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Nancy Collins Johnson

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CAN FERTILIZATION OF SOIL SELECT LESS MUTUALISTIC MYCORRHIZAE?¹

NANCY COLLINS JOHNSON²

Department of Ecology, Evolution and Behavior, University of Minnesota,
Minneapolis, Minnesota 55455 USA

Abstract. It has been noted previously that nutrient-stressed plants generally release more soluble carbohydrate in root exudates and consequently support more mycorrhizae than plants supplied with ample nutrients. Fertilization may select strains of vesicular-arbuscular mycorrhizal (VAM) fungi that are inferior mutualists if the same characteristics that make a VAM fungus successful in roots with a lowered carbohydrate content also reduce the benefits that the fungus provides a host plant. This two-phase study experimentally tests the hypothesis that fertilizing low-nutrient soil selects VAM fungi that are inferior mutualists. The first phase examines the effects of chemical fertilizers on the species composition of VAM fungal communities in long-term field plots. The second phase measures the effects of VAM fungal assemblages from fertilized and unfertilized plots on big bluestem grass grown in a greenhouse. The field results indicate that 8 yr of fertilization altered the species composition of VAM fungal communities. Relative abundance of *Gigaspora gigantea*, *Gigaspora margarita*, *Scutellispora calospora*, and *Glomus occultum* decreased while *Glomus intraradix* increased in response to fertilization. Results from the greenhouse experiment show that big bluestem colonized with VAM fungi from fertilized soil were smaller after 1 mo and produced fewer inflorescences at 3 mo than big bluestem colonized with VAM fungi from unfertilized soil. Fungal structures within big bluestem roots suggest that VAM fungi from fertilized soil exerted a higher net carbon cost on their host than VAM fungi from unfertilized soil. VAM fungi from fertilized soil produced fewer hyphae and arbuscules (and consequently provided their host with less inorganic nutrients from the soil) and produced as many vesicles (and thus provisioned their own storage structures at the same level) as fungi from unfertilized soil. These results support the hypothesis that fertilization selects VAM fungi that are inferior mutualists.

Key words: big bluestem grass; Cedar Creek Natural History Area; effectivity; fertilization; fungal communities; mutualists; parasites; vesicular-arbuscular mycorrhizae.

INTRODUCTION

Mycorrhizae are symbiotic associations believed to be as ancient as land plants (Pirozynski and Malloch 1975, Wagner and Taylor 1981, Stubblefield et al. 1987). Vesicular-arbuscular mycorrhizal (VAM) fungi can be found within the roots of most plant species and are obligate plant symbionts. Mycorrhizae are often assumed to be solely beneficial; however, the effects that VAM fungi have on their host plants, or "effectivity," differs greatly between fungal strains, and may range from mutualistic to parasitic (O'Bannon et al. 1980, Miller et al. 1985, Bethlenfalvai et al. 1989, Modjo and Hendrix 1986). For the past decade there has been an interest in inoculating crops with VAM fungi to improve yields and decrease reliance on chemical fertilizers. But before this goal can be realized the effectivities of indigenous and inoculant VAM fungi must be assessed and the factors controlling whether mycorrhizal associations are mutualistic or parasitic must be understood.

There are theoretical reasons to speculate that fertilizing soil may select for VAM fungal strains that are inferior mutualists or even parasites. It has been noted that mildly nutrient-stressed plants tend to release more soluble carbohydrate in root exudates, and make better VAM hosts, than unstressed plants (Sylvia and Neal 1990, Schwab et al. 1991). A strong selective pressure will be exerted on VAM fungal populations when fertilization causes host plants to allocate less carbohydrate to root exudates. In response to fertilization, the abundance of VAM fungal strains that most aggressively acquire host carbohydrate will increase at the expense of less aggressive strains. It is likely that the same characteristics that make a VAM fungus successful in a low carbohydrate environment also reduce its mutualistic effects. Namely, a successful fungus could acquire carbohydrates that the host plant has not allocated to it, and thus, parasitically provision its own growth without contributing to the fitness of the plant. In this way, less beneficial, or even detrimental, VAM fungi could be selected in fertilized soils where plants gain little from VAM associations.

The virulence of many parasites lessens with time, and over a long enough period of time, many parasitic

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² Present address: 212 Spruce Street, Santa Fe, New Mexico 87501 USA.

associations evolve into more stable mutualistic associations (Law 1985, Ahmadjian and Paracer 1986). Perhaps the ultimate result of VAM selection in undisturbed systems is the creation of coadapted mycorrhiza-soil complexes. A mycorrhiza includes both a plant and a fungus, so a "coadapted mycorrhiza-soil complex" can be defined as a dynamic system in which both plant and fungal communities have adjusted, and continue to adjust, to the soil conditions and to one another so that the mycorrhizal associations within the complex become increasingly more mutualistic over time.

