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Niche Partitioning Among Arbuscular Mycorrhizal Fungi and Consequences for Host Plant Performance

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NICHE PARTITIONING AMONG ARBUSCULAR MYCORRHIZAL FUNGI AND

CONSEQUENCES FOR HOST PLANT PERFORMANCE

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and Consequences for Host Plant Performance

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Jennifer H. Doherty

Dedication

This dissertation is dedicated to Craig, my husband. Without Craig's encouragement,

assistance, and love I would still be picking spores. This degree belongs to both of us.

Craiger, we are now CJDD, Ph.D.

Acknowledgments

ABSTRACT

NICHE PARTITIONING AMONG ARBUSCULAR MYCORRHIZAL FUNGI AND

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Jennifer H. Doherty

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We understand little about the factors that determine and maintain local species

diversity of arbuscular mycorrhizal fungi (AMF), the reasons why a single plant has

multiple AMF partners, and how that diversity influences host plant performance. The

extent to which co-occurring AMF species occupy different niche space, based on their

ability to tolerate different soil conditions or differentially promote host plant growth in

those differing conditions, offers possible explanations for the maintenance of diversity.

AMF community composition was examined in relation to soil variability in a

naturally metalliferous serpentine grassland and along a Cu, Cd, Pb, and Zn soil

contamination gradient. Both field surveys demonstrated that AMF community

composition is strongly influenced by soil factors and provide evidence that local

diversity of AMF communities is at least partially maintained by environmental niche

partitioning among fungal species.

Because there is some evidence that AMF species can be non-additive in their

effects on plant growth, the appropriate measure of AMF function may be how much

plant growth is affected when that particular AMF species is deleted from the

community. Greenhouse experiments using this deletion approach, and the traditional

approach of evaluating host plant growth with a single AMF species, were performed.

The experiments involved two grass species: *Andropogon gerardii* and *Sorhastrum*

nutans and a subset of their natural AMF community grown in soils differing in nitrogen, phosphorus, and nickel, which is naturally high in the plants' native serpentine soils. This deletion method revealed that functional redundancy, with regards to host plant growth promotion, was the most common consequence of multiple species infecting one root. Functional complementarity and functional synergy, which may help explain why plants support multiple partners, were also demonstrated. Each of these interactions was found to be soil context dependent for most fungal species. These results demonstrate that the composition of the AMF community colonizing a host plant is important for plant performance and the consequences of colonization change with soil condition. They also suggest an explanation for why any one plant species supports several species of these fungi.

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Introduction

The ultimate goal of my dissertation is to improve our understanding of how arbuscular mycorrhizal (AM) fungal diversity is maintained at a local scale, and to explore the consequences of that diversity on plant performance. An estimated two-thirds of the known 250,000 vascular plant species support AM fungi (Fitter and Moyersoen 1996) yet there are currently only 214 described fungal species (www.amf-phylogeny.com). Despite this low global diversity, local diversity of AM fungi is similar to plant diversity (Bentivenga and Hetrick 1992, Bever et al. 1996, Stutz and Morton 1996, Johnson and Wedin 1997, Eom et al. 2000, Castelli and Casper 2003) and a given mycorrhizal plant may be colonized by many species of fungi (Smith and Read 1997, DeBellis and Widden 2006).

To investigate the factors maintaining this AM fungal diversity, we must consider the possibility that co-occurring AM fungal species occupy different niche space and are not just stochastically assembled (Abbott and Gazey 1994). Evidence for niche partitioning among AM fungi based on abiotic conditional differentiation has been found in a variety of field surveys (Jacobson 1997, Miller and Bever 1999, Whitfield et al. 2004, Lekberg et al. 2007, Wu et al. 2007). AM fungal species also have been shown to directly exhibit great inter- and intra-specific diversity in fitness with regards to different abiotic environments such as the amount of heavy metals (Li et al. 2009), water (Auge 2001, 2004), salt (Juniper and Abbott 2004, 2006) and soil temperature (Tommerup 1983, Klironomos et al. 2001, Rillig et al. 2002). These studies have been carried out using a variety of fitness measures such as spore germination (Tommerup 1983), hyphal growth (Heinemeyer and Fitter 2004), plant colonization success (Li et al. 2009), and even

hyphal wound repair (de la Providencia et al. 2007). However, many of the studies of this type compare fungal species from different locations and soil origins, limiting the ecological implications of these differences in fitness in relation to local habitat heterogeneity.

As an obligate mutualist (Smith and Read 1997), the fitness of an AM fungal species is dependent not only on the suitability of the fungus to its abiotic soil environment but also its relationship with the host plant. Plants may be able to choose when and with which partners they associate. This statement is based on the fact that plants have been shown to decrease the amount of carbon available to partner fungi in conditions where the mutualism would be less beneficial to plants, such as in soils with high amounts of P (Thomson et al. 1986, Graham and Eissenstat 1994), light-limited environments (Heinemeyer et al. 2004), or under herbivory (Klironomos et al. 2004). Additionally, Bever et al. (2009) has shown that plants can preferentially allocate C to the more beneficial fungal partner within a spatially structured community. Therefore the fitness of an AM fungal species may also rely on its ability to confer a needed benefit to the host plant.

Ecological explanations of taxonomic diversity rely on functional diversity within the AM fungi. There is growing evidence for such functional diversity within this group. Differences in such factors as nutrient or water uptake or protection from pathogens or heavy metals (Smith and Read 1997, Borowicz 2001, Entry et al. 2002, Auge 2004, Vogel-Mikus et al. 2005) and the importance to the host plant of any one AMF species may be conditional on the soil environment (Medeiros et al. 1994, Jansa et al. 2005, Lee and George 2005, Vogelsang et al. 2006).

Though there is limited evidence that AMF species differ in their ability to promote plant growth in different environments and can provide different functions to plants (Bever et al. 2001, Heinemeyer and Fitter 2004), I am aware of no study that assesses whether the amount of growth promotion and type of function provided by naturally co-occurring species changes with soil condition.

In Chapter 1, I describe a field study examining the composition of AM fungal communities of a naturally metalliferous serpentine grassland in Nottingham County Park in relation to soil variability using field spore surveys and cultures of field soil in the greenhouse. If there is niche partitioning among AM fungi in relation to soil environment, then AM fungal community composition will change with soil environment.

In Chapter 2, I describe a large greenhouse experiment to investigate the core question of my dissertation, "Is there niche partitioning, as measured by variation in host plant performance, fungal sporulation, and root colonization, among arbuscular mycorrhizal fungi from a natural plant community?" I used a natural plant/fungal community and soil from the naturally metalliferous serpentine grassland in Nottingham County Park.

For Chapter 3, I investigated many of the same issues as in Chapters 1 and 2 but using AM fungal communities from a natural grassland heavily contaminated by the activities of a Zn smelter. I examined how a Cu, Cd, Pb, and Zn soil contamination gradient affects the composition the AM fungal community and how the AM fungal communities from along the gradient influence host plant performance.

Chapter 1: Soil factors influence arbuscular mycorrhizal fungal communities in an Eastern Serpentine grassland

1.1. Introduction

A single arbuscular mycorrhizal (AM) fungal species can infect a wide variety of plant species (Smith and Read 1997, Smith et al. 2000, DeBellis and Widden 2006) and several species normally occur together on the same plant. A major goal in the study of arbuscular mycorrhizae is determining the ecological factors that structure the taxonomic composition of fungal communities. Recent work has shown AM fungi to be more specific to particular host plant species than was previously thought (Eom et al. 2000, Johnson et al. 2005, King-Salter et al. 2007), and different plant species can alter AM fungal community composition (Mummey et al. 2005, Hawkes et al. 2006). But there is increasing evidence that abiotic soil characteristics have the ultimate impact on the AM community (Lekberg et al. 2007, An et al. 2008, Appoloni et al. 2008, West et al. 2009).

Serpentine sites are a mosaic of microhabitats created by a combination of widely varying soil depths and chemical properties that differ greatly over a small area (Brady et al. 2005) thus can serve as a model system for exploring the effects of soil heterogeneity on maintaining AM fungal diversity. Previous studies have shown that intense changes in soil characteristics, such as large changes in heavy metal content (Del Val et al. 1999, Schechter and Bruns 2008, Zarei et al. 2008), pH (Lekberg et al. 2007, An et al. 2008), water content (Miller and Bever 1999) and fertilization (Egerton-Warburton et al. 2001) have been shown to change AM fungal diversity and richness. Studies of AM fungal community diversity in sites with less dramatic variation have also showed a correlation between various abiotic soil factors and AM community composition, but many of these

studies find patterns over large areas (Ji 2007, West et al. 2009) or vegetation gradients (Fitzsimons et al. 2008) and do not focus on local microhabitats that can explain diversity within a single plant root system.

To better understand the factors shaping the composition of local AM fungal communities beneath a single host plant we performed two field surveys, each focusing on variation on a different scale, of AM fungi associated with the perennial bunch grasses, *Andropogon gerardii*, *Sorghastrum nutans* and *Schizachyrium scoparium* (added for the second survey), in an Eastern serpentine grassland. With both surveys I asked if the presence of AM fungal species in soil beneath the host plant is dependent on abiotic soil factors or host plant identity. I measured soil nutrients, such as P and NO_3^-, and soil metals, such as Ni, Cr, and Mg, which have all been shown to affect AM function and abundance.

In the first survey, I used trap cultures and spore identification to determine what was in root-only or whole soil communities. Both of these are proxy measures of which fungi are available in the soil to colonize plants. Identifying fungi inside roots, whether by using molecular methods or trap cultures from root-only inocula is an attempt to measure which species of AM fungi are active in a plant at any given time. By surveying both root-only and whole soil communities we hoped to determine if soil factors influence the subset of available AM fungi that colonize roots.

In the second survey, we considered the influence of proximity to the rooting zone of other plants in addition to abiotic factors. We did so by characterizing the field spore AM fungal communities at the center and edge of the bunch grass clumps (and at point in between). These grasses are long-lived perennials that may be able to modify the abiotic

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and biotic factors of their soil. In particular, we wondered if the edge of the clump, which could be influenced by neighbors, and be trained by target plant for less time, had a different AM community composition.

1.2. Methods

1.2.1. Site description

The study was performed in the serpentine barrens in Nottingham County Park located in Chester County in the Piedmont Plateau of southeastern Pennsylvania (39° 44' N, 76° 02' W). The Nottingham serpentine barrens are part of a group, the State Line Serpentine Barrens, distributed across a 60-square mile area in southeastern Pennsylvania and northern Maryland. The park is approximately 200-ha of serpentine grassland surrounded by a pine-oak forest (Fig. 1.1). The regional climate is humid temperate, with an annual mean temperature of 11°C and mean precipitation of 1200 mm (Arabas 1997). Soils are shallow, ranging from 15 cm deep in the grassland to 75 cm in the forest and have low levels of several macronutrients, including P and K, and high, potentially toxic, levels of Mg, Cr, Ni, and Fe (Casper et al. 2008). Three C_4 bunch grass species targeted in this study, *Andropogon gerardii* Vitman, *Schizachyrium scoparium* (Michx.) Nash, and *Sorghastrum nutans* L. Nash, and a fourth, *Sporobolus heterolepis* (A. Gray) A. Gray, dominate the grassland vegetation at the site. The surrounding forest is composed of pitch pine (*Pinus rigida* Mill.) and oak (*Quercus* spp.), with greenbrier (*Smilax rotundifolia* L. and *Smilax glauca* Walter) abundant in the understory.

1.2.2. 2006 Survey

1.2.2.1. Field collection

For our first survey I examined soil heterogeneity first and then, based on that soil survey, sampled the AM fungal community under plants growing in widely distinct soils. To do this, we collected soil cores (6 cm in diameter and 10 cm long) from under 50 clumps of *A. gerardii* and 50 of *S. nutans* in three distinct areas of the park in June 2006 and stored them at 4°C until analysis for gravimetric soil water content (SWC), P, Ca, Mg, Ni, NO3- + NO2+ -N, and NH_4^+-N (see methods below). Then in August 2006, we returned to sample 45 clumps, 25 of *Sorghastrum nutans* and 20 of *Andropogon gerardii*, with very high and very low values of each abiotic soil factor. To compare AM fungi colonizing roots with fungal species in the soil, we set up pairs of trap cultures to determine the AM species composition of both root-only and whole soil communities, which included roots. We also re-measured for SWC, $NO_3^- + NO_2^+$ -N, and NH_4^+-N to examine possible correlations between soil factors and the AM fungal communities.

1.2.2.2. Greenhouse cultures

As many roots as possible were removed from each core, cut into 2 cm segments and suspended in water. We randomly removed 0.5 g portions for each trap culture. To construct trap cultures from soils, we homogenized the soil from each core and measured out two 30-ml volumes. One volume was steam pasteurized for 2 hours at 100 °C, 1 atm to kill AM fungi in the soil. We mixed each volume 1:4 with sterilized white bar sand and one portion of roots, and put the mixture into a cone-tainer (Stuewe and Sons, Inc.; 160 mL tapered cylindrical pots 3.8 cm in diameter and 21 cm in depth). Thus one of

each pair of cultures consisted of sterile soil with roots and the other was whole soil and roots.

Seeds of *A. gerardii* and *S. nutans* to be used as hosts for trap culture were collected at Nottingham, surfaced sterilized with 70% ethanol for 5 min, and germinated in sterilized vermiculite and sand. When seedlings were three weeks old, one was planted in each cone-tainer, with the species matching the grass clump under which the soil was collected. Cultures were grown for 4 months in temperature controlled greenhouses at the University of Pennsylvania that averaged 25 °C between 0600 and 1800 h and 21 °C otherwise. They received a minimum PAR of 430 μmol m^{-2} s^{-1} supplied by either active greenhouse lighting or ambient sunlight for 14 h each day.

After 4 months, AM fungal spores were extracted from a 50-mL soil sample from each culture using the wet sieve method (McKenney and Lindsey 1987) and identified based on morphological characters such as size and color, cell wall structure and texture, and differential staining with Melzer's reagent (Morton 1986). Since the relative abundance of AM fungal species in cultures grown in the greenhouse does not accurately reflect field relative abundances, samples were scored for the presence or absence of fungal species only.

As some AM fungal species require long growth periods to produce spores (Stutz and Morton 1996, Oehl et al. 2004), a second generation of trap culture was started using the remaining soil and roots from each culture. These were mixed with 330 ml of sterilized sand and 70 ml of sterilized field soil and used to fill a 600-ml pot. Each of these second generation cultures were planted with five seedlings of the appropriate species, grown for another 4 months, harvested, and the spore communities characterized.

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As the root inoculum was evenly distributed in both pots, we expected that all species found in a root pot would also be in the whole soil pot. If there is a selection process between the AM fungal community in the soil and in the roots we expected the whole soil pot to contain more species than the root pot.

1.2.3. 2007 Survey

For our second survey, we examined AM fungal diversity at two different spatial scales: among sites in the park and within an individual grass clump. We sampled at four sites in June 2007. Two of the sites were the same as in the 2006 survey, and the others were new (Fig. 1.1). Within each site we sampled at five points separated by at least 5 m. At each point, we sampled under the nearest monospecific clump of each of the three grass species (3 individuals per point, 5 points per site at 4 sites, a total of 60 individuals, 20 of each species) by collecting approximately 300 ml of soil from the center of each clump. We also recorded the latitudinal and longitudinal coordinates of each clump so we could consider spatial patterning in our analysis. At sites 1 and 3, we also sub-sampled within clumps that were approximately 30 cm in diameter. To subsample, in addition to collecting soil from the center of a clump, we collected soil at the edge of the clump and halfway between the center and edge, about 8 cm from the center. To characterize the soil properties in this second survey, we analyzed all soil samples for SWC, P, K, Ca, Mg, Ni, Zn, Cr, NO_3^- + NO_2^+ -N, and NH_4^+-N. In this survey, we quantified the number of each morphospecies making up the AM fungal spore community in each field sample.

1.2.4. Soil analyses

For all soil elemental analyses we used a weak acid, Mehlich-3 (0.2 N CH_3COOH + 0.25 N NH_4NO_3 + 0.015 N NH_4F + 0.013 N HNO_3 + 0.001 M EDTA), as the extractant (Mehlich 1984). We used this extractant instead of a strong acid to obtain total element concentrations because we were most interested in obtaining a proxy for bioavailabilty (Mehlich 1984). For these extractions, 40 ml of Mehlich-3 extractant was added to 4 g of fresh soil. The mixture was shaken at 180 rpm for 5 min, then filtered through a P8 paper filter (Fisher Scientific) to remove soil particles and frozen until analysis. Samples collected in 2006 were analyzed on a Perkin Elmer Optima 3000 ICP-OES according to EPA Method 6010C. Samples collected in 2007 were similarly analyzed on a Spectro Genesis ICP-OES. No standard reference materials (SRM) were extracted and analyzed so while data are internally consistent within year it cannot be determined if they represent absolute values.

