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Controls on soil carbon accumulation during woody plant encroachment: Evidence from physical fractionation, soil respiration, and $\delta^{13}C$ of respired CO_2

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ABSTRACT

Woody plant encroachment into grasslands and savannas is a globally extensive land-cover change that alters biogeochemical processes and frequently results in soil organic carbon (SOC) accrual. We used soil physical fractionation, soil respiration kinetics, and the isotopic composition of soil respiration to investigate microbial degradation of accrued SOC in sandy loam soils along a chronosequence of C_3woody plant encroachment into a C_4-dominated grassland in southern Texas. Our previous work in this system demonstrated significant changes in the chemistry and abundance of lignin and aliphatic biopolymers within particulate soil fractions during the first 40 yrs of woody plant encroachment, indicating selective accrual of purportedly more recalcitrant plant chemicals. However, during the long-term soil laboratory incubation presented herein, a greater proportion of SOC was mineralized in soils from older woody stands (34–86 yrs) than in soils from younger woody stands (14–23 yrs) and grasslands, providing no evidence for greater biochemical recalcitrance as a controlling mechanism for SOC accrual. In addition, $\delta^{13}C$ values of respired CO_2 indicate that the mineralized SOC was predominately of C_3 origin from all woody stands along the chronosequence, and that respired CO_2 was primarily derived from the free light fraction (density <1.0 g/cm^3) and macroaggregate-sized soil fraction. Our data suggested that the location of SOC among soil fractions was more important than plant polymer chemistry in determining SOC turnover rates during incubation. Surprisingly, estimates of the size and turnover rate of the active SOC pool based on respiratory kinetics did not increase with woody encroachment, and the turnover rate of the slower SOC pool decreased, again supporting the notion that increases in biochemically recalcitrant biopolymers did not hinder decomposition in the lab. These data indicate environmental conditions that may allow for C accrual in the field were alleviated during the controlled incubation. Therefore, C accrual in these sandy loam soils following woody encroachment should not be assumed stable, and this factor should be taken into account when considering responses of SOC to climate change or when making management decisions regarding land cover impacts on SOC.

1. Introduction

Grasslands and savannas cover 20% of the Earth's land surface (Lieth, 1975) and store 30% of global soil organic carbon (SOC) (Field et al., 1998). Woody plant encroachment into grasslands and savannas is a significant global change phenomenon (Archer, 1995) driven primarily by shifts in land use, that impacts biogeochemical cycling of SOC and nutrients in sometimes unpredictable ways. Contrasting results have been reported regarding the extent and even direction of SOC accrual in response to woody encroachment (Boutton et al., 1998; Jackson et al., 2002). As some estimates attribute 20–40% of the US terrestrial C sink to woody encroachment (Pacala et al., 2001), it is important to evaluate the impacts of this land cover change on soil carbon storage and dynamics.

SOC is stabilized by a combination of three interacting factors: (i) chemical protection, (ii) physical protection, and (iii) inherent structural resistance to degradation (Sollins et al., 1996). SOC can be chemically protected by organo–mineral complexes to silt and clay particles that limit exposure to SOC-degrading enzymes (Hassink, 1997; Six et al., 2002). Physical protection occurs when SOC is held within stable soil aggregates, limiting water and oxygen diffusion and reducing physical access by decomposer organisms (Jastrow et al., 1996). Additionally, depending on the type of soil

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microorganisms present, biochar and forms of lignin, tannins, and aliphatics have been shown to be more resistant to degradation and exhibit inherently slower decay rates (Derenne and Largeau, 2001; Kalbitz et al., 2003). In concert, these factors help to determine the overall biogeochemical stability of microbial and plant-derived SOC, although the dominant mechanisms will vary in response to interactions between microbes, available substrates, and abiotic driving variables.

Woody plant encroachment has been well-characterized in the Rio Grande Plains region of Texas where C_3 subtropical thorn woodlands have invaded areas that were once C_4-dominated grasslands (Archer, 1990; Boutton et al., 1998; Whittaker et al., 1979), resulting in linear accrual of both SOC and soil total nitrogen with woody stand age (Archer et al., 2001; Boutton et al., 1998). Research conducted at the Texas AgriLife La Copita Research Area has demonstrated that increases in SOC in this region outpace concurrent increases in microbial biomass, resulting in lowered efficiencies of respiration (Liao and Boutton, 2008). This effect could result from the restructuring of soil organic pools that enhance physical protection, greater inputs of biochemically recalcitrant biomolecules in response to changing plant community compositions, and/or changes in environmental conditions that suppress microbial decay rates relative to plant input rates. However, in the sandy loam upland portions of this landscape, C accrual is largely in non-aggregated particulate fractions (Liao et al., 2006a) which have relatively short mean residence times ($\sim$18–24 years, Liao et al., 2006b). Therefore, it is unlikely that physical protection is the dominant control over SOC storage and dynamics in this system. Changing litter chemistry may play an important role in slowing decomposition of woody tissue input, as previous work has shown that particulate C undergoes a dramatic shift in lignin and aliphatic polyesters in the first 40 years of woody stand development (Filley et al., 2008). From these data, it has been suggested that increased lignin and suberin and cutin polyesters could allow for plant C input to outpace microbial decomposition, especially within non mineral-bound soil fractions (Filley et al., 2008; Liao and Boutton, 2008; McCulley et al., 2004). Therefore, both biochemical recalcitrance and environmental conditions that influence microbial decay rates remain viable mechanisms for C accrual in this region.

The purpose of this study was to determine controls facilitating the accumulation of SOC following woody plant invasion into grasslands of the Rio Grande Plains region of Texas. At the Texas AgriLife La Copita Research Area we assessed the relative influence of increasing woody plant input upon SOC storage and dynamics by: (i) characterizing the distribution of SOC within soil physical fractions; and (ii) utilizing laboratory soil incubations coupled with measurements of the stable carbon isotopic composition of respired CO_2. We hypothesized that if newer (C_3) and older (C_4) SOC is equally accessible and degradable, then the magnitude of the difference between the $\delta^{13}C$ values of CO_2 and SOC should be identical among woody and grassland soils at each time point through the incubation. If, however, microbes are preferentially respiring newer C_3-derived SOC located within poorly protected soil physical fractions, the $\delta^{13}C$ values of respired CO_2 should reflect those fractions rather than bulk SOC. Also, if SOC accrual is controlled primarily by a progressive shift to proportionately more biochemically recalcitrant materials, the relative proportion of SOC respired during the incubation should be lowest in the oldest woody plant stands. Alternatively, if environmental conditions (*e.g.* water stress, nutrient limitation, or plant controlled allelopathy) are restricting microbial decay rates below C-input rates, the relative proportion of C respired from woody stands should surpass the grasslands during the idealized conditions of the incubation.

2. Materials and methods

2.1. Site description

Soils for this experiment were sampled from the Texas AgriLife La Copita Research Area in the Rio Grande Plains region of the Tamaulipan Biotic Province in southern Texas (27°40′N; 98°12′W) (Blair, 1950). The climate is subtropical, with mean annual temperature of 22.4 °C and an average annual rainfall of 716 mm that is delivered mostly during 2 rainy seasons (fall and spring). The topography of the site is fairly level at 75–90 m above sea level. The natural vegetation at La Copita is *Prosopis–Acacia–Andropogon–Setaria* savanna (Küchler, 1964), but fire suppression combined with livestock grazing has resulted in woody plant encroachment over the past 150 years, causing substantial replacement of the native grassland vegetation with subtropical thorn woodlands (Archer, 1990; Archer et al., 2001; Boutton et al., 1998). Discrete woody clusters are initiated with the establishment of a single *Prosopis glandulosa* (Torr.) var. *glandulosa* (mesquite), a drought-tolerant, nitrogen-fixing tree legume. Subsequently, other tree/shrub species colonize the *Prosopis* understory (Archer et al., 1988). The woody vegetation is dominated by *P. glandulosa* and *Zanthoxylum fagara* and the remnant grasslands are dominated by species of *Chloris, Panicum, Bouteloua,* and *Tridens.* Soils under the remnant grasslands and discrete woody clusters are sandy loams (Typic and Pachic Argiustolls). For more detailed site descriptions see Archer (1995), Boutton et al. (1998) and Scifres and Koerth (1987).

