

# Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland

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## Abstract

Woody plant invasion of grasslands is prevalent worldwide. In the Rio Grande Plains of Texas, subtropical thorn woodlands dominated by  $C_3$  trees/shrubs have been replacing  $C_4$  grasslands over the past 150 yr, resulting in increased soil organic carbon (SOC) storage and concomitant increases in soil total nitrogen (STN). To elucidate mechanisms of change in SOC and STN, we separated soil organic matter into specific size/density fractions and determined the concentration of C and N in these fractions. Soils were collected from remnant grasslands (Time 0) and woody plant stands (ages 10–130 yr). Rates of whole-soil C and N accrual in the upper 15 cm of the soil profile averaged  $10\text{--}30\text{ g C m}^{-2}\text{ yr}^{-1}$  and  $1\text{--}3\text{ g N m}^{-2}\text{ yr}^{-1}$ , respectively, over the past 130 yr of woodland development. These rates of accumulation have increased soil C and N stocks in older wooded areas by 100–500% relative to remnant grasslands. Probable causes of these increased pool sizes include higher rates of organic matter production in wooded areas, greater inherent biochemical resistance of woody litter to decomposition, and protection of organic matter by stabilization within soil macro- and microaggregates. The mass proportions of the free light fraction ( $<1.0\text{ g cm}^{-3}$ ) and macroaggregate fraction ( $>250\text{ }\mu\text{m}$ ) increased linearly with time following woody plant invasion of grassland. Conversely, the mass proportions of free microaggregate ( $53\text{--}250\text{ }\mu\text{m}$ ) and free silt + clay ( $<53\text{ }\mu\text{m}$ ) fractions decreased linearly with time after woody invasion, likely reflecting stabilization of these fractions within macroaggregate structures. Carbon and N concentrations increased in all soil fractions with time following woody invasion. Approximately half of the C and N accumulated in free particulate organic matter (POM) fractions, while the remainder accrued in stable macro- and microaggregate structures. Soil C/N ratios indicated that the organic C associated with POM and macroaggregates was of more recent origin (less decomposed) than C associated with the microaggregate and silt + clay fractions. Because grassland-to-woodland conversion has been geographically extensive in grassland ecosystems worldwide during the past century, changes in soil C and N storage and dynamics documented here could have significance for global cycles of those elements.

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## 1. Introduction

Woody plant encroachment is prevalent in many grass-dominated ecosystems worldwide. This globally extensive phenomenon has been documented in North and South America, Africa, Asia, and Australia and appears to be driven primarily by land-use practices, especially livestock grazing and fire suppression (Van Auken, 2000; Archer et al., 2001). This widespread shift in ecosystem structure has strong potential to alter key ecosystem processes that could

affect C and N cycles at ecosystem, regional, and global scales. In fact, it has been estimated that woody encroachment into grasslands may represent as much as 34% of the terrestrial C sink strength of the USA alone (Houghton et al., 1999; Tilman et al., 2000; Pacala et al., 2001).

During the past 150 yr, trees and shrubs have invaded areas previously dominated by grasses in the subtropical Rio Grande Plains of Southern Texas (Archer et al., 1988, 2001; Boutton et al., 1998, 1999). These invasive woodlands, which are dominated by N-fixing trees/shrubs (Zitzer et al., 1996), have higher rates of net primary productivity than the grasslands they replace (Archer et al., 2001, 2004; Hibbard et al., 2001, 2003), leading to

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increased C and N storage in biomass (Archer et al., 2001) and soils (Boutton et al., 1998; Archer et al., 2004; McCulley et al., 2004). This shift in vegetation structure from grasslands to woodlands over the last 100–130 yr provides a unique opportunity to examine how changes in ecosystem structure (i.e. grassland to woodland) and function (increased productivity and N-inputs) affect soil organic carbon (SOC) and soil total N (STN) storage and dynamics. Furthermore, the gradients created by the elevated C and N inputs caused by this vegetation shift can be used to investigate the mechanisms contributing to the stabilization and accumulation of soil organic matter (SOM).

Recently, the role of soil physical structure as a determinant of SOM sequestration and turnover has generated much interest (Tisdall and Oades, 1982; Ladd et al., 1993; Jastrow and Miller, 1998; Christensen, 2001; Six et al., 2004). SOM occurs throughout the soil matrix in a variety of different sizes, shapes, levels of degradation, and degrees of association with soil minerals. The positions of SOM with respect to pores and aggregated structures causes differential accessibility to decomposers, and results in a range of SOM pools that differ in stability and dynamics (Ladd et al., 1993). The conceptual view of aggregate hierarchy suggests that the process by which SOM is incorporated into the soil matrix occurs with some degree of order (Tisdall and Oades, 1982; Oades, 1993; Golchin et al., 1994; Six et al., 2004). In areas where biotic factors are the main agents of aggregation, soil primary particles (sand, silt, clay) are stabilized into secondary and higher orders of aggregates. Primary particles are cemented together by clay–organic matter complexes incorporating highly decomposed aromatic and aliphatic substances and polysaccharides. Microaggregates often have cores of partially decomposed particulate organic matter (POM) and are considered relatively stable against disturbance (Oades and Waters, 1991).

Microaggregates generally contain a greater proportion of SOM that is older and more resistant to decay (Six and Jastrow, 2002). Microaggregates together with primary particles are often found within macroaggregates bound together by roots and fungal hyphae and by polysaccharides and other byproducts generated by the decomposition of organic inputs. Because of the relatively labile, ephemeral nature of these binding agents and the size of pores being bridged, macroaggregates are usually less stable than microaggregates to physical disturbance (Tisdall and Oades, 1982).

Thus, SOM is comprised of a gradient of pools that differ in physical and chemical properties, decomposability, and turnover. Physical fractionation procedures utilize differences in the size and density of organic matter to isolate pools that differ in structure and function and, hence, storage mechanisms and turnover. Although physical fractionation of SOC has been widely employed in the study of land cover/land use effects in agricultural systems, application of these techniques to the study of SOC and

soil total N storage and dynamics in natural systems that have never been cropped or cultivated is less frequent. The specific objectives of this study were to investigate the role of soil aggregate structure in C and N sequestration following woody invasion of grassland and to quantify the dynamics and longevity of C and N in organic matter fractions isolated from specific physical locations within the soil structure.

## 2. Materials and methods

### 2.1. Study area

Field sampling was conducted in December 2001 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 mean annual temperature of 22.4 °C and mean annual precipitation of 716 mm (peaks in May–June and September). The topography consists of nearly level uplands that grade (1–3% slopes) into lower-lying drainage woodlands. Elevation ranges from 75 to 90 m. Evidence from sequential aerial photography, tree ring analyses, vegetation dynamics modeling, and the isotopic composition of soils all indicate that this region was once relatively open grassland and that woody plant encroachment into grasslands has occurred over the past 150 yr (Archer et al., 1988, 2001, 2004; Boutton et al., 1998, 1999). Domestic livestock have grazed this research area over the past century.

Upland surface soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic (B<sub>1</sub>) horizon that includes non-argillic inclusions. Uplands are dominated by C<sub>4</sub> grasslands interspersed with small, discrete clusters of woody plants. Dominant C<sub>4</sub> grasses in uplands include species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis*. Clusters expand laterally, fusing to form larger groves of woody vegetation where the argillic horizon is absent. In lower-lying portions of the landscape, soils are finer-textured clay loams (Pachic Argiustolls) and support closed-canopy drainage woodlands.

*Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite) is the dominant plant species in all wooded landscape elements. Mesquite is the first to colonize in grasslands and subsequently facilitates recruitment of other woody plant species beneath its canopy. Understorey species common to both uplands and lowlands include *Condalia hookeri* (M.C. Johnst.), *Celtis pallida* (Torr.), *Zanthoxylum fagara* (L.), *Diospyros texana* (Scheele.), *Zizyphus obtusifolia* (T.&G.), and *Berberis trifoliolata* (Moric.). Plant nomenclature follows Correll and Johnston (1979). Additional details on plant communities, soils, and successional dynamics have been presented elsewhere (Scifres and Koerth, 1987; Archer et al., 1988; Boutton et al., 1998).

## 2.2. Chronosequence approach

A chronosequence approach was used to quantify C and N pool sizes and their rates of change in soils and soil fractions following woody plant encroachment into areas that were previously open grasslands. Ten sites were sampled within remnant grasslands, clusters, and groves (upland sites); 11 sites were sampled within lower-lying drainage woodlands. All sites were located within an area of approximately 2 km<sup>2</sup>. Remnant grasslands were sampled to characterize C and N pool sizes at time zero (i.e. prior to woody encroachment), whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in C and N 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 grasslands (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 using those values to predict tree ages based on regression equations specific to each landscape element (Stoker, 1997). Woody plant stands sampled in this study were selected to encompass the full range of *P. glandulosa* basal diameters, corresponding to tree ages ranging from approximately 10–130 yr.

## 2.3. Collection of soil samples

Soil samples were collected from a grazing exclosure that was established in 1985. Four soil cores (5-cm diameter × 30-cm length) were taken, one in each cardinal direction within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site. This sampling depth allowed us to sample the entire A-horizon at each site (Boutton et al., 1998). Sampling was identical in each remnant grassland site, but cores were taken in each cardinal direction from the base of a randomly selected perennial C<sub>4</sub> grass plant. Soil surface litter was removed gently to expose mineral soil prior to taking each soil core. All soil cores were sectioned into 0–15 and 15–30 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. Then, the 4 cores from each site were composited by depth increment.

## 2.4. Soil chemical and physical characterization

An aliquot of field-moist soil was passed through a 2-mm sieve to remove large organic matter fragments and used for physical, chemical, and elemental 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 analyses.

## 2.5. Soil physical fractionation

An aliquot of field-moist soils was first passed thru a 15-mm sieve followed by an 8-mm sieve to remove large roots and then thoroughly mixed and air-dried. Soils were physically fractionated (Fig. 1) into 2 size classes of water-stable aggregates by using the disruptive forces of slaking and wet-sieving, and stable aggregates were further dispersed into POM and mineral components by shaking in sodium hexametaphosphate (HMP) by following a procedure modified from Elliott (1986) and Six et al. (1998).

After oven-drying overnight at 65 °C, a 30 g subsample of soil from each site was immersed in deionized water on top of a 250-μm sieve. Organic debris and root pieces that floated in the water (density <1.0 g cm<sup>-3</sup>) were aspirated onto a 20-μm nylon filter. This material was quantified as the free light fraction (Free LF) and considered to be the litter component of whole-soil C. After 5 min of slaking, the sieve was manually moved up and down 3 cm, 50 times over a 2 min period. The fraction remaining on the 250-μm sieve was oven-dried at 65 °C and collected in a pre-weighed aluminium pan. Water plus soil <250 μm was poured through a 53-μm sieve and the sieving procedure

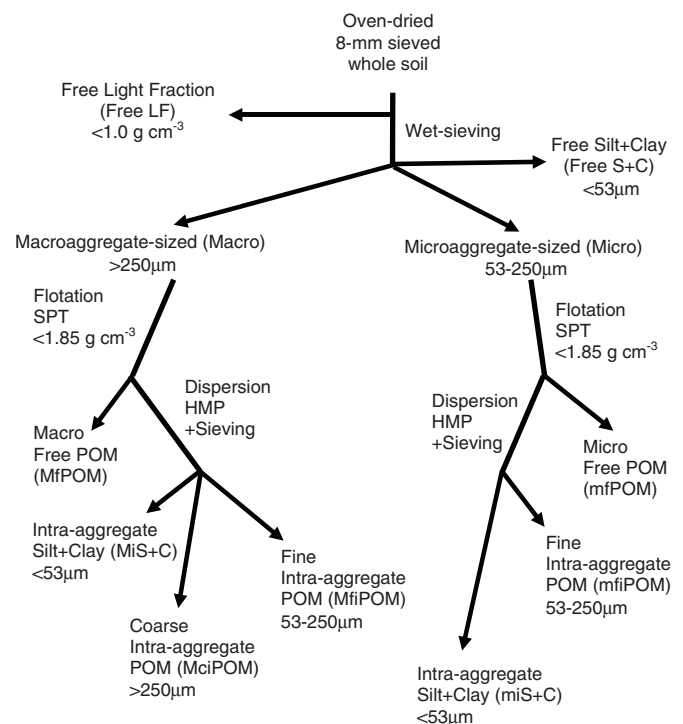


Fig. 1. Flow chart for separation of soil organic matter into different size and density fractions (adapted from Elliott, 1986; Six et al., 1998). POM = particulate organic matter, SPT = sodium polytungstate, HMP = sodium hexametaphosphate.

was repeated. Unaggregated (free) silt and clay that passed through the 53- $\mu\text{m}$  sieve was collected by centrifugation. This procedure yielded a macroaggregate-sized fraction  $>250\ \mu\text{m}$  (Macro), a microaggregate-sized fraction 53–250  $\mu\text{m}$  (Micro), and a free silt + clay fraction  $<53\ \mu\text{m}$  (Free S + C). Silt and clay were not quantified separately because of the small percentage of silt and clay sized particles relative to whole soil at all sites (Table 1).

Both aggregate size fractions consisted of intact aggregates, sand of the same size class, and inter-aggregate POM of the same size class released during slaking and sieving. Inter-aggregate POM was separated from intact aggregates and sand by flotation of 5-g subsamples in sodium polytungstate (SPT) at a density of  $1.85\ \text{g cm}^{-3}$ . This material (MfPOM and mfPOM) was aspirated onto a 20- $\mu\text{m}$  nylon filter, washed with deionized water, and dried. The remaining heavy fractions were washed with deionized water 3 times to thoroughly remove SPT. The heavy fractions were dispersed by shaking for 16 h on a reciprocating shaker at low speed (180 oscillations per minute) in  $5\ \text{g L}^{-1}$  of HMP solution. Dispersed macroaggregates were passed sequentially through 250 and 53- $\mu\text{m}$  sieves and rinsed with deionized water to yield coarse intra-aggregate POM  $>250\ \mu\text{m}$  (MciPOM), fine intra-aggregate POM 53–250  $\mu\text{m}$  (MfiPOM), and macroaggregated silt + clay (MiS + C). Dispersed microaggregates were passed through a 53- $\mu\text{m}$  sieve to yield fine intra-aggregate POM 53–250  $\mu\text{m}$  (mfiPOM) and microaggregated silt + clay (miS + C). Intra-aggregate silt + clay  $<53\ \mu\text{m}$  (iS + C) was collected after addition of 5.0 ml of 0.25 M  $\text{CaCl}_2$  + 0.25 M  $\text{MgCl}_2$  to flocculate clays.

