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PLANT WINNERS AND LOSERS DURING GRASSLAND N-EUTROPHICATION DIFFER IN BIOMASS ALLOCATION AND MYCORRHIZAS

Nancy Collins Johnson,[1,5] Diane L. Rowland,[1,2] Lea Corkidi,[3,4] and Edith B. Allen[3]

[1]*Center for Environmental Sciences and Education, Northern Arizona University, P.O. Box 5694, Flagstaff, Arizona 86011-5694 USA*
[2]*USDA-ARS, National Peanut Research Laboratory, 1011 Forrester Drive S.E., Dawson, Georgia 31742-0509 USA*
[3]*Department of Botany and Plant Sciences, University of California, Riverside, California 92521-0124 USA*
[4]*Tree of Life Nursery, P.O. Box 635, San Juan Capistrano, California 92693 USA*

Abstract. Human activities release tremendous amounts of nitrogenous compounds into the atmosphere. Wet and dry deposition distributes this airborne nitrogen (N) on otherwise pristine ecosystems. This eutrophication process significantly alters the species composition of native grasslands; generally a few nitrophilic plant species become dominant while many other species disappear. The functional equilibrium model predicts that, compared to species that decline in response to N enrichment, nitrophilic grass species should respond to N enrichment with greater biomass allocation aboveground and reduced allocation to roots and mycorrhizas. The mycorrhizal feedback hypothesis states that the composition of mycorrhizal fungal communities may influence the composition of plant communities, and it predicts that N enrichment may generate reciprocal shifts in the species composition of mycorrhizal fungi and plants. We tested these hypotheses with experiments that compared biomass allocation and mycorrhizal function of four grass ecotypes (three species), two that gained and two that lost biomass and cover in response to long-term N enrichment experiments at Cedar Creek and Konza Long-Term Ecological Research grasslands. Local grass ecotypes were grown in soil from their respective sites and inoculated with whole-soil inoculum collected from either fertilized (FERT) or unfertilized (UNFERT) plots. Our results strongly support the functional equilibrium model. In both grassland systems the nitrophilic grass species grew taller, allocated more biomass to shoots than to roots, and formed fewer mycorrhizas compared to the grass species that it replaced. Our results did not fully support the hypothesis that N-induced changes in the mycorrhizal fungal community were drivers of the plant community shifts that accompany N eutrophication. The FERT and UNFERT soil inoculum influenced the growth of the grasses differently, but this varied with site and grass ecotype in both expected and unexpected ways suggesting that ambient soil fertility or other factors may be interacting with mycorrhizal feedbacks.

Key words: Agropyron repens; *allocation plasticity;* Andropogon gerardii; *arbuscular mycorrhizas; Cedar Creek Natural History Area; functional equilibrium model; Konza Prairie Research Natural Area; LTER site; mycorrhizal feedback; nitrogen eutrophication;* Panicum virgatum; *root : shoot ratio*.

Introduction

Anthropogenic nitrogen inputs currently equal or exceed natural N inputs in many ecosystems (Vitousek et al. 1997). Nitrogen eutrophication of grasslands often increases productivity, reduces diversity, and changes community composition (Berendse et al. 1993). The changes in grassland structure caused by long-term fertilization may last decades, or may even be irreversible (Semelová et al. 2008). Generally, a few fast-growing "nitrophilic" species become dominant while other species disappear from N-enriched systems. Many grasslands are naturally N limited and their native flora have evolved mechanisms to cope with low N availability (Chapin 1980). Removing N limitation from these systems changes the competitive landscape so that fast-growing species with high N requirements overshadow slow-growing species adapted to infertile soil (Bobbink 1991). Consequently, N eutrophication may cause grasslands to change from being N limited to light limited as the dominant plant species grow denser and taller (Tilman 1988). Traits related to a plant's ability to compete for N or light are expected to be predictive of their responses to N enrichment.

The functional equilibrium model predicts that the relative availability of light and soil resources influences plant allocation to above- and belowground structures; reducing light availability will cause increased allocation to shoots, and adding soil nutrients will cause reduced allocation to roots (Brouwer 1983, Tilman 1988, Ericsson 1995). Grassland plant species that respond to N eutrophication by rapidly shifting their biomass

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[5] E-mail: Nancy.Johnson@nau.edu

PLATE 1. The roots of *Agropyron repens* (left), the winner species at Cedar Creek, are much finer than those of *Andropogon gerardii* (right). Plants with fine roots are generally less mycotrophic than those with coarse roots. Photo credit: Rick Johnson, Jana Johnson, and Claire Johnson.

allocation aboveground to favor light-harvesting structures are expected to outcompete species with a less responsive root : shoot ratio (Tilman 1988). The functional equilibrium model may also apply to plant allocation to root symbionts that help plants acquire limited soil resources. If fertilization eliminates limitation of soil nutrients but increases light limitation, then these symbioses may become more of a cost than a benefit and plants may reduce their carbon allocation to these partnerships. For example, it is well known that nodule formation by rhizobium bacteria in legumes can be inhibited by N fertilization (Kiers et al. 2002). Also, as predicted by the functional equilibrium model, N enrichment can reduce plant allocation to mycorrhizas in P-rich soil, but increase allocation to mycorrhizas in P-poor soil because higher N availability exacerbates P limitation and increases the value of mycorrhizas for P uptake (Johnson et al. 2003).

Arbuscular mycorrhizal (AM) fungi are among the most abundant soil organisms in grasslands (Olsson et al. 1999). These fungi generally form mutualisms with plants by trading soil resources for photosynthates, but not all AM partnerships are equally beneficial for plants; neutral and parasitic AM symbioses are known to occur (Johnson et al. 1997, Klironomos 2003). Plant species vary in the degree to which they forage with mycorrhizas, ranging from obligate mycotrophs that require mycorrhizas to survive and reproduce to non-mycotrophic species that never form functional mutualisms. In between these extremes are facultative mycotrophs that benefit from mycorrhizas only when soil resources are in limiting supply. Most plant species are facultative mycotrophs. We hypothesize that plant species that increase in abundance in eutrophied grasslands may be less mycotrophic than species that decrease in abundance because nitrophilic plants generally grow tall quickly and are excellent competitors for light; consequently, they allocate relatively more biomass aboveground to shoots and relatively less to roots and mycorrhizas.

