

Soil Carbon Sequestration and the Greenhouse Effect

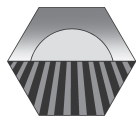
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Belowground Carbon Storage and Dynamics Accompanying Woody Plant Encroachment in a Subtropical Savanna

T.W. Boutton and J.D. Liao

Dep. of Ecosystem Science and Management, Texas A&M University, College Station, TX

T.R. Filley

Dep. of Earth and Atmospheric Sciences and Purdue Climate Change Research Center, Purdue University, West Lafayette, IN

S.R. Archer

School of Natural Resources, University of Arizona, Tucson, AZ

Woody plant encroachment into grass-dominated ecosystems during the past century has been documented in North and South America, Australia, Africa, and Southeast Asia (Archer et al., 2001) and appears to be a consequence of human land-use activities, primarily livestock grazing and fire suppression (Archer et al., 1995; Van Auken, 2000). Major functional consequences of increased woody plant abundance in grasslands may include alterations of above- and belowground productivity, changes in the quality of litter inputs, modifications to rooting depth and distribution, altered hydrology, and changes in microclimate and energy balance (Scholes and Hall, 1996; Connin et al., 1997; Gill and Burke, 1999; Jackson et al., 2000, 2002; Jobbágy and Jackson, 2000; Chapin et al., 2005; Huxman et al., 2005; Weintraub and Schimel, 2005; Hughes et al., 2006). Additionally, many of the woody plant species encroaching into grasslands are capable of symbiotic N_2 -fixation, adding fixed N to N-limited ecosystems (Rundel et al., 1982; Johnson and Mayeux, 1990; Stock et al., 1995; Zitzer et al., 1996; Baer et al., 2006). These alterations in ecosystem characteristics following woody plant encroachment into grasslands have strong potential to modify C dynamics and storage and may influence regional and global climate through feedback interactions (Schlesinger et al., 1990; Ojima et al., 1999; Asner et al., 2004). Despite the possibility that woody plant invasion could influence the carbon cycle at ecosystem and global scales, mechanisms and processes associated with C dynamics and storage in the affected systems are poorly understood (Jackson et al., 2000, 2002; Archer et al., 2001, 2004; Hudak et al., 2003; Wessman et al., 2004).

Increased storage of soil organic carbon (SOC) is likely to occur if the rate of net primary productivity (NPP) increases relative to the rate of organic matter decomposition following woody plant encroachment into grassland. However, the extent to which SOC storage occurs will be strongly dependent on the degree to which new organic matter inputs are stabilized and protected from decomposition. The primary mechanisms by which organic matter may be protected from decay are (i) biochemical recalcitrance inherited from original plant chemistry or acquired through postdepositional modification, (ii) physical protection by aggregates of biological and mineral particles held together by exudates, roots, and fungal hyphae, and (iii) physicochemical associations with silt and clay surfaces (Baldock et al., 2004; Huang et al., 2005).

One region where woody plant encroachment has been particularly significant is the southwestern United States and northern Mexico (Rappole et al., 1986; Asner et al., 2003; Gibbens et al., 2005). In this chapter, attention is focused on the Rio Grande Plains of southern Texas, where C_3 subtropical thorn woodlands dominated by highly productive N-fixing tree legumes have largely replaced C_4 grasslands over the past 100 to 150 yr (Archer et al., 1988, 2001; Boutton et al., 1998, 1999). This vegetation change has increased rates of above- and belowground productivity in wooded areas relative to remnant grasslands (Archer et al., 2001). This increase in NPP is also accompanied by significant changes in plant tissue chemistry (e.g., high-quality herbaceous tissue vs. poor-quality lignified woody tissue) that have strong potential to alter ecosystem C storage and dynamics.

Because the current plant community comprises C_3 trees and shrubs ($\delta^{13}C \approx -27\%$), and grasslands are dominated by C_4 species ($\delta^{13}C \approx -13\%$), this grassland-to-woodland conversion affords a unique opportunity for isotopically tracing rates of SOC accumulation and turnover in native rangeland systems that have never been cropped or cultivated. Prior studies have demonstrated the usefulness of $\delta^{13}C$ natural abundance for estimating turnover and dynamics of SOC in soils where the photosynthetic pathway of the original vegetation has shifted (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Martin et al., 1990). The $\delta^{13}C$ values of SOC reflect the plant material from which it was derived with minimum isotopic fractionation associated with decomposition (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Nadelhoffer and Fry, 1988; Martin et al., 1990; Boutton, 1996). A vegetation shift from a C_4 -dominated plant community to a C_3 -dominated plant community or vice versa provides an *in situ* label allowing quantification of the loss rate of C derived from the previous plant community and the simultaneous accumulation of new C derived from the current plant community (Martin et al., 1990).

In this chapter, the impact of grassland-to-woodland conversion on the soil carbon cycle in the Rio Grande Plains of southern Texas is assessed by (i) quantifying rates of SOC sequestration following grassland-to-woodland conversion, (ii) evaluating the role of physical and biochemical mechanisms of SOC stabilization, (iii) assessing the relative importance of residual grassland-derived C_4 -C versus C_3 woodland-derived C inputs to total SOC content in bulk soil, and (iv) estimating turnover rates of SOC in bulk soil using the natural isotopic difference between C_4 grasses and C_3 woody plants.

Approach

Study Area

The data presented are based on a series of studies conducted at the Texas Agricultural Experiment Station LaCopita Research Area (27° 40'N; 98° 12'W), located 65 km west of Corpus Christi, Texas, in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical, with a mean annual precipitation of 715 mm and mean annual temperature of 22°C. Topography consists of nearly level uplands that grade (1–3% slopes) into lower-lying drainage woodlands and playas. The elevation ranges from 75 to 90 m.

Although originally classified as a *Prosopis–Acacia–Andropogon–Setaria* savanna (Kuchler, 1964), the contemporary vegetation is subtropical thorn woodland due to significant woody plant expansion in recent history. The area was heavily grazed by domestic livestock over the past century, which has likely been a causal factor in succession from grassland to woodland in this region (Archer et al., 2001).

Upland soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface (B) horizon with nonargillic inclusions and are characterized by a two-phase vegetation pattern consisting of discrete clusters of woody vegetation embedded within a matrix of remnant C₄ grasses. Species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis* dominate in the grasslands. Formation of clusters is initiated when grasslands are colonized by *Prosopis glandulosa* Torr. var. *glandulosa* (honey mesquite), which then facilitates recruitment of other woody plant species in the understory (Archer et al., 1988). Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation. Lower-lying portions of the landscape consist of clay loam soils (Pachic Argiustolls) dominated by continuous closed-canopy drainage woodlands. The vegetation composition of these drainage woodlands is similar to that in upland clusters and groves. *Prosopis glandulosa* is the dominant tree species in all wooded landscape elements, with the shrubs *Condalia hookeri* (M.C. Johnst.) and *Zanthoxylum fagara* (L.) typically dominating the understory.

Chronosequence Approach

A space-for-time, chronosequence approach was used to quantify C pool sizes and their rates of change in litter, roots, and soils following woody plant proliferation in areas that were previously grassland. Sites were sampled within remnant grasslands, clusters, and groves ($n = 10$ each), and within drainage woodlands ($n = 11$). All sites were located within an area of approximately 2 km². Remnant grasslands located on sandy loam uplands were sampled to characterize C pool sizes at the time woody encroachment began, whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in C pools at different points in time following woody invasion.

Ages of clusters, groves, and drainage woodlands were based on the fact that the formation of these wooded landscape elements is initiated only after the establishment of *Prosopis glandulosa* in grassland (Archer et al., 1988). Thus, the age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand. The ages of *P. glandulosa* trees were determined by measuring their basal diameters and then substituting those values into regression equations to predict tree ages, using equations specific to each landscape element (Stoker, 1997).

Woody plant stands sampled in each of the landscape elements were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from approximately 10 to 130 yr. Mean ages (\pm SD) of the woody stands sampled in clusters, groves, and drainage woodlands were 48 ± 28 yr, 62 ± 38 yr, and 61 ± 33 yr, respectively.

