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# Soil microbial biomass response to woody plant invasion of grassland

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## Abstract

Woody plant proliferation in grasslands and savannas has been documented worldwide in recent history. To better understand the consequences of this vegetation change for the C-cycle, we measured soil microbial biomass carbon ( $C_{mic}$ ) in remnant grasslands (time 0) and woody plant stands ranging in age from 10 to 130 years in a subtropical ecosystem undergoing succession from grassland to woodlands dominated by N-fixing trees. We also determined the ratio of SMB-C to soil organic carbon ( $C_{mic}/C_{org}$ ) as an indicator of soil organic matter quality or availability, and the metabolic quotient ( $qCO_2$ ) as a measure of microbial efficiency. Soil organic carbon ( $C_{org}$ ) and soil total nitrogen (STN) increased up to 200% in the 0–15 cm depth increment following woody plant invasion of grassland, but changed little at 15–30 cm.  $C_{mic}$  at 0–15 cm increased linearly with time following woody plant encroachment and ranged from 400 mg C kg<sup>-1</sup> soil in remnant grasslands up to 600–1000 mg C kg<sup>-1</sup> soil in older (>60 years) woody plant stands.  $C_{mic}$  at 15–30 cm also increased linearly with time, ranging from 100 mg C kg<sup>-1</sup> soil in remnant grasslands to 400–700 mg C kg<sup>-1</sup> soil in older wooded areas. These changes in  $C_{mic}$  in wooded areas were correlated with concurrent changes in stores of C and N in soils, roots, and litter. The  $C_{mic}/C_{org}$  ratio at 0–15 cm decreased with increasing woody plant stand age from 6% in grasslands to <4% in older woodlands suggesting that woody litter may be less suitable as a microbial substrate compared with grassland litter. In addition, higher  $qCO_2$  values in woodlands ( $\geq 0.8$  mg CO<sub>2</sub>-C g<sup>-1</sup>  $C_{mic}$  h<sup>-1</sup>) relative to remnant grasslands (0.4 mg CO<sub>2</sub>-C g<sup>-1</sup>  $C_{mic}$  h<sup>-1</sup>) indicated that more respiration was required per unit of  $C_{mic}$  in wooded areas than in grasslands. Observed increases in  $C_{org}$  and STN following woody plant encroachment in this ecosystem may be a function of both greater inputs of poor quality C that is relatively resistant to decay, and the decreased ability of soil microbes to decompose this organic matter. We suggest that increases in the size and activity of  $C_{mic}$  following woody plant encroachment may result in: (a) alterations in competitive interactions and successional processes due to changes in nutrient dynamics, (b) enhanced formation and maintenance of soil physical structures that promote  $C_{org}$  sequestration, and/or (c) increased trace gas fluxes that have the potential to influence atmospheric chemistry and the climate system at regional to global scales.

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## 1. Introduction

Woody plant proliferation has been documented in many grass-dominated ecosystems throughout the world in recent history (Van Auken, 2000; Archer et al., 2001; Asner et al., 2004; Tape et al., 2006). This geographically extensive vegetation change is often attributed to land-use activities such as livestock grazing and fire suppression, but may also be related to changes in atmospheric composition, atmospheric deposition, and climate (Polley et al., 1996; Bond and Midgley, 2000; Archer et al., 2001;

Tape et al., 2006). Because plant species composition exerts strong control over soil organic matter storage and dynamics via the quantity and quality of litter inputs to soil (Dijkstra et al., 2006; Russell et al., 2007), woody plant encroachment has a strong potential to modify key C-cycle processes at ecosystem and global scales (Schlesinger et al., 1990; Asner and Martin, 2004).

In the Rio Grande Plains of Southern Texas, subtropical woodlands dominated by N-fixing tree legumes have largely replaced grassland over the last century primarily as a result of livestock grazing and fire suppression (Archer et al., 1988; Boutton et al., 1998). Previous studies in this region have documented significant increases in above- and below- ground primary productivity (Archer et al., 2001;

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Hibbard et al., 2001) and in soil C and N storage (McCulley et al., 2004; Liao et al., 2006; Boutton et al., 2008) following woody plant proliferation in areas that were once grassland.

These changes in rates of organic matter input to the soil which have resulted in increased soil C and N storage are likely to influence the pool size and activity of the soil microbial biomass ( $C_{mic}$ ). Since soil microbes are often limited by C and/or N (Wardle, 1992; Zak et al., 1994; Kaye and Hart, 1997), increased C and N input could provide more resources to support a larger  $C_{mic}$  pool. On the other hand, the transition from grassland to woodland may result in lower-quality organic matter inputs that are resistant to biological decay, thereby supporting less  $C_{mic}$ . Woody plants are generally considered to be more decay-resistant as a result of lignins, tannins, and other secondary compounds that are either absent or present in lower concentrations in herbaceous grassland plants (Horner et al., 1988; Hobbie, 1996; Kraus et al., 2003). Furthermore, changes in the quantity and/or quality of organic matter inputs may shift the composition of the soil microbial community towards organisms that are either more or less efficient at converting organic substrates into  $C_{mic}$ , with potential consequences for the size of  $C_{mic}$  pool.

