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# Organic matter turnover in soil physical fractions following woody plant invasion of grassland: Evidence from natural $^{13}$C and $^{15}$N

J.D. Liao[a], T.W. Boutton[a,*], J.D. Jastrow[b]

[a] *Department of Rangeland Ecology and Management, Texas A&M University, College Station, TX 77843-2126, USA*
[b] *Biosciences Division, Argonne National Laboratory, Argonne, IL 60439-4843, USA*

## Abstract

Soil physical structure causes differential accessibility of soil organic carbon (SOC) to decomposer organisms and is an important determinant of SOC storage and turnover. Techniques for physical fractionation of soil organic matter in conjunction with isotopic analyses ($\delta^{13}$C, $\delta^{15}$N) of those soil fractions have been used previously to (a) determine where organic C is stored relative to aggregate structure, (b) identify sources of SOC, (c) quantify turnover rates of SOC in specific soil fractions, and (d) evaluate organic matter quality. We used these two complementary approaches to characterize soil C storage and dynamics in the Rio Grande Plains of southern Texas where $C_3$ trees/shrubs ($\delta^{13}C = -27‰$) have largely replaced $C_4$ grasslands ($\delta^{13}C = -14‰$) over the past 100–200 years. Using a chronosequence approach, soils were collected from remnant grasslands (Time 0) and from woody plant stands ranging in age from 10 to 130 years. We separated soil organic matter into specific size/density fractions and determined their C and N concentrations and natural $\delta^{13}$C and $\delta^{15}$N values. Mean residence times (MRTs) of soil fractions were calculated based on changes in their $\delta^{13}$C with time after woody encroachment. The shortest MRTs (average = 30 years) were associated with all particulate organic matter (POM) fractions not protected within aggregates. Fine POM (53–250 μm) within macro- and microaggregates was relatively more protected from decay, with an average MRT of 60 years. All silt + clay fractions had the longest MRTs (average = 360 years) regardless of whether they were found inside or outside of aggregate structure. $\delta^{15}$N values of soil physical fractions were positively correlated with MRTs of the same fractions, suggesting that higher $\delta^{15}$N values reflect an increased degree of humification. Increased soil C and N pools in wooded areas were due to both the retention of older $C_4$-derived organic matter by protection within microaggregates and association with silt + clay, and the accumulation of new $C_3$-derived organic matter in macroaggregates and POM fractions.

*Keywords:* Woody plant invasion; Soil aggregates; Particulate organic matter; Organic matter stabilization; Stable isotopes; Carbon cycle; Nitrogen cycle; Chronosequence

## 1. Introduction

Organic C storage in soils is the net effect of organic matter inputs to soil and losses through decomposition (Schlesinger, 1997; Amundson, 2001). Because of the structural complexity and the heterogeneous nature of soil organic matter, rates of turnover vary along a continuum from labile organic materials that decay rapidly to more recalcitrant materials that remain in the soil for potentially thousands of years (Agren and Bosatta, 1996; Cadisch and Giller, 1997; Hassink, 1997; Baldock and Skjemstad, 2000). Soil organic matter dynamics and storage are affected by the physical form of the organic materials, the chemistry of the inputs, and the position they occupy within the soil matrix (Oades, 1988; Jastrow and Miller, 1998). In particular, the role of soil structure in organic matter stabilization is of great importance because even labile organic materials may be sorbed to clay surfaces or protected from decomposer organisms by incorporation into aggregate structure resulting in long-term storage of soil organic matter (Tisdall and Oades, 1982; Ladd et al., 1993).

Physical fractionation of soil organic matter has revealed that soil size and density separates differ in chemistry, rates of turnover, and dynamics (Tiessen and Stewart, 1983; Schulten, 1996; Golchin et al., 1997; Puget et al., 2000;

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*Corresponding author. Tel.: +1 979 845 8027; fax: +1 979 845 6430.
*E-mail address:* boutton@neo.tamu.edu (T.W. Boutton).

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Chefetz et al., 2002). For example, greater organic matter concentrations and higher mineralization rates are often reported to be associated with macroaggregate fractions. Conversely, organic matter associated with microaggregates may be more protected physically and more recalcitrant biochemically (Jastrow, 1996; Six et al., 2000). Because the location of organic matter within the soil structure is related to organic matter storage and dynamics, physical fractionation methods have been utilized to identify mechanisms controlling changes in C and N pools associated with land-use/land-cover changes (Elliott, 1986; Jastrow, 1996; Christensen, 2001; Six et al., 2002a; Jolivet et al., 2003).

Land uses and land cover changes have been shown to have significant impacts on soil physical structure that often result in changes in soil organic matter storage and turnover (Jastrow, 1996; Six et al., 2002a, b; Conant et al., 2003). One of the most geographically extensive land cover changes occurring around the world today is woody plant invasion of grass-dominated ecosystems. Woody plant encroachment has been documented in grasslands and savannas in North and South America, Australia, Africa, and Southeast Asia over the past century, most likely due to livestock grazing and fire suppression (Jackson et al., 2000; Van Auken, 2000; Archer et al., 2001). Since grass-dominated ecosystems cover approximately 40% of the terrestrial surface and store more than 30% of global soil organic carbon (SOC), alterations to ecosystem structure and function due to woody encroachment could have significant consequences for ecosystem biogeochemistry and potential implications for global C and N cycles and climate (Schlesinger et al., 1990; Schlesinger, 1997). Many studies have shown that woody encroachment results in increased carbon storage in soils (Mordelet et al., 1993; Archer et al., 2001, 2004; Jessup et al., 2003). Current extrapolations and model results suggest that woody encroachment into grasslands and savannas may result in the sequestration of 0.10–0.13 $\mathrm{Pg\,C\,yr^{-1}}$ in the USA, which represents approximately 20–40% of the current USA carbon sink strength (Tilman et al., 2000; Houghton et al., 2000; Pacala et al., 2001). Nonetheless, these regional estimates have a high degree of uncertainty, and some ecosystem-level studies have suggested that woody encroachment may result in no change (McCarron et al., 2003; Smith and Johnson 2003) or even net loss (Jackson et al., 2002) of soil carbon. Additional studies are needed to quantify the direction, magnitude, and mechanisms of change in soil carbon storage following woody encroachment into grass-dominated ecosystems.

In the Rio Grande Plains of southern Texas, grasslands dominated by $C_4$ grasses have undergone succession to subtropical thorn woodlands dominated by nitrogen-fixing $C_3$ trees and shrubs, resulting in increased soil C and N storage (Boutton et al., 1998, 1999; Archer et al., 2001, 2004; Hibbard et al., 2001; McCulley et al., 2004). This vegetation change provides a unique opportunity to utilize the natural abundance of $^{13}C$ and $^{15}N$ to evaluate changes in C and N cycles following woody plant invasion of grassland. Differences in $^{13}C/^{12}C$ ratios of plants utilizing the $C_3$ and $C_4$ photosynthetic pathways provide a natural tracer when a $C_4$ community type ($\delta^{13}C \approx -14‰$) is replaced by a $C_3$ community ($\delta^{13}C \approx -27‰$) or vice versa, permitting the quantification of the loss rate of C derived from the original vegetation and the simultaneous accumulation of new C derived from the current vegetation (Balesdent et al., 1988; Balesdent and Mariotti, 1996). Mean residence times (MRTs) of soil organic matter can be determined as a function of the rate at which $\delta^{13}C$ values of soil change over time following the change in plant cover. Relatively few estimates of MRTs exist for soil organic matter under natural plant communities relative to agricultural systems (Paul et al., 1997).

