

# Conversion of Tallgrass Prairie to Woodland: Consequences for Carbon and Nitrogen Cycling

R. L. MCCULLEY<sup>1</sup>

*Department of Biology, Duke University, Durham, North Carolina 27708*

AND

R. B. JACKSON

*Nicholas School of the Environment and Earth Sciences, Duke University, Durham, North Carolina 27708*

**ABSTRACT.**—Woody plant encroachment and proliferation in grasslands is occurring worldwide and has significant but variable effects on ecosystem processes, including primary production, litter decomposition, and N cycling. In mesic systems, recent reports suggest aboveground net primary production (ANPP) is stimulated by grass-to-woodland conversion; however, significant losses of soil C may accompany this land cover change in these environments. This study aimed to quantify how changes in plant production inputs (both above- and belowground), litter decomposition, and N cycling may feed back to reduce soil C pools in a mesic tallgrass prairie in central Texas, USA, that has undergone grass-to-woodland conversion over the past 60–70 y. Belowground net primary production (BNPP) in woodland was half that of adjacent grassland, and contrary to recent reports, no significant differences in ANPP between the two vegetation types were observed. Decomposition rates of aboveground woody and grass material were surprisingly similar. However, higher N concentrations in woodland leaves and stems meant woodland received more than 3× the amount of N via litterfall than adjacent grassland, and woody litter mineralized N when decomposing, while grassland litter tended to retain it. Losses of soil C accompanying grass-to-woodland conversion at this site (woodland soil C was ~20% less than that of adjacent grassland) may be the result of both reduced BNPP inputs to the soil C pool and increased rates of N cycling stimulating soil organic matter decomposition. Given that the processes controlling whether grasslands accrue or lose C and N when they are replaced by woody species vary across time and depend on the species involved and the biotic and edaphic conditions and management history of the site, more complex models that incorporate these parameters may be required to understand and predict when gains and losses of C will accompany vegetation change.

## INTRODUCTION

Worldwide, grassland is being converted to woodland via either direct planting of woody plantations in grasslands or indirectly from alterations in climate or land use (*e.g.*, grazing and fire regimes), which promote woody plant encroachment and proliferation in grasslands (Archer *et al.*, 1995; Jackson *et al.*, 2002; Briggs *et al.*, 2005; Jackson *et al.*, 2007). Regardless of the mechanism, such alterations in land cover impact many ecological processes. The effect this vegetation change has on ecosystem nutrient cycling and storage has been a major research focus for decades (*e.g.*, Schlesinger *et al.*, 1990). Interest in this topic remains high because conversion of grassland to woodland is occurring extensively worldwide, with implications for both current and future human land use (Archer *et al.*, 2001). Furthermore, this vegetation change has the potential to sequester significant amounts of carbon (C) from the atmosphere into ecosystem pools (Pacala *et al.*, 2001).

<sup>1</sup>Corresponding author's Present address: Department of Plant & Soil Sciences, N-222D Ag. Sci. North, University of Kentucky, Lexington 40546; Telephone: (859) 257-6388; FAX: (859) 323-1952; e-mail: rebecca.mcculley@uky.edu

Recent studies indicate that aboveground net primary production (ANPP) increases with woody plant encroachment and proliferation in mesic grasslands (Harcombe *et al.*, 1993; Reich *et al.*, 2001; Hughes *et al.*, 2006; Knapp *et al.*, 2008). These large increases in plant production are likely to promote C accumulation over time, and increases in aboveground C pools (biomass and litter) are frequently reported at mesic sites undergoing woodland conversion (Hibbard *et al.*, 2001; Jackson *et al.*, 2002; McKinley and Blair, 2008). However, the response of belowground C pools, especially soil C, to this vegetation change has been harder to predict (Wessman *et al.*, 2004). Woody plant encroachment and proliferation in grasslands can result in soil C accumulation (Boutton *et al.*, 1998; Archer *et al.*, 2001; Brantley and Young, 2010), no net change (McCarron *et al.*, 2003; Smith and Johnson, 2003; Hughes *et al.*, 2006), or loss (Jackson *et al.*, 2002). Interestingly, mesic systems, where ANPP is thought to be most stimulated by grass-to-woodland conversion (Knapp *et al.*, 2008), are also where soil C losses are more commonly observed with woody encroachment and plantation forestry (Guo and Gifford, 2002; Jackson *et al.*, 2002).

How might woody plant dominance in mesic grasslands reduce soil C pools? There are several possible explanations: (1) Belowground net primary production (BNPP), a more direct source of C to soil pools than ANPP, may be reduced under woody plants (thereby reducing C inputs to soil C pools; Steinaker and Wilson, 2005); and/or (2) Woody plant dominance, through many possible mechanisms, may stimulate soil organic matter decomposition, for instance through changes in nitrogen (N) availability (Brantley and Young, 2008; Kirschbaum *et al.*, 2008), alterations in microclimate (Vetaas, 1992), detritivore and microbial communities (Waldrop and Firestone, 2004), and/or a soil 'priming' effect (Fontaine *et al.*, 2004).