We extracted exchangeable $NO_3^- + NO_2^+$ -N, and NH_4^+-N by adding 20 ml of 2 M KCl to 4 g of fresh soil (Keeney and Nelson 1982, Griffin 1995). Each sample was then shaken for 60 min at 100 rpm, filtered and frozen until analysis. Samples collected in 2006 were analyzed colorimetrically on an automated ion analyzer (Lachat Quikchem 8000, Zellweger analytics, Milwaukee, WI) using protocols 12-107-04-1-B and 12-107-06-2-A for NH_4^+ and $NO_3^- + NO_2^+$ respectively. Samples collected in 2007 were analyzed by the Rutgers University Pinelands Field Station using a Technicon autoanalyzer and Methods 350.1 and 353.2 for NH_4^+ and $NO_3^- + NO_2^+$ respectively (EPA 1983b, a).

1.2.5. Statistical analyses

We compared the AM species in each culture within a pair and reported frequencies of species' presence and absence. We then pooled data for both pots in each pair when comparing AM fungal communities with soil factors.

Canonical analysis of principal coordinates (CAP, Anderson and Robinson 2003, Anderson and Willis 2003) was used to analyze differences in AM communities among sites, host plant species, and in relation to soil factors across collection sites and plant species. First we used CAP to perform discriminant analysis on AM communities by site and host plant and then we used it to perform a canonical correlation analysis comparing AM communities with soil factors. The analyses were based on Bray-Curtis dissimilarities calculated from square-root transformed AM community data and untransformed soil factor data. Species that were found in less than 5% of cultures or samples were not used for CAP analysis. The canonical correlations in each case were tested using 999,999 unrestricted random permutations of the raw data.

The AM fungal species that obtained a correlation of $|r| > 0.35$ with given canonical axes were then investigated with regards to the soil properties ($|r| > 0.20$) also correlated with that axis. For the 2006 survey, t-tests were performed to determine differences in soil factors according to AM species presence or absence, while for the 2007 survey, correlation analysis was used. Spatial autocorrelation in the 2007 survey was investigated by using principal coordinates analysis (PCA) and correlation. Two PCA were performed: one with latitude and longitude and another with the 10 soil factors. The first axis of each analysis was then used in a correlation analysis across all sites and within each site.

All the 2007 analyses described above were performed using the 60 samples from the center of the grass clumps. To investigate proximity to the rooting zone of other plants on the AM community we performed a CAP discriminant analysis comparing the AM communities in the center, edge, and middle samples of those plants in which we performed all three collections. If there was some influence of other plants we would expect edge collections to be different than center collections. CAP analyses were done using CAP (Anderson 2004). PCA, correlations, and t-tests were performed in JMP 7.0.1 (SAS Institute Inc. 2007).

1.3. Results

1.3.1. 2006 Survey

Thirteen morphospecies were identified in the first harvest of the trap cultures: *Gigaspora gigantea, Scutellospora calospora, S. pellucida, Glomus aggregatum, G. claroidium, G. etunicatum, G. mosseae, G. rubiforme,* an unidentified brown *Glomus* with a thick wall, *Acaulospora mellea, A. morrowiae, A. spinosa, and Entrophospora infrequens*. *G. mosseae* and *A. spinosa* were not found in the second harvest. This species list was very similar to the list from other Nottingham field and greenhouse studies (Castelli and Casper 2003, Casper et al. 2008). Although we found a brown *Glomus* species with a thicker wall than usually observed, no new species were identified. The most common species found were *G. aggregatum* (present in 42 paired cultures), *G. claroidium* (38), and *G. etunicatum* (29) followed by a group of species approximately half as common: *Gi. gigantea* (22), *S. calospora* (22), and *A. mellea* (18) (Table 1.1).

Presence in the first round of a trap cultures did not ensure a species' presence in the second round. We observed 81 instances where a species was observed in the first harvest of a whole soil inoculum pot and not the second and 69 instances where the reverse was true (Table 1.1). Iin the root only inocula pots there were only 8 instances where a species was observed in the first harvest but not the second and 38 instances of the reverse. In general, *Glomus* species were more common or equally present in the second round of trap cultures (Table 1.1, except *G. mosseae* and thick *Glomus*). Members of the family Gigasporaceae and the genera *Acaulospora* and *Entrophospora* (except *A. mellea*) were more often observed after the first round of trap cultures (Table 1.1).

AM fungal communities in root-only cultures were largely made up of *G. aggregatum*, *G. claroidium*, and *G. etunicatum*. Except in two cases, cultures started with only roots were missing the five large-spored species (mean diameter > 125 µm) present in cultures started with whole soil inocula: *Gi. gigantea*, *S. calospora*, *S. pellucida*, *A. spinosa*, and *G. mosseae* (Table 1.1). The non-Gigasporaceae species, *A. spinosa*, and *G. mosseae*, were also very rare in whole soil cultures while the members of Gigasporaceae were frequently found in whole soil trap cultures but not the root only cultures.

In all but two pairs, the whole soil cultures produced a greater number of AM fungal species producing spores (Fig. 1.2b). Whole soil cultures contained between one and nine species whereas root only cultures contained between zero and five (Fig. 1.2a). Half of root only cultures contained just one sporulating species while the median of whole soil cultures was five.

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When the cultures were considered in pairs, there were 23 instances where a particular species was present in the root trap culture but not in the paired whole soil culture which included a similar sample of roots (Table 1.1). These cases indicate a great inconsistency in culturing AMF from roots.

The CAP analysis showed a significant effect of site on AM community composition (visualized in Fig. 1.3), with a squared canonical correlation of $\delta^2 = 0.3171$ ($p = 0.0036$). There was not, however, a significant effect of plant species on AM community composition ($p = 0.4295$). AM communities from sites B and C were separated from each other, but communities from site A were less distinguishable. The relative distinctiveness of the communities was reinforced by differences in success of the leave-one-out allocation from the CAP analysis (Anderson and Willis 2003). Leave-one-out analysis in CAP involves using a single observation as validation data, and the remaining observations as the model creation data. This is repeated such that each observation in the sample is used once as the validation data. Communities from site A had a much lower classification success, 0%, than communities from sites B (83% success) and C (31%).

CAP analysis also showed a significant correlation between AM community composition and soil factors ($\delta^2 = 0.4105$, $p = 0.0458$). Canonical axis one explained 34% of the variation and was also the best axis at separating communities by site. *Gi. gigantea*, *S. calospora*, thick *Glomus*, and $NO_3^- + NO_2^+$ were all relatively well positively correlated with axis one and each other, while Ni and SWC were negatively correlated (see Table 1.2 for values of r and less strongly correlated factors). Canonical axis two explained another 24.5% of the variation in AM community composition and was

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relatively well correlated with *G. aggregatum* (positive), *G. claroidium*, *G. etunicatum*, *A. mellea*, P, and Ca (negative).

Univariate analysis revealed that the presence of some AM species is related to specific soil factors. Communities with *Gi. gigantea* and *S. calospora* had significantly more extractable $NO_3^- + NO_2^+$ in the soils they were collected in (Fig. 1.3). Communities containing *Gi. gigantea* also had less Ni, as did communities containing the *thick* Glomus species. Communities with *S. calospora* were wetter on average. Communities with *G. aggregatum* had less P while communities with *A. mellea* had more (Fig. 1.5). Communities with *A. mellea* also had more Ca.

3.2. 2007 Survey

A similar set of species were identified from field soils collected in 2007. The ten morphospecies identified were: *Gi. gigantea, S. calospora*, *S. pellucida*, *G. aggregatum, G. claroideum, G. etunicatum, G. rubiforme,* an unidentified brown *Glomus, A. mellea* and *E. infrequens*. Among the most frequently found species were again *G. aggregatum* and *G. claroideum* while *G. etunicatum* and *S. calospora* were also very common (Table 1.3). Samples contained between two and nine species (Fig. 1.6). Most samples contained 5 species.

Correlation analysis of the first principal component from the spatial location and soil factors showed a weak, yet significant correlation between spatial location and soil factors ($r^2 = 0.15$, $p = 0.0021$, Fig. 1.7). However, there was no correlation between location within a site and soil factors; that is, there were no evident soil gradients within a site.

There was no effect of site on AM community composition (visualized in Fig. 1.8, $p = 0.3790$) or plant species on AM community composition ($p = 0.8661$). We also found no effect of location of collection within a clump of grass on the AM fungal community ($p = 0.2262$).

CAP analysis did show a highly significant correlation between AM fungal community composition and soil factors ($\delta^2 = 0.5384$, $p = 0.0001$). Canonical axis one explained 47.1% of the variation and was also the best axis at separating communities by site. *G. aggregatum* was very negatively correlated with axis one, while *A. mellea*, the brown *Glomus*, P, Mg, Zn, and SWC were all relatively well positively correlated with axis one and each other (see Table 1.4 for values of r and less strongly correlated factors). Canonical axis two explained another 19.8% of the variation in AM community composition and was relatively well correlated with *Gi. gigantea*, *S. calospora*, and SWC (positive) and *G. claroidium* and Mg (negative).

Univariate analysis revealed that the number of *G. aggregatum* spores in a sample was significantly, negatively correlated with SWC ($r^2 = 0.11$, $p = 0.0011$, results are from analysis with log transformed data), Mg ($r^2 = 0.25$, $p < 0.0001$), P ($r^2 = 0.28$, $p < 0.0001$), and Zn ($r^2 = 0.15$, $p = 0.0001$). In contrast, the number of *A. mellea* spores in a sample was significantly, positively correlated with each of those soil factors: SWC ($r^2 = 0.15$, $p = 0.0001$), Mg ($r^2 = 0.18$ $p < 0.0001$), P ($r^2 = 0.29$, $p < 0.0001$), and Zn ($r^2 = 0.17$, $p < 0.0001$). The brown *Glomus* species was also sensitive to the presence of P; like *A. mellea*, the number of brown *Glomus* spores increased as P increased P ($r^2 = 0.24$, $p < 0.0001$). The number of *S. calospora* spores in a sample was significantly, positively correlated with SWC ($r^2 = 0.24$, $p < 0.0001$) and Mg ($r^2 = 0.7$ $p = 0.0099$).

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1.4. Discussion

1.4.1. Soil factor effects

Soil factors were strongly and significantly correlated with the composition of AM fungal communities in soil in both surveys. The two surveys were confounded by year and growing season in addition to differences in what aspect of the community was measured, so direct comparisons are difficult. In 2006 we measured which species were available in the soil for colonization the next year, whether they were present as spores or not. In 2007 we measured what spores were in the soil, which presumably will be correlated with what species will be available for future colonization but will not be exactly the same, due to spore viability, etc. We did find that AM fungal communities in each survey were equally rich with about 5 fungal species in 50 ml of soil. Additionally, three patterns of association between AM fungal species and soil factors were robust and consistent over both surveys. In both August 2006 and June 2007, *A. mellea* was found to be positively associated with levels of P in the soil while *G. aggregatum* was found to be negatively associated. *S. calospora* was positively associated with SWC.

Significant site differences in AM fungal community composition were observed in August 2006 but not in June 2007, even though two of the same sites were revisited. The relationship between site location and soil factors found in 2006 can confound any soil factor- AM community relationship found. For example, instead of soil factor structuring the AM community, it could be some other factor associated with site, such as slope or surrounding vegetation. However, sites in 2006 were only separated by canonical axis 1 which did not explain all the variation. In fact, two of the three

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significant associations between AM species and soil factors found in both 2006 and 2007, were associated with axis 2.

The importance to the host plant of any one AM fungal species may be conditional on the environment (Jansa et al. 2005, Lee and George 2005, Vogelsang et al. 2006). AM fungal abundance, growth, and AM fungal-mediated host plant performance has been found to vary with abiotic factors such as soil water, P, and heavy metal content (Auge 2004, Cuenca et al. 2004, Lee and George 2005) suggesting that distinct species of AM fungi may be adapted to different conditions (Fitter et al. 2000). It is possible that AM local diversity is maintained as fungal species occupy different niches by functioning differently in different environments (Bever et al. 2001, Heinemeyer and Fitter 2004). Our results indicate that inocula availability of AM fungal species differ in accordance with soil factors. This pattern in inocula availability may lead to differences in the community actually colonizing plant roots within a heterogeneous soil environment.

1.4.2. Host plant effects

Host plants have been found to influence both the AM fungal spore community in the soil and the community within roots (Johnson et al. 1991, Koske and Gemma 1997, Helgason et al. 2002, Pivato et al. 2007, Croll et al. 2008). In contrast to one previous study with *A. gerardii*, *S. nutans*, and *S. scoparium* at Nottingham (Castelli and Casper 2003) and in support of another (Ji 2007), we did not observe a host plant effect on AM fungal spores present in the soil. The growth form and phenology of these three bunchgrass species are generally very similar and host plant species may play a larger role in shaping AM communities if host plants with contrasting growth forms or phenologies were considered (Bever et al. 1996).

Proximity of other plant species has also been shown to change the community colonizing roots of a target plant, most especially in studies of invasive species (Mummey et al. 2005, Hawkes et al. 2006). However, since we found no effect of host plant species, it was not suprising that we found no differences in AM fungal communities as a function of location within clump. We found that location within a clump of grass, and hence proximity to neighboring roots, did not affect the AM spore community in the soil. However, most neighbors to target plants in this study and Nottingham as a whole are other C_4 bunchgrasses. A more sensitive measure of the AM community associated and functioning with a given host plant is to observe exactly what species are colonizing the roots of the plant.

Though we found no measurable differences in AM fungal species composition among host plants, that does not necessarily mean there was no genetic or functional differences among the communities. Many researchers have found genetic and functional differences within an AM fungal morphospecies both among widely disparate ecotypes and clones from the same field (Munkvold et al. 2004, Jansa et al. 2005, Kelly et al. 2005, Bedini et al. 2009). At Nottingham, we have found that spores of *Gi. gigantea* and other species collected under different grasses differ in their ability to promote growth in the same grasses (Casper Lab unpublished, Ji et al. 2007).

1.4.3. Root-only inocula

Strong discrepancies have been reported between the taxa present as spores in the field or produced in the trap cultures using field soil, and the fungal community currently detected in the roots, often using molecular methods (e.g. Clapp et al. 1995, Kowalchuk et al. 2002, Wubet et al. 2003, Renker et al. 2005, Ahulu et al. 2006, Borstler et al. 2006,

Sykorova et al. 2007b). It may be that A. *gerardii* and *S. nutans* at Nottingham are colonized in August by only a subset of the available AM fungal species. Several studies have demonstrated that AM fungal species found in the roots of a plant may not be well represented by either the spore or extraradical hyphal community in the soil (Clapp et al. 1995, Renker et al. 2005, Hempel et al. 2007, Sykorova et al. 2007a). This has been interpreted as seasonal, host plant, and successional differences in what fungi are active within a plant (Heinemeyer and Fitter 2004, Sykorova et al. 2007a). Peaks in sporulation tend to occur after the species of fungus is physiologically active (Douds and Schenck 1990, Gazey et al. 1992, Abbott and Gazey 1994). The general lack of *G. gigantea* spores in June samples may indicate that *Gi. gigantea* is active in June and not August and that the three abundant species of *Glomus* species are the fungi that are active in August. That *Gi. gigantea* spores are much more abundant at Nottingham later in the growing season (Casper lab unpublished) also supports this interpretation.

Another interpretation of the sporulation discrepancies between cultures is that individual AM fungal species may be better than others in colonizing plants from root inocula. Much of the fungal biomass in roots is made up of hyphae. Species in the suborder Gigasporineae have been shown to not colonize plants from intra- and extra-radical hyphae as well as species from the Glomineae (Klironomos and Hart 2002). Besides a potential taxonomic inocula bias, another difficulty in using root-only trap cultures as a measure of which fungal species are active in the roots of a host plant is the relatively high incidence of cases when the root-only culture in a pair contained species the whole soil culture did not. It is possible that there was inconsistent infection from root inocula even within fungal species that colonize well from root inocula. This could

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be due to differences in the health or life stage of individual fungi, the chance absence of the fungal species in one of the paired root samples, or that species common in the whole soil, germinating from spores, could colonize the roots of the culture plant and keep fungi found in the root incoculum, hyphae, from colonizing. Therefore, we must conclude that root-only trap cultures should not be relied upon to give a good representation of the AM fungal community colonizing a plant's roots.

Table 1.1: The number of various types of cultures in which each AM fungal species appears. 45 pairs of whole soil cultures and root only cultures were harvested after growing for 4 months, repotted, and harvested again in another 4 months.

	Gi. gigantea	*S. calospora*	*S. pellucida*	*G. aggregatum*	*G. claroideum*	*G. etunicatum*	*G. mosseae*	*G. rubiforme*	Thick *Glomus*	*A. mellea*	*A. morrowiae*	*A. spinosa*	*E. infrequens*
Whole soil culture													
1st harvest only	8	13	3	8	9	6	2	5	9	6	8	1	3
2nd harvest only	5	2	1	14	17	11	0	5	4	8	0	0	2
Both harvests	9	6	2	19	10	2	0	3	0	4	1	0	0
Total appearances	22	21	6	41	36	19	2	13	13	18	9	1	5
Root only culture													
1st harvest only	0	1	0	0	0	7	0	0	0	0	0	0	0
2nd harvest only	0	0	0	12	15	10	0	0	0	0	0	0	1
Both harvests	0	0	0	14	8	1	0	1	1	4	3	1	3
Total appearances	0	1	0	26	23	18	0	1	1	4	3	1	4
Exclusively in													
Whole soil cultures	22	21	6	16	15	11	2	13	13	14	8	1	5
Root only cultures	0	1	0	1	2	10	0	1	1	0	2	1	4
Number of pairs	22	22	6	42	38	29	2	14	14	18	11	2	9

Table 1.2: Correlation coefficients for individual species or soil factors ($|r| > 0.20$) with canonical axes 1 and 2 from CAP analysis of 2006 survey data.