## 2.6. Recovery of soil mass and C and N concentrations following fractionation

Approximately 99.5% of whole-soil mass was recovered in soil fractions isolated by wet-sieving. Following dispersion, 94.1% of macro- and microaggregate mass was recovered in soil fractions. Lower recovery was likely related to loss of sand that was close to the size of sieve openings during collection and washing of fractions from

sieves. Recovery of C and N in fractions from wet-sieving was 98% and 92% of whole-soil C and N, respectively. However, recovery of C and N in fractions following dispersion indicated 25–30% of whole-soil C and N was lost. C and N may have been lost following flotation of macro- and microaggregate-sized fractions in SPT to remove the free POM fraction and/or following dispersion of the remaining heavy fraction in HMP. Chan (2001) found that as much as 20% of total organic C was lost and recovered as soluble organic C in the HMP solution following dispersion of soil.

## 2.7. Elemental analyses

Soil fractions isolated by wet-sieving and dispersion were dried at  $65^\circ\text{C}$  in a convection oven, weighed, and ground in a SPEX mill (SPEX CertiPrep, Metuchen, NJ). Whole-soil and soil fractions were analyzed for C and N concentrations on a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ). None of the samples contained  $\text{CaCO}_3$ . Concentrations of C in Macro and Micro free POM 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  $>250\ \mu\text{m}$ . 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  $\text{kg}^{-1}$  soil).

## 2.8. Statistical analyses

ANOVA was used to determine differences between soil physical and chemical characteristics of grassland and woodland landscape elements (0–15 and 15–30 cm). Fisher's protected least significant difference (LSD) method was used to distinguish differences among landscape elements within a soil depth following ANOVA. Linear regression was used to determine relationships between the size distributions of aggregate mass and increasing woody plant stand age (i.e. time following woody plant invasion)

Table 1

Soil physical and chemical characteristics of landscape elements (grassland, cluster, grove, drainage) at LaCopita Research Area

	0–15 cm				15–30 cm			
	Grassland	Cluster	Grove	Drainage	Grassland	Cluster	Grove	Drainage
pH	6.5(0.1) <sup>a</sup>	6.4(0.1) <sup>a</sup>	6.3(0.1) <sup>a</sup>	6.2(0.1) <sup>a</sup>	6.9(0.1) <sup>a</sup>	6.4(0.1) <sup>b</sup>	6.7(0.1) <sup>a</sup>	6.3(0.1) <sup>b</sup>
Texture	Loamy sand	Loamy sand	Loamy sand	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy clay loam
Sand (%)	80.5(0.4) <sup>a</sup>	81.3(0.5) <sup>a</sup>	81.4(0.6) <sup>a</sup>	66.0(3.0) <sup>b</sup>	74.1(1.5) <sup>a</sup>	78.8(0.9) <sup>a</sup>	78.0(0.8) <sup>a</sup>	56.1(4.1) <sup>b</sup>
Silt (%)	10.5(2.0) <sup>ab</sup>	9.6(1.6) <sup>b</sup>	12.0(1.3) <sup>ab</sup>	15.4(2.7) <sup>a</sup>	16.9(1.9) <sup>ab</sup>	12.5(2.2) <sup>bc</sup>	8.9(2.2) <sup>c</sup>	21.0(2.1) <sup>a</sup>
Clay (%)	9.0(2.0) <sup>b</sup>	9.1(1.6) <sup>b</sup>	6.6(1.3) <sup>b</sup>	18.6(1.9) <sup>a</sup>	9.1(2.3) <sup>b</sup>	8.7(2.0) <sup>b</sup>	13.1(1.8) <sup>b</sup>	22.9(2.8) <sup>a</sup>
Bulk Density ( $\text{g cm}^{-3}$ )	1.2(0.02) <sup>a</sup>	1.0(0.03) <sup>b</sup>	1.0(0.02) <sup>b</sup>	1.0(0.04) <sup>b</sup>	1.3(0.03) <sup>a</sup>	1.3(0.02) <sup>a</sup>	1.2(0.02) <sup>a</sup>	1.2(0.02) <sup>a</sup>

Different letters represent significant differences between means within a row for each depth interval from Fisher's LSD following ANOVA. Standard errors of the mean are in parentheses.

and the relationships between C and N concentrations and woody plant stand age. C and N concentrations were converted to C and N stocks by using soil bulk density estimates for each soil sample at each site. The slopes of regression lines fit to C and N stocks were used to determine soil C and N accumulation rates ( $\text{g C}$  or  $\text{N m}^{-2} \text{yr}^{-1}$ ). Because the mass distribution of soil fractions at 15–30 cm was unaffected by woody plant encroachment, regression analyses were only performed for fractions from the upper 15 cm of the profile. ANOVA was used to determine differences among landscape elements for C:N ratios. However, because no significant differences were detected, C:N ratios were averaged across landscape elements to obtain a mean value for each soil fraction. ANOVA was also used to determine differences among landscape elements for concentrations of protected C (POM within macro- and microaggregates and associated with silt+clay), unprotected C (POM external to aggregates including MfPOM, mfPOM, and the Free LF), and the proportion of protected C. Mean separations were performed using Fisher's protected LSD. Linear regression analyses were performed using SigmaPlot 2001 (SPSS Inc., 2001). ANOVAs were run using Number Cruncher

Statistical Systems 6.0 (NCSS, 1995). Significance was declared at  $p < 0.05$ .

### 3. Results

#### 3.1. Soil physical and chemical characteristics

Soil pH was approximately 6.5 in the upper 15 cm of the profile in remnant grasslands and was not altered following woody plant encroachment into grasslands (Table 1). Upland soils beneath grasslands, clusters, and groves were loamy sands, with a particle-size distribution of approximately 80% sand, 10% silt, and 10% clay. In contrast, soils in lower-lying drainage woodlands were sandy loams with a particle-size distribution of 60% sand, 20% silt, and 20% clay (Table 1). Patterns were similar at 15–30 cm, although all soils at this depth contained slightly more silt and clay relative to 0–15 cm. Bulk density of the 0–15 cm depth interval decreased significantly from  $1.2 \text{ g cm}^{-3}$  in grasslands to  $1.0 \text{ g cm}^{-3}$  in wooded landscape elements (clusters, groves, and drainage woodlands); at 15–30 cm, bulk densities ranged from 1.2 to  $1.3 \text{ g cm}^{-3}$ .