The species composition of plant and AM fungal communities reciprocally influence each other (Bever et al. 1997, van der Heijden et al. 1998) and may be important in structuring plant communities (Klironomos 2002, 2003, Sigüenza et al. 2006*a*). It is possible that feedbacks between plants and their associated AM fungi may contribute to the plant community changes that accompany N enrichment. Previous experiments indicate that N fertilization changes the species composition of AM fungal communities in grasslands (Egerton-Warburton et al. 2000, 2007). If plants grow differently when associated with AM fungi from N-enriched soil compared with those in un-enriched soil, then mycorrhizas may play a role in the plant composition changes that accompany terrestrial N eutrophication. There is support for this idea; compared to AM fungi in unfertilized soil, those from N-fertilized sites have been shown to be inferior mutualists to certain native grasses in mesic (Johnson 1993) and semiarid (Corkidi et al. 2002) grasslands and shrublands (Sigüenza et al. 2006*a*). This suggests that negative feedback between AM fungi and their plant hosts may be a driver of plant community changes with eutrophication. Alternatively, positive feedback could drive community shifts if nitrophilic plants grow best with AM fungi in N-fertilized soil.

The purpose of this research is to compare the biomass allocation and mycotrophy of grass species that are winners and losers during N eutrophication and examine their responses to AM fungi and associated soil

TABLE 1. Soil properties, disturbance history, and winner and loser grass species used in experiments from each of the LTER sites.

LTER site	Soil properties					Disturbance history	Grass species	
	Soil NH_4-N (mg/kg)	NO_3-N (mg/kg)	PO_4-P (mg/kg)	N:P	pH		Winner	Loser
Cedar Creek	8.8 (1.6)	2.1 (1.6)	39.8 (2.8)	0.3 (0.02)	6.4 (0.5)	old field, 40 years post agriculture	*Agropyron repens*	*Andropogon gerardii*
Konza	33.7 (1.5)	5.4 (1.6)	11.1 (1.0)	3.5 (0.35)	6.6 (0.7)	undisturbed tallgrass prairie	*Panicum virgatum*	*Andropogon gerardii*

Note: Data are from Johnson et al. (2003), values are means (with SE in parentheses) of early, mid-season and late-season samples.

organisms from N-fertilized (FERT) or unfertilized (UNFERT) soils. We selected dominant grass species in FERT and UNFERT plots at two grasslands that have experienced significant plant community shifts in response to long-term N enrichment. Our experiments test four hypotheses linking mycorrhizal structure and function with plant community shifts associated with N eutrophication:

H_1: Winner grass species allocate relatively more biomass to shoots and less to roots and mycorrhizas than loser species;

H_2: Winner grass species are less responsive to mycorrhizas (i.e., mycotrophic) than loser species;

H_3: Loser grass species grow better when they are colonized by AM fungi from UNFERT soil than those from FERT soil (evidence for negative feedback); and

H_4: Winner grasses grow better when they are colonized by AM fungi from FERT soil than those from UNFERT soil (evidence for positive feedback).

Insights from this research will help us better understand the mechanisms causing the community shifts that often accompany terrestrial eutrophication.

MATERIALS AND METHODS

Study sites

Materials for these experiments were collected from long-term N enrichment experiments at two grasslands within the Long-Term Ecological Research (LTER) network: Cedar Creek Natural History Area, 50 km north of Minneapolis, Minnesota, USA, and Konza Prairie Research Natural Area, 20 km south of Manhattan, Kansas, USA. Detailed information about each site is provided in Johnson et al. (2003), therefore, we will only summarize some of the important site characteristics. The climate at both sites is mid-continental, with warm summers and cold winters. Mean annual precipitation is 660 mm/yr at Cedar Creek and 835 mm/yr at Konza. Characteristics of the soil, disturbance history, and dominant grass species at each of these sites are summarized in Table 1.

Biological materials

Two grass species, a "winner" and a "loser" with N enrichment, were selected from each LTER site based upon the results of previous studies. Tilman (1988) showed that long-term fertilization at Cedar Creek decreased the abundance of *Andropogon gerardii* and increased the abundance of *Agropryon repens* (see Plate 1). Gibson et al. (1993) found that N enrichment at Konza also reduced *A. gerardii* abundance and *Panicum virgatum* took its place as a dominant plant. Consequently, the Cedar Creek experiment used a local genotype of *A. gerardii* from Prairie Restorations in Princeton, Minnesota, USA, near Cedar Creek. *Agropyron repens* is a nonnative invasive species at Cedar Creek that spreads primarily through vegetative reproduction so we acquired *A. repens* seeds from V&J Seed farms in Woodstock, Illinois, USA. The Konza experiment used *A. gerardii* and *P. virgatum* seeds that were collected on the Konza Prairie.

Live soil inoculum was collected from N-enriched (FERT) and control (UNFERT) plots. At each LTER site, fertilizer had been applied as granular NH_4NO_3 to replicate plots that were randomly arranged among unfertilized control plots. At the time of this research, 170 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ and 200 kg $P{\cdot}ha^{-1}{\cdot}yr^{-1}$ had been added for 9 years at Cedar Creek; and 100 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ had been added for 11 years at Konza. Communities of AM fungi are known to differ significantly in FERT and UNFERT soils at both of these sites (Egerton-Warburton et al. 2007). Shifts in the community composition of other soil organisms are also likely, but have not been measured.

Experimental setup

Factorial greenhouse experiments were used to examine the main effects and interactions of host plant species (winner or loser), origin of soil organisms (from fertilized [FERT] or unfertilized [UNFERT] field plots plus sterilized controls), and level of N application in the greenhouse (HIGH N or LOW N). Each treatment combination was replicated 10 times with two harvests (6 and 12 weeks) for a total of 480 plants.

Soil from unfertilized areas at each site was mixed 1:1 with silica sand and steam sterilized for three hours on two consecutive days. Pots (6.4 cm diameter × 25 cm deep DeePot, Steuwe & Sons, Corvallis, Oregon, USA) were filled with 650 mL of the sterile soil–sand mixture. A band of live soil inoculum was layered 8 cm from the top of each pot and covered with more sterile soil–sand. This inoculum consisted of fresh soil collected from a composite of the 10 FERT or 10 UNFERT plots at each

site. Care was taken to ensure that the nutrients were the same across inoculum treatments. To do this, each pot received a combination of living and sterilized soils from both treatments: FERT inoculum consisted of 30 g of living FERT soil and 30 g of sterilized UNFERT soil, UNFERT inoculum consisted of 30 g of living UNFERT soil and 30 g of sterilized FERT soil, and CONTROL inoculum consisted of 30 g of sterilized soil from both FERT and UNFERT plots. Differences in microbial communities among treatments were reduced by applying 5 mL of a microbial wash to each pot (Koide and Li 1989). This wash was prepared by mixing 3.5 kg of FERT soil and 3.5 kg of UNFERT soil with 2 L of deionized water. The supernatant was filtered through a 25-μm sieve three times.