Canonical Axis 1

Positive		Negative	
Gi. gigantea	0.8608	*A. mellea*	-0.3463
S. calospora	0.5303	*G. aggregatum*	-0.2012
Thick *Glomus*	0.5148		
S. pellucida	0.2495		
G. rubiforme	0.2004		
$NO_3^- + NO_2^-$	0.4686	SWC	-0.3490
		Ni	-0.2554
		NH_4^+	-0.2162

Canonical Axis 2

Positive		Negative	
G. aggregatum	0.4469	*G. claroideum*	-0.6347
S. calospora	0.3205	*A. mellea*	-0.4807
		G. etunicatum	-0.4435
		A. morrowiae	-0.3816
		G. rubiforme	-0.3193
		Gi. gigantea	-0.2211
		S. pellucida	-0.2168
		E. infrequens	-0.2017
		P	-0.3271
		Ca	-0.2709
		Mg	-0.2032

Table 1.3: Frequency and abundance of the different AM fungal species in collections from the center of grass clumps.

AM fungal species	Present in center of clump (%)	Mean number of spores when present (± SE)
Gi. gigantea	58	4 ± 1
S. calospora	85	12 ± 1
S. pellucida	28	4 ± 1
G. aggregatum	78	21 ± 2
G. claroideum	82	4 ± 1
G. etunicatum	83	115 ± 10
G. rubiforme	18	33 ± 8
Brown *Glomus*	68	41 ± 4
A. mellea	33	13 ± 3
E. infrequens	12	15 ± 1

Table 1.4: Correlation coefficients for individual species or soil factors ($|r| > 0.20$) with canonical axes 1 and 2 from CAP analysis of 2007 survey data.

Canonical Axis 1			
Positive		Negative	
G. aggregatum	0.9414	*A. mellea*	-0.5435
		Brown *Glomus*	-0.5434
		P	-0.6257
		Mg	-0.5118
		Zn	-0.4685
		SWC	-0.3664
		Ca	-0.2271
Canonical Axis 2			
Positive		Negative	
Gi. gigantea	0.3875	*G. claroidium*	-0.7936
S. calospora	0.3733		
G. etunicatum	0.2590		
A. mellea	0.2084		
SWC	0.2699		
Mg	0.2637		
$NO_3^- + NO_2^-$	0.2122		
Zn	0.2075		
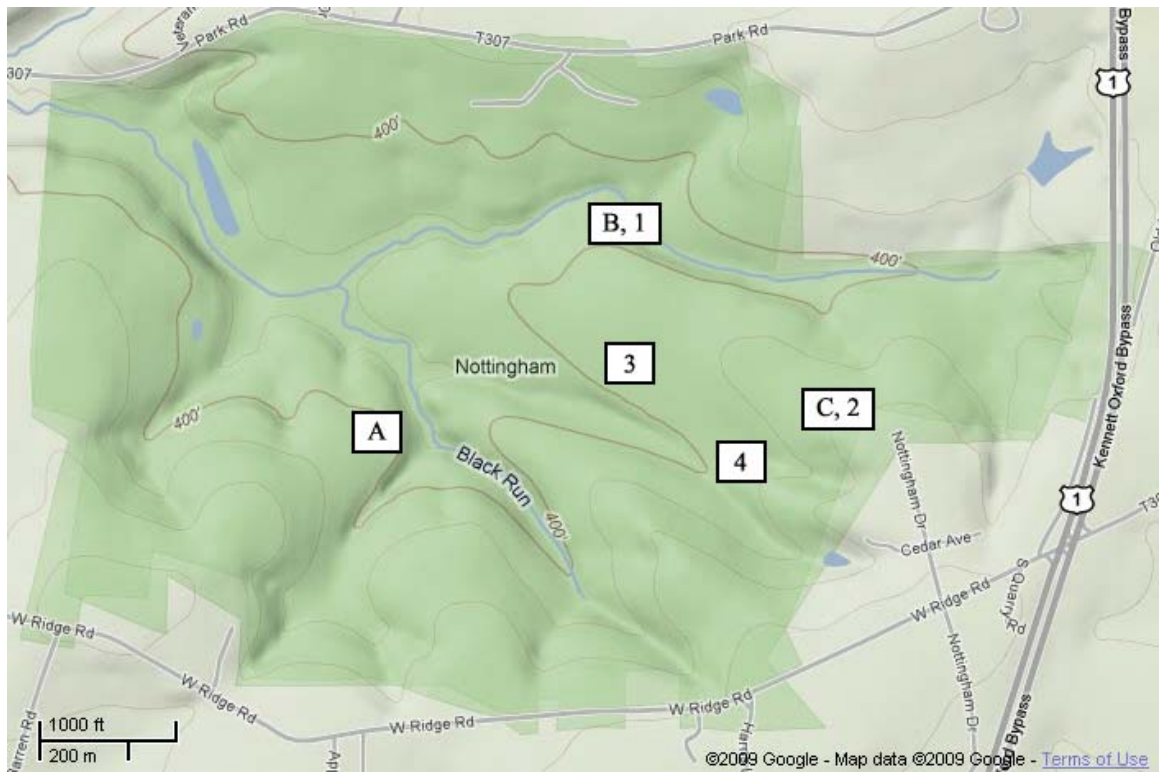
Figure 1.1: Map of Nottingham Country Park with collection locations indicated: 2006 survey locations A, B, and C; 20007 survey locations 1, 2, 3, 4. Sites B (1) and C (2) were surveyed in both 2006 and 2007.
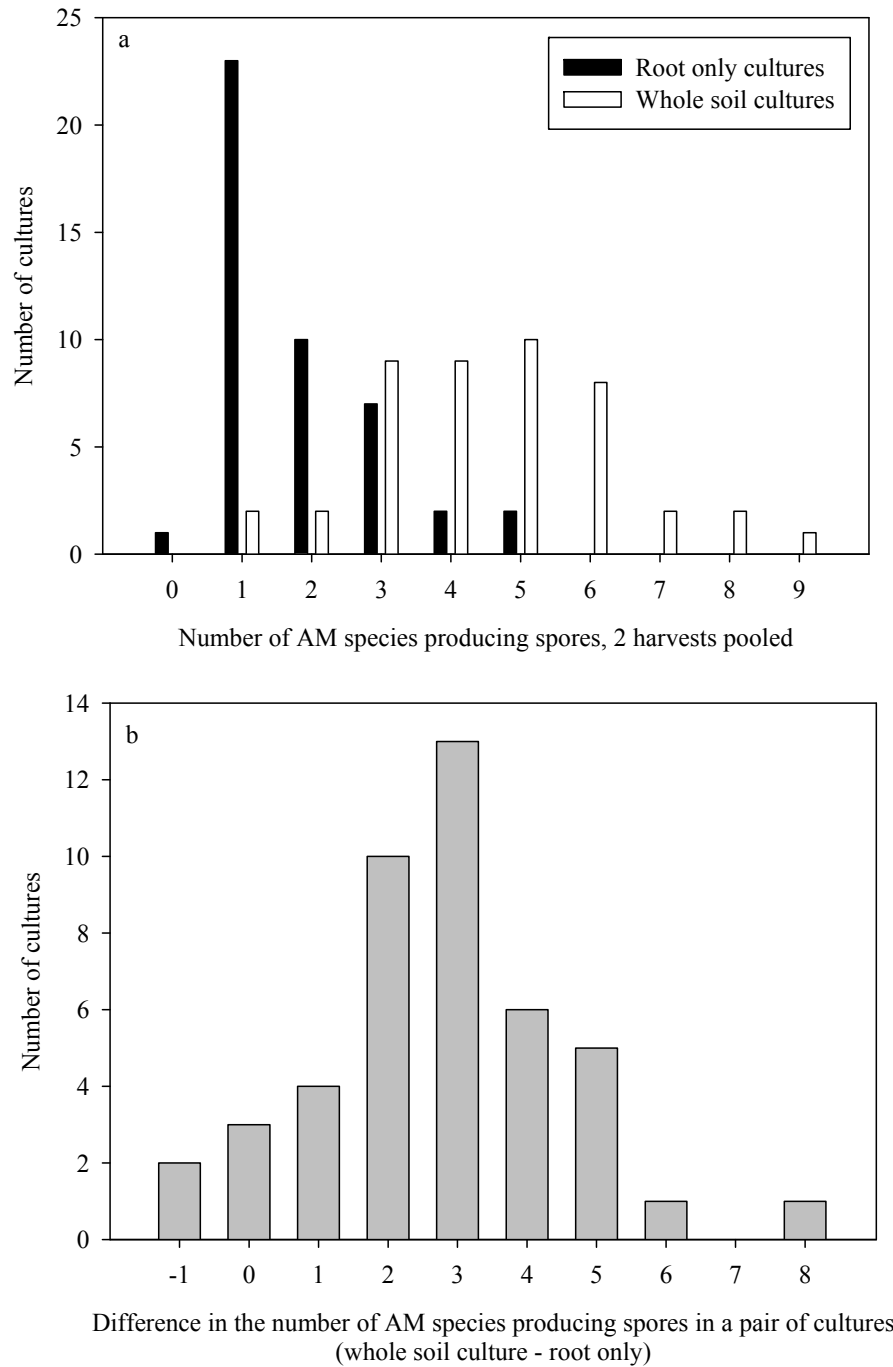
Figure 1.2: a) Histogram of the number of AM species producing spores in a root only culture after 2 harvests. b) Histogram of the difference in the number of AM species producing spores of the whole soil culture and root only culture in a pair.
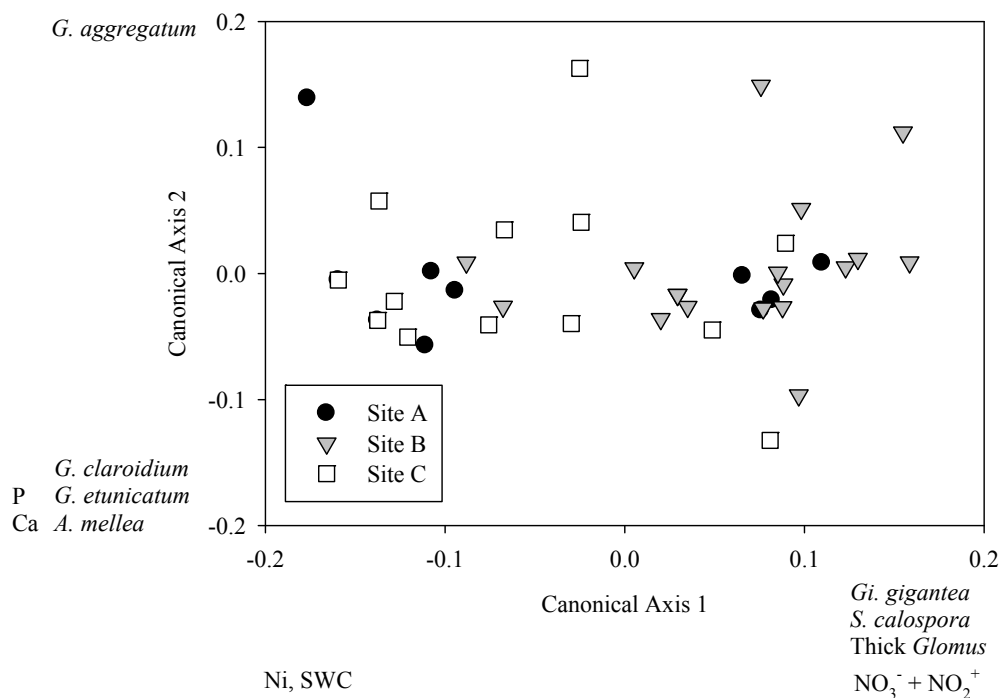
Figure 1.3: Two-dimensional scatter plot generated by the CAP analysis of the 2006 survey AM spore communities grouped by site. Analysis included 11 AM species and 7 soil factors. Factors relatively highly correlated to each canonical axis are indicated in the figure (AM species ($|r| > 0.40$), soils properties ($|r| > 0.25$)).
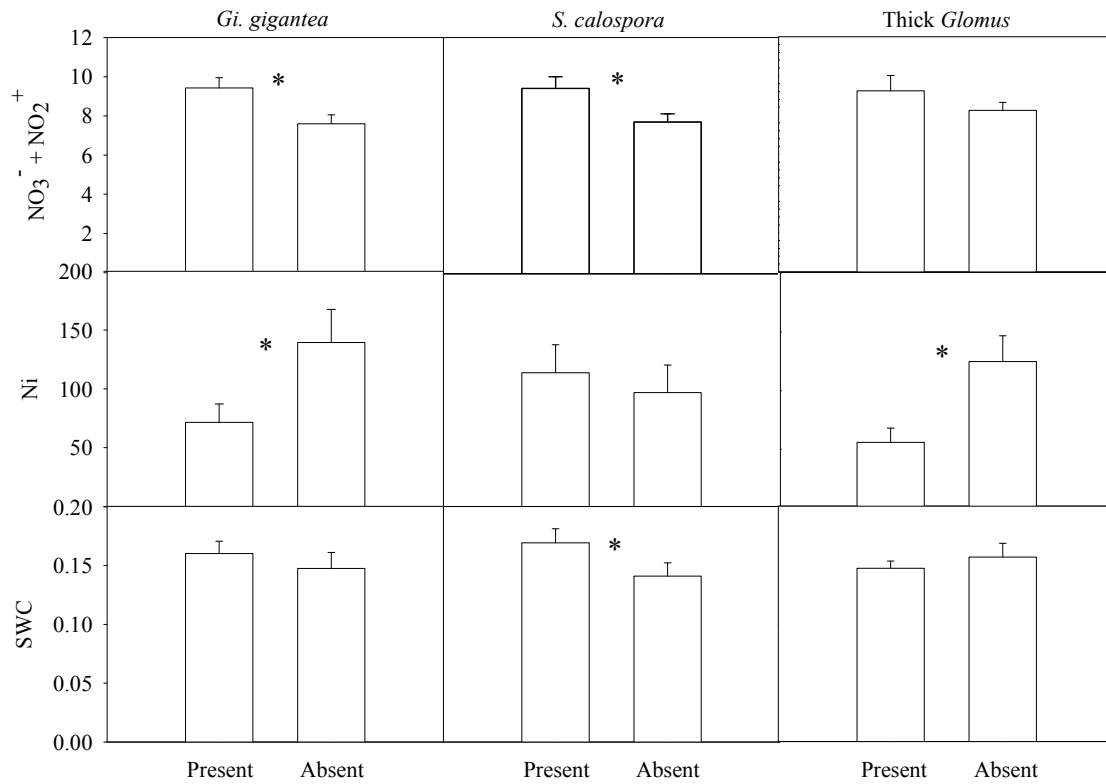
Figure 1.4: 2006 survey soil factor values (mean + SE) for collections with and without each AM fungal species that obtained a correlation of $|r| > 0.35$ with given canonical axis 1 (visualized in Fig. 1.3). * indicates a $p < 0.05$ from a t-test.
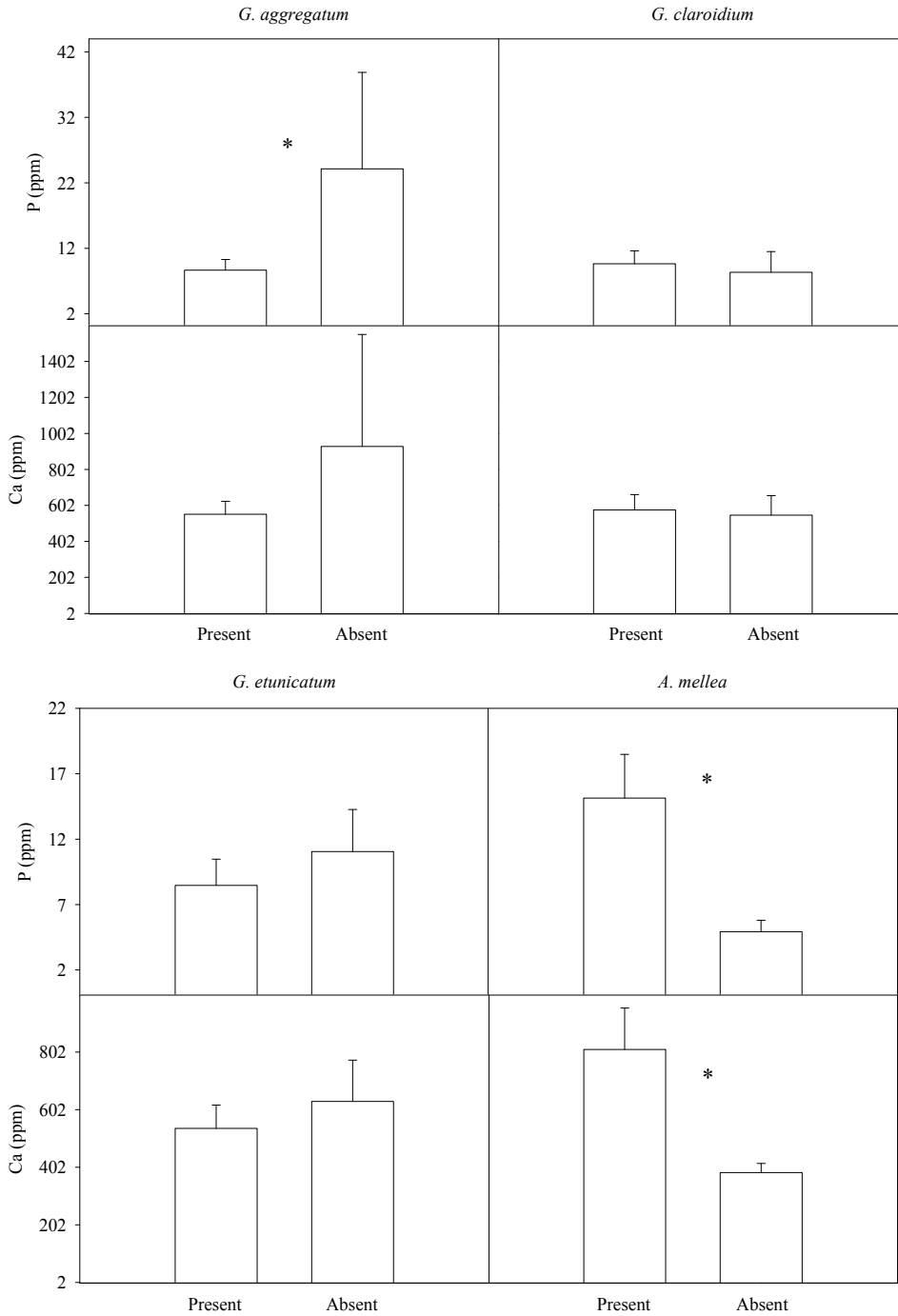
Figure 1.5: 2006 survey soil factor values for samples with and without each AM fungal species that obtained a correlation of $|r| > 0.35$ with given canonical axis 2 (visualized in Fig. 1.3). * indicates a $p < 0.05$ from a t-test.
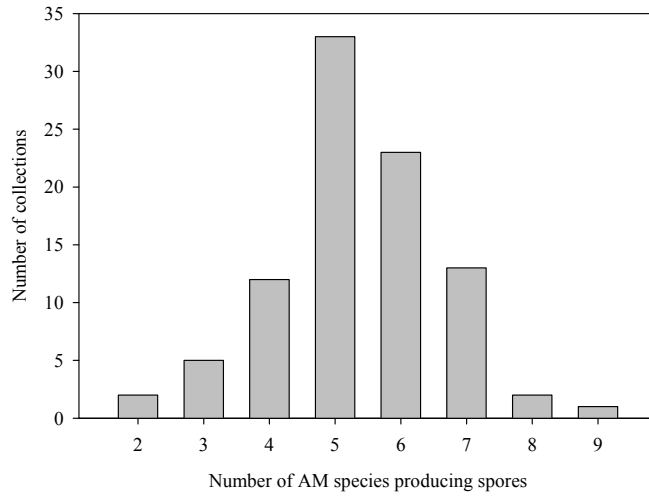
Figure 1.6: Histogram of the number of AM species found in each 2007 survey sample.
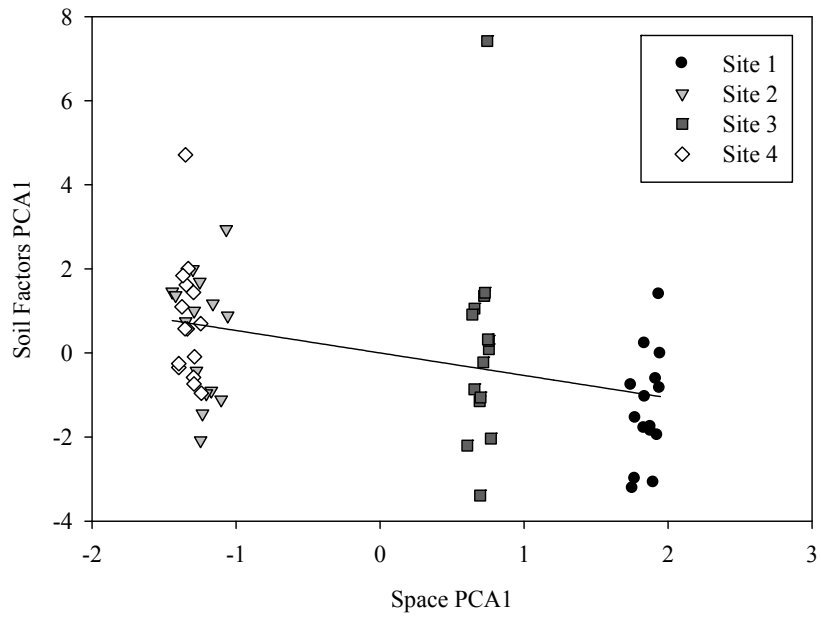
Figure 1.7: Two-dimensional scatter plot of PCA axis 1 from analysis of latitude and longitude and PCA axis 1 from analysis 10 soil factors from the 2007 survey. Points grouped by collection site and lines indicate linear correlation. $r^2 = 0.15$
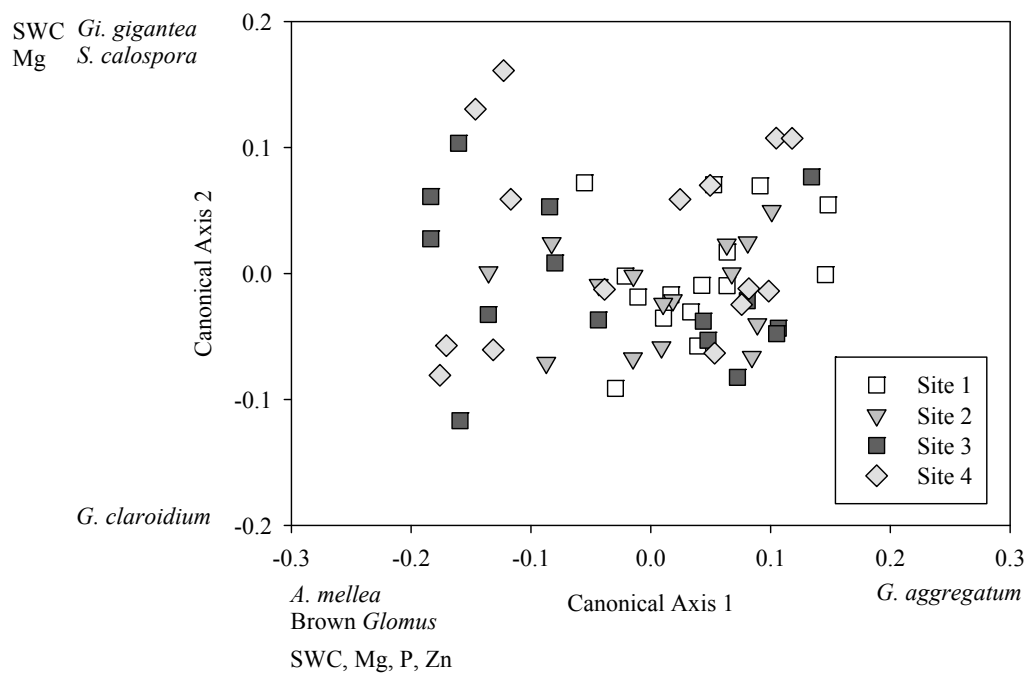
Figure 1.8: Two-dimensional scatter plot generated by the CAP analysis of the 2007 survey AM spore communities grouped by site. Analysis included 10 AM species and 10 soil factors. Factors relatively highly correlated to each canonical axis are indicated in the figure (AM species (|r| > 0.35), soils properties (|r| > 0.20)).
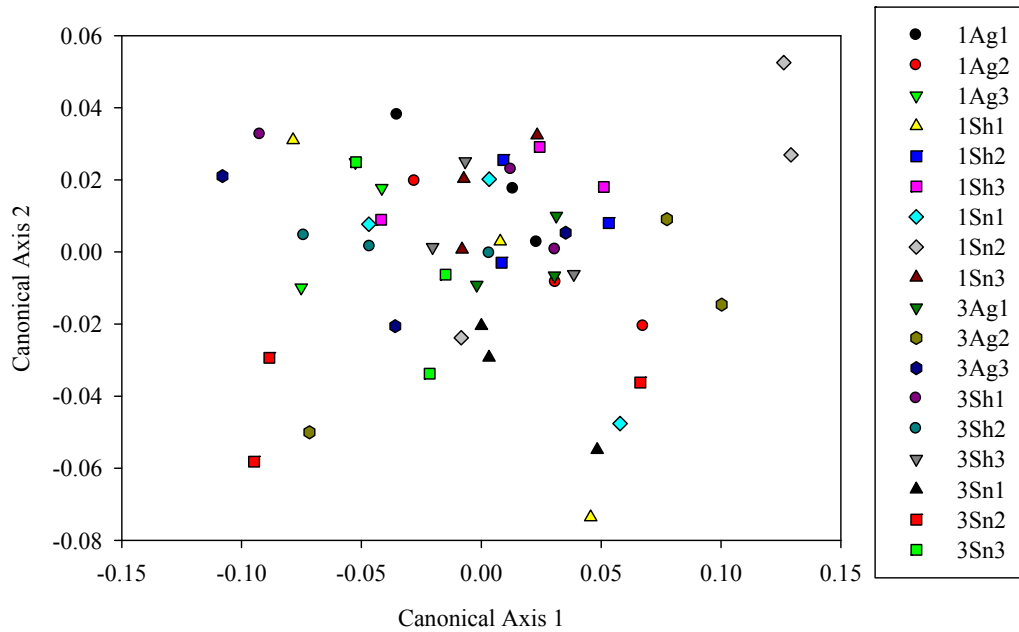
Figure 1.9: Two-dimensional scatter plot generated by the CAP analysis of the 2007 survey of AM spore communities within a clump. Clumps are identified by site number (1, 3), plant species (Ag = *Andropogon gerardii*, Sn = *Sorghastrum nutans*, Sh = *Sporobolus heterolepis*), and individual ID number (1, 2, 3). Analysis included 10 AM fungal species.

