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The Impacts of Fertilization on Mycorrhizal Production and Investment in Western Gulf Coast Grasslands

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ABSTRACT.—Mycorrhizal fungi are ubiquitous components of terrestrial ecosystems that can influence plant performance, abundance and diversity. Patterns of allocation to specific mycorrhizal fungal structures could provide a useful context for understanding belowground dynamics in response to changing resources. Specifically, increased soil nutrient availability has been predicted to favor plants with lower rates of mycorrhizal association. In addition, fertilization has been predicted to favor mycorrhizae with higher investment in storage structures relative to uptake and exchange structures.

A 3 y field experiment was conducted in coastal tallgrass prairie to examine how fertilization affects mycorrhizal abundance and investment patterns. Fertilization significantly increased total mycorrhizal abundance. Specifically, mycorrhizal investment in hyphae, coils and vesicles were significantly increased 53%, 252% and 440% in fertilized plots, respectively. Together these results suggest that mycorrhizae in high fertility soils allocate more to internal fungal storage at a potential cost to plant hosts. Thus, fertilization could have important indirect effects on the aboveground plant community by potentially altering the costs and benefits of mycorrhizae to host plants.

INTRODUCTION

Mycorrhizal fungi are soil organisms that provide soil resources to plants in exchange for reduced carbon used for growth and reproduction (Allen, 1991; Smith and Read, 1997). Arbuscular mycorrhizal fungal associations are ubiquitous components of terrestrial ecosystems that can influence plant performance, abundance and diversity (Johnson, 1993; Eom *et al.*, 1999; Hartnett and Wilson, 1999; Smith *et al.*, 2003; Johnson *et al.*, 2006). They are estimated to associate with 82% of angiosperms (Brundrett, 2002). Mycorrhizal associations can have other important benefits in the form of root protection, disease resistance, enhanced water and inorganic ion uptake, soil aggregation and stimulation of plant growth through auxin production (*e.g.*, Allen, 1991; Read, 1991; Ganade and Brown, 1997; van der Heijden *et al.*, 1998; Eom *et al.*, 2000). Most importantly, arbuscular mycorrhizae may play a role in plant uptake of phosphorus (Hetrick *et al.*, 1989a; Koide, 1991; Read, 1991; Wilson *et al.*, 2001).

In grassland ecosystems, phosphorus availability is often limited because of its rapid fixation as iron, aluminium or calcium phosphates and it's weak ability to be transported by mass flow (Allen, 1991; Ganade *et al.*, 1997; Smith and Read, 1997). However, nitrogen availability is also frequently limited in grassland ecosystems because of poor parent material or volatization of aboveground biomass and detritus in frequently burned areas (Tilman, 1987; Ojima *et al.*, 1990; Seastedt *et al.*, 1991; Eom *et al.*, 1999). Arbuscular mycorrhizae possess external hyphae that reach beyond the root zone of depletion as well as specialized structures within the root to increase plant nutrient acquisition and exchange (Hetrick *et al.*, 1989b; Hetrick *et al.*, 1989c; van der Heijden *et al.*, 1998). Therefore, arbuscular mycorrhizal

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fungi may be particularly important in grassland systems with high phosphorus and nitrogen limitation.

Understanding how plants allocate resources in response to resource fluctuations is well studied and has important implications for our knowledge of how plants respond to changes in resource availability. Focusing attention on the allocation shifts of specific mycorrhizal fungal structures could provide a powerful context for understanding belowground dynamics in response to changing resources and better integrate the functional ecology of plants with other important members of terrestrial ecosystems. Mycorrhizal allocation patterns are expected to be closely linked with resource availability in grasslands because mycorrhizal associations are obligatory for many plant species and have been suggested to be controlled by carbon supplied from the host plant (Johnson, 2003).

