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Long-term incubations of size and density separated soil fractions to inform soil organic carbon decay dynamics

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ABSTRACT

Soil organic matter in coarse-textured soils is more vulnerable to environmental disturbances due to reduced potential for soil organic carbon (SOC) stabilization in aggregates or organo-mineral complexes. In sandy loam soils from the Rio Grande Plains region of southern Texas, woody encroachment has resulted in the rapid accrual of root and leaf tissues derived from trees and shrubs into poorly physically protected (macroaggregate >250 μm) and non-mineral associated (free light fraction <1.0 g cm^{-3}) soil fractions. To determine the impact of changing plant input chemistry on potential degradability of accumulating SOC fractions, we measured the quantity and isotopic composition of respired CO_2 from year-long incubations of the macroaggregate and free light soil fractions along a grassland to woodland successional chronosequence. During incubation of both fractions, the proportion of SOC respired from older woody stand soils (~40–90 yrs) relative to recently established woody stands (<40 yrs) and remnant grassland soils decreased. We interpreted this decrease with woody stand age to result from a change to plant input chemistry with more lignin and aliphatic structures combined with a progressive shift to more non-hydrolyzable, poorly accessible forms of soil organic nitrogen, resulting in a system with slower short-term decay dynamics. The $\delta^{13}C$ values of respired CO_2 from all landscape elements indicated a selective release of older grassland-derived SOC in the first month of the macroaggregate incubation, possibly due to the disruption and rapid microbial utilization of grassland SOC after the soil fractionation process. Due to the sensitivity of these rapidly-cycling soil fractions to environmental disturbance and their capacity to influence longer-term SOC dynamics, understanding their decay dynamics is essential for understanding mechanisms of SOC stabilization. This is especially important in coarse-textured soils where large SOC stocks may be present in physical fractions that are relatively unprotected from decomposition.

1. Introduction

Soil physical fractionation schemes aim to isolate conceptually-defined soil organic carbon (SOC) pools that vary in size, density, and aggregate associations, and therefore differ in turnover times and stabilization potentials. Despite frequent emphasis on long-term SOC pools for C stabilization, the average age of respired CO_2 (Trumbore, 2000) and average turnover time of bulk SOC (Raich and Schlesinger, 1992) is on decadal time scales, which highlights the significance of soil organic matter (SOM) pools with intermediate turnover rates to the global C cycle. Carbon held within shorter- and intermediate-term decadal pools, such as particulate organic matter (POM) and litter-dominated soil fractions (Krull et al., 2005; Liao et al., 2006b; Bol et al., 2009), are highly sensitive to disturbance and management (Six et al., 1998; Paustian et al., 2000; Haynes, 2005; Leifeld and Fuhrer, 2009) and potentially to future climate change (Trumbore, 1997; Fang et al., 2005; Jones et al., 2005).

In POM and litter, chemical structure dictates short-term decay dynamics so that material with high C:N, lignin:N, and lignin:cellulose ratios, or with high lignin, phenol, and wax contents degrade more slowly (Melillo et al., 1982; Taylor et al., 1989; Heal et al., 1997). Compounds containing aliphatic and aromatic structures have been shown to be more resistant to microbial decay (Marschner and Kalbitz, 2003; Feng et al., 2008), and fire-derived (charcoal) C can persist in soils for hundreds or even thousands of years (Bird et al., 1999; Schmidt et al., 2002; Preston and Schmidt, 2006), suggesting that chemical composition is an important determinant of SOC turnover.

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However, recent reviews have suggested that organic matter chemistry is not particularly important for long-term SOC stabilization (Amelung et al., 2008; Schmidt et al., 2011). Instead, physical protection of SOM on organo-mineral complexes and in soil aggregates plays the dominant role (Marschner et al., 2008; Kleber et al., 2010; Schmidt et al., 2011). The presence of a so-called abiotic "regulatory gate" (Kemmitt et al., 2008) which serves to release previously unavailable SOM into bioavailable forms through processes such as chemical oxidation and desorption from mineral surfaces supports such observations. It stands as a compelling hypothesis that the interaction of soil microbial biota with SOM is not the rate-limiting step in SOM decomposition (Kemmitt et al., 2008), and that it is not the chemical nature but rather the quantity of input to soil that governs SOM accrual (Paterson, 2009; Gentile et al., 2011; Carrington et al., 2012). By examining the decomposition of soil fractions comprised of fresher plant material, fewer mineral surfaces, and lower aggregate protection than whole soil, one might bypass (or greatly minimize) some of the constraints imposed by such abiotic gate controls on SOC decomposition.