Soil $\delta^{15}N$ values reflect the net effect of N-cycling processes as influenced by the biotic and abiotic environment (Robinson, 2001; Dawson et al., 2002). Globally, soil $\delta^{15}N$ values are positively correlated with mean annual temperature and negatively correlated with mean annual precipitation (Amundson et al., 2003). At the ecosystem level, soil $\delta^{15}N$ values are also influenced by a number of factors such as quantity and quality of organic matter inputs, soil N sources, and isotopic fractionation resulting from N transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994). In whole soil, $\delta^{15}N$ is related to degree of organic matter humification, increasing with higher degree of decomposition (Shearer et al., 1978; Turner et al.,1983; Nadelhoffer et al., 1996; Koba et al., 1998). However, few studies have evaluated patterns of $\delta^{15}N$ values in soil physical fractions. Kramer et al. (2003) found that $\delta^{15}N$ values of separated density fractions increased with increasing aliphaticity, which reflects greater microbial processing. Other researchers have also shown that microbial processing results in $^{15}N$ accumulation (Andreux et al., 1990; Piccolo et al., 1996) although mechanisms of N fractionation associated with organic matter decomposition are still unclear. Because $\delta^{15}N$ tends to become enriched with increasing humification, $\delta^{15}N$ values of soil fractions may be related to MRTs and may be a potential indicator of soil organic matter quality.

The purpose of this study was to evaluate mechanisms of C and N storage and changes in C and N dynamics in relation to soil physical structure following woody plant invasion of grassland. To accomplish this, we utilized the natural abundance of $^{13}C$ and $^{15}N$ to: (1) quantify amounts of C derived from $C_4$ vs. $C_3$ sources; (2) estimate the MRTs of the organic matter residing in soil physical fractions; and (3) assess the potential relationship between $^{15}N$ and MRTs of soil physical fractions.

## 2. Materials and methods

### 2.1. Study area

Research was conducted at the Texas Agricultural Experiment Station La Copita 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 temperature of 22.4 °C. Mean annual precipitation is 716 mm, with peaks in May–June and September. The topography consists of nearly level uplands which grade (1–3%) into lower-lying drainages and playas. Elevations range from 75 to 90 m. Domestic livestock have grazed this site over the past century.

Upland surface soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic horizon (depth ≈ 30–40 cm) which contains non-argillic inclusions. $C_4$ grasslands interspersed with small, discrete clusters of woody plants dominate uplands. Species of *Paspalum, Bouteloua, Chloris,* and *Eragrostis* dominate in the grasslands. *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite) is the dominant plant species in all wooded landscape elements. Discrete clusters are characterized by a *Prosopis* overstory with species of *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory. Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation. Soils in lower-lying portions of the landscape are finer-textured clay loams (Pachic Argiustolls) and support closed-canopy woodlands. *Condalia hookeri* (M.C. Johnst.), *Celtis pallida* (Torr.), *Zanthoxylum fagara* (L.), *Diospyros texana* (Scheele.), and *Zizyphus obtusifolia* (T.&G.) dominate the understory beneath *Prosopis* in these lower-lying drainage woodlands. Evidence based on current vegetation patterns, sequential aerial photography, tree ring analysis, and the isotopic composition of soils all indicate conclusively that this entire landscape was once open $C_4$ grassland, and that woody plants have encroached into the grassland during the past 100 years (Archer et al., 1988, 2001; Boutton et al., 1998).

### 2.2. Chronosequence approach

Soils were collected from the LaCopita Research Area using a chronosequence approach. Ten sites were sampled within remnant grassland, cluster, and grove upland landscape elements. In lower-lying drainage woodlands, 11 sites were sampled. All sites were located within an area of approximately 2 km$^2$. Soils from remnant grasslands represented time zero (i.e. prior to woody invasion), whereas soils from woody clusters, groves, and drainage woodlands were sampled in stands of known age.

Because the formation of cluster, grove, and drainage woodland landscape elements are initiated only after the establishment of *P. glandulosa* in grassland (Archer et al. 1988), the age of a woody plant stand corresponds to the age of the largest (presumably oldest) *P. glandulosa* in that stand. Ages of *P. glandulosa* were determined by measuring their basal diameters, and then converting those values to tree ages based on regression equations specific to each landscape element (Stoker, 1997). In this study, woody plant stands were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from 10 to 130 years.

### 2.3. Collection of soil samples

Four soil cores (5-cm diameter × 30-cm depth) were taken beneath the largest *P. glandulosa* at each site, one in each cardinal direction from the bole. This sampling depth allowed us to sample the entire A-horizon at each site (Boutton et al., 1998). Sampling in remnant grassland sites was identical to woodlands with the exception that cores were taken in each cardinal direction from the base of a randomly selected perennial $C_4$ grass plant. Prior to coring, soil surface litter was gently removed to expose mineral soil. Soil cores were sectioned into 0–15 and 15–30 cm increments and stored at 4 °C. In the lab, each soil sample was thoroughly mixed and a subsample dried at 105 °C to determine bulk density using the core method (Culley, 1993). Then, the four cores from each site were pooled by depth increment and mixed. Only data for the 0–15 cm depth increment are reported in this study.

### 2.4. Soil physical and chemical characterization

An aliquot of soil was passed through a 2-mm sieve to remove large organic matter 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.

### 2.5. Soil physical fractionation

Soils were separated into specific size and density fractions using methods modified from Elliott (1986) and Six et al. (1998) as described in Liao et al. (2006). A total of 12 different soil fractions were isolated: (1) whole soil, (2) free light fraction with density $<1.0\,\mathrm{g\,cm^{-3}}$ (Free LF), (3) free silt + clay fraction $<53\,\mu\mathrm{m}$ (Free S + C), (4) macroaggregate-sized fraction $>250\,\mu\mathrm{m}$ (Macro), (5) macroaggregate-sized free particulate organic matter $>250\,\mu\mathrm{m}$ with density $<1.85\,\mathrm{g\,cm^{-3}}$ (MfPOM), (6) coarse intra-macroaggregate organic matter $>250\,\mu\mathrm{m}$ (MciPOM), (7) fine intra-macroaggregate particulate organic mattter 53–250 μm (MfiPOM), (8) intra-macroaggregate silt + clay $<53\,\mu\mathrm{m}$ (MiS + C), (9) microaggregate-sized fraction 53–250 μm (Micro), (10) microaggregate-sized free particulate organic matter 53–250 μm with density $<1.85\,\mathrm{g\,cm^{-3}}$ (mfPOM), (11) fine intra-microaggregate particulate organic matter 53–250 μm (mfiPOM), and (12) intra-microaggregate silt + clay $<53\mu\mathrm{m}$ (miS + C).

### 2.6. Elemental and isotopic analyses

Soil fractions were analyzed for $\delta^{13}$C, $\delta^{15}$N, %C, and %N 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 and N isotope ratios are presented in $\delta$ notation:

$$\delta = \left[(R_{\mathrm{SAMPLE}} - R_{\mathrm{STD}})/R_{\mathrm{STD}}\right] \times 10^3, \qquad (1)$$

where $R_{\mathrm{SAMPLE}}$ is the $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N ratio of the sample and $R_{\mathrm{STD}}$ is the $^{13}$C/$^{12}$C ratio of the V-PDB standard (Coplen, 1996) or $^{15}$N/$^{14}$N ratio of atmospheric $N_2$ (Mariotti, 1983). Precision of duplicate measurements was 0.1‰ for $\delta^{13}$C and 0.15 ‰ for $\delta^{15}$N.

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

$$F_C = (\delta_T - \delta_{WL})/(\delta_G - \delta_{WL}), \qquad (2)$$

where $\delta_T$ is the $\delta^{13}$C value of the SOC in some soil fraction at time T, $\delta_G$ is the average $\delta^{13}$C value of SOC in that same fraction from remnant grasslands, and $\delta_{WL}$ is the average $\delta^{13}$C value of organic matter inputs (roots, litter) in woodlands. Because the $\delta^{13}$C of woodland SOC at equilibrium is not known (i.e. the $\delta^{13}$C of soil in wooded areas is still changing), it is assumed that woodland soils will ultimately achieve a $\delta^{13}$C value similar to that of woody plant inputs (roots and litter). The average $\delta^{13}$C value of all root and litter samples from wooded areas in this study was −26‰, so we used this value for $\delta_{WL}$.