The literature on woody plant encroachment and proliferation in grasslands contains many site-specific studies where one or more of these possible controlling factors on soil C gain or loss have been measured (*e.g.*, Wessman *et al.*, 2004). However, the majority of research has been done in xeric systems where either gains or no net change in soil nutrient pools have been observed (*e.g.*, Hibbard *et al.*, 2001; Lett *et al.*, 2004; Smith and Johnson, 2004; Hughes *et al.*, 2006; McClaran *et al.*, 2008; McKinley and Blair, 2008). In an effort to identify some of the potential mechanisms governing soil C loss with woody plant proliferation in wetter grasslands, we measured plant inputs of C and N (ANPP and BNPP) and litter decomposition losses within a paired tallgrass prairie and adjacent woodland site in the southern Great Plains of North America shown previously to have experienced soil C losses with grass-to-woodland conversion (Jackson *et al.*, 2002). We hypothesized that while ANPP might be higher in woody than grass-dominated areas at this site, woodland BNPP would be significantly lower than native grassland. In addition, given that some studies have shown grass-to-woodland conversion in mesic habitats increases N availability (Wilson and Kleb, 1996; Reich *et al.*, 2001; McCulley *et al.*, 2004; Brantley and Young, 2008), combined with the fact that a potential N-fixing species [*Prosopis glandulosa*; (Johnson and Mayeux, 1990)] is dominant at this site, we hypothesized that N inputs via plant production would be greater in the woodland than the grassland. Finally, despite higher N inputs in the woodland, we predicted that alterations in litter chemistry (*e.g.*, more lignin/woody contributions) in the woodland would result in woody litter decomposing more slowly than grass material, as has been observed at other sites (Kochoy and Wilson, 1997; Norris *et al.*, 2001). These production and decomposition trends might mean that over time C pools are shifted from primarily belowground in grassland to aboveground in woodland (into plant material and a developing litter layer; Jackson *et al.*, 2002). Increasing N availability can stimulate decomposition of relatively labile material but may retard decomposition of

TABLE 1.—Woodland vegetation characteristics. The basal diameter of all stems >5 cm dbh (diameter at breast height) and the number of understory saplings (taller than 50 cm but with dbh <5 cm) found in the 30 m × 30 m plot were recorded by species in Sept. 2003. Basal diameter data were converted to basal area for each stem and species (total woody basal area for the stand was 18.4 m<sup>2</sup> ha<sup>-1</sup>)

Species name	Proportion of total basal area (%)	Number of understory saplings
<i>Prosopis glandulosa</i>	53.1	0
<i>Gleditsia triacanthos</i>	24.9	147
<i>Celtis laevigata</i>	13.0	153
<i>Ulmus crassifolia</i>	4.2	143
<i>Melia azedarach</i>	2.4	0
<i>Sideroxylon lanuginosum</i>	1.9	31
<i>Zanthoxylum hirsutum</i>	0.6	0

recalcitrant litter or soil organic matter pools (Knorr *et al.*, 2005). If soil C at this site is relatively labile in nature, the observed reductions in soil C pools that accompany woody plant establishment and dominance in this mesic grassland ecosystem might be the result of both reductions in belowground C inputs and increases in N input and availability, which may stimulate organic matter decomposition and loss.

## MATERIALS & METHODS

### SITE DESCRIPTION

We conducted this study in central Texas at the USDA-ARS field station near the city of Riesel, (31°29'N, 96°53'W), where annual precipitation is 896 mm (41 y mean on site) and mean annual temperature is 18.9 C. The annual precipitation at the site was 903 mm and 1171 mm for study years, 2003 and 2004, respectively. Using historical aerial photographs, supplemented with personal landowner accounts, we identified a location where native, uncultivated tallgrass prairie existed adjacent to (within 100 m) an uncultivated area that was originally grassland but that has become dominated by woody plant species (mesquite, *Prosopis glandulosa*, and others) over time, presumably due to changes in land management (*e.g.*, cessation of mowing and burning). Carbon stable isotope signatures of the soil in the woodland confirm that the site was previously dominated by C<sub>4</sub> vegetation (*i.e.*, grasses: Jackson *et al.*, 2002). We established a 30 m × 30 m plot in each vegetation type based on similarities in soil type (Heiden clay; Udic Haplusterts) and topography [relatively flat (1–3% slopes) upland landscape positions].

The native grassland site has been managed as a hay pasture for many years and contains a plant community considered representative of native, remnant tallgrass prairie. Dominant grassland plant species included: *Schizachyrium scoparium*, *Sorghastrum nutans*, *Desmanthus illinoensis*, and *Arnoglossum plantagineum* (Polley *et al.*, 2007). Long-term management of the site consists of annual Jun. mowing followed by hay removal, but the site receives no fertilizer applications. During the year of sampling, the grassland was hayed in Jun. of 2003 (and material was removed), immediately before this study began.

In contrast, the woodland has not been actively managed in >60 y and is dominated by seven woody species [Table 1; taxonomy according to (Diggs *et al.*, 1999)]. Herbaceous vegetation in the woodland was relatively sparse and patchily distributed in the understory. An inventory of the woodland plot was performed in Sept. 2003 and consisted of recording the basal diameter of all live stems with dbh (diameter at breast height) >5 cm and counting the number of saplings >50 cm tall but with dbh <5 cm by species. *Prosopis*

*glandulosa* was the dominant woody species at the site comprising >50% of the woody basal area (Table 1), although the two individuals with the largest basal diameters (33 and 34 cm) were both *Gleditsia triacanthos*. No *Prosopis* saplings were encountered in the woodland understory, suggesting this species may eventually be replaced by other co-occurring species. Using regression equations derived from *Prosopis* stands from a relatively nearby site (~400 km from Riesel) to relate basal diameter to age, the *Prosopis* stems measured in this study were 18–70 y old (Hughes *et al.*, 2006). Using the oldest year in common of the three oldest trees [the method employed by Hughes *et al.* (2006)], stand age was estimated at 61 y old, which is in line with estimates from aerial photographs that indicate trees were present on site before 1941 (at least 62 y old at the time this study was conducted).

