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## Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition—evidence from rDNA data

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**Abstract** Intracellular arbuscular mycorrhizal (AM) colonization was compared between nitrogen ( $\text{NH}_4\text{NO}_3$ ) fertilized ( $10 \text{ g N m}^{-2}$ ) and nonfertilized tallgrass prairie plots. In the microscopic analyses of host roots, only intracellular coils showed an increasing trend as a result of N fertilization, whereas intracellular colonization by arbuscules, hyphae, or vesicles did not differ between the N treatments. Clone libraries established from pooled PCR products of AM fungi contained exclusively species of *Glomus*; no other genera were detected indicating that *Glomus* spp. dominated the host roots. Comparisons between observed and random topologies indicated that cloned sequence placement covaried with N treatment: unique clades within *Glomus* originated exclusively from N-fertilized or nonfertilized treatments. We conclude that the communities of dominant and most commonly occurring AM fungi changed in response to N amendment, although the root colonization showed minimal or no response.

**Keywords** Arbuscular mycorrhiza · Nitrogen deposition · Ribosomal DNA · Tallgrass prairie

### Introduction

Human activities have changed biogeochemical cycles globally. Human  $\text{N}_2$  fixation has added at least as much N to the global N cycle as all natural sources combined (Vitousek et al. 1997a,b). The human additions into the global N cycles impact terrestrial ecosystems and net primary productivity by changing the environment, increasing plant growth, and reducing plant species diversity (Fenn et al. 1998; Kochy and Wilson 2001; Vitousek et al. 1997a).

Changes in N availability also impact fungal communities. Studies on ectomycorrhizal (EM) fruiting bodies have

shown that some EM species are favored by N additions, whereas others may decline and disappear from the community (Avis et al. 2003; Lilleskov et al. 2001; Peter et al. 2001; Wallenda and Kottke 1998). It is not only the production of fruiting bodies, however, that is affected. Studies that directly enumerated EM on the roots of host plants have confirmed that, indeed, the N-induced changes in fruiting bodies aboveground are also reflected by fungal community shifts belowground (Avis et al. 2003; Lilleskov et al. 2002; Peter et al. 2001; Wallenda and Kottke 1998).

Arbuscular mycorrhizal (AM) fungi do not produce aboveground fruiting bodies. However, their functional structures (hyphae, arbuscules, vesicles, and coils) can be microscopically enumerated in the host roots. Depending on the intrinsic nutrient availability and ratios of available nutrients, the occurrence of typical AM structures may change as a result of N amendments. Results and conclusions from field and greenhouse experiments vary greatly and indicate that soil N enrichment may increase, decrease, or have no effect on root colonization by AM fungi (Aerts 2002; Bentivenga and Hetrick 1992a; Hayman 1982; Johnson et al. 2003; Rillig et al. 2002; Sylvia and Neal 1990). In addition to quantification of root colonization, the community composition of the AM fungi can be assessed by survey and identification of spores collected from soil samples or baited in trap cultures. Thus far, these studies have shown that N additions likely result in the overall reduction of AM spore abundance (Egerton-Warburton and Allen 2000; Hayman 1970) and shift the AM spore community composition (Bentivenga and Hetrick 1992a; Egerton-Warburton and Allen 2000; Eom et al. 1999; Johnson 1993). It is uncertain, however, how well the community estimates based on spore abundance reflect the colonization of host roots by particular AM taxa, the AM species composition, or the species abundance (Clapp et al. 1995; Merryweather and Fitter 1998; Morton et al. 1995). Furthermore, even highly divergent taxa cannot be reliably distinguished by their morphological characteristics (Morton and Redercker 2001), whereas some taxa with distinct morphologies show close, overlapping similarities in molecular studies (Helgason et al. 2002; Lloyd-MacGilp et al. 1996).

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In this study, we aimed to use recently developed molecular approaches (Helgason et al. 1998, 1999) to test whether or not anthropogenic N deposition alters AM fungal community composition. We utilized an N-fertilization experiment in a tallgrass prairie ecosystem at Konza Prairie Biological Station (KPBS) in Kansas. Our specific goals were to (1) evaluate the effect of N amendment on host root colonization by AM fungi and (2) test whether the molecular tools could be used to detect changes in AM communities resulting from N amendments. Our results indicate that the root colonization by AM fungi was minimally affected, but the fungal community structure changed as a result of N amendment.

## Materials and methods

Konza Prairie Biological Station (39°05'N, 96°35'W) is a 3,400-ha tallgrass prairie reserve in the Flint Hills region in northeastern Kansas. Soils are mainly Pachic Argiustolls with silty clay loam structure. July mean temperature is 26.6°C, and January mean temperature is -2.7°C. Climate is mesic and annual mean precipitation is 835 mm, ~75% of which falls during the growing season.

We utilized an N-deposition experiment established in 1999, when a total of 12 experimental 1-m<sup>2</sup> plots were located at a homogeneous lowland site and N treatments (no additional N and annual N amendment of 10 g N m<sup>-2</sup> as NH<sub>4</sub>NO<sub>3</sub>) randomly assigned to the study plots. The N amendments were applied annually during early growing season. The area was annually burned to mimic high fire frequency typical to the tallgrass prairie ecosystem. The resultant plant communities were typical to high fire frequency areas and were dominated by native grasses including *Andropogon gerardii*, *Panicum virgatum*, and *Sorghastum nutans*.

Two soil cores (2.54 cm in diameter, 20 cm in depth) were collected and pooled for each plot to minimize within-plot variation. Because of the limited plot size, we did not excavate individual plants but aimed to collect a sample that fairly represented the community at the time of sampling. Although decoupling the effects of N amendment and plant community shifts would have been desirable, separation of the effects would likely require a multifactorial study with individual host plants exposed to various N treatments in a greenhouse. We emphasize that this study utilized field manipulations focusing on the overall effects of N amendments, either directly or via plant community responses. Finally, Sanders and Fitter (1992) emphasize the importance of whole community analyses to elucidate ecological phenomena in natural environments.

The sampling was conducted twice, in June and July of 2002, 3 years after initiation of the N amendments. The roots were washed from the cores onto a 2-mm sieve under running tap water and stored in deionized water until further processing. A subsample of roots was cleared with 10% KOH, stained with Trypan Blue (Phillips and Hayman 1970), and root colonization by AM hyphae, arbuscules, coils, and vesicles was estimated by magnified intercept method under 200× magnification (McGonigle et al. 1990). Another sub-

sample was rinsed with distilled water, ground under liquid N<sub>2</sub>, and genomic DNA-extracted with Ultra Clean Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions.

Fungal 18S rDNA was PCR-amplified in a 50-μl reaction using a nested PCR protocol. The initial reaction contained final concentrations or absolute amounts of reagents as follows: 400 nM of each of the forward (NS1, White et al. 1990) and reverse primers (nu-SSU-1196-3', Borneman and Hartin 2000), 2 μl of the extracted template DNA, 200 μM of each deoxynucleotide triphosphate, 2.5 mM MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase (Promega, Madison, WI), and 5 μl of manufacturer's PCR buffer. The PCR cycle parameters consisted of an initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 3 min, followed by a final extension step at 72°C for 10 min. The nested PCR reaction was also performed in 50 μl and contained final concentrations or absolute amounts of reagents as follows: 400 nM of each of the forward (NS31, Simon et al. 1992) and reverse primers (AM1, Helgason et al. 1998), 2 μl of tenfold diluted PCR product from the initial reaction, 200 μM of each deoxynucleotide triphosphate, 2.5 mM MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase (Promega), and 5 μl of manufacturer's PCR buffer. The PCR cycle parameters consisted of an initial denaturation at 94°C for 3 min, then 40 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 2 min, followed by a final extension step at 72°C for 10 min. All PCR reactions were performed in a Hybaid OmniCycler (Hybaid Ltd., Middlesex, UK). Possible PCR amplification of contaminants was determined using a blank sample run through the extraction protocol simultaneously with the actual samples and a negative PCR control in which the template DNA was replaced with ddH<sub>2</sub>O. These remained free of PCR amplicons in all trials.

The mixed populations of PCR products were pooled for each N treatment within a sampling time resulting in four pools of mixed PCR products, one for each N treatment in June and July. The pooled products were ligated into a linearized pGEM-T vector (Promega). The circularized plasmids were transformed into competent JM109 cells (Promega) by heat shock, and the putative positive transformants were identified by α-complementation (Sambrook 1989). Fifty putatively positive transformants from each clone library were randomly sampled, and the presence of the target insert was confirmed by PCR amplification in 15-μl reaction volume under the same reaction conditions as described above for the nested PCR reaction. To select different plasmids for sequencing, these PCR products were digested with endonucleases (*Hinf*I, *Alu*I; New England BioLabs, Beverly, MA) and resolved on 3% agarose gels (Gardes and Bruns 1996). Sequences from each different restriction fragment length polymorphism (RFLP) phenotype in all clone libraries were obtained by use of fluorescent dideoxy-terminators (ABI Prism BigDye; Applied Biosystems, Foster City, CA) and an automated ABI Prism 3700 DNA Analyzer (Applied Biosystems) at the DNA Sequencing and Genotyping Facility at Kansas State University (GenBank

accession numbers AY499469–AY499506). Vector contamination was removed with the automated vector trimming function in Sequencher (Version 4.1, GeneCodes, Ann Arbor, MI). The similarities to existing rDNA sequences in the GenBank database were determined at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>, Altschul et al. 1997) by standard nucleotide BLAST (version 2.2.1) without limiting queries.

The environmental sequences (37 in total) and sequences from GenBank (38 in total) were aligned in 566 positions using Sequencher and manually adjusted to maximize conservation. Regions adjacent to the priming sites were omitted due to high frequency of ambiguous sites. The taxonomic relationships among the fungal sequences were inferred by neighbor joining (NJ) and maximum parsimony (MP) analyses in PAUP\* (Swofford 2001). *Geosiphon pyriforme* was used as the outgroup. For the NJ analyses, data matrices were left uncorrected, rates for variable sites were assumed equal, and no sites were assumed invariable. Sites with missing data, ambiguous nucleotides, or gaps were randomly distributed among taxa. The robustness of the inferred NJ topologies was tested by 1,000 bootstrap replicates. The most parsimonious trees were obtained using random addition sequence and a branch swapping algorithm with tree bisection reconnection. The number of equiparsimonious trees was expected to be high attributable to several closely related sequences in the clone libraries. As a result, the maximum number of retained trees was restricted to 1,000 (option MAXTREES=1,000). Robustness of the MP topologies was tested with 1,000 bootstrap replicates with maximum number of equiparsimonious trees set to 1,000 at each step (option MAXTREES=1,000).

Aboveground, plant biomass was analyzed by one-way analysis of variance ( $n=12$ ). Root colonization by various AM structures was analyzed as two-way analysis of variance ( $n=24$ ) with sampling month, N treatment, and their interaction as explanatory variables. To normalize data and to homogenize variances, all percent data were arcsine square root transformed prior to analyses. The effects of sampling month and nitrogen amendment on the AM fungus community structure were tested using a phylogenetic test (P-test) as described in Martin (2002). We tested whether the distribution of unique sequences from different

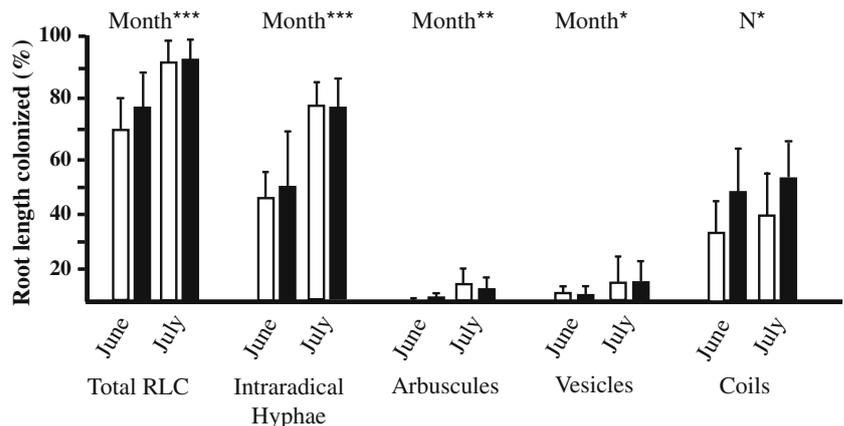
sampling months or N treatments covaried with obtained NJ and MP topologies. Presence and absence of sequences obtained from each sampling month and N treatment were optimized on the topologies in MacClade (version 4.06, Maddison and Maddison 2001). The NJ and MP topologies provided an estimate of the minimum number of changes in the sampling month and N treatment to explain their observed distribution in the obtained topologies. The significance of this observed covariation with the topologies was established by determining the expected number of changes under the null hypothesis that no covariation exists (Martin 2002). The expected distribution was obtained using 1,000 randomly generated topologies (see Maddison and Slatkin 1991). Fewer changes in observed distributions for the treatments than in random distributions were interpreted to indicate community level differences among the treatments.

## Results

Nitrogen amendment increased the aboveground productivity (analysis of variance,  $P<0.05$ ); the peak season biomass in the N-amended plots was nearly double the biomass in the nonamended controls. However, the effect of N amendment on AM root colonization was minimal. Only intracellular coil colonization was affected; the intracellular coils were more abundant in N-amended treatments (Fig. 1, Table 1). Root length colonized by different AM structures increased from June to July with the exception of coils (Fig. 1, Table 1).

Although N amendment had minimal effects on the root colonization, our phylogenetic tests (P-tests) indicated that the distribution of sequences obtained from the two N treatments was not random but affected by the N treatment. The NJ topology and consensus of 1,000 most parsimonious trees had significantly ( $P<0.05$ ) fewer changes in N treatment than did the random topologies (Fig. 2a). Similarly, equiparsimonious MP trees had fewer changes in N treatment ( $P<0.10$ ) than did the random topologies. These results indicate that the distribution of the N treatments in these topologies was not derived from a random population of trees, but that the distribution of sequences in the observed topologies was affected by the N treatments. In con-

**Fig. 1** Intraradical root colonization (mean $\pm$ 1 standard deviation) by arbuscular mycorrhizal fungi in June and July 2002. Open bars are colonization in nonfertilized plots, and filled bars are colonization in N-fertilized ( $10 \text{ g m}^{-2}$ ) plots. Legend above the bars indicates the significant parameters and their  $P$  values: \* $P\leq 0.05$ , \*\* $0.001 < P \leq 0.01$ , and \*\*\* $P \leq 0.001$ . RLC Root length colonized



**Table 1** Results of two-way ANOVA for intraradical arbuscular mycorrhizal (AM) structures: total colonization, intraradical hyphae, arbuscules, vesicles, and coils

Source of variation	Total intraradical colonization			Intraradical hyphae			Arbuscules			Vesicles			Coils		
	MS	$F_{1,23}$	$P$ value	MS	$F_{1,23}$	$P$ value	MS	$F_{1,23}$	$P$ value	MS	$F_{1,23}$	$P$ value	MS	$F_{1,23}$	$P$ value
Month	0.388	26.44	0.001	0.549	27.31	0.001	0.17	12.27	0.002	0.16	5.35	0.031	0.02	0.73	ns
Nitrogen	0.028	1.93	ns	<0.01	0.01	ns	<0.01	0.20	ns	0.01	0.40	ns	0.12	4.84	0.040
Interaction	0.013	0.89	ns	0.01	0.30	ns	0.03	1.92	ns	0.02	0.56	ns	<0.01	<0.01	ns

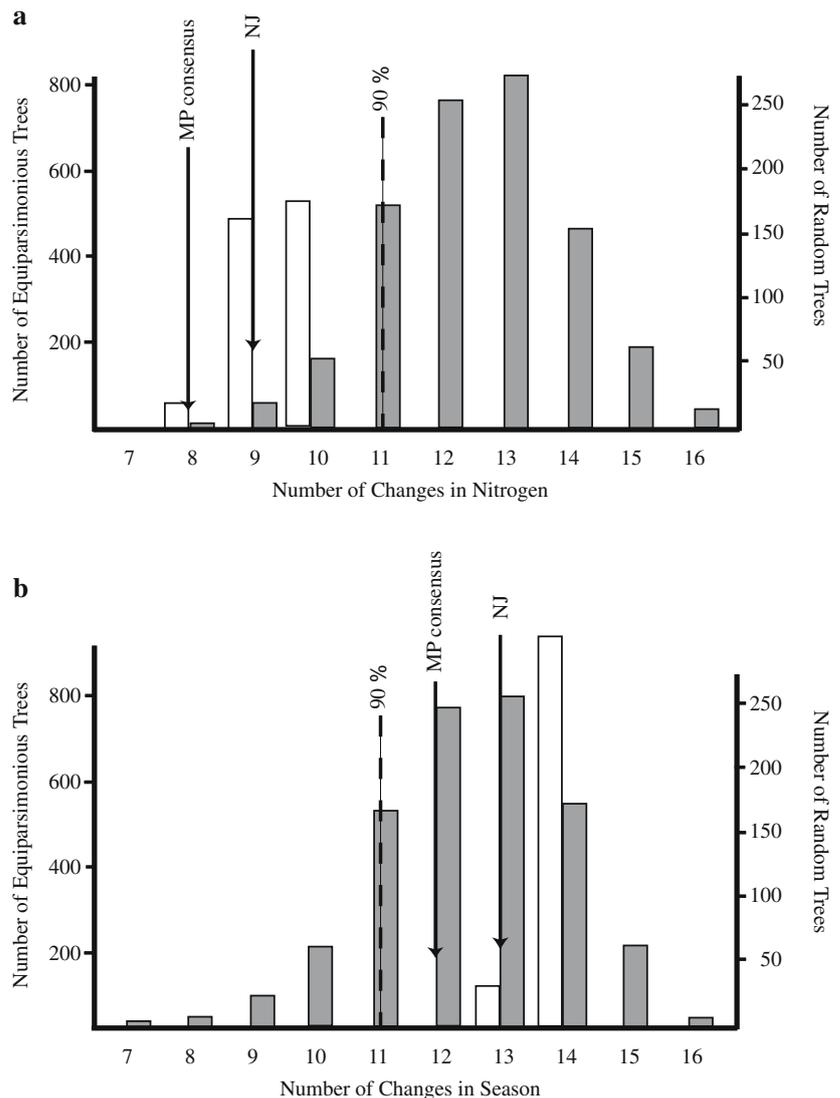
ANOVA was performed on arcsine square-root-transformed data. For comparison of means, see Fig. 1  
 ns Not significant

trast, the 2 months sampled here did not differ significantly (Fig. 2b).

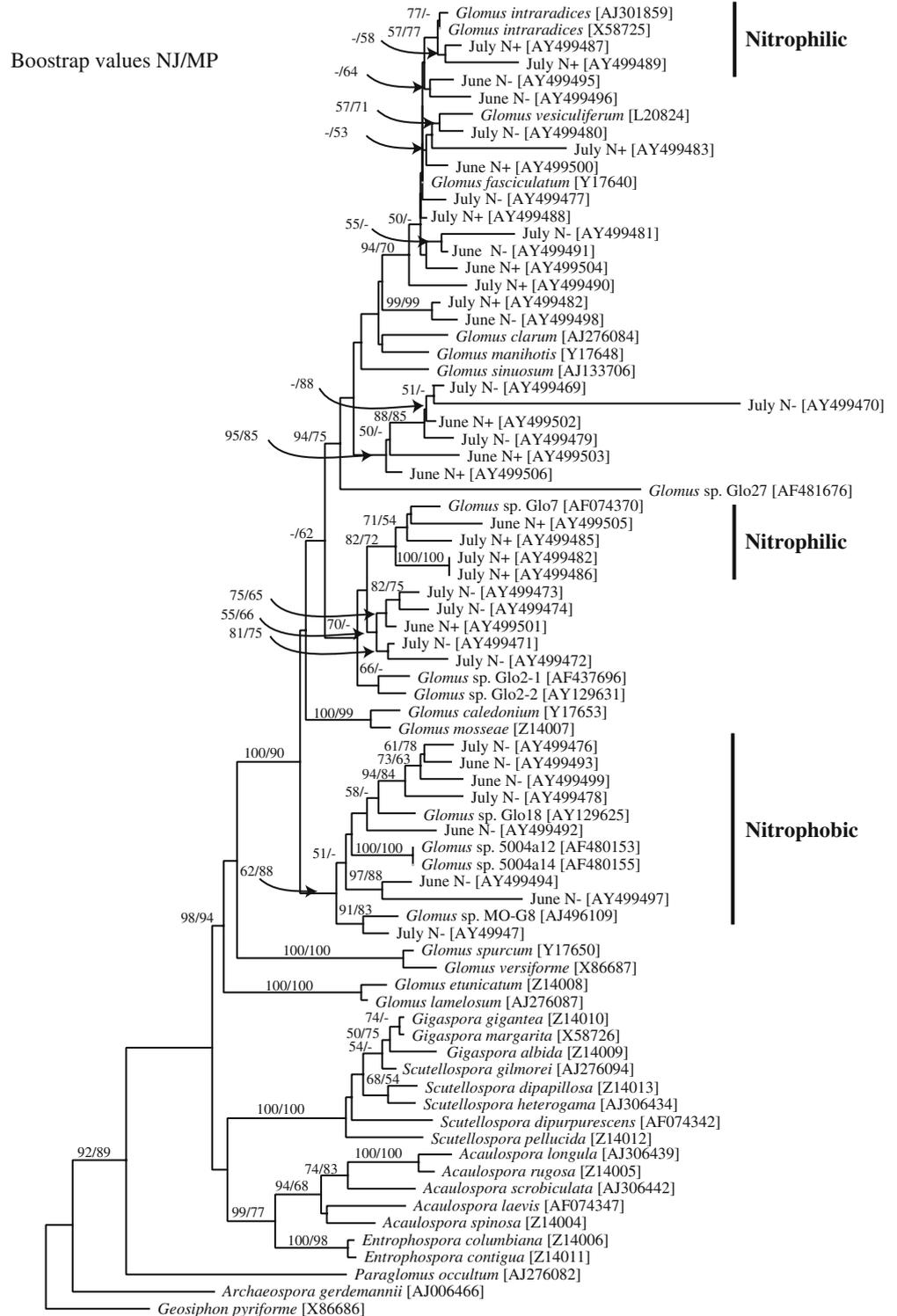
Our clone libraries contained PCR amplicons pooled for six plots representing four different treatment combinations. The clone libraries contained exclusively species of *Glomus* (Fig. 3). No representatives of *Acaulospora*, *Entrophospora*, *Gigaspora*, or *Scutellospora* were observed. Regardless, as indicated by the P-tests, the most parsimonious distribution of sequences obtained from different N treat-

ments was not derived from a random population of trees. This is attributable to three (two nitrophilic and one nitrophobic) clades within *Glomus* (highlighted in Fig. 3). These nitrophilic or nitrophobic clades contained sequences exclusively observed in only one of the two N treatments. A majority of the obtained sequences remain unidentified and can only be related to sequence data from other cloning studies (Helgason et al. 1998; Vandenkoornhuysen et al. 2002). However, one nitrophilic group seemed to be com-

**Fig. 2** Frequency distributions of required changes to describe covariation between obtained topologies and N enrichment treatment (a) and sampling (b) in observed most parsimonious (open bars, Y-axis on the left) and randomly generated topologies (filled bars, Y-axis on the right) (so-called phylogenetic or P-tests). Observed frequency distributions are based on topologies of 1,000 equiparsimonious trees. Arrows show one neighbor joining tree (NJ) and 50% majority rule consensus of the most parsimonious (MP) topologies. Frequency distributions in randomly generated topologies were obtained from MacClade (version 4.06). Dashed lines indicate the 90% lower confidence limit for the randomized data. The sampling dates did not differ (MP topologies likely derived from a random population of trees), whereas N enrichment treatments were different at  $\alpha=0.10$  (MP topologies and the distribution of N treatments were unlikely to be derived from a random population of trees)



**Fig. 3** Neighbor joining phylogram of cloned AM sequences obtained from N-enriched and nonenriched plots in June and July 2002. Numbers above the nodes indicate bootstrap support in neighbor joining (*above slash*) and maximum parsimony (*below slash*) analyses. Nitrophilic clades include sequences that were exclusively obtained from N-fertilized plots; nitrophobic clades include sequences that were exclusively obtained from nonfertilized plots



prised of *Glomus intraradices* or closely related AM taxa (Fig. 3).

## Discussion

The responses of intraradical AM structures to N enrichment have been shown to be variable across grassland ecosystems

and may either respond positively or negatively or show no clear response (Bentivenga and Hetrick 1992a; Egerton-Warburton and Allen 2000; Hayman 1982; Johnson et al. 2003; Sylvia and Neal 1990). We observed no or few changes in the intraradical root colonization as a result of N enrichment. Our data contrast with earlier reports from Konza tallgrass prairie, which have shown that N enrichment can increase total root colonization and intraradical hyphae

(Johnson et al. 2003) or decrease total root colonization (Bentivenga and Hetrick 1992a). Variability among seasons and years (Bentivenga and Hetrick 1992b) as well as spatial variability likely accounts for these differences. Our limited data support a dynamic temporal nature of the intraradical AM structures: overall AM colonization, intraradical hyphae, arbuscules and vesicles increased from June to July.

In the present study, we assessed the effects of N fertilization directly on root-colonizing AM communities using 18S rDNA data. It is also possible that our sampling included fungi associated with root surfaces that did not actively colonize roots at the time of sampling. However, this experiment was established in 1999 and had been running for 3 years prior to our sampling. Accordingly, residual DNA from spores or extrametrical mycelia not colonizing sampled root tissue are also likely to be adapted to the present N-amended or nonamended environment.

Our approach to pool PCR products prior to cloning allows conclusions only on the dominant components of the AM community; the likelihood of obtaining clones for infrequent and rare taxa decreases when multiple PCR products dominated by the same organisms are combined. The root colonizing communities in this study exclusively comprised species with affinities to *Glomus*; no species of *Acaulospora*, *Entrophospora*, *Gigaspora*, or *Scutellospora* were observed. Previous studies at KPBS have observed these taxa in the sporulating AM communities, although *Glomus* spp. have invariably been shown to be most common and dominate the AM communities (Bentivenga and Hetrick 1992a,b; Eom et al. 1999, 2000, 2001). The primers we used may be biased towards genus *Glomus*, although they have been shown to amplify taxa beyond Glomaceae (Helgason et al. 1998, 1999, 2002; Husband et al. 2002). Taken together, we conclude that our observations reflect the most frequent taxa in our samples. Additional genera and families are likely to be present as well but were not detected because of the great abundance of dominant *Glomus* spp. and limited number of clones sampled from each library.

Regardless of the small responses in root colonization, our rDNA data indicated that AM fungi respond on a community level to N enrichment. Although many infrequent taxa may have important ecosystem functions (see van der Heijden et al. 1998a,b, 2003), we emphasize the importance of the detected shifts among dominant components of root-colonizing communities. For example, soil eutrophication may select AM fungi that are less efficient in nutrient uptake or exert a greater carbon cost on their host (Johnson 1993). Such fungi do not reciprocate in the mutualistic association as well as the fungi that are dominant under lesser nutrient availability. Our data support earlier studies from grassland and coastal sage scrub ecosystems, where dominant field-collected spore communities changed as a result of fertilization treatment (Bentivenga and Hetrick 1992a; Egerton-Warburton and Allen 2000; Eom et al. 1999; Johnson 1993). In an N fertilization study in California coastal sage scrub (Egerton-Warburton and Allen 2000), more than 80% of all spores belonged to genus *Glomus* and more than 60% were identified as a single taxon (*Glomus*

*aggregatum*), whose sporulation was significantly reduced by N enrichment. Similarly, in a study at Cedar Creek Natural History Area in Minnesota (Johnson 1993), more than 96% of all field-collected spores belonged to a single species, again *G. aggregatum*. In that study, another *Glomus* species, *Glomus occultum*, sporulated more frequently under N enrichment. Finally, two studies at KPBS (Bentivenga and Hetrick 1992a; Eom et al. 1999) concluded that more than 90% of all spores belonged to genus *Glomus* and that *Glomus mosseae* (Eom et al. 1999) and *Glomus ambisporum* (Bentivenga and Hetrick 1992a) responded positively to N fertilization. Although the direct correlation between sporulation and root colonization remains somewhat uncertain (Clapp et al. 1995; Merryweather and Fitter 1998; Morton et al. 1995), we conclude that our study supports community level shifts as a result of *Glomus* species responses to N enrichment.

The observed changes in the fungal community composition coincided with changes in the host plant community composition. Nitrogen enrichment increased the abundance *P. virgatum*, whereas it decreased the abundance of *A. gerardii* (L. Johnson, personal communication), which invariably dominates annually burned areas in tall-grass prairie. Complex interactions among the hosts, their root-associated fungal communities, and the environmental conditions complicate interpretation of results and identification of the driving mechanisms. In the current study (and others focusing on the changes in AM communities resulting from N enrichment), it remains unclear whether the fungal community changes are mainly driven by chemical and physical alterations in soil environment and carbon availability resulting from N enrichment or by plant community responses to altered competitive abilities among component species in the N-enriched environment. Although AM fungal communities have been believed to have no host specificity, recent molecular studies have indicated that different AM fungi are abundant in the roots of some hosts whereas absent in others (Helgason et al. 2002; Husband et al. 2002; Vandenkoornhuyse et al. 2002). These findings suggest that the AM community composition responses in our study may have resulted from changes either in the environmental conditions or in the host community composition. Alternatively, it is possible that the AM community responses to environmental manipulation drive the plant community structure and its responses to environmental conditions (see van der Heijden et al. 1998a,b, 2003). To exemplify, Hartnett and Wilson (1999) showed that fungicidal treatment used to eliminate AM fungi in soil resulted in a host community shift similar to that observed in our study. Uncoupling the host effects and the alterations of environmental conditions are subjects of our forthcoming research.

Observational and experimental studies have suggested that N enrichment may also reduce species richness and diversity of mycorrhizal fungi. Reconstruction of fungal communities over an extended anthropogenic N deposition has indicated losses of species and genera from soil AM communities (Egerton-Warburton et al. 2001). Although our approach of pooling PCR products prior to cloning allows detection of changes in only the major components

in the AM community, it provides little information on the diversity of the community. However, we argue that diversity estimates based on studies of cloned PCR products may suffer from various biases. The determination of species richness may be subjective in an rDNA data set because it may depend on the chosen cutoff for an operational taxonomic unit. Furthermore, as outlined in Helgason et al. (1999), species richness estimates rely on various assumptions that no bias occurs during DNA extraction, PCR amplification, or downstream processing of the amplicons.

Previous studies describing efforts to test effects of environmental parameters on AM communities have designated clades in phylograms into operational taxonomic units, used the frequencies of individual clones in the clone libraries as a proxy for true occurrence in the sample, and analyzed these data using logistic or generalized linear regression (e.g., Helgason et al. 1999; Husband et al. 2002). Such approaches provide means to statistically analyze the effects of environmental or experimental parameters on the fungal communities. However, PCR-based applications are prone to various biases including differential DNA extraction, amplification, ligation, or cloning of various templates and PCR products (Ford et al. 1994; Helgason et al. 1999; Jumpponen 2003; Kopczynski et al. 1994; von Wintzingerode et al. 1997; Wang and Wang 1996). Accordingly, the frequencies in the clone libraries may not truly represent frequencies in the original samples. To reduce these sources of biases, we eliminated our reliance on the clone frequencies in the rDNA clone libraries. Instead, we chose to pool samples within a treatment, analyze the distribution of AM sequences from different treatments in our inferred topologies, and compare those topologies to randomly obtained ones (Maddison and Slatkin 1991; Martin 2002). Although compromising the ability to acquire species diversity estimates, this approach minimizes the cost of sequencing individual clones from each clone library. Yet, it seems robust enough to detect community level changes among the dominant components.

Our results indicate that root-colonizing AM communities shift as a result of N enrichment, although the root colonization did not show marked responses. Larger sampling would allow us to follow seasonal patterns in these AM communities as well as to determine which are the most responsive AM species. Although observations of species and community level responses to environmental change are an essential initial step, the function of various components in the AM community needs to be compared to determine the overall ecosystem impacts of the community change.

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