Chapter 2: Niche partitioning in a natural community of arbuscular mycorrhizal fungi

2.1. Introduction

Arbuscular mycorrhizae are among the world's most common generalized mutualisms. Any of the approximately 214 described arbuscular mycorrhizal (AM) fungal species (www.amf-phylogeny.com) is able to colonize many different plant species (Smith and Read 1997, Klironomos 2003), and any colonized plant normally supports more than one AM fungal species (Smith and Read 1997, DeBellis and Widden 2006). As in most mutualistic guilds, we understand little about the factors that determine and maintain local species diversity of AM fungi, the reasons why a single plant has multiple AM fungal partners, and how that diversity influences host plant performance (Abbott and Gazey 1994, Palmer et al. 2003, Stanton 2003, Alkan et al. 2006, van der Heijden and Scheublin 2007).

There are four classes of coexistence mechanisms proposed for maintaining community diversity in general: spatial or temporal niche partitioning within a heterogeneous environment (Tokeshi 1999), patch dynamics (e. g. colonization/ competition trade-offs) (Palmer et al. 2003), recruitment limitation and aggregation (Rees et al. 1996, Rejmanek 2002), and tritrophic interactions (Janzen 1970, Connell 1971). Maintenance of diversity within mutualism guilds includes a fifth mechanism; as the patch the community is living in is actually a living organism, and that species might coevolve to promote coexistence (Palmer et al. 2003). In the case of AM mutualism, diversity within the fungal community may be to the advantage of a host plant if fungal species differ in their ability to provide different services to the host.

Multiple species of fungi might be especially beneficial in a temporally and spatially heterogeneous soil environment. AM fungal species have been shown to differ greatly in their fitness response to abiotic environments such as the amount of heavy metals or soil water content or temperature (Tommerup 1983, Klironomos et al. 2001, Rillig et al. 2002). Traditionally, AM fungi were thought to exhibit functional redundancy with regards to the services they provide the plant with taxonomically distinct AM fungal species providing similar functions—such as increasing nutrient or water uptake or protection from pathogens or heavy metals (Smith and Read 1997, Borowicz 2001, Leake et al. 2004). However, there is growing evidence that different AM fungal species can provide different functions or services for the host plant (Smith and Read 1997, Borowicz 2001, Entry et al. 2002, Auge 2004, Vogel-Mikus et al. 2005) and the importance of any one AM fungal species, as measured by its ability to promote plant growth, may be conditional on the environment (Medeiros et al. 1994, Jansa et al. 2005, Lee and George 2005, Vogelsang et al. 2006). Therefore, a plant might preferentially associate with different AM fungal species in different microhabitats, thereby helping to maintain a greater local diversity of AM fungal species within a plant population or root system (Bever et al. 2009). The extent to which co-occurring AM fungal species occupy different niche space, based on their ability to tolerate different soil conditions or their ability to provide different services to the host, could offer possible explanations for why diversity is maintained (Bever et al. 2001, Heinemeyer and Fitter 2004).

Related to the idea that different AMF species do occupy different niche space, several possible benefits to the host for the presence of multiple fungal partners have

been proposed: temporal partitioning of benefit such that different fungal species are active at different times (Pringle and Bever 2002, Sanders 2003); complementary benefits, where multiple species, representing a range functions, will best benefit the plant (Koide 2000); synergistic benefits, where a species affects the plant negatively or neutrally alone but is a neutral or positive partner when other species are present (Gustafson and Casper 2006, Jansa et al. 2008); or buffering against environmental variation or change because different AM fungi are adapted to different conditions (Abbott and Gazey 1994). Indirect interactions, such as complementarity and synergism, are now recognized as central to community dynamics (McCann 2000), but the study of generalized mutualisms continues to focus on pair-wise interactions with one species on each side of the mutualism (Hoeksema and Bruna 2000).

The benefit to the host plant of a particular AM fungal species is normally evaluated by comparing plant performance with that AM fungal species alone to plant performance without any AM fungi (Helgason et al. 2002, Cornejo et al. 2007). This approach has revealed that different AM fungal species result in different amounts of host plant growth with some species producing no or negative amounts of growth (Castelli and Casper 2003, Klironomos 2003). There is also some evidence that the ability of any given AM fungal species to promote growth is conditional on the presence of co-occurring AM fungal species (Gustafson and Casper 2004, Reynolds et al. 2006). On the other hand, many studies have found that a community of four or five AMF species does not benefit a plant more than the most beneficial species alone, implying a level of functional redundancy within the AMF community (Reynolds et al. 2006).

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I, therefore, suggest that the appropriate means of evaluating the role of a particular AM fungal species in the community in a given environment is to examine the consequences of deleting that AM fungal species from the community. As a comparison, I also evaluated the role of a particular AM species traditionally by applying it alone to the host plant. As it is impossible to remove only one species of AM fungi from a soil community, I used a synthetic community whereby I removed all fungal species from the soil and added back: a community of five species, all possible combinations of four species with one species left out, each of the species applied individually or no AMF species at all. To my knowledge, no one has taken the approach of eliminating a single AMF species from the community although the technique has been used to study plant community diversity and function (Diaz et al. 2003).

Using different fungal inocula, I performed a pair of greenhouse experiments with a natural plant/fungal community from a naturally metalliferous serpentine grassland to investigate three questions: 1) Is plant growth enhanced by colonization with multiple partners in a way that suggests complementarity or synergy within the AM fungal community? 2) Does environmental niche partitioning among AM fungi help maintain local AM fungal diversity? That is, does soil condition, represented by different levels of N, P, and Ni, modify the fitness among fungal species differentially? Here fungal fitness is measured by spore production and root colonization and the ability of individual fungal species to promote plant growth. 3) Does colonization with multiple species of AM fungi buffer the host plant against environmental variation? 4) Do different AMF species provide distinctly different services for the host plant, as revealed by measures of plant biomass and levels of nutrients and heavy metals in plant tissue?

Though there is limited evidence that AMF species differ in their ability to promote plant growth and can provide different services to plants, I am aware of no study that assesses whether the amount of host plant growth promotion and fungal fitness by naturally co-occurring species changes with soil conditions.

2.2. Methods

In two separate experiments, I examined the relationship between two C_4 grass species, *Andropogon gerardii* Vitman and *Sorghastrum nutans* (L.) Nash, and subsets of the AM fungal community found within their rhizospheres in nature. Plant and fungal material came from a naturally occurring serpentine grassland. The AM fungal community differed in the two experiments: Experiment 1: *Glomus etunicatum, Gl. mosseae, Gigaspora gigantea* (Thaxter) Gerd. & Trappe emend. Walker & Sanders, *Entrophospora infrequens* (Hall) Ames & Schneid., and *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders and Experiment 2: *Gl. aggregatum*, *Gl. claroidium* Schenk & Smith, *Gi. gigantea*, *Acaulospora spinosa* Walker & Trappe, and *Scutellospora pellucida* Koske & Walker. Due to recent evidence that functional diversity among AMF might be related to taxonomy (Hart and Reader 2002, van der Heijden et al. 2004, Voets et al. 2006), I chose a taxonomically diverse sets of AM species. Because only *Gl. aggregatum* and *Gi. gigantea* consistently colonized the host plants in the second experiment, the second experiment focuses exclusively on those two species.