Concentrations of free POM (density $< 1.85\,\mathrm{g\,cm^{-3}}$) were subtracted from macro and microaggregate-sized fractions so that reported concentrations for these fractions only reflect organic matter contained within intact water-stable aggregates. Macroaggregate-sized fractions contained little to no sand $> 250\,\mu$m. Because microaggregate-sized fractions contained sand of the same size class as the microaggregates, all concentrations are reported on a whole-soil basis (g C or N in fraction $\mathrm{kg^{-1}}$ soil).

### 2.7. Statistical analyses

ANOVA was used to test for statistical differences between soil chemical and physical characteristics attributable to landscape element (NCSS, 1995). To distinguish differences between measurements where ANOVA indicated significant effects due to landscape element, Fisher's protected least significant difference (LSD) was used for mean separation. Fisher's protected LSD was also used for mean separation of concentrations of protected C (POM within macro- and microaggregates and associated with silt + clay) and unprotected C (POM external to aggregates including MfPOM, mfPOM, and the Free LF fractions) and proportion of protected C following ANOVA. Significance level was $p < 0.05$. To describe changes in $\delta^{13}$C vs. time, non-linear regressions of the form $y = a + e^{-kt}$ were fit to the data using SigmaPlot 2001 (SPSS Inc., 2001). Non-linear regression equations of the form $y = e^{-kt}$ were fit to the $F_C$ data vs. time; $F_C$ was forced to be equal to 1.0 at time zero. $R^2$ values are not reported since they are not a legitimate descriptive statistic for nonlinear regression analyses (Neter et al., 1996).

## 3. Results

### 3.1. Soil chemical and physical characteristics

Particle size distribution in upland soils (grasslands, clusters, and groves) 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 (Table 1). Soil pH was approximately 6.5 in remnant grasslands and was not altered following woody plant encroachment into grasslands (Table 1). Bulk density of the 0–15 cm depth interval decreased significantly from $1.2\,\mathrm{g\,cm^{-3}}$ in grasslands to $1.0\,\mathrm{g\,cm^{-3}}$ in wooded landscape elements (clusters, groves, and drainage woodlands).

### 3.2. Carbon and nitrogen concentrations of whole soil and soil fractions

Whole soil C in the upper 15 cm of the profile increased on average by 80–118% in upland clusters and groves and by 240% in drainage woodland landscape elements relative to remnant grasslands (Table 2). In woodlands, highest C concentrations were found in the unprotected free POM fraction, accounting for 34–53% of total SOC. In contrast, the unprotected free POM fraction of grasslands was only 20% of total SOC. In grasslands, macro- and microaggregates together constituted the largest proportion of total SOC (48–53%). Partitioning of C in macro- and microaggregates differed between grasslands and woodlands. In grasslands, microaggregates comprised the largest proportion of total SOC (44%). Microaggregates in woodlands ranged from 19% to 22% of total SOC. Macroaggregates were only 9% of total SOC in grasslands but ranged from

Table 1
Comparison of soil chemical and physical characteristics (0–15 cm) from grassland and woodland landscape elements of a subtropical savanna parkland

| | Grassland | Cluster | Grove | Drainage |
|---|---|---|---|---|
| Texture | Loamy sand | Loamy sand | Loamy sand | Sandy loam |
| Sand (%) | 80.5 (0.4)$^{a}$ | 81.3 (0.5)$^{a}$ | 81.4 (0.6)$^{a}$ | 66.0 (3.0)$^{b}$ |
| Silt (%) | 10.5 (2.0)$^{ab}$ | 9.6 (1.6)$^{b}$ | 12.0 (1.3)$^{ab}$ | 15.4 (2.7)$^{a}$ |
| Clay (%) | 9.0 (2.0)$^{b}$ | 9.1 (1.6)$^{b}$ | 6.6 (1.3)$^{b}$ | 18.6 (1.9)$^{a}$ |
| pH | 6.5 (0.1)$^{a}$ | 6.4 (0.1)$^{a}$ | 6.3 (0.1)$^{a}$ | 6.2 (0.1)$^{a}$ |
| Bulk density ($\mathrm{g\,cm^{-3}}$) | 1.20 (0.02)$^{a}$ | 1.00 (0.03)$^{b}$ | 1.00 (0.02)$^{b}$ | 1.00 (0.04)$^{b}$ |

Standard errors of the mean are in parentheses. Values within a row followed by the same letter are not significantly different at $p < 0.05$.

Table 2
Absolute carbon and nitrogen concentrations (g kg $^{-1}$ soil) of whole-soil and size/density fractions from the 0–15 cm depth interval in grassland, cluster, grove, and drainage woodland landscape elements of a subtropical savanna ecosystem

| | Grassland | | Cluster | | Grove | | Drainage | |
|---|---|---|---|---|---|---|---|---|
| | C | N | C | N | C | N | C | N |
| Whole soil | 6.20 (0.28) | 0.55 (0.02) | 11.17 (1.31) | 1.03 (0.10) | 13.30 (1.58) | 1.18 (0.12) | 21.09 (3.07) | 1.81 (0.27) |
| Free POM | 1.21 (0.14) | 0.07 (0.01) | 5.91 (1.21) | 0.37 (0.07) | 7.09 (1.38) | 0.50 (0.10) | 7.25 (1.67) | 0.51 (0.10) |
| density $<1.0\,\mathrm{g\,cm^{-3}}$ | 0.47 (0.04) | 0.02 (0.001) | 2.63 (0.60) | 0.14 (0.03) | 3.66 (0.91) | 0.24 (0.06) | 3.05 (0.93) | 0.20 (0.07) |
| density $<1.85\,\mathrm{g\,cm^{-3}}$ ($>250\,\mu$m) | 0.53 (0.13) | 0.03 (0.01) | 2.57 (0.59) | 0.17 (0.04) | 2.58 (0.43) | 0.19 (0.03) | 3.37 (0.66) | 0.23 (0.04) |
| density $<1.85\,\mathrm{g\,cm^{-3}}$ (53–250 $\mu$m) | 0.21 (0.04) | 0.02 (0.003) | 0.72 (0.09) | 0.05 (0.01) | 0.85 (0.11) | 0.07 (0.01) | 1.00 (0.21) | 0.08 (0.02) |
| Macroaggregate | 0.57 (0.18) | 0.05 (0.01) | 2.48 (0.43) | 0.19 (0.06) | 3.01 (1.10) | 0.20 (0.07) | 4.00 (0.91) | 0.36 (0.09) |
| MciPOM | 0.14 (0.01) | 0.01 (0.001) | 0.48 (0.13) | 0.03 (0.01) | 0.37 (0.06) | 0.03 (0.01) | 0.55 (0.13) | 0.05 (0.02) |
| MfiPOM | 0.07 (0.01) | 0.004 (0.001) | 0.21 (0.05) | 0.01 (0.003) | 0.19 (0.02) | 0.02 (0.004) | 0.38 (0.08) | 0.02 (0.01) |
| MiS + C | 0.19 (0.04) | 0.02 (0.004) | 0.52 (0.13) | 0.05 (0.01) | 0.45 (0.07) | 0.05 (0.01) | 1.83 (0.44) | 0.17 (0.04) |
| Microaggregate | 2.70 (0.19) | 0.25 (0.04) | 3.39 (0.22) | 0.30 (0.10) | 3.50 (0.15) | 0.34 (0.10) | 6.44 (0.70) | 0.47 (0.16) |
| mfiPOM | 0.39 (0.02) | 0.07 (0.001) | 0.60 (0.07) | 0.06 (0.002) | 0.62 (0.04) | 0.07 (0.002) | 0.61 (0.12) | 0.06 (0.01) |
| miS + C | 1.10 (0.09) | 0.12 (0.01) | 1.34 (0.09) | 0.16 (0.02) | 1.50 (0.10) | 0.17 (0.02) | 3.23 (0.60) | 0.28 (0.05) |
| Free silt + clay | 1.53 (0.08) | 0.15 (0.01) | 1.09 (0.08) | 0.11 (0.01) | 1.13 (0.08) | 0.11 (0.01) | 1.93 (0.13) | 0.16 (0.02) |

Standard errors are in parentheses.