Woody plant encroachment into grasslands and savannas is a globally extensive land-cover change (Archer, 1995; Dickie et al., 2011) that can alter the quantity and chemistry of plant inputs, thereby influencing the biogeochemical cycling of SOC and nutrients (Hibbard et al., 2001; Boutton et al., 2009). In the Rio Grande Plains region of southern Texas, encroachment of C_3 woody plants into C_4 dominated grasslands over the last 150 yrs has been accompanied by increases in soil C and N (Boutton et al., 1998; Archer et al., 2001; Boutton and Liao, 2010) predominately in more physically unprotected soil fractions (Liao et al., 2006a; Creamer et al., 2011). The percentage of whole soil carbon held within the more physically unprotected free light fraction (FLF) and macroaggregate-sized fraction increases from 28% in grasslands to 38% in woody clusters <40 yrs of age to 61% in woody clusters >40 yrs of age (Creamer et al., 2012). Due to these large increases in soil C and N in more physically unprotected soil fractions in response to woody encroachment, their decay dynamics are integral to understanding mechanisms of SOC accrual in these soils.

Accumulation of SOC in these more physically unprotected soil fractions is thought to be driven, at least in part, by the changing carbon input chemistry in response to woody encroachment, as the isotopic composition of these soil fractions resembles woody C_3 inputs after about 40 yrs of woody stand development (Liao et al., 2006a, 2006b; Boutton et al., 2009; Creamer et al., 2011). Microbial biomass carbon decreases relative to total soil C and respired CO_2, suggesting SOC in woodland soils is more difficult to degrade (Liao and Boutton, 2008). Higher proportions of cutins, waxes, and more difficult to degrade syringyl and vanillyl lignin in woody plant litter are reflected in the accumulation of these compounds in the FLF and in particulate organic matter (POM) (Filley et al., 2008). However, incubations of the whole soil revealed that physical accessibility to soil C dictated degradation dynamics more than changes in soil carbon chemistry (Creamer et al., 2011). In addition, recent studies have indicated that progressively accumulating soil N under woody stands is less extractable as amino compounds by wet chemical means than in the grasslands, potentially facilitating soil C accrual (Creamer et al., in press) due to the importance of N in C cycling (*e.g.* Knicker, 2011). Therefore the actual importance of changing SOC chemistry to the decay dynamics within the soil fractions is unknown.

The purpose of this study was to determine if increases in plant tissues with purportedly slower decay rates facilitate the observed SOC accrual in soil fractions with low physical protection capacity and decadal to sub-decadal mean residence times (the FLF and macroaggregate-sized soil fraction). The low clay content of these soils (approximately 10%) and absence of dense mineral matter in the FLF prevents any substantial stabilization of SOC on clay particles (Hassink, 1997; Six et al., 2002) and allows for the examination of SOC dynamics and bioavailability without the constraints of abiotic "regulatory gate" controls. We hypothesized that the lower quality of organic matter inputs in older woody plant stands would cause a decrease in the fraction of C mineralized in the rapidly accruing soil fractions. More broadly, this experiment also allowed us to determine differences in C stabilization mechanisms between these soil fractions and the whole soil in order to gain insights into short term SOC dynamics and long-term C stabilization that might be applicable to other ecosystems with coarse-textured soils.

2. Materials and methods

2.1. Site description

The Texas AgriLife La Copita Research Area (La Copita) is in the Rio Grande Plains region of southern Texas (27°40′N; 98°12′W). This area has a mean annual temperature of 22.4 °C and an average annual rainfall of 716 mm. Over the past 150 yrs, fire suppression combined with livestock overgrazing has resulted in progressive encroachment of C_3 woody plants into the native C_4-dominated grasslands (Archer, 1990; Boutton et al., 1998; Archer et al., 2001). The grassland is dominated by grass species such as *Chloris cucullata*, *Panicum hallii*, *Bouteloua rigidiseta*, and *Tridens muticus*. Woody encroachment begins with the establishment of the leguminous *Prosopis glandulosa* (Torr.) var. *glandulosa* (honey mesquite). Other tree/shrub species, such as *Zanthoxylum fagara*, then enter the understory, resulting in the formation of discrete C_3 woody clusters that may coalesce into closed-canopy groves (Archer et al., 1988). The soils under both the remnant grassland and the invading woody clusters are sandy loams (Typic and Pachic Argiustolls). Additional information regarding the study area has been published elsewhere (Scifres and Koerth, 1987; Archer, 1995; Boutton et al., 1998).

2.2. Soil sampling and physical fractionation

Soils were sampled from the site and physically fractionated as described in Creamer et al. (2011). Briefly, soil cores of 5 cm diameter and 30 cm depth were taken in October 2006 from 15 discrete woody clusters and 15 grassland sites. Samples within the discrete woody clusters were taken in the four cardinal directions within 50 cm from the trunk of the largest mesquite tree. The ages of these trees are equal to the ages of the woody clusters (Archer et al., 1988), and were determined by using the basal diameter in site-specific regression equations (Stoker, 1997). The ages (in years) of the fifteen woody clusters sampled were: 14, 16, 23, 34, 37, 41, 41, 52, 56, 63, 65, 70, 73, 82, and 86. For the paired grassland sites, soil cores were taken in the four cardinal directions around a randomly selected C_4 plant located adjacent to, but not within 3 m, of the canopy edge of the sampled woody clusters.

