Soil Biology & Biochemistry 41 (2009) 1943–1950

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland

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ARTICLE INFO

Article history:
Received 31 March 2009
Received in revised form
17 June 2009
Accepted 28 June 2009
Available online 10 July 2009

Keywords:
Faunal analysis
Decomposition pathways
Chronosequence
Prosopis glandulosa
Texas
USA

ABSTRACT

Woody plant encroachment is an important land cover change in dryland ecosystems throughout the world, and frequently alters above and belowground primary productivity, hydrology, and soil microbial biomass and activity. However, there is little known regarding the impact of this geographically widespread vegetation change on the biodiversity and trophic structure of soil fauna. Nematodes represent a major component of the soil microfauna whose community composition and trophic structure could be strongly influenced by the changes in ecosystem structure and function that accompany woody encroachment. Our purpose was to characterize nematode community composition and trophic structure along a grassland to woodland chronosequence in the Rio Grande Plains of southern Texas. Research was conducted at the La Copita Research Area where woody encroachment has been documented previously. Soil cores (0–10 cm) were collected in fall 2006 and spring 2007 from remnant grasslands and woody plant stands ranging in age from 15 to 86 years, and nematodes were extracted by sugar centrifugation. Neither nematode densities (3200–13,800 individuals kg^{-1} soil) nor family richness (15–19 families 100 g^{-1} soil) were altered by woody encroachment. However, family evenness decreased dramatically in woody stands >30 years old. This change in evenness corresponded to modifications in the trophic structure of nematode communities following grassland to woodland conversion. Although root biomass was 2–5× greater in wooded areas, root-parasitic nematodes decreased from 40% of all nematodes in grasslands to <10% in the older wooded areas, suggesting the quality (C:N or biochemical defenses) of woody plant root tissue could be limiting root-parasites. In contrast, bacterivores increased from 30% of nematodes in grasslands to 70–80% in older woody patches. This large increase in bacterivores may be a response to the 1.5–2.5× increase in soil microbial biomass (bacteria + fungi) following woody encroachment. Therefore, while energy flow through grassland nematode communities appears to be distributed nearly equally among herbivory, fungivory and bacterivory, the energy flow through nematode communities in wooded areas appears to be based primarily on bacterivory. We speculate that these shifts in nematode community composition and trophic structure could have important implications for ecosystem patterns and processes. First, the low abundance of root-parasitic nematodes (and presumably root herbivory) under woody plants may be one mechanism by which woody plants are able to establish and compete effectively with grasses during succession from grassland to woodland. Second, the large increase in bacterivores following woody encroachment likely accelerates microbial turnover and the mineralization of N, thereby providing a feedback that enables the persistence of N-rich woody plant communities.

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1. Introduction

Woody plant encroachment into grass-dominated ecosystems is a globally extensive shift in plant community structure that has strong potential to alter key processes (such as primary productivity, allocation patterns, biogeochemical cycles, and hydrology) at ecosystem, regional, and global scales (Hibbard et al., 2001; Pacala et al., 2001; Jackson et al., 2002; Zavaleta and Kettley, 2006). Although many studies have focused on the impacts of woody plant encroachment on ecosystem processes, little is known regarding the potential for this vegetation change to influence the biodiversity and trophic structure of belowground biological communities. Soil microfauna, such as protozoa and nematodes, are important constituents of soil food webs (Bonkowski, 2004). Their activities regulate the size and function of fungal and bacterial populations in the soil

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0038-0717/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2009.06.019

(Ingham et al., 1985; Poll et al., 2007), plant community composition (De Deyn et al., 2003) and rates of carbon (Bradford et al., 2007) and nitrogen (Standing et al., 2006) turnover. Nematodes are of particular interest because they are the most numerous soil mesofauna and occupy all consumer trophic levels within the soil food web. Therefore, their community structure can provide important insights regarding many aspects of ecosystem function (De Ruiter et al., 2005).

The development of woody vegetation in grasslands is known to alter nematode communities (Jackson et al., 2002). For example, the development of tea plantations in areas once dominated by herbaceous vegetation initially reduced nematode density and temporarily increased the relative density of bacterivore nematodes (Li et al., 2007). The expansion of *Prosopis glandulosa* [Torr.] *var. glandulosa* (honey mesquite), a nitrogen-fixing shrub, into new habitats in the southwestern USA also changes nematode communities. Sites with recent woody plant invasions have reduced nematode species richness, particularly among root-parasite species (Jackson et al., 2002). In the Chihuahuan Desert, nematode density increased with *P. glandulosa* root biomass and soil nitrogen (Freckman and Virginia, 1989) and the trophic structure was altered with age of the *P. glandulosa* habitat; older systems had larger relative densities of root-parasitic nematodes (Virginia et al., 1992), suggesting that the root consumers did not migrate as fast as their plant hosts (Freckman and Virginia, 1989). Although *P. glandulosa* has expanded its range and increased its cover throughout southwestern North America (Johnson and Mayeux, 1990), it is unknown if similar changes in nematode community structure occur elsewhere in this region following *P. glandulosa* invasion.