2.2. Soil sampling and chronosequence approach

Soil samples were taken in October 2006 from 15 discrete woody clusters and 15 native grassland sites; each woody cluster was paired with an open grassland site immediately adjacent to it but not closer than 3 m from the boundary of the canopy. Soil cores of 5 cm diameter and 30 cm depth were taken from the mineral soil in the four cardinal directions within 50 cm of the trunk of the mesquite tree within each of the woody stands. Soil cores were then sampled from the adjacent grasslands to account for site-specific variations, and were taken in the four cardinal directions around a randomly selected C_4 grass. Cores were fractionated into 0–5 cm, 5–10 cm, 10–15 cm, and 15–30 cm depths. The four cores were combined and homogenized within each depth to obtain a composite sample. After sampling, the cores were placed on ice until arrival at the lab for processing. The homogenized field-moist samples were passed through an 8-mm sieve and then oven-dried at 50 °C until soils reached constant weight. Any fine roots $<$8 mm were not removed from the soil. A portion of the soil from the 0–10 cm depth was subjected to physical fractionation (as described below). Only the 0–10 cm depth was used in this study as it has been shown to exhibit the greatest changes in carbon accrual rate, organic chemistry, and soil physical structure.

Because *P. glandulosa* initiates cluster formation (Archer et al., 1988), the age of the mesquite tree in each cluster can be considered equivalent to woody stand age. The ages of the trees were estimated by measuring the basal diameter and using these values in site-specific regression equations developed by Stoker (1997). Soils were sampled from woody clusters ranging in age from 14 to 86 yrs to create the chronosequence.

2.3. Soil physical fractionation

Bulk soil was fractionated based upon density and size following a procedure modified from Cambardella and Elliott (1993) and Six et al. (1998). Briefly, the soil was first immersed in water and all floating fragments were aspirated and subsequently oven dried at

50 °C to constant weight. This fraction, with density <1.0 g/cm^3, is defined as the free light fraction (FLF). All remaining soil was subjected to wet sieving using a progression from 250 μm to 53 μm sieves to yield water-stable aggregate fractions (Elliott, 1986). Soil >250 μm compromised the macroaggregate-sized fraction, soil 53–250 μm the microaggregate sized fraction, and soil <53 μm the free silt and clay fraction. Respiration on whole soils only is presented in this study.

2.4. Microrespiration measurements

2.4.1. Microcosm design

Methods for the incubations are similar to those described previously (Crow et al., 2006; Swanston et al., 2002). Soil microcosms were created in 12 mL vials with septa caps (Labco, UK). The bottoms of the vials were packed with ~1 cm^3 of ashed glass wool to prevent anaerobic conditions. Two grams of oven-dried whole soil were added to each vial and mixed by gentle rolling with 2 g of ashed quartz sand (grain size 53–250 μm) to minimize caking and anaerobic conditions. Care was taken to ensure equal distributions of the soil throughout the sand and to prevent excessive shaking or disruption of the microcosms. The experiment was initiated after wetting the soil to 60% water holding capacity with an inoculum (described below).

2.4.2. Inoculum preparation

Because the soil was oven-dried after sieving and stored for 2 years, only sporulating microorganisms may have survived in the soil. This potentially incomplete microbial community was considered undesirable, and an inoculum was used to introduce a greater variety and number of microorganisms. Moreover, microbial communities may differ significantly between grasslands and woody clusters, and the inoculation was an attempt to homogenize the starting communities.

The inoculum was created from a mixture of frozen, field-moist soils that were sampled in October 2006 along with the soils used in the incubation (14.3, 33.9, 41.2, 64.7, and 85.8 year-old woody clusters and corresponding grasslands). This mixture of soil was incubated at 60% water holding capacity (WHC) for one week at 30 °C in the dark and then made into a slurry using a 1:10 ratio of soil:water (g:mL). The slurry was shaken for 1 h at room temperature, and subsequently filtered on Whatman GF/F filter paper (pore size 0.7 μm) for isolation of the inoculum (Crow et al., 2009; Swanston et al., 2002). Seven days was chosen as the incubation time for inoculum generation due to preliminary experiments that displayed the highest respiration rates after 7 days (data are not shown). To account for added carbon from the inoculum, the quantity and isotopic composition of CO_2 respired from the inoculum added to quartz sand were measured throughout the duration of the experiment. The amount of CO_2 released from the inoculum was negligible compared to the cumulative CO_2 respired over the course of the experiment.

2.4.3. CO_2 sampling procedure

After the initiation of the experiment by adding the inoculum, the whole soil was incubated in the dark at 30 °C and maintained at 60% WHC by periodic weighing and addition of small amounts (~10–100 μL) of sterile water. The quantity and isotopic composition of CO_2 respired from soil microorganisms were determined directly from the microcosm vials on days 1, 3, 5, 7, 10, 14, 21, 28, and subsequently every 28 days for one year after initiation of the experiment using a PDZ-Europa trace gas analyzer (TGA) interfaced to a 20/20 PDZ-Europa isotopic ratio mass spectrometer (IRMS) (Sercon, Crewe, UK). All reported carbon isotope ratios measured using the TGA–IRMS are expressed in standard δ notation. Average instrumental analytical precision, based upon gas standards, was 0.34‰.

Prior to each sampling event, vials were flushed with 10x their volume of humidified, CO_2-free air created by passing atmospheric air through a NaOH filled trap then bubbling through sterile water. A flushed blank vial accounted for any CO_2 not removed with the NaOH filled trap; for all sampling days this contribution was negligible. CO_2 respired by microorganisms was then allowed to accumulate in vials for subsequent measurement on the TGA–IRMS. For the first month of the incubation, CO_2 accumulated for 24 and 35 h for woodland and grassland soils, respectively. After one month, the vials needed to accumulate CO_2 for 2–9 days for woodland and grassland soils, respectively, to obtain measurable CO_2 concentrations. A trial experiment was conducted to test if the isotopic composition of CO_2 changed significantly in response to the varying CO_2 accumulation times used in this experiment. Within the first month of the incubation, accumulation times up to 74 h had no significant effect upon the isotopic composition of respired CO_2 (P values ranged from 0.14 to 0.90). During the second month of incubation, accumulation times up to 10 days (240 h) had no significant effect upon the isotopic composition of CO_2 (P values ranged from 0.09 to 0.82) (Supplementary Fig. 1). Immediately after sampling, vials were automatically flushed by the TGA with air to prevent anaerobic conditions. When not accumulating CO_2 the caps with septa were replaced with GF/F filters to permit normal gas exchange. After the last sampling day, vials were kept frozen prior to oven drying and elemental analysis (see below).

2.5. Soil carbon and nitrogen

After incubation, the frozen soils were oven-dried for 2 days at 50 °C and ground to a fine powder with a stainless steel mixer mill (Retsch, Haan, Germany). Percent C, percent N, $\delta^{13}C$ and $\delta^{15}N$ were measured on the soil using a CHN elemental analyzer (EA) (Sercon Ltd., Crewe, UK) interfaced to the 20/20 IRMS. This measurement was also performed on whole soil prior to incubation, and on all four soil physical fractions.

2.6. Calculations and statistical analyses

Statistical groupings of respiration rates and $\delta^{13}C$ values of respired CO_2 ($\delta^{13}C_{CO_2}$) justified grouping clusters aged 14–23 years (younger clusters) separately from clusters 34 to 86 years (older clusters). One-way ANOVA was used to test for differences between $\delta^{13}C$ values of respired CO_2 among different landscape elements (younger clusters, older clusters, grasslands) and differences in cumulative respiration for each sampling day during the incubation. Differences between individual days were not analyzed. Tukey's studentized range (HSD) test determined statistically significant means ($\alpha = 0.05$). Two-tailed, unpaired t-tests ($\alpha = 0.05$) were used to test for significant differences between carbon content of soil fractions.