After sampling, the cores were placed on ice and then the 0–10 cm sections from each of the four cores were homogenized into a single sample. After homogenization the field-moist soil was passed through an 8 mm sieve, oven-dried at 50 °C, and then subjected to size and density fractionation following a procedure modified from Cambardella and Elliott (1993) and Six et al. (1998). Briefly, the FLF was first obtained by immersing oven-dried soils in water, then aspirating and drying all floating fragments. The soil was then wet-sieved to isolate the three remaining size fractions (Elliott, 1986): the macroaggregate-sized fraction (>250 μm), the microaggregate-sized fraction (53–250 μm) and the free silt and

clay fraction (<53 μm). All soil fractions were oven-dried at 50 °C. Percent C, percent N, and $\delta^{13}C$ was measured on the bulk soil and soil fractions using a CHN elemental analyzer (EA, Sercon, Crewe, UK) interfaced to a 20/22 isotope ratio mass spectrometer (IRMS, Sercon, Crew, UK); this data is reported in Creamer et al. (2011). Portions of the bulk soil, FLF, and macroaggregate-sized fractions were subjected to long-term laboratory incubations. The results from the bulk soil incubation are reported in Creamer et al. (2011).

2.3. Soil fraction incubation

Methods for the FLF and macroaggregate-sized fraction incubations are nearly identical to those described previously for the bulk soil (Creamer et al., 2011) and for similar experiments (Swanston et al., 2002; Crow et al., 2006). All soil incubations were carried out in 12 mL vials with septum caps (Labco, UK) that were packed on the bottom with ~1 cm^3 of glass wool to prevent anaerobic conditions. For the macroaggregate incubation, 2 g of the macroaggregate-sized fraction from the woody clusters and paired grassland soils were mixed in equal weight with ashed quartz sand (grain size 53–250 μm). This mixture was wet with an inoculum (described below) to 18% by weight. Similar to the whole soil incubation, three replicates for each age of the sampled cluster and grassland soils were created. Therefore, for the two woody clusters of the same age (41 yrs), only one was used in the incubations. For the FLF incubation, 750 mg of the FLF from the woody cluster and grassland soils was mixed with 3 g of ashed quartz sand. Only 750 mg of the FLF was used for the incubation due to the small amount of material isolated from the fractionation procedure. The low density of the FLF compared to the macroaggregate fraction and whole soil required a larger amount of sand to homogenize the sample and prevent caking and anaerobic conditions. The amount of sand chosen was within the range of values for incubations of light fractions and plant material reported by others (Swanston et al., 2002; Crow et al., 2006, 2007). Three replicates of each age were made for all woody clusters except ages 14 and 16 yrs, which were made in duplicate. As a result of the minimal amount of material isolated during the fractionation procedure, there was only one replicate for each grassland sampling location, for a total of 14 samples. The FLF was then wet to 20% by weight with an inoculum (described below).

The inoculum was prepared from a mixture of frozen, field moist soils (14, 34, 41, 65, and 86 year old cluster soils and corresponding grassland soils). This mixture was wet to 60% water holding capacity and incubated for 7 days at 30 °C in the dark. After incubation, the inoculum was obtained by adding sterile nanopure H_2O until a 1:10 ratio of soil:water was obtained (g:mL). This mixture was agitated on a rotary shaker at 120 rpm for 1 h at room temperature, vacuum filtered on ashed Whatman GF/F filter paper, and then added to the soil fractions as described previously (Creamer et al., 2011). A sample blank containing only 4 g ashed quartz sand accounted for CO_2 released from dissolved organic matter or microbial biomass from the inoculum; the amount of CO_2 released from this sand blank was negligible.

Soil fractions were incubated in the dark at 30 °C for 1 yr. Soils were kept at constant moisture by periodically weighing and adding 10–100 μL of sterile, nanopure water. Prior to CO_2 sampling, the respiration vials were flushed with 10× their volume of humidified, CO_2-free air created by passing atmospheric air through a NaOH trap and then bubbling through sterile water. Over the course of the year-long incubations the vials were then allowed to accumulate CO_2 for 6–130 h (macroaggregate-sized fraction) and 2–77 h (FLF); these times were adjusted during the course of the experiment to allow the buildup of measurable amounts of CO_2. Varying the CO_2 accumulation times did not affect the $\delta^{13}C$ values of respired CO_2 (Creamer et al., 2011). The quantity and isotopic composition of the accumulated CO_2 was then measured directly from the microrespiration vials using a trace gas analyzer (Sercon, Crewe, UK) interfaced to a 20/22 IRMS (Sercon, Crewe, UK) on days 1, 3, 5, 7, 10, 14, 21, 28 of the incubation and then every 28 days for one year. Vials were automatically flushed with CO_2 free air by the trace gas analyzer after sampling. After each sampling batch, samples were returned to the incubator and the septa caps were replaced with caps containing GF/F filters to permit normal gas exchange. Following the last sampling day, the soil fractions were dried at 50 °C for 2 days and then ground to a fine powder using a steel ball mill (Retsch, Haan, Germany). Percent N, percent C, and $\delta^{13}C$ were measured on an EA-IRMS on all samples after incubation.