Few studies have looked specifically at how mycorrhizae allocate resources to specific fungal structures under different levels of soil fertility; such assessments can provide a more detailed understanding of mycorrhizal structure and function (Johnson, 1993; Klironomos *et al.*, 1996; Johnson *et al.*, 2003a, 2006). For example, across a range of mesic and semiarid grasslands in the United States nitrogen enrichment tended to decrease or increase allocation among mycorrhizal structures in sites of ample or deficient soil phosphorus, respectively (Johnson *et al.*, 2003a). A few studies have quantified mycorrhizal allocation patterns and effectiveness in field settings. For example, field studies have shown decreases in vesicles under high fertility (Egerton-Warburton *et al.*, 2000; Titus *et al.*, 2000; Treseder *et al.*, 2002), decreases or increases in spore numbers of particular mycorrhizal species (Thomson *et al.*, 1992; Egerton-Warburton *et al.*, 2000; Kahiluoto *et al.*, 2001), reductions in arbuscules and coils (Johnson *et al.*, 2003a), decreases in extraradical hyphae (Johnson *et al.*, 2003a) and increased mycorrhizae and vesicles (Pietikainen *et al.*, 2005). However, how ecological factors affect arbuscular mycorrhizal allocation to different fungal structures in the field is not yet well resolved.

Mycorrhizal colonization frequency may change under different levels of soil fertility that may alter the net costs and benefits received by plant hosts (Hetrick *et al.*, 1989a; Johnson, 1993; Johnson *et al.*, 1997; Marler *et al.*, 1999). Changes in the relative benefits of mycorrhizae to different plant hosts are important because they may influence plant species' coexistence, biodiversity and ecosystem function of grassland communities (Hetrick *et al.*, 1988; Hartnett *et al.*, 1993; Johnson, 1993; van der Heijden *et al.*, 1998; Callaway *et al.*, 2003). Fertilization may decrease or eliminate the net benefits that mycorrhizae provide to infected plants (manifested as increased growth and survival) because the plant's cost of carbon allocation to the fungus may not be offset by the benefits of hosting fungi when soil nutrients are less limiting to the plant (Johnson *et al.*, 1992, 1997; Johnson, 1993). Thus, we predict that if fertilization reduces the benefits of mycorrhizae to plants, then plants will decrease their frequency of association as fertilization increases (''Production Hypothesis'' hereafter, developed by Hetrick, 1991).

In addition, the overall benefit of mycorrhizae to plants may decrease with fertilization if the fungal symbionts allocate more resources to growth and reproduction at the expense of nutrient exchange with the plant host. If plants in high fertility soils allocate less carbon to root exudates, mycorrhizal strains that are more aggressive colonizers may proliferate because they convert more of the plant's resources to internal fungal growth and respiration instead of nutrient gathering and exchange (Peng *et al.*, 1993; Graham *et al.*, 1996; Johnson *et al.*, 1997). For example, high fertility may promote shifts in fungal community composition that promote the proliferation of less generous fungi. Rapid fungal colonizers that can overcome plant control of mycorrhizal colonization rates and carbon allocation

would be at an advantage over slower fungal colonizers sensitive to plant regulation (Johnson *et al.*, 1997). Rapid utilization of non-structural carbohydrates by aggressive fungal colonizers would result in increased belowground costs to plants associated with the construction and maintenance of intra- and extraradical fungal structures (Peng *et al.*, 1993; Graham *et al.*, 1996). We predict that if fertilization reduces plant exudates and favors shifts towards a more aggressive, mycorrhizal community composition, then fertilization will increase the production of structures associated with growth and storage (hyphae and vesicles) relative to those associated with the plant host (arbuscules and coils) (''Investment Hypothesis'' hereafter, developed by Johnson, 1993; Johnson *et al.*, 1997, 2006).

If high fertility favors mycorrhizal fungi that reduce allocation to structures for nutrient exchange between the fungi and plant (arbuscules and coils) and increase allocation to structures for internal storage and growth (vesicles and intraradical hyphae), then the benefits of the mycorrhizal symbionts for plant nutrition are anticipated to decrease (Johnson *et al.*, 1997, 2003a).