#### SOIL NUTRIENT POOLS

To verify changes in soil C and N pools with grass-to-woodland conversion at this site, we used a hydraulic soil probe to take ten 120 cm deep soil cores (4.0 cm i.d.) from random locations within each of the two 30 m × 30 m plots in the grassland and the woodland in Jun. of 2003. We used only non-compacted soil cores (n = 8 and 5, for grassland and woodland, respectively) for the following analyses. We sectioned the soil into five depth increments: 0–10, 10–30, 30–60, 60–90, 90–120 cm. We air-dried and sieved (2 mm) the soils, removing root fragments by hand. The soil was weighed and a sub-sample used for gravimetric water determinations. Bulk density was calculated as the water- and root-free mass of the soil divided by the volume. Additional sub-samples were ball-milled to powder, acid-treated to remove inorganic C from these marine sediment derived soils using a vapor method (Harris *et al.*, 2001), and analyzed for % C on a CE Instruments NC 2100 elemental analyzer (ThermoQuest Italia). Percent N was determined in a similar manner, except on un-acidified samples, as acidification has been shown to alter measured N concentrations (Ryba and Burgess, 2002).

#### PLANT NUTRIENT INPUTS

We measured aboveground production (ANPP) using two techniques: peak standing crop in the grassland and litter tray collections in the woodland. We clipped five 50 × 50 cm quadrats in Sept. 2003, which gave us an estimate of aboveground grassland production since the Jun. 2003 haying event. We then clipped eight 50 × 50 cm quadrats in late Aug. 2004, and subtracted the Sept. 2003 average biomass from the individual Aug. 2004 quadrat biomass data for aboveground grassland production estimates (Sept. 2003–Aug. 2004). Although the peak standing crop technique may be less accurate than more frequent sampling methods that track changes in live and standing dead material throughout the year, it gives accurate results in humid temperate grassland systems, such as the site measured in this study (Scurlock *et al.*, 2002). We coupled this once-a-year grassland sampling scheme with litter collections that occurred every 2–3 mo from 10 trays (34 cm × 50 cm each) placed randomly throughout the woodland site. Trays were installed Jul. 2003, and all material in them (*e.g.*, seed pods, leaves, twigs, etc.) was collected in Sept. and Nov. 2003 and in Feb., May, Jun., and Sept. 2004. For the annual total woody litter production estimates, we summed the Nov. 2003 (which included Oct. and Nov. litter) through Sept. 2004 values. We also clipped four 50 × 50 cm quadrats in the woodland in late Aug. 2004 for estimates of understory herbaceous production. The average biomass and elemental content of this understory vegetation (on a g m<sup>-2</sup> basis) was added to that of the woody litter production estimates to derive total woodland aboveground production values. We were unable to estimate wood production on the main trunk and limbs of the trees in this study; however, we checked our litter tray productivity estimates with allometry-derived

aboveground productivity equations developed for *Prosopis* stands at a nearby site (Hughes *et al.*, 2006) and found surprising similarity between the two (*see* Results for more details). Other studies have also shown that annual radial growth of woody stems accounts for a small proportion of aboveground tree litter production (<5%, Stienaker and Wilson, 2005).

Harvested litter and biomass was oven-dried for 5 d (50 C) and weighed. The entire sample was ground in a cyclone mill, and a sub-sample was then ball milled and analyzed for C and N content on the same elemental analyzer used for the soil analysis. We calculated the nitrogen use efficiency (NUE) of both vegetation types as the aboveground plant production ( $\text{g m}^{-2}$ ) divided by the amount of N in litterfall (a.k.a., the N contained in the Aug. harvest period for the grassland and the combined total of the litter collections for the woodland).

We used a modified root in-growth technique to measure belowground production in both the grassland and woodland systems (Lauenroth, 2000). In Jul. 2003, we excavated eight randomly placed 0–30 cm deep holes in both the grassland and woodland sites using a bucket augur (11 cm i.d.). We refilled these holes with root-free silty clay soil (Austin soil series containing 1.84% organic C and 0.16% N) collected from a nearby site. We collected root in-growth samples in Sept. 2003, Jun. 2004, and Sept. 2004, by driving a 4 cm i.d. soil corer into the center of the excavated hole and extracting one 0–30 cm depth increment. After each sample extraction, more root-free soil was used to fill the holes.

Extracted root in-growth samples were oven-dried (5 d at 50 C) for storage and transportation purposes. Within 6 mo of field collection, roots were removed using a flotation and hand-picking procedure, where DI water was added to sub-samples of each 0–30 cm core to soften the soil and was then sorted and picked until all root segments >2 mm in length were extracted from the total core. The roots were then dried, weighed, ground in a ball mill, and analyzed for C and N content. Samples that contained enough mass were further subdivided for ash determination (combusted at 500 C for 5 h in a muffle furnace). Belowground production values were calculated as the ash-corrected sum of the Jun. and Sept. 2004 sampling dates.