2.4. Calculations and statistical analyses

For all statistical analyses, grasslands were treated as time 0 in the chronosequence. A cluster analysis was performed with Unscrambler (v10.1×) on the cumulative respiration data to determine if woody stands of particular ages released similar proportions of CO_2 during the incubation. For the macroaggregate sized fractions the younger woody stands included ages 14, 16, and 23 yrs ($n = 9$); all other ages were grouped into older woody stands ($n = 33$) and the grassland soils grouped together ($n = 42$). For the FLF, the younger woody stands included ages 14, 16, 23, 34, and 37 ($n = 13$). All other woody stand ages were grouped into older woody stands ($n = 27$) and the grassland soils grouped together ($n = 14$). One-way ANOVA, using the GLM procedure (SAS, v9.2) was used to test for differences in respiration and isotopic data between grassland, younger woody cluster and older woody clusters. Each day of the incubation was examined separately. Tukey's studentized range (HSD) test determined statistically significant means ($\alpha = 0.05$). The isotopic composition of the soil fractions, measured at the beginning of the experiment, was subtracted from the isotopic composition of respired CO_2 to determine the extent of C depletion or enrichment relative to the source during incubation.

3. Results

3.1. Fraction SOC and N content before and after incubation

Prior to incubation, the SOC content of macroaggregates in older woody stands was significantly higher than in the grasslands, although neither was different than the younger woody stands (Table 1). The N content of macroaggregates in older woody stands was significantly higher than both younger woody stands and grasslands, resulting in a significantly lower C/N ratio in older woody stands (~12) compared to younger woody stands and grassland macroaggregates (~15–16). The isotopic composition of the macroaggregates in older woody stands was also significantly depleted with respect to younger woody stands and grasslands (Table 1). Prior to incubation of the FLF, C content was similar between all landscape elements, while N content differed slightly, with the FLF in older woody stands containing significantly more N than the FLF in grasslands (Table 1). Similar to the macroaggregate fraction, this resulted in the FLF in the older woody stands having a significantly lower C/N ratio than younger woody stand and grassland FLF (~15 versus 18–19, respectively). The carbon isotopic composition of the FLF became progressively depleted in ^{13}C moving along the chronosequence from the grassland to younger woody stands to older woody stands.

After the year-long incubation SOC content decreased significantly in older woody stands and grasslands in both the FLF and macroaggregates, although SOC losses in younger woody stands were not significant. Due to the larger error in measuring C with the

Table 1
C and N content and C isotopic composition by landscape element for the macroaggregate (macro) and free light fraction (FLF), before and after year-long incubation. Standard deviations are given.

	C content mg C g soil^{-1}		N content mg N g soil^{-1}		C/N		δ^{13}C (‰)	
	Before	After	Before	After	Before	After	Before	After
Macro								
Grass	18.4 ± 8.6b	13.7 ± 5.0a*	1.19 ± 0.45a	1.31 ± 0.47a	15 ± 2.0a	10 ± 1.1ab*	−20.1 ± 1.2a	−20.5 ± 1.5a
Young	24.6 ± 10.3ab	19.8 ± 8.1b	1.52 ± 0.18a	1.72 ± 0.35b	16 ± 5.0a	11 ± 2.9a*	−20 ± 2.8a	−20.2 ± 2.6a
Old	30.5 ± 10.9a	24.6 ± 7.0c*	2.48 ± 0.56b	2.44 ± 0.55c	12 ± 1.8b	10 ± 0.8b*	−25.1 ± 0.3b	−24.9 ± 0.6b
FLF								
Grass	186 ± 50.3a	150 ± 56.4a*	9.7 ± 2.5a	9.7 ± 2.6a	19.3 ± 2.4a	15.2 ± 2.4a*	−20.2 ± 2.5a	−20.3 ± 2.1a
Young	185 ± 40.8a	178 ± 60.5a	11.3 ± 1.0ab	12.7 ± 3.6b	18.2 ± 2.5a	13.8 ± 0.9b*	−23.9 ± 1.7b	−24.0 ± 1.9b
Old	204 ± 21.5a	153 ± 50.0a*	12.6 ± 2.7b	12.2 ± 3.4b	14.7 ± 1.2b	12.4 ± 0.9c*	−25.6 ± 0.7c	−25.8 ± 0.9c

Asterisks indicate significant differences before and after incubation for a particular parameter. Lower-case letters indicate significant differences between landscape elements of a soil fraction (down columns).

elemental analyzer compared to the trace gas analyzer, the average C loss calculated from Table 1 does not always exactly match values given by the cumulative CO_2 respiration curves (Fig. 1a), although they are well within the range of error shown in Table 1. The C/N ratios of all landscape elements also decreased significantly during both soil fraction incubations. However, there were differences in the C/N ratios between landscape elements in both fractions, where macroaggregates in older woody stands had significantly lower C/N ratios than macroaggregates in younger woody stands, and where the C/N ratio in the FLF decreased significantly from grasslands to younger woody stands to older woody stands. Despite substantial C losses for all landscape elements, there were no significant differences between the isotopic composition of SOC before and after the incubation. Additionally, there were no significant changes in weight % of nitrogen during the incubation.