Perhaps the variability in the effectivity of VAM fungi is not random in nature. Might inferior or detrimental VAM associations be the result of human activities (such as cultivation and fertilization) that disrupt coadapted mycorrhiza-soil complexes? The present study experimentally tests the hypothesis that fertilizing low-nutrient soil selects mycorrhizae that are inferior mutualists.

METHODS

This experiment was conducted in two phases. The first phase assessed the influence of chemical fertilizers on the species composition of VAM fungal communities in long-term field plots at Cedar Creek Natural History Area (CCNHA) in central Minnesota. The second phase measured the effects of VAM fungal assemblages from fertilized and unfertilized field plots on big bluestem grass (*Andropogon gerardi* Vitm.) grown in a greenhouse.

Phase 1

On 6 May 1990, composite soil samples were collected from 12 4 × 4 m experimental plots in a field abandoned from agriculture for 22 yr. Six of these plots had been fertilized annually for 8 yr with: 50 g/m² NH₄NO₃; 20 g/m² P₂O₅; 20 g/m² K₂O; 40 g/m² CaCO₃; 30 g/m² MgSO₄; 18 μg/m² CuSO₄; 37.7 μg/m² ZnSO₄; 15.3 μg/m² CoCl₂; 322 μg/m² MnCl₂; and 15.1 μg/m² NaMoO₄. The remaining six plots had been left unfertilized. The fertilized (FERT) soils contained an average of 8.6 mg/kg (NO₂ + NO₃) and 62.2 mg/kg P (Bray-1 analysis; NH₄F + HCl extractable P), the unfertilized (UNFERT) soils contained an average of 1.6 mg/kg (NO₂ + NO₃) and 26.5 mg/kg P (Bray-1). Each soil sample was a composite created by combining nine cores (25 cm deep × 5 cm diameter) taken at 90-cm intervals along two transects diagonally crossing each plot. Samples were mixed gently by hand and stored for 6 wk in plastic bags at ≈5°C.

Analysis of field soils

Species of VAM fungi are characterized by the morphology of their soil-borne spores. To assess the species composition of VAM fungal communities in the experimental plots, spores were extracted from 25-g subsamples from each of the 12 soil samples and spread

evenly on a membrane filter using the technique of McKenney and Lindsey (1987). All spores within the central 27% of the filter paper were removed using a fine forceps and a dissecting microscope (50×) and mounted on permanent slides. These slides were examined with a compound microscope (100 to 1000×) and spores were counted, and identified based on wall structure (Schenck and Perez 1990). The relative density (%) of each species in each sample was calculated as: $n_i/N_i \times 100$, where n_i = the number of spores from the i^{th} species and N_i = the total number of spores examined from the sample.

Analysis of cultures

It is likely that many of the infective VAM propagules in the soil samples collected in May were in the form of hyphae or mycorrhizal root fragments and would not be detected in a spore analysis. To more effectively assess the species composition of the VAM fungal community, cultures of the 12 soils were established in an effort to induce all VAM propagules (spores, hyphae, and VAM roots) to infect a host plant and subsequently sporulate. These cultures were established in sterilized 23 cm diameter clay pots by mixing 100 g of sample soil with a 1:1 mixture of steamed sand from CCNHA + river-washed sand. Each pot was sown with surface-sterilized (70% ethanol for 3 min) seeds of *Sorghum sudanense* (Piper) Stapf. and kept in a greenhouse (22°–27°C) for 4 mo. The rapidly developing, fibrous root system of *S. sudanense* has made it a standard host plant for VAM cultures (Ferguson and Woodhead 1982). Plants were watered on alternate days with a nutrient solution that was modified from Sylvia and Hubbell's (1986) formulation for aeroponic culture of mycorrhizae, and contained: 433 mg/L KNO₃; 8.4 mg/L Ca(NO₃)₂·4H₂O; 199 mg/L CaSO₄·2H₂O; 130 mg/L K₂SO₄; 72 mg/L MgSO₄; 0.86 mg/L H₃BO₃; 0.54 mg/L MnCl₂·4H₂O; 0.07 mg/L ZnSO₄·7H₂O; 0.02 mg/L CuSO₄·5H₂O; and 0.03 mg/L NaCl. Watering was stopped and tops of the sorghum plants were cut to a height of ≈10 cm 1 wk before terminating the cultures. Spores were extracted from duplicate 25-g subsamples of each culture, counted and identified as described above.