#### LITTER DECOMPOSITION

We measured decomposition rates of the dominant vegetation in both the grassland and woodland using litterbags filled with aboveground plant material collected in Sept. 2003. Aboveground material in the grassland was collected by clipping five 50 × 50 cm randomly placed quadrats. All clipped material was dried (5 d at 50 C), cut into 2–4 cm pieces with grass shears, and mixed well throughout litterbag construction. For the woodland, we removed ~1 m long healthy limbs from the dominant species. These limbs were sectioned into smaller pieces, dried (5 d at 50 C), and then separated into leaf and wood components (by species). Small (<2-cm diameter) twigs and stems were cut into discs and small pieces. Although we did not use senesced material for our decomposition experiment, litter bag C:N ratios (51 for grassland and 25 for woodland) were reflective of overall above- and belowground production C:N differences between the two litter types (averaged above- and belowground C:N values were 72 vs. 32 for grassland and woodland, respectively; Fig. 2).

Litterbags (10 × 10 cm<sup>2</sup>) constructed of fiberglass-nylon mesh (1 × 2 mm<sup>2</sup> openings) were packed with 3.79 g of material. Both grassland and woody litterbags contained a mixture of species present in each site. The grassland bags were constructed from the well-mixed composite clipped material. Woody litterbags contained a mixture of the dominant species leaf and wood material based on the proportion of total basal diameter covered by each species (Table 1; material from species with <3% basal area coverage was not included) and from a previous study in *Prosopis*-dominated woodland that showed litterfall

was 60% leaf material and 40% wood (McCulley, 1998). We assumed a 60/40 leaf-to-wood litter production ratio for all woody species.

A reciprocal transplant experimental design was used with the litterbags to determine if differences in microclimate or decomposer communities between the two vegetation types were important in controlling litter decomposition rates. Grass and woody litterbags ( $n = 66$  of each) were randomly placed on the soil surface in Nov. 2003 in both the grassland and woodland locations and were harvested in Feb., May, Jun., and Sept. 2004 (4–6 litterbags of each grass and woody litter type were randomly collected per location and harvest date). Initial mass and elemental content was determined from litterbags ( $n = 8$  and  $9$  for grass and woody bags, respectively) taken to the field in Nov. 2003 but brought immediately back to the lab. Initial and harvested litterbags were dried (5 d at 50 C); litter was removed from the bags and weighed. We ground the material using a cyclone and ball mill and determined C, N, and ash content using previously described methods. Because litterbag samples can accumulate significant quantities of soil over time, we also soil-corrected our C & N litter values (Hunt *et al.*, 1999). We calculated the decomposition constant [on a mass ( $k$ ,  $y^{-1}$ ) and carbon ( $k_c$ ,  $y^{-1}$ ) basis] over the study period by fitting the following equation to the data:

$$\ln(X_t/X_o) = -kt$$

where  $X_t$  and  $X_o$  are the ash-free mass or carbon content of the litter at the final sampling period (Sept. 2004;  $t = 0.83 y^{-1}$ ) and time 0 (Nov. 2003) (Olson, 1963). In order to evaluate differences in N dynamics, we calculated the ratio of N remaining in the litter at each harvest period to that of the initial litter (on a mass basis;  $N_t/N_o$ ).

#### STATISTICAL ANALYSES

We used several statistical approaches to assess the effect of grassland conversion to woodland on the measured parameters. Because depth increments within individual soil cores are correlated, we ran a multivariate analysis of variance (MANOVA) test using a general linear model (SAS, 1996) to determine vegetation type differences in the soil profile % C, % N, and bulk density data. For the total 120-cm-deep soil profile C and N content ( $g C$  or  $N m^{-2}$ ) and the above- and belowground production data, we utilized a simple paired *t*-test procedure employing the Satterthwaite variance calculation to test for differences between vegetation types. To assess litter and habitat type effects on litter decomposition rates ( $k$  and  $k_c$ ) and N dynamics accompanying decomposition, we ran a repeated-measures mixed model using harvest date, litter type (grass or woody material), and location (incubated in grassland or woodland) as fixed effects and the rep (litter type  $\times$  location) effect as a repeated component. For all statistics, individual soil cores, quadrats, litter collection trays, and/or litter bags from each vegetation type were considered replicates. Ideally, multiple paired sites of woodland and adjacent grassland would have been identified and used as replicates in this study; however, in this geographic region (an area where intensive agriculture has historically and still is widely practiced), finding adjacent sites of unplowed native grassland and >50 y old mesquite dominated woodland was not possible. Normality and equality of variance assumptions were checked prior to analyses of all datasets, and appropriate transformations and/or selection of statistical methods were made accordingly.