The use of space-for-time substitutions in chronosequences is common in ecological studies aimed at understanding long-term and strongly directional dynamics and assumes that spatial and temporal variation are equivalent and that important processes are independent of space and time (Hargrove and Pickering, 1992; Kratz et al., 2003). The most obvious risk in this approach is that past and present ecological conditions in different locations on the landscape may be similar but are never identical (Ghosh and Wildi, 2007).

Collection and Preparation of Soil, Root, and Litter Samples

Surface litter was collected from a 0.25-m² plot at each site. In wooded landscape elements, the plot was located within 0.5 m of the bole of the largest *P. glandulosa* tree. Woody debris ≤ 2 cm in diameter and all foliar litter were collected from these plots; no effort was made to separate foliar from woody litter. Litter samples were washed with water over a 2-mm sieve to remove adhering soil particles, dried at 60°C to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and saved for elemental and isotopic analyses.

Four soil cores (5-cm diameter \times 30-cm depth) were taken within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site, one in each cardinal direction. Sampling was identical in each remnant grassland site, but instead centered around the base of a large perennial C₄ grass plant. Soil surface litter was gently removed to expose mineral soil before taking each core. Cores were sectioned into 0- to 15- and 15- to 30-cm increments and stored at 4°C. In the lab, each soil sample was mixed thoroughly, and a subsample was dried at 105°C to determine bulk density. The four cores from each cardinal location of a given tree or plot were then pooled by depth increment and mixed.

One aliquot of each soil sample was set aside for determination of root biomass, and another was set aside for soil physical fractionation. A third aliquot was passed through a 2-mm sieve to remove large organic fragments and used for physical, chemical, and isotopic analyses. Soil pH was determined on a 1:2 (soil/water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipette method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was dried at 60°C, pulverized in a centrifugal mill, and used for elemental and isotopic analyses. Root biomass was quantified on well-mixed aliquots (100 g) from each pooled soil sample using hydropneumatic elutriation (Smucker et al., 1982) (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410- μ m screen. Recovered roots were dried at 60°C, weighed, pulverized with a mortar and pestle, and saved for elemental and isotopic analyses.

Physical fractionation of soil organic matter was conducted on a 30-g aliquot of each bulked soil sample as described by Liao et al. (2006a). This procedure used wet sieving and density flotation to separate soil organic matter into four physical fractions: (i) macroaggregates (>250 μ m), (ii) microaggregates (53–250 μ m), (iii) free silt + clay (<53 μ m), and (iv) free particulate organic matter (POM) not associated with aggregate structure. Each fraction was dried at 60°C, pulverized, and analyzed for C concentration. Organic C associated with macroaggregates,

microaggregates, and free silt + clay was defined as being physically protected, while free POM was defined as being physically unprotected.

Elemental and Isotopic Analyses

Litter, root, and soil samples were analyzed for C concentrations and $\delta^{13}\text{C}$ using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Carbon isotope ratios are presented in δ -notation:

$$\delta^{13}\text{C} = [(R_{\text{SAMPLE}} - R_{\text{STD}})/R_{\text{STD}}] \times 10^3 \quad [1]$$

where R_{SAMPLE} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample, and R_{STD} is the $^{13}\text{C}/^{12}\text{C}$ ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate measurements was 0.1‰. None of the samples contained CaCO_3 or other forms of inorganic C.

The relative proportion of SOC derived from the original C_4 grassland vegetation (F_C) versus the more recent C_3 woodland vegetation was estimated by mass balance:

$$F_C = (\delta_T - \delta_{\text{WL}})/(\delta_{\text{SOCG}} - \delta_{\text{WL}}) \quad [2]$$

where δ_T is the $\delta^{13}\text{C}$ value of SOC at time T , δ_{WL} is the average $\delta^{13}\text{C}$ value of organic matter inputs (litter and roots) in woodlands (-26%), and δ_{SOCG} is the average $\delta^{13}\text{C}$ value of SOC in remnant grasslands (-17.6% at 0–15 cm, and -14.9% at 15–30 cm). All isotopic end-members in this mass balance model were based on direct measurements obtained in this study.

Organic Chemistry of Litter and Roots

Roots isolated from the 0- to 15-cm depth increment, and surface litter from a remnant grassland and from young (14 yr) and old (80 yr) woody clusters were analyzed for lignin and cutin- and suberin-derived substituted (e.g., hydroxyl and carboxylic acid-substituted) fatty acids (SFAs), using alkaline cupric oxide (CuO) oxidation (Hedges and Mann, 1979; Goñi and Hedges, 1990; Rumpel et al., 2002). All samples were powdered to a flour consistency with a liquid N_2 SPEX CertiPrep (SPEX CertiPrep, Metuchen, NJ) freezer mill to ensure homogeneity in analysis. Briefly, lignin phenols were quantified by analysis of the trimethylsilane derivatives of vanillyl (V)-based (i.e., vanillin, acetovanillone, vanillic acid), syringyl (S)-based (i.e., syringaldehyde, acetosyringone, syringic acid), and cinnamyl (C)-based (i.e., *p*-hydroxycinnamic acid and ferulic acid) phenols using extracted ion internal calibration curves relative to the internal standard ethyl vanillin. The trimethylsilane derivatives of nine SFAs were assessed by extracted ions based on proxy standard calibration curves relative to the internal reference standard DL-12, hydroxystearic acid (Goñi and Hedges, 1990). As SFA proxy standards were used, calibration curves represent reasonable assessments of concentration by selected ions. A Hewlett-Packard 5971 quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA) interfaced to a 5890 Series II gas chromatograph was used along with a Restek Corp. (Bellefonte, PA) Rtx-5 capillary gas chromatograph column. The CuO extractions were run in batches of 12, and all samples were run in duplicate. The percent relative standard deviation (%RSD) of the total of S, V, or C monomers ranged from 1.0 to 7.3%. For SFA, the %RSD ranged from 1.5 to 33%, with the compound 9, 10, 18-trihydroxyoctadec-12-enoic acid always exhibiting the highest deviation but simultaneously nearly the lowest abundance (<5% of total SFA quantified). The %RSD among

duplicates for 9, 16 and 10, 16 dihydroxyhexadecanoic acid, typically the most abundant compound, was 8.4%.

Statistical Analyses

ANOVA was used to test for differences in soil pH, bulk density, and particle size distribution with respect to landscape element and soil depth when averaged across all age states within each landscape element. Regression analyses were performed using Sigma Plot 4.0 (SPSS, 1997) to determine the significance of relationships between C storage in surface litter biomass, root biomass, and soil with woody plant stand age. ANOVA (NCSS, 1995) was used to analyze differences in litter, root, and SOC stocks (g C m^{-2}) and $\delta^{13}\text{C}$ values attributable to the effects of landscape element and soil depth when averaged across all age states within each landscape element. Fisher's LSD method was used to distinguish differences among landscape elements within each soil depth following ANOVA analysis. Exponential relationships between soil $\delta^{13}\text{C}$ and F_c and woody plant stand age were established by nonlinear curve fitting. Fractional rate constants (k -values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the F_c data, and mean residence times (MRTs) were computed as the inverse ($1/k$) of the fractional rate constants. Relationships between F_c and stand age were forced through $F_c = 1$ at time zero. All statistical results are reported as significant when $p < 0.05$.

Results

Soil Characterization

Mean values for all age states within each landscape element are provided for soil pH, bulk density, and particle size distribution in Fig. 12-1. Soil pH was approximately 6.5 in the upper 15 cm of the profile in remnant grasslands and was not altered following woody plant establishment (Fig. 12-1). Bulk density of the 0- to 15-cm depth interval decreased from 1.18 in grasslands to 1.02 in pooled wooded landscape elements (clusters, groves, and drainage woodlands); at 15 to 30 cm, bulk densities ranged from 1.20 to 1.25 g cm^{-3} but differed only between grassland and drainage woodland. Particle size distribution in upland soils (grasslands, clusters, and groves) at 0 to 15 cm was approximately 80% sand, 10% silt, and 10% clay. In contrast, the particle size distribution of lower-lying drainage woodland soils was 60% sand, 20% silt, and 20% clay (Fig. 12-1). Patterns were similar at 15 to 30 cm, although all soils at this depth contained slightly more silt and clay relative to 0 to 15 cm.