Many studies have assessed how the experimental reduction of mycorrhizal fungi and fertilization may affect mycorrhizal colonization and abundance in central to northern grasslands of the United States, which may in turn affect host plant competitive abilities, species composition and plant diversity in greenhouse and field studies (Grime *et al.*, 1987; Hetrick *et al.*, 1988; van der Heijden *et al.*, 1998; Hartnett *et al.*, 1999; Eom *et al.*, 2000; Johnson *et al.*, 2003b). However, no information exists on how mycorrhizal fungi allocate resources to specific fungal structures in Western Gulf Coastal grasslands. Coastal grasslands and coastal tallgrass prairie specifically, are a threatened ecosystem in the United States with less than 1% remaining (Grace, 1998). The purpose of this study was to assess how multiple years of anthropogenic fertilization impact mycorrhizal investment patterns at the plant-root interface. We address the following questions: (1) Does increased nutrient availability decrease mycorrhizal colonization frequency? (2) Does fertilization favor mycorrhizae with higher investment in storage structures relative to uptake and exchange structures?

METHODS

Field experiment.—In Jun. 2001, 32 2 m × 2 m plots were established at the University of Houston Coastal Center, a research area located approximately 50 km southeast of Houston, TX, USA in the Western Gulf Coastal Prairie (Harcombe *et al.*, 1993). This site is jointly phosphorus and nitrogen limited (Smeins *et al.*, 1991). The Western Gulf Coastal prairie is considered a distinct ecosystem that is not included in the north-south continuum of tallgrass prairie because of its more semitropical climate, proximity to the Gulf coast, distinct natural processes and geological origin with subsequent succession since the Pleistocene (Diamond and Smeins, 1984; Diamond and Fulbright, 1990; Bailey, 1994). The regional climate is semitropical with 1200 mm annual mean rainfall and a 250 d growing season (Harcombe *et al.*, 1993). Soils are Lake Charles clay (fine, montmorillonitic, theric Typic Pelludert) containing a 40–60% clay content. The primary vegetation in the field where the experiment was established included the C_4 grass dominants *Schizachyrium scoparium* (Michx.) (little bluestem), *Muhlenbergia capillaries* (Lam.) (Muhly grass) and *Andropogon glomeratus* (Walt.) (bushy bluestem) (unpublished data). A diverse assemblage of graminoids, perennial forbs and shrubs comprise the remaining plant cover in the field.

Each plot was randomly assigned to a NPK treatment in a randomized design (16 control and 16 fertilized plots). Plots in the fertilized treatment received 4 g/m^2 of nitrogen, 1.3 g/m^2 of phosphorus, 2.6 g/m^2 of potassium and micronutrients (Vigoro Ultra Turf fertilizer) twice per growing season. Fertilizer was broadcast in a day prior to forecasted rain.

In late Sept. 2003, four background soil cores (2 cm diameter by 16 cm deep) were collected from each experimental plot. Samples were stored at 4 C until they could be processed. Roots were washed on a 500-micrometer sieve and stored in 50% ethanol until processed for staining. Roots were cleared in 10% KOH at 80 C for 45 min then rinsed and soaked in 10% bleach for additional clearing. Roots were rinsed and acidified in concentrated HCl for 5 min (Bray *et al.*, 2003). Fine roots from each core were stained and dyed in trypan blue and ten 1 cm root fragments were mounted on microscope slides and measured for arbuscular mycorrhizal colonization rates following modification of the gridline intersect method (McGonigle *et al.*, 1990; Brundrett *et al.*, 1996). A total of 100 intersections, 10 per root fragment, were viewed on each slide. Mounted roots were scored for the presence of vesicles, arbuscules, coils and hyphae of arbuscular mycorrhizal fungi under a compound microscope at 400× magnification. Nonmycorrhizal fungi and microbes were also noted. Arbuscular mycorrhizae were distinguished from nonmycorrhizal fungi by the methods of Callaway *et al.* (2003).