Plant and fungal material and field soil were all collected from the serpentine barrens in Nottingham County Park located in Chester County in the Piedmont Plateau of southeastern Pennsylvania, described in detail by Castelli and Casper (2003). The Nottingham serpentine barrens are part of the State Line Serpentine Barrens, distributed

across a 15,500-ha area in southeastern Pennsylvania and northern Maryland. Briefly, the park is approximately 200 ha of mostly serpentine grassland dominated by C_4 grasses. Soils have low levels of several macronutrients, including P and K, and high, potentially toxic, levels of Mg, Cr, Ni, and Fe (Casper et al. 2008).

2.2.1. Experimental design and set-up

To investigate the benefit of a particular AMF species in specific conditions, I examined the role of a particular AMF species in two ways. First, as a traditional measure of benefit, I compared the biomass of a plant grown without AM fungi to a plant grown with only the target species (e.g. *Gl. etunicatum*). The difference in biomass of the two (biomass $_{Gl. e.}$- biomass $_{no\ AMF}$) is the AM fungal effect on plant biomass due to the target AM fungal species. Second, I used a deletion method by comparing the plant biomass of a plant grown with the entire community of AM fungi to plant biomass when grown with all AM fungal species except the target species (e.g. all AM fungal species minus *Gl. e.*). The difference in biomass of the two (biomass $_{all\ AMF}$ - biomass $_{all - Gl. e.}$) is the effect of that one AM fungal species on plant biomass within the AM fungal community. Both *A. gerardii* and *S. nutans* are highly mycorrhizal dependent and, hence, very small when grown without mycorrhizal partners.

The potentially large size difference between the two sets of control plants, those without mycorrhizae and those with all species added, made quantitative comparisons between the traditional and deletion approach difficult. Therefore, when comparing the two methods I concentrated on whether the target fungal species was measured to be a mutualist, neutral partner, or parasite based on whether there was either a significant increase or decrease in plant biomass compared to the respective control plants. Using

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these two methods for determining plant benefit with a community of five AM fungi resulted in 12 fungal treatments: no AM fungi (1), one of the five AMF species (5), all possible combinations of four fungal species, where one of the five AM species was deleted (5), or all five AMF species (1).

To examine whether the benefit of a particular AMF species was conditional on the soil environment, I repeated the entire experiment in four different soil treatments: one treatment with levels of P, N, and Ni comparable to those at the lower range found at Nottingham, one treatment with added P, one with added N, and one with added Ni. Each soil treatment consisted of two parts field soil and one part sand with P, N, Ni, or nothing added. The full factorial experiment of 12 AMF treatments, 2 host plant species, and 4 soil treatments produced 96 different treatment combinations. Because of the time involved and the time sensitive nature of harvesting AMF spores from soil to set up one replicate of these 96 treatment combinations, it was impossible to set up all replicates at one time. Therefore, two replicate groups of the 96 treatment combinations were set up at a time with planting date used as blocking factor in statistical analyses. Each experiment was replicated 4 times with 2 planting times for each set. Experiment 1 was conducted in 2006-7 and Experiment 2 in 2008-9.

Different methods were used for the soil treatments in the two experiments in order to achieve different soil conditions within the natural range of soil variation at Nottingham. In Experiment 1, 50 ml of super triple phosphate (0.77 g L^{-1} water), ammonium nitrate (1.43 g L^{-1}), or nickel chloride (6 g L^{-1}) was applied weekly for 12 weeks starting one month after transplant. N application was the same for the two experiments. For Experiment 2, P and Ni was mixed into the soil before the start of the

experiment due to concerns that P and Ni applied after planting were not available throughout the pot. To accomplish this, super triple phosphate powder or equal parts nickel sulfate and nickel acetate dissolved in water were added to pots along with non-AM soil microbes (see below) and all pots (including those with no soil amendments, only microbes) were moistened and allowed to incubate moist for 7 days. The incubation lessens the acidification that sometimes accompanies additions of large amounts of nickel by allowing the soil microbes time to oxidize the acetate (Chaney personal communication, Chaney et al. 2007). After 7 days, about 30 pot volumes of water were added to each pot to flush out the excessive salts.

Seeds of *A. gerardii* and *S. nutans* were surface sterilized in 70% ethanol for 5 min, washed in running tap water for 10 min, and germinated in a 1:1 sterilized mixture of sand and vermiculite in the University of Pennsylvania greenhouse. Four weeks after planting, seedlings were inoculated with the spores as they were transplanted individually into standard 11.5 cm square pots containing a 1:2 sterilized mixture of sand and field soil. Soil was autoclaved for one hour (100 C, 1 atm) on two consecutive days and then mixed with autoclaved sand.

AMF spores were suspended in water and delivered with a microliter pipet over the seedlings' naked root system during transplant. Mature spores were isolated from field soil (experiment 1) or greenhouse cultures (experiment 2) by wet-sieving and a sucrose gradient (McKenney and Lindsey 1987). Spores were identified to morphospecies level under both dissecting and compound microscopes based on their color, size, hyphal attachment, Melzer's reaction, and wall structure. Spores that were judged to be healthy under a dissecting microscope were individually picked using a

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microliter pipet and stored at 4°C until planting. For Experiment 1, the inoculum of each

AMF species was standardized by spore volume, which was estimated by assuming a

spherical volume of $4/3\pi r^2$ with r being the average radius: *Gi. gigantea* (no. of spores:

2), *G. etunicatum* (45), *G. mosseae* (9), *E. infrequens* (28), and *Sc. calospora* (19).

The second experiment used a different set of AM species. Between the two

experiments, new greenhouse facilities were completed for the Penn Biology Department,

and several AM fungi had unreliable plant colonization in the new facility: *G.

etunicatum*, *G. mosseae*, *E. infrequens*, and *Sc. calospora*. For the second experiment I

chose species that were shown to grow very well in the new greenhouse (Doherty

unpublished data). To ensure I had viable spores for Experiment 2 and to standardize

inoculation potential by percent colonization instead of volume, primary colonization

rates (colonization three weeks after planting) were measured for the set of 5 species in a

5 replicate experiment using *S. nutans* seedlings. The number of spores required to

produce 14% primary colonization were used in Experiment 2: *Gi. gigantea* (4), *Sc.

pellucida* (6), *G. claroidium* (10), *Ac. spinosa* (14), and *G. aggregatum* (20).

Seedlings in the non-mycorrhizal treatments were treated with water without

spores. Naturally occurring non-mycorrhizal microbes were added to each pot by the

addition of 3 ml non-sterile soil solution created by adding 1 L of water to 500 ml of field

soil, mixing thoroughly, and passing the slurry through a 20-µm sieve to remove any

AMF inocula.

Despite our initial assessments of colonization success, only two of the five

species of AM fungi germinated consistently in the control soil or with P or N added, *Gi.

gigantea* and *G. aggregatum*, resulting in *de facto* four mycorrhizal treatments in these

soil treatments: *Gi. gigantea* alone or with *G. aggregatum*, *G. aggregatum* alone, or no AM fungi.   The Ni added treatment was also removed because only *G. aggregatum* was found in the those pots. The reduced number of mycorrhizal treatments increased the number of replicates for any fungal species/soil combination by as much as a factor of 5, depending on the treatment.

2.2.2. Harvest

Plants were harvested 16 weeks after transplanting. Aboveground biomass clipped at the soil surface, dried at 60 $^{\circ}$ C for 48 h and weighed. Aboveground plant tissue from Experiment 2 was analyzed for N, Ni, Cr, P, K, Ca, and Mg. Dry tissue was ground using a mortar and pestle and liquid nitrogen. For N and C determination, 3.5 mg of ground sample was analyzed using a Carlo-Erba NA 1500 analyzer (Thermo Scientific, Massachusetts, USA). For Ni, Cr, P, K, Ca, and Mg determination, tissue was digested following EPA method 3050 for microwave assisted hot acid digestion in a MARSXpress closed vessel microwave (CEM Corp., North Carolina, USA). Nine to 10 mL of HNO_3 was added to 0.15 - 0.40 g of sample (depending on plant size) in a Teflon vessel 30 min before heating. Sample vessels were then capped, ramped to the target temperature of 200 ºC over a period of 25 min, and maintained at 200 ºC for 20 min. Digests were then analyzed on a Spectro Genesis ICP-OES (AMTEK, Germany).

The amount of tissue available for analysis from the no AM fungal added, control treatments was insufficient for analysis, so the plant tissue from control treatments was pooled for Ni, Cr, P, K, Ca, and Mg analysis and not analyzed for N. At the time of this report, I only have preliminary results from the digestions (all elements but N).

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Therefore, I only report data for Ni, K, Ca, and Mg; these data reported are pooled for the two host plant species and do not include the non-mycorrhizal treatments.

Roots were removed from the soil and washed. Root colonization by AM fungi was measured from 0.2 – 0.3 g (wet mass) of roots collected from each pot. The remaining root material was dried and weighed. A subset of the root samples was weighed before drying and the wet mass: dry mass ratio was used to convert the roots examined for colonization to dry mass. Root samples were cleared for 20 min in hot 10% KOH, acidified for 10 min in 5% HCl, and stained with hot 0.1% Trypan blue/lactoglycerol for 4 min (Phillips and Hayman 1970). For Experiment 1, roots were stored in tap water at 4 °C until mounted on microscope slides in polyvinyl lactic acid glycerol. Each sample was represented by 10 root pieces of at least 1 cm length, and colonization was scored at 200X using the modified line-intersect method with 100 intersections per sample (McGonigle et al. 1990). For Experiment 2, the gridline-intersect method (Giovannetti and Mosse 1980) was used to obtain total mycorrhizal root length. As only *Gi. gigantea* and G. *aggregatum*, two species with very different root colonization morphologies, I was able to distinguish between them when both were present on the plant. *G. aggregatum* colonization is distinguished by thinner hyphae and intra-radical spores, while *Gi. gigantea* colonization has hyphal coils.

Soil from each pot was thoroughly homogenized and stored at 4 °C until spores were extracted and counted using a dissecting scope. Soil samples from the treatment without any AM fungi and from the treatment with the whole community AM fungi were tested for nutrient and metal concentrations both at planting and harvest to quantify how the soil amendments actually changed bioavailable P and Ni (Table 2.1). Ni and P were

extracted from soil using Mehlich-3 because the interest was in obtaining a proxy for bioavailabilty (Mehlich 1984). The extractant (25 ml) was added to 2.5 g of soil and shaken for 5 min at 200 rpm (Wolf and Beegle 1995). Extractions were filtered using P8 coarse paper filter (Fisher Scientific) and frozen until analysis on the Spectro Genesis ICP-OES.

2.2.3. Statistics

ANOVA using restricted maximum likelihood (REML) method was used to examine several dependent variables: plant biomass, concentrations of N, Ni, Ca, Mg and K in aboveground tissue, root colonization, and spore abundances, as a function of host plant species, soil treatment, and AM fungal treatment, all fixed effects. Planting date was included as a random effect. Post-hoc tests used Tukey's HSD. To compare the traditional and deletion measures of benefit, one analysis was performed using the five species alone and the non-mycorrhizal control as the fungal treatments. Then the same was repeated for the deletion method, the five treatments with four fungal species and the five species control. Planned comparisons were used to determine whether each target treatment was different from the respective control in a given soil type. If host plant biomass was larger than the control for the traditional treatments, then the target AM species was acting as a mutualist and if the biomass was smaller, a parasite. Similarly, if host plant biomass was smaller with four species than in the five species control, then the missing AM species acted as a mutualist in the community context. If plant biomass was larger with four species than with five, then the target species was acting as a parasite in the community context.

To determine whether variance in aboveground biomass across the different soil treatments differed among the fungal treatments with zero, one, four, or five species, variances were compared using Bartlett's test.

Spore communities were examined to determine if host plant species, soil treatment or the composition of co-occurring AM fungal species affected spore production of a target species. This analysis was performed using ANOVA; the different levels of fungal treatment consisted of the six treatments that included the target species were included (the species alone, the five member community, and four four-member communities). AM fungal spore communities produced in species mixtures were also analyzed using linear function discriminant analysis (LDFA) to determine if soil treatment affected their composition.

All data were log transformed before analysis; biomass was then subsequently arcsin transformed. JMP 7.0.1 (SAS Institute Inc. 2007) was used for all analyses.

2.3. Results

2.3.1. Experiment 1

2.3.1.2. Plant growth

Host plant species, AM fungal treatment, and soil treatment each had a significant effect on plant biomass in examining the traditional measurement treatments (Table 2.2). Mycorrhizal plants were generally larger than plants without mycorrhizae (Fig. 2.1). Plants grown in P-added soil were largest, and those growing in Ni-added soil were smallest. The significant interaction between host plant species and soil treatment reflects the larger size of *S. nutans* in the control soil compares to other soil treatments.

The effectiveness of a given AM species in promoting plant growth, measured in the traditional way, depended on both soil type and host plant species (Table 2.2). *Gi. gigantea* increased the growth of both host plant species in all soil treatments (Fig. 2.1). *Sc. calospora* was a positive partner in five of eight cases. With *S. nutans* growing in N added soil, *Sc. calospora* had no effect on biomass and with *A. gerardii* growing in Ni added or control soil, it was a negative partner. *G. mosseae* was also a positive partner in five of eight cases but had no effect on biomass in three: control soil with both plant species and with *A. gerardii* in the Ni added soil. *E. infrequens* was always a positive partner with *S. nutans*. With *A. gerardii*, *E. infrequens* was a positive partner only in the control and N added soils. It was a negative partner in the Ni added soil and a neutral partner with added P. *G. etunicatum* was always a neutral partner with S. *nutans*. It was also a neutral partner in P added and control soil with *A. gerardii*; in N added soil it increased *A. gerardii* growth and in Ni added soil, decreased it.

Only the main effects of soil and mycorrhizal treatments explain differences in plant biomass for the deletion method fungal treatments (Table 2.2). As in the traditional method, plants were largest in P added soil and smallest with the addition of Ni. Deleting one species from the five member community usually had no affect on plant biomass, but there were a few exceptions. The omission of *Gi gigantea* in soils with N added, with P added and the control decreased growth for *S. nutans*, and deleting it from N, P, or Ni added soil decreased growth for *A. gerardii*. Deleting *E. infrequens* in Ni added soil decreased biomass for *S. nutans*, and deleting *Sc. calospora* from P added soil decreased biomass for *A. gerardii* (Fig. 2.1).

The experimental design produced 40 different cases for comparison between the traditional and deletion methods in evaluating the effectiveness of an AM fungal species. In 16 cases, a positive effect on growth found using the traditional approach was undetectable with the deletion approach (Fig. 2.2). In 20 cases, the two methods produced the same results. In the remaining 4 cases, all with *A. gerardii*, a fungal species that decreased plant biomass using the traditional method was measured as having a neutral affect using the deletion method. In general, *Gi. gigantea* was measured as a positive partner regardless of whether it was evaluated in the traditional way or by the deletion method, while *G. etunicatum* was generally a neutral partner (Fig. 2.2).

Variance in plant biomass did not differ among fungal treatments with no, one, four, or five fungal species (Bartlett's p = 0.7034).

2.3.1.2. Fungal growth

Percent root colonization was generally low (18 ± 5%) and differed among soils but not among fungal treatments (Table 2.3). Plants in Ni added soil had less colonization (17 ± 7%) than plants in P added soil (19 ± 8%).

The quantity of spores produced by a particular fungal species differed by host plant, soil treatment and the composition of co-occurring fungal species. Spore production of *Gi. gigantea* was affected only by AM fungal treatment (Table 2.4). *Gi. gigantea* produced more spores when other species were present than when alone, and the identity of those co-occurring species mattered. In four species mixtures, *Gi. gigantea* produced the most spores when *G. mosseae* was absent from the community (421 ± 42 per 50 ml of soil); this number was significantly more than when *Gi. gigantea* was present alone (282 ± 39), when all AM species were present (226 ± 25), and when *E.*

infrequens (280 ± 33) had been deleted from the community. Spore production in *G. mosseae* was affected only by soil treatment (Table 2.4), producing more spores in the P and Ni added treatments (13 ± 0.5) than in the Control or N added treatments (6 ± 0.3). Spore production of *E. infrequens* was affected by host plant species, soil treatment, and AM fungal species (Table 2.4). *E. infrequens* produced the most spores in P added soil treatments as the lone species (167 ± 21) and produced the least in the P added soil treatment without *Sc. calospora* (55 ± 14) and as the only species in the control soil treatment (80 ± 19). Spore production by *Sc. calospora* (6 ± 0.2) and *G. etunicatum* (4 ± 0.2) was very low, and did not differ among any treatment factors.