Carbon Storage in Litter, Roots, and Soils

Carbon storage in litter, roots, and soils averaged across all age states within each landscape element is presented in Fig. 12-2. Surface litter C stocks ranged from 100 to 175 g C m^{-2} and were not significantly different among grassland, cluster, and drainage woodland landscape elements (Fig. 12-2). In contrast, the litter C stock in groves (310 g C m^{-2}) was significantly greater than the litter C stocks in all of the other landscape elements. Carbon stocks in roots (0-30 cm) were significantly greater in all wooded landscape elements (1000-1500 g C m^{-2}) compared with remnant grasslands (< 400 g C m^{-2}). Total SOC in the upper 15 cm of the soil profile increased from 1000 g C m^{-2} in remnant grasslands to 3000 g C m^{-2}

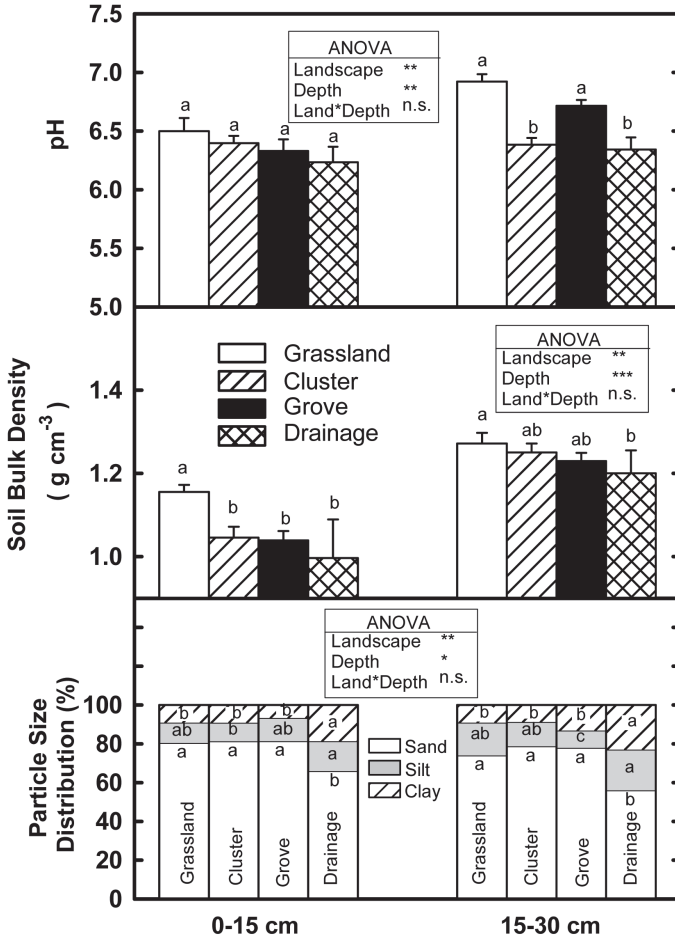


Fig. 12-1. Mean values (\pm SE) for texture, bulk density, and pH of soil in grassland, cluster, and drainage woodlands at the LaCopita Research Area. Data are averaged across all age states within each landscape element ($n = 10$ for grasslands, clusters, and groves; $n = 11$ for drainage woodlands). Mean ages (\pm SD) for clusters, groves, and drainage woodlands were 48 ± 28 , 62 ± 38 , and 61 ± 33 yr, respectively. Asterisks denote level of significance from ANOVA (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., not significant at $p < 0.05$). Different letters above/within bars indicate differences between means within a depth from Fisher's LSD.

in drainage woodlands. Total SOC at 15 to 30 cm was not significantly different among the upland landscape elements, but drainage woodlands contained twice as much C as upland portions of the landscape (Fig. 12-2).

Rates of Carbon Sequestration in the Soil System

Carbon stocks in surface litter increased linearly over time following woody plant encroachment, ranging from approximately 100 g C m^{-2} in woody plant stands <30 yr old to $>400 \text{ g C m}^{-2}$ in stands >60 yr old (Fig. 12-3). Similarly, C

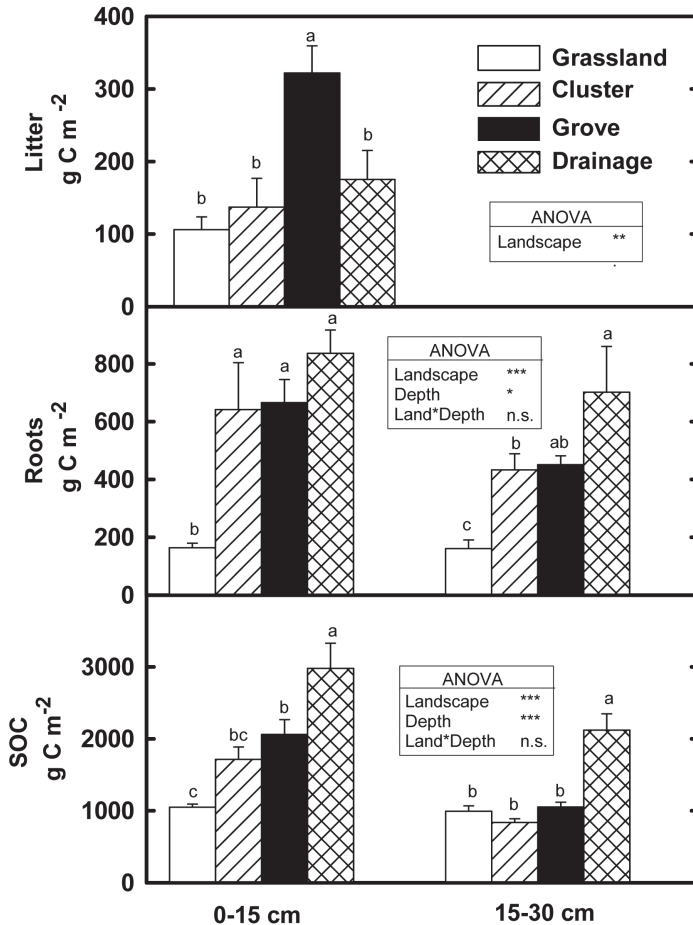


Fig. 12-2. Mean values (\pm SE) for litter, root, and soil organic carbon (SOC) mass of soils in different landscape elements at the LaCopita Research Area by soil depth (0–15 cm and 15–30 cm). Data are averaged across all age states within each landscape element ($n = 10$ for grasslands, clusters, and groves; $n = 11$ for drainage woodlands). Mean ages (\pm SD) for clusters, groves, and drainage woodlands were 48 ± 28 , 62 ± 38 , and 61 ± 33 yr, respectively. Asterisks denote level of significance from ANOVA (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., not significant at $p < 0.05$). Different letters above bars indicate significant differences among means within a depth from Fisher's LSD.

stocks in root biomass (0–15 cm) increased linearly from 200 g C m⁻² in remnant grassland to >1000 g C m⁻² in some of the older (i.e., 60–130 yr) woody plant stands. These dramatic increases in litter and root C are associated with equally dramatic increases in SOC stocks at 0 to 15 cm from 1000 g C m⁻² in grasslands to >4000 g C m⁻² in drainage woodlands. Over the past century, SOC accumulation rates (upper 30 cm of profile) have ranged from 11.5 g C m⁻² yr⁻¹ in upland clusters to 43.2 g C m⁻² yr⁻¹ in low-lying drainage woodlands, and most of this soil C accumulation (70–90%) was stored in the upper 15 cm of the profile (Fig. 12-3, Table 12-1).