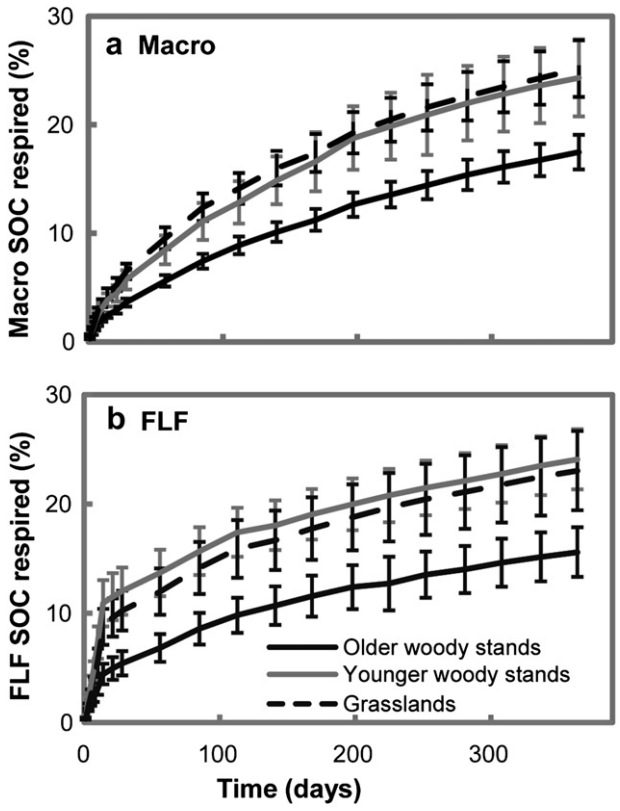

Fig. 1. Percentage of (a) macroaggregate SOC lost during the macroaggregate incubation and (b) FLF SOC lost during the FLF incubation. Vertical bars are the standard error of each measurement.

3.2. Carbon mineralization during incubation

After one year of incubation, the macroaggregate fraction lost (±1 standard error) 25 ± 3%, 24 ± 4%, and 17 ± 2% of initial fraction OC as C–CO_2 from grassland, younger woody stand, and older woody stand soils, respectively (Fig. 1a). Similarly, 23 ± 4%, 24 ± 3%, and 16 ± 2% of initial fraction OC was lost from the FLF of grassland, younger woody stand, and older woody stand soils (Fig. 1b). For both soil fractions, C–CO_2 losses were significantly higher from younger woody stand and grassland soils relative to older woody stand soils ($P < 0.0001$). This effect was immediate and seen by the first day of the incubation in the macroaggregate fraction incubation and by the 10th day of the FLF incubation.

Despite losing similar proportions of initial C, the patterns of C–CO_2 loss through time were different between the two soil fractions (Fig. 1a, b). Respiration rates were high for the first 14 days of the FLF incubation, so that 30–50% of the C–CO_2 lost during the entire incubation was respired in these two weeks. In contrast, in the macroaggregate fraction only 14–17% of the total C–CO_2 respired was lost in the first two weeks.

3.3. Isotopic composition of mineralized CO_2

3.3.1. Macroaggregate incubation

On the first day of the macroaggregate incubation the δ^{13}C value of respired CO_2 ($\delta^{13}C_{CO2}$) was significantly lower from older woody stands than from younger woody stands and grassland soils (Fig. 2a). From days 3–10 all landscape elements released a pulse of CO_2 that was very similar in isotopic composition (−19 to −21‰) and higher than the δ^{13}C value of their respective macroaggregate fractions ($\delta^{13}C_{MACRO}$) (Fig. 2c). This enrichment of CO_2 relative to $\delta^{13}C_{MACRO}$ ($\delta^{13}C_{CO_2}-\delta^{13}C_{MACRO}$) was up to 6‰ in older woody stands and was significantly higher than the $\delta^{13}C_{CO_2}-\delta^{13}C_{MACRO}$ values of younger woody stand and grassland soils.

After this initial enriched CO_2 pulse, the $\delta^{13}C_{CO_2}$ respired from all landscape elements stabilized, although at slightly different values, so that CO_2 mineralized from grassland soils was significantly more enriched than CO_2 from younger and older cluster soils until the end of the incubation (Fig. 2b), but depleted with respect to the $\delta^{13}C_{MACRO}$ value of grassland soils (Fig. 2d). The extent of this depletion increased from ~1‰ on day 30 to ~2‰ on day 365. The $\delta^{13}C_{CO_2}$ values from younger woody stand soils were similarly depleted with respect to $\delta^{13}C_{MACRO}$, although the extent of depletion started at ~2‰ and increased to ~3‰ by day 365. In contrast, the $\delta^{13}C_{CO_2}$ values from older woody stand soils stabilized around a value that was generally not significantly different than the $\delta^{13}C_{MACRO}$ of older woody stand soils. Due to these slight differences between landscape elements, CO_2 from younger woody stand soils was significantly more depleted with respect to the