Over the last 150 years, woody plant communities dominated by *P. glandulosa* and other N-fixing shrubs (Zitzer et al., 1996) have invaded areas previously dominated by grasses in the subtropical Rio Grande Plains of South Texas (Archer et al., 1988, 2001; Boutton et al., 1998, 1999). Woody encroachment in this region has increased above- and belowground primary productivity (Archer et al., 2001), the density and biomass of roots (Watts, 1993; Hibbard et al., 2001; McCulley et al., 2004), the storage of C and N in soil organic matter (Liao et al., 2006b; Boutton et al., 2009), and the size of the soil microbial biomass pool (Liao and Boutton, 2008). Since root and microbial tissue represent key energy sources for nematode communities, it seems likely that the increased quantity of these resources could alter the community composition and trophic structure of nematodes. However, intrinsic characteristics of plant roots and total soil microbial biomass (e.g., nutritional quality, biochemical composition, physical attributes, etc.) may be different in grasslands vs. wooded areas, thereby influencing the availability of these resources to nematode consumers. For example, root tissue is more enriched in the phenolic biopolymer suberin in wooded areas than in grasslands (Boutton et al., 2009; Filley et al., 2008), which may represent a mechanical and/or biochemical deterrent to root-parasitic soil fauna including nematodes (Galeano et al., 2003; Bernards and Bastrup-Spohr, 2008).

We used the shift in vegetation structure from grasslands to woodlands known to have occurred in the Rio Grande Plains of southern Texas during the past century as an opportunity to determine how and when the nematode community composition and trophic structure respond to this change. We used a chronosequence approach to address the following hypotheses: (1) The biodiversity and population sizes of all nematode functional groups will increase with time following woody plant encroachment into grassland due to apparent increases in resource availability in the form of higher root biomass and larger soil microbial biomass pools that develop in wooded areas; (2) Alternatively, roots in wooded areas may have intrinsic biological characteristics (such as higher concentrations of suberin, or increased woodiness) that may deter root-parasitic nematodes, thereby reducing population sizes of this functional group and shifting nematode community structure towards greater dependence on microbivory with time following woody encroachment.

2. Methods

2.1. Site description

Research was conducted at the Texas AgriLife La Copita Research Area (27° 40′N; 98° 12′W) located 65 km west of Corpus Christi, Texas on the eastern edge of the Central Rio Grande Plains of the Tamaulipan Biotic Province. The climate is subtropical, with a mean annual temperature of 22 °C. The mean annual precipitation is 715 mm, with peaks in May–June and September.

The topography of this area consists of nearly level uplands that grade (1–3% slopes) into lower-lying drainage woodlands. Elevation ranges from 75 to 90 m. Upland surface soils are sandy loams (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic (Bt) horizon at 30–40 cm. Evidence from sequential aerial photography, tree ring analyses, vegetation dynamics modeling, and the isotopic composition of soils indicate that this region was once relatively open grassland and that woody plant encroachment into grasslands has occurred over the past 150 years (Archer et al., 1988, 2001, 2004; Boutton et al., 1998, 1999). Domestic livestock have grazed this research area over the past century.

The uplands are dominated by C_4 grasslands interspersed with small, discrete clusters, or stands, of woody plants. Dominant C_4 grasses in uplands include species of *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis*. *P. glandulosa* is the dominant plant species in all wooded landscape elements and its establishment in the grassland facilitates the development of woody stands through the recruitment of other woody plant species beneath its canopy. The most common understory species 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 (1970). Additional details on plant communities, soils, successional dynamics, and changes in ecosystem processes have been presented elsewhere (Archer et al., 1988, 2001; Boutton et al., 1998; McCulley et al., 2004; Liao et al., 2006a,b, Liao and Boutton, 2008).

2.2. Experimental design

All sample sites in this study were located in a grazing exclosure established in 1985 on an upland portion of the landscape on fine sandy loam soils of the Runge series. A space-for-time, chronosequence approach was used to characterize nematode communities following woody plant encroachment into remnant grasslands. The ages of the woody clusters correspond to that of the largest *P. glandulosa* within it, as stands are initiated only following the establishment of *P. glandulosa* in grasslands (Archer et al., 1988). The ages of *P. glandulosa* trees were determined by regression equation using basal diameter (Stoker, 1997). Remnant grasslands were assumed to represent time zero conditions prior to woody encroachment.

After the seasonal precipitation peaks in October 2006 and May 2007, eight woody clusters containing *P. glandulosa* trees ranging in age from 14 to 86 years and the remnant grassland areas adjacent to each woody cluster were sampled. Because the fall 2006 data showed significant changes in nematode community structure during the first 30 years of woody cluster development, two additional clusters <30 years of age and their adjacent grasslands were added to the sampling regime in May 2007 in an effort to improve our temporal resolution. The woody vegetation in each of the sampled clusters was characterized by measuring the basal diameter of all trees and then calculating the basal area (m^2) of each species within the cluster (Fig. 1).