Discriminant analysis showed that soil treatment affected the composition of the spore community in the deletion method but only when *Gi. gigantea* was absent (Wilks' Lambda, p = 0.03). Canonical axis 1 accounted for 56% of variation and separated communities with N added and P added from one another (Fig. 2.3). Axis 1 was well correlated with the number of *G. mosseae* spores; P added treatments had more *G. mosseae,* supporting ANOVA results. Canonical axis 2 accounted for an additional 37% of the variation and mostly separated Ni added communities from the control; Ni added treatments were associated with more spores of *Sc. calospora*, *E. infrequens*, and *G. etunicatum*.

2.3.2. Experiment 2

2.3.2.1. Plant growth

As in Experiment 1, plants were generally significantly larger in mycorrhizal treatments and nutrient amended soil treatments (Table 2.5, Fig. 2.4). Plant growth increased in the control and N added soil treatments with *G. aggregatum* and *Gi.*

gigantea, alone or in combination. There was also an interaction between the mycorrhizal and soil treatments. *A. gerardii* grown with *G. aggregatum* alone or in combination with *Gi. gigantea* were larger than those with *Gi. gigantea* alone in both the control and N added soil treatments. The same was true for *S. nutans* only in the N added treatment. (Table 2.5). Mycorrhizal treatment had no effect on plant biomass in the P added soil treatment.

Element concentrations in aboveground plant tissue did not differ by mycorrhizal treatment (*G. aggregatum* alone, *Gi. gigantea* alone, or the combination) except in the case of K (Table 2.6a,b). The concentration of K (ppm) in plants infected with *Gi. gigantea* was generally greater than in plants infected with *G. aggregatum* or both species (Fig. 2.6). In the control soil treatment, plants with both *Gi. gigantea* and *G. aggregatum* had K intermediate to that of plants with *Gi. gigantea* only or *G. aggregatum* only (Fig. 2.6). Soil treatment and plant species affected the concentration of N (%) in plant tissue. Adding N to the soil increased N in aboveground tissue in *S. nutans* but not in *A. gerardii* (Table 2.5, Fig. 2.5). While there was no significant overall main effect of mycorrhizal treatment on % N for both host species combined, Tukey's HSD test showed that in control soils, % N in *A. gerardii* was greater in both the *Gi. gigantea* and combination treatments, than in the *G. aggregatum* treatment (Fig. 2.5).

2.3.2.2. *Fungal* growth

Unsurprisingly, *G. aggregatum* produced a vastly higher number of spores than *Gi. gigantea* in every treatment combination with no differences by soil treatment, host plant species, or whether *Gi. gigantea* was present or not (Table 2.7, Fig. 2.7). *Gi. gigantea*, on the other hand, produced a larger spore volume owing to its larger spore

diameter (approximately 340 µm) compared to *G. aggregatum* (70 µm in this experiment; Fig. 2.7). Sporulation by *Gi. gigantea* was also not affected by soil treatment or host plant species, but it was reduced in combination with *G. aggregatum*.

Total root length colonized, the length of roots colonized by either *Gi. gigantea* or *G. aggregatum*, was influenced by all three main factors: host plant species, mycorrhizal treatment, and soil treatment (Table 2.8). Total root length colonized was greatest in treatments with *G. aggregatum* alone or in combination with *Gi. gigantea* (Fig. 2.8). Plants in the P added soil had more root length colonized than those in the control soil, while colonization in soils with N added were intermediate between the two (Table 2.8 Tukey's HSD). *G. aggregatum* specific root length colonization decreased dramatically in the combination treatment (Table 2.8, Fig. 2.8) while *Gi. gigantea* colonization was highest when alone on *A. gerardii* and lowest when alone on *S. nutans* (Table 2.8, Fig. 2.8). Thus, the presence of *G. aggregatum* increased colonization of *Gi. gigantea* when *S. nutans* was the host but decreased it when *A. gerardii* was the host. There was no interaction between mycorrhizal treatment and soil treatment.

2.4. Discussion

2.4.1. Is plant growth enhanced by colonization with multiple partners in a way that suggests complementarity or synergy within the AM fungal community?

The deletion approach allowed me to identify several examples of functional complementarity, cases where the absence of a species results in the loss of benefit from the plant, and of functional synergy, where a species affects the plant negatively or neutrally alone but is a neutral or positive partner when viewed in a community context. These findings add to a small list of studies that show either synergism (Gustafson and

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Casper 2006, Jansa et al. 2008, Antunes et al. 2009) or complementarity (Maherali and Klironomos 2007, Janouskova et al. 2009). However, my results also indicate a great deal of functional overlap, or redundancy, among AM fungal species. This may help to explain why many researchers find a community of 4 or 5 AMF species does not benefit a plant more than the most beneficial species alone (van der Heijden et al. 2003, Reynolds et al. 2006).

Apparent functional redundancy among AM fungal species might occur for three reasons: because multiple AM fungal species are present but do not differ appreciably in function, because a mixed species community is dominated by one or a few species, or because there is not sufficient resolution to detect differences. In the first experiment, it was impossible to know the relative colonization rates of co-occurring fungal species, but Experiment 2 provides evidence that species are actually functioning redundantly. Plants infected with both *Gi. gigantea* and *G. aggregatum* were not different in size than plants with only *G. aggregatum*, even though a large portion of the root was colonized was by *Gi. gigantea*.

Few other studies have addressed the consequences for the host plant of having multiple fungal partners while also quantifying species-specific root colonization. Recent studies by Alkan et al. (2006) and Jansa et al. (2008) have utilized qPCR systems to monitor root colonization by multiple species, but only Jansa et al. also reported the consequences to the host. Alkan et al. (2006) found inoculation with both *G. mosseae* and *G. intraradices* changes the distribution of colonization on the root system but not the amount of colonization by either species. The Jansa et al. (2008) study showed that *G. intraradices* dominated in a mixed community with *G. claroidium* and *G. mosseae*.

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The possibility that some fungal species might dominate in mixture must also be considered when viewing apparent functional synergy. In the current study, this applies to cases where a particular fungal species had a negative effect on host plant biomass when considered alone, but had no impact when deleted from the whole community. It is conceivable that the root colonization by that species is so low when other AM species are present that its usual negative effect on growth is not observed. A more compelling case for functional synergy would be made by a species acting as a negative or neutral partner alone and but a positive partner when viewed in the community context such as found by Gustafson and Casper (2006) or Atunes et al. (2009). Atunes et al. (2009) found that inoculation of *G. intraradices* promoted plant growth only when grown with a native community of fungi. Gustafson and Casper (2006) showed *G. etunicatum* to be a neutral partner alone but a positive partner in the presence of *G. intraradices*. I also found that *G. etunicatum* was mostly a neutral partner but exhibited functional synergy in the presence of other fungal species.

The study also demonstrates that the importance of an AM fungal species within a natural community depends on the make-up of that community. *Gi. gigantea* was viewed as a positive partner in 75% of cases calculated by the deletion method in Experiment 1, apparently playing a large role in the benefit the plant received from the mycorrhizal community but in Experiment 2, its presence did not increase plant growth over *G. aggregatum* alone.

Calculating how much an AM species benefits the host plant in the traditional way can significantly overestimate the role of a particular species within a natural community. These results are ecologically relevant because AM fungi are rarely found in

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monocultures outside of severely disturbed areas (Chapter 3) or some agricultural fields (Richter et al. 2002). Only in limited cases, such as when choosing the best species for an agricultural or horticultural application, is measuring the benefit of species singly appropriate.

2.4.2. Does environmental niche partitioning among AM fungi help maintain local AM fungal diversity?

2.4.2.1. Does soil condition differentially modify the ability of individual fungal species to promote plant growth?

The amount of growth promotion provided by AM fungi varied with soil treatment in all fungal species in both experiments, except for *G. etunicatum* and *Sc. calospora* growing on *S. nutans* and *Gi. gigantea* in Experiment 1. In several cases (e.g. P added soils in Experiment 2) the benefit from an AM species changed with soil type. More interesting, however, are the cases of relative change in host plant benefit. In Experiment 1, the rank benefit from *G. etunicatum* and *Sc. calospora* with *A. gerardii* and from *G. mosseae* and *E. infrequens* with *S. nutans* changed across different soil types, although *Gi. gigantea* always produced the most growth. Rank reversals in benefit provided across soil types could have ecological significance esspecially since only half of field collections at Nottingham contain *Gi. gigantea* (Chapter 1). AM fungal-mediated host plant performance has been found to vary with abiotic factors such as soil water potential and P and heavy metal soil content (Tonin et al. 2001, Auge 2004, Cuenca et al. 2004, Lee and George 2005) by other researchers but most of these studies are performed for the purpose of finding the most beneficial fungal partner for agricultural or

bioremediation applications and do not investigate functional variation within a natural community across soil types.

The extent that growth promotion by AM fungi is conditional on the soil environment differs between the traditional vs. deletion approach. For example, in the traditional method, the biomass of *A. gerardii* with fungal monocultures was enhanced by each separate AM fungal species in the N added treatments, but in the control soil treatments, only *Gi. gigantea* and *E. infrequens* enhanced growth and *Sc. calospora* actually reduced it. The deletion method showed only the removal of *Gi. gigantea* from N added soil depressed plant growth while the rest of the fungal species appeared to be functionally redundant.

There are at least two factors impacting relative to the benefit provided by different AM fungal species in different soil environments–(1) how well a particular species tolerates *and* functions in a particular soil environment and (2) how much species that are particularly well matched to the soil environment might outcompete other fungal species. The potential for these two factors means that niche partitioning among AM fungi may occur even in the presence of functional redundancy within the community. It could be that a particular species enhances plant growth more in one soil than another (e.g. *G. etunicatum* in N added soil compared to the control) but when that species, and any competitive effects provided by that species, is removed, another species can take its place in providing plant benefit when it could not before.

2.4.2.2. Does soil condition modify the spore production and root colonization of fungal species differentially?

While all fungal treatments had more root colonization in P added treatments and were negatively affected by adding Ni, relative fitness among the AM species, as measured by spore production and root colonization (Miller and Bever 1999), was only affected by soil treatment in a few cases. Most convincingly, in communities without *Gi. gigantea* in Experiment 1, discriminant analysis separated spore communities by soil treatment. *Sc. calospora* and *E. infrequens* were more common in Ni added soils and *G. mosseae* produced more spores in P added treatments. Similarly, the number of *Sc. calospora* spores in natural communities found at Nottingham negatively correlated with the amount of P in the soil (Chapter 1).

The changes in experimental methodology between the first and second experiment produced insights into the ecology of AM fungi with respect to soil composition. Adding all of the Ni all at once in Experiment 2 decreased the survival of AM fungi, particularly *Gi. gigantea*. There were no pots with Ni where *Gi. gigantea* survived while a small number of pots still had *G. aggregatum*. This observation is also consistent with the Nottingham field survey (Chapter 1), where *Gi. gigantea* was found to be negatively related to the amount of Ni in soils while Ni had no affect on *G. aggregatum*.

Adding P at the beginning of the experiment increased P availability and appears, unsurprisingly, to have decreased the importance of AM fungi to host plant performance. Providing a plant with increased amounts of P, a relatively immobile nutrient in soil, is one of the main services provided by AM fungi and it is often found that when P soil

availability is higher, the growth promotion due to AM fungi decreases (Smith and Read 1997). In Experiment 1 P added treatments, AM fungi were mostly found to promote plant growth using the traditional method and *Gi. gigantea* was a positive partner using either method of measurement. In contrast, in the P added treatment in Experiment 2, neither fungal species increased host plant performance while they did provide benefit in the other treatments.

The fitness of individual fungal species was also affected by other members of the AM community independent of soil type. In Experiment 1, *Gi. gigantea* had the greatest sporulation, not when grown by itself, but in a community that lacked its close relative *Sc. calospora*. Mahelari and Klironoms (2007) also found evidence of competitive exclusion of fungi in the same family. In Experiment 2, the presence of the proliferative *G. aggregatum* decreased *Gi. gigantea* sporulation. This suggests *Gi. gigantea* sporulation is enhanced by the presence of *E. infrequens*, *G. mosseae*, or *G. etunicatum* and limited by *G. aggregatum*. In contrast, *E. infrequens* sporulated most when alone and least when in the community without *Sc. calospora.* Host plant identity also modulated interactions between root colonization in Experiment 2, where *Gi. gigantea* root length colonized responded positively to the presence of *G. aggregatum* when on *A. gerardii* but negatively when on *S. nutans.*

2.4. 3. Does colonization with multiple species of AM fungi buffer the host plant against environmental variation?

As presented above, some fungal species are better suited for some soil environments, however, it does not appear that colonization by multiple AM fungal species is a buffer against environmental variation at Nottingham (Abbott and Gazey

1994). Colonization by four or five species of fungi did not decrease variation in plant size across the four soil treatments as compared with one species.

2.4.4. Do different AM fungal species provide distinctly different services for the host plant?

Evidence for functional diversity has been found at multiple scales, including AM fungal species-specific exploitation of different types of nutrient patches and soil types (Cavagnaro et al. 2005, Drew et al. 2006, Lekberg et al. 2007); differences in sugars produced in the hyphosphere (Hooker et al. 2007); and differential activation of plant phosphate transporters by specific AMF species (Burleigh et al. 2002). Based on elemental analysis of plant tissues, there is little evidence that the AM species in Experiment 2 differ in the measured services they provide the plant (they might differ in unmeasured services). In control soils, *A. gerardii* infected with *Gi. gigantea* had higher % N and % K in its aboveground tissue. Percent N in leaf tissue is known to be a good indicator of maximum photosynthetic rate, due to the large amount of N-containing compounds in the photosynthetic machinery (Reich et al. 2009). It is possible that colonization by *Gi. gigantea* increases % N in the host plant by actively providing the plant with more N (Talbot et al. 2008); however, plants infected with *G. aggregatum* are larger and contain significantly more N and K in total.

2.4.5. Conclusions

The amount of growth promotion provided by AM fungi and some measures of fungal fitness varied with soil treatment, supporting the existence of niche partitioning among AM species from Nottingham. This variation is most evident when using the traditional method of evaluating individual species; the deletion approach used here

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suggests that such differences are less important when any one fungal species is viewed

in the context of the whole fungal community. Functional redundancy, complementarity,

and synergy, are all operating within this fungal community and are not describable using

fungal monocultures alone. Both functional complimentarity and synergy may help

explain why plants might support multiple fungal partners.

Table 2.1: Ni and P (µg/g) in autoclaved, amended soils, before planting and after harvest.

	Before		After	
Experiment 1	Ni	P	Ni	P
Control	29.7 ± 0.9	17.2 ± 0.8	27.7 ± 0.3	15.2 ± 0.7
P added	-	-	26.4 ± 1.2	20.3± 2.1
N added	-	-	26.3 ± 0.4	16.8 ± 1.3
Ni added	-	-	69.6 ± 5.4	16.1 ± 0.9
Experiment 2				
Control	33.8 ± 1.7	16.5 ± 0.2	32.3 ± 0.6	15.5 ± 0.8
P added	30.1 ± 0.8	24.9 ± 0.3	31.8 ± 0.7	23.1 ± 1.2
N added	31.4 ± 1.9	16.4 ± 0.4	33.8 ± 1.9	14.9 ± 0.7
Ni added	100.8 ± 2.3	16.5 ± 0.5	87.4 ± 1.1	16.0 ± 0.2

Table 2.2: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment on total biomass in subset 1 for each set of treatments, traditional and deletion.

Source	DF	F Ratio	Prob > F
Traditional measure treatments			
AMF	**5, 59**	**14.6264**	**<0.0001**
Soil	**3, 59**	**52.5138**	**<0.0001**
AMF x Soil	15, 59	1.2798	ns
Plant species	**1, 59**	**4.6705**	**0.0323**
AMF x Plant species	5, 59	1.7574	ns
Soil x Plant species	**3, 59**	**7.7296**	**<0.0001**
AMF x Soil x Plant	**15, 59**	**2.0615**	**0.0150**
Deletion method treatments			
AMF	**5, 59**	**8.1645**	**<0.0001**
Soil	**3, 59**	**29.7150**	**<0.0001**
AMF x Soil	15, 59	0.7594	ns
Plant species	1, 59	0.5171	ns
AMF x Plant speices	5, 59	0.3381	ns
Soil x Plant species	3, 59	1.4096	ns
AMF x Soil x Plant	15, 59	1.0921	ns

Table 2.3: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment on percent root colonization in subset 1.

Source	DF	F Ratio	Prob > F
Plant species	1, 131	19.7531	ns
Soil	3, 131	5.2296	ns
Plant species x Soil	3, 131	1.5739	ns
AMF	**11, 131**	**22.6938**	**<0.0001**
Plant species x AMF	11, 131	0.4837	ns
Soil x AMF	33, 131	0.6524	ns
Plant species x Soil x AMF	33, 131	0.5590	ns

Table 2.4: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment on sporulation of *Gi. gigantea*, *G. mosseae*, and *E. infrequens* in subset 1. Only those treatments with a target species added were considered.