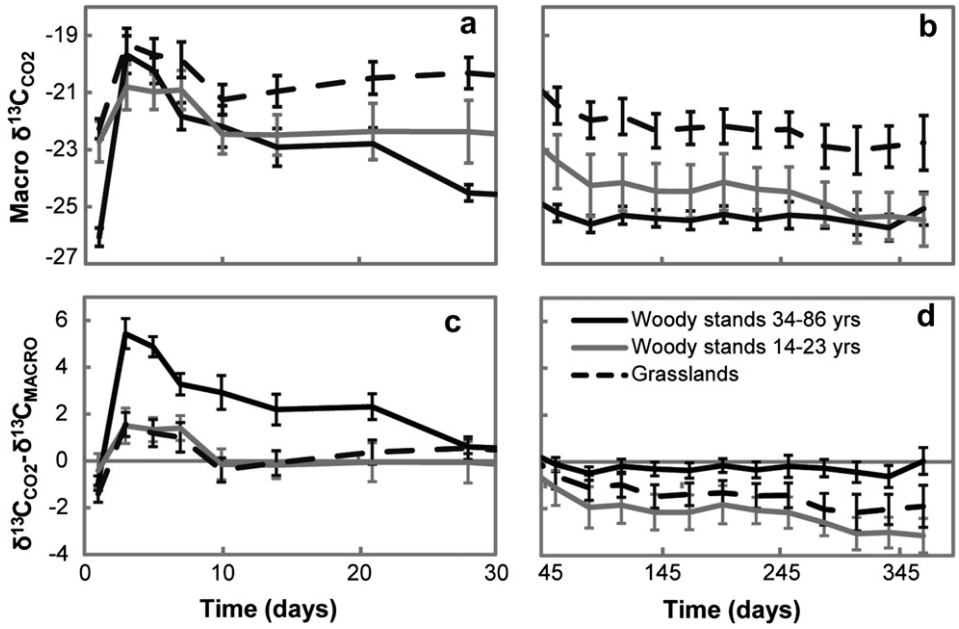

Fig. 2. (a–b) Isotopic composition of CO_2 respired during the macroaggregate incubation and (c–d) relative to the isotopic composition of the macroaggregate fraction. Each sampling point is indicated by the standard error.

macroaggregate fraction than older woody stand soils from day 28 of the incubation on. However, neither landscape element was different from grassland soils until day 225, when CO_2 from both younger woody and grassland soils was more depleted with respect to the macroaggregate fraction than older woody stand soils.

3.3.2. FLF incubation

During the FLF incubation, CO_2 released from grassland soils was significantly more enriched in ^{13}C than CO_2 released from either of the woody stand soils for every sampling point except day 5 (Fig. 3a,b). Although there were 3 sampling points (days 7, 85, 113) where CO_2 from younger woody stand soils was significantly more enriched than CO_2 from older woody stand soils, for the majority of sampling points they were not different.

Relative to the isotopic composition of the FLF carbon ($\delta^{13}C_{FLF}$), mineralized CO_2 was enriched by as much as 3‰ during the first month of incubation (Fig. 3c). The extent of this enrichment was similar between the three landscape elements except on day 5, when CO_2 from grassland soils was less enriched relative to the FLF than CO_2 from the woody stand soils. After the first month, $\delta^{13}C_{CO_2}$ values from all landscape elements remained relatively constant until the end of the incubation (Fig. 3b). In grassland soils, $\delta^{13}C_{CO_2}$

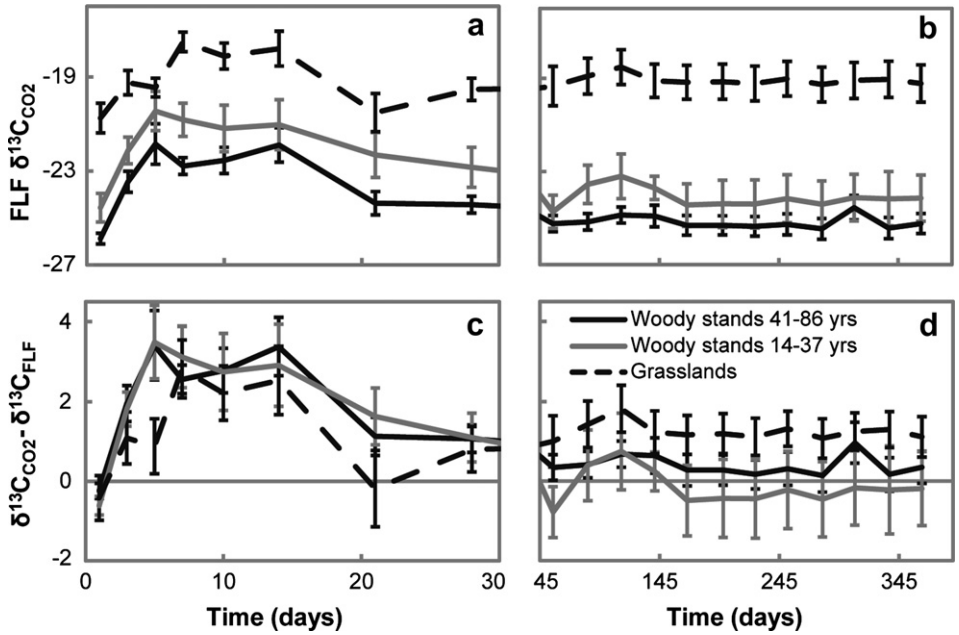

Fig. 3. (a–b) Isotopic composition of CO_2 respired during the FLF incubation and (b) relative to the isotopic composition of the FLF. Each sampling point is indicated by the standard error.

stabilized around −19‰, which was about 1‰ more enriched than $\delta^{13}C_{FLF}$ (Fig. 3d). In contrast, the $\delta^{13}C_{CO_2}$ values from the two woodland soils, which stabilized at −24 to −26‰, did not differ significantly from $\delta^{13}C_{FLF}$ for most days of the incubation.