## RESULTS

### SOIL NUTRIENT POOLS

Consistent with previous sampling at this site (Jackson *et al.*, 2002), total soil C pools were reduced in woodland compared to adjacent grassland by  $\sim 20\%$  (Fig. 1). This trend, while

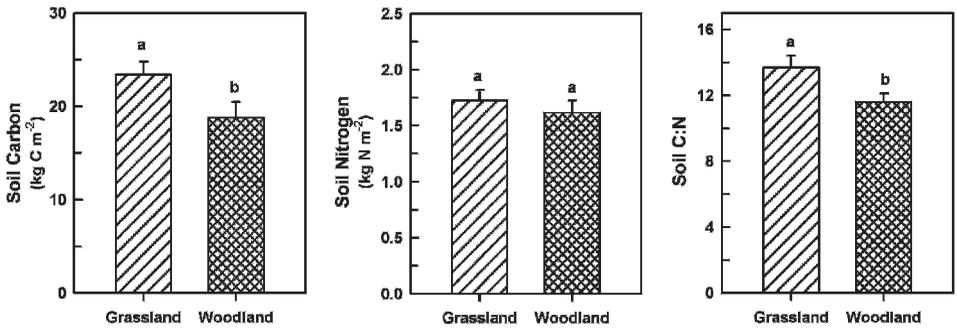


FIG. 1.—The 0–120 cm depth total soil C and N pools and C:N data for each vegetation type. Values on all graphs are means  $\pm$  1 SE. Different letters indicate significant differences between vegetation types as determined by paired *t*-tests ( $P < 0.05$ , except for the soil C panel where  $P = 0.0609$ )

marginally insignificant ( $P = 0.06$ ), reflects the fact that organic C concentrations tended to be reduced in woodland soils, and grassland soil bulk densities were significantly greater than adjacent woodland, perhaps resulting from the mechanized haying practices employed in the grassland (Fig. 2). There was no significant difference between vegetation types in total soil N pools (Fig. 1), but the concentration of N was higher in the woodland surface soil (0–10 cm; Fig. 2). C:N ratios of both the surface soil and the total 0–120 cm depth profile were significantly lower in woodland than adjacent grassland (Figs. 1 & 2).

#### PLANT PRODUCTION

Consistent with the traditional dogma that grasses allocate proportionally more C belowground than woody species, root production in woodland was less than half that of adjacent grassland (Table 2) and accounted for a smaller proportion of the total C inputs to the ecosystem (10.4% vs. 23.2% for woodland and grassland, respectively). However, %C of woodland roots (35.7%) tended to be higher than that of grassland (32.3%) ( $P = 0.0557$ ). Total carbon inputs via plant production were surprisingly similar in grassland and woodland vegetation types at this site: net primary production was 330 and 342 g C m<sup>-2</sup> y<sup>-1</sup> for grassland and woodland, respectively. There was no significant difference in aboveground production between the vegetation types (Table 2; Fig. 3), regardless of whether or not understory herbaceous aboveground production (64 g C m<sup>-2</sup> y<sup>-1</sup>) was included in the woodland estimate. Because the litter tray technique we employed to measure woody aboveground production does not quantify radial wood production or production of new stems or branch material, we compared our data with estimates derived from regression equations relating stand age to aboveground productivity from a relatively nearby *Prosopis*-dominated site (Hughes *et al.*, 2006). Our aboveground woody production values were surprisingly similar to those derived from the allometric equations [244 vs. 206 g C m<sup>-2</sup> y<sup>-1</sup>, this study vs. Hughes *et al.* (2006), respectively].

In contrast to the C input patterns measured between the two vegetation types, N inputs were significantly greater in the woodland than the grassland, consistent with the fact that mesquite is a N-fixing species. Nitrogen inputs measured in woodland total production were 3 $\times$  that of adjacent grassland (Fig. 3), and significant differences between vegetation types were more apparent above- than belowground. Woodland litterfall peaked in the fall (Sept.–Nov.), with woody litter N concentrations also lowest at this time of the year (~1.1%N) though still significantly higher than grassland fall biomass %N (0.6%; data not shown).

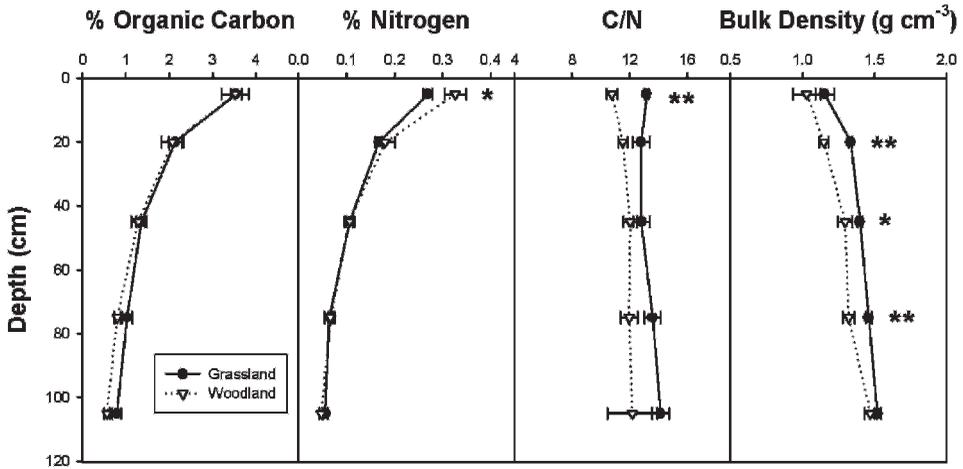


FIG. 2.—Depth distribution of concentrations of soil organic carbon and total nitrogen, C:N ratios, and bulk density from grassland and woodland sites at Riesel, Texas. Values are means ( $\pm 1$  SE). Significant differences between grassland and woodland values by depth are indicated by asterisks (\* for  $P < 0.05$  and \*\* for  $P < 0.01$ ). Bulk density is the only parameter where MANOVA revealed significant differences between grassland and woodland soils for the whole depth profile ( $P = 0.0032$ )

Percent N in woodland roots (1.37%) was significantly higher than that of grassland (0.51%;  $P < 0.0001$ ). As a consequence of these trends, C:N ratios of both above- and belowground production inputs in woodland were half that of grassland (Fig. 3), and the NUE of grassland ANPP (g biomass/g N in litterfall) was 2 $\times$  that of woodland ( $P < 0.0001$ ; Table 2).