19% to 22% in wooded landscape elements. Within macro- and microaggregates, the intra-aggregate silt + clay fraction had the highest C concentration (15–50%) across all landscape elements. Free silt + clay was 25% of total SOC in remnant grasslands. In contrast, in woodlands, free silt + clay was only 8–10% of total SOC (derived from Table 2).

The proportion of protected C (POM within aggregates and C associated with silt + clay) was greater than 60% for all landscape elements and was highest in grasslands (Fig. 1). However, absolute concentrations of protected and unprotected C were higher in woodlands relative to remnant grasslands. The concentration of protected C increased 40–140% from 5 g C kg$^{-1}$ soil in remnant grasslands to 7–12 g C kg$^{-1}$ soil in woodlands. Concentrations of unprotected C increased 400–600% from 1 gC kg$^{-1}$ soil to 5–7 gC kg$^{-1}$ soil in wooded landscape elements (Fig. 1).

Similar to whole soil C, N concentrations in whole soil showed increases of 87–230% following woody plant invasion of grassland (Table 2). Nitrogen concentrations in soil fractions also paralleled C concentrations. The free POM fraction was only 13% of soil total N in grasslands but increased to 28–42% of soil total N in woodlands. Microaggregates in grasslands comprised the largest proportion of soil total N (45%). Microaggregates in woodlands ranged from 26% to 29% of whole-soil N. Macroaggregates were 10% of soil total N in grasslands and 17–20% of soil total N in wooded landscape elements. As with C concentrations, the intra-aggregate silt + clay fraction within macro- and microaggregates (MiS + C and miS + C) had the highest N concentration (25–60%) across all landscape elements. The free silt + clay fraction was 27% of total SOC in grasslands but only 9–11% of SOC in woodlands (Table 2).

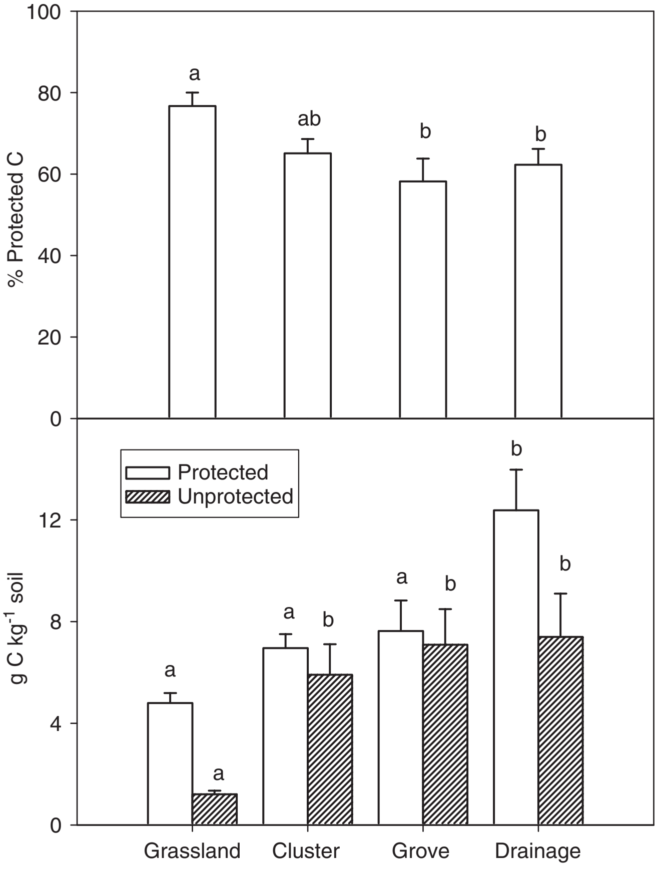

Fig. 1. Percent protected C (%) and C concentrations (g C kg $^{-1}$ soil) of protected and unprotected soil C (0–15 cm depth) in grassland, cluster, grove, and drainage woodland landscape elements. Different letters above bars within a category (protected vs. unprotected) indicate significant differences among means from Fisher's LSD.

### *3.3. Changes in the natural abundance of $^{13}$C in soil physical fractions*

$\delta^{13}$C values of all soil fractions in remnant grasslands ranged from approximately –16‰ to –20‰, typical of C derived from a grassland dominated by $C_4$ species (Fig. 2). Following woody invasion, $\delta^{13}$C values began to decrease in negative exponential fashion as grassland C decayed out of the system and woody plant-derived C accumulated. In all cases, the shifts in $\delta^{13}$C followed exponential decay kinetics although the fractions differed in rates of change. The most rapid rates of isotopic change (steeper curves) were the free light fraction (density $< 1.0\,\mathrm{g\,cm^{-3}}$), Macro and Micro free POM (density $< 1.85\,\mathrm{g\,cm^{-3}}$), and macro coarse and fine iPOM fractions (MciPOM, MfiPOM). In contrast, the silt and clay fractions (MiS + C, miS + C, free S + C) showed slower rates of change (shallower curves).

An isotopic mass balance model was utilized to compute $F_C$, the fraction of SOC derived from grassland (Eq. 2; Fig. 3). Fractional rate constants ($k$-values) for organic C decay were obtained by fitting exponential equations of the form $y = e^{-kt}$ to the $F_C$ vs. time data in Fig. 3. Similar to changes in $\delta^{13}$C values, the free light fraction and free POM fractions showed the most rapid decay of grassland-derived C out of the fractions, whereas the silt + clay fractions indicated that 20–80% of grassland-derived C remained following 100 years of woody plant invasion.

MRTs of C in each fraction were computed as the inverse ($1/k$) of the fractional rate constants (Table 3). The shortest MRTs (generally < 50 years) were associated with the unprotected free POM and macroaggregate-associated coarse and fine iPOM fractions. The C in intact macroaggregates was also relatively dynamic with MRTs ranging from 27 to 111 years. In contrast, intact microaggregate C exhibited longer MRTs ranging from 71 to 476 years. Silt + clay (free and intra-aggregate) associated C had the longest MRTs (80–1111 years). In groves and drainage woodlands (but not clusters), the longest MRTs were associated with silt + clay C within microaggregates. Whole-soil MRTs ranged from 36 to 115 years, similar to the MRTs for C in intact macroaggregates. In addition to differences between soil fractions, there were also significant differences between landscape elements. More specifically, the longest MRTs were associated with drainage woodlands, which have higher silt + clay contents than the upland landscape elements.