Seeds were soaked for 10 minutes in 10% bleach, rinsed with water, and sown in sterilized vermiculite. Five days after germination 120 seedlings per species per site were transplanted into the pots. Care was taken to select seedlings that were uniform in size and development. Any plants that died during the first week were replanted. Plants were randomly arranged and regularly rotated in the greenhouse and maintained at a mean temperature of 25°C days and 18°C nights. The free-draining pots were watered with deionized water and on alternate days with a dilute nutrient solution containing 8.4 mg/L $Ca(NO_3)_2{\cdot}4H_2O$; 199 mg/L $CaSO_4{\cdot}2H_2O$; 130 mg/L K_2SO_4; 72 mg/L $MgSO_4$; 0.86 mg/L H_3BO_3; 0.54 mg/L $MnCl{\cdot}4H_2O$; 0.07 mg/L $ZnSO_4$; 0.02 mg/L $CuSO_4{\cdot}5H_2O$; and 0.03 mg/L NaCl. Pots receiving the HIGH N treatment were given the same solution plus 433 mg/L KNO_3, and those receiving the LOW N treatment did not receive supplemental N.

The experiments were maintained in the greenhouse at full sunlight from June through August. Ten randomly selected replicates were harvested at 6 and 12 weeks after initiation of the study. At each harvest, plants were removed from their pots, roots were carefully washed, and shoots and roots were separated, dried at 60°C for 48 hours, and weighed. A subsample of roots was cleared and stained using the technique of Koske and Gemma (1989), and percentage AM colonization was measured using the magnified intersect method (McGonigle et al. 1990).

Data analysis

The influence of plant species, inoculum source, and N availability on plant height, biomass, root : shoot ratio, and AM colonization was analyzed using three-factor ANOVA. A natural-logarithm transformation was used to improve the normality of the plant biomass data and root colonization data were arcsine square-root transformed. Tukey hsd multiple-comparison tests were used to compare means. All analyses were conducted using JMP 4.0 (SAS 1997).

Plant response to AM fungi and other soil organisms was calculated as the natural logarithm of the ratio of the total dry mass (TDM) of each species with living or dead AM fungal inoculum using the following equation: $\ln (TDM_{+AM}/TDM_{-AM})$. For these calculations, the means of +AM plants (inoculated with FERT or UNFERT soil) were paired with the means of –AM plants (inoculated with CONTROL soil) within the same species and N treatments.

Results

Plant height and biomass allocation

Winner and loser grass species responded significantly differently to soil inoculum and N availability. At both LTER sites and both harvests, the winner species was significantly taller than the loser species ($P < 0.001$). In the Cedar Creek experiment at 6 weeks, the height of *Agropyron repens* was 37 ± 0.6 cm [mean ± SE] vs. 22 ± 0.6 cm for *Andropogon gerardii*; and at 12 weeks, the height of *A. repens* was 41 ± 1.0 cm vs. 35 ± 1.0 cm for *A. gerardii*. Similarly in the Konza experiment at 6 weeks, the height of *Panicum virgatum* was 40 ± 1.1 cm vs. 19 ± 0.5 cm for *A. gerardii*; and at 12 weeks, the height of *P. virgatum* was 65 ± 2.0 cm vs. 45 ± 1.7 cm for *A. gerardii*. Also, *A. gerardii* had lower shoot biomass and higher root : shoot ratio than the winner species at both sites and both harvest dates (Figs. 1 and 2). At 6 weeks at both sites there was a significant interaction between host and N in the responses of shoot biomass and root : shoot ratio. The loser, *A. gerardii,* was insensitive to N application, while the corresponding winner species (*A. repens* at Cedar Creek and *P. virgatum* at Konza) significantly increased shoot biomass and reduced root : shoot ratio in the HIGH N treatment (Figs. 1 and 2). By 12 weeks all of the plant species adjusted their allocation above- and belowground so root : shoot ratio was lower in the HIGH N treatment.

There were significant interactions between host species and N level, and host species and inoculum source in biomass allocation. At Cedar Creek, shoot biomass of the winner (*A. repens*) was always larger at HIGH N irrespective of inoculum source; it grew equally with the CONTROL, FERT, and UNFERT treatments. In contrast, shoot biomass of the loser (*A. gerardii*) was unresponsive to N level at 6 weeks, and the degree to which it responded to N at 12 weeks depended on the inoculum—HIGH N caused shoot biomass to increase the most in plants grown with UNFERT inoculum and least with those grown with CONTROL inoculum. Shoot biomass of the loser at Konza (*A. gerardii*) was also unresponsive to N level at 6 weeks; and at 12 weeks it responded to N the least in plants grown without mycorrhizas (CONTROL treatment). Shoot biomass of the winner at Konza (*P. virgatum*) increased significantly with HIGH N at both harvests and was also influenced by inoculum source; plants grown with the CONTROL or UNFERT inocula had the largest increase in shoot biomass with HIGH N (Figs. 1 and 2).