Phase 2

Big bluestem seedlings were inoculated with three types of soil: fertilized (FERT), unfertilized (UNFERT), and a sterilized control (NONVAM), and were grown with and without supplemental P, and with and without supplemental N. This 3 × 2 × 2 factorial experiment had 10 replicates. Five randomly selected replicates were harvested at 1 mo, and the remaining five replicates were harvested at 3 mo.

Big bluestem was chosen as the indicator plant in the bioassay because it is native to CCNHA and is well adapted to the nutrient-poor soils in the area. Thus, if coadapted mycorrhiza-soil complexes exist at CCNHA,

TABLE 1. Mean total spore counts and relative spore densities of individual VAM fungal species in FERT and UNFERT inoculum soils from field samples and from cultures ($N = 6$).

	Field samples		Cultures	
	FERT	UNFERT	FERT	UNFERT
Total spore density (no./25 g soil)	1316	1215	3935	2147**
VAM fungal species present (% of total spores)				
<i>Acaulospora scrobiculata</i> Trappe	0.78	0.71	0.02	0.05
<i>Acaulospora spinosa</i> Walker & Trappe	0.01	0.02	0.05	0.01
<i>Gigaspora gigantea</i> (Nicol. & Gerd.) Gerd. & Trappe	0.22	0.32	0.03	0.40***
<i>Gigaspora margarita</i> Becker & Hall	0.21	0.34†	0.00	0.45***
<i>Glomus aggregatum</i> Schenck & Smith emend. Koske	96.4	96.1	24.3	28.0
<i>Glomus fasciculatum</i> (Thaxter) Gerd. & Trappe emend. Walker & Koske	0.33	0.22	1.90	0.01
<i>Glomus</i> "immature"		NP‡	7.00	9.30
<i>Glomus intraradix</i> Schenck & Smith		NP	46.5	27.0**
<i>Glomus macrocarpum</i> Tul. & Tul.	0.85	0.60	0.13	0.14
<i>Glomus mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	0.08	0.06		NP
<i>Glomus occultum</i> Walker	0.27	0.64*	20.1	34.5†
<i>Scutellispora calospora</i> (Nicol. & Gerd.) Walker & Sanders	0.64	1.04	0.02	0.11**
<i>Scutellispora persica</i> (Koske & Walker) Walker & Sanders	0.09	0.03		NP

†, *, **, ***, indicate that mean spore densities in FERT and UNFERT samples were different at $P \leq .10$, .05, .01, and .001, respectively.

‡ NP = spores of this species were not present.

then big bluestem should be coadapted with the VAM fungi present in the UNFERT soils. The four nutrient treatments (-N-P, -N+P, +N-P, +N+P) used in the greenhouse study were designed to assess the role of mycorrhizae in N and P uptake. Application rates of N and P were chosen to correspond with available levels of N and P in FERT soils. Bioassay plants given ample nutrients (+N+P) should benefit less from mycorrhizae than nutrient-stressed plants. Since VAM fungi are most likely to be parasitic to well-nourished plants, differences in the effectivities of VAM fungi from FERT and UNFERT soils should be most obvious in the +N+P treatment.

Subsurface sand from CCNHA (containing 0.7 mg/kg $\text{NO}_2\text{-N}$ and 32 mg/kg P (Bray-1)) was mixed (1:1) with river-washed sand (No. 19 granusil, Unimin Corporation LeSuer, Minnesota) and steam sterilized ($\approx 85^\circ\text{C}$) for 1 h on two consecutive days. Square pots (11.5 cm on a side) were filled with 980 g of this sand mixture and leached with deionized water. A 6 cm deep \times 3 cm wide hole was pushed into the center of each pot and filled with 30 g of "inoculum-soil."

Thirteen different inoculum-soils were used: 6 FERT, 6 UNFERT, and one NONVAM composed of a steamed composite of soils from all 12 plots. To equalize the fertility of FERT and UNFERT inocula, 15 g of "live" FERT soil was combined with 15 g of a steamed composite of UNFERT soil, and 15 g of "live" UNFERT soil was combined with 15 g of a steamed composite of FERT soil. Communities of soil microbes ($< 25 \mu\text{m}$ in size) were equalized across treatments by applying 15 mL of "microbial-wash" to each pot. This microbial-wash was prepared by blending 800 mL of a composite of all 12 soils with 4000 mL of deionized water and filtering it three times through a 25- μm mesh screen.