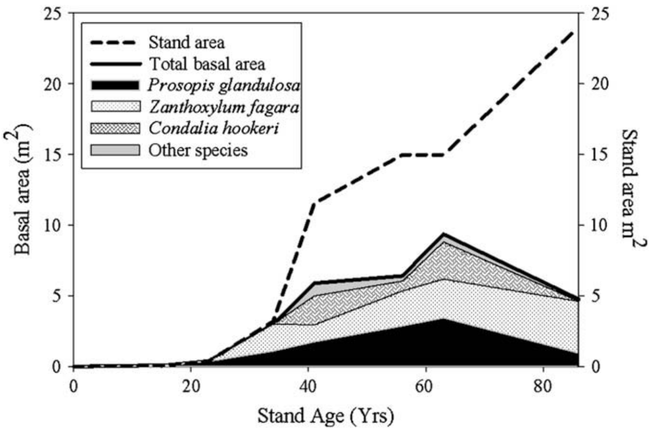

Fig. 1. Woody plant basal area (m^2) by species and total stand size (m^2) with respect to stand age at La Copita Research Area. Values for grasslands are shown as Time 0.

2.3. Soil collection and analysis

Soil samples were collected from the upper 10 cm of the soil profile near the center of each woody cluster within 0.5 m of the bole of the *P. glandulosa* tree. This depth and location within clusters was selected because it has the largest fine and coarse root biomass (Boutton et al., 1998; Millard et al., 2008) and the highest amounts of soil microbial biomass and activity (McCulley et al., 2004; Liao and Boutton, 2008) compared to deeper soil depths and other locations within the woody clusters and in the grasslands. Since roots and microbes are primary resources for soil nematode communities, this maximized the potential for detecting differences in nematode community composition following woody encroachment into grassland. For each woody cluster sampled, an adjacent remnant grassland located at least 5 m from any woody vegetation was also sampled.

From each sample point, four soil cores (10 cm deep × 2.5 cm diameter) were collected and bulked into a single sample, mixed thoroughly, and placed in a cooler for transport. Gravimetric water content was determined on an aliquot of each soil sample to express nematode densities by soil dry weight (per kg of soil). Soil organic carbon (SOC) and soil total N (STN) were determined from sieved aliquots that were dried at 60 °C and ground with a centrifugal mill. Soils were analyzed using a Carlo Erba EA-1108 elemental analyzer (ThermoElectron, Woburn, MA).

Within 24 h of soil collection, nematodes were extracted from approximately 100 g of soil using sugar centrifugation (Coleman et al., 1999) and preserved in 5% formalin. All nematodes within a sample were identified to family and enumerated using an inverted microscope at 40× magnification. Feeding habits were assigned to nematode families according to Yeates et al. (1993), with the exception of root-hair feeders, which were placed in the fungal feeders group as recommended by Forge et al. (2003).

2.4. Nematode community indices

Nematode communities were characterized using trophic group distribution (Yeates et al., 1993) and several different indices that quantified the structure and function of their communities (Table 1) (Ritz and Trudgill, 1999). These indices included nematode diversity, which was evaluated using family richness and the Simpson index of dominance (Simpson, 1949); ratio of fungivore to microbivore nematodes [fungivore/root-hair feeding nematodes divided by ((fungivore/root-hair feeding) + bacterivore nematodes)] (Neher and Campbell, 1994); the total maturity index (ΣMI) (Bongers, 1990; Yeates, 1994); the enrichment index (EI) and structure index (SI) (Ferris et al., 2001). These indices have been used successfully to differentiate the effects of vegetation change (Yeates et al., 1997; Kardol et al., 2005; Chen et al., 2007; Li et al., 2007) and land management practices (Freckman and Ettema, 1993; Neher and Campbell., 1994; Todd, 1996; Fu et al., 2000) on nematode communities.

2.5. Statistical analysis

Linear regression was used to determine relationships between nematode community indices and increasing woody plant stand age (i.e., time following woody plant invasion). SOC and STN were also analyzed using a linear regression with stand age.

3. Results

In grasslands, SOC was 6.1 ± 0.6 g C kg^{-1} soil and STN was 0.5 ± 0.03 g N kg^{-1} soil. Both SOC and STN increased linearly with stand age in soils sampled in 2006 and 2007 (Fig. 2) Woody clusters older than 50 years had SOC and STN concentrations that were 2–3×

Table 1
Summary of nematode indices used in this study.

Nematode index	Conditions described	Formula and citation
Simpson's index of dominance	Family diversity and dominance Larger values indicate high family diversity Smaller values indicate community dominated by few families	$D = 1/\Sigma p_i^2$ Simpson, 1949
Fungivore to microbivore ratio	Decomposition pathway Larger values indicate greater fungivore dominance	Fungivore:Microbivore = (FV)/(FV + BV) Yeates et al., 1993
Total maturity index (ΣMI)	Successional stage of community Range: rapid colonizers (1) to persisters (5) dominate	$\Sigma MI = \Sigma v_i f_i$ Bongers, 1990; Yeates, 1994
Enrichment index (EI)	Availability of nutrients following disturbance Range: low (1) to high (100) nutrient availability	$EI = 100\ (\Sigma k_e n_e/(\Sigma k_e n_e + \Sigma k_b n_b))$ Ferris et al., 2001
Structure index (SI)	Connectedness of nematode community Range: few (1) to many (100) trophic links	$SI = 100\ (\Sigma k_s n_s/(\Sigma k_s n_s + \Sigma k_b n_b))$ Ferris et al., 2001

p_i = proportion of nematode family to total density.
v_i = c–p value of the ith taxa.
f_i = frequency of the ith taxa.
k = the weight assigned to each nematode guild.
n = the density of nematodes within each guild.
b = basal guild.
e = enrichment guild.
s = structure guild.