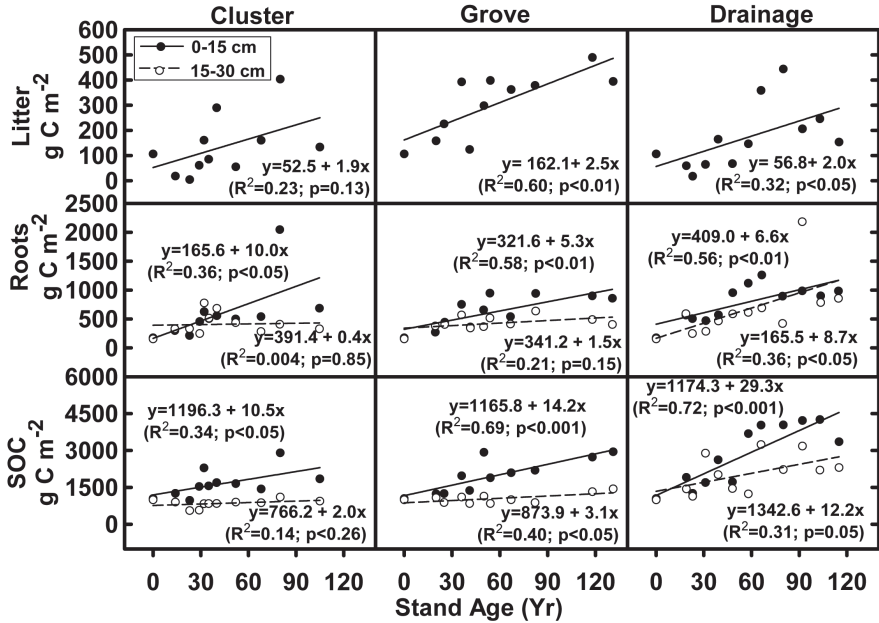


Fig. 12-3. Changes in litter, roots, and soil organic carbon (SOC) of soils (0–15 cm and 15–30 cm depths) with respect to woody plant stand age in landscape elements at the LaCopita Research Area. In each frame, time zero is based on data from upland grasslands and is assumed to represent values before woody encroachment.

Table 12-1. Accumulation rates of C in litter, roots, soil, and the total soil system (litter + roots + soil).

	Accumulation rates†							Total
	Litter	Roots			Soil			
		0–15 cm	15–30 cm	0–30 cm	0–15 cm	15–30 cm	0–30 cm	
g C m ⁻² yr ⁻¹								
Cluster	1.9*	10.0*	0.4	9.2*	10.5*	2.0	11.5*	20.5*
Grove	2.5*	5.3*	1.5	9.6*	14.2*	3.1*	17.2*	29.4*
Drainage	2.0*	6.6*	8.7*	16.1*	29.3*	12.2*	43.2*	61.9*

* Significant at the 0.05 probability level.

† Rates of accumulation in litter, roots, and soil are derived from the slopes of the regression lines in Fig. 12-3. Rates from 0 to 30 cm for the total soil system (litter + roots + soil) were obtained by performing separate regressions on summed data.

For the soil system (litter + roots + soil), rates of C sequestration ranged from 21 to 62 g C m⁻² yr⁻¹ in wooded landscape elements. Less than 10% of C accumulation rates in the whole-soil system could be attributed to C storage in litter, whereas 25 to 45% of C accumulation rates in the whole-soil system could be accounted for by C storage in roots. Most of the C accumulation in the soil system (55–70%) was due to storage in soil (Table 12-1).

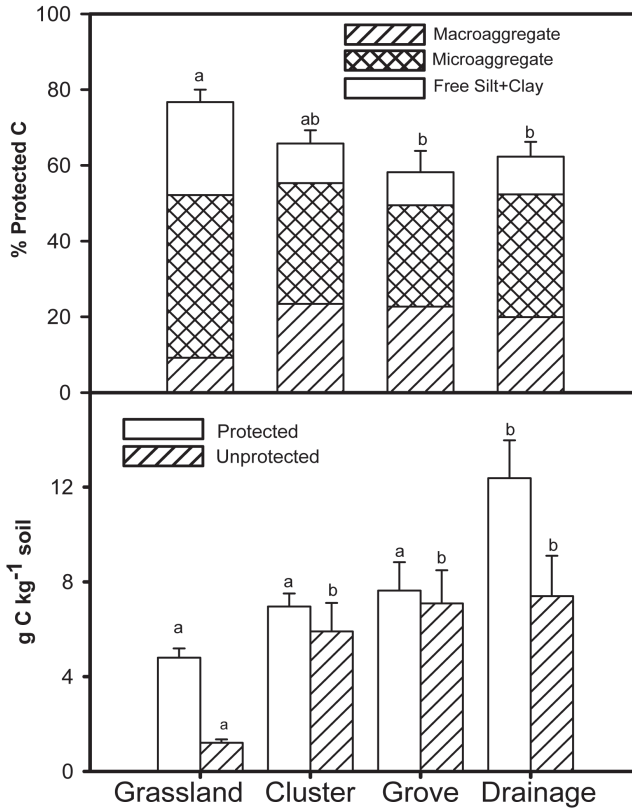


Fig. 12–4. Physically protected and unprotected soil organic carbon in the upper 15 cm of the soil profile in grassland, cluster, grove, and drainage landscape elements. The top panel shows the relative proportion of C protected by a given physical mechanism. The bottom panel shows the concentration of C in protected versus unprotected pools. Error bars are standard errors of the mean. Different letters above bars within a category (protected vs. unprotected) indicate significant differences between means based on Fisher’s LSD. (Data modified from Liao et al., 2006b.)

Physical and Chemical Attributes of Organic Carbon

The proportion of protected C (POM within aggregates and C associated with silt + clay) was approximately 78% in remnant grasslands and declined to 58 to 65% in the wooded landscape elements (Fig. 12–4). Following woody encroachment, the percentage of C stored in macroaggregates approximately doubled, while the relative importance of microaggregates and free silt + clay as protection mechanisms declined.

Absolute concentrations of both protected and unprotected C were higher in woodlands relative to remnant grasslands (Fig. 12–4). The concentration of protected C increased 40 to 140%, from 5 g C kg⁻¹ soil in remnant grasslands to 7 to 12 g C kg⁻¹ soil in wooded areas, although only the drainage woodlands stored significantly more C in protected form than the remnant grasslands. Concentrations

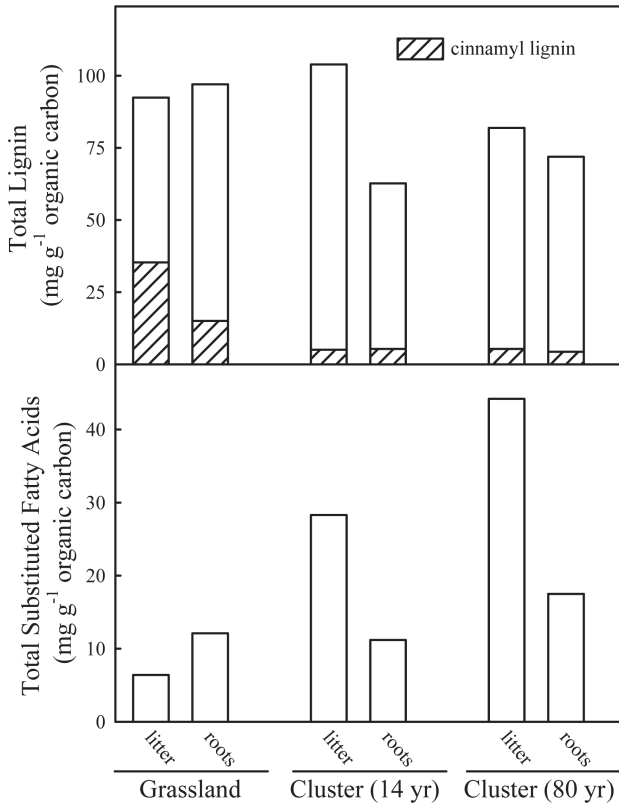


Fig. 12-5. Total SVC lignin phenol content (a combination of syringyl, vanillyl, and cinnamyl phenols) (top panel) and substituted fatty acids (bottom panel) in litter, roots, and soil in a remnant grassland, and in a young (14 yr) and an old (80 yr) woody cluster. Data for roots and soils are based on the upper 15 cm of the profile.

of unprotected C increased five- to sevenfold, from 1 g C kg⁻¹ soil in grasslands to 5 to 7 g C kg⁻¹ soil in wooded landscape elements (Fig. 12-4). All wooded landscape elements stored significantly more C in unprotected form than the grasslands. Approximately 30 to 55% of the C accruing following woody encroachment was stored in protected form; the remainder of the new C was stored as free POM outside of physical protection mechanisms.