### *3.4. Natural abundance of $^{15}$N in soil fractions*

$\delta^{15}$N values of soil fractions showed no relationship with time following woody plant encroachment into grassland (data not shown). $\delta^{15}$N values of soil fractions were not significantly different between the three wooded landscape elements. Therefore, $\delta^{15}$N values for soil fractions were

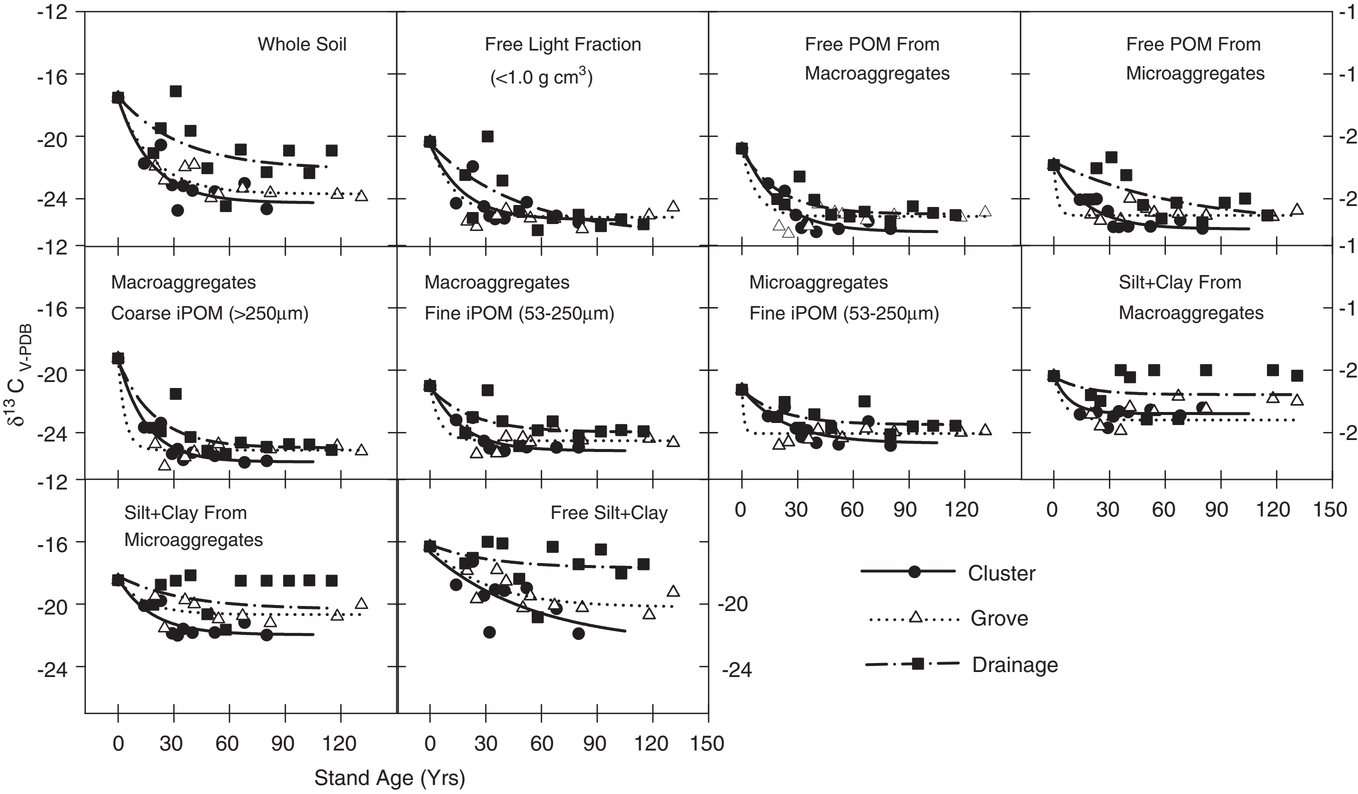

Fig. 2. Changes in $\delta^{13}$C (‰ vs. V-PDB) values of whole soil and soil fractions (0–15 cm depth increment) with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at time zero and respresent baseline starting values. The data were fit to an exponential decay model of the form $y = a + e^{-kt}$.

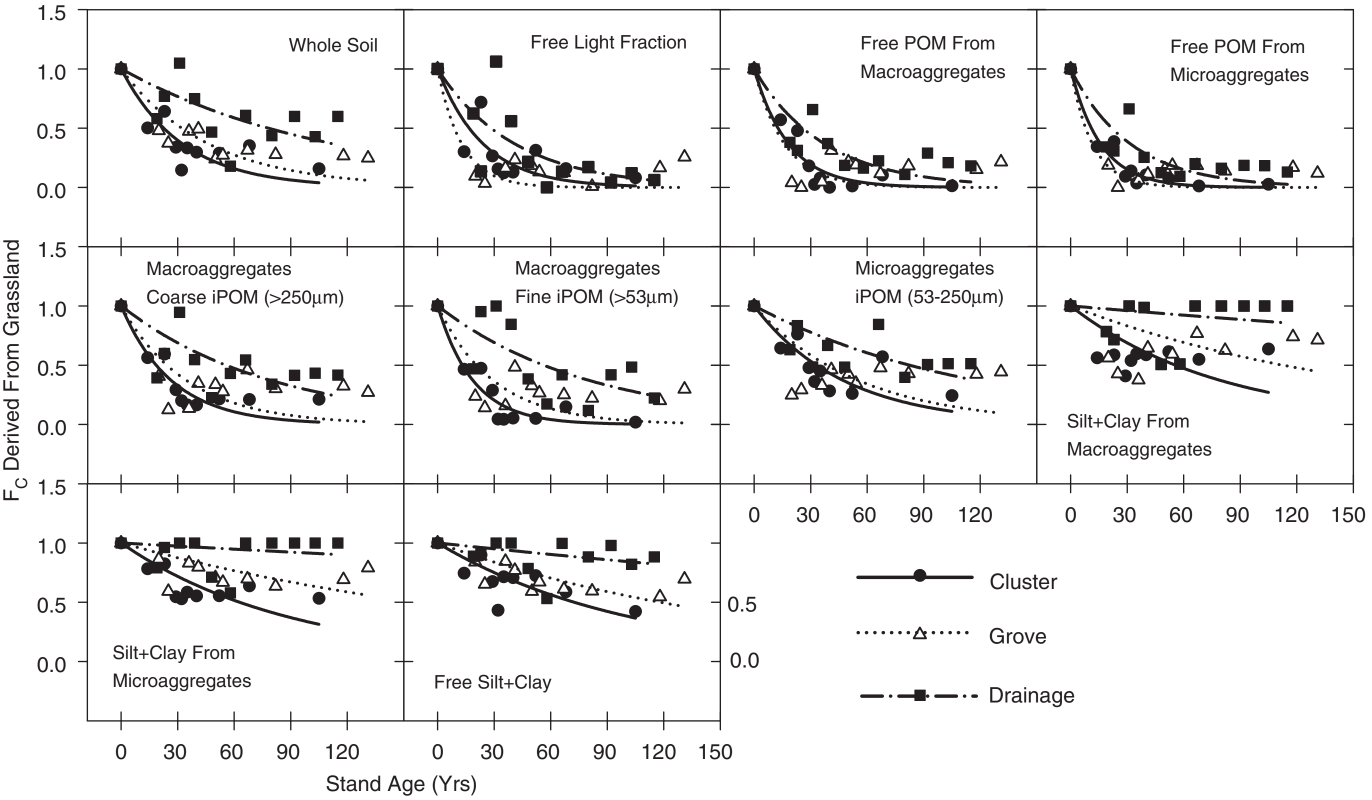

Fig. 3. The fraction of C derived from grassland ($F_C$) for whole soil and soil fractions (0–15 cm depth) with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at one, indicating that all soil C was derived from grassland. $F_C$ was calculated by mass balance using the $\delta^{13}C$ values of whole soil and soil fractions from the original grassland and −26‰ as the $\delta^{13}C$ value of woodland C (litter and roots).