Source	DF	F Ratio	Prob > F
Gi. gigantea			
Plant species	1, 131	19.7531	ns
Soil	3, 131	5.2296	ns
Plant species x Soil	3, 131	1.5739	ns
AMF	**11, 131**	**22.6938**	**<0.0001**
Plant species x AMF	11, 131	0.4837	ns
Soil x AMF	33, 131	0.6524	ns
Plant species x Soil x AMF	33, 131	0.5590	ns
G. mosseae			
Plant species	1, 59	0.0605	ns
Soil	**3, 59**	**26.7404**	**0.0115**
Plant species x Soil	3, 59	0.5324	ns
AMF	**5, 59**	**0.4574**	ns
Plant species x AMF	5, 59	0.2036	ns
Soil x AMF	15, 59	0.8306	ns
Plant species x Soil x AMF	15, 59	0.8891	ns
E. infrequens			
Plant species	1, 59	0.7996	ns
Soil	**3, 59**	**13.0742**	**0.0315**
Plant species x Soil	3, 59	0.1871	ns
AMF	5, 59	1.1328	ns
Plant species x AMF	**5, 59**	**9.7800**	**0.0128**
Soil x AMF	**15, 59**	**2.8359**	**0.0260**
Plant species x Soil x AMF	15, 59	0.9265	ns

Table 2.5: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment (no mycorrhizae, *G. aggregatum* alone, *Gi. gigantea* alone, and the combination) on total biomass in subset 2.

Source	DF	F Ratio	Prob > F
Total biomass			
AMF	**2**	**30.2634**	**<0.0001**
Soil	**2**	**19.2394**	**<0.0001**
AMF x Soil	**4**	**4.0730**	**0.0010**
Plant species	1	2.5526	ns
AMF x Plant species	2	2.1449	ns
Soil x Plant species	**2**	**4.0314**	**0.0205**
AMF x Soil x Plant	4	1.3211	ns

Table 2.6a: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment (*G. aggregatum* alone, *Gi. gigantea* alone, and the combination) on % N in aboveground tissue from subset 2.

Source	DF	F Ratio	Prob > F
%N in aboveground tissue			
AMF	2	0.1497	ns
Soil	**2**	**3.5613**	**0.0347**
AMF x Soil	4	1.1984	ns
Plant species	**1**	**12.0740**	**0.0010**
AMF x Plant species	2	1.4267	ns
Soil x Plant species	2	1.4573	ns
AMF x Soil x Plant	4	0.6389	ns

Table 2.6b: ANOVA for the effects of soil treatment and mycorrhizal treatment (*G. aggregatum* alone, *Gi. gigantea* alone, and the combination) on the concentration of Mg, Ni, Ca, and K in aboveground tissue from subset 2.

Source	DF	F Ratio	Prob > F
Mg			
AMF	2	0.3628	ns
Soil	2	1.3545	ns
AMF x Soil	4	0.8244	ns
Ni			
AMF	2	0.3628	ns
Soil	2	1.3545	ns
AMF x Soil	4	0.8244	ns
Ca			
AMF	1	0.0308	ns
Soil	2	2.1994	ns
AMF x Soil	4	2.0024	ns
K			
AMF	**1**	**5.2834**	**0.0108**
Soil	2	0.4838	ns
AMF x Soil	4	1.6290	ns

Table 2.7: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment on species specific spore production.

Source	DF	F Ratio	Prob > F
Gi. gigantea spore number			
AMF	**1**	**10.2004**	**0.0028**
Soil	2	0.5876	ns
AMF x Soil	2	0.6946	ns
Plant species	1	2.2174	ns
AMF x Plant species	1	0.2693	ns
Soil x Plant species	2	1.5167	ns
AMF x Soil x Plant	2	0.11307	ns
G. aggregatum spore number			
AMF	1	1.9169	0.1727
Soil	2	0.0872	0.9166
AMF x Soil	2	0.2136	0.8085
Plant species	1	0.1047	0.7477
AMF x Plant speices	1	0.0306	0.8618
Soil x Plant species	2	0.6100	0.5476
AMF x Soil x Plant	2	0.6162	0.5443

Table 2.8: ANOVA for the effects of host plant species, soil treatment, and mycorrhizal treatment on total and species specific root length colonized.

Source	DF	F Ratio	Prob > F
Total root length colonized			
AMF	**2**	**4.5911**	**0.0138**
Soil	**2**	**5.5668**	**0.0059**
AMF x Soil	4	0.6724	ns
Plant species	**1**	**7.1047**	**0.0098**
AMF x Plant species	2	2.1063	ns
Soil x Plant species	2	0.5652	ns
AMF x Soil x Plant	4	1.3211	ns
Gi. gigantea root length colonized			
AMF	1	0.6611	ns
Soil	2	1.6695	ns
AMF x Soil	4	0.6441	ns
Plant species	1	0.0629	ns
AMF x Plant species	**2**	**27.4191**	**<0.0001**
Soil x Plant species	2	0.1990	ns
AMF x Soil x Plant	4	0.1948	ns
G. aggregatum root length colonized			
AMF	**1**	**32.3775**	**<0.0001**
Soil	2	0.9238	ns
AMF x Soil	4	0.1077	ns
Plant species	1	0.1100	ns
AMF x Plant speices	2	0.1377	ns
Soil x Plant species	2	1.4187	ns
AMF x Soil x Plant	4	0.1286	ns
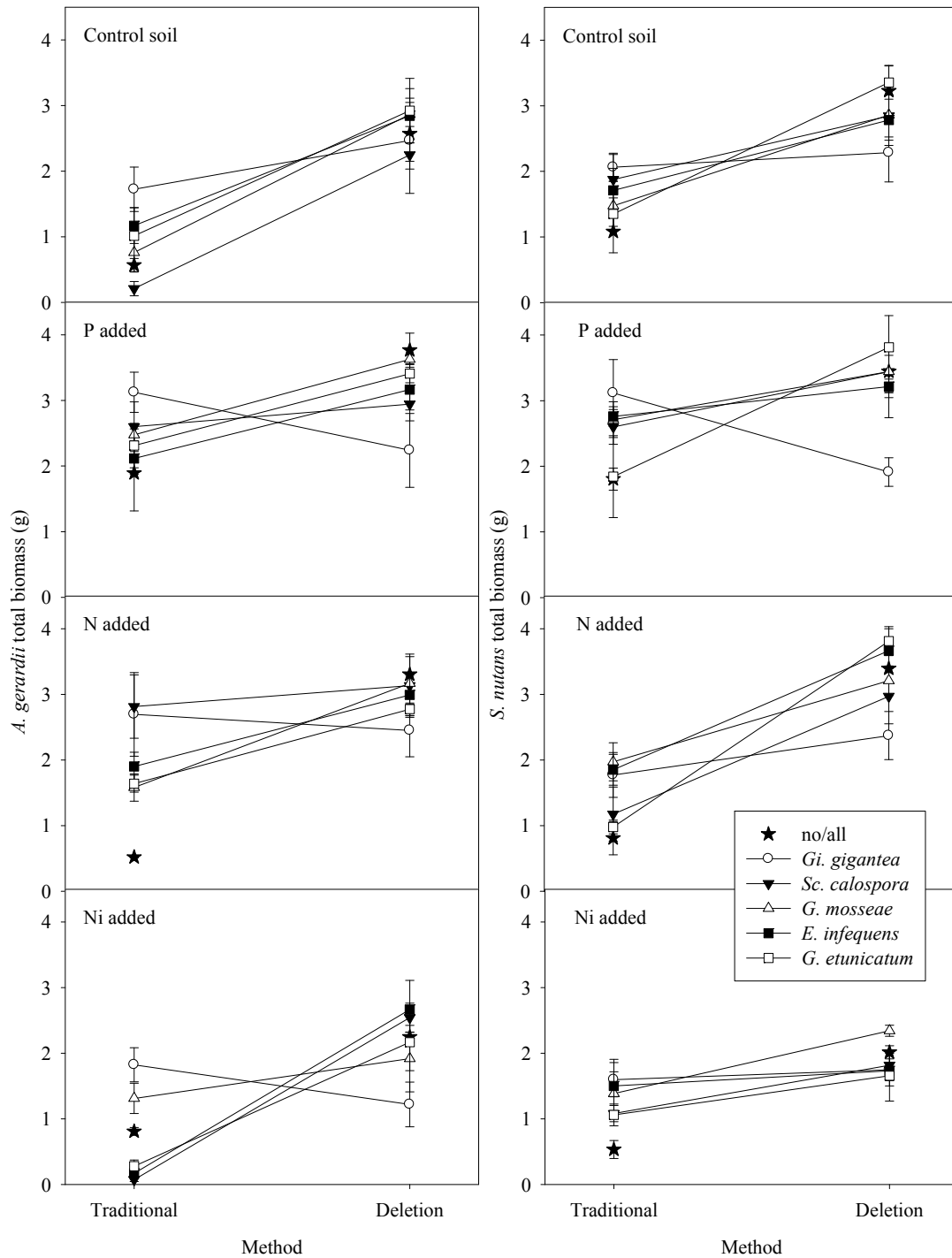
Figure 2.1: Mean ± SE total biomass of *A. gerardii* and *S. nutans* grown in control, P added, N added, or Ni added soil. Mycorrhizal treatments are grouped by traditional (all species grown singly plus the non-mycorrhizal control) or deletion method (all species and all species minus the target species.

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Sorghastrum nutans

Traditional Method

Deletion method	Negative	Neutral	Positive
Negative			
Neutral		C: Gm, E, Ge N: S, Ge P: Ge Ni: Ge	C: S N: Gm, E P: S, Gm, E Ni: Gi, S, Gm, E
Positive			C: Gi N: Gi P: Gi Ni: E

Andropogon gerardii

Traditional Method

Deletion method	Negative	Neutral	Positive
Negative			
Neutral	C: S Ni: S, E, Ge	C: Gm, Ge P: E, Ge Ni: Gm	C: Gi, E N: S, Gm, E, Ge P: Gm
Positive			N: Gi P: Gi, S Ni: Gi

Figure 2.2: Grid comparing the qualitative effect of method on plant growth of each AMF species/soil combination. Data from the traditional method are presented in columns and the deletions method in rows. Soil treatment and AMF identity are abbreviated as follows: four soil treatments: (C) control soil with nothing added, (N, P, Ni) soil with N, P, or Ni added. Five AMF species: Gi = *Gi. gigantea*, S = *Sc. calospora*, Gm = *G. mosseae*, E = *E. infrequens*, Ge = *G. etunicatum*. An AMF species/soil combination in the positive or negative column/row represents a treatment that was significantly different than the control. Presence in the neutral column/row represents a treatment that was not significantly different to the control. If the methods of approach are equivalent for an AMF species/soil combination the abbreviation will be in the cells on the diagonal.