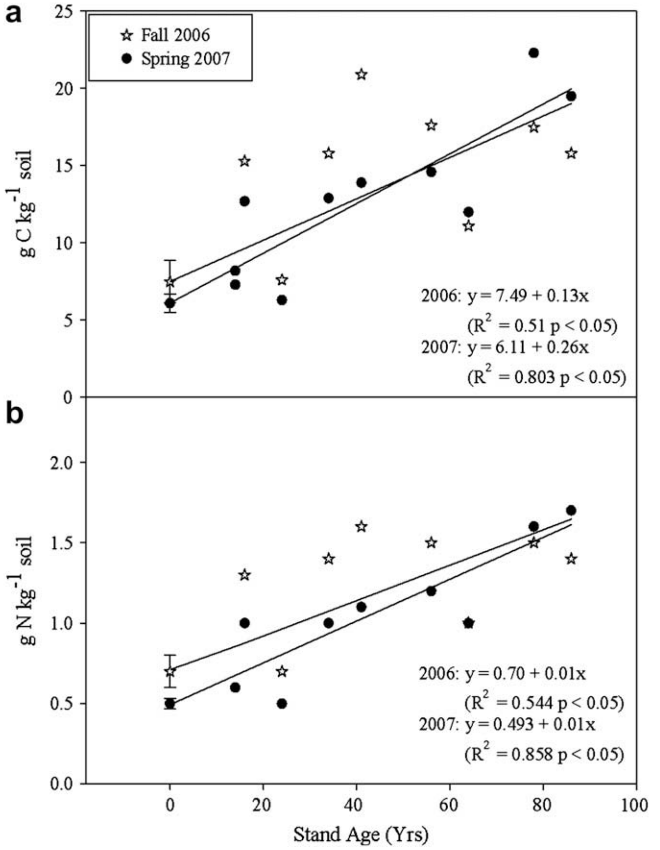

Fig. 2. Soil organic carbon concentration (g C kg^{-1} soil) (a) and total soil nitrogen concentration (g N kg^{-1} soil) (b) with respect to stand age at La Copita Research Area in fall 2006 and spring 2007. Values for grasslands (±1 S.D.) are shown as Time 0.

higher than those of remnant grasslands. Total nematode density ranged from 3200 to 10,500 nematodes kg^{-1} soil in fall 2006 and 1700 to 19,600 nematodes kg^{-1} soil in spring 2007. There was no relationship between the total density of nematodes and the age of woody plant clusters during either sample period (Fig. 3a). Likewise, nematode family richness, which varied from 15 to 19, was not affected by the age of woody clusters (Fig. 3b). At both sample periods there was a positive linear relationship with time and nematode family evenness, as measured by Simpson's index of dominance (Fig. 3c).

The trophic composition of the nematode community changed with stand age. The density of bacterivore nematodes ranged from 1000 to 5800 nematodes kg^{-1} soil in fall 2006 and 2900 to 9400 nematodes kg^{-1} soil in spring 2007. The relative density of bacterivore nematodes increased significantly with stand age in both years (Fig. 4a); the grasslands had an average of 27.2 ± 4.3% in fall 2006 and 34.7 ± 1.9% in spring 2007. In the 86-year-old stands the bacterivore proportion of the community was 72.4% in 2006 and 66.1% in 2007. At both sample periods, there was no change in the relative proportion of fungivore/root-hair feeding nematodes with respect to stand age (Fig. 4b). The relative density of root-parasitic nematodes decreased significantly with stand age in both years (Fig. 4c); the grasslands had an average of 39.1 ± 5.5% in fall 2006 and 29.9 ± 3.1% in spring 2007. In the 86-year-old stands the proportion of root-parasitic nematodes was 7.8% and 2.6% respectively. The relative density of omnivorous nematodes decreased with stand age in the spring sample (Fig. 4d).

Predatory nematode populations were too small to analyze statistically, but in fall 2006 there were 12.2 ± 9.4 nematodes kg^{-1} soil in the grasslands (time 0) while nematodes of this group were absent from woody stands of all ages. In spring 2007, there were 21.9 ± 16.6 predator nematodes kg^{-1} soil in the grasslands. At this time, predatory nematodes were present in the 14, 15 and 16-year-old woody clusters (with 20.4, 10.2 and 10.8 predator nematodes kg^{-1} soil, respectively), but they were absent from clusters older than 16 years.