Differences in lignin and SFA chemistry were evident among litter and roots in grasslands vs. woody clusters. Cutin- and suberin-derived SFA concentrations in bulk surface litter increased from 6 mg g⁻¹ organic C in the grassland to 28 to 44 mg g⁻¹ organic C in the clusters (Fig. 12-5). Also, the relative proportion of the predominantly ester-bound lignin compounds, in the form of cinnamyl phenols, decreased in the litter and roots of both woody clusters compared to remnant grassland. Cinnamyl phenols represented ~38% of total lignin in grassland litter, but only ~5% of total lignin in litter from the two woody clusters (Fig. 12-5). Cinnamyl concentrations were also substantially lower in the roots from clusters (6-8% of total lignin) compared to grasslands (16% of total lignin). In addition, the

absolute concentration of SV-lignin was higher in the litter of woody clusters (77–99 mg g⁻¹ organic C) compared to the remnant grassland (57 mg g⁻¹ organic C).

Changes in the Natural Abundance of Carbon-13 in Litter, Roots, and Soil Organic Carbon

Litter and root $\delta^{13}\text{C}$ values reflected the predominance of C₃ plant inputs in the wooded landscape elements, and C₄ plant inputs in the grasslands. The average $\delta^{13}\text{C}$ value of grassland litter (-17‰) was significantly higher than that of woodland litter (-26‰) (Fig. 12-6). Litter $\delta^{13}\text{C}$ values in wooded landscape elements had strongly C₃-dominated signatures (-25 to -26‰) and did not differ significantly among cluster, grove, and drainage woodland. Root $\delta^{13}\text{C}$ values at both soil depths averaged -18‰ in grasslands and -26‰ in woodlands. The mean $\delta^{13}\text{C}$ of woodland SOC at 0 to 15 cm (-23‰) was significantly lower than that of remnant grassland (-18‰); at 15 to 30 cm, $\delta^{13}\text{C}$ values reflected greater C₄ plant inputs in both grassland (-15‰) and wooded landscape elements (-17‰) (Fig. 12-6).

$\delta^{13}\text{C}$ values of SOC in the upper 15 cm of the soil profile decreased exponentially over time following woody plant encroachment (i.e., with increasing woody plant stand age) from approximately -18‰ in remnant grasslands (time 0) to -24‰ in upland clusters and groves (Fig. 12-7). In drainage woodlands, $\delta^{13}\text{C}$ values of SOC (0–15 cm) decreased over time from -18 to -22‰. These isotopic changes largely occurred within 60 yr of woody plant encroachment into grasslands and are a consequence of the simultaneous loss of grassland-derived C and the gain of woody plant-derived C. In the 15- to 30-cm depth increment, $\delta^{13}\text{C}$ values of SOC decreased exponentially from -15‰ in remnant grasslands to approximately -19‰ in upland clusters and groves. In contrast, $\delta^{13}\text{C}$ values at 15 to 30 cm in drainage woodlands changed little with time following woody encroachment.

An isotopic mass balance model was utilized to compute the relative proportions of SOC derived from grassland vs. woodland (Eq. [2]; Fig. 12-7). According to this model, the fraction of SOC derived from grassland (F_c) in the 0- to 15-cm increment decreased from 1.0 at time 0 to approximately 0.2 within 100 yr after cluster and grove establishment, and from 1.0 to approximately 0.4 in drainage woodlands over the same time period. In the 15- to 30-cm depth increment, the fraction of soil C derived from grassland decreased to 0.5 in clusters and groves and to 0.8 in drainage woodlands.

Fractional rate constants (k values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the F_c data in Fig. 12-7, and mean residence times (MRTs) were computed as the inverse (1/ k) of the fractional rate constants (Table 12-2). The MRTs of SOC at 0 to 15 cm in upland clusters (36 yr) and groves (47 yr) were lower than those for drainage woodlands (115 yr). The MRTs were substantially greater at 15 to 30 cm in the profile, ranging from 156 yr in clusters to 476 yr in drainage woodlands.

Tracing Sources of Soil Organic Carbon Following Grassland-to-Woodland Conversion

Changes in the density (g C m⁻²) of whole-soil organic C resulting from the decay of C inherited from the original C₄-dominated grassland and the simultaneous accumulation of C derived from C₃ woodland over the past century (Fig. 12-8) were revealed by multiplying age-specific fractions of C derived from

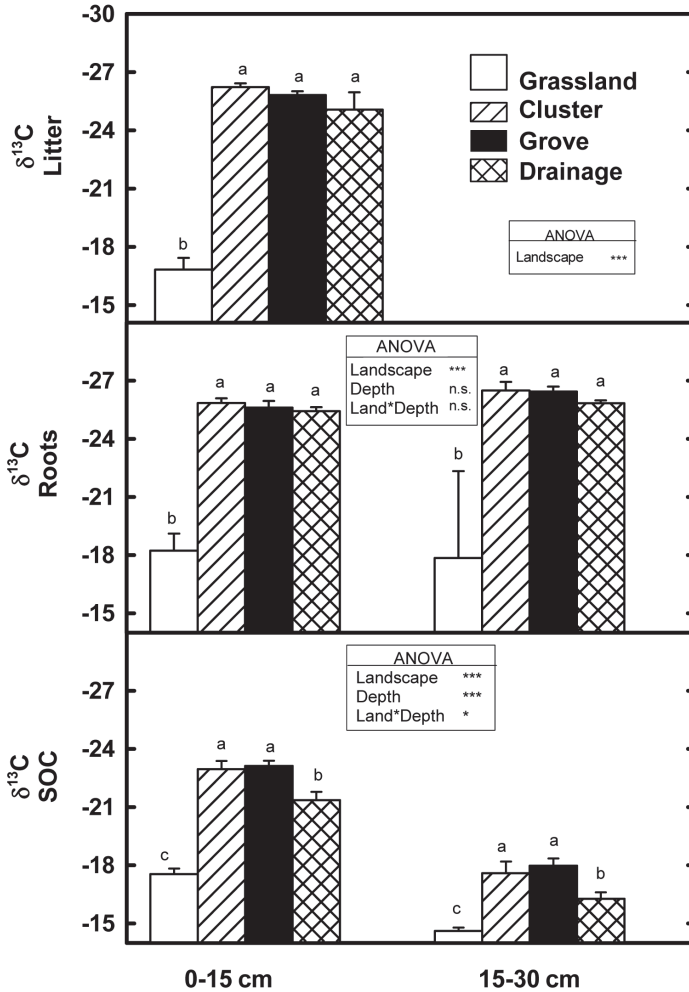


Fig. 12-6. Mean $\delta^{13}\text{C}$ values ($\pm\text{SE}$) of litter, roots, and soil organic carbon in different landscape elements at the LaCopita Research Area by soil depth (0–15 cm and 15–30 cm). Data are averaged across all age states within each landscape element ($n = 10$ for grasslands, clusters, and groves; $n = 11$ for drainage woodlands). Mean ages ($\pm\text{SD}$) for clusters, groves, and drainage woodlands were 48 ± 28 , 62 ± 38 , and 61 ± 33 yr, respectively. Asterisks denote level of significance from ANOVA (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., nonsignificant at $p < 0.05$). Different letters above bars indicate significant differences among means within a depth from Fisher’s LSD.

grassland (F_{C}) (Fig. 12-7) by the age-specific soil C pool sizes (Fig. 12-3). The rate of accretion of SOC derived from C_3 woody plants was greater than the rate of loss of organic C derived from the original grassland, so that overall SOC increased by 100 to 200% in upland clusters and groves, and by >300% in drainage woodlands (Fig. 12-8).