As a first step towards evaluating these scenarios, we quantified soil microbial biomass carbon ( $C_{mic}$ ), the ratio of  $C_{mic}$  to total  $C_{org}$  ( $C_{mic}/C_{org}$ ), and the metabolic quotient or  $qCO_2$  (respiration rate per unit of  $C_{mic}$ ) in soils from the Rio Grande Plains where grasslands have been invaded by woody plants. We utilized a chronosequence approach in which remnant grasslands represented time before woody invasion (time 0), and woody plant stands ranging in age from 10 to 130 years represented time following woody encroachment. We hypothesized that: (1) increased soil C and N content would result in increased  $C_{mic}$  with time following woody plant encroachment; (2) the  $C_{mic}/C_{org}$  ratio would decrease with increasing woody plant stand age due to the inherently more recalcitrant nature of woody plant litter compared with herbaceous litter; and (3) the  $qCO_2$  would increase with time after tree/shrub invasion reflecting lower microbial efficiency in processing low-quality woody plant litter.

## 2. Materials and methods

### 2.1. Study area

Soils were collected from the Texas Agricultural Experiment Station LaCopita Research Area (27°40'N; 98°12'W) located 65 km West of Corpus Christi, Texas in the Eastern Rio Grande Plains of the Tamaulipan Biotic Province. Climate is subtropical, with a mean annual precipitation of 715 mm (bimodally distributed) and a mean annual temperature of 22.4 °C. Topography consists of nearly level uplands which grade (1–3%) into lower-lying drainage woodlands and playas. The elevation ranges from 75 to 90 m.

Originally classified as a *Prosopis–Acacia–Andropogon–Setaria* savanna (Kuchler, 1964), significant woody plant expansion in recent history has resulted in a landscape largely dominated by subtropical thorn woodland. Over the past 100–200 years, this area was heavily grazed by domestic livestock, which has been identified as a causal factor in succession from grassland to woodland (Archer et al., 1988).

Upland soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface ( $B_t$ ) horizon with non-argillic inclusions, and are characterized by a two-phase vegetation pattern consisting of discrete clusters of woody vegetation embedded within a grassland matrix. Species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis* dominate the grasslands. Formation of clusters is initiated when grasslands are colonized by *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite, an N-fixing tree legume), which then facilitates recruitment of other woody plant species in the understory (Archer et al., 1988). Where the argillic horizon is absent, clusters expand laterally and fuse to form larger groves of woody vegetation (Archer, 1995). Soils in lower-lying drainage areas have clay loam soils (Pachic Argiustolls) and are characterized by continuous closed-canopy drainage woodlands. These drainage woodlands appear to have originated from the same successional processes currently underway in uplands, and their vegetation composition is similar to that in upland clusters and groves. *P. glandulosa* is the dominant species in all wooded landscape elements with *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory.

### 2.2. Chronosequence approach

To quantify changes in soil microbial biomass following woody plant invasion of areas that were formerly grasslands, a chronosequence approach was utilized. Within each upland landscape element (grasslands, clusters, groves), 10 sites were sampled; eleven sites were sampled in the lower-lying drainage woodlands. All sites were located within an area of approximately 2 km<sup>2</sup>. To characterize  $C_{mic}$  at time zero (i.e. prior to woody encroachment), soils were sampled in remnant grasslands. Clusters, groves, and drainage woodland landscape elements were sampled to evaluate changes in  $C_{mic}$  at known points in time following woody plant encroachment.

The age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand since the formation of these wooded landscape elements is initiated only after the establishment of *P. glandulosa* in grassland (Archer et al., 1988). Therefore, ages of *P. glandulosa* trees were determined by measuring their basal diameters, and then using those values to predict tree ages based on regressions between basal diameter and tree age; equations specific to each wooded landscape element were developed previously (Stoker, 1997). In this study, the woody plant

stands sampled were selected to encompass a wide range of *P. glandulosa* basal diameters and corresponded to tree ages ranging from approximately 10 to 130 years.

### 2.3. Collection and analyses of litter, roots, and soil

At each site, all surface litter within a 0.25 m<sup>2</sup> quadrat was collected. In wooded landscape elements, the quadrat was located within 0.5 m of the bole of the largest *P. glandulosa* tree. To remove adhering soil particles, litter samples were washed with deionized water over a 2 mm sieve, oven-dried at 60 °C to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and retained for elemental analyses.

At each site within the wooded landscape elements, 4 soil cores (5 cm diameter × 30 cm depth) were taken beneath the largest *P. glandulosa*, one in each cardinal direction from the bole. Sampling in remnant grassland sites was identical, but cores were taken around the base of a large perennial C<sub>4</sub> grass plant instead. Prior to soil coring, soil surface litter was gently removed to expose the mineral soil. Each soil core was sectioned into 2 depth increments (0–15 and 15–30 cm) and stored at 4 °C. In the lab, soils were thoroughly mixed to homogenize the samples. A subsample of each soil was dried at 105 °C to determine bulk density. The four soil cores from each site were pooled by depth increment and again thoroughly mixed.

An aliquot (approximately 25%) of each soil sample was set aside for determination of root biomass prior to subsequent analyses. For determination of physical and chemical properties, another aliquot was passed through a 2 mm sieve to remove large organic fragments. Soil pH was determined on a 1:2 (soil:water) mixture using a glass electrode (McLean, 1982). Soil texture was determined by the pipet method (Sheldrick and Wang, 1993). The remainder of the sieved aliquot was oven-dried at 60 °C, pulverized in a centrifugal mill, and used for elemental analyses.

Root biomass was quantified on well-mixed, aliquots (100 g) from each pooled soil sample using a hydropneumatic elutriation system (Smucker et al., 1982) (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410 µm screen. Roots were then dried at 60 °C, weighed, pulverized with a mortar and pestle, and saved for elemental analyses. C and N concentrations of litter, roots, and soil were determined on a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ).