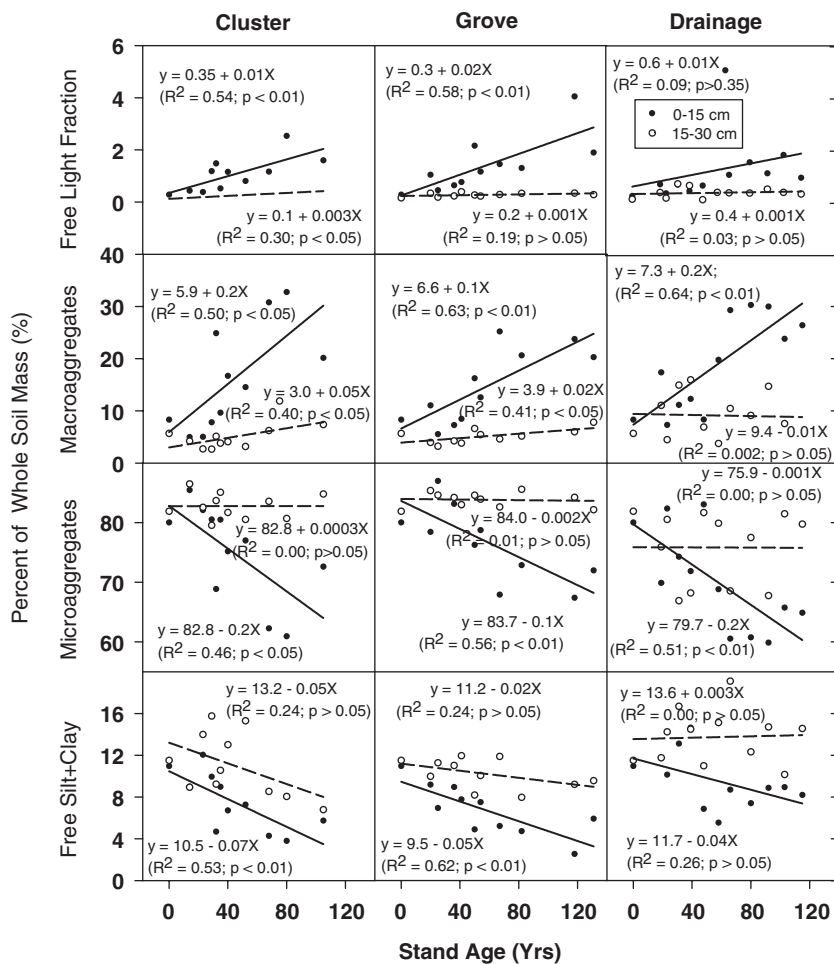


Fig. 2. Soil physical fractions (as a percentage of whole-soil weight) relative to woody plant stand age and soil depth (0–15 and 15–30 cm). Fractions were isolated according to the procedure in Fig. 1. Values for time zero in each frame are remnant grasslands.

3.2. Distribution of soil fractions by mass

The distribution of soil fractions (from wet-sieving) as a proportion of whole-soil mass increased in the order Free LF < Free S+C < Macro < Micro (Fig. 2). With time following woody plant invasion of grasslands, relative proportions of the free light fraction and macroaggregate-sized fraction increased linearly in the upper 15 cm of the soil. The free light fraction (density < 1.0 g cm<sup>-3</sup>) increased from 1% of whole-soil weight in grasslands to 2–4% of whole-soil weight in wooded landscape elements. The macroaggregate-sized fraction (>250 μm) in the 0–15 cm depth increment increased from <10% of whole-soil weight in grasslands to >30% of whole-soil weight in older (>80 yr) clusters, groves, and drainage woodlands. Consequently, the relative proportions of the microaggregate-sized fraction (53–250 μm) and free silt + clay fraction (<53 μm) decreased linearly with time after woody establishment. Microaggregates decreased from 80% of whole-soil weight in grasslands to approximately 60% of whole-soil weight after 80–130 yr of woodland develop-

ment. The free silt + clay fraction decreased from 11% of whole-soil weight in grasslands to 3–8% in older woodlands (>80 yr). Unlike the results from wet-sieving, soil fractions as a proportion of total macro- and microaggregate mass showed no significant trends with time following woody plant invasion of grassland. At 15–30 cm, distribution of soil fractions remained largely unaffected by woody plant invasion of grassland. Thus, the 15–30 cm depth interval is not discussed further in this paper.

3.3. C and N concentrations in whole soil and soil fractions

Total SOC and STN (0–15 cm) increased approximately 1–5 fold along the chronosequence following woody plant invasion of grassland (Figs. 3 and 4). Carbon concentrations of all fractions (0–15 cm) isolated from wet-sieving, except free silt + clay, increased linearly with woodland age (Fig. 3). The greatest increase of C was in organic matter external to aggregates (free POM). Carbon concentrations in these non-protected free POM fractions (Free

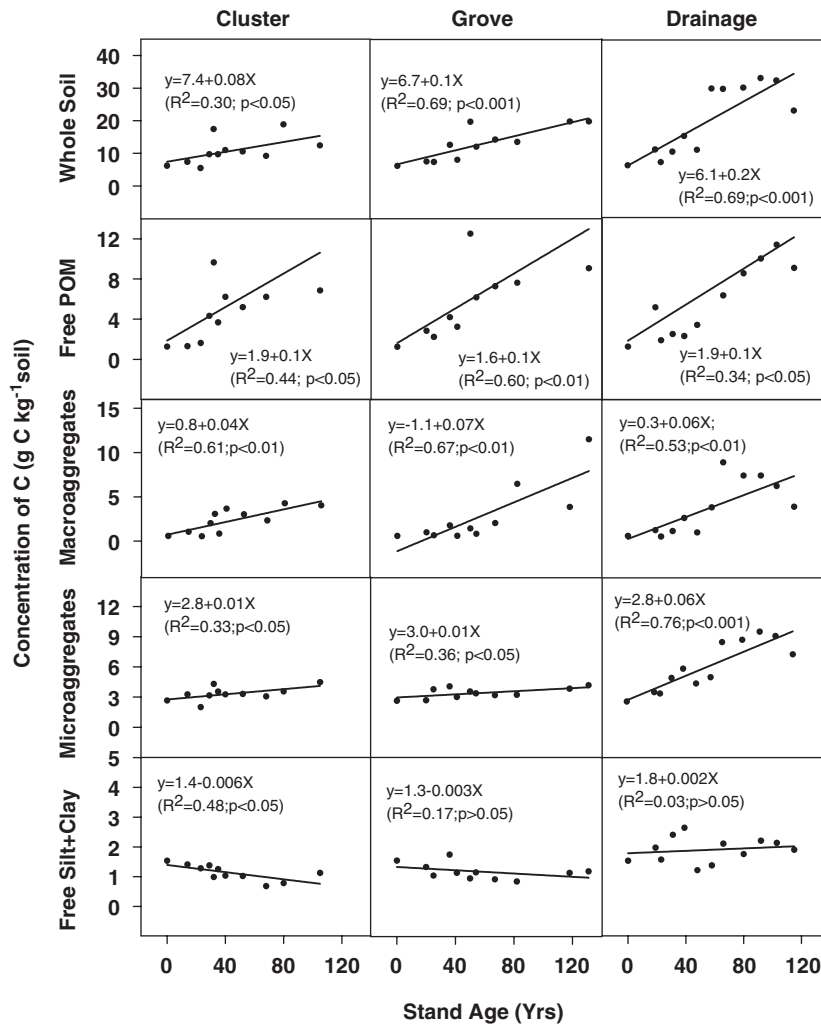


Fig. 3. Carbon (g C kg<sup>-1</sup> soil) in whole soil and soil fractions (0–15 cm soil depth) relative to woody plant stand age at LaCopita Research Area in southern Texas. Fractions were isolated according to the procedure in Fig. 1. In each frame, values for time zero are remnant grasslands.