Linear regression was used to determine the accrual rate of carbon with woody stand age. Nonlinear regression, using the linear plateau and response model, was used to determine the woody stand age at which the isotopic composition of SOC reached a steady state. Nonlinear regression of CO_2 data from the respiration experiments was used to determine the turnover rates and sizes for active (C_a) and slow pools (C_s) with the following two-pool model (Paul et al., 2001):

$$dC/dt = C_a e^{(-k_a * t)} + C_s e^{(-k_s * t)} \quad (1)$$

where dC/dt is the daily mineralization rate (μg C [g soil]$^{-1}$ day^{-1}), C_a and C_s are the active and slow pool sizes, respectively (mg C [kg soil]$^{-1}$) and k_a and k_s are the active and slow pool turnover rates, respectively. The size and MRT of the resistant pool was not determined separately,

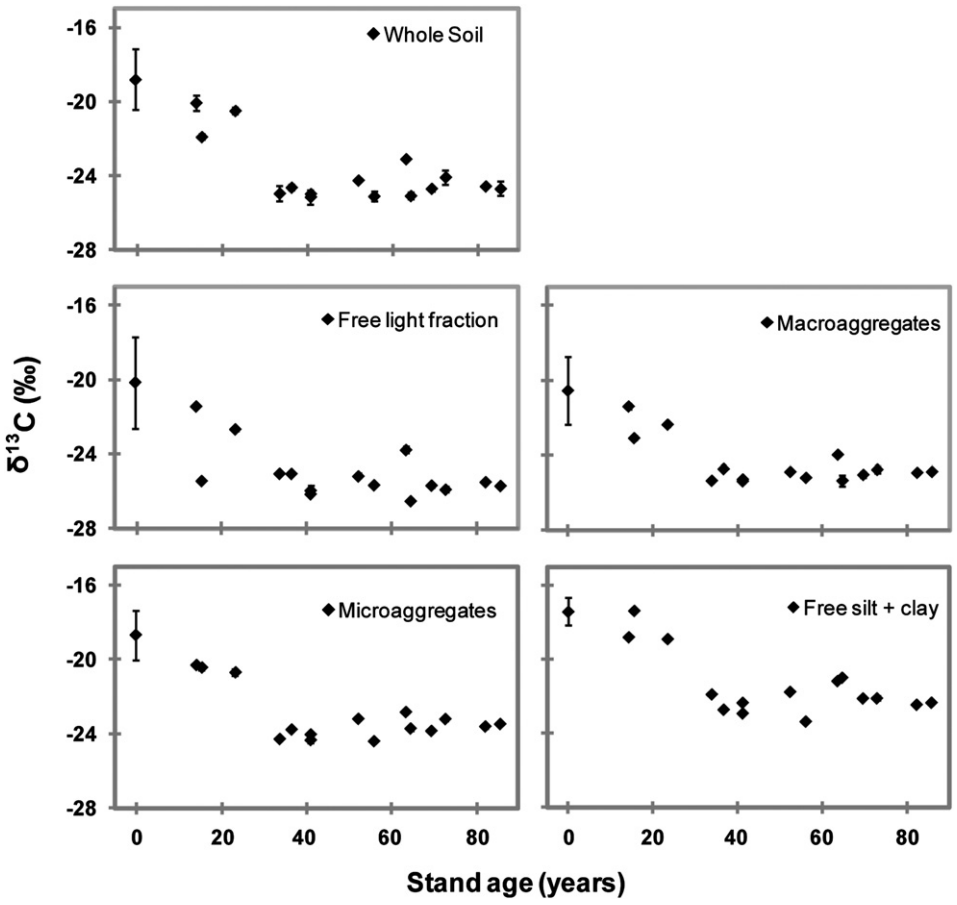

Fig. 1. The $\delta^{13}C$ (‰) values of the 0–10 cm depth of whole soil and four soil physical fractions in relation to woody stand age. Time zero represents the average of all grassland samples. Error bars indicate ± one standard deviation.

therefore C_s should be interpreted as the resistant pool and the slow pool combined. Without including the resistant pool, the two-pool model can underestimate the size and MRT of C_s, but the size the MRT of C_a is unchanged (Paul et al., 2001). Although this model can be run unconstrained, here C_s was constrained by assuming it was equal to total SOC minus C_a.

3. Results

3.1. Changes to SOC with woody plant encroachment

Within the whole soil, $\delta^{13}C$ values ranged from −16.6 to −20.3‰ in the grasslands, while in the woody clusters the $\delta^{13}C$ values decreased

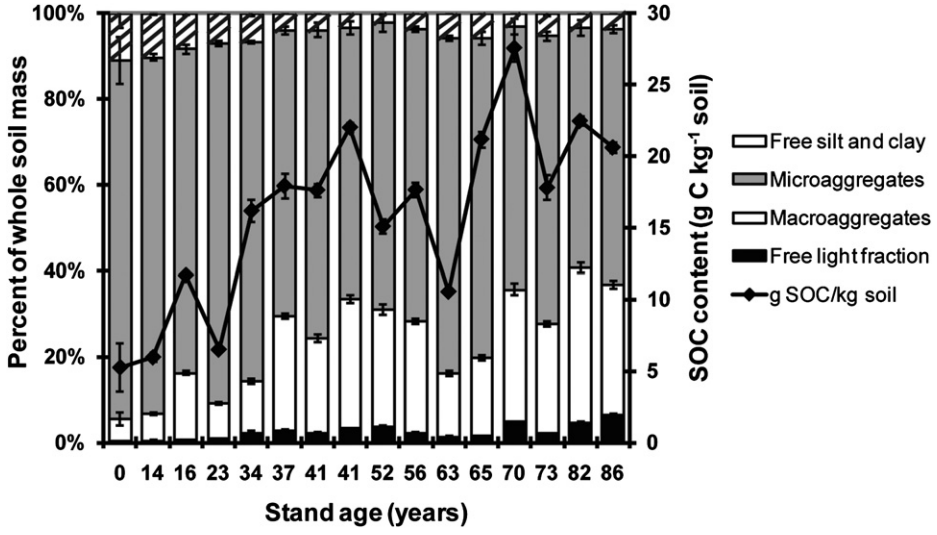

Fig. 2. Soil physical fractions (as percentage of whole soil weight, left-hand *y*-axis) and the amount of C (g C kg^{-1} soil) held in whole soil (right-hand *y*-axis) expressed relative to woody stand age. Stand age of 0 years represents the average of all grassland samples. Error bars indicate ± one standard deviation.

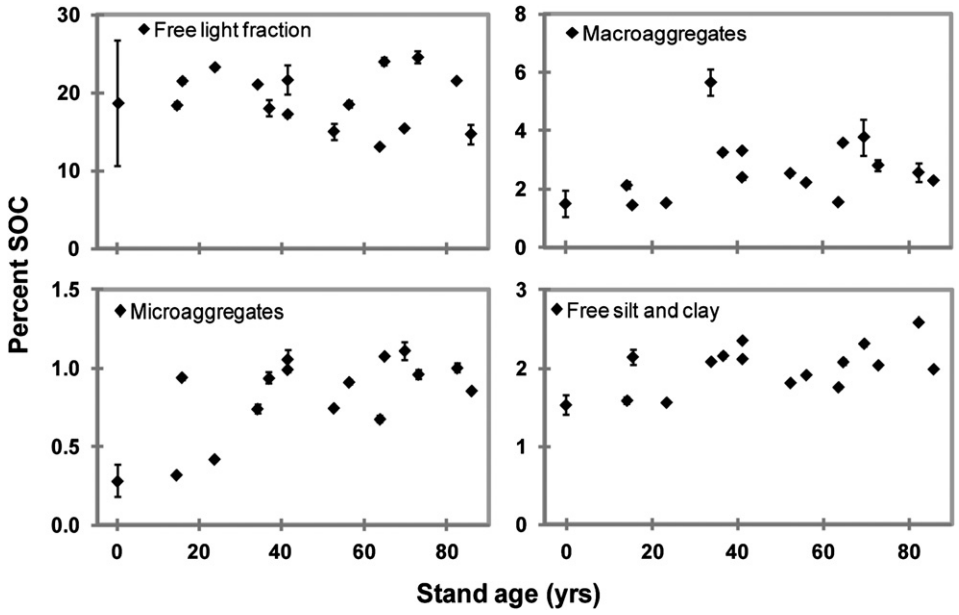

Fig. 3. Percent of soil organic carbon (SOC) held within the 0–10 cm depth of four soil physical fractions in relation to woody stand age. Error bars indicate ± one standard deviation.

until ~40 years and then remained relatively constant. However, the age at which $\delta^{13}C$ values reached constant values, and the actual $\delta^{13}C$ values, varied slightly between the whole soil and soil fractions (Fig. 1). The whole soil, FLF and macroaggregate fraction reached similar constant $\delta^{13}C$ values of −24.6‰, −25.6‰, and −25.0‰, and reached these values at 37, 39, and 36 yrs, respectively. The microaggregate and free silt and clay fractions reached higher constant $\delta^{13}C$ values of −18.2‰ and −16.4‰ at 36 and 38 yrs, respectively.