Table 3
Rate constants (*k*) and mean residence times (MRT) of whole-soil and size/density fractions from the 0-15 cm depth increment in cluster, grove, and drainage woodland landscape elements of a subtropical savanna ecosystem

| | Cluster | | Grove | | Drainage | |
|---|---|---|---|---|---|---|
| | *k*-value | MRT (*y*) | *k*-value | MRT (*y*) | *k*-value | MRT (*y*) |
| Whole soil | 0.0280 (0.0053) | 36 | 0.0213 (0.0029) | 47 | 0.0087 (0.0018) | 115 |
| Free POM | 0.0479 (0.0078) | 21 | 0.0673 (0.0204) | 15 | 0.0239 (0.0047) | 42 |
| density $<1.0\,g\,cm^{-3}$ | 0.0415 (0.0079) | 24 | 0.0776 (0.0229) | 13 | 0.0245 (0.0059) | 41 |
| density $<1.85\,g\,cm^{-3}$ ($>250\,\mu m$) | 0.0547 (0.0074) | 18 | 0.0619 (0.0210) | 16 | 0.0269 (0.0041) | 37 |
| density $<1.85\,g\,cm^{-3}$ (53-250 μm) | 0.0523 (0.0094) | 19 | 0.0336 (0.0078) | 30 | 0.0123 (0.0028) | 81 |
| Macroaggregates | 0.0369 (0.0043) | 27 | 0.0402 (0.0117) | 25 | 0.0090 (0.0021) | 111 |
| MciPOM | 0.0630 (0.0058) | 16 | 0.0865 (0.0257) | 12 | 0.0317 (0.0050) | 32 |
| MfiPOM | 0.0372 (0.0046) | 27 | 0.0286 (0.0069) | 35 | 0.0125 (0.0024) | 80 |
| MiS + C | 0.0124 (0.0029) | 80 | 0.0061 (0.0022) | 162 | 0.0014 (0.0011) | 714 |
| Microaggregates | 0.0141 (0.0023) | 71 | 0.0084 (0.0020) | 119 | 0.0021 (0.0008) | 476 |
| mfiPOM | 0.0209 (0.0033) | 48 | 0.0179 (0.0045) | 56 | 0.0084 (0.0015) | 119 |
| miS + C | 0.0110 (0.0018) | 91 | 0.0045 (0.0009) | 224 | 0.0009 (0.0007) | 1111 |
| Free silt + clay | 0.0095 (0.0016) | 105 | 0.0059 (0.0009) | 170 | 0.0017 (0.0007) | 589 |

MRTs of macro- and microaggregates were calculated based on weighted averages of the C concentrations of the fractions comprising the aggregates. Standard errors are in parentheses.

averaged for cluster, grove, and drainage woodlands to obtain a composite woodland value for each soil fraction (Fig. 4). $\delta^{15}N$ values of roots, the free light fraction, macro free POM, macro coarse iPOM, macro iS + C, microaggregates, micro iS + C, and whole soil were less enriched in woodlands than remnant grasslands. In both grasslands

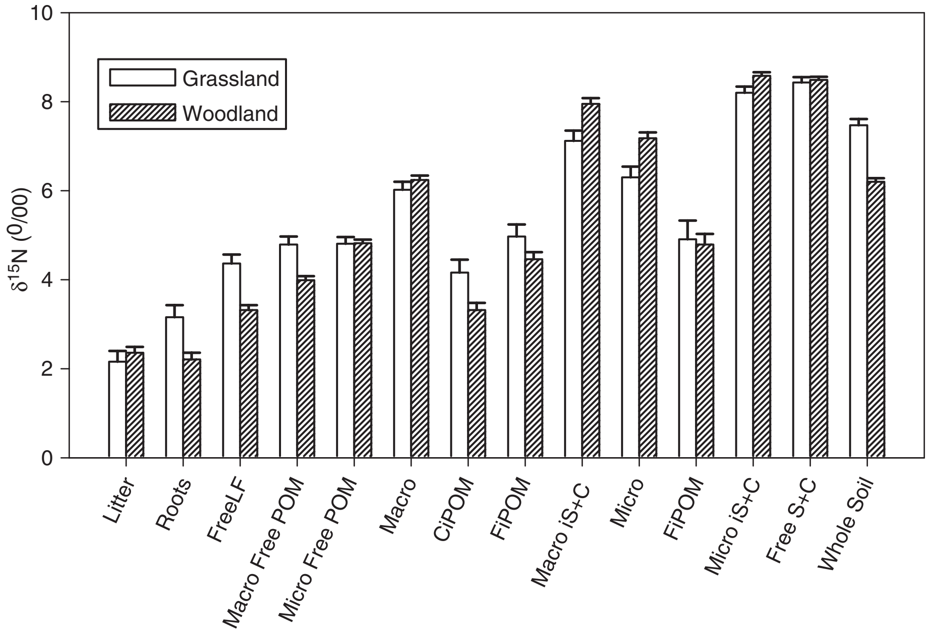

Fig. 4. $\delta^{15}N$ (‰) values of whole soil, surface litter, roots, and soil fractions (0–15 cm depth) from remnant grassland and woodland landscape elements at LaCopita Research Area. Woodland values are the mean of cluster, grove, and drainage woodlands.

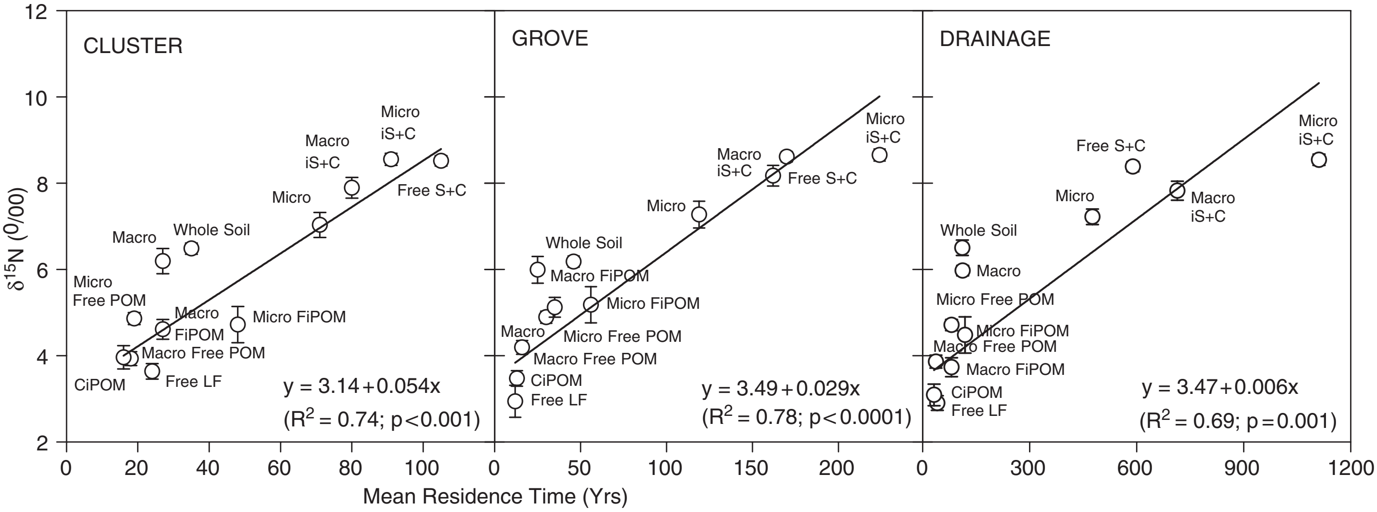

Fig. 5. $\delta^{15}N$ (‰) values of soil fractions vs. mean residence times of the fractions derived from $\delta^{13}C$ dynamics for cluster, grove, and drainage woodland landscape elements.

and woodlands, surface litter and roots had the lowest $\delta^{15}N$ values (Fig. 4). All other fractions exhibited higher $\delta^{15}N$ values than litter and roots with the silt + clay fractions being the most enriched. Most POM fractions were not significantly different from each other with respect to $\delta^{15}N$ values for both grasslands and woodlands. Whole-soil $\delta^{15}N$ values of both grasslands and woodlands were not significantly different from macroaggregates.