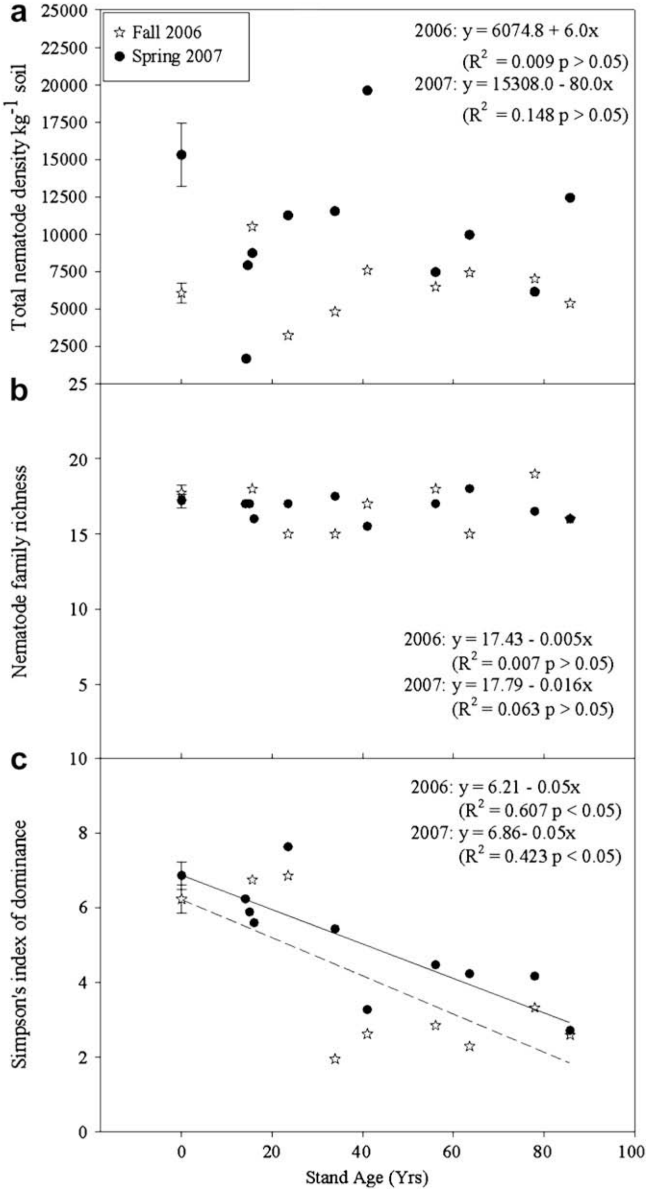

Fig. 3. Total nematode density (kg^{-1} soil) (a), nematode family richness (b) and Simpson's index of dominance (c) with respect to stand age at La Copita Research Area in fall 2006 and spring 2007. Values for grasslands (±1 S.D.) are shown as Time 0.

The ratio of fungivore/root-hair feeding to bacterivore nematode density declined with stand age (Fig. 5a). Total maturity index (ΣMI) decreased with stand age during both sampling dates (Fig. 5b). EI values range from 31.2 to 41.4 in fall 2006 with the exception of the 34-year-old stand, which had an EI value of 6.4 (Fig. 6a). In spring 2007, there was a wider range of EI values (25.7–72.1), but again, there was no pattern with stand age (Fig. 6b). SI values generally indicated that nematode communities in woody plant stands older than 24 years had reduced trophic structure and redundancy.

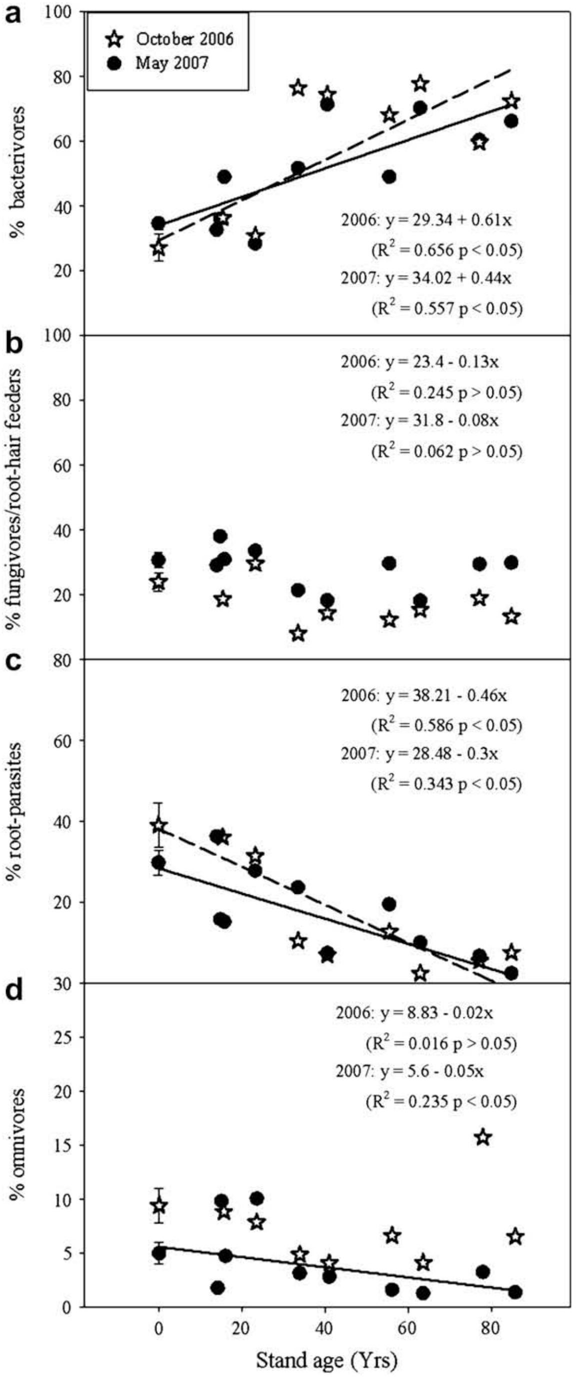

Fig. 4. Relative density of bacterivore nematodes (a), fungivores/root-hair feeding nematodes (b), root-parasitic nematodes (c) and omnivorous nematodes (d) with respect to stand age at La Copita Research Area fall 2006 (open stars) and spring 2007 (filled circles). Values for grasslands (±1 S.D.) are shown as Time 0.