An additional subsample of each soil was sieved <4 mm for determination of C<sub>mic</sub> by the chloroform fumigation–incubation method (Jenkinson and Powlson, 1976; Horwath and Paul, 1994) using a *k<sub>c</sub>* value of 0.41 (Anderson and Domsch, 1978; Voroney and Paul, 1984). C<sub>mic</sub> values are presented without the subtraction of an unfumigated control because this approach has been shown to yield estimates of C<sub>mic</sub> that are more highly correlated with rates of key microbial processes (such as C- and N-mineralization) and with potential microbial resources (such as C<sub>org</sub>

and soil total N (STN)), and is more responsive to land use/land cover changes than C<sub>mic</sub> estimates derived via subtraction of a control (Franzuebbers et al., 1999a, b).

### 2.4. Statistical analyses

ANOVA (NCSS, 1995) was used to analyze soil physical and chemical characteristics, C<sub>mic</sub>, C<sub>mic</sub>/C<sub>org</sub>, and *q*CO<sub>2</sub> for potential differences due to landscape element and soil depth. Fisher's LSD method was used to identify significant differences among measurements due to the main effect of landscape element within each soil depth increment following ANOVA. Regression analyses were performed using Sigma Plot 4.0 (SPSS, 1997) to determine significance of relationships between C<sub>mic</sub> and soil characteristics (C<sub>org</sub>, STN, litter C and N, and root C and N). Regression analyses were also utilized to determine the significance of relationships between woody plant stand age and C<sub>mic</sub>, C<sub>mic</sub>/C<sub>org</sub>, and *q*CO<sub>2</sub>.

## 3. Results

### 3.1. Chemical and physical characteristics of soil, roots, and litter

Soil pH in the upper 0–15 cm of the soil profile was 6.5 in remnant grasslands and was not significantly altered following woody plant establishment in grasslands (Table 1). Bulk density in the 0–15 cm depth increment decreased significantly from 1.2 in remnant grasslands to 1.0 in wooded landscape elements (clusters, groves, and drainage woodlands), but did not change at 15–30 cm in response to woody encroachment. In upland grasslands, clusters, and groves, particle size distribution was approximately 80% sand, 10% silt, and 10% clay in surface soils (0–15 cm). In contrast, the particle size distribution of lower-lying drainage woodlands was 60% sand, 20% silt, and 20% clay (Table 1). C<sub>org</sub> and STN in wooded landscape elements increased up to 200% relative to remnant grassland (Table 1). C/N ratios of whole soil (0–15 and 15–30 cm depth increments) ranged from 10 to 13.

Pool sizes of C and N in roots were significantly greater in all wooded areas relative to grasslands in both the 0–15 and 15–30 cm depth increments (Table 1). Litter C stores were significantly greater in groves compared with all other landscape elements, while litter N stores were significantly greater in groves and drainage woodlands compared with grasslands (Table 1).

### 3.2. Changes in C<sub>mic</sub> following woody plant invasion of grassland

There was a significant interaction between landscape element and soil depth that influenced C<sub>mic</sub> (Fig. 1). This interaction appears to be due to much greater differences in C<sub>mic</sub> between remnant grasslands vs. wooded areas at the 15–30 cm depth than at the 0–15 cm depth. In general,



Table 1  
Characteristics of soils, roots, and litter in grasslands and wooded landscape elements

|   | 0–15 cm                  |                           |                           |                           | 15–30 cm                 |                          |                          |                           |
|---|--------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
|   | Grassland                | Cluster                   | Grove                     | Drainage                  | Grassland                | Cluster                  | Grove                    | Drainage                  |
| Soil 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>b</sup>   | 6.4 (0.1) <sup>b</sup>   | 6.7 (0.1) <sup>a</sup>   | 6.3 (0.1) <sup>b</sup>    |
| 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>   |
| Soil bulk density (g cm <sup>-3</sup> ) | 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>   |
| Soil organic C (g C m <sup>-2</sup> )   | 1050 (43) <sup>c</sup>   | 1715 (172) <sup>bc</sup>  | 2062 (207) <sup>b</sup>   | 2981 (350) <sup>a</sup>   | 994 (75) <sup>b</sup>    | 838 (52) <sup>b</sup>    | 1054 (54) <sup>b</sup>   | 2121 (228) <sup>a</sup>   |
| Soil total N (g N m <sup>-2</sup> )     | 94.2 (2.8) <sup>c</sup>  | 157.8 (13.4) <sup>b</sup> | 179.9 (14.9) <sup>b</sup> | 256.7 (30.0) <sup>a</sup> | 87.1 (5.1) <sup>b</sup>  | 80.5 (4.6) <sup>b</sup>  | 100.7 (5.4) <sup>b</sup> | 157.3 (13.2) <sup>a</sup> |
| Soil C/N                                | 11.3 (0.2) <sup>ab</sup> | 10.8 (0.2) <sup>b</sup>   | 11.3 (0.3) <sup>ab</sup>  | 11.6 (0.2) <sup>a</sup>   | 11.1 (0.4) <sup>b</sup>  | 10.4 (0.2) <sup>c</sup>  | 10.4 (0.2) <sup>c</sup>  | 13.3 (0.4) <sup>a</sup>   |
| Root C (g C m <sup>-2</sup> )           | 164 (16) <sup>b</sup>    | 642 (162) <sup>a</sup>    | 666 (79) <sup>a</sup>     | 837 (81) <sup>a</sup>     | 161 (30) <sup>c</sup>    | 433 (56) <sup>b</sup>    | 451 (30) <sup>ab</sup>   | 702 (159) <sup>a</sup>    |
| Root N (g N m <sup>-2</sup> )           | 4.1 (0.5) <sup>b</sup>   | 34.2 (7.8) <sup>a</sup>   | 35.3 (4.9) <sup>a</sup>   | 48.9 (6.9) <sup>a</sup>   | 4.1 (0.7) <sup>c</sup>   | 15.5 (2.1) <sup>b</sup>  | 18.5 (1.9) <sup>b</sup>  | 30.6 (7.3) <sup>a</sup>   |
| Litter C (g C m <sup>-2</sup> )         | 106 (18) <sup>b</sup>    | 137 (40) <sup>b</sup>     | 322 (37) <sup>a</sup>     | 175 (40) <sup>b</sup>     |                          |                          |                          |                           |
| Litter N (g N m <sup>-2</sup> )         | 2.4 (0.4) <sup>c</sup>   | 5.8 (1.8) <sup>bc</sup>   | 14.1 (1.8) <sup>a</sup>   | 7.8 (1.8) <sup>b</sup>    |                          |                          |                          |                           |