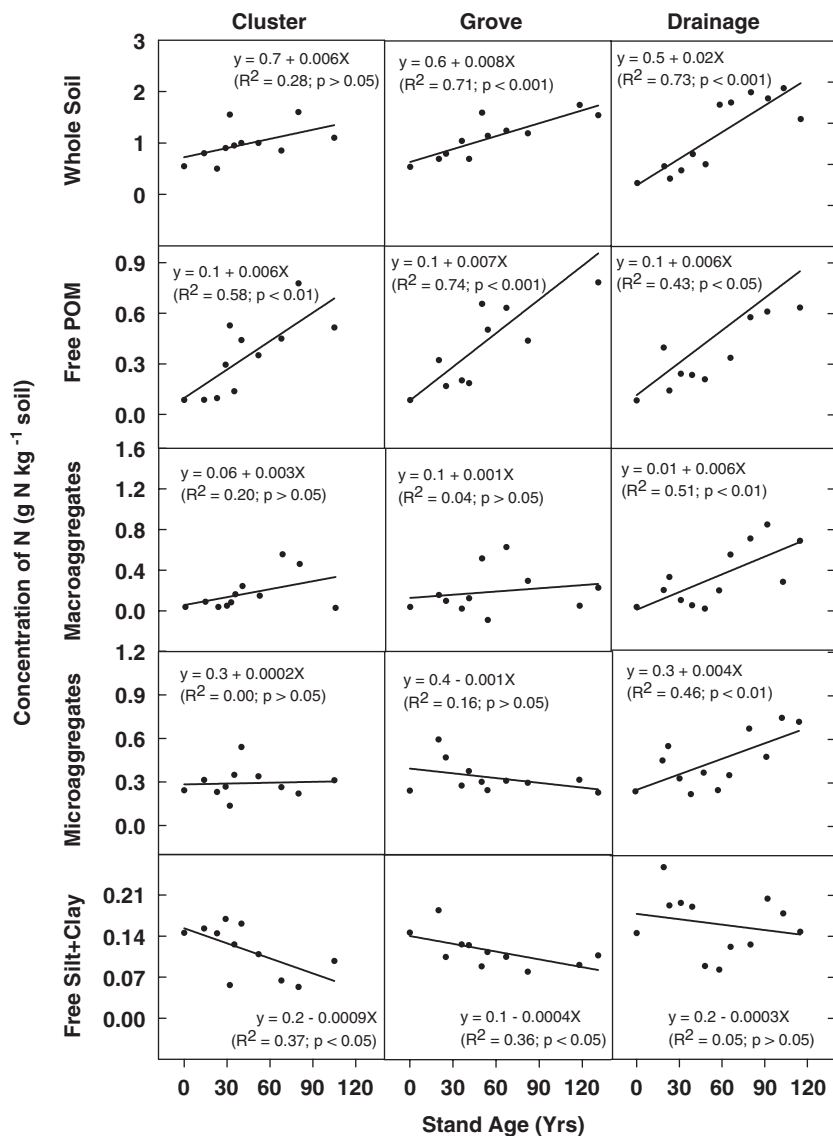


Fig. 4. Nitrogen ( $\text{g N kg}^{-1}$  soil) in whole soil and soil fractions (0–15 cm soil depth) relative to woody plant stand age at LaCopita Research Area. Fractions were isolated according to the procedure in Fig. 1. In each frame, values for time zero are remnant grasslands.

LF + MfPOM + mfPOM) increased from approximately  $2 \text{ g C kg}^{-1}$  whole soil in grasslands to  $12 \text{ g C kg}^{-1}$  whole soil in older woodlands. Carbon concentration in macroaggregate-sized fractions increased from  $1 \text{ g C kg}^{-1}$  soil in grasslands to  $4\text{--}8 \text{ g C kg}^{-1}$  soil in older woodlands. Microaggregate C concentrations increased from  $3 \text{ g C kg}^{-1}$  whole soil to  $5 \text{ g C kg}^{-1}$  whole soil in clusters and groves and up to  $10 \text{ g C kg}^{-1}$  whole soil in older drainage woodlands. C concentrations of the free silt + clay fractions tended to decrease following woody plant invasion although trends were generally not significant (Fig. 3).

Macroaggregate-associated fractions (MciPOM, MfiPOM, MiS+C) in all wooded landscape elements increased in C concentration with time following woody plant invasion of grassland (Fig. 5). All microaggregate-associated fractions (mfiPOM, miS+C) in drainages

increased in C concentration with time following woody plant invasion of grassland. However, for clusters and groves, although intact microaggregates increased linearly, none of the individual microaggregate-associated fractions increased in C concentration with time.

Changes in N paralleled those of C with the free POM (0–15 cm) having the greatest increase with time after woody plant invasion (Fig. 4). Nitrogen concentrations in the free POM fraction (external to aggregates) increased from  $0.1 \text{ g N kg}^{-1}$  whole soil in grasslands to  $0.9 \text{ g N kg}^{-1}$  whole soil in older groves and drainage woodlands. For both macro- and microaggregate-sized fractions, only drainage woodlands showed significant increases in N concentration with time after woody invasion (Fig. 4). Nitrogen concentration of the free silt + clay fraction in clusters and groves decreased with time, but was not

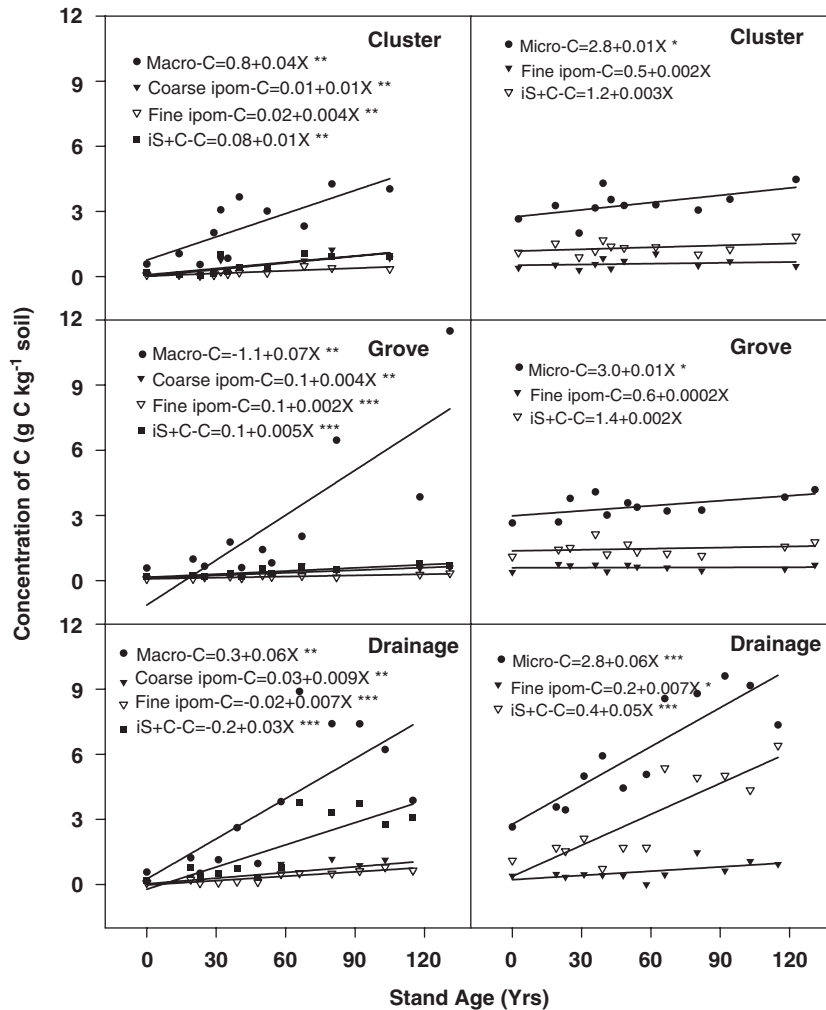


Fig. 5. Carbon ( $\text{g C kg}^{-1}$  soil) in macro- and microaggregates and aggregate-dispersed fractions (0–15 cm soil depth) relative to woody plant stand age at LaCopita Research Area. Values for time zero in each frame are remnant grasslands.

affected in drainages. Macroaggregate-associated fractions (MciPOM, MfiPOM, MiS + C) increased in N concentration with increasing time following woody plant invasion of grassland although the trends were not all significant (Fig. 6). Microaggregate-associated fractions (mfiPOM, miS + C) showed no consistent trends (Fig. 6).