4. Discussion

4.1. Relevance of C mineralization from soil fractions to SOC stabilization

The significantly greater proportional respiration of soil fraction OC in the younger woody stands and grassland of both soil fractions compared to the older woody stands (Fig. 1) suggest, as hypothesized previously (McCulley et al., 2004; Filley et al., 2008; Liao and Boutton, 2008), that C inputs from the invading woody plants may be inherently more difficult to degrade than C inputs from the grasslands. However, as the proportion of whole soil carbon held within these soil fractions more than doubles in response to woody encroachment, from 27% of total soil carbon in grasslands to 61% in older woody clusters (Creamer et al., 2012), the differences measured in respiration within the soil fractions themselves (Fig. 1) would not be observed during an incubation of the whole soil (Creamer et al., 2011). Assuming the rates of degradation seen during the incubation of the FLF only (Fig. 1b) could be sustained within the FLF during a whole soil incubation, multiplying the percentage of C lost during the FLF incubation by the amount of whole soil C held in the FLF calculates the amount of whole soil carbon that could potentially be lost from the FLF. In this way, the proportion of whole soil C that could be lost from the FLF increases from 1.4% in grassland soils to 3.4% and 8.9% in younger and older woody cluster soils, respectively. A similar calculation for the macroaggregate fraction also reveals that the amount of whole soil carbon potentially lost from the macroaggregate fraction increases from 3.3% in grasslands to 4.0% and 11.3% in younger and older woody cluster soils, respectively. This confirms, as hypothesized by Creamer et al. (2011), that the allocation of SOC into physically unprotected soil fractions strongly influences SOC dynamics in the whole soil and can mask the effects of changing plant chemistry that slowed degradation in the isolated soil fractions. As these soils provide an optimal circumstance in which biochemical recalcitrance could be expressed (low clay content, decreased physical protection, increases in biochemically recalcitrant biopolymers), this supports, as other authors have suggested (Marschner et al., 2008; Kleber, 2010; Schmidt et al., 2011) that in this study environmental constraints upon degradation and physical protection within aggregates and on organo-mineral complexes play a dominant role in long-term whole soil C stabilization.

In these soils, then, what are the mechanisms controlling SOC accrual? Plant litter quality impacts short-term increases in SOC, but in the long-term the quantity of litter input is more important (Paterson, 2009; Gentile et al., 2011; Carrington et al., 2012). Increases in litter production, fine and coarse root biomass, and aboveground net primary productivity (Hibbard, 1995; Archer et al., 2001; Hibbard et al., 2001) could be facilitating increases in SOC. Coupled with changes to organic N extractability (Creamer et al., in press), and potentially with water or other nutrient limitations (Jackson et al., 2002; Huxman et al., 2005), changes to microbial community structure (Jastrow et al., 2007), or plant-driven allelopathy (Weidenhamer and Callaway, 2010), these woody systems create conditions under which SOM can accrue.

Although the effects of changing plant chemistry seen in the more physically unprotected soil fractions are likely are not the main mechanism for long-term SOC stabilization at this site, this change is not inconsequential. Carbon held within POM (in macroaggregates) and in the FLF responds rapidly to disturbances and land cover/land use practices (Six et al., 1998; Paustian et al., 2000; Leifeld and Fuhrer, 2009). As the proportion of whole soil carbon held within the FLF and macroaggregate-sized fraction doubles (Liao et al., 2006a; Creamer et al., 2011) they likely would represent significant sources of CO_2 if the ecosystem were to be disturbed, for example, as a result of attempts to restore the native grassland. This work also has implications for other coarse textured soils where the C dynamics are dominated by allocation to more physically unprotected soil fractions. For example, if changes in plant input chemistry occurred without the increases in plant input seen at this site, we would expect modest increases in soil carbon in physically unprotected soil fractions. Conversely, if productivity increased without decreases in soil carbon quality, increased degradation of physically unprotected soil fractions, unconstrained by carbon quality, may promote SOC stabilization within more physically protected soil fractions.