Values are means with standard errors in parentheses ( $n = 10$  or  $11$ ). Fisher's LSD was used to separate differences between landscape elements within a depth interval following ANOVA. Different letters represent significant differences between means within a row for each depth interval.

$C_{mic}$  (mg C kg<sup>-1</sup> soil) showed a trend towards higher values in the wooded landscape elements relative to remnant grassland across both soil depth increments (Fig. 1).  $C_{mic}$  increased 75% in drainage woodlands and 25% in groves relative to remnant grassland in the upper 15 cm of the profile. However,  $C_{mic}$  in clusters (0–15 cm) was not significantly greater than the remnant grassland (Fig. 1).  $C_{mic}$  estimates in all wooded landscape elements were significantly greater (300–400%) than grassland  $C_{mic}$  at the 15–30 cm depth (Fig. 1).

Relative to woody plant stand age,  $C_{mic}$  in the surface 0–15 cm increased linearly from approximately 400 mg C kg<sup>-1</sup> soil in woody plant stands <30 years old up to as high as 900 mg C kg<sup>-1</sup> soil in woody plant stands >60 years (Fig. 2).  $C_{mic}$  at 15–30 cm in the profile followed a similar pattern to  $C_{mic}$  in surface soils, increasing from approximately 100 mg C kg<sup>-1</sup> soil in younger woody plant stands (<30 years) up to 500–700 mg C kg<sup>-1</sup> soil in older woodlands (>60 years) (Fig. 2). Rates of accumulation of  $C_{mic}$  ranged from 1.6 to 5.4 mg C kg<sup>-1</sup> soil year<sup>-1</sup> at 0–15 cm, and from 1.6 to 2.9 mg C kg<sup>-1</sup> soil year<sup>-1</sup> at 15–30 cm (Fig. 2).

When  $C_{mic}$  values were calculated by subtracting an unfumigated control, values for  $C_{mic}$  were approximately 25% lower than those when controls were not subtracted. In addition, differences in  $C_{mic}$  between remnant grasslands vs. wooded landscape elements were greatly reduced, and there was no relationship between  $C_{mic}$  and the time following woody plant encroachment (data not shown).

### 3.3. Relationships between $C_{mic}$ and characteristics of the plant–soil system

$C_{mic}$  in cluster and drainage woodland soils (0–15 cm) was significantly correlated with  $C_{org}$ , STN, litter C and N, and root C and N, with correlation coefficients ranging from 0.70 to 0.96 (Table 2). In grove soils (0–15 cm), only

$C_{org}$  ( $r = 0.79$ ), STN ( $r = 0.71$ ), and root N ( $r = 0.60$ ) were significantly correlated with  $C_{mic}$ .  $C_{mic}$  in grassland soils (0–15 cm) was not significantly correlated with any of the measured characteristics, likely due to the much lower variability in soil, litter, and root C and N pools in grasslands than in wooded landscape elements (Table 1).

At the 15–30 cm depth, correlations of  $C_{mic}$  with characteristics of the plant–soil system were variable. In grasslands and drainage woodlands (15–30 cm)  $C_{mic}$  was significantly correlated with  $C_{org}$  and STN. In contrast,  $C_{mic}$  in clusters at depth was correlated only with STN. In grove soils at 15–30 cm, litter and root C and N concentrations were significantly correlated with  $C_{mic}$ , whereas  $C_{org}$  and STN were not.

Estimates of  $C_{mic}$  from grassland and woodland landscape elements computed by subtracting an unfumigated control were not significantly correlated with any of the characteristics of the plant–soil system included in Table 2 (data not shown).

### 3.4. Changes in $C_{mic}/C_{org}$ ratio following woody plant invasion of grassland

There was a significant interaction between landscape element and soil depth for the  $C_{mic}/C_{org}$  ratio (Fig. 1). The  $C_{mic}/C_{org}$  ratio of grassland soils in the surface 0–15 cm of the profile was approximately 6% and was 1.5 times greater than  $C_{mic}/C_{org}$  ratios of wooded landscape elements (4%). At 15–30 cm, the  $C_{mic}/C_{org}$  ratios for cluster and grove soils (9%) were higher than those of both grasslands (3%) and drainage woodlands (4.5%).