Big bluestem seeds were surface sterilized in 5% sodium hypochlorite for 10 min and sown in flats of steamed sand. Five days following germination, uniform seedlings were transplanted into the inoculum-soil plugs in the center of each pot. Plants were randomly arranged in a greenhouse and maintained at $27^\circ/22^\circ\text{C}$ (day/night) without supplemental lighting from 16 June through 12 September 1990. On alternate days every plant was watered with Sylvia and Hubbell's (1986) modified nutrient solution (without N or P) described above. Enough nutrient solution (50–150 mL, depending on plant size and weather) was applied to each pot so that excess solution drained from the bottom. The +N treatments received 433 mg/L KNO_3 , and the +P treatments received 44 mg/L KH_2PO_4 . The pH of all nutrient solutions was brought to 6.5 using 0.01 mol/L NaOH.

Five randomly chosen plants from each treatment were harvested after 4 wk and the remaining plants were harvested when inflorescences were fully mature and the plants had begun to senesce after 12.5 wk. Plant heights and shoot and root masses (oven dry) were measured at both harvests and the number of inflorescences per plant were counted at the second harvest. At the 1 mo harvest, a subsample of roots (≤ 0.25 g) from each plant was stained with trypan blue in lactoglycerin using the technique of Phillips and Hayman (1970), and specific root length (metres per gram of root) and the percentage of root length colonized with VAM fungi were assessed using the magnified line-intercept method of Ambler and Young (1977).

Statistical analysis

Two sample t tests were used to compare the relative spore densities of VAM fungal species in FERT and UNFERT field soils and cultured soils. Plant and fun-

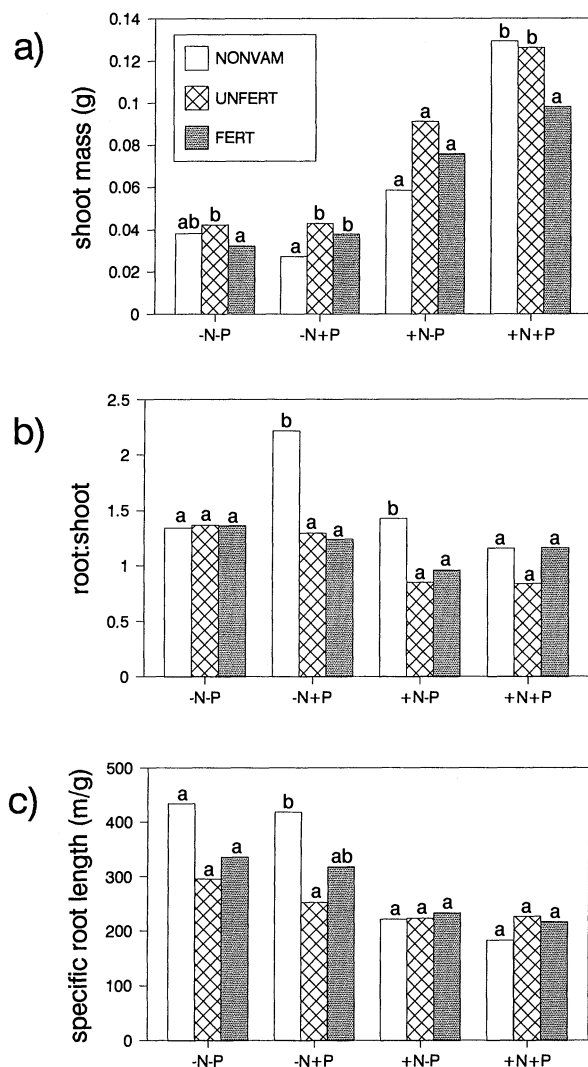


FIG. 1. Responses of big bluestem to inoculum and nutrient treatments at 1 mo: (a) shoot mass, (b) root : shoot mass ratio, (c) specific root length. Data represent average values of five plants. Within each nutrient treatment, bars with different letters differ at a .05 level.

gal responses to the experimental treatments were assessed using two-way ANOVA with the 12 inoculum sources nested into two (FERT and UNFERT) inoculum treatments. NONVAM plants were not included in the ANOVAs, but within each of the four nutrient treatments, mean responses of the NONVAM plants were compared with mean responses of plants inoculated with FERT and UNFERT soils using least significant difference multiple comparisons at a 95% confidence level. Prior to statistical analysis, shoot and root masses were log transformed, number of inflorescences per plant were square-root transformed, and percent VAM root length and relative spore densities were arcsine-transformed. Analyses were performed with SAS (SAS 1988) and Statgraphics (STSC 1986).