A positive linear relationship was observed between $\delta^{15}N$ values of soil fractions and the MRTs derived from changes in $^{13}C$ dynamics (Fig. 5). Both $\delta^{15}N$ and MRTs increased in the sequence-free light fraction < POM fractions < macroaggregates < microaggregates < S + C(free and intra-aggregate). Whole-soil values were intermediate between macro- and microaggregates in terms of $\delta^{15}N$ values and MRTs.

## 4. Discussion

Whole-soil C and N concentrations increased 80–240% following woody plant invasion of grasslands (Table 2). These increases are within the range of whole-soil increases in C and N previously estimated in this subtropical savanna ecosystem (Archer et al., 2001, 2004; Hibbard et al., 2001, 2003) and in other dryland ecosystems (Tiedemann and Klemmedson, 1973; Rundel et al., 1982; Virginia and Jarrell, 1983; East and Felker, 1993; Geesing et al., 2000) experiencing woody encroachment over comparable periods of time. Increases in whole-soil C and N may be in part a consequence of the greater above- and belowground productivity of woodlands relative to remnant grasslands. Aboveground NPP at this site increased from 1.9–3.4 $\mathrm{Mg\,ha^{-1}\,yr^{-1}}$ in remnant grasslands to 5.1–6.0 $\mathrm{Mg\,ha^{-1}\,yr^{-1}}$ in woodlands (Archer et al., 2001). Belowground productivity was also accelerated significantly as increases of up to 1000% for coarse and fine root biomass were found in woodlands compared with the original grassland (Hibbard et al., 2001).

Although whole-soil increases in C and N in this subtropical savanna ecosystem have been well-quantified, mechanisms of organic matter storage and rates of soil organic matter turnover have not been described. Concentrations of C and N in all soil fractions were greater in woodlands relative to remnant grasslands suggesting that the potential for C and N storage in these fractions is not currently saturated (Table 2). High C and N concentrations were found in the unprotected free POM fraction. Because the organic matter in this fraction is not protected either within stable aggregate structure or by association with silt and clay particles, accumulations of C and N in this fraction are probably being sustained by greater organic matter inputs and by decreased decomposition, perhaps due to the poorer quality of woody plant inputs. The chemical composition of shrub leaf litter is considered to be more resistant to decay due to the increased presence of lignins, tannins, and other secondary compounds that are not present in herbaceous grassland litter (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Sharma et al., 1995; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). Thus, slower decomposition rates due to lower-quality substrates coupled with higher organic matter inputs may be responsible for the dramatic accumulations in C and N following woody plant invasion of grassland.

Nevertheless, physical protection of organic matter by stable soil structure and association with silt and clay may be contributing to SOC and total N storage in this dryland ecosystem. Protected C in this study was more than 60% of whole-soil C for all landscape elements (Fig. 1). However, absolute values of protected C in woodlands were greater than remnant grasslands mainly due to increased C storage in macroaggregates. In woodlands, less than 10% of total SOC and N were stored in the free silt + clay fraction (Table 2).

The increased fine and coarse root biomass and higher microbial biomass following woody plant invasion of grassland in this ecosystem (Boutton et al., 1998; Hibbard et al., 2001; McCulley et al., 2004) have likely fostered increased aggregation of soils in wooded areas. Following woody plant invasion of grassland, there was an increase in the proportion of macroaggregates in wooded landscape elements and concurrent decreases in proportions of free microaggregates. This suggests that either microaggregates are being incorporated into stable macroaggregates or that there is a decrease in the release of microaggregates formed within stable macroaggregates. Decreases in the free silt and clay fraction following woody invasion is also likely due to their incorporation into macroaggregate structure. Greater root biomass (coarse and fine) and associated rhizosphere activity in woodland soils likely promoted the formation of macroaggregates since roots and fungal hyphae are important binding agents operating at this scale (Tisdall, 1991; Jastrow and Miller, 1998). Bird et al. (2002) found that aggregate stability was higher beneath mesquite than in between mesquite canopies due to higher litter inputs, roots, and microbial activity. They also found higher concentrations of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungal hyphae, beneath mesquite. Glomalin appears to be highly correlated with soil aggregate stability (Wright et al., 1999; Bird et al., 2002). Greater soil microbial biomass in this system also suggests that glomalin and other residues of microbial activity may be contributing to the stabilization of smaller macroaggregates and microaggregates (Tisdall, 1991).

Separation of soil organic matter into physical fractions in conjunction with the use of the natural abundance of $^{13}$C and $^{15}$N helped elucidate mechanisms of soil organic matter accumulation and turnover following grassland-to-woodland succession. Prior to woody invasion, organic matter inputs were primarily of $C_4$ origin (Fig. 2). Following woody plant invasion, all soil fractions showed an exponential decline in $\delta^{13}$C values, reflecting the decay of grassland C out of the system and the accumulation of woodland C. The $F_C$ data showed that following 100 years of woodland development, 10–30% of C derived from grasslands remained in whole soil (Fig. 3). This remaining C pool appears to be relatively resistant to decay and is likely an important component of long-term C storage. The proportion of $C_4$-derived C in the free light fraction and POM fractions declined rapidly following woody plant invasion indicating rapid turnover in these fractions. Unprotected organic matter fractions are often relatively labile with high concentrations of carbohydrates and nitrogen compounds (Skjemstad et al., 1996; Golchin et al., 1994, 1995). Because the organic matter in these fractions is not protected within aggregates or associated with clay minerals, it is readily accessible to microbes as reflected by their initial rapid loss following woody plant encroachment. With increasing degree of decomposition, organic matter may be transferred to more stabilized soil fractions. In contrast to the rapid decomposition of $C_4$-derived C from unprotected soil fractions, a substantial amount of $C_4$-derived C remained in silt + clay fractions reflecting slower turnover rates. The long-term protection of soil organic matter by silts and clays has been well-established (Anderson and Paul, 1984; Ladd et al., 1985; Feller and Beare, 1997; Hassink, 1997). Silts and clays are often associated with strongly humified organic residues of high molecular weight that are resistant to decomposition.

However, clays may also react with labile organic matter constituents resulting in longer turnover of those otherwise labile fractions (Schmidt and Kögel-Knabner, 2002; Six et al., 2002b).

Radiocarbon dating of silts and clays indicate the importance of organo-mineral complexing in protection of organic matter from decomposition (Anderson and Paul, 1984). Anderson and Paul (1984) found MRTs ranging from 170 years for fine clay-associated organic C, 800–965 years for coarse and fine silt C, and 1255 years for coarse clay C. Balesdent et al. (1988) reported MRTs of 400 years for silt C and 1000 years for clay C. Monreal et al. (1997) found that the mineral fraction ($<50\,\mu$m) was comprised of resistant organic C that was highly humified with an MRT time of 275 years (Monreal et al., 1997). The MRTs of soil fractions derived from $^{13}$C dynamics following woody encroachment corroborate that silt+clay-associated fractions had the longest residence times (average = 361 years), reflecting the combination of residence times of the various silt- and clay-sized fractions. This silt+clay fraction is a long-term pool for C storage.

