC, Zhu Y (2011) Soil organic carbon variability in croplands: implications for sampling design. Soil Sci 176:367–371. doi:10.1097/SS.0b013e31821eb7d2