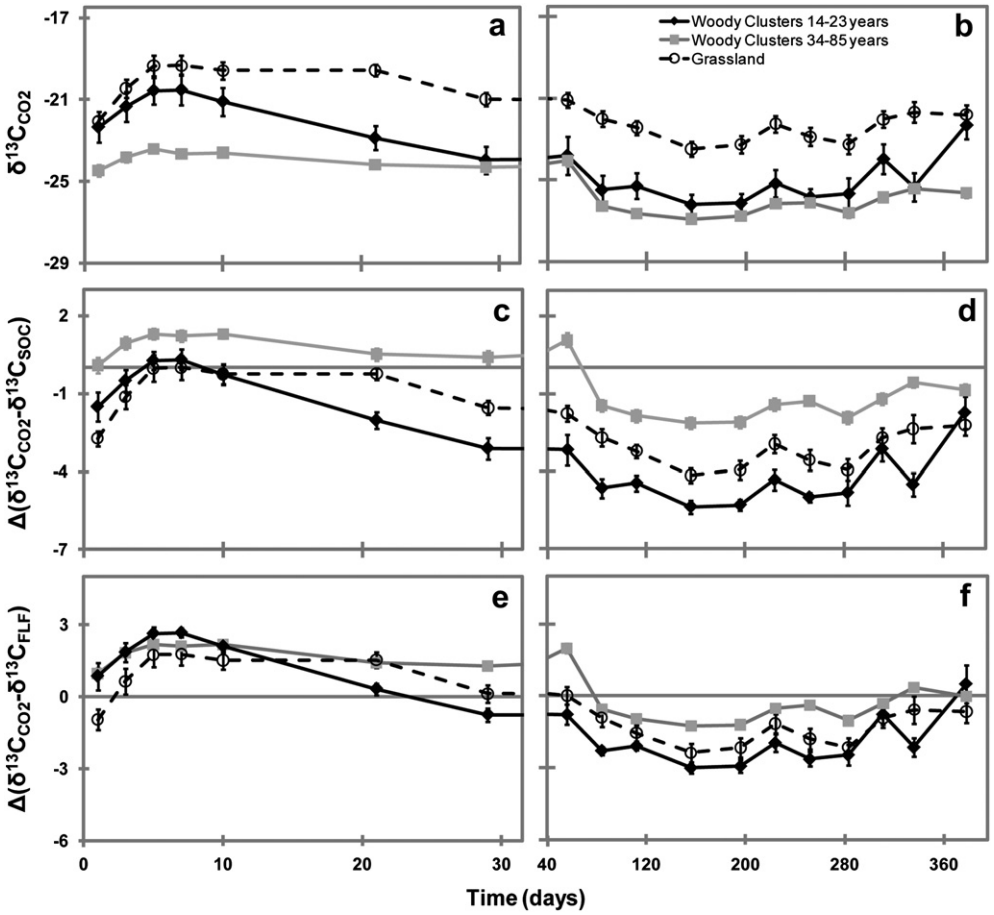

Fig. 4. (a–b) The $\delta^{13}C$ value of respired CO_2 along the course of the incubation and the difference in $\delta^{13}C$ values of respired CO_2 and (c–d) bulk SOC, (e–f) and free light fraction. For graphs c–f, negative values indicate a lower $\delta^{13}C$ value for CO_2 relative to bulk SOC or the FLF, respectively. Error bars indicate ± one standard error.

Based upon a linear regression of SOC content versus woody cluster age, woody plant encroachment resulted in SOC accrual at a rate of 15.6 g Cm^{-2} yr^{-1} ($R^2 = 0.62$) in the upper 10 cm of soil. This accrual was predominately in the FLF and macroaggregate fraction, as the weight contribution of these fractions to total soil increased with woody encroachment (Fig. 2). All soil fractions, except the FLF, showed significant %C increases in cluster soils relative to grassland soils (Fig. 3). The C/N ratio increased significantly ($P < 0.0001$) from ~9.6 in grassland soils to ~11 in woody cluster soils due to C accrual (data are not shown).

3.2. Stable carbon isotope composition of respired CO_2

During the 1-year incubation period, $\delta^{13}C_{CO_2}$ values from grassland soils were typically significantly higher than $\delta^{13}C_{CO_2}$ values from the woody cluster soils (Fig. 4a–b). The $\delta^{13}C_{CO_2}$ values for all landscape elements exhibited a complex but similar pattern as the incubation progressed; initially the $\delta^{13}C_{CO_2}$ value was less than the isotopic composition of bulk SOC ($\delta^{13}C_{SOC}$), but with time it shifted to values greater than $\delta^{13}C_{SOC}$ and then back to values less than $\delta^{13}C_{SOC}$ (Fig. 4c–d). The inflection points occurred at distinct times in the incubation depending upon the age of the cluster. Specifically, for grassland and younger cluster soils (14–23 yrs), CO_2 was most enriched in ^{13}C with respect to SOC (+0.2‰) at around day 5 of the incubation and then was depleted with respect to SOC (−1‰) by day 21 (Fig. 4c). In contrast, CO_2 from older cluster soils (34–86 yrs) was more enriched in ^{13}C on day 5 (+1.2‰) and did not become depleted with respect to SOC (1.5‰) until day 84 (Fig. 4c–d). From day 28 on, CO_2 from younger cluster and grassland soils was significantly more depleted with respect to SOC than older cluster soils (by about 3‰, Fig. 4c–d).

3.3. Transformations of SOC during incubation

The maximum and minimum percentage of SOC released as CO_2 over the course of the incubation ranged from 20.2% (52-year old cluster soil) to 8.6% (average grassland soil). In general, 15–25% of the total CO_2 respired was released within the first 30 days of the experiment. By day 84 the younger cluster soils had liberated significantly less CO_2 than older cluster soils, although neither was significantly different from the grassland soils. By day 112, however, both younger cluster and grassland soils had mineralized a significantly smaller proportion of carbon than older cluster soils, and this pattern continued throughout the course of the incubation (Fig. 5).

After incubation the mean C/N ratio of the woody cluster soils decreased significantly ($P < 0.001$) from 11 to 9.3. These changes were driven primarily by C losses. There was no significant change of the mean C/N ratio within grassland soils during the incubation (mean 9.6, $P = 0.14$) (data are not shown).

3.4. Pool sizes and turnover rates

The size of the active pool for all landscape elements varied from 1.5 to 1.9% of total SOC (Fig. 6a) and the size of the slow plus resistant pool varied from 96 to 99% of total SOC (Fig. 6b). The size and MRT of the active pool were not well correlated with stand age ($R^2 = 0.13$ and 0.27, respectively) and did not differ significantly between landscape elements (Fig. 6a). Neither the size nor the MRT of the active pool was correlated with the size of the FLF ($R^2 = 0.03$ and 0.10, respectively). The size of the slow pool did not differ significantly with stand age, although the MRT of the slow pool decreased in a linear fashion from the grassland to the 37 yr old cluster, and then remained relatively constant until 86 yrs (Fig. 6b).