In contrast to the longer MRTs of the silt+clay fraction, C in free POM fractions had the shortest MRTs (average = 26 years). Aggregate-protected C had an average MRT of 47 years (Table 3). MRTs calculated from natural $^{13}$C dynamics following conversion of native forest to cultivation (Besnard et al., 1996) were 12 years for free POM (external to macro- and microaggregates) and 33 years for occluded POM (within aggregates), reflecting the decreased turnover of organic matter protected within stable aggregates. Light fraction or free POM is comprised of younger C (i.e. more recently added residues) that contains recognizable plant material (Gregorich et al., 1995; Puget et al., 2000; Baisden et al., 2002). Average turnover for C in intact macroaggregates (54 years) was faster than turnover for microaggregate-associated C (222 years) (Table 3). MRTs for Macro fine iPOM (MfiPOM) was longer than MRTs for Macro coarse iPOM (MciPOM) and free POM fractions suggesting that this fraction is more protected by aggregate structure. Some of the MfiPOM is likely POM within microaggregates that have been incorporated or formed within the macroaggregate structure, hence the longer MRTs of the MfiPOM fraction relative to MciPOM. However, MfiPOM had faster turnover rates than Micro fine iPOM (mfiPOM), indicating that microaggregates have a greater protective capacity than macroaggregates, likely due to small pore spaces and slower diffusion of oxygen into microaggregates (Sexstone et al., 1985). Faster turnover of MfiPOM may also be indicative of new microaggregates being formed within macroaggregates whereas free microaggregates may reflect a greater proportion of more stabilized material remaining intact following release as macroaggregates turnover (Six et al., 2002b).

Buyanovsky et al. (1994) found that macroaggregates had approximately the same life span as vegetative fragments reflecting a labile C pool. Unlike macroaggregates that are held together by temporary, labile binding agents (roots and fungal hyphae), microaggregates are held together by microbial polysaccharides and more persistent binding agents (aromatic humic material) that have slower turnover (Tisdall and Oades, 1982; Elliott and Coleman, 1988). Monreal et al. (1997) found via chemical characterization that the rapid turnover of organic matter in macroaggregates was due to the presence of plant residues and the high activities of soil microorganisms. Microaggregates had molecular properties and decay kinetics intermediate between macroaggregates and silt+clay, and had an average MRT of 61 years (Monreal et al., 1997). Other studies indicate that turnover of macroaggregates is accelerated relative to microaggregates although rates are extremely variable ranging from a few years for macroaggregates to a century or more for microaggregates (Skjemstad et al., 1990; Buyanovsky et al., 1994; Jastrow et al., 1996; Monreal et al., 1997; Puget et al., 2000). The results of this and other studies all confirm that turnover of organic matter appears to be dependent on its position within soil structure.

However, the long MRTs of the silt+clay fractions in this study irrespective of location within or exterior to soil macro- or microaggregates suggests that soil texture plays an important role in C and N accumulation somewhat independent of soil structure. Evidence for additional protection of silt+clay-protected organic matter is reflected in the fact that microaggregate-associated silt+clay in groves and drainages were longer than any other silt+clay fraction. Generally though, MRTs of silt+clay fractions in aggregates were variable and not consistently longer than estimated MRTs of free silt+clay external to aggregates. It appears that organic materials protected via associations with silts and clays have long MRTs regardless of position within or external to aggregates. The longer MRTs of soil fractions from drainage woodlands could be due to the protective effects of soil texture since this lower-lying portion of the landscape contained higher concentrations of silt+clay relative to uplands. Longer MRTs of POM fractions in drainages may be due to more coating with mineral matter. Longer MRTs may also be due to reductions in the diffusion of oxygen into finer-textured soils. Prior studies at LaCopita Research Area indicated that drainage woodland soils retained the greatest memory of the original $C_4$ grassland in the clay fraction reflecting the slow turnover associated with clay-associated organic matter (Boutton et al., 1998, 1999). The range of MRTs across soil fractions in this study is consistent with the concept of a range of organic matter fractions that differ in dynamics and turnover.

The natural abundance of $^{15}$N in soil fractions may help elucidate the quality of organic matter that has accumulated following grassland-to-woodland succession. Controls on soil $\delta^{15}$N values are complex and include the quantity and quality of organic matter inputs, soil N sources, and isotopic fractionations resulting from N transformations (Nadelhoffer and Fry, 1988; Piccolo et al., 1994; Hopkins et al., 1999).

Despite this complexity in the interpretation of $\delta^{15}N$ values, there are some generalities. Plant and litter inputs are generally less enriched in $^{15}N$ relative to soil due to plant uptake of mineral N which is isotopically depleted compared to soil total N (Gebauer and Schulze, 1991; Handley and Raven, 1992). $\delta^{15}N$ values increase with increasing microbial transformation of residues in litter inputs since microbial discrimination of $^{15}N$ occurs during N-catabolic processes in the soil (Andreux et al., 1990; Christensen, 1992). Thus, successive microbial decomposition of N-containing substrates results in the progressive increase in $^{15}N$ of soil organic matter.

Several studies have shown increasing $\delta^{15}N$ values with decreasing particle size (Ledgard et al., 1984; Tiessen et al., 1984; Kerley and Jarvis, 1997) although few studies have evaluated patterns of $\delta^{15}N$ values in soil aggregate fractions. Baisden et al. (2002) showed increasing $\delta^{15}N$ values with increasing density. Higher-density organic matter fractions are associated with increasing degree of organic matter decomposition (Golchin et al., 1995). Kramer et al. (2003) found that increased humification due to greater microbial processing was correlated with increases in $\delta^{15}N$ values of soil organic matter fractions. The results of this study are in agreement with Kramer et al. (2003). Lower $\delta^{15}N$ values were associated with recent organic matter inputs (litter, roots, POM fractions) (Fig. 4). At the other extreme, higher $\delta^{15}N$ values in silts + clays indicate that the organic matter in these fractions is older and more humified. Results from a related study at LaCopita Research Area showed increasing $\delta^{15}N$ values with increasing lignin degradation in soil fractions, consistent with the notion that $\delta^{15}N$ values become more enriched as decomposition proceeds (Gamblin et al., 2003).

Because $\delta^{15}N$ tends to become enriched with increasing humification, $\delta^{15}N$ values of soil fractions may be related to MRTs of the same fractions and thereby provide additional information about soil organic matter quality. MRTs were positively correlated with $\delta^{15}N$ values of soil physical fractions suggesting that fractions with higher $\delta^{15}N$ values and longer MRTs are more humified (Fig. 5). Longer MRTs and more enriched $\delta^{15}N$ values in microaggregates and in silt + clay fractions relative to macroaggregates and free POM fractions which had shorter MRTs and lower $\delta^{15}N$ values suggests that the organic matter associated with microaggregates and silt + clay fractions may be not only physically protected, but also highly decomposed and biochemically recalcitrant.

## 5. Conclusions

Woody plant invasion of grasslands in this subtropical ecosystem in southern Texas has resulted in increased SOC and soil total N pools. Increased soil C and N pools in wooded areas were due to both the retention of older $C_4$-derived organic matter by protection within microaggregates and association with silt + clay, and the accumulation of new $C_3$-derived organic matter in macroaggregates and POM fractions. The MRTs of the soil fractions derived from $^{13}C$ dynamics indicated that the C and N associated with microaggregate (222 yr) and silt + clay fractions (360 yr) was physically protected from decay and/or biochemically recalcitrant. Much of the C and N in woodlands was stored in free POM with an average MRT of 30 years. Average MRTs of fine POM within aggregates was 60 years, reflecting the importance of stable aggregates in organic matter protection. Soil texture also appeared to play an important role in organic matter sequestration independent of soil structure since organic matter associated with silts and clays had long residence times regardless of position within or external to soil aggregates. $\delta^{15}N$ values of soil fractions corroborated that the organic matter associated with microaggregates and silt + clay was more humified ($^{15}N$-enriched), while organic matter associated with macroaggregates and POM fractions was of more recent origin ($^{15}N$-depleted). Because similar grassland-to-woodland conversions have been geographically extensive in grasslands and savannas worldwide, processes, patterns, and mechanisms documented here through the use of the natural abundance of $^{13}C$ and $^{15}N$ could have significance for understanding regional and global C and N cycles.

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