C and N concentrations in unprotected free POM and macroaggregates as a proportion of total whole soil C and N increased with time following woody plant invasion of grassland (data not shown). In contrast, C and N as a proportion of total C and N decreased in microaggregates and free silt + clay with time following woodland development. Carbon and N within macroaggregate-associated fractions (MciPOM, MfiPOM, MiS + C) as a proportion of total macroaggregate C and N did not change following woody plant invasion of grassland. Relative to total microaggregate C and N, C and N in microaggregate-associated fractions (mfiPOM, miS + C) also showed no significant proportional changes following woody plant establishment in grasslands.

### 3.4. Unprotected vs. aggregate-protected C

Relative proportions of protected C (silt + clay and aggregate-associated) were higher in grasslands (80%) than woodlands < 50 yr old (65–70%) (Fig. 7). In woodlands > 50 yr, relative proportions of protected C were lower than those of younger woodlands, ranging from 55% to 65%. Partitioning by fraction (data not shown), microaggregates accounted for 50% of the total amount of protected C across landscape elements. In grasslands, most of the protected C was occurring in microaggregates (56%) and silt + clay (32%). In woodlands, protection was occurring mainly in microaggregates (50%) and macroaggregates (35%). When looking at absolute amounts of protected C, values for grasslands were similar to those of younger woodlands. For older woodlands (> 50 yr), the amount of protected C was generally higher compared to remnant grasslands and younger woodlands. For all wooded areas irrespective of age, amounts of unprotected C were greater relative to amounts in remnant grasslands (Fig. 7).



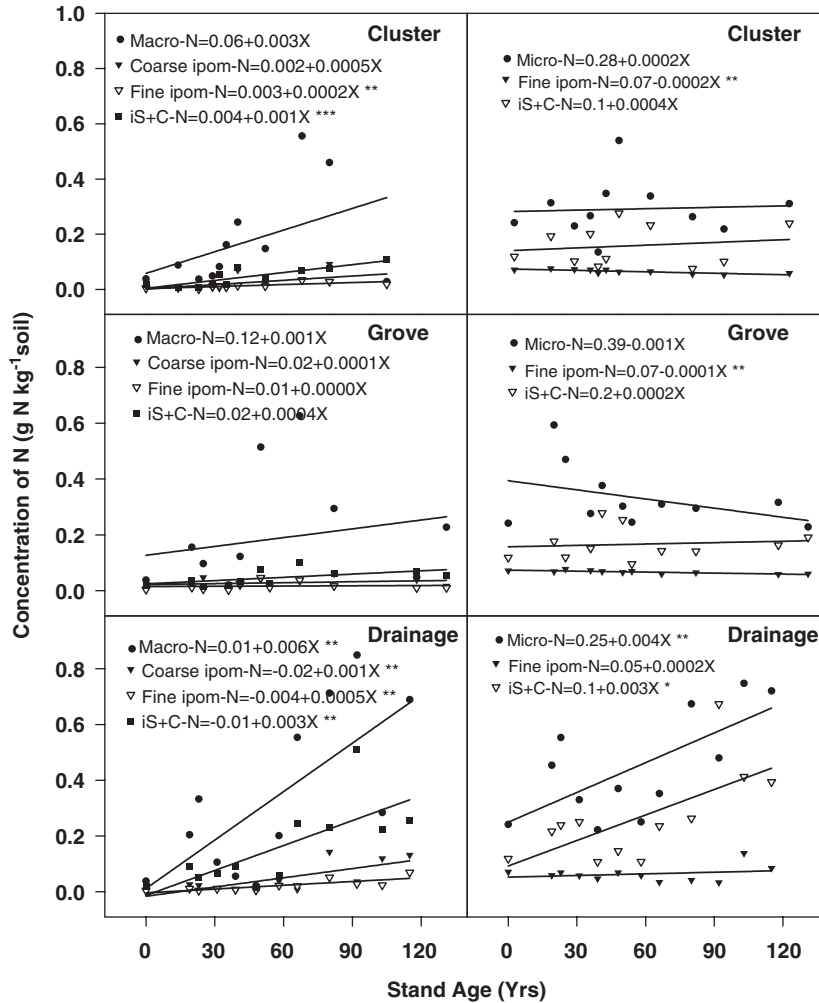


Fig. 6. Nitrogen (g N kg<sup>-1</sup> soil) in macro- and microaggregates and aggregate-dispersed fractions (0–15 cm soil depth) relative to woody plant stand age at LaCopita Research Area. In each frame, values for time zero are remnant grasslands.

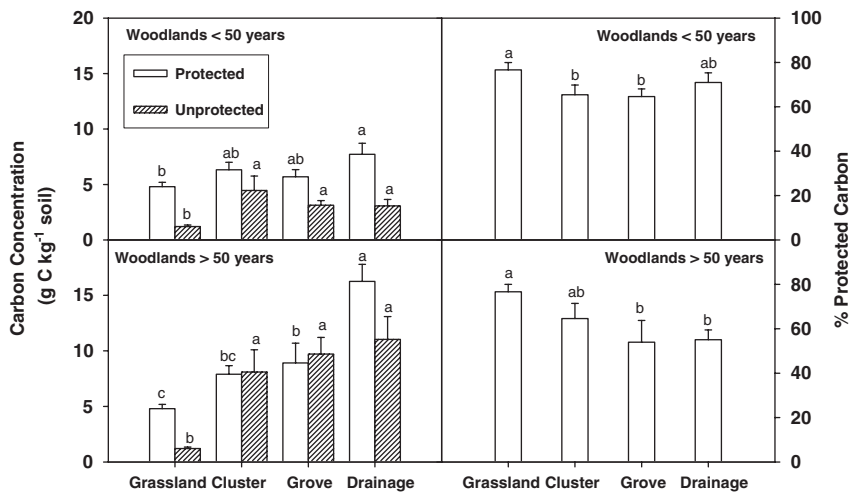


Fig. 7. Protected and unprotected carbon (g C kg<sup>-1</sup> soil) and proportion of protected carbon in younger (<50 yr) and older (> 50 yr) woodlands (0–15 cm soil depth) relative to remnant grasslands. Different letters above bars within a category (protected vs. unprotected) indicate significant differences among means from Fisher's protected LSD following ANOVA.

### 3.5. C and N accumulation rates

Concentrations of C and N ( $\text{g kg}^{-1}$  soil) were converted to C and N stocks ( $\text{g C m}^{-2}$ ) using soil bulk densities. Regression equations were fit to the data, and the slopes of the regression lines are the accumulation rates reported in Tables 2 and 3 for C and N, respectively. Rates of whole-soil C accumulation in the upper 15 cm of the profile averaged  $10\text{--}30 \text{ g C m}^{-2} \text{ yr}^{-1}$  (Table 2). Most of the SOC accumulation appeared to be a consequence of higher rates of accrual associated with the free POM fraction (Table 2).