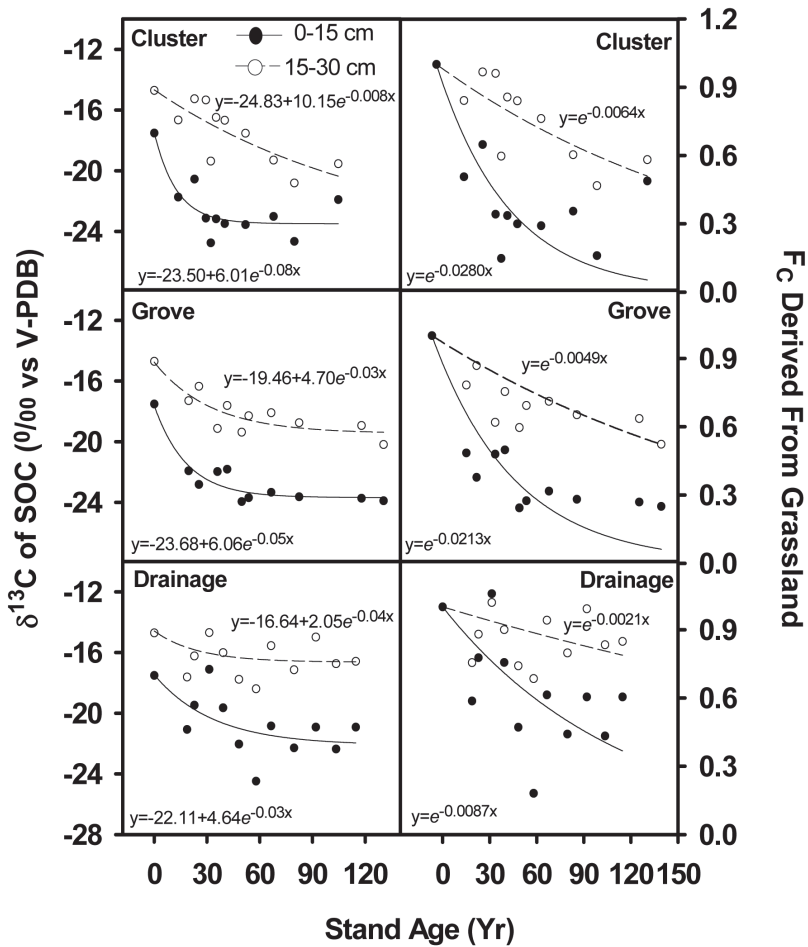


Fig. 12-7. δ¹³C values of soil organic carbon (SOC) (left axis) and fraction of SOC (F_C) derived from grassland (right axis) by soil depth (0–15 cm and 15–30 cm) and woody plant stand age. In each frame, time zero is based on data from upland grasslands and is assumed to represent values before woody encroachment.

Discussion

Linear increases in C storage in litter, roots, and soil demonstrate that woody plant invasion of grassland has resulted in significant C sequestration over the past century (Fig. 12-3). Most of the C accumulation in roots and SOC was in the upper 15 cm of the soil profile. Values for grassland SOC were 1000 g C m⁻², while values for the oldest wooded areas ranged from 3000 to 4500 g C m⁻², representing an increase of 200 to 350% in the upper 15 cm of the soil profile (Fig. 12-3).

In other studies, responses of SOC to woody encroachment into grasslands have ranged from decreases to no change to significant increases (Wessman et al.,

Table 12–2. Comparison of k -values and mean residence times of SOC in cluster, grove, and drainage landscape elements at LaCopita Research Area.

	k values		Mean residence times			
			$^{13}\text{C}\dagger$		$^{14}\text{C}\ddagger$	
	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
Cluster	0.0280 (0.0053)§	0.0064 (0.0010)	36	156	nm¶	nm
Grove	0.0213 (0.0029)	0.0049 (0.0005)	47	204	52 (17)	282 (77)
Drainage	0.0087 (0.0018)	0.0021 (0.0006)	115	476	54 (12)	516 (188)

† Mean residence times are computed as the inverse of the fractional rate constants (k) in the exponential equations in Fig. 12–7 derived from ^{13}C isotope data.

‡ Mean residence times obtained from ^{14}C radiocarbon dating are from Boutton et al. (1998).

§ All numbers in parentheses are the standard errors of the estimates.

¶ nm, not measured.

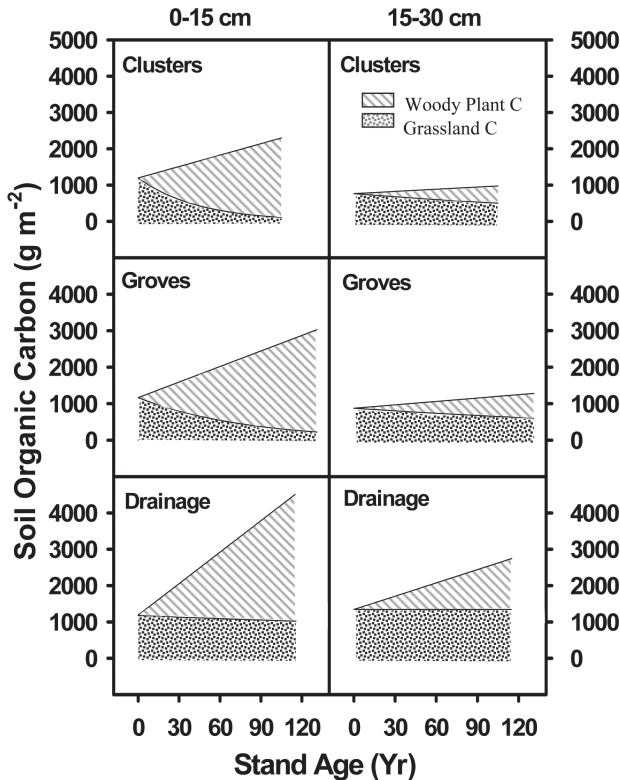


Fig. 12–8. Changes in the mass of total soil organic carbon (SOC) resulting from the decay of SOC inherited from the original C_4 grassland and the simultaneous accumulation of SOC derived from C_3 woodland development over the past century. Values of total SOC were obtained by direct measurement. The mass of SOC from grassland was calculated as the product of total SOC and F_c from Fig. 12–7. The mass of SOC from C_3 woodland was calculated as the difference between total SOC and the mass of SOC from grassland.

2004). Most studies across comparable arid or semiarid ecosystems have shown that woody plant invasion of grasslands results in increased C storage in soil beneath woody plant canopies (Virginia and Jarrell, 1983; Mordelet et al., 1993; Stock et al., 1995; San Jose et al., 1998; Geesing et al., 2000; Burrows et al., 2002; Reyes-Reyes et al., 2002; Zavaleta and Kettleby, 2006). In contrast, a few recent studies have shown no changes in SOC (McCarron et al., 2003; Smith and Johnson, 2003; Dai et al., 2006; Hughes et al., 2006) or actual decreases in SOC with woody plant encroachment (Jackson et al., 2002).

It is not yet clear why responses to woody encroachment are variable and range from net losses to net gains in soil C. Documented variable effects of woody plant impacts on SOC may be due to several factors such as species-dependent changes in microclimate, litter production, litter quality, and rooting depth and biomass (Bush and Van Auken, 1986; Lugo and Brown, 1993; Pugnaire et al., 1996; Jackson et al., 2000), and their interactions with edaphic properties such as soil depth and texture. In many of the studies where significant increases in SOC were observed, encroaching woody plant species were often N-fixing tree legumes (Virginia and Jarrell, 1983; Bush and Van Auken, 1986; Stock et al., 1995; Pugnaire et al., 1996). Greater inputs of fixed N to N-limited ecosystems reduce N limitation of plants and microbes and results in greater plant productivity (Wardle, 1992). Because N is limiting in most ecosystems, the presence of N-fixing species may be an important driving factor in the observed increases in SOC in many aridland ecosystems. However, the discrepancies among studies suggest that current understanding of the effects of woody plant species on grassland biogeochemistry is still limited. These discrepancies highlight the need for additional ecosystem-level studies of SOC storage and dynamics following grassland-to-woodland conversion.