With increasing woody plant stand age, the  $C_{mic}/C_{org}$  ratios of grove and drainage woodland soils (0–15 cm) decreased linearly from 6% in woody plant stands <30 years old to less than 4% in wooded landscape elements >60 years. This relationship was not significant in soils from woody clusters. At the 15–30 cm depth, there was no

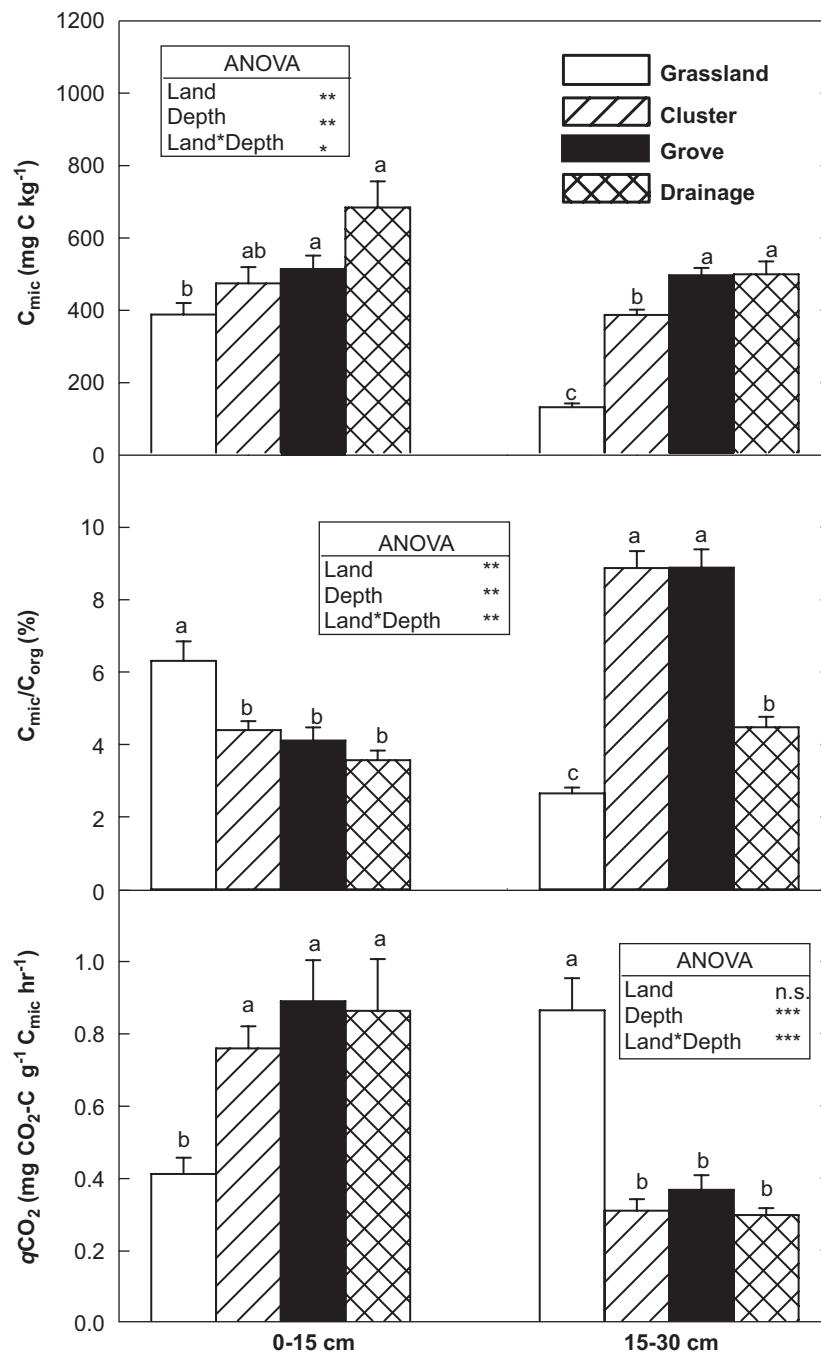


Fig. 1.  $C_{mic}$ ,  $C_{mic}/C_{org}$ , and  $qCO_2$  of soils in different landscape elements at LaCopita Research Area by soil depth (0–15 and 15–30 cm). Error bars are standard errors of the mean ( $n = 10$  or  $11$ ). In the ANOVA tables, Land = landscape element, and asterisks indicate degree of significance ( $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ ). Different letters above bars indicate significant differences among means within a depth from Fisher's LSD.

correlation between  $C_{mic}/C_{org}$  ratios and stand age in any of the wooded landscape elements (Fig. 2).

### 3.5. Changes in $qCO_2$ following woody plant invasion of grassland

There was a significant interaction between landscape element and soil depth for  $qCO_2$  (Fig. 1). This interaction occurred because  $qCO_2$  was higher in all of the wooded landscape elements than in grasslands at 0–15 cm depth,

but the reverse was true at 15–30 cm. The  $qCO_2$  was up to 125% higher in wooded landscape elements relative to remnant grassland in the upper 15 cm of the profile (Fig. 1). At 15–30 cm, the trend was reversed and woodland  $qCO_2$  values were significantly lower (200%) than grassland values. Values for clusters, groves, and drainage woodlands were similar within each depth increment (Fig. 1).

With time following woody plant invasion of grassland,  $qCO_2$  increased linearly in the upper 15 cm of the profile from  $0.4 \text{ mg CO}_2\text{-C g}^{-1} \text{ C}_{mic} \text{ h}^{-1}$  in grasslands to 1.2 to