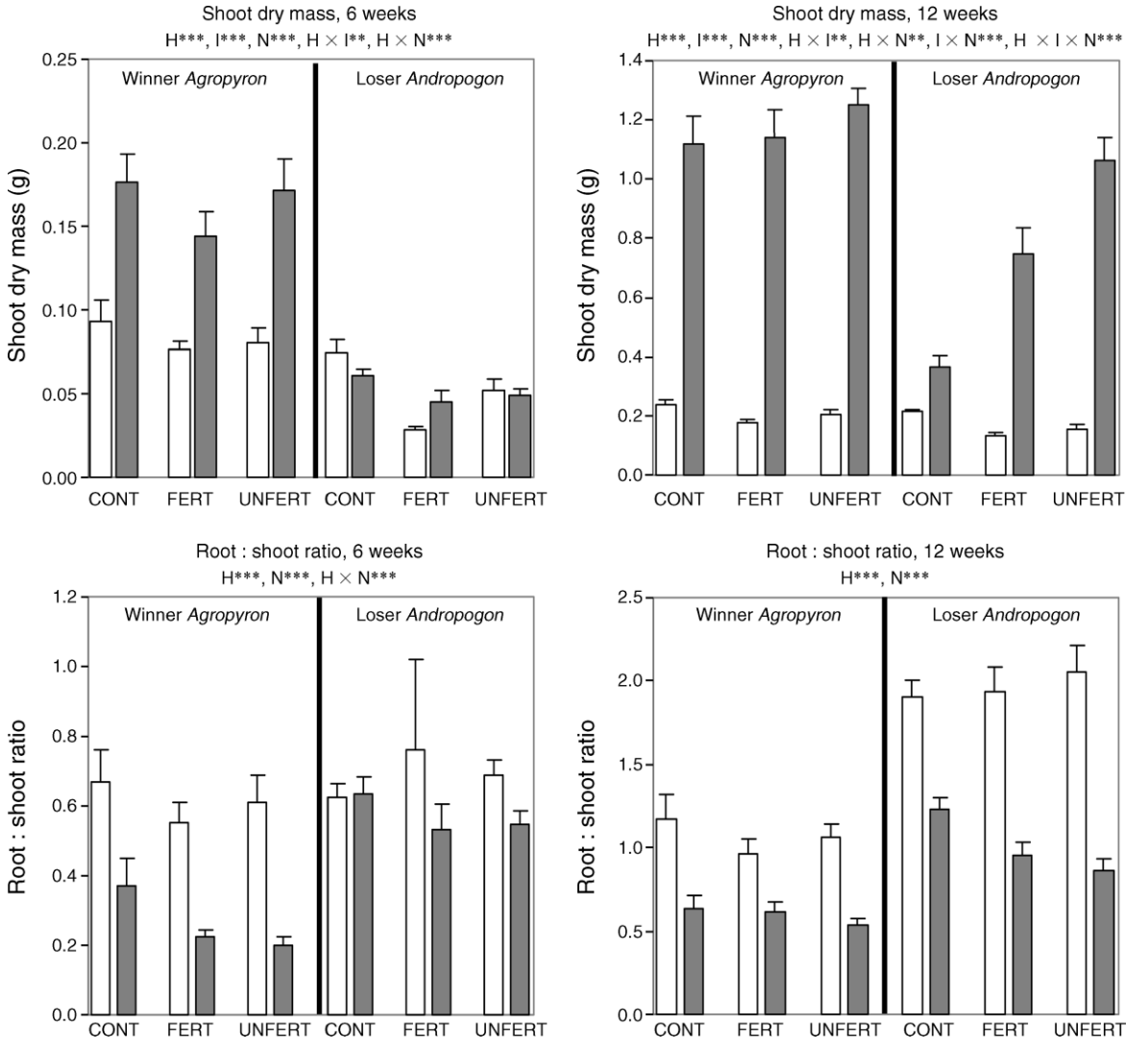

FIG. 1. Shoot dry mass and root : shoot ratio of *Agropyron repens* and *Andropogon gerardii* from the Cedar Creek LTER (Minnesota, USA) experiment at 6 and 12 weeks. Data are means ± SE; $n = 10$ replications. Open bars represent plants grown with LOW N, and shaded bars represent plants grown at HIGH N availability in the greenhouse. Plants were inoculated with sterilized soil (control, CONT) or soil from fertilized (FERT) and unfertilized (UNFERT) field plots. The significance levels of the host species (H), inoculum (I), or nitrogen (N) treatments and interactions, as determined by ANOVA, are indicated as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. The *y*-axis scales vary among graphs.

Total biomass responses to soil inoculum

The total dry mass of loser and winner plant species responded to AM fungi and other soil organisms differently as measured by the ratio: ln (TDM_{+AM}/TDM_{-AM}), where TDM = total dry mass, AM = arbuscular mycorrhizal fungi. The loser species, *A. gerardii*, responded more positively to mycorrhizas than the winner species in both harvests in the Konza trial and at the second harvest in the Cedar Creek trial (Fig. 3). In both trials, plant response to mycorrhizas was generally greater (more positive) in the HIGH N than the LOW N treatments (Fig. 3). Furthermore, *A. gerardii* grown in the Cedar Creek system and *P. virgatum* in the Konza system had a significantly more positive (or less negative) growth response to UNFERT inoculum compared to FERT inoculum.

Mycorrhizal colonization

Plants inoculated with CONTROL soil had very low or no colonization, while those inoculated with FERT and UNFERT soils became colonized (Figs. 4 and 5). Percent root length colonized by all AM fungal structures (total colonization) and percentage root length containing arbuscules doubled from week 6 to week 12 in the Cedar Creek experiment, but not in the Konza experiment. Host plant species was a significant factor in determining root colonization in both sites. At 6 weeks, the winner grass species had significantly less