RESULTS

Spore communities in field soils

Total spore densities in FERT soils were not significantly different from total spore densities in UNFERT soils (Table 1). Spores collected directly from field soils frequently had broken or degraded walls and were probably inviable. Samples were collected late enough in the spring so that most of the viable spores had already germinated, but not so late that young spores would have been produced. Thus, due to the timing of sample collection, it is likely that the species and densities of VAM fungal spores observed in the field samples reflected the resistance of the spores to degradation more than the species composition of the VAM fungal community. Eleven species of VAM fungi were observed in the 12 samples; however, >96% of the spores encountered belonged to a single species, *Glomus aggregatum* (Table 1), a species known for copious production of tiny, recalcitrant spores. There were significantly more *Glomus occultum*, and there tended to be more *Gigaspora margarita* spores ($P = .09$), in UNFERT soils than in FERT soils.

Spore communities in cultures

Cultures of FERT soil contained significantly more spores than cultures of UNFERT soil (Table 1). Spores of 10 species of VAM fungi plus unidentifiable immature *Glomus* spores were observed in the cultures (Table 1). Spore communities in the cultures were more evenly divided between several species than spore communities in the field samples. It is likely that the species composition of the field plots was more accurately represented by the cultures than the field samples, because as explained previously, the field samples were collected after many of the viable spores had already germinated.

An unexpected taxonomic dilemma arose while analyzing the cultures. Spores of *Glomus intraradix*, a species which generally sporulates inside root cortices, were common within minute root fragments present in the spore extractions. The morphology (wall structure, hyphal attachment, reaction with Melzer's Reagent (chloral hydrate, iodine, and potassium iodide), and size) of these spores formed a continuum with the morphology of *G. aggregatum* spores. This situation has raised questions about the taxonomic integrity of these two "species." As a working solution to this dilemma, spores were classified as *G. intraradix* if they occurred inside a root fragment, and as *G. aggregatum* if they were not inside a root.

Composition of the spore communities in cultures of FERT soils were different from those of UNFERT soils. Relative spore densities of *G. gigantea*, *G. margarita*, and *S. calospora* were significantly higher in UNFERT soil than FERT soil (Table 1). Conversely, relative densities of *G. intraradix* were significantly higher in FERT soil than UNFERT soil.

TABLE 2. *F* ratios from two-way ANOVA tests of inoculum and nutrient effects on plant responses and vesicular-arbuscular mycorrhizal (VAM) colonization.

Response variable	Source of variance				
	Main effects			Interactions†	
	Inoculum	Nitrogen	Phosphorus	Inoculum × N	N × P
Plant responses					
Harvest 1					
Shoot mass	8.85**	176.54***	5.56*	1.88	0.75
Root mass	2.16	23.84***	0.66	1.22	1.84
Root : shoot ratio	1.58	25.09***	2.15	0.31	2.63
Height	6.16*	186.17***	3.37	2.06	4.96*
Specific root length	1.65	19.77***	0.79	0.68	0.26
Harvest 2					
Shoot mass	0.29	6265***	30.93***	0.61	4.74*
Root mass	0.05	1182***	12.93***	0.49	2.57
Root : shoot ratio	2.69	743***	0.23	3.94*	0.002
Height	0.15	2985***	5.44*	1.13	1.01
Inflorescences/plant	11.21**	2860***	18.77***	1.00	18.77***
VAM colonization					
% hyphae	13.19**	30.09***	1.71	0.86	0.42
% arbuscules	22.76***	111.00***	1.22	1.06	5.57*
% vesicles	0.52	18.33***	8.14**	0.85	0.15

*, **, *** indicate that *F* ratios were significant at $P \leq .05$, $.01$, and $.001$, respectively.

† None of the inoculum × P or three-way interactions were significant at $P \leq .05$.

Plant responses at one month

Inoculum (FERT vs. UNFERT), N, and P each explained a significant amount of the variance in shoot mass, while only N explained a significant amount of the variance in root mass, root : shoot ratio, and specific root length (Table 2). Both inoculum and N accounted for a significant amount of the variance in shoot height (Table 2). Plants inoculated with UNFERT soil were consistently larger than plants inoculated with FERT soil, but this difference was only significant in the -N-P and +N+P treatments (Fig. 1a). It is interesting to note that within the +N+P treatment, NONVAM plants weighed significantly more than plants inoculated with FERT soil but not plants inoculated with UNFERT soil. This contrasts with the trend from the other three nutrient treatments (-N-P, -N+P, +N-P) in which NONVAM plants were consistently (but not always significantly) smaller than plants inoculated with UNFERT soil (Fig. 1a).