4. Discussion

4.1. Response of SOC to woody plant encroachment

Similar to previous studies (Liao et al., 2006b), our data shows soils at La Copita are accruing SOC rapidly (15.6 g Cm^{-2} yr^{-1}) in the upper 10 cm of soil, although this accrual rate is about 50% faster than the accrual ratein the upper 15 cm (10.5 g Cm^{-2} yr^{-1}) reported by

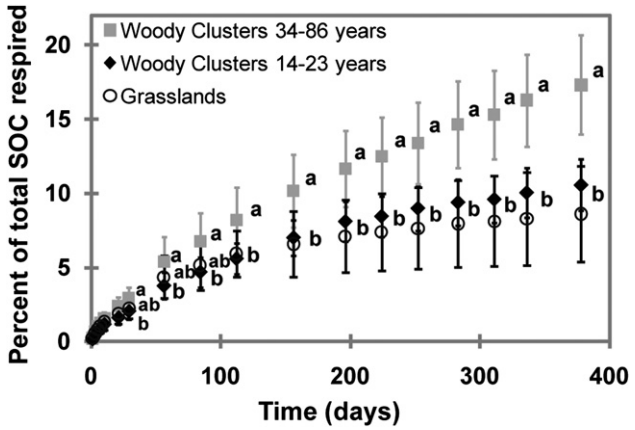

Fig. 5. The cumulative percentage of total C respired as CO_2 during the incubation. Different letters indicate statistical significance between samples for each day. Error bars indicate ± one standard deviation.

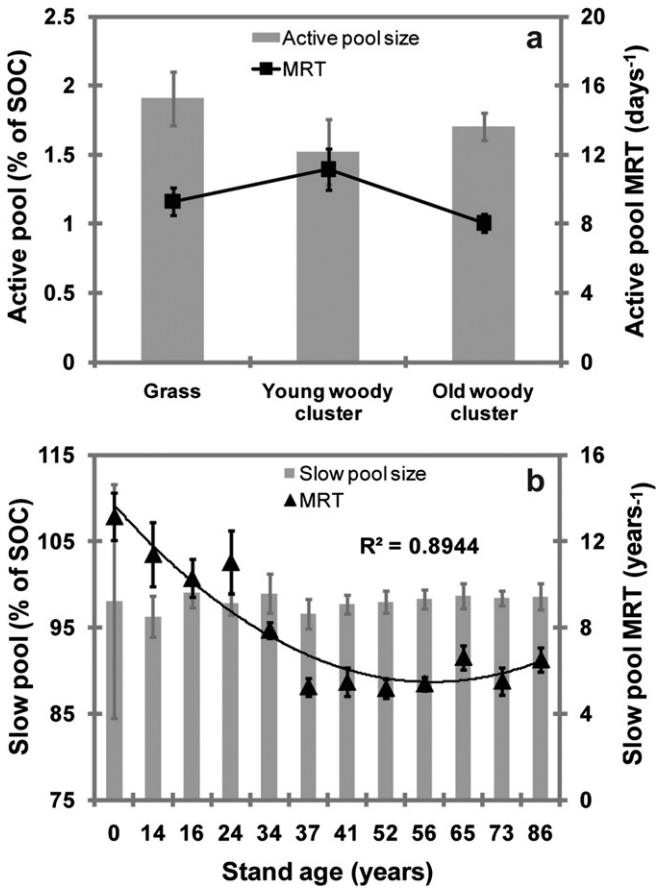

Fig. 6. The size and MRT of the (a) active pool for the three landscape elements and (b) the slow pool by stand age. Stand age of 0 years represents the average of all grassland samples. Error bars indicate ± one standard error.

Liao et al. (2006b). The extrapolation of these SOC accumulation rates to large regions should be done with caution, as this space for time chronosequence approach assumes the nature of woody development between discrete woody clusters of differing ages is equivalent to the development of one stand through time. In addition, soils were sampled within 50 cm of the *P. glandulosa* trunk, and the lateral distribution of SOC and nutrients from the base of the tree is heterogeneous and dependent upon the sampling distance from the trunk, as well as the size (and hence age) of the tree, as changes in canopy and rooting structure, understory density, the extent of nutrient cycling, and net primary productivity (NPP) can alter SOC distributions (Hibbard et al., 2003; Liu et al., 2011). These changes with age are observed at this site; for example, previous work has shown increases in aboveground NPP from 87 to 340 g Cm^{-2} yr^{-1} in the grasslands to 510–600 g Cm^{-2} yr^{-1} in encroaching woodlands (Archer et al., 2001; Hibbard et al., 2003). As samples were taken close to the base of the tree, and as fine roots were not removed from the soil prior to C analysis, the calculated C accrual rate likely reflects near maximal values.

As shown in previous studies, the distribution of size separated soil fractions was altered with woody encroachment. The greatest increases in mass occurred in the more physically unprotected FLF and macroaggregate-sized fractions (Fig. 2), resulting in greater microbial accessibility to SOC (Liao et al., 2006b). This restructuring of soil particles into water-stable aggregate structures does not represent a change in soil texture, but rather a reallocation of previously free silt and clay particles into micro- and macroaggregate structures that is potentially facilitated by increases in root biomass with woody encroachment (Liao et al., 2006b).

The observed trend of increasing SOC (Figs. 2 and 3) and ^{13}C depletion with stand age (Fig. 1) is indicative of new C_3–C input from the encroaching woody plants (Archer et al., 2001; Boutton et al., 1998; Liao et al., 2006b). The average $\delta^{13}C$ value of grassland soils (−18.8‰) indicates that C_3 forbs were a significant contributor to SOC (Boutton et al., 1998). In the grassland FLF ($\delta^{13}C = -21.5‰$), the contribution is C_3–forb C is even more pronounced. Using a two end member isotope mass balance approach (Balesdent and Mariotti, 1996), and defining the C_4 end member as −14‰ (C_4 plant material, Boutton et al., 1998) and the C_3 end member as −29‰ (average $\delta^{13}C$ of C_3 forbs in grasslands, Boutton et al., 1998), the average proportion of C_3–forb C in the FLF is roughly 50%. However, only a rough estimate can be obtained as the isotopic composition of degraded plant residues can be altered during degradation (*e.g.* Schweizer et al., 1999), the extent and direction of which was not determined separately in this experiment.

4.2. Influence of biochemical recalcitrance on SOC degradation

The cumulative amount of CO_2 respired from the grassland soils (3–11%) was within range (Pendall and King, 2007) or higher (Feng and Simpson, 2009) than respiration from other laboratory incubated grassland soils. The woody cluster soils generally liberated a greater proportion of SOC as CO_2 (9.8–20.2%) than laboratory incubations of North American corn-belt agricultural soils (~3–9%, Collins et al., 2000) and coniferous and deciduous forest soils (~2–9%, Crow et al., 2009; 4%, Haile-Mariam et al., 2000), but were within range of the SOC lost as CO_2 during a 4 yr laboratory incubation of coarse textured Australian soils (5–42%; Wynn and Bird, 2007). The greater SOC degradation observed in this study could be due to our use of only the upper 10 cm of the soil profile, the high sand content (~80%) of La Copita soils, a 5–10 °C warmer incubation temperature compared to other studies, the presence of fine roots (<8 mm) in the soil, and/or a stronger presence of potentially degradable substrates within woody clusters.