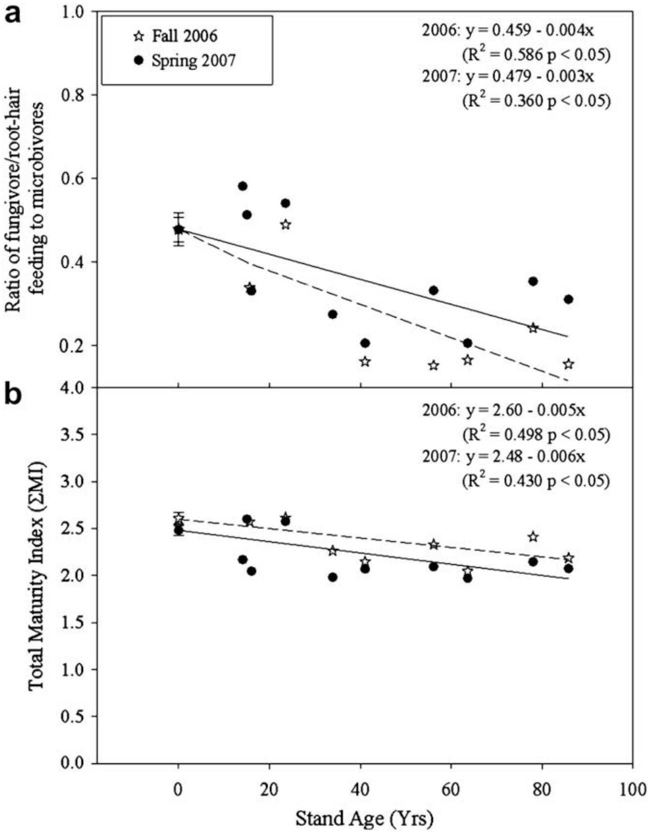

Fig. 5. The ratio of fungivores/root-hair feeding to microbivore (fungivores/root-hair feeding + bacterivore) nematodes (a) and Total Maturity Index (ΣMI) (b) with respect to stand age at La Copita Research Area in fall 2006 (open stars) and spring 2007 (filled circles). Values for grasslands (±1 S.D.) are shown as Time 0.

4. Discussion

4.1. Trophic group changes with stand development

Although total nematode densities and taxonomic diversity were unaffected by the development of woody plant stands in areas that were once grassland, this vegetation change reduced the evenness component of taxonomic diversity (Fig. 3). The Simpson index decreased from approximately 6–7 in grasslands to 2–4 in woody plant stands older than 30 years, indicating that nematode communities are dominated by fewer taxonomic groups in wooded areas compared to grasslands. Wittebolle et al. (2009) showed recently that communities with a high degree of evenness were better able to maintain the functional stability of ecosystems exposed to environmental stresses than communities with identical richness, but lower evenness. Hence, following woody plant encroachment into grassland, the less even nematode communities (and the functions they perform) that characterize these wooded areas may be less resistant to the effects of disturbance and environmental changes.

The changes responsible for this reduction in nematode community evenness suggest that there may be significant differences in how incoming organic matter is processed by soil organisms following transition from grassland to woodland. More specifically, the relative density of root-parasitic nematodes decreased from 40% of the total nematode population in the remnant grasslands to less than 10% in the older woody areas (Fig. 4c). In contrast to root-parasitic nematodes, the relative density of bacterivore nematodes increased following woody stand development: from 30% of all nematodes in grasslands to 70–80% in older woody stands (Fig. 4a). Therefore, while energy flow through grassland nematode communities appears to be distributed nearly equally among herbivory, fungivory and bacterivory, the energy flow through nematode communities in wooded areas appears to be based primarily on bacterivory.

The major decline in root-parasitic nematodes occurs despite a two to five-fold increase in root biomass over this same time interval following woody invasion (Hibbard et al., 2001; Boutton et al., 2009). There are several potential explanations for this paradox. First, concentrations of suberin-derived substituted fatty