Table 2

Soil organic carbon accumulation rates from linear correlations between woody stand ages and carbon stocks of soil fractions in cluster, grove, and drainage landscape elements at LaCopita Research Area

	C accumulation rates ( $\text{g C m}^{-2} \text{ yr}^{-1}$ )		
	Cluster 0–15 cm	Grove 0–15 cm	Drainage 0–15 cm
Whole soil	10.5(4.9)	14.2(3.2)	29.3(5.7)
Free POM	12.7(4.5)	12(3.2)	11.1(4.7)
Density $< 1.0 \text{ g cm}^{-3}$	5.5(2.5)	7.4(2.2)	4.0(3.0)
Density $< 1.85 \text{ g cm}^{-3} (> 250 \mu\text{m})$	6.5(2.0)	3.6(1.2)	4.9(1.9)
Density $< 1.85 \text{ g cm}^{-3} (53\text{--}250 \mu\text{m})$	0.7(0.4)	1.1(0.2)	2.2(0.4)
Macro	5.3(1.4)	10.1(2.5)	7.9(2.4)
MciPOM	1.5(0.4)	0.5(0.1)	1.1(0.3)
MfiPOM	0.6(0.1)	0.2(0.1)	0.9(0.1)
MiS + C	1.5(0.4)	0.6(0.1)	4.5(1.0)
Micro	1.4(1.4)	5.7(1.5)	6.5(1.4)
mfiPOM	0.2(0.3)	-0.1(0.2)	0.7(0.4)
miS + C	0.3(0.4)	-0.03(0.40)	6.0(1.3)
Free S + C	-1.2(0.4)	-0.7(0.3)	-0.5(0.7)

Numbers in parentheses are the standard errors of the estimates.

Table 3

Soil nitrogen accumulation rates from linear correlations between woody stand ages and nitrogen stocks of soil fractions in cluster, grove, and drainage landscape elements at LaCopita Research Area

	N accumulation rates ( $\text{g N m}^{-2} \text{ yr}^{-1}$ )		
	Cluster 0–15 cm	Grove 0–15 cm	Drainage 0–15 cm
Whole soil	0.8(0.4)	1.1(0.2)	2.6(0.4)
Free POM	0.8(0.2)	0.9(0.2)	0.8(0.3)
Density $< 1.0 \text{ g cm}^{-3}$	0.3(0.1)	0.5(0.1)	0.3(0.2)
Density $< 1.85 \text{ g cm}^{-3} (> 250 \mu\text{m})$	0.5(0.1)	0.3(0.1)	0.3(0.1)
Density $< 1.85 \text{ g cm}^{-3} (53\text{--}250 \mu\text{m})$	0.05(0.03)	0.1(0.02)	0.2(0.04)
Macro	0.4(0.3)	0.1(0.3)	0.7(0.3)
MciPOM	0.07(0.04)	0.01(0.02)	0.1(0.04)
MfiPOM	0.04(0.01)	0.003(0.020)	0.06(0.02)
MiS + C	0.1(0.03)	0.05(0.03)	0.4(0.1)
Micro	-0.02(0.2)	-0.2(0.1)	0.3(0.3)
mfiPOM	-0.05(0.01)	-0.03(0.01)	-0.001(0.040)
miS + C	0.03(0.10)	-0.07(0.07)	0.3(0.1)
Free S + C	-0.2(0.1)	-0.09(0.03)	-0.1(0.1)

Numbers in parentheses are the standard errors of the estimates.

This non-protected fraction accounted for 40 to nearly 100% of the whole-soil C accumulation rate. Macro-aggregates also play an important role in C-accumulation as C accrual within macroaggregates accounted for 30–70% of total SOC. Although the microaggregate fraction and all of its component parts appeared to be contributing much less to the overall rate of whole-soil C accumulation, this fraction still potentially accounted for 10–40% of total SOC. Intra-aggregate silt + clay may be an important component in aggregate-sized fractions, especially in drainages, as 60% and 90% of C accumulation in macro- and microaggregate-sized fractions in drainages, respectively, was due to this occluded silt + clay fraction.

Rates of whole-soil N accumulation averaged  $1\text{--}3 \text{ g N m}^{-2} \text{ yr}^{-1}$  (Table 3). As with SOC, much of the N accumulation (30–80%) in whole-soil was due to the higher rates of N sequestration in free POM (Table 3). The macroaggregate-sized fraction accounted for 5–30% of whole-soil N accumulation. Generally, the microaggregate-sized fraction and the free silt + clay fraction contributed little ( $< 10\%$ ) to the overall rate of whole-soil N accumulation.

### 3.6. C:N ratios of whole soil and soil fractions

C:N ratios of whole soil and soil fractions were not altered subsequent to woody plant invasion of grassland (data not shown). The C:N ratios of whole-soil and soil fractions were not statistically different among wooded landscape elements so values were averaged and compared to grassland values. Grassland and woodland C:N ratios were also not statistically different so values were averaged to obtain composite values for soil fractions (Fig. 8). The free light fraction, macro free POM, and macro-associated POM fractions (MciPOM, MfiPOM) had the highest C:N ratios (16–25). In contrast, microaggregates and microaggregate-associated fractions along with silt + clay fractions and whole soil had the lowest C:N ratios (10–13).

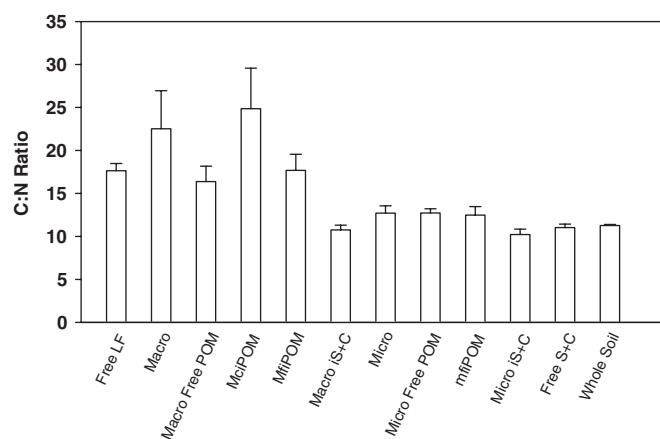


Fig. 8. Average C:N ratios of whole soil and soil fractions (0–15 cm soil depth) of remnant grassland and wooded landscape elements at LaCopita Research Area.

#### 4. Discussion

Whole-soil C and N concentrations increased by as much as five-fold following woody plant invasion of grassland (Figs. 3 and 4). In a system where low protection of organic matter was expected due to low silt+clay content, accumulation of organic matter resulted from a sustained increase in primary production relative to decomposition, and/or inputs of residues with high chemical resistance to decay (Baldock and Skjemstad, 2000). The accumulations of whole-soil C and N in woodlands are certainly a consequence of greater rates of primary production that has increased inputs through surface litter and roots in the wooded areas (Boutton et al., 1999; Archer et al., 2001; Hibbard et al., 2001). Organic matter inputs may also have been of lower quality due to greater lignin concentrations, tannins, and other plant secondary compounds associated with woody plant species (Horner et al., 1988; Enríquez et al., 1993; Gillon et al., 1994; Hobbie, 1996; Köchy and Wilson, 1997; Kraus et al., 2003). Increased aggregation resulting from greater organic matter inputs following woody plant proliferation may also affect C and N sequestration because the protection afforded by the aggregates can slow organic matter turnover. A direct relationship between aggregation and SOM content has been observed in soils where organic materials are the dominant aggregate stabilizing agents (Oades and Waters, 1991).