It appears that there are at least three important mechanisms driving C accumulation in the soil system following woody encroachment into grassland in this subtropical savanna ecosystem: (i) higher rates of NPP in wooded areas relative to grasslands; (ii) physical protection of organic matter inputs within the soil structure; and (iii) increased biochemical recalcitrance of litter inputs in wooded areas.

First, these dramatic increases in SOC are likely fueled by higher rates of NPP in the wooded landscape elements compared to remnant grasslands. At the La Copita site, aboveground NPP has increased from 1.9 to 3.4 Mg ha⁻¹ yr⁻¹ in remnant grasslands to 5.1 to 6.0 Mg ha⁻¹ yr⁻¹ in areas now dominated by woody vegetation (Archer et al., 2001). Although belowground productivity has not been quantified, other studies at the La Copita site have shown significantly greater root biomass and seasonal fluctuations in root biomass in wooded areas than in remnant grasslands, providing strong circumstantial evidence for higher belowground productivity in wooded portions of the landscape (Boutton et al., 1999; Hibbard et al., 2001).

Second, evidence was presented to show that SOC is accumulating in this system in part due to physical protection within soil structure. Approximately 30 to 55% of the new SOC that accrued following woody encroachment was protected within macro- and microaggregates or by association with the silt + clay fraction. The relative importance of SOC storage in macroaggregates increased while that of microaggregates and silt + clay decreased, probably because microaggregates and silt + clay fractions were being incorporated into macroaggregate structures (Liao et al., 2006a). Increased root biomass (Fig. 12–2, Fig. 2–3) together

with a larger and more active soil microbial biomass pool (McCulley et al., 2004) in the wooded areas likely afforded greater potential for fine roots and fungal hyphae to act as binding agents and promote the formation of macroaggregates (Jastrow and Miller, 1998). The relative importance of microaggregates external to macroaggregates decreased over time following woody encroachment; however, it is possible that absolute importance of microaggregates may have increased as a result of microaggregate formation within macroaggregates. Although the soil physical fractionation scheme used in this study did not permit evaluation of this possibility, several previous studies have highlighted the importance of this mechanism of SOC stabilization (Six et al., 2002; Denef et al., 2004; Kong et al., 2005).

And third, data from this study showed that approximately 45 to 70% of the SOC stored after woody plants replace grassland was found outside of physical protection mechanisms in the free POM fraction, and that this physically unprotected free POM may be able to accumulate and persist in the soil due to its biochemical recalcitrance. Organic matter recalcitrance is often attributed to slowly degrading lignin or aliphatic biopolymers that vary in abundances and chemistries in plants (Kogel-Knabner, 2002). Litter and roots in the woody clusters were chemically distinct and enriched in recalcitrant biopolymers relative to grasslands. Although the total lignin (S + V + C) concentrations were comparable in litter and roots of grasslands versus wooded areas, the total SVC phenols in grassland litter and roots contained 16 to 38% cinnamyl compounds. In contrast, total SVC phenols in litter and roots of wooded areas contained only 5 to 8% cinnamyl and was comprised largely of the more resistant S and V subunits. These are important differences because cinnamyl phenols are bound by easily hydrolyzed ester linkages, while V- and S-based phenols are predominantly carbon-carbon bonded and ether linked making them harder to decompose (Opsahl and Benner, 1997).

Furthermore, litter in wooded areas contained 4 to 7 times higher concentrations of SFAs than grassland litter. Total SFAs are proxies for the relatively resistant aliphatic biopolymers suberin and cutin, and their greater abundance provides further evidence that litter in wooded areas is biochemically more resistant to decomposition compared to grassland litter. Many studies have now shown that aliphatic biopolymers like suberin and cutin comprise a particularly important subset of recalcitrant material in soils (e.g., Nierop, 2001; Nierop and Verstraten, 2003; Mikutta et al., 2006) and may form part of the core of organic materials tightly bound to mineral surfaces (Kleber et al., 2007).

Although several studies have addressed changes in pool sizes of SOC following grassland-to-woodland conversion, few have quantified rates of C accumulation in soil or in the total soil system (litter + roots + soil). Carbon accumulation in the total soil system ranged from 21 to 62 g C m⁻² yr⁻¹ (Table 12-1). These rates reflect the impact of the higher quantity of organic matter inputs to soil as a result of increased above- and belowground productivity of woodlands relative to remnant grasslands. SOC accumulation rates in this study ranged from 12 to 43 g C m⁻² yr⁻¹ and accounted for 55 to 70% of C accumulation in the belowground system. Carbon accumulation rates in litter accounted for less than 10% of C accumulation in the total soil system, whereas roots accounted for 25 to 45% of the observed C accumulation rates in the total soil system (Table 12-1). This is consistent with the observation that root productivity is greater than litterfall in wooded areas at this site (Hibbard et al., 2001).

Rates of soil C accumulation were greatest in drainage woodlands and lower in clusters and groves. This higher accumulation rate of SOC in drainage woodlands may be related to the higher rates of NPP due to the more favorable soil moisture regimes in this lower-lying portion of the landscape (Wu and Archer, 2005). Additionally, the finer-textured soils (higher silt and clay content) in drainage woodlands (Fig. 12-1) may better stabilize organic matter, allowing greater protection from decomposition by soil microbes through the formation of macro- and microaggregates and organomineral complexes (Ladd et al., 1993; Christensen, 1996). Conversely, coarse-textured soils of upland clusters and groves have less potential to form stable aggregates and protect organic matter from decomposition, resulting in slower SOC accumulation rates in the upland woodlands.

An alternate explanation for the higher rates of SOC accumulation in drainage woodlands is that it is an artifact of using upland remnant grasslands as time zero in the regression analyses. Although the use of remnant grasslands as a substitute for preencroachment conditions appears to be appropriate in the case of woody clusters and groves in the uplands because all three of these landscape elements have similar chemical and physical properties in the surface soil (Fig. 12-1) (McCulley et al., 2004; Liao et al., 2006a), they may not be entirely appropriate as a time zero proxy for drainage woodlands for reasons discussed in the previous paragraph. The grasslands that once occupied the lower-lying drainages before woody encroachment may have been more productive, and the soils in those drainages more able to protect and stabilize SOC due to their higher clay content. Hence, it is possible that grasslands in the drainages before woody encroachment stored more SOC compared to upland grasslands. Unfortunately, there are no remnant grasslands in the drainages to provide a more appropriate pre-encroachment assessment of SOC stores. Although the use of upland remnant grasslands as a proxy for time zero SOC in the drainages might be expected to bias the regressions in Fig. 12-3 toward a steeper slope and a lower y intercept, removal of the upland grassland data point at time zero has no significant effect on the relationship between time and SOC in the drainage woodlands. Thus, estimates of SOC accumulation rates in drainage woodlands are not biased by the use of upland grasslands as a substitute for time zero SOC stocks in drainage woodlands.

Rates of C accumulation documented in this study are comparable to those obtained from prior field and modeling studies at this same site. Model output from Century simulations estimated a relatively narrow range of accumulation rates (11–19 g C m⁻² yr⁻¹) for SOC in wooded landscape elements (0–20 cm) (Hibbard et al., 2003). However, results based on field measurements to a depth of 20 cm showed rates ranging from 12 to 47 g C m⁻² yr⁻¹ (Archer et al., 2001, 2004), which are virtually identical to the accumulation rates found in this study (12–43 g C m⁻² yr⁻¹). These rates are also similar to SOC accumulation rates found in other ecosystems experiencing woody plant encroachment or afforestation. An average accumulation rate of 30 g C m⁻² yr⁻¹ was determined for soils (0–30 cm) of a savanna ecosystem in Africa experiencing grassland-to-woodland conversion (Scholes and van der Merwe, 1996). Carbon accumulation rates documented in the present study are also within the range of observed rates for abandoned agricultural land in both temperate and tropical regions. A review by Silver et al. (2000) showed SOC accumulation rates of approximately 41 g C m⁻² yr⁻¹ following 100 yr of forest reestablishment on abandoned agricultural and pasture lands

in the tropics. Schlesinger (1997) reported rates of SOC accumulation of 21 to 55 g C m⁻² yr⁻¹ in abandoned agricultural soils and other disturbed sites allowed to return to native vegetation in temperate regions.