Data analysis.—We performed two sets of analyses. The first set tested whether different structures responded significantly to fertilization and the second set tested whether their magnitudes of changes in response to fertilization varied significantly.

We performed ANOVAs to test whether fertilization treatement changed the frequency of occurrence of different structures. For these analyses, the four background cores were averaged to give one percentage value per plot for total mycorrhizal root colonization, arbuscules, vesicles, coils, hyphae per plot for mycorrhizal fungi and non-mycorrrhizal fungi and endophytic microbes respectively. Percentage total root colonization and mycorrhizal hyphae had non-normal residuals distributions and were transformed with a squareroot function to achieve normality and homogeneity of variances. Data for arbuscules, vesicles, coils and endophytic microbes could not be transformed to approximate a normal distribution of the residuals. These data were ranked and tested using a non-parametric version of ANOVA (Conover and Iman, 1981, SAS Institute, Cary, NC). Data were also tested with a Mann-Whitney non-parametric test and results were consistent with the ANOVA.

A second analysis examined how strongly each mycorrhizal structure responded to fertilization in comparison to other structures. This ANOVA included fertilization, plot (nested within fertilization), the factor ''structure type'' (with five levels: arbuscules, hyphae, vesicles, coils and endophytic hyphae) and the structure type*fertilization interaction (SAS Institute, Cary, NC). A significant interaction in this model indicates that structures differed in the magnitude of their response to fertilization. Finally, we constructed *a priori* planned contrast tests for each mycorrhizal structure that compared the fertilization treatment to the control for that structure.

RESULTS

Total mycorrhizal colonization ($F_{1,30} = 5.8$, $P = 0.0226$), hyphae ($F = {}_{1,30} = 4.4$, $P = 0.0444$), coils ($F_{1,30} = 4.9$, $P = 0.0349$) and vesicle production ($F_{1,30} = 18.1$, $P = 0.0002$) were significantly increased by fertilization (Fig. 1a–d). Allocation to hyphae, coils and vesicles were increased under fertilization 53%, 253%and 440%, respectively. Mycorrhizal arbuscules ($F_{1,30} = 0.2$, $P = 0.6520$) and endophytic microbes ($F_{1,30} = <0.1$, $P = 0.8750$) were not significantly affected by fertilization (Fig. 1e–f).

The magnitude of response to fertilization differed among the mycorrhizal structures as shown by a significant structure*fertilization interaction (Table 1). Planned contrasts revealed significant increases in coils and vesicles with fertilization (Table 2) with no