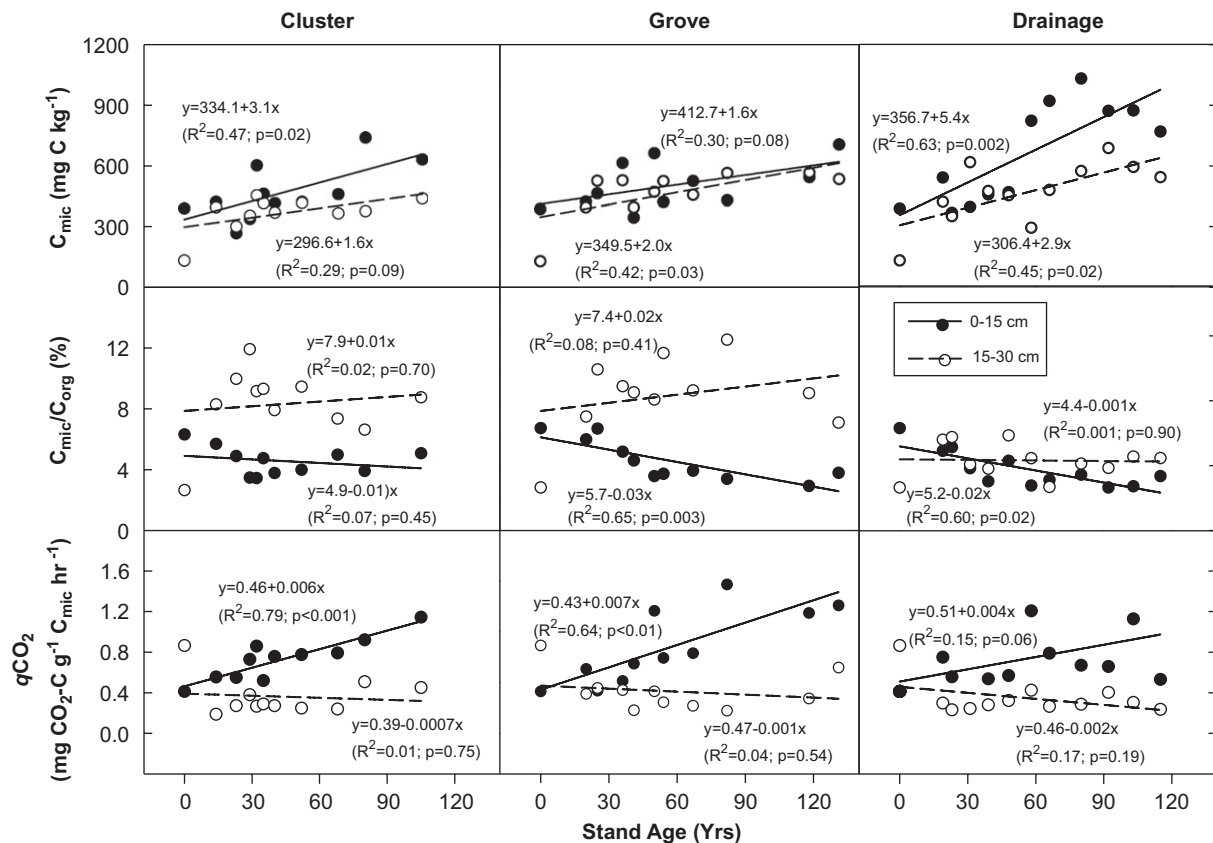


Fig. 2. Changes in  $C_{mic}$ ,  $C_{mic}/C_{org}$ , and  $qCO_2$  in soils (0–15 cm and 15–30 cm) with respect to woody plant stand age in landscape elements at LaCopita Research Area. In each frame, grassland values are at time 0 and represent baseline starting values.

Table 2  
Correlation coefficients ( $r$ ) between  $C_{mic}$ -C and pool sizes of C and N (g m<sup>-2</sup>) in soil, litter, and roots

|           | $C_{mic}$      |       |              |       |            |        |               |         |
|-----------|----------------|-------|--------------|-------|------------|--------|---------------|---------|
|           | Grassland (cm) |       | Cluster (cm) |       | Grove (cm) |        | Drainage (cm) |         |
|           | 0–15           | 15–30 | 0–15         | 15–30 | 0–15       | 15–30  | 0–15          | 15–30   |
| $C_{org}$ | 0.21           | 0.67* | 0.89***      | 0.55  | 0.79**     | 0.26   | 0.95***       | 0.79**  |
| STN       | 0.25           | 0.38* | 0.89***      | 0.69* | 0.71*      | 0.38   | 0.96***       | 0.88*** |
| Litter C  | 0.14           | 0.34  | 0.70*        | 0.11  | 0.58       | 0.78** | 0.86***       | 0.38    |
| Litter N  | 0.01           | 0.27  | 0.71*        | 0.18  | 0.53       | 0.79** | 0.87***       | 0.41    |
| Root C    | 0.23           | 0.38  | 0.81**       | 0.59  | 0.47       | 0.85** | 0.79**        | 0.57    |
| Root N    | 0.55           | 0.41  | 0.84**       | 0.54  | 0.60*      | 0.76** | 0.93***       | 0.50    |

Asterisks indicate degree of significance ( $p < 0.05^*$ ,  $p < 0.01^{**}$ ,  $p < 0.001^{***}$ ).

1.5 mg CO<sub>2</sub>-C g C<sub>mic</sub><sup>-1</sup> h<sup>-1</sup> in older clusters, groves, and drainage woodlands. At 15–30 cm, there was no significant correlation between  $qCO_2$  and time following woody plant invasion of grassland (Fig. 2).