Older woody cluster soils (34–86 yrs) respired a significantly greater proportion of total SOC then younger cluster and grassland soils after day 112 of the incubation (Fig. 5). This finding indicates that either a greater proportion of SOC in the older woody cluster soils is accessible to microbes or is more labile than the grassland or younger woody cluster soils. From previous studies it is known that the poorly protected particulate organic matter (POM) fractions in woody cluster soils from this region accumulate proportionately more of what are considered to be refractory biopolymers than POM in grassland soils (Filley et al., 2008). In addition, other studies have demonstrated that respiration is lower in the presence of refractory biopolymers, specifically, in the presence of aromatic compounds (Kalbitz et al., 2003). But, if the input of increasingly refractory biopolymers was the main control impeding microbial decay rates and leading to SOC accrual at La Copita, we would not expect higher respiration from older woody cluster soils but rather a decrease in respiration with increasing cluster age. In addition, while the grassland soil C/N ratio remained constant during the incubation (~9.5), the C/N ratio of the cluster soils dropped significantly ($P < 0.0001$) from 11 to 9, indicating a greater degree of degradation in cluster versus grassland soils during the incubation. These data indicate that although there are dramatic changes to the chemistry of particulate carbon fractions (Boutton et al., 2009; Filley et al., 2008), toward what are typically considered as more refractory biopolymers, these pools are easily accessible to soil microbes because of their lack of physical protection. It is likely that some concomitant environmental or microbial changes suppress the capability of soil microbes to degrade the new input. Therefore we question, as others have at different sites (Kleber et al., 2010; Marschner et al., 2008), the importance of biochemical recalcitrance as a mechanism for C stabilization at this site.

In the field, a variety of environmental conditions could limit microbial activity and result in SOC accrual. Possible mechanisms for microbial suppression with woody encroachment could include the production of microbial inhibitors by invading plants (*e.g.*Weidenhamer and Callaway, 2010), changes to microbial community structure (Jastrow et al., 2006), enzyme suppression (Waldrop et al., 2004), and nutrient or water limitations (Huxman et al., 2005; Jackson et al., 2002). Due to low mean annual precipitation (720 mm), high mean annual temperature, (22.4 °C), and altered hydrology from the high water need by the understory (Boutton et al., 1999), there is potential that microorganisms within woody clusters are more water-stressed than their grassland counterparts. McCulley et al. (2004) demonstrated that although respiration rates were higher in woody cluster versus grassland soil, the respiration did not keep up with input, resulting in an increased MRT of SOC in woody clusters. Moreover, the links between lowered water availability with woody plant encroachment and C accrual have been established for similar ecosystems (Jackson et al., 2002; Huxman et al., 2005).

4.3. Selective respiration of accessible (unprotected) SOC pools

Published trends in $\delta^{13}C_{CO_2}$ values during the course of laboratory incubations show great variability, with studies exhibiting both higher (*e.g.* Crow et al., 2006; Schweizer et al., 1999) and lower (*e.g.* Pendall and King, 2007; Wynn et al., 2006) values relative to source C. Our system has an imbedded complexity of multiple isotopically distinct sources, with both grassland and cluster soils possessing contributions of C_3 (forb or mesquite) and C_4 (grass) derived C. Our advantage, however, is that these sources vary in proportional input with time and we have documented accrual over that same time period, allowing us to glean insight into selective C-utilization.

During the incubation, each landscape element exhibited a trend of brief enrichment in ^{13}C followed by an extended depletion (Fig. 4).

Higher $\delta^{13}C_{CO_2}$ values relative to source-C observed during other laboratory incubations have been attributed to the degradation of ^{13}C enriched biopolymers such as sugars, acids, and microbial products, the quality of available C substrates, and to shifts in microbial community composition (Schweizer et al., 1999; Fernandez et al., 2003; Crow et al., 2006). The shift from enriched to depleted ^{13}C values took place at different times and to different extents in the older cluster soils versus younger cluster and grassland soils, so that CO_2 from older cluster soils was about ~3‰ less depleted relative to SOC than younger clusters and grasslands (Fig. 4d). The smaller relative depletion in older clusters could arise from different degrees of isotopic fractionation (Fernandez et al., 2003) or from the degradation of different C sources (depleted biopolymers; C_3 versus C_4-derived C) between the three landscape elements.

The degradation of ^{13}C depleted biopolymers, such as lignin, lipids, and aliphatic biopolymers, has been proposed by others as a mechanism for CO_2 ^{13}C depletion relative to source C during incubation (Crow et al., 2006; Pendall and King, 2007). In our study the greatest potential for this isotope effect is in the oldest clusters as they contain the greatest amount of lignin and substituted fatty acids (Boutton et al., 2009; Filley et al., 2008). We observe, however, the opposite trend, where CO_2 from older cluster soils is the least depleted relative to source C (Fig. 4d–f). Although we are not eliminating lignin degradation as a mechanism of overall CO_2 depletion, we suggest that the degradation of C_3–C subpools within the more unprotected soil fractions, such as the FLF, is the dominant mechanism of the greater depletion of older cluster CO_2 relative to SOC.

Two pieces of data presented thus far are particularly important in determining the source of the respired CO_2 during the incubation: (i) for most of the incubation respired CO_2 is isotopically depleted with respect to bulk SOC (Fig. 4c–d), and (ii) the FLF is the physical fraction with the lowest $\delta^{13}C$ values, and is lower than bulk SOC (Fig. 1). We propose that the $\delta^{13}C$ values of respired CO_2 might closely reflect $\delta^{13}C$ value of the SOC in unprotected accruing fractions. Our results indicate that the most reasonable source of the majority of the respired CO_2 from the clusters is the poorly protected FLF. In fact, Fig. 4f shows that the difference between the $\delta^{13}C$ value of respired CO_2 and C source is much less for all landscape elements when considered this way. Due to similar $\delta^{13}C$ values between the FLF and the macroaggregate fraction, a comparable isotopic relationship exists between $\delta^{13}C_{CO_2}$ values and the isotopic composition of the macroaggregate fraction. This relationship is not maintained for the microaggregate and free silt plus clay fractions, as they are enriched relative to bulk SOC (Fig. 1). These data suggest that C protected within microaggregates and bound on silt and clay particles is generally being preserved during a rapid mineralization episode such as the incubation study here, while portions of the physically unprotected fractions, containing predominantly C_3 carbon in the woodland and approximately 50% C_3 carbon in the grasslands, are being preferentially degraded.

Within grasslands, 14% of total SOC is held within the FLF while 19% is held within the macroaggregate fraction. After ~30 years of woody plant encroachment these proportions nearly double to 29% and 38%, respectively (calculated using Figs. 2 and 3). This provides further evidence that these fractions contributed greatly to CO_2 release during the incubation. To assess the potential contribution of these unprotected fractions to CO_2 release, the cumulative percent of SOC respired during incubation was plotted against the percentage weight of each soil fraction to the whole soil (Fig. 7). Positive significant correlations were observed between the percent cumulative C respired and both the macroaggregate fraction and FLF, while negative correlations were observed for the silt plus clay fraction and the microaggregate fraction. As it was previously demonstrated (Filley et al., 2008), purportedly biochemically recalcitrant biopolymers accrue in particulate soil fractions, which are found in the FLF and macroaggregate fractions, therefore a positive correlation should not be observed if biochemical recalcitrance is preventing degradation during the incubation. These data, along with positive correlations between cumulative respiration and initial SOC ($R^2 = 0.55$, $P < 0.0001$) suggest that it is the amount and location of C, rather than its biochemical composition, that dictates total respiration during the incubation. Our results are consistent with previous studies that attribute C loss during respiration to soil structure, where disruption of soil aggregates increases CO_2 efflux (Elliott, 1986; Gregorich et al., 1989) and the proportions of silt and clay are frequently correlated with SOC storage (Hassink, 1997; Six et al., 2002).