Root : shoot ratios of NONVAM plants were significantly greater than mycorrhizal plants in the -N+P and +N-P treatments; otherwise there were no significant differences between root : shoot ratios of the treatments (Fig. 1b). Specific root length was influenced by N. Plants deficient in N produced much finer roots than plants receiving supplemental N. This pattern was most pronounced in NONVAM plants and least pronounced in plants inoculated with UNFERT soil (Fig. 1c).

Plant responses at three months

Nitrogen accounted for a significant amount of the variance in all variables measured, and P accounted

for a significant amount of the variance in all variables except root : shoot ratio. Inoculum accounted for a significant amount of the variance in the number of inflorescences per plant (Table 2). NONVAM plants and plants that received no supplemental N were severely stunted and had higher root : shoot ratios compared to mycorrhizal plants that received N (Fig. 2a, b). In contrast to the results at 1 mo, after 3 mo NONVAM plants across all nutrient treatments were significantly smaller than plants inoculated with either FERT or UNFERT soils (Fig. 2a). Within the +N+P treatment, plants inoculated with UNFERT soil produced significantly more inflorescences per plant than those inoculated with FERT soil (Fig. 2c). Only plants receiving supplemental N produced inflorescences.

VAM colonization

Both inoculum and N accounted for a significant amount of the variance in hyphal and arbuscular colonization (Table 2). Plants inoculated with UNFERT soil consistently contained more hyphae and arbuscules than plants inoculated with FERT soil (Fig. 3a, b). Supplemental N also increased the amount of hyphal and arbuscular colonization (Fig. 3a, b). Variance in vesicular colonization was not related to inoculum; however, both N and P accounted for a significant amount of variance (Table 2). In general, addition of N increased vesicular colonization while addition of P decreased it (Fig. 3c). NONVAM plants generally remained uncontaminated by VAM fungi; however, hyphae were observed in several "NONVAM plants" and arbuscules were observed in one "NONVAM" plant.

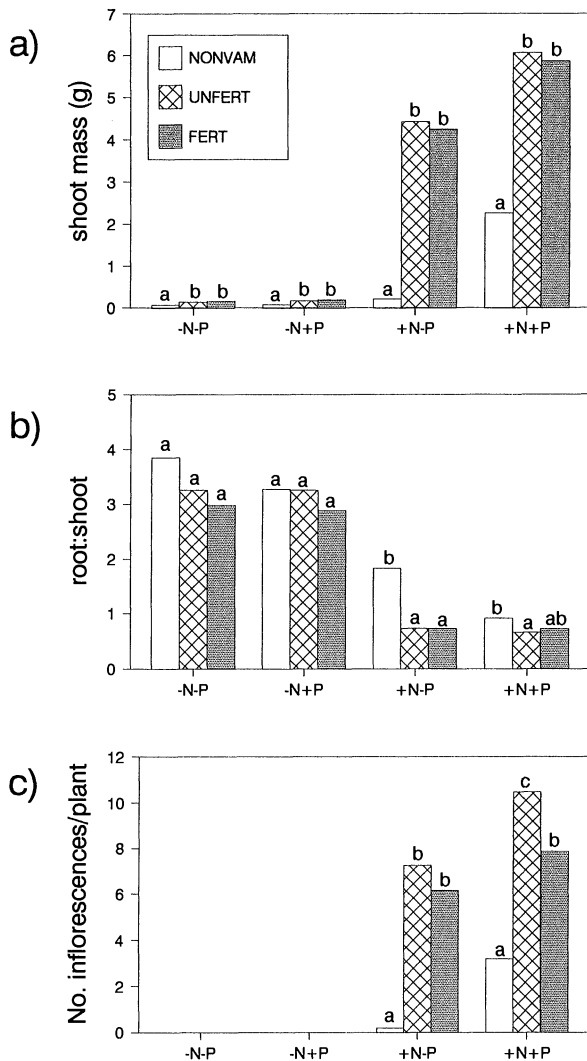


FIG. 2. Responses of big bluestem to inoculum and nutrient treatments at 3 mo: (a) shoot mass, (b) root : shoot mass ratio, (c) number of inflorescences per plant. Data represent average values of five plants. Within each nutrient treatment, bars with different letters differ at a .05 level.

It is impossible to determine the origin of this contamination.

DISCUSSION

Fertilization concomitantly changed both plant and fungal communities at Cedar Creek. Previous studies have shown that long-term fertilization of experimental grasslands at Cedar Creek changed the composition of plant communities (Tilman 1988); and the present study showed that fertilization also changed the composition of VAM fungal communities. These findings suggest that coadapted mycorrhiza-soil complexes may exist, and fertilizing low-nutrient soils disrupts these complexes.