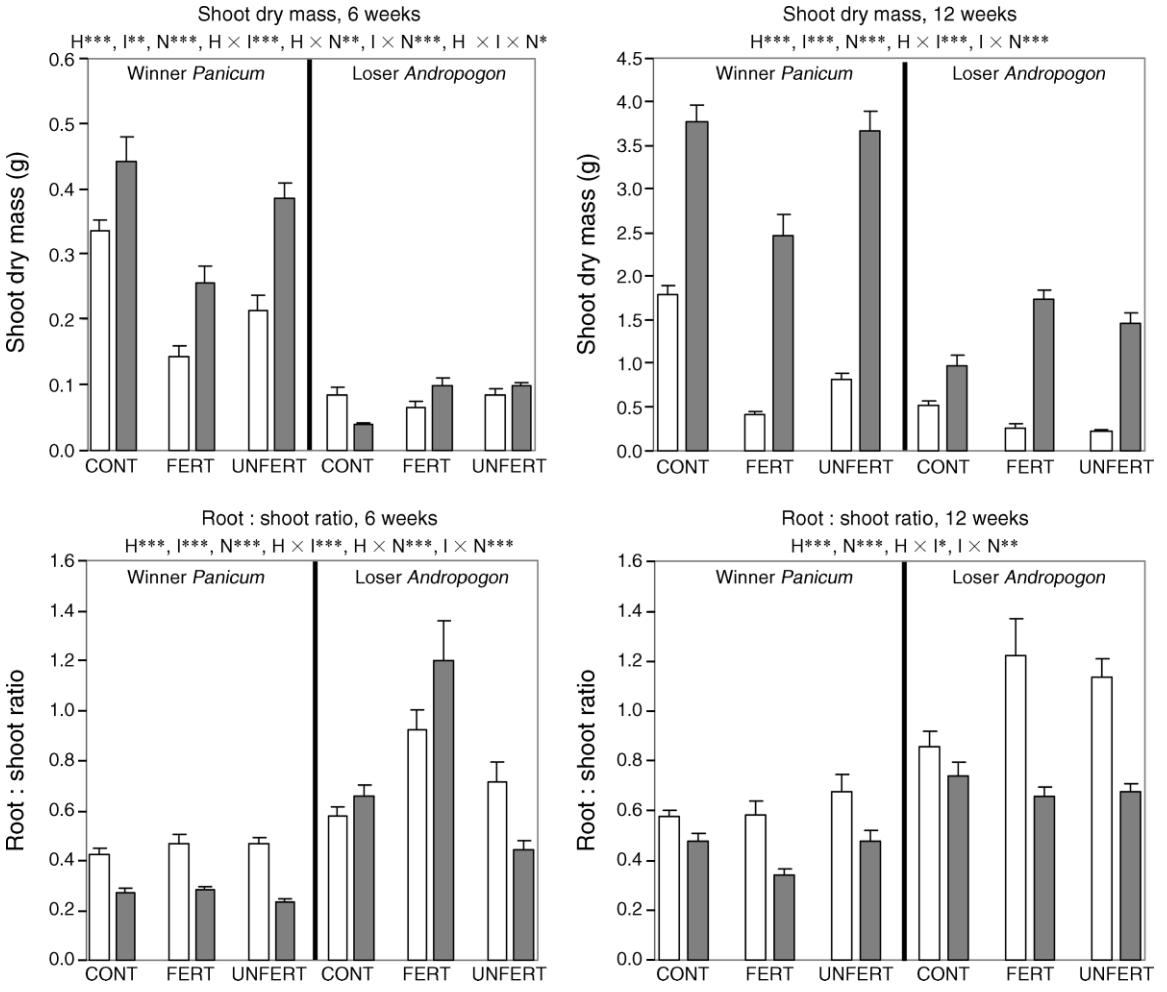

FIG. 2. Shoot dry mass and root : shoot ratio of *Panicum virgatum* and *Andropogon gerardii* from the Konza LTER experiment (Kansas, USA) at 6 and 12 weeks. Data are means ± SE; $n = 10$ replications. Format is as in Fig. 1.

AM colonization and fewer arbuscules compared to *A. gerardii*; however, this difference disappeared in the 12-week-old plants at Konza. In both experiments at 12 weeks there was a significant interaction between N and host species on AM colonization. In the Cedar Creek experiment, HIGH N significantly increased total and arbuscular colonization of *A. gerardii* (Fig. 4). In contrast, in the Konza experiment, arbuscular colonization of *P. virgatum* was significantly lower in the HIGH N treatment compared to the LOW N treatment (Fig. 5). At Cedar Creek, inoculum source (i.e., FERT or UNFERT soil) did not influence colonization in either host species, but in the Konza experiment, *P. virgatum* had higher total AM colonization at 6 weeks, and lower arbuscular colonization at 12 weeks if it was inoculated with FERT soil.

DISCUSSION

Our findings strongly support our first two hypotheses. In both experiments, the winner grass species was taller, allocated less biomass to roots, and benefited less from mycorrhizas compared to the loser species. Also, the winner species responded to N enrichment with greater plasticity in biomass allocation to shoots, roots, and mycorrhizal fungi than the loser species. All of these traits are predicted by the functional equilibrium model because N enrichment is expected to reduce limitation of belowground resources so that plant taxa that grow tall and allocate less to roots and mycorrhizae and more to light-harvesting shoots will be at an advantage compared to those that are not as plastic in their carbon allocation.

Our results provide evidence for negative feedback (H_3) at the Cedar Creek LTER site, but not at the Konza LTER site. The loser at Cedar Creek *Andropogon gerardii*, grew larger when inoculated with UNFERT soil than when inoculated with FERT soil (Figs. 1 and 3). This suggests that long-term fertilization changed the functioning of communities of arbuscular mycorrhizal (AM) fungi and other associated soil organisms. Inferior AM mutualisms may be selected by fertilization if plants reduce C allocation belowground in response to nutrient