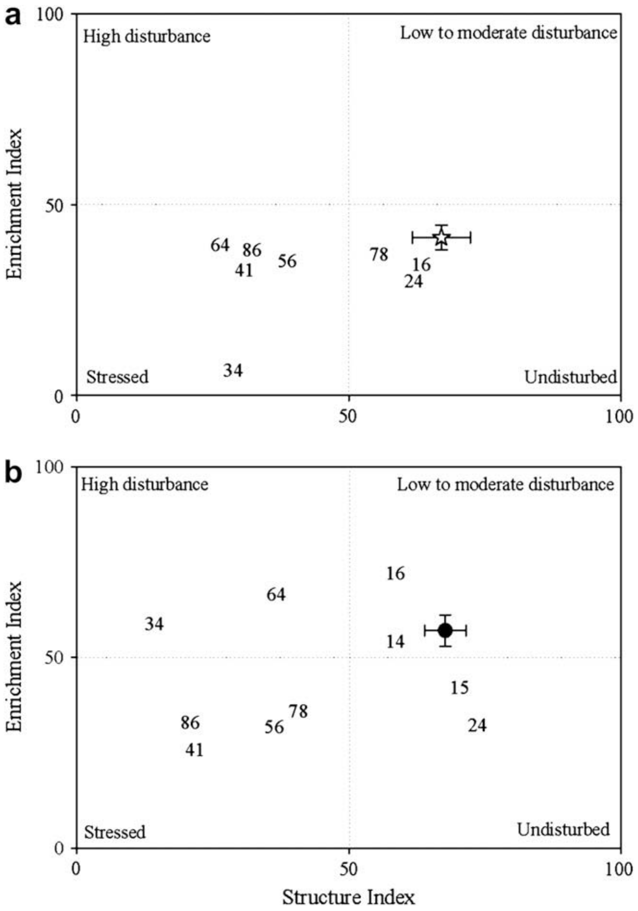

Fig. 6. Mean nematode weighted faunal profiles (see Ferris et al., 2001) of each woody cluster (labeled with stand age on graph) compared to remnant grasslands (symbols with error bars) along axes of structure (SI) and enrichment (EI) (mean ± SE) in fall 2006 ($n = 8$) (a) and spring 2007 ($n = 10$) (b) at La Copita Research Area. Error bars on the grassland points represent ±1 S.D.

acids in root tissues are approximately 50% higher in roots from wooded areas (18 mg g^{-1} tissue) than those from remnant grasslands (12 mg g^{-1} tissue) (Filley et al., 2008; Boutton et al., 2009), which may represent a mechanical and/or biochemical deterrent to root-feeding soil fauna including nematodes (Galeano et al., 2003; Bernards and Bastrup-Spohr, 2008). Second, defensive secondary chemicals in tissues of the woody plants, such as *Z. fagara* (a member of the Rutaceae or citrus family), may limit nematode populations (Joymati et al., 2003). Members of the *Zanthoxylum* genus are known for their anti-biotic properties, including anti-bacterial (Obi et al., 2002), anti-fungal (Steenkamp et al., 2007), anti-nematode (Joymati et al., 2003) and anti-protozoal (Ahua et al., 2007) compounds. Third, the woody plants may be expanding their ranges and colonizing habitats faster than their belowground predators, e.g., root-parasitic nematodes (Virginia et al., 1992). However, many of the wooded patches in our study area have been present on the landscape for at least the past 100 years, seemingly adequate time for the range expansion of root-parasites following their host plants. Finally, predators of nematodes in other soil taxa that we did not quantify (such as amoebae and mites) may be limiting the abundance of root-parasitic nematodes in soils beneath woody patches but not in the remnant grasslands. We are not able to evaluate the merits of each of these arguments at this time.

In contrast to root-parasitic nematodes, the relative density of bacterivore nematodes increased following woody stand development: from 30% of all nematodes in grasslands to 70–80% in older woody stands (Fig. 4a). The ratio of fungivore/root-hair feeders to microbivore nematodes did decrease significantly through time (Fig. 5a) and also suggests that there was an alteration in decomposition pathway with woody stand development at the site. The bacterivorous nematode family Cephalobidae experienced the greatest increase with woody stand age (Biederman, unpublished data). It is unclear if this change in bacterivorous nematode density is due to changes in resource availability (bottom-up forces) or the loss of predators (top-down control) (Wardle and Yeates, 1993). There is evidence supporting each of these scenarios. Carbon resource availability does increase with woody stand development: there is a 150–250% increase in soil microbial biomass carbon, and concomitant increases in metabolic quotient values (qCO_2) suggest that the composition of the microbial biomass becomes increasingly dominated by bacteria as woody stands age (Liao and Boutton, 2008). Similarly, *P. glandulosa* has also been shown to increase bacteria populations relative to fungi in the semi-arid woodlands of Mexico (Reyes-Reyes et al., 2002) and in the Chihuahuan Desert (Herman et al., 1995). Furthermore, due to the presence of *P. glandulosa* and other N-fixing tree legumes (Zitzer et al., 1996), the woody stands at our site are associated with higher concentrations of STN (Fig. 1b), and this enrichment can also promote the productivity of opportunistic soil bacteria and their nematode consumers (Todd, 1996; Yeates and King, 1997; Viketoft et al., 2009).

Conversely, there is some evidence indicating that bacterivore nematodes may be released from top-down control with woody stand development. Predatory nematodes are completely absent from older woody plant stands, while omnivorous populations generally decrease with stand age (Fig. 4d). However, populations of other nematode predators, such as predatory mites or other arthropods, would need to be quantified before we could assert confidently that a reduction in top-down forces contribute to the observed population increases of bacterivore nematodes in wooded areas.

4.2. Nematode community indicators

Indices commonly used to describe and quantify nematode community structure and function suggest that some aspect of the soil environment important to the ecology of nematodes is significantly altered following N-fixing woody plant encroachment. Nematode family dominance decreases with time following woody stand initiation: older stands are dominated by only a few nematode families, particularly members of the bacterivore Cephalobidae family.