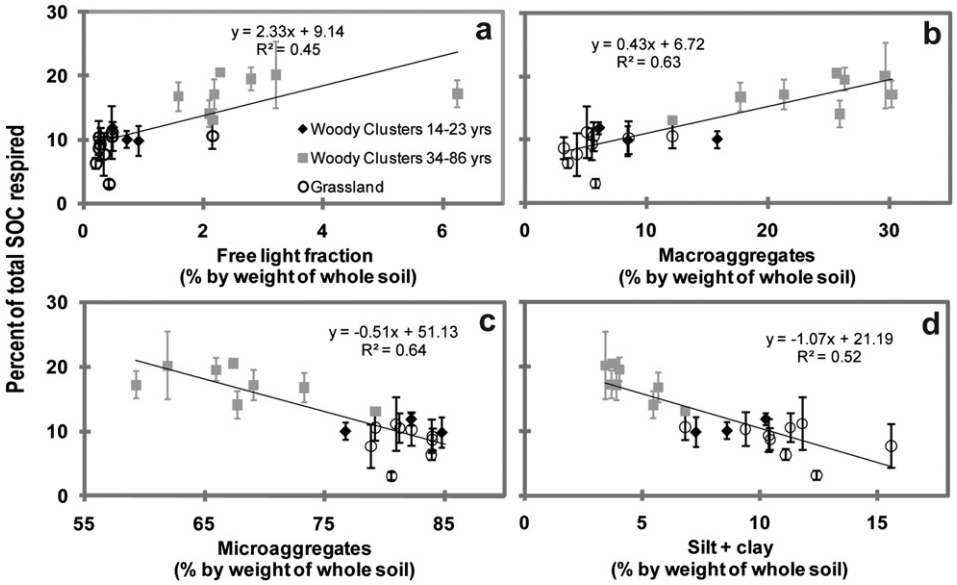

Fig. 7. The relationships between the cumulative C lost during the incubation and the percentage by weight of whole soil of each soil fraction. Open circles represent the native grasslands while filled diamonds and squares represent woody stands. Error bars indicate ± one standard deviation.

4.4. Relationships between landscape elements and conceptual SOC pools

The size of the active pool (C_a) for the grassland soils (1.5%) is within the range of C_a values determined for other grassland soils (0.7–1.4% Haile-Mariam et al., 2000; 0.5–1.5% Pendall and King, 2007) and the size of C_a for the cluster soils (0.95–4%) is within range of published values for woodland soils (0.7–9%) (Collins et al., 2000; Paul et al., 2006). The range of calculated C_a MRT (5–35 days) is similar to those determined for air-dried corn-belt soils (8–18 days, (Collins et al., 2000)). Contrary to our hypothesis where we expected the size and the MRT of C_a to increase with woody plant encroachment, as predicted from field-based respiration studies at La Copita (McCulley et al., 2004), we found no significant changes in C_a with increasing stand age or between landscape elements. Moreover, we found no correlations between the size of the active pool and the amount SOC held within the FLF or macroaggregate-sized fraction. These data appear to be in contrast the respiration correlations (Fig. 7) and isotopic data (Fig. 4f) that suggest that the FLF and macroaggregate fraction are major C sources during the incubation. A possible explanation is that changes to the MRT of conceptual soil pools is promoted by the optimal conditions of the incubation, allowing for increased overall degradation in woody cluster soils. Alternatively, the active pool may be comprised of varying proportions of the FLF and macroaggregate-sized fraction, independent of the fraction size or cluster age. Or, it may be that the model itself does not properly represent active C. Indeed, others have debated the relevance of such calculations to field conditions, especially as drying of soils can affect the estimation of active SOC MRTs (Collins et al., 2000).

Similar to previous studies (Collins et al., 2000; Haile-Mariam et al., 2000; Paul et al., 2006; Pendall and King, 2007), we found that most of the SOC in La Copita soils was held in slow plus resistant (C_s) pool. The calculated MRTs of the slow pool in this study (5–13 yr) are shorter than results from Paul et al. (2006) for agricultural and pine forest soils (27–45 yr) but were similar to incubations on soils from corn-belt agroecosystems (~9–20 yr, Collins et al., 2000) and grassland soils (~2–6 yr, Pendall and King, 2007). As described by Pendall and King (2007), the optimal conditions for decomposition created by the incubation, and a lack of adjustment of MRT to field temperatures results in slightly shorter estimated MRT for the slow pool than the MRT measured under field conditions. Our MRT calculations of C_s could be particularly skewed to faster turnover because of our omission of the resistant (or passive) C pool from the model (Paul et al., 2001). Interestingly, the MRT of the slow pool decreased in a linear fashion from the grassland to the 37 yr old cluster, and then stabilized (Fig. 6b). This pattern of more rapid turnover of the slow pool along the chronosequence supports the idea that the C accruing with woody plant encroachment is not necessarily stable C, although these results should be interpreted with caution as we did not isolate the refractory (passive) C pool. We attempted to reduce this problem by constraining the slow pool, setting it equal to total SOC minus C_a. As our results for the size and MRT of the slow (plus resistant) pools are similar to other published studies, and confirm the other results of the experiment, we feel that these are accurate estimations.

5. Conclusions

Through soil physical fractionation and soil respiration kinetics combined with isotopic data, we sought to determine the controls facilitating accumulation of SOC following woody plant invasion into grasslands of the Rio Grande Plains region of Texas. Although previous work in this area has shown that purportedly biochemically recalcitrant compounds accrue with woody encroachment, our data suggested that the packaging of SOC into more unprotected soil fractions was more important than plant polymer chemistry in determining SOC turnover rates during incubation. This notion was supported by correlations between the size of more unprotected SOC pools and cumulative respiration (Fig. 7) and the similarity of the isotopic composition of respired CO_2 to these fractions (Fig. 4c–f). These data indicate that the increased input of biochemically recalcitrant biomolecules with woody encroachment is not the primary mechanism for SOC accrual. Therefore, we hypothesize that changes in microbial activity, perhaps as a result of changes in microbial community structure or changing environmental conditions that suppress decay rates below input rates, are the dominant mechanisms for SOC accrual.

The fact that the C_3-derived carbon was rapidly respired from all landscape elements under the optimal conditions of the laboratory incubation indicates that if environmental conditions were to change, resulting in a reduction of NPP or an increase in microbial activity in the woody clusters, the accrued C_3-derived SOC could be lost through decomposition. This idea is further supported by the decreasing MRT of the slow pool over the first 40 yrs of woody stand development. These results should be taken into consideration when making management decisions regarding woody plant control on rangelands or when treating woody plant encroachment as a C sink in modeling scenarios. Further work should be directed towards understanding the biological and environmental mechanisms responsible for altering soil C storage and dynamics following woody encroachment in this region.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at "doi:10.1016/j.soilbio.2011.04.013".

References

Archer, S.R., 1990. Development and stability of grass/woody mosaics in a subtropical savanna parkland, Texas, U.S.A. Journal of Biogeography 17, 453–462.

Archer, S.R., 1995. Tree–grass dynamics in a Prosopis-thornscrub savanna parkland: reconstructing the past and predicting the future. Ecoscience 2, 83–99.

Archer, S.R., Scifres, C., Bassham, C., Maggio, R., 1988. Autogenic succession in a subtropical savanna: conversion of grassland to thorn woodland. Ecological Monographs 58, 111–127.

Archer, S.R., Boutton, T.W., Hibbard, K., 2001. Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schulze, E.-D., Harrison, S.P., Heimann, M., Holland, E.A., Lloyd, J., Prentice, I.C., Schimel, D. (Eds.), Global Biogeochemical Cycles in the Climate System. Academic Press, San Diego, pp. 1–46.

Balesdent, J., Mariotti, A., 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. In: Boutton, T.W., Yamasaki, S. (Eds.), Mass Spectrometry of Soils. Marcel Dekker, New York, pp. 83–111.

Blair, W., 1950. The biotic provinces of Texas. The Texas Journal of Science 2, 93–117.

Boutton, T.W., Archer, S.R., Midwood, A.J., 1999. Stable isotopes in ecosystem science: structure, function and dynamics of a subtropical savanna. Rapid Communications in Mass Spectrometry 13, 1263–1277.

Boutton, T.W., Archer, S.R., Midwood, A.J., Zitzer, S., Bol, R., 1998. $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. Geoderma 82, 5–41.

Boutton, T.W., Liao, J.D., Filley, T.R., Archer, S.R., 2009. Belowground carbon storage and dynamics accompanying woody plant encroachment in a subtropical savanna. In: Lal, R., Follett, R. (Eds.), Soil Carbon Sequestration and the Greenhouse Effect. Soil Science Society of America, Madison, WI, pp. 181–205.