LITTER DECOMPOSITION

Litterbags composed of grass and woody material had initial C:N ratios of 51 and 25, respectively, reflecting the observed differences in C:N of the plant production measurements from the different vegetation types. Yet, despite this large difference in initial litter chemistry, ~40–50% mass remained in both types of litterbags after almost a year in the field (Fig. 4), and there was no significant ‘litter type’ main effect on calculated litter decomposition rates ( $k$  or  $k_c$ ) (Table 3), indicating that these two very different types of litter decomposed from a mass and C perspective in a surprisingly similar manner. This was not the case with regard to N. The ‘litter type’ main effect was highly significant in the

TABLE 2.—Plant production (above- and belowground) and ecosystem-level nitrogen use efficiency (NUE) of aboveground production in grassland and adjacent woodland (see methods for calculation of NUE). Data are means ( $\pm 1$  SE). Significant differences between vegetation types (paired  $t$ -test  $P < 0.05$ ) are illustrated by different superscript letters within a measured parameter

Parameter	Grassland	Woodland
Plant Production ( $\text{g m}^{-2}$ )		
Aboveground	599 (75) <sup>a</sup>	703 (52) <sup>a</sup>
Belowground	241 (43) <sup>a</sup>	98 (24) <sup>b</sup>
NUE ( $\text{g biomass g litterfall N}^{-1}$ )		
Aboveground	181 (9) <sup>a</sup>	81 (3) <sup>b</sup>

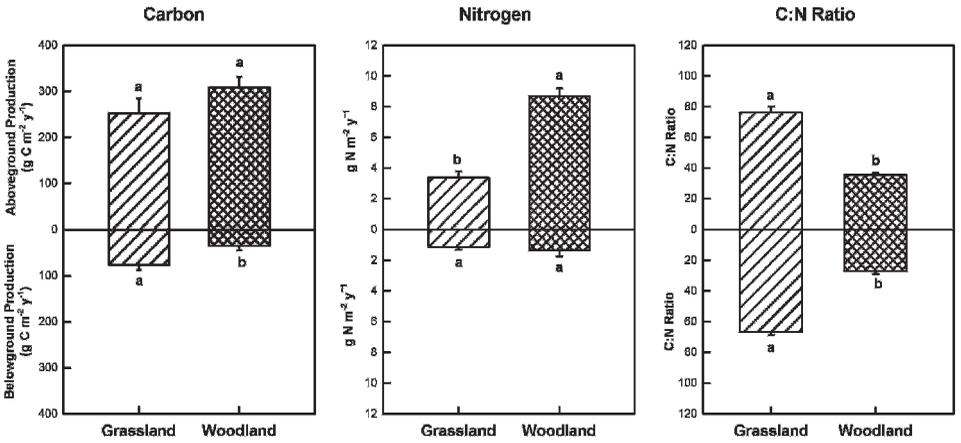


FIG. 3.—Carbon and nitrogen inputs and C:N ratio of above- and belowground production from adjacent grassland and woodland at Riesel, Texas. Values on all graphs are means  $\pm$  1 SE. Different letters indicate significant differences between vegetation types as determined by paired *t*-tests ( $P < 0.05$ )

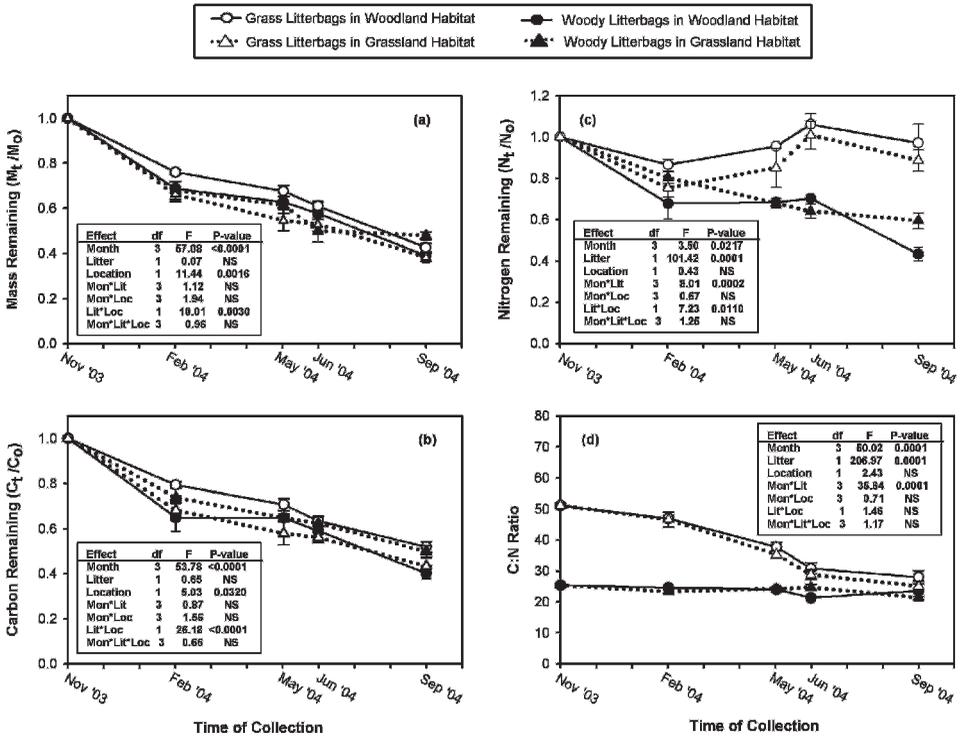


FIG. 4.—Mass (a), Carbon (b), Nitrogen (c), and C:N ratio (d) of litter remaining in litterbags over the course of the experiment. Litterbags were constructed of two types of litter material (grass or woody) and both types of bags were placed in grassland and woodland locations/habitat. Values on all graphs are means  $\pm$  1 SE