## 4. Discussion

### 4.1. Soil C and N storage following woody encroachment

The results of this study and others at the same site (Hibbard et al., 2001; Archer et al., 2004; McCulley et al.,

2004; Liao et al., 2006; Boutton et al., 2008) indicate that woody plant invasion has altered soil chemical and physical properties and resulted in substantial increases in  $C_{org}$  and STN beneath woody plant canopies over the past century. Increases of up to 200% for  $C_{org}$  and STN in wooded landscape elements relative to remnant grassland in this study (Table 1) are almost certainly a direct consequence of higher rates of net primary productivity (NPP) and N-fixation in wooded landscape elements relative to remnant grasslands. Rates of above-ground NPP in remnant grasslands are approximately

1.9–3.4 Mg ha<sup>-1</sup> year<sup>-1</sup>; in contrast, rates of above-ground NPP in areas currently dominated by woody vegetation are approximately 5.1–6.0 Mg ha<sup>-1</sup> year<sup>-1</sup> (Archer et al., 2001; Hibbard et al., 2001). Although rates of belowground NPP have not been quantified, coarse and fine root biomass are 2–5 times greater and show significantly larger seasonal fluctuations in wooded areas than remnant grasslands (Boutton et al., 1998, 1999; Hibbard et al., 2001), suggesting that belowground productivity is also significantly accelerated following woody plant establishment in grasslands. Plant productivity has been cited as the most important process influencing organic matter accumulation and soil microbial biomass dynamics (Zak et al., 1990).

Additionally, woody species that invade grasslands are often capable of symbiotic N-fixation (Virginia and Jarrell, 1983; Johnson and Mayeux, 1990; Stock et al., 1995; Zitzer et al., 1996). In our study area, Zitzer et al. (1996) showed that six dominant tree legumes present in wooded landscape elements produced active root nodules with nitrogenase activity, but did not quantify ecosystem-level rates of N-fixation. However, rates of N-fixation in other comparable mesquite woodlands in the southwestern USA have been estimated to range from 4–15 g N m<sup>-2</sup> year<sup>-1</sup> (Rundel et al., 1982; Johnson and Mayeux, 1990). The addition of fixed-N undoubtedly stimulates primary productivity in this ecosystem, likely accounting for some of the observed increases in NPP, C<sub>org</sub>, and STN following woody plant encroachment into grassland.

#### 4.2. Soil microbial biomass carbon

As hypothesized, these accumulations of C<sub>org</sub> and STN in wooded landscape elements were associated with greater C<sub>mic</sub> beneath woody plant canopies. This pattern of higher C<sub>mic</sub> in wooded areas relative to grassland is consistent with results from other savanna and shrubland ecosystems around the world (Belsky et al., 1989; Kirchmann and Eklund, 1994; Kieft et al., 1998; Reyes-Reyes et al., 2002). Significant correlations between C<sub>mic</sub> and C and N stores in soil, litter, and roots suggest strongly that linear increases in C<sub>mic</sub> with time following woody encroachment are related to concomitant increases in inputs and stores of C and N beneath woody canopies. C<sub>mic</sub> is often highly correlated with C<sub>org</sub> content at ecosystem (Insam and Domsch, 1988; Insam et al., 1989) and continental scales (Zak et al., 1994; Franzluebbers et al., 2001; Booth et al., 2005) reflecting the strong dependence of C<sub>mic</sub> on plant productivity and organic C inputs. Changes in C<sub>mic</sub> observed in this study could also be influenced by possible microclimatic modifications induced by woody plant cover, such as increases in soil moisture due to reduced evaporation or hydraulic redistribution, and reductions in soil temperature due to increased shading (Belsky et al., 1989; Kirchmann and Eklund, 1994; Kieft et al., 1998; Reyes-Reyes et al., 2002; Zou et al., 2005).

#### 4.3. Significance of changes in C<sub>mic</sub>/C<sub>org</sub> and qCO<sub>2</sub>

Although organic matter inputs and soil C and N storage have increased following grassland-to-woodland conversion, little is known regarding the quality of organic matter inputs under grassland vs. wooded areas. The C<sub>mic</sub>/C<sub>org</sub> and qCO<sub>2</sub> are indices of microbial activity that might be helpful in assessing the quality of the C that has accrued over the past century following woody plant invasion of grassland, and in assessing possible mechanism(s) of C sequestration in this ecosystem (Sparling, 1992; Wardle and Ghani, 1995; Webster et al., 2001; Anderson, 2003).

We hypothesized that the C<sub>mic</sub>/C<sub>org</sub> ratio would decrease with increasing woody plant stand age due to the inherently more recalcitrant nature of woody litter compared with herbaceous litter. At 0–15 cm, the pattern of decreasing C<sub>mic</sub>/C<sub>org</sub> ratios with time following woody plant invasion (Figs. 1 and 2) supported our hypothesis and suggested a decrease in the relative availability of organic matter inputs with time (i.e. less microbial biomass is supported per unit C<sub>org</sub> with time following woody invasion).

Two lines of evidence suggest that C<sub>org</sub> becomes more decay-resistant as a result of woody encroachment. First, lab and field studies of soil respiration at this site indicate longer mean residence times for C<sub>org</sub> and larger pools of resistant C in wooded areas than in grasslands (Boutton et al., 2002; McCulley et al., 2004), suggesting that a larger proportion of woodland-derived C inputs is resistant to decomposition. Second, litter and roots in wooded areas contain 4–7 × higher concentrations of aliphatic biopolymers (primarily cutin and suberin) than those in grasslands (Boutton et al., 2008). These aliphatic biopolymers comprise a particularly important subset of recalcitrant material in soils (e.g. Nierop, 2001; Nierop and Verstraten, 2003; Mikutta et al., 2006), and their greater abundance provides evidence that organic matter inputs in wooded areas are biochemically more resistant to decomposition than those in grassland. In addition, relatively labile cinnamyl subunits comprised 16–38% of total lignin phenols in litter and roots of grasslands, but only 5–8% of lignin phenols in wooded areas (Boutton et al., 2008). Collectively, these prior studies on respiration kinetics and organic matter chemistry support the notion that lower C<sub>mic</sub>/C<sub>org</sub> ratios in wooded areas are due to poorer quality organic matter inputs and stores that are less accessible to serve as substrate for the production of microbial biomass.