The greatest change in the diversity indicators for the nematode community corresponds to an interval of rapid understory establishment and growth during the development of woody plant clusters (Archer et al., 1988). *P. glandulosa* trees in this region that are <30 years of age have mean understory shrub diversity ($\exp[H']$) values $<$ 2.0, whereas *P. glandulosa* trees $>$ 30 years typically have a mean understory shrub diversity ($\exp[H']$) value $>$ 2.0 (Archer et al., 1988). In the present study, woody clusters <30 years old lacked a woody understory, while clusters $>$ 30 years had acquired an understory dominated by *Z. fagara* (Fig. 1). Likewise, forb diversity increased from $\leq$4 species in stands younger than 30 years to $\geq$7 species in stands older than 30 years (Boutton et al., unpublished data). Asynchronous changes in above and belowground biodiversity, such as this, can indicate declining stability within an ecosystem (Hooper et al., 2005).

SI (Fig. 5) also suggests that ecological conditions relevant to nematode community structure have deteriorated following woody encroachment. SI values indicate reduced complexity in stands older than 30 years. The ΣMI decline with stand age also suggests that the structure of the nematode community is deteriorating with age and becoming dominated by nematode families that are thought to increase with disturbance or stress. This change corresponds to the interval of sub-canopy development and plant community diversification under the founding *P. glandulosa* tree. As discussed above, *Zanthoxylum* tissues are well defended with a diverse array of anti-biotic chemicals (Obi et al., 2002; Steenkamp et al., 2007; Joymati et al., 2003; Ahua et al., 2007), and compounds from *Z. fagara*, or other understory species, may contribute to the biological changes in stands older than 30 years.

4.3. Potential consequences of altered nematode trophic structure

Quantifying the magnitude of belowground herbivory on plants is difficult in the field (Freckman and Virginia, 1989) and was not done in this study. However, based on population sizes of root-parasitic nematodes, it seems reasonable to conclude that levels of belowground nematode herbivory are much reduced in woody areas compared to remnant grasslands. We hypothesize that the differential belowground herbivory may have contributed to the conversion of the grassland to woodland (Weltzin et al., 1998; Partel and Helm, 2007) by accentuating competitive differences between woody plants and grasses created by increased aboveground grazing pressure, such as that experienced at this site over the past century (Archer et al., 1988). Many studies suggest that root-parasitic nematodes can be significant consumers of plant productivity. For example, root-parasitic nematodes can consume up to 40% of standing root biomass in temperate pastures (Verschoor, 2002), from 7 to 26% of primary production in native short grass prairie (Scott et al., 1979), and can reduce aboveground productivity up to 59% in mixed grass prairies (Smolik, 1974). Differential herbivory by root-parasitic nematodes has been shown to be a significant driver of secondary plant succession (Korthals et al., 2001; Verschoor et al., 2002; De Deyn et al., 2003). Although aboveground grazing pressure has been eliminated in the study area, the dramatic decline in root-parasitic nematode populations in woody patches may be reinforcing the persistence of *P. glandulosa* and other woody plants in this system. Engelkes et al. (2008) have suggested that successful range-expanding plants often differ in defense trait characteristics and experience less belowground herbivory compared to the original native species growing in the same area.

The biogeochemical consequences of this trophic shift to higher relative densities of bacterivore nematodes have not been investigated. However, grazing by bacteria-feeding nematodes generally increases soil microbial activity (Standing et al., 2006), and through differences in body chemistry, their activity can increase nitrogen mineralization and stimulate the growth of plants and microflora (Ingham et al., 1985; Fox et al., 2006; Osler and Sommerkorn, 2007). As a single adult bacterivore nematode can consume up to 106 bacterial cells day^{-1} (Blanca et al., 2006), bacteria-based energy channels are often associated with more rapid turnover in bacteria populations and accelerated nutrient transformations (Freckman, 1988; Wardle, 2002). This is consistent with prior studies at this site showing that soil C- and N-mineralization rates are significantly higher in woody plant stands than in remnant grasslands (Hibbard et al., 2001; McCulley et al., 2004). This feedback may be important for the maintenance of N-rich woody plant communities in this system (Barnes and Archer, 1999).

5. Conclusion

Dramatic changes in nematode community structure occur following encroachment of woody plant communities dominated by N-fixing tree legumes in this subtropical savanna ecosystem, suggesting that there may be significant alterations in how incoming organic matter is processed by soil organisms. Most importantly the energy flow through grassland nematode communities appears to be based nearly equally on herbivory and bacterivory. In contrast, the flow of energy through the nematode fauna in wooded areas appears to be based primarily on bacterivory. We speculate that these shifts in nematode community composition and trophic structure could have important implications for ecosystem patterns and processes. First, the low abundance of root-parasitic nematodes (and presumably root herbivory) under woody plants may be one mechanism by which woody plants are able to establish and compete effectively with grasses during succession from grassland to woodland. Second, the large increase in bacterivore nematodes following woody encroachment corresponds to increases in soil microbial biomass, and may accelerate microbial turnover and the mineralization of N. This may also provide a feedback that enables the persistence of N-rich woody plant communities in this system.

Acknowledgements

This research was funded by the NSF Biogeosciences Program (EAR-0525349), Texas AgriLife Research (Project H-6945), and Texas A&M University. We thank James Starr, Ilsa Kantola, Darrin Moore, Helen Rowe, Tim Filley, Julia Liao and Elizabeth Morgan for their assistance with this project.

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