TABLE 3.—Litter decomposition rates [mass (k) and carbon ( $k_c$ ) based] are given for both types of litter (grass or woody material) incubated in either grassland or woodland locations. Superscript letters within each dataset, k or  $k_c$ , indicate significant differences between litter types and locations ( $P < 0.05$ ). Smaller k and  $k_c$  values indicate slower decomposition rates

Litter decomposition rates ( $\text{yr}^{-1}$ )	Incubated in grassland	Incubated in woodland
Grass k	$1.04 \pm 0.12^a$	$1.03 \pm 0.05^a$
Woody k	$0.89 \pm 0.05^a$	$1.14 \pm 0.09^a$
Grass $k_c$	$1.00 \pm 0.003^a$	$0.79 \pm 0.05^b$
Woody $k_c$	$0.84 \pm 0.05^b$	$1.10 \pm 0.07^a$

repeated measures ANOVA on N retention ( $P = 0.0001$ ), as was the ‘month\*litter type’ interaction ( $P = 0.0002$ ). Grass litter retained significantly more N than the woody litterbags (Fig. 4c), and the two litter types exhibited different patterns of N retention over time. Woody litter mineralized N throughout the experiment; whereas, grass material tended to retain it. These trends were also reflected in the C:N ratio of the remaining litter material (Fig. 4d). Grass litter C:N ratios declined throughout the experiment, while woody litter C:N ratios remained relatively constant.

The ‘location’ of litterbags in the field (*i.e.*, whether litterbags were incubated in the grassland or woodland site) was not significant for k,  $k_c$ , N retention, or C:N ratios; however, ‘location’ was significant in the mass and C loss repeated measures ANOVA, and the ‘location\*litter type’ interaction was significant for both of these parameters and  $k_c$  and N retention. For mass, C loss and  $k_c$ , the significant interaction term was primarily the result of each litter type losing mass and C more quickly when incubated in its ‘native’ location (Table 3; Fig. 4a, b). The grass litterbags incubated in the woodland location decomposed the slowest from both a mass and C perspective, especially early on in the decomposition study (Fig. 4a, b), and contributed to the significant ‘location’ effect in the repeated measures ANOVA. With N retention, both litter types retained more N when incubated in the ‘non-native’ location (Fig. 4c). These results suggest that litter decomposition as a process has differentiated between these two vegetation types, despite their close proximity and similar macroclimate and soil type, to favor the decomposition of the dominant vegetation type.

#### DISCUSSION

Reduced belowground plant production and enhanced N cycling rates, as evidenced by higher surface soil %N concentrations, higher N content and lower NUE of aboveground litter inputs, and less N retention in decomposing woody litter, may contribute to the loss of soil C observed to accompany woody plant proliferation and dominance in this mesic tallgrass prairie. Reductions in soil C pools, while marginally insignificant, were comparable with the scale of the reduction (~20% less soil C in woodland than grassland) previously reported from this site (Jackson *et al.*, 2002), and represent a large amount of C ( $4.6 \text{ kg C m}^{-2}$ ). Soil C represents the balance of C inputs via plant production and C outputs via soil organic matter and litter decomposition and dissolved organic C loss integrated over time, all of which likely vary as woody stands age, and species composition and/or climate and land use changes. This temporal variability in the controlling parameters presents a major challenge to understanding grass-to-woodland conversion effects on soil C pools. While we examined only one site, our data, showing that woodland belowground plant production, at least in the top 30 cm of soil, is half that of the adjacent grassland and that

aboveground litter C deposition and decomposition rates are similar between the two vegetation types, suggests that woodland soil C pools are unlikely to increase in the near future, and they may continue to decline. Similar to prior studies (*e.g.*, Jackson *et al.*, 2002), these results suggest that ecosystem C storage is shifting aboveground in the conversion of grassland-to-woodland at this site. It is likely that woody species have deeper root production than was quantified in this study (30 cm). Additional work at the site with stable C isotopes has shown significant woody plant contributions to soil organic C pools to ~2 m in depth but not below (Jackson *et al.*, 2002); however, both this prior work and our study indicate that despite these deeper woody plant root contributions, total soil organic C pools at depth are similar between grassland and woodland sites.