We also hypothesized that qCO<sub>2</sub> would increase with time following woody plant invasion of grassland reflecting lower microbial efficiency in processing low-quality woody litter. In fact, qCO<sub>2</sub> increased with woody plant stand age at 0–15 cm. Hence, more respiration was required to sustain a unit of microbial biomass in wooded areas. The reduced efficiency of soil microbes in wooded areas may be another manifestation of the lower quality of organic matter inputs derived from woody plants. For example, microbial efficiency of utilization of lignin-C for microbial



biomass production is low, with most of the carbon being evolved as  $\text{CO}_2$  (reflected by a higher  $q\text{CO}_2$ ) or incorporated into soil organic matter (Wagner and Wolf, 2005). The higher  $q\text{CO}_2$  values in woodlands relative to grassland may also indicate a shift towards a less efficient microbial community composition, and studies of microbial community composition in this landscape are needed to evaluate this possibility.

At 15–30 cm, there was no relationship between either  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  or  $q\text{CO}_2$  vs. time following woody encroachment (Fig. 2), although  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  was generally greater and  $q\text{CO}_2$  was generally smaller in wooded areas vs. grasslands (Fig. 1). At this greater depth, soil organisms are potentially more isolated from aboveground litter inputs, and likely more dependent on roots for carbon substrate. However, roots are recognized as more resistant to decay than aboveground litter (Rasse et al., 2005) and would be unlikely to cause the patterns in  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  and  $q\text{CO}_2$  observed at 15–30 cm. Instead, we speculate that patterns of  $q\text{CO}_2$  and  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  values at 15–30 cm may be driven by: (a) hydrologic transport of relatively labile dissolved organic matter from the litter layer into deeper soil layers, and/or (b) larger quantities of labile root exudates in wooded areas due to the much larger root masses that occur there (Hibbard et al., 2001; Boutton et al., 2008).

#### 4.4. Potential significance of a larger soil microbial biomass pool

Changes in the size of the  $\text{C}_{\text{mic}}$  pool following woody plant encroachment could have implications at both ecosystem and global scales. Previous field studies at this study site have shown that soil respiration and net N-mineralization are greater in wooded areas than in remnant grasslands (Hibbard et al., 2001; McCulley et al., 2004). Thus, the larger pool sizes of  $\text{C}_{\text{mic}}$  observed in this study and in McCulley et al. (2004) are in fact accompanied by higher rates of key processes in the C- and N-cycles. Higher absolute rates of C and N mineralization should enhance soil nutrient availability, thereby altering plant–microbe and plant–plant interactions, and potentially influencing competitive interactions and successional dynamics in this ecosystem.

The size and activity of the  $\text{C}_{\text{mic}}$  pool could also influence soil structure. Microbial secretions and residues are critical in the formation and stabilization of soil aggregates (Tisdall and Oades, 1982; Oades and Waters, 1991; Jastrow and Miller, 1998), which are important in the protection and storage of soil organic matter (Christensen, 2001; Six et al., 2002). In fact, Liao et al. (2006) showed that the mass of water stable aggregates increased substantially following woody plant encroachment, and that approximately 50% of the new  $\text{C}_{\text{org}}$  accrued following woody encroachment was protected within macroaggregates, microaggregates, and silt/clay organomineral complexes. Hence, the larger pool of  $\text{C}_{\text{mic}}$  in wooded areas may be playing an important role in the formation and maintenance of soil physical

structures that promote the sequestration of  $\text{C}_{\text{org}}$  in this system.

Finally, the larger pools of  $\text{C}_{\text{mic}}$  in wooded areas could also result in higher rates of trace gas fluxes from microbial processes (Lloyd, 1995). For example, it has been shown that  $\text{NO}_x$  fluxes (Archer et al., 2001) are approximately  $15\times$  greater from soils in wooded areas than in remnant grasslands in this study area.  $\text{NO}_x$  is an important reactant in the formation of tropospheric  $\text{O}_3$ , which is an important air pollutant and a climatically active trace gas. If our observations on  $\text{C}_{\text{mic}}$  are representative of other ecosystems around the world where woody encroachment has occurred, then woody encroachment into grasslands is an important phenomenon that can alter trace gas fluxes at the ecosystem scale that then have the potential to influence atmospheric chemistry and the climate system at regional and perhaps even global scales (Schlesinger et al., 1990; Asner et al., 2004).

## 5. Conclusions

The absolute size of the  $\text{C}_{\text{mic}}$  pool increased with time following woody plant invasion of grassland. The increases in  $\text{C}_{\text{mic}}$  are likely due to the greater above- and below-ground productivity and larger pools of  $\text{C}_{\text{org}}$  and STN in wooded areas relative to remnant grassland.  $\text{C}_{\text{mic}}$  was generally correlated with C and N stores in litter, roots, and soils, indicating that microbes may have been C- and/or N-limited prior to woody plant proliferation in this dryland ecosystem. Although absolute values of  $\text{C}_{\text{mic}}$  increased following the woody plant invasion of grassland, simultaneous linear decreases in the  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  ratio with time indicate that the quality of the C available to microbes may have decreased over time as less microbial biomass is supported per unit of  $\text{C}_{\text{org}}$ . Increases in  $q\text{CO}_2$  with time following woody invasion also suggest that organic matter quality may be reduced since microbes in wooded areas appear to be less efficient in converting available C to biomass. Therefore, the observed increases in  $\text{C}_{\text{org}}$  and STN following woody plant encroachment in this ecosystem may be a function of both greater inputs of poor quality C that is relatively resistant to decay, and the decreased ability of soil microbes to decompose this organic matter.

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