Although storage of SOC often changes following woody plant invasion of grassland, little is known about the turnover rates of SOC following this vegetation change. The $\delta^{13}\text{C}$ natural abundance method is useful for estimating turnover and dynamics of SOC in soils and in elucidating mechanisms of SOC accumulation where the photosynthetic pathway of the original vegetation has shifted (Balesdent and Mariotti, 1987; Balesdent et al., 1988; Martin et al., 1990). Before woody plant invasion, grassland soils had $\delta^{13}\text{C}$ values ranging from -17 to -18‰, typical of C derived from C₄ grassland (Boutton et al., 1999). Following woody plant invasion of grassland, $\delta^{13}\text{C}$ of SOC declined in negative exponential fashion, reflecting the simultaneous loss of C derived from the original C₄ grassland and the accumulation of C derived from the current C₃ woody vegetation. Mass balance calculations indicate that after 130 yr of woodland development at the La Copita site, 10 to 30% of C₄-derived C remained in the soil. This fraction of grassland-derived SOC appears to be relatively resistant to decay, as the magnitude of this pool changed little in woody plant stands older than about 60 yr (Fig. 12-8). At 15 to 30 cm, 60 to 80% of C derived from grassland remained present in the soil even after 130 yr of woodland development, suggesting that this fraction is very resistant to decomposition and is an important pool for long-term C storage. These results are comparable to the findings of Martin et al. (1990) for a tropical savanna ecosystem in Ivory Coast where C₃ woody plants have invaded grasslands dominated by C₄ grasses. Martin et al. (1990) found that 30 to 50% of the original grassland-derived SOC in the upper 10 cm of the profile remained following 16 yr of woody plant development, whereas 60 to 70% of the original grassland-derived SOC remained at 10 to 25 cm in the profile.

The rate of change of the $\delta^{13}\text{C}$ values of SOC following woody invasion is a direct function of the SOC turnover rate. Mean residence times were computed as the inverse (1/k) of the fractional rate constants for organic C decay obtained by fitting exponential equations to the F_c data in Fig. 12-7. The MRTs for SOC ranged from 36 to 115 yr for SOC at 0 to 15 cm in woodlands. At 15 to 30 cm, MRTs were much longer, ranging from 156 to 476 yr. The longer MRTs associated with depth may be related in part to the higher silt and clay contents deeper in the soil profile (Fig. 12-1). Organic C associated with silts and clays generally have slower turnover rates than those associated with sand-size fractions (Christensen, 1992). Carbon that is adsorbed onto mineral surfaces is relatively inaccessible to microbial decay and may thus persist in the soil, resulting in increased MRTs (Baldock and Skjemstad, 2000). Previous studies at LaCopita Research Area indicated that a large proportion of old C₄-derived C has persisted in the clay fraction of woodland soils, likely due to protection of the organic matter from microbial decomposition by association with the soil mineral fraction (Boutton et al., 1998; Liao et al., 2006b).

The MRTs for SOC in woodlands in this study were comparable to estimates obtained from ¹⁴C dating of woodland derived SOC in an earlier study (Boutton et al., 1998). The natural abundance of ¹³C thus appears to be a feasible and relatively accurate means of estimating residence times of SOC in whole soil pools following woody plant invasion of grassland. This is contrary to results obtained by Paul et al. (2003), which showed that MRTs obtained by natural ¹³C appeared

to grossly underestimate SOC turnover rates derived from radiocarbon dating. Mean residence times could not be determined for SOC in grasslands using the natural abundance of ^{13}C method due to the lack of a tracer; thus, direct comparison of MRTs between grasslands and woodlands is currently not feasible using natural ^{13}C . However, prior results from soil respiration kinetics during long-term incubation studies (Boutton et al., 2002) and from field studies of soil respiration (McCulley et al., 2004) showed that turnover of SOC was slower in woodlands than in remnant grasslands. This slower turnover is consistent with our findings of a greater proportion of more refractory lignin and polymeric aliphatic components in the organic matter inputs in wooded areas.

Overall, evidence from ^{13}C dynamics shows that the rate of accretion of C derived from C_3 woody plants is greater than the loss of organic C derived from the original grassland. Accordingly, SOC in woodlands has increased significantly relative to remnant grassland (Fig. 12–3, Fig. 12–8). Thus, it appears that soils at the La Copita site have been actively sequestering C over the past century following woody plant invasion of grassland.

Although the findings of this study along with many others show increasing SOC with woody plant encroachment into grasslands, the consequences of woody encroachment on soil C pools and fluxes in affected ecosystems remain controversial. Current estimates suggest that woody plant encroachment into grasslands and savannas may result in the sequestration of 0.10 to 0.13 Pg C yr^{-1} in the United States, which represents 20 to 40% of the current U.S. carbon sink strength (Houghton et al., 2000; Tilman et al., 2000; Pacala et al., 2001). If these estimates are correct, then woody encroachment is certainly playing a significant role in the global carbon cycle. However, neither the geographic extent nor the ecosystem level impacts on C storage are well constrained at this time. There remains a need for local and regional assessments of SOC pools and fluxes to extrapolate results from the ecosystem level to the broader scales needed to predict the role of terrestrial ecosystems in the global C budget, including the potential of terrestrial ecosystems for long-term C storage.

Conclusions

Although woody plant invasion of grasslands has been geographically extensive over the past century, few robust generalizations regarding the biogeochemical consequences of this land cover change have emerged. The case study presented here indicates that woodlands have replaced grasslands over the past century in the Rio Grande Plains of southern Texas. Following this grassland-to-woodland transition, increased C storage in surface litter and roots was accompanied by significant increases in SOC (0–30 cm) from 2000 g C m^{-2} in grasslands to >5000 g C m^{-2} in older woodlands. Accumulation rates of SOC ranged from 12 to 43 $\text{g C m}^{-2} \text{yr}^{-1}$. This is consistent with prior results obtained from field estimates and modeling at this same site and is comparable to estimates reported for other ecosystems experiencing woody plant encroachment or from reforestation of agricultural lands.

Evidence is presented for three important mechanisms driving C accumulation in the soil system following woody encroachment: (i) higher rates of NPP in wooded areas relative to grasslands, (ii) shifts to biopolymer composition that should result in greater biochemical recalcitrance of litter inputs in wooded areas,

and (iii) physical protection of organic matter inputs within the soil structure. Approximately 30 to 55% of the SOC that accumulates following woody encroachment is stabilized by physical protection in macro- and microaggregates, and by association with silt and clay. The remainder of the newly accrued SOC accumulates as free POM, which likely persists in the soil as a result of its biochemical resistance to decay, as indicated by the composition of its lignin fraction and its high concentrations of substituted fatty acids.

A large portion of SOC in woodlands developing on grasslands (10–20% in surface soils and 60–80% at deeper depths) comprised older C derived from the previous grassland community. This older grassland C is relatively resistant to decay, as little change was detected in the size of this pool in wooded areas older than 60 yr. Hence, accumulations of SOC are due to both increased inputs of newer woodland-derived C and the retention of older, stabilized grassland-derived C. The significant increases in SOC documented in this study suggest that those portions of the Rio Grande Plains in southern Texas undergoing grassland-to-woodland conversion have been acting as a net sink for atmospheric CO₂ over the past century. Grassland-to-woodland conversions have been geographically widespread in the world's drylands during this same time period, suggesting that changes in SOC storage and dynamics documented here could have significance for regional and global carbon cycles and potentially our climate system.

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