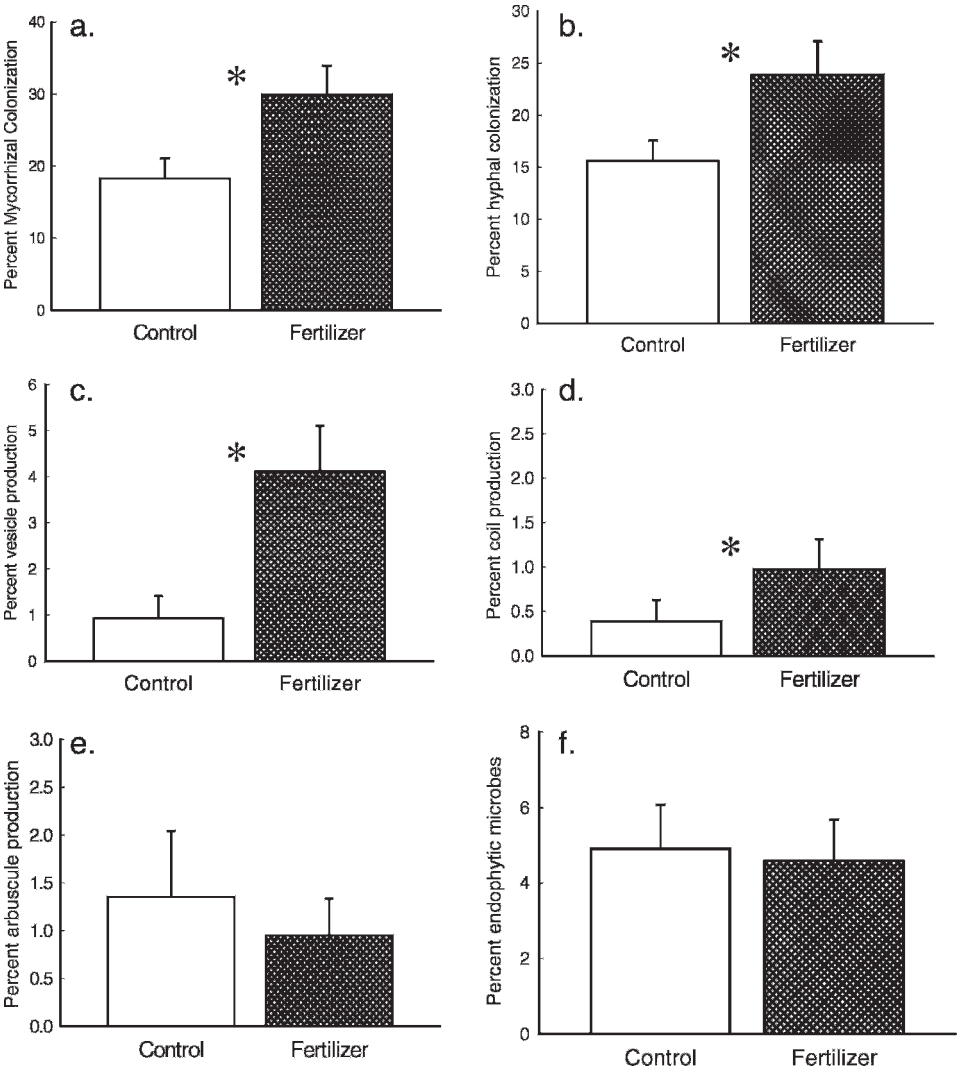

FIG. 1.—The dependence of (a) percent total mycorrhizal colonization, (b) hyphae, (c) vesicles, (d) coils, (e) arbuscules and (f) endophytic microbes on fertilization. +1 standard errors are presented. Asterisks denote significant results ($P < 0.05$)

significant response by hyphae or arbuscules. This suggests that coils and vesicles are increasing particularly strongly in response to fertilization.

DISCUSSION

Fertilization significantly altered mycorrhizal allocation patterns in this coastal tallgrass prairie. Results of this study strongly supported the Investment Hypothesis. Fertilization significantly increased mycorrhizal growth and storage structures (hyphae, Fig. 1b and

TABLE 1.—Dependence of mycorrhizal structure type to experimental treatments. Significant treatment effects are highlighted in bold

Terms	df	SS	F-value	P-value
Model	39	328,080	6.39	<0.0001
Error	120	157,990		
Fertilization	1	15,524	11.79	0.0008
Structure	4	239,323	45.44	<0.0001
Fertilization*Structure	4	14,531	2.76	0.0309
Plot(Fertilization)	30	58,703	1.49	0.0697

vesicles, Fig. 1c). Although coil production also increased significantly with fertility (253% increase, Table 2, Fig. 1d) and coils are thought to function in nutrient exchange, the much greater allocation to vesicles in higher fertility soils (440% increase, Table 2, Fig. 1c), suggests that these fungi are increasing internal storage reserves at the potential cost of nutrient exchange with the plant.