Cambardella, C., Elliott, E., 1993. Carbon and nitrogen distribution in aggregates from cultivated and native grassland soils. Soil Science Society of America Journal 57, 1071–1076.
Collins, H.P., Elliott, E.T., Paustian, K., Bundy, L.G., Dick, W.A., Huggins, D.R., Smucker, A.J.M., Paul, E.A., 2000. Soil carbon pools and fluxes in long-term corn belt agroecosystems. Soil Biology and Biochemistry 32, 157–168.
Crow, S.E., Lajtha, K., Filley, T., Swanston, C., Bowden, R., Caldwell, B.A., 2009. Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. Global Change Biology 15, 2003–2019.
Crow, S.E., Sulzman, E.W., Rugh, W.D., Bowden, R.D., Lajtha, K., 2006. Isotopic analysis of respired CO_2 during decomposition of separated soil organic matter pools. Soil Biology and Biochemistry 38, 3279–3291.
Derenne, S., Largeau, C., 2001. A review of some important families of refractory macromolecules: composition, origin, and fate in soils and sediments. Soil Science 166, 833–847.
Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal 50, 627–633.
Feng, X., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804–812.
Fernandez, I., Mahieu, N., Cadish, G., 2003. Carbon isotopic fractionation during decomposition of plant materials of different quality. Global Biogeochemical Cycles 17, 1075. doi:10.1029/2001GB001834.
Field, C., Behrenfeld, M., Randerson, J., Falkowski, P., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281, 237–240.
Filley, T.R., Boutton, T.W., Liao, J.D., Jastrow, J.D., Gamblin, D.E., 2008. Chemical changes to nonaggregated particulate soil organic matter following grassland-to-woodland transition in a subtropical savanna. Journal of Geophysical Research 113 (G03009). doi:10.1029/2007JG000564.
Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. European Journal of Soil Science 40, 649–659.
Haile-Mariam, S., Cheng, W., Johnson, D., Ball, J., Paul, E., 2000. Use of carbon-13 and carbon-14 to measure the effects of carbon dioxide and nitrogen fertilization on carbon dynamics in ponderosa pine. Soil Science Society of America Journal 64, 1984–1993.
Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77–87.
Hibbard, K., Schimel, D., Archer, S.R., Ojima, D., Parton, W., 2003. Grassland to woodland transitions: integrating changes in landscape structure and biogeochemistry. Ecological Applications 13, 911–926.
Huxman, T.E., Wilcox, B.P., Breshears, D.D., Scott, R.L., Snyder, K.A., Small, E.E., Hultine, K., Pockman, W.T., Jackson, R.B., 2005. Ecohydrological implications of woody plant encroachment. Ecology 86, 308–319.
Jackson, R.B., Banner, J.L., Jobbagy, E.G., Pockman, W.T., Wall, D.H., 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418, 623–626.
Jastrow, J.D., Miller, R.M., Boutton, T.W., 1996. Carbon dynamics of aggregate-associated organic matter estimated by carbon-13 natural abundance. Soil Science Society of America Journal 60, 801–807.
Jastrow, J.D., Amonette, J.E., Bailey, V.L., 2006. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80, 5–23.
Kalbitz, K., Schmerwitz, J., Schwesig, D., Matzner, E., 2003. Biodegradation of soil-derived dissolved organic matter as related to its properties. Geoderma 113, 273–291.
Kleber, M., Nico, P., Plante, A., Filley, T., Kramer, M., Swanston, C., Sollins, P., 2010. Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. Global Change Biology.
Küchler, A.W., 1964. Potential Natural Vegetation of the Conterminous United States. American Geographical Society, New York.
Liao, J.D., Boutton, T.W., 2008. Soil microbial biomass response to woody plant invasion of grassland. Soil Biology and Biochemistry 40, 1207–1216.
Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006a. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. Soil Biology and Biochemistry 38, 3184–3196.
Liao, J.D., Boutton, T.W., Jastrow, J.D., 2006b. Organic matter turnover in soil physical fractions following woody plant invasion of grassland: evidence from natural 13C and 15N. Soil Biology and Biochemistry 38, 3197–3210.
Lieth, H., 1975. Modeling the primary productivity of the world. In: Lieth, H., Whittaker, R.H. (Eds.), Primary Productivity of the Biosphere. Springer-Verlag, New York, pp. 237–263.
Liu, F., Wu, X.B., Bai, E., Boutton, T.W., Archer, S.R., 2011. Quantifying soil organic carbon in complex landscapes: an example of grassland undergoing encroachment of woody plants. Global Change Biology 17, 1119–1128.
Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L., Wiesenberg, G.L.B., 2008. How relevant is recalcitrance for the stabilization of organic matter in soils? Journal of Plant Nutrition and Soil Science 171, 91–110.
McCulley, R., Archer, S.R., Boutton, T.W., Hons, F., Zuberer, D., 2004. Soil respiration and nutrient cycling in wooded communities developing in grassland. Ecology 85, 2804–2817.
Pacala, S.W., Hurtt, G.C., Baker, D., Peylin, P., Houghton, R.A., Birdsey, R.A., Heath, L., Sundquist, E.T., Stallard, R.F., Ciais, P., Moorcroft, P., Caspersen, J.P., Shevliakova, E., Moore, B., Kohlmaier, G., Holland, E., Gloor, M., Harmon, M.E., Fan, S., Sarmiento, J.L., Goodale, C.L., Schimel, D., Field, C.B., 2001. Consistent land- and atmosphere-based U.S. carbon sink estimates. Science 292, 2316–2320.
Paul, E.A., Morris, S.J., Böhm, S., 2001. The determination of soil C pool sizes and turnover rates: biophysical fractionation and tracers. In: Lal, R., Kimble, J.M., Eswaran, H., Stewart, B.A. (Eds.), Assessment Methods for Soil Carbon. Lewis Publications, Boca Raton, FL, pp. 193–205.
Paul, E.A., Morris, S.J., Conant, R.T., Plante, A.F., 2006. Does the acid hydrolysis-incubation method measure meaningful soil organic carbon pools? Soil Science Society of America Journal 70, 1023–1035.
Pendall, E., King, J.Y., 2007. Soil organic matter dynamics in grassland soils under elevated CO_2: insights from long-term incubations and stable isotopes. Soil Biology and Biochemistry 39, 2628–2639.
Schweizer, M., Fear, J., Cadisch, G., 1999. Isotopic (^{13}C) fractionation during plant residue decomposition and its implications for soil organic matter studies. Rapid Communications in Mass Spectrometry 13, 1284–1290.
Scifres, C., Koerth, B., 1987. Climate, Soils, and Vegetation of the La Copita Research Area. Texas Agricultural Experimental Station, MP-1626, College Station, TX.
Six, J., Conant, R.T., Paul, E.A., Paustian, K., 2002. Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant and Soil 241, 155–176.
Six, J., Elliott, E.T., Paustian, K., Doran, J., 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Science Society of America Journal 62, 1367–1377.
Sollins, P., Homann, P., Caldwell, B.A., 1996. Stabilization and destabilization of soil organic matter: mechanisms and controls. Geoderma 74, 65–105.
Stoker, R., 1997. Object-Oriented, Spatially Explicit Simulation Model of Vegetation Dynamics in a South Texas Savanna. PhD Dissertation, Texas A&M University, College Station, TX. UMI Dissertation Number 9729281.
Swanston, C.W., Caldwell, B.A., Homann, P.S., Ganio, L., Sollins, P., 2002. Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. Soil Biology and Biochemistry 34, 1121–1130.
Waldrop, M.P., Zak, D.R., Sinsabaugh, R., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological Applications 14, 1172–1177.
Weidenhamer, J.D., Callaway, R.M., 2010. Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. Journal of Chemical Ecology 36, 59–69.
Whittaker, R.H., Gilbert, L.E., Connell, J.H., 1979. Analysis of two-phase pattern in a mesquite grassland, Texas. Journal of Ecology 67, 935–952.
Wynn, J.G., Harden, J.W., Fries, T.L., 2006. Stable carbon isotope depth profiles and soil organic carbon dynamics in the lower Mississippi Basin. Geoderma 131, 89–109.
Wynn, J.G., Bird, M.I., 2007. C4-derived soil organic carbon decomposes faster than its C3 counterpart in mixed C3/C4 soils. Global Change Biology 13, 2206–2217.