Allocation patterns of C and N to aggregate fractions did not change with time following woody plant invasion of grasslands. Proportions of C and N within macro- and microaggregate-sized fractions were not significantly altered with grassland-to-woodland conversion. However, alterations to soil structure were evidenced by the lower bulk densities in wooded areas relative to remnant grassland (Table 1) and by the changes in aggregate size distribution and aggregate-associated organic matter following woody plant invasion. Increases in free POM (external to aggregates) reflected the higher organic matter inputs from woody plants. More stable macroaggregates were also formed following woody plant encroachment probably as a consequence of greater organic matter inputs. Once woody plants establish in grasslands, the increase in macroaggregates and the simultaneous decrease in microaggregates suggests that either microaggregates are being bound into stable macroaggregates, or that the slowed turnover of macroaggregates minimizes the release of microaggregates formed inside of the macroaggregates (Fig. 2). The decline in free silt and clay following woody invasion is also likely a consequence of their incorporation into macroaggregated soil. However, despite the increase in macroaggregates following woody plant invasion of grassland, approximately half of the accumulated C and N occurred in free POM external to aggregates and was therefore not actually protected within aggregate structures. These increases in whole-soil C and N were probably sustained by greater inputs and relatively slower turnover

of POM, perhaps due to the biochemical recalcitrance of POM materials derived from woody vegetation or lack of sufficient nutrients and water for microbes to keep up with the additional inputs.

Although much of the C and N accumulation was in unprotected free POM fractions, the formation and stabilization of macroaggregates played an important role in the protection and subsequent accumulation of organic matter in this ecosystem. Woodlands contained a greater proportion of water-stable macroaggregates than remnant grasslands. Macroaggregates accounted for 30–70% of SOC accumulation rates and 5–30% of whole-soil N accumulation rates in woodlands. The greater formation of macroaggregates following grassland-to-woodland conversion may have been facilitated or mediated by the significantly greater root biomass (Boutton et al., 1998, 1999; Archer et al., 2001; Hibbard et al., 2001) and soil microbial biomass (McCulley et al., 2004) in wooded landscape elements compared to remnant grassland. Macroaggregates are held together by temporary binding agents such as plant roots and fungal hyphae, and by transient agents (e.g., polysaccharides) (Elliott, 1986; Oades and Waters, 1991). Because of the labile nature of the organic binding agents, macroaggregates are considered less stable than microaggregates that are bound together by more persistent biochemicals (Tisdall and Oades, 1982).

The relatively high C:N ratio (23) of macroaggregates in this study (Fig. 8) lends support to the idea that macroaggregates are held together by labile binding agents. Oades et al. (1987) suggested that organic matter with C:N ratios close to 20 are composed of plant materials in the early stages of decomposition. Buyanovsky et al. (1994) found that macroaggregates had higher C:N ratios than microaggregates, attributable to incompletely humified organic material, suggesting that active binding agents are root hairs and fungi. In contrast, they found lower C:N ratios in microaggregates, indicative of organic matter that was more highly processed and persistent (longer mean residence time) (Buyanovsky et al., 1994). Clay fractions also had narrower C:N ratios (10) (Oades and Waters, 1991). Other studies have indicated that the organic matter associated with macroaggregates and POM is less processed than that associated with microaggregates and silt and clay (Elliott, 1986; Oades et al., 1987; Beare et al., 1994; García-Oliva et al., 1994).

In this ecosystem, C and N concentrations in macroaggregates represented an important contribution to whole-soil C and N accumulation even though this pool generally does not exhibit long-term stability. For example, much of the POM associated with macroaggregates is lost upon cultivation (Beare et al., 1994). Others have shown that macroaggregates are sensitive to changes in SOM because these structures are dependent on live binding agents (Tisdall and Oades, 1979; Christensen, 2001; Six et al., 2004). Therefore, disturbance or any changes in land-use practices may cause rapid destruction of stable

macroaggregates and a decrease in associated soil C and N pools, particularly POM.

Turnover rates of organic matter in macroaggregates have been found to be variable ranging from a few years to decades (Skjemstad et al., 1990; Jastrow et al., 1996; Puget et al., 2000). It is generally agreed that protection afforded to organic matter in microaggregates is greater than that provided by macroaggregates (Krull et al., 2003). In this study, microaggregates accounted for approximately 50% of the total amount of protected C (C within aggregates and associated with silt + clay). The shift from grassland to woodland resulted in a shift in fractions responsible for C-protection. In grasslands, microaggregates and associations with free silt + clay accounted for most of the protected C. In woodlands, 85% of protected C is found in microaggregates plus macroaggregates. Macroaggregates may yet play a significant role in long-term organic matter storage and dynamics in this ecosystem since recent evidence suggests that microaggregates form within stable macroaggregates (Six et al., 2004). While macroaggregates may not afford long-term protection of organic matter, they may slow rates of decomposition of initial inputs and facilitate formation of stable microaggregate structures within macroaggregates (Gale et al., 2000; Six et al., 2002).

Although a larger proportion of the C and N is accumulating in the Free POM and macroaggregate-sized fractions, microaggregates still accounted for 10–40% of whole-soil C accumulation. Therefore, stabilization of C and N within microaggregates likely constitutes an important long-term sink for soil C and N in this ecosystem since the microaggregate sized fraction may have longer residence times as suggested by narrower C:N ratios.

It has been hypothesized that soils may have a finite capacity to protect organic matter from decay, and that the maximum protective capacity is related to the maximum amount of organic matter that can be associated with silt and clay particles (Hassink, 1997). Franzluebbers and Arshad (1996, 1997) showed that physical protection of organic matter within aggregates increased with increasing clay content. However, despite the low silt + clay content of the soils in this subtropical savanna parkland, accumulations of C and N have been linear over the past century, suggesting that these soils have not yet reached their maximum storage capacity.

## 5. Conclusions

Whole-soil C and N increased 100–500% during 130 yr of woodland development in areas that were once grassland. Potential causes of these increased pool sizes include higher rates of organic matter production in wooded areas, greater inherent biochemical resistance of woody litter to decomposition, and protection of organic matter by stabilization within soil macro- and microaggregates. Woody plant invasion of grassland resulted in increased formation of water-stable macroaggregates, likely as a consequence of greater above- and belowground produc-

tivity, which increased organic matter inputs to the soil. The increase in soil structural stability and SOM storage was also evident in decreased soil bulk density in wooded areas. The unprotected Free POM fraction accounted for approximately half of C and N accumulation in whole-soil. Carbon and N in macro- and microaggregates collectively accounted for the other half of C and N accumulation in whole soil. Although only a small proportion of the increase in C and N was found in free microaggregates (<10%) following woody plant invasion, this pool is known to be comprised of C and N that is relatively more stabilized than that stored in macroaggregates. However, if microaggregates are formed within macroaggregates as recent studies suggest, then stable macroaggregates may also contain an appreciable amount of C and N that is stabilized within constituent microaggregates. The results of this study suggest that grassland ecosystems undergoing woody plant invasion may be potentially large sinks for atmospheric CO<sub>2</sub> given the linear increases in soil C over periods of time exceeding 100 yr and the widespread geographic extent of this change in vegetation structure.

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