Reduced belowground production in woodland compared to grassland, as we measured here, is consistent with previous studies showing greater root biomass in grass dominated systems (Jackson *et al.*, 1996). Though our BNPP values are considerably lower than those reported for prairie and adjacent aspen forests in Canada (Steinaker and Wilson, 2005), both our study and that of Steinaker and Wilson (2005) found no significant difference in the amount of N in BNPP between the vegetation types but measured 3× larger N inputs via ANPP in woodlands than adjacent grassland. Alterations in N cycling have been implicated as a potential mechanism enhancing plant invasions, such as trees moving into grassland (Laungani and Knops, 2009). Increased rates of N cycling with woody plant dominance in grasslands has been reported for several woody species: mesquite (*Prosopis glandulosa*, Schlesinger and Pilmanis, 1998; Hibbard *et al.*, 2001), aspen (*Populus tremuloides*, Wilson and Kleb, 1996), oaks (*Quercus spp.*, Reich *et al.*, 2001), and wax myrtle (*Morella cerifera*, Brantley and Young, 2008). Some of these species, including the dominant tree in our study, mesquite (*Prosopis*), are capable of forming symbiotic N-fixing associations with various microbes (Johnson and Mayeux, 1990; Brantley and Young, 2008). In the case of oaks (and potentially junipers), reductions in fire frequency that typically co-occur when these woody species invade grassland may reduce N volatilization losses and lead to a gradual build up of N pools and eventual increases in N availability in developing woodlands (Reich *et al.*, 2001; McKinley and Blair, 2008). Although we did not assess N-fixation in the mesquite present at our site, elevated %N in surface soils, lower C:N ratios throughout the soil profile, higher %N in both roots and aboveground litter, lower aboveground NUE, and less N retained in decomposing litter, all suggest that N is more available and cycling more quickly in the woodland at this site. Enhanced N availability can have varying effects on litter and soil organic matter decomposition, depending on the chemistry of the material being degraded, overall nutrient availability at the site, and the type of microbes involved in the process (Janssens *et al.*, 2010). It is probable that enhanced N availability directly or indirectly alters ecosystem processes that ultimately control soil C pools (Kirschbaum *et al.*, 2008). Additional process oriented studies that further explore these possibilities at this site seem warranted.

The fact that aboveground litter decomposition rates were similar, on a mass and C loss basis, between the two vegetation types was surprising; given the large and significant differences in initial C:N ratios of the litter. This result contradicts basic decomposition theory that higher C:N litter should decompose more slowly than lower C:N material (Aerts, 1997), and differs from previous work that has shown grass litter decomposes more quickly than juniper and aspen litter (Kochy and Wilson, 1997; Norris *et al.*, 2001). We did not measure lignin content of our litter, and given that wood was included in our woody litter bags, it is possible that higher lignin content in woody litter bags interacted with lower C:N ratios to keep overall decomposition rates similar between the two litter types. The significant 'location' effect in the repeated measures ANOVA for mass and C loss concurs

with prior studies on this subject that decomposition tends to occur more rapidly in grass-dominated systems (Kochy and Wilson, 1997; Norris *et al.*, 2001). However, it is notable that this 'location' effect was primarily driven by the slow rate of grass litter decomposition in woodland and was not observed in the statistical analysis of  $k$  or  $k_c$  parameters, perhaps suggesting that differences in decomposition rates between the two vegetation type locations were not that strong. We did not quantify differences in biotic (arthropod or microbial) communities or environmental conditions between the two vegetation types, but such differences may have contributed to the observed result that each litter type tended to decompose more rapidly when incubated in the 'native' habitat/location. Interactions between litter quality and habitat-specific biota and microclimate may promote similar litter decomposition rates in grassland and woodland at this site.

Perhaps the most surprising result of our study is that ANPP was similar in our grassland and woodland sites. This finding differs markedly from recent reports of dramatic increases in ANPP accompanying woody plant dominance in other mesic grasslands of North America (Knapp *et al.*, 2008). According to the Knapp *et al.* (2008) regression of mean annual precipitation versus increases in ANPP with woody plant dominance in grassland, we should have seen  $\sim 800 \text{ g m}^{-2}$  more production in the woodland than grassland at our site. Instead, we measured  $\sim 100 \text{ g m}^{-2}$  difference between the two, which was not significantly different from zero. While our measured woodland ANPP value is within the range reported in Knapp *et al.* (2008) for other mesquite dominated sites, our grassland ANPP is significantly higher than the mesic grasslands included in that work. Yet, our grassland ANPP data agree with other independent estimates of ANPP at this Riesel, TX location (Polley *et al.*, 2007) and are within the range of other humid temperate grasslands worldwide (Scurlock *et al.*, 2002). Riesel occurs in the 'Blackland Prairie' of Texas, an area characterized by high clay, deep, fertile soils, warm temperatures, and relatively high rainfall (Smeins and Diamond, 1983). The large amount of grassland ANPP at this site may be the product of both the abiotic conditions and the relatively low intensity haying management practices employed. It is possible that ANPP between the two vegetation types may have differed more in unusually dry or wet years. It seems likely that differences in abiotic and edaphic conditions, ages of the woody stands, species involved, and/or management practices of the various sites account for this discrepancy between our work and that of Knapp *et al.* (2008).

Effects of woody plant encroachment and proliferation in grassland on ecosystem processes can be significant and vary over time and on a site-by-site basis. This variability is probably related to differences in species characteristics (of both the woody invaders and the grassland species they are invading) and the abiotic and edaphic conditions and management history of the sites. More complicated, multifactor models, that take these parameters into account, may be required to accurately represent changes in ecosystem parameters that accompany woody plant encroachment and proliferation in a variety of grassland systems and to help develop a better understanding of when gains and losses of C will accompany this type of vegetation change.

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