To our knowledge, this is the first study to document increases in both intraradical hyphae and vesicle production following fertilization in grassland field soils. Although a number of empirical greenhouse and field studies have shown changes in mycorrhizal colonization with increased fertility (*e.g.*, Menge *et al.*, 1978; Bentivenga and Hetrick, 1992; Johnson, 1993; Olsson *et al.*, 1997; Cordiki *et al.*, 2002), few have quantified changes in allocation to specific mycorrhizal structures in field conditions as a relative measure of mycorrhizal functioning (Klironomos *et al.*, 1996). Pietikainen *et al.* (2005) others showed increased mycorrhizal hyphae and vesicles of *Solidago virgaurea* in subarctic meadows, but did not document significant increases for any other species with fertilization.

Our study cannot disentangle whether changes in mycorrhizal allocation reflect different allocation patterns of individual mycorrhizal fungi species or overall shifts in the mycorrhizal community composition. However, the dramatic increase in vesicle numbers between fertilized and unfertilized soils suggests that mycorrhizae in fertilized soils could yield lower net benefits or perhaps a net cost to their hosts because they exchange similar soil resources (no change in arbuscule number) while increasing their own storage structures (vesicles) (Fig. 1, Johnson, 1993). However, it is also possible that the increased vesicle numbers found in fertilized soils are due solely to greater nutrient abundance. If so, mycorrhizal fungi in high fertility would be able to increase storage reserves without decreasing benefits to the plant host. Future studies will need to be conducted to verify if such changes in mycorrhizal production and investment are indeed indicative of costs and benefits to aboveground plant fitness.

TABLE 2.—Planned *a priori* contrasts for each mycorrhizal structure type contrasting unfertilized versus fertilized plots. Significant treatment effects are highlighted in bold

Contrast	df	Contrast SS	F-value	P-value
Arbuscules	1	237	0.18	0.6724
Coils	1	6815	5.18	**0.0247**
Endophytic Microbes	1	32	0.02	0.8764
Hyphae	1	657	0.50	0.4813
Vesicles	1	22,313	16.95	**<0.0001**

Contrary to support for the Investment Hypothesis, the predicted decrease in mycorrhizal association under high fertility (Production Hypothesis) was not supported because fertilization significantly increased total mycorrhizal colonization (Fig. 1a). Patterns here are opposite of results expected if plants control mycorrhizal growth via root exudation because when nutrient supply is less limiting, plants that allocate carbohydrates to growth, reproduction, or defense should outperform those with greater root exudation (Treseder *et al.*, 2002).

In the coastal grassland we studied, these changes in allocation to specific mycorrhizal structures may be one factor influencing the decline in percent cover of the obligately mycorrhizal C_4 grasses (*Schizachyrium scoparium, Muhlenbergia capillaries* and *Andropogon glomeratus* control = 40% cover, fertilized = 23% cover) and increase in woody species (*Sapium sebiferum, Rubus louisianus, Myrica cerifera* and *Ilex vomitoria* control = 54%, fertilized = 70%) (unpublished data). While each of these woody genera can form endomycorrhizal associations, it is not known to what extent these species utilize (facultative association) or require (obligate association) mycorrhizae in field conditions (Sylvia, 1990; Poole and Sylvia, 1991; Semones and Young, 1995; Taylor and Harner, 2000; Nijjer *et al.*, 2004). Overall changes in mycorrhizal allocation may reduce the ability of obligately mycorrhizal C_4 dominants to compete with woody species.

In conclusion, nitrogen and phosphorus fertilization altered patterns of mycorrhizal allocation in this coastal grassland. Under fertilization, strong increases in hyphal and vesicle investment combined with no change in arbuscule production suggest that mycorrhizal growth and storage reserves expanded at the potential expense of nutrient exchange with plant hosts. Although measurements of costs and benefits for aboveground plant fitness will be necessary to elucidate the broader ecological impacts of these changes, increased mycorrhizal production and investment under elevated fertility could have important consequences for the structure and function of the aboveground plant community in this endangered ecosystem.

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