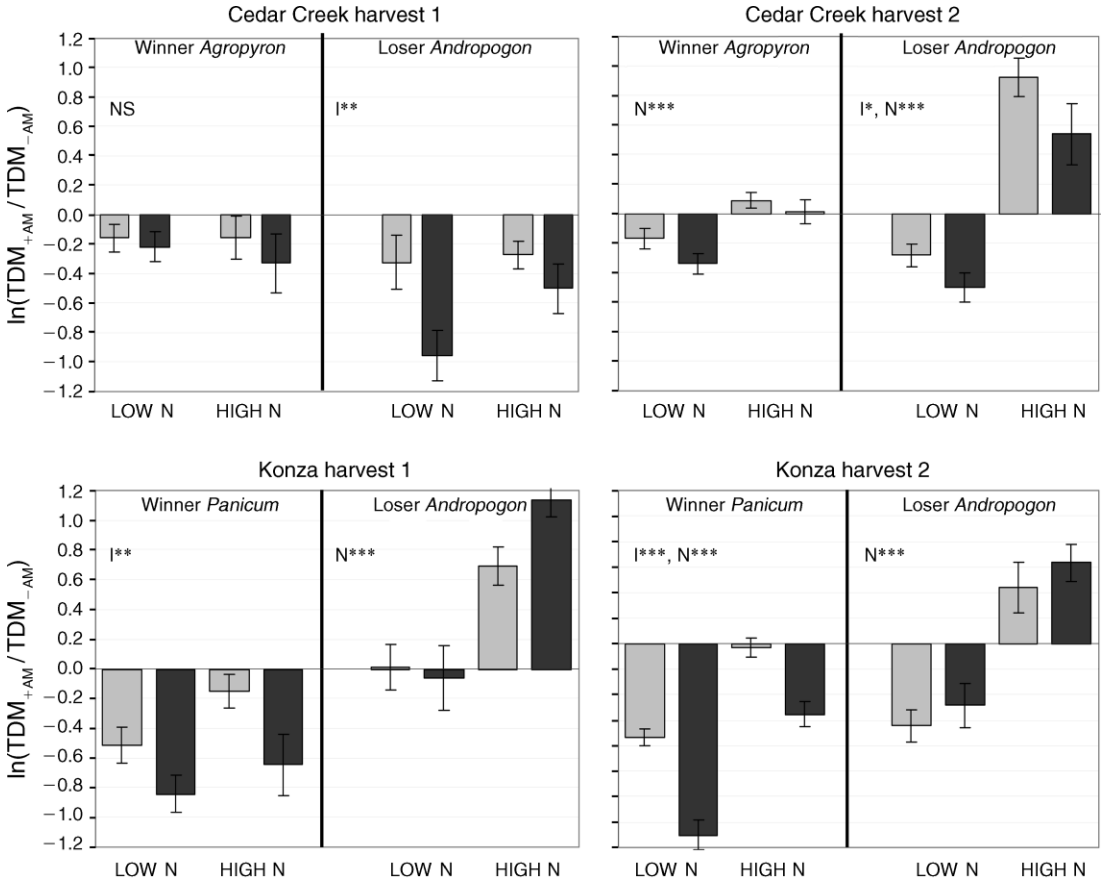

FIG. 3. Influence of arbuscular mycorrhizal (AM) fungi and other soil organisms on plant biomass in plants growing in Cedar Creek and in Konza soils. The bars represent $\ln(TDM_{+AM}/TDM_{-AM})$ where TDM = total dry mass, and the error bars represent ±SE. Light gray bars indicate UNFERT soil inoculum; dark bars indicate FERT soil inoculum. Positive values indicate beneficial effects of AM fungi and other soil organisms, and negative values indicate reduced biomass compared to non-mycorrhizal controls. Significance is indicated as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS = not significant.

enrichment. This plant response will be a strong selection pressure on AM fungi so that fungal genotypes that best extract C from plants that are "unwilling hosts" will be most fit. We expect that these fungal taxa will exert higher net C costs on their host plants, and in this way, the AM fungal communities within fertilized (FERT) soils can be expected to have a higher proportion of parasitic species and genotypes compared to those in unfertilized (UNFERT) soil (Johnson 1993).

In contrast to the Cedar Creek ecotype of *A. gerardii*, which grew smaller with FERT soil inoculum, the growth of the Konza ecotype of *A. gerardii* was not significantly different when inoculated with FERT or UNFERT inocula. This difference between sites could be caused by differences in plant ecotypes, soil characteristics, and/or AM fungal communities at Cedar Creek and Konza. Our data as well as the findings of Schultz et al. (2001) show that *A. gerardii* ecotypes differ significantly in their dependence on mycorrhizas. Also, soil properties are very different at Cedar Creek and Konza (Table 1). The nature of the parent material at Konza makes the soil strongly P limited, and N fertilization of the FERT plots at this site exacerbates P limitation and increases the value of AM symbioses for P uptake. In contrast, Cedar Creek soil is rich in P and organisms living in the long-term FERT plots were probably not limited by either P or N so AM symbioses are expected to be of lesser value to plants in the FERT treatment.

The edaphic differences among sites correspond to different responses of AM fungal communities to N enrichment. The species composition of spore communities of AM fungi shifted significantly in response to N fertilization at both Cedar Creek and Konza, but the direction of change was opposite at the two sites. Nitrogen enrichment at Cedar Creek reduced the species diversity of AM fungal spores and nearly eliminated Gigasporaceae (a family of AM fungi); but at Konza, N enrichment increased species diversity of spores and the abundance of Gigasporaceae (Egerton-Warburton et al. 2007). Species of AM fungi vary in the degree to which they associate with different plant species (Johnson et al. 1992, Bever et al. 1996, Sigüenza et al. 2006*b*) and improve plant fitness (Sylvia et al. 1993). Consequently,