4.2. Sources of mineralized C during fraction incubations

Both soil fractions displayed a pulse of ^{13}C-enriched CO_2 (relative to the source material) in the first 10 days of the incubation (Figs. 2 and 3c). However, the same mechanisms may not be controlling this seemingly identical response among the two soil fractions. In the macroaggregate fractions, all landscape elements released CO_2 with a similar isotopic composition (−19 to −23‰; Fig. 3a), despite having large differences in the $\delta^{13}C$ values of macroaggregate fractions (Table 1). This suggests a similar C source was degraded by all three landscape elements during this event. In addition, the extent of enrichment of CO_2 relative to $\delta^{13}C_{MACRO}$ from older woody stand soils (6‰) also suggests this pulse of CO_2 is from an enriched SOC source, rather an effect of isotopic fractionation, which is usually minor relative to the mineralization of different SOC sources (Blagodatskaya et al., 2011).

As macroaggregate-sized free POM is isolated with the macroaggregate-sized fraction in addition to water stable aggregates and sand (Liao et al., 2006a), and as the re-wetting of soil can disrupt soil structure and release labile SOC (*e.g.* Franzluebbers et al., 2000), it is likely that the soil fractionation scheme, or the re-wetting of the soil itself, disrupted the soil structure to release of a mix of fresh, easily degradable material with an enriched component that was previously stabilized in the macroaggregates. The enriched C source destabilized by soil fractionation is likely a mix of C_4 and C_3-derived SOC, which in the older woody stands represents a loss of older (>40 yrs) SOC. However, the degradation of enriched biopolymers such as polysaccharides (Hobbie and Werner, 2004; Teece and Fogel, 2007) or enriched microbial biomass derived from C_4–C (Dijkstra et al., 2006) may have also occurred, especially as polysaccharides aid in forming and binding macroaggregates together (Tisdall and Oades, 1982; Elliott, 1986; Oades and Waters, 1991) and as microbial biomass may be stabilized in soil and comprise a large proportion of slower-cycling SOC (Kiem and Kögel-Knabner, 2003; Simpson et al., 2007; Liang and Balser, 2011). The sensitivity of the macroaggregate fraction to disturbance highlights how disturbance of this ecosystem, perhaps in efforts to restore the native grassland, could potentially result in great losses of older SOC.

Instead of respiring sources of similar isotopic compositions, during the first 10 days of the FLF incubation there was mineralization of isotopically distinct sources in the landscape elements (Fig. 3a) but a similar extent of isotopic fractionation of CO_2 relative to source (Fig. 3c; ~3‰). This could result from isotopic fractionation during degradation, differences in microbial community structure, or the mineralization of ^{13}C-enriched biopolymers such as sugars, amino acids, or microbial biomass (Schweizer et al., 1999; Lerch et al., 2010; Werth and Kuzyakov, 2010).

After the first month, in the macroaggregate fractions of younger woody stand and grassland soils there was a slight and progressive depletion of $\delta^{13}C_{CO_2}$ relative to $\delta^{13}C_{MACRO}$ that was not present in older woody stand soils (Fig. 2d). The progressive depletion could result from the preferential degradation and release of CO_2 from C_3-sources, differences in isotopic fractionation during degradation, or the ongoing degradation of lignin or other ^{13}C depleted biopolymers (*e.g.* Crow et al., 2006; Pendall and King, 2007). The extent of isotopic fraction has been shown to differ between C_3 and C_4 plants and between plants with differing C/N ratios (Werth and Kuzyakov, 2010); this could potentially result in differences seen between the landscape elements. In addition, the degradation of lignin also could be more extensive in younger cluster and grassland soils, resulting in the sustained release of depleted CO_2, as grass lignin (and monomers more prevalent in grass lignin) is more readily degraded than wood lignin (Ertel and Hedges, 1984; Rodriguez et al., 1996) and the type and cross-linking of lignin with other polymers impacts residue decomposition (Machinet et al., 2011). Also, due to the wide variation in the isotopic composition of different lignin components (*e.g.* greater depletion of syringyl lignin) (Dignac et al., 2005), differences in the composition of lignin may result in differences in the isotopic composition of respired CO_2.

The enrichment of the CO_2 respired from grassland soils ($\sim 1‰$) relative to $\delta^{13}C_{FLF}$ after the first month of incubation (Fig. 3d) indicates that in the grassland FLF there may have been a slight preference for C_4 over C_3-derived C (*e.g.* Wynn and Bird, 2007), or a slight isotopic enrichment by the microbial community that was not present in the woodland soils. However, in general for all landscape elements there was minimal fractionation of CO_2 relative to SOC, suggesting there was no to minimal use of different sources during decomposition of the FLF (*e.g.* Blagodatskaya et al., 2011).

5. Conclusions

During the year-long incubations of size (macroaggregates, >250 μm) and density (FLF, density <1.0 g cm^{-3}) separated soil fractions, we measured smaller proportional losses of soil fraction C from older woody stands relative to younger woody stands and grassland soils, suggesting that changes in litter chemistry, potentially combined with decreases in extractable organic N, are slowing down short-term decay dynamics in these more physically unprotected C pools. Pools with decadal turnover times, such as those examined in this study, are the dominant players in SOC losses through respiration, and this work suggests that although SOC chemistry plays a role in decay dynamics within soils fractions that possess fewer abiotic "regulatory gate" controls upon decomposition (higher C content and fewer mineral associations), the relevance to stabilization in whole soils will be largely dependent on other factors.

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