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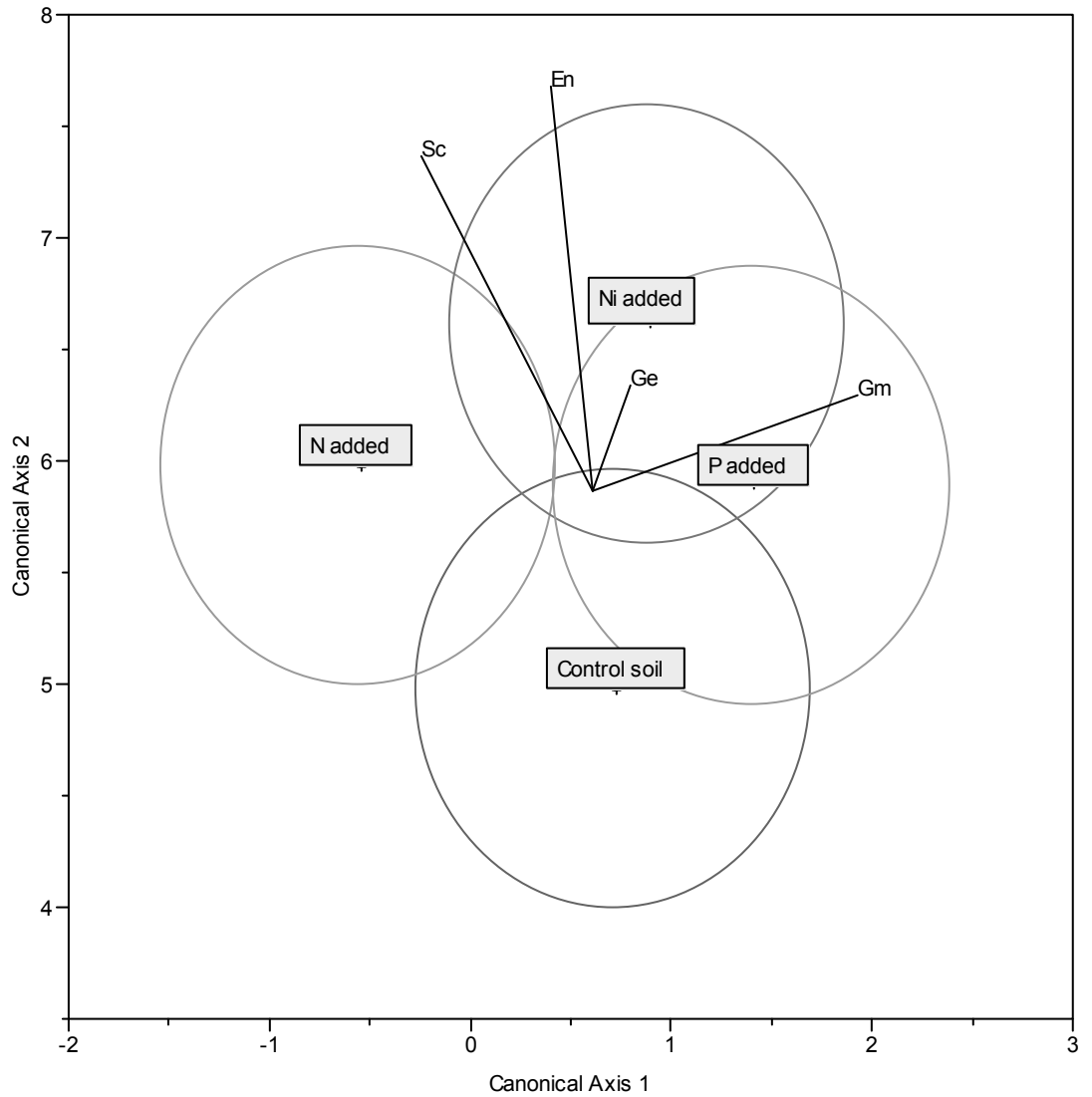
Figure 2.3: Canonical plot scores and 95% confidence ellipses from linear discriminant function analysis of fungal spore communities without *Gi. gigantea*. Ellipses are labeled with the soil treatment. Canonical axis 1 accounts for 56% of variation, while axis 2 accounts for 37%. Biplot rays are annotated with fungal species Sc = *Sc. calospora*, Gm = *G. mosseae*, En = *E. infrequens*, and Ge = *G. etunicatum*.
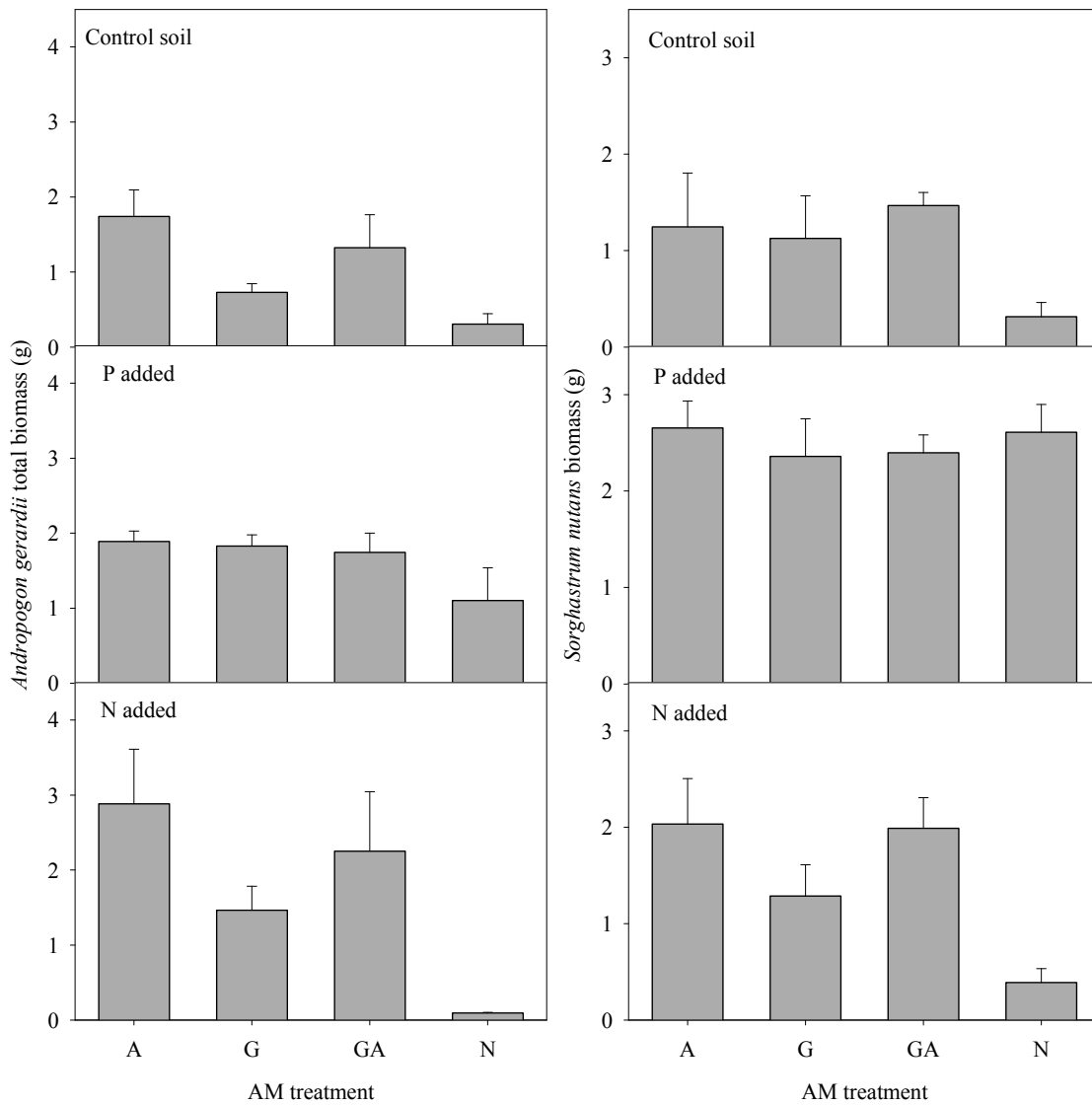
Figure 2.4: Mean + SE total biomass of *A. gerardii* and *S. nutans* grown in control, P added, or N added soil grown with *G. aggregatum* (A), *Gi. gigantea* (G), the combination (GA) or no fungi (N).
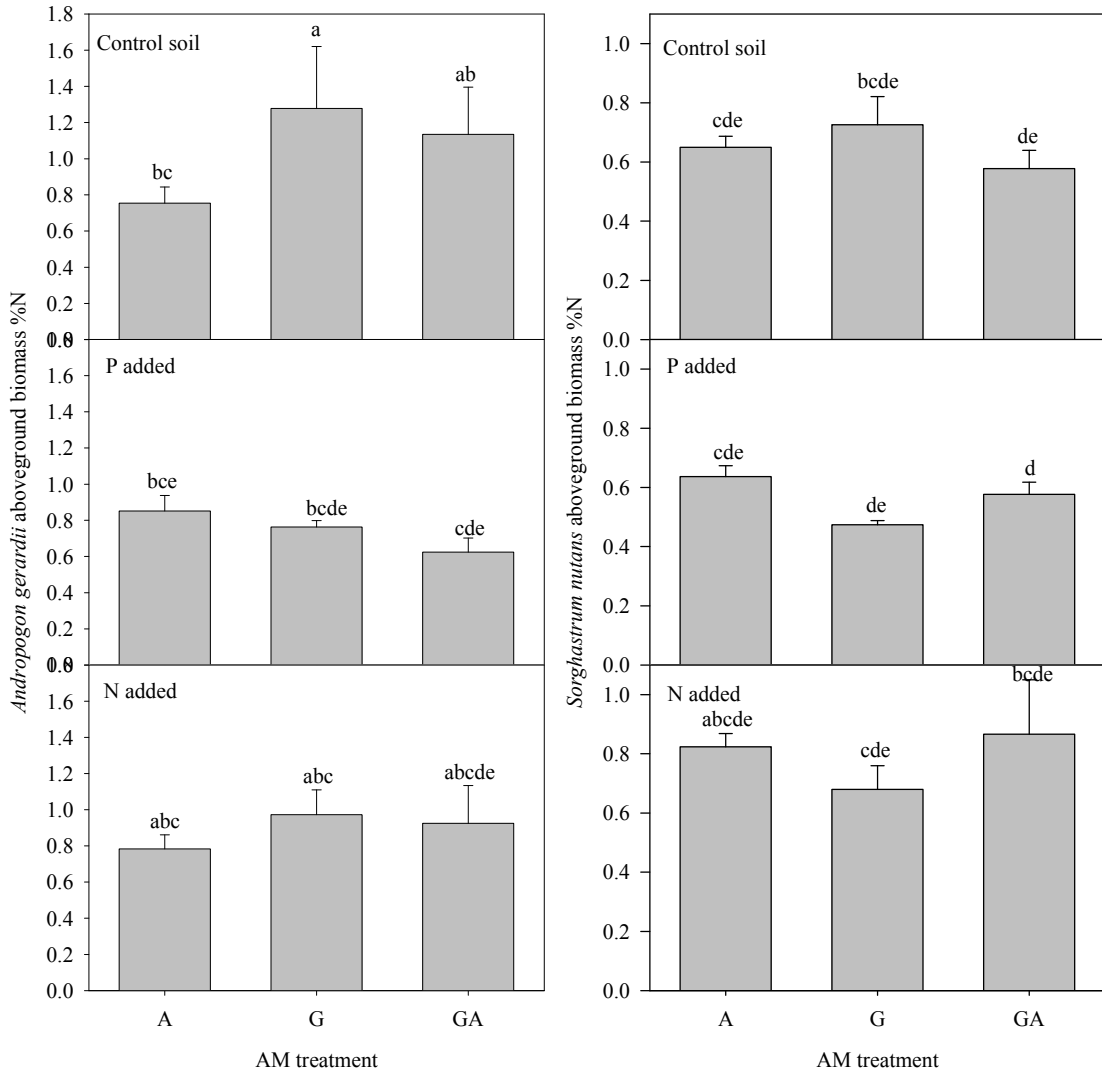
Figure 2.5: Mean + SE % N in aboveground tissue of *A. gerardii* and *S. nutans* grown in control, P added, or N added soil grown with *G. aggregatum* (A), *Gi. gigantea* (G) or the combination (GA). Levels not connected by letters are significantly different (Tukey's HSD).
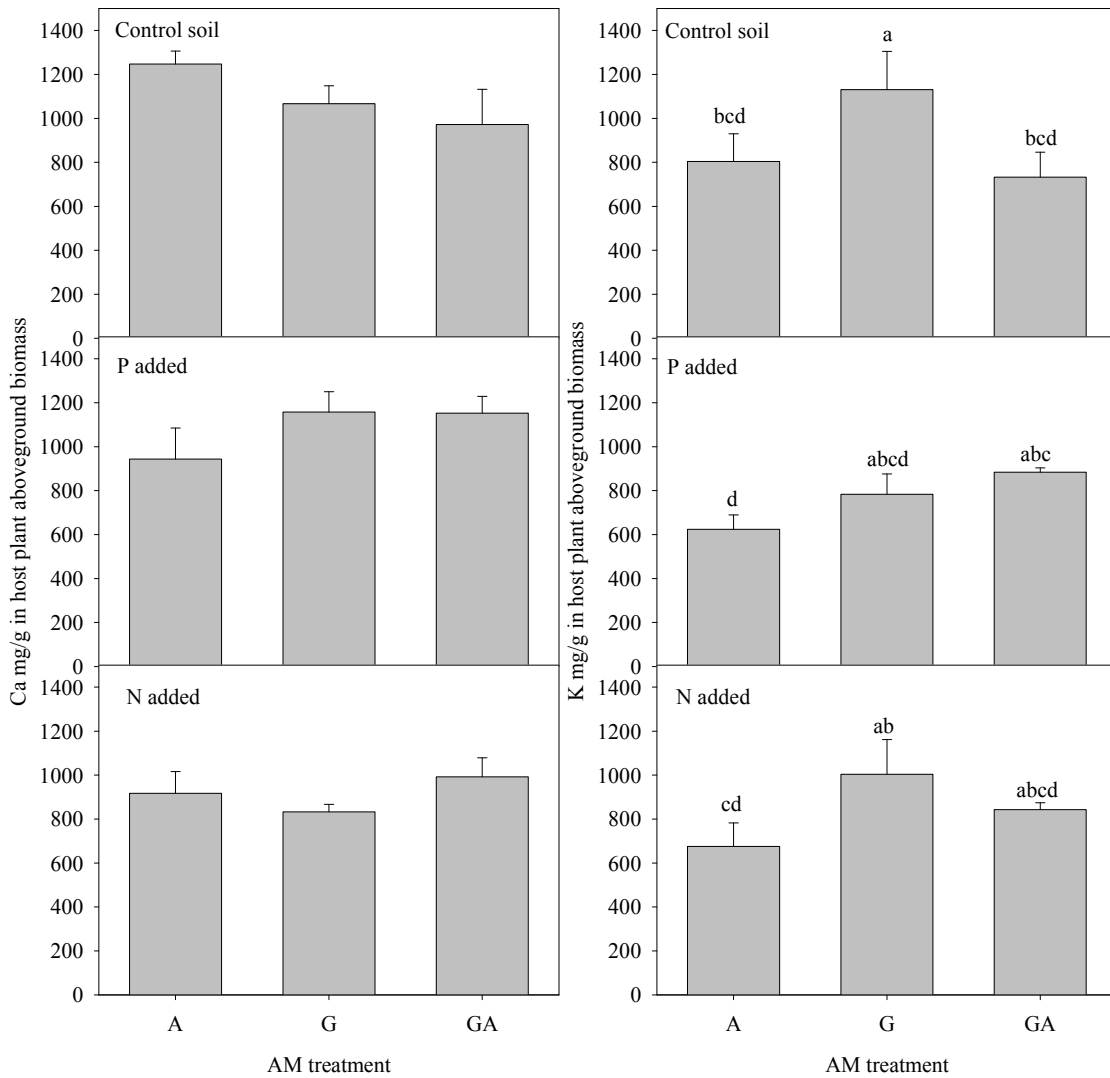
Figure 2.6: Mean + SE Ca and K (µg/g) in aboveground tissue of host plants grown in control, P added, or N added soil grown with *G. aggregatum* (A), *Gi. gigantea* (G) or the combination (GA). Levels not connected by letters are significantly different (Tukey's HSD).
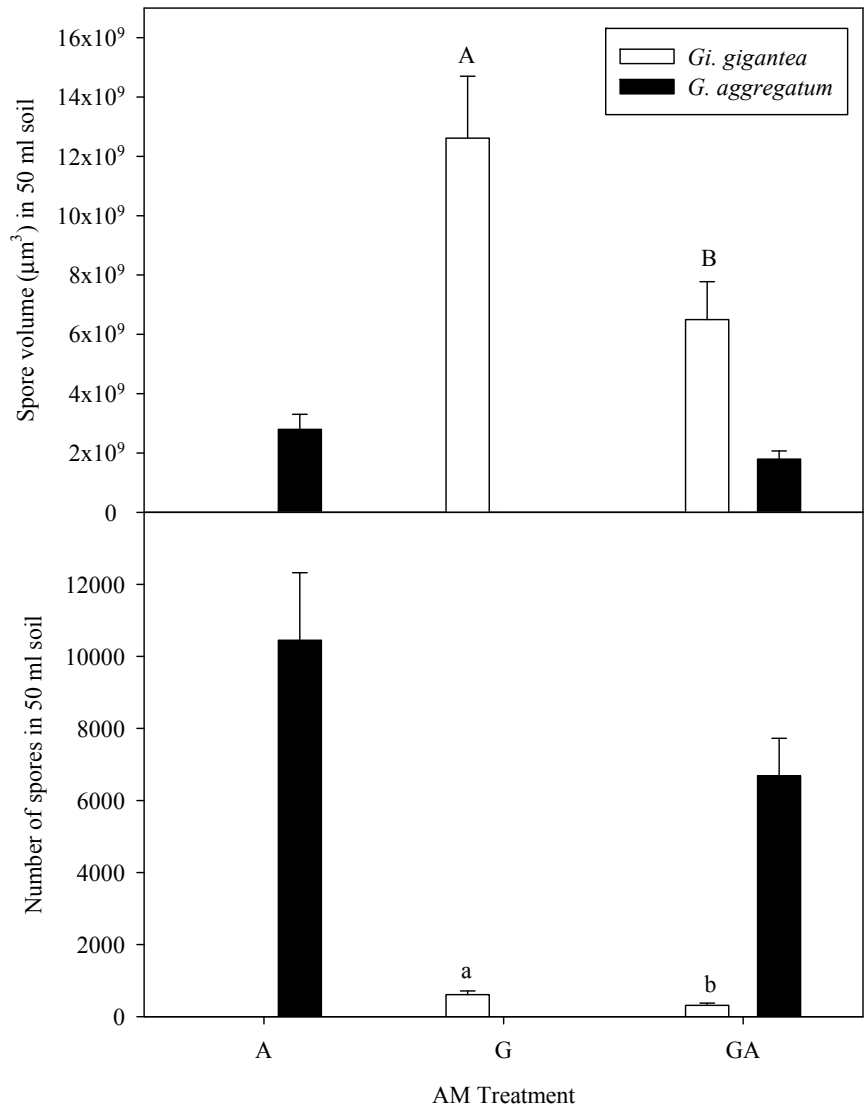
Figure 2.7: Mean + SE species specific a) volume and b) number of spores in the *G. aggregatum* (A), *Gi. gigantea* (G), and the combination (GA) treatments. Levels not connected by letters are significantly different (Tukey's HSD).
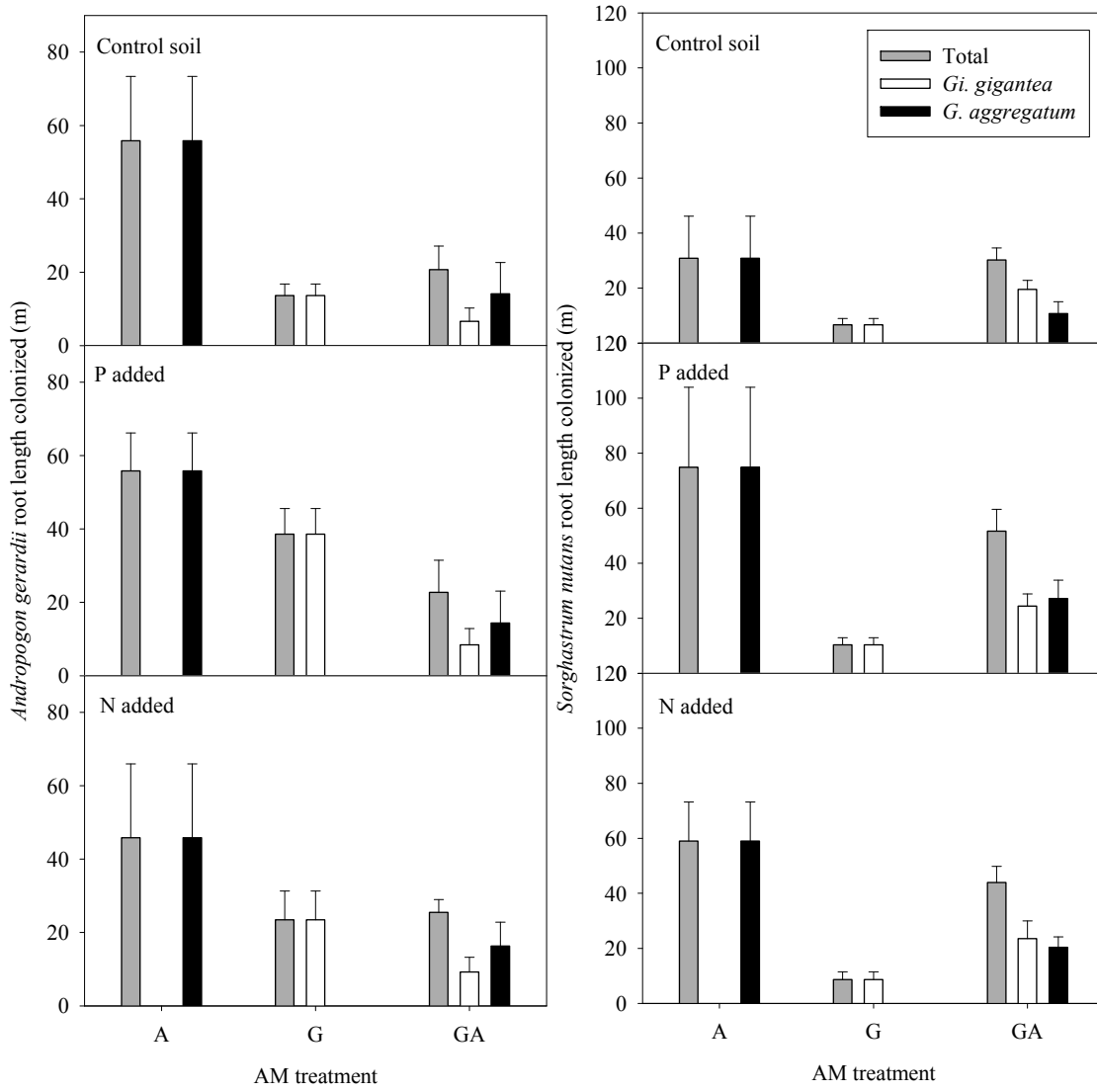
Figure 2.8: Mean + SE total and species specific root length colonized of *A. gerardii* and *S. nutans* grown in control, P added, or N added soil with *G. aggregatum* (A), *Gi. gigantea* (G), or the combination (GA).

Chapter 3: Effects of an extreme heavy metal contamination gradient on arbuscular fungal community structure and function

3