Eight years of fertilization reduced relative spore densities of *Gigaspora gigantea*, *G. margarita*, *Scutel-*

lispora calospora, and *G. occultum*, and increased densities of *G. intraradix*. These findings are interesting because they are results of an 8-yr field experiment that corroborate results of several growth chamber and greenhouse studies. It has been shown that *G. margarita* (Douds and Schenck 1990) and *S. calospora* (Thomson et al. 1986, 1990) are sensitive to P fertilization. In contrast, sporulation by *G. intraradix* has been shown to be insensitive to P fertilization (Sylvia and Schenck 1983) and even positively correlated with concentrations of N and P in host plant tissues (Douds and Schenck 1990). Differences in fungal sensitivities to fertilization may result from differences in require-

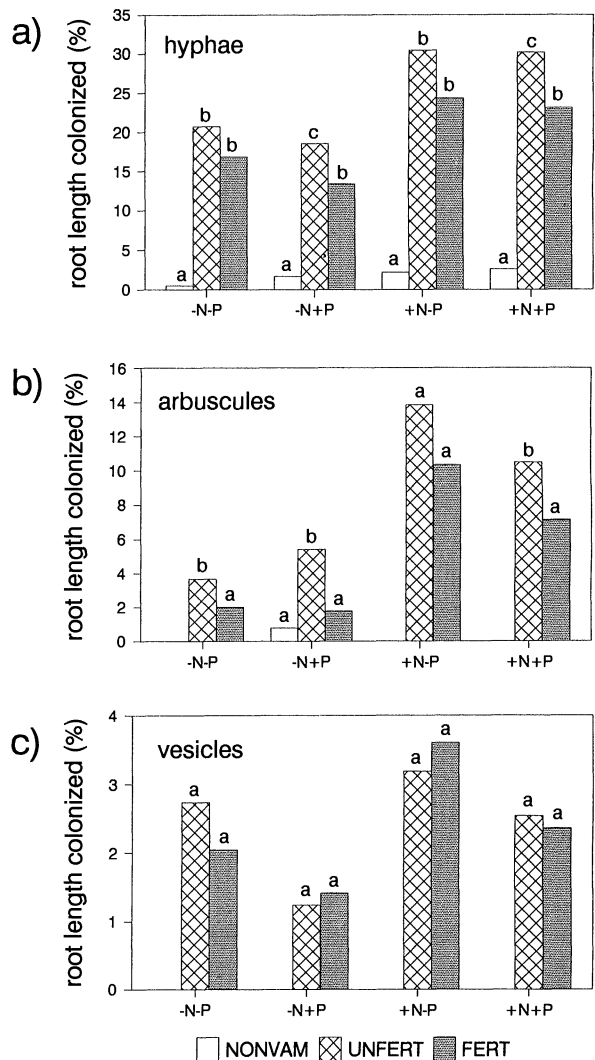


FIG. 3. Fungal structures in big bluestem roots grown under three inoculum treatments and four nutrient treatments: (a) percent root length colonized with hyphae, (b) percent root length colonized with arbuscules, (c) percent root length colonized with vesicles. Data represent average values of five plants. Within each nutrient treatment, bars with different letters differ at a .05 level.

ments for soluble carbohydrates in root exudates. Phosphorus fertilization has been shown to reduce concentrations of soluble carbohydrates in root exudates (Ratnayake et al. 1978, Graham et al. 1981). Douds and Schenck (1990) suggest that intracortical sporulation by *G. intraradix* shortens the path of carbon flow and may contribute to the insensitivity of this species to P fertilization.

Results of this study support the hypothesis that fertilizing low nutrient soil favors the proliferation of inferior VAM mutualists. Big bluestem inoculated with FERT soil were significantly smaller at 1 mo (Fig. 1a), and produced significantly fewer inflorescences at 3 mo (Fig. 2c), than big bluestem inoculated with UNFERT soil. Other workers have also found VAM fungi isolated from nutrient-rich soil to be inferior mutualists compared to VAM fungi isolated from nutrient-poor soil. Louis and Lim (1987) reported that a strain of *G. clarum* isolated from low P soils improved growth and nitrogenase activity of soybean (*Glycine max* var. Mik-iwashima) while a strain isolated from high P soils did not. Similarly, Henkel et al. (1989) found assemblages of VAM fungi from P-deficient ridge-top soils improved P uptake by *Agropyron smithii* to a greater extent than VAM fungi from adjacent P-rich soil at the base of the slope. It is interesting to note that *G. intraradix*, the only species to increase in response to fertilization, has been shown to depress growth of citrus grown in high P soils (Peng et al. 1993). Big bluestem inoculated with FERT and UNFERT soil were colonized differently by VAM fungi. Plants inoculated with UNFERT soils contained significantly more hyphae and arbuscules than plants inoculated with FERT soils. Mycorrhizal hyphae exploit soil more thoroughly than root hairs and thus increase the absorptive surface area of the host's roots. Arbuscules are the organs through which VAM fungi provide their host inorganic nutrients in exchange for organic carbon. Graham et al. (1982) showed that effectivity of *Glomus* isolates on citrus was related to production of external hyphae. Furthermore, an isolate of *Glomus fasciculatum* from high P soil produced less external hyphae, and was consequently less beneficial, than isolates from low P soils. The higher levels of hyphae and arbuscules observed in big bluestem roots inoculated with UNFERT soil suggests that the VAM fungi in these soils were more effective at acquiring nutrients from the soil, and were also more actively exchanging resources with their host plants, than the VAM fungi in the FERT soils.