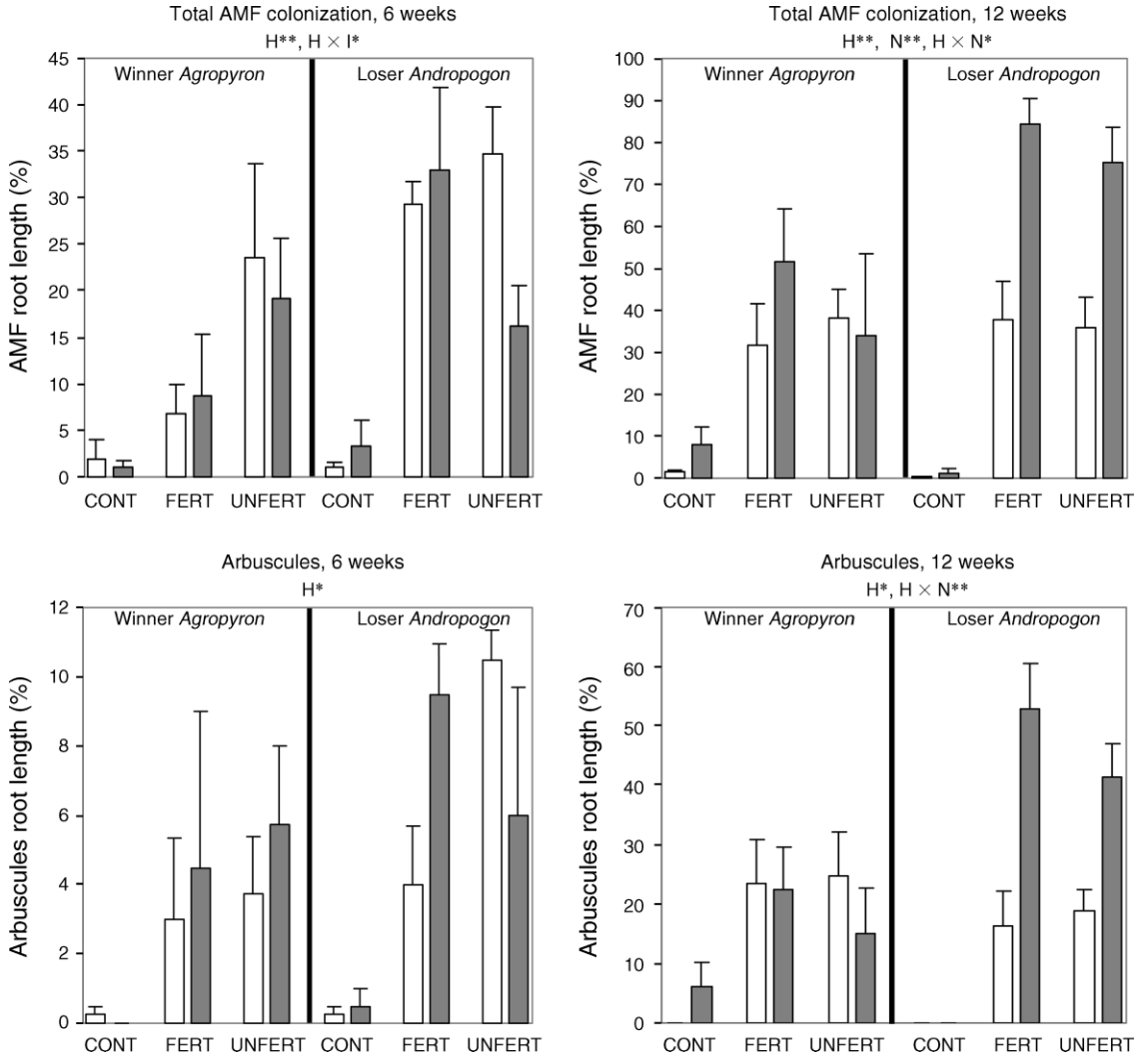

FIG. 4. Percentage of root length colonized by AM fungi (all structures) and percentage of root length colonized with arbuscules of *Agropyron repens* and *Andropogon gerardii* from the Cedar Creek experiment at 6 and 12 weeks. Data are means ± SE; $n = 10$ replications. Open bars represent plants grown with LOW N, and shaded bars represent plants grown at HIGH N availability in the greenhouse. Plants were inoculated with sterilized soil (CONT) or soil from fertilized (FERT) and unfertilized (UNFERT) field plots. The significance levels of the host species (H), inoculum (I), or nitrogen (N) treatments and interactions, as determined by ANOVA, are indicated as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Control plants were not included in the ANOVA because of no, or very low, levels of colonization; consequently, the inoculum treatment compared only the FERT and UNFERT treatments. The *y*-axis scales vary among graphs.

differences in the species composition of communities of AM fungal spores are likely to translate to functional differences (Klironomos 2003). Future studies are necessary to elucidate the roles of plant ecotype, soil characteristics and AM fungal community composition in controlling the mycorrhizal function in the FERT and UNFERT plots at Cedar Creek and Konza.

There was no evidence for positive feedback (H_4) at either site: the winner grass species never grew better with FERT compared to UNFERT inoculum. Mycorrhizal individuals of the winner species were not larger than the non-mycorrhizal (CONTROL) plants, suggesting that *Agropyron repens* at Cedar Creek and *Panicum virgatum* at Konza were not mycotrophic in our experimental system. Thus, there is no reason to expect that mycorrhizal feedbacks facilitate the success of these grass species in dominating grasslands undergoing N eutrophication.

Mycorrhizas only enhanced plant growth in the HIGH N treatments, compared to the non-mycorrhizal (control) plants. Initially, this may seem counterintuitive because the FERT inoculum was often less beneficial than the UNFERT inoculum; however, differences in the temporal and spatial scales of the treatments accounts for the apparent paradox. The FERT treatment involved fertilization of field plots for a period of 9

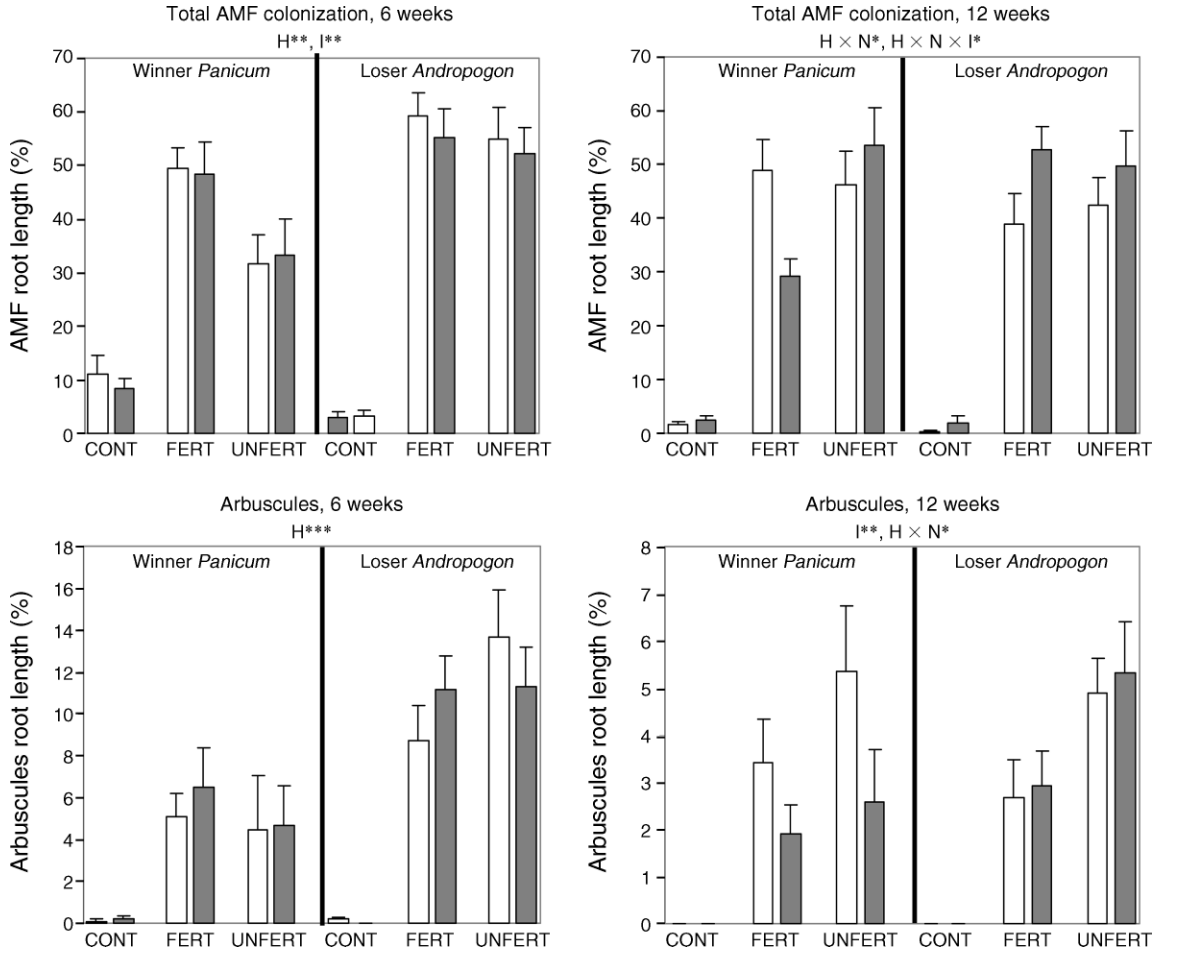

FIG. 5. Percentage of root length colonized by AM fungi (all structures) and percentage of root length colonized with arbuscules of *Panicum virgatum* and *Andropogon gerardii* from the Konza experiment at 6 and 12 weeks. Data are means ± SE; $n = 10$ replications. Format is as in Fig. 4.

or 11 years while the HIGH N treatment involved supplying N to individual plants in pots during the 6- or 12-week duration of the experiment. The FERT treatment manipulated the species composition of communities of AM fungi and associated soil organisms, the HIGH N treatment manipulated resource availability of the individual grasses used in the experiments. The reason that mycorrhizal benefits were not manifested in the LOW N treatments is likely because the plants were too N limited to benefit from AM symbioses. Although AM fungi can increase N uptake (He et al. 2003, Govindarajulu et al. 2005), if N levels are so low that the AM fungus is too N limited to grow, then mycorrhizal benefits will not be manifested in the host (Treseder and Allen 2002). The HIGH N treatment removes N limitation for both the plant and fungal partners and thus increases the value of mycorrhizas for P uptake.

Our results correspond with earlier findings (McGonigle 1988) and indicate that there is not a simple relationship between the total amount of AM root colonization and plant growth enhancement. Although *P. virgatum* in the Konza experiment grew significantly larger when it was inoculated with UNFERT inoculum, at 6 weeks plants inoculated with UNFERT soil had significantly lower total colonization compared to those inoculated with FERT soil. Total colonization is a measure of all fungal structures in roots, hyphae, vesicles, and arbuscules. Relative allocation to these structures may be related to mycorrhizal function (Johnson et al. 2003). Closer examination of AM colonization of *P. virgatum* shows that the arbuscules, which are the haustorium structures involved with resource trading, were significantly more abundant in the 12-week-old *P. virgatum* inoculated with UNFERT soil (Fig. 5). This suggests that the AM symbioses in UNFERT inoculated plants were more actively exchanging limited mineral resources for carbon. At 12 weeks there was a significant host × N interaction in arbuscule colonization at both sites. Nitrogen enrichment increased or did not change arbuscules in the loser species and decreased or did not change arbuscule colonization in the winner grasses. This suggests high N

availability increases mycorrhizal commerce in loser plant species and decreases mycorrhizal commerce in winner species. Thus, although total colonization data may be too coarse to infer mycorrhizal function, closer examination of fungal allocation to arbuscules may reveal some functional patterns.

Aerts and Chapin (2000:18) state that: "*the adaptive significance of root biomass allocation patterns is probably less important than root morphology (i.e., specific root length) in explaining species adaptations to habitats with different levels of nutrient availability*." In other words, on a per gram basis, fine roots have a much greater capacity for resource absorption than coarse roots. Also, there seems to be an evolutionary trade-off between allocation to mycorrhizas and root morphology; plant taxa with coarse roots tend to be much more mycotrophic than those with fine roots (Baylis 1975). Although we did not measure specific root length in our experiments, we do have these measurements from a common-garden study at Cedar Creek (see Plate 1). Interestingly, *A. gerardii*, the highly mycotrophic loser with N enrichment, had significantly lower specific root length (0.6 ± 1.2 cm/mg) than did the winner species *A. repens* (2.9 ± 1.0 cm/mg; N. C. Johnson and D. Wedin, *unpublished data*). This result suggests that N fertilization changes the relative limitation of resources and tips the trade balance between grasses and mycorrhizal fungi such that fine-rooted grass species that can directly absorb soil resources without investing carbon in a fungal partnership will have an advantage over coarse-rooted species that are more mycotrophic.

Our experiments indicate that the functional equilibrium model is a useful predictor of plant community dynamics in grasslands undergoing eutrophication; and mycorrhizal symbioses should be considered extensions of root systems when applying this model. Grass species that allocate less carbon to mycorrhizal symbioses will be at an advantage when eutrophication makes mineral resources no longer limiting. Our experiments provide less support for the hypothesis that mycorrhizal feedbacks are driving plant community shifts associated with eutrophication. There was some evidence for negative feedback because the Cedar Creek ecotype of *Andropogon gerdarii* grew better with UNFERT compared to FERT soil inoculum, but there was no evidence for positive feedback. Future experiments are needed to more thoroughly test the interaction among edaphic properties and AM fungal ecotypes in generating feedbacks. Application of molecular methods that permit identification of AM fungi inside plant roots (Aldrich-Wolfe 2007, Lekberg et al. 2007) will facilitate these studies.

Mycorrhizal feedbacks probably occur at a larger scale than was tested by our experiment. If fertilization causes the composition of plant communities to shift towards less mycotrophic species that allocate less C belowground, then we would expect the overall abundance of AM fungi to decrease over time. Field studies show that AM root colonization, extraradical hyphae, and spore densities are all significantly lower in FERT plots than in UNFERT plots at Cedar Creek, the site with high availability of soil P (Johnson et al. 2003). These changes could have important ecosystem-scale ramification because we know that AM hyphae play an important role in the formation of stable aggregates and soil stability (Miller and Jastrow 2000). Reduction of extraradical hyphae caused by N eutrophication may reduce soil aggregation and increase soil loss in erosion-prone ecosystems. Thus, changes in the biomass of AM hyphae in the soil may generate long-term effects on community and ecosystem structure and function. Once soil properties and biotic communities shift to a eutrophied state, it may be difficult to reverse these changes. Differences between fertilized and control plots have persisted for 62 years following the cessation of fertilizer application in the 200-year-old Grass Garden experiment in the Czech Republic (Semelová et al. 2008). Similarly, differences in soil properties and plant communities in FERT and UNFERT have persisted for over 20 years following cessation of fertilization at the Shortgrass Steppe LTER site in Colorado, USA (Milchunas and Lauenroth 1995). These differences are associated with different AM spore communities (Egerton-Warburton et al. 2007) and mycorrhizal function (Corkidi et al. 2002). Thus, mycorrhizas may play a role in maintaining alternate stable states in grassland communities over the long term.

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