Vesicles are intracortical structures that serve a storage and possibly reproductive function for VAM fungi. Unlike hyphae and arbuscules, there was no difference in the amount of vesicles formed by plants inoculated with FERT and UNFERT soil. This suggests that fungi from FERT soil exerted a higher net carbon cost on their hosts than fungi from UNFERT soils because FERT fungi were providing their host with less soil resources (had fewer arbuscules) but were provisioning

their own storage structures at the same level as the UNFERT fungi. Perhaps vesicle : arbuscule ratios may be a useful index of VAM effectivity. Future research should be aimed at identifying characteristics of beneficial vs. detrimental VAM associations.

One might hypothesize that plants inoculated with UNFERT soils were more fit than plants inoculated with FERT soil because UNFERT soils contained higher densities of infective VAM propagules and caused more extensive early VAM colonization. If this alternative hypothesis is true, and extensive early colonization is the key to high fitness, then mycorrhizal plants should always be larger than NONVAM plants. However, this prediction is not supported by the results presented in Fig. 1a. Well-nourished (+N+P) plants inoculated with FERT soil were significantly smaller than NONVAM plants, suggesting that the VAM fungi in FERT soils were actually detrimental to young big bluestem plants. Shoot mass of plants inoculated with UNFERT soil were not significantly different from the NONVAM plants, suggesting that VAM fungi in these soils were neither beneficial nor detrimental to 1-mo-old plants receiving N and P. It is well known that VAM fungi can be a significant carbon drain on plants (Buwalda and Goh 1982). If the benefit of increased access to soil resources does not outweigh the carbon cost, then VAM plants will be smaller than NONVAM plants. Bethlenfalvai et al. (1982) showed that even ultimately beneficial VAM fungi may be parasitic early in the ontogeny of the symbiosis when growth of the host plant is not limited by soil resources. In the present experiment, providing young plants with N and P appears to compensate for the absence of VAM. Even though VAM colonization was greater in plants inoculated with UNFERT soils than FERT soils, a parasitic response was caused only by the VAM fungi in FERT soil.

It might also be argued that because soil inoculum was used in this experiment, the effects of VAM fungi are confounded with the effects of "other soil organisms" that inhabit fertilized soils. A composite microbial-wash was applied to each plant in an effort to remedy such confounding. Nevertheless, the unlikely possibility remains that soil inhabitants other than VAM fungi that were too large to be included in the microbial wash could be responsible for the observed results. Also, the potential for soil microorganisms to antagonize VAM fungi must be recognized. Hetrick et al. (1986) showed that mycorrhizal growth response of big bluestem was eliminated in the presence of non-sterile prairie soil or soil microorganisms. More recently, Hetrick and Wilson (1991) showed that soil microflora may regulate root colonization and suppress mycorrhizal effectivity of a wide variety of VAM fungal species. Although it is clear that soil microorganisms are able to suppress mycorrhizal responses, the mechanisms responsible for this phenomenon remains a mystery.

Mycorrhizae are important components of nearly all terrestrial ecosystems. A basic understanding of the mechanisms controlling whether VAM associations are mutualistic or parasitic is necessary for understanding and managing ecosystems. The composition of VAM fungal communities are known to change as ecosystems change (Schenck et al. 1989, Wacker et al. 1990, Johnson et al. 1991). It is important to consider how changes in VAM fungal communities may influence plant communities. For example, there is good evidence that inferior mycorrhizae may be involved in the yield decline associated with continuous cropping of corn and soybean (Johnson et al. 1992). If heavily fertilized agricultural systems have the potential to develop inferior mycorrhizal associations, then effective management of mycorrhizae in agriculture may require manipulation of VAM fungal communities through inoculation or cultural practices that favor proliferation of the most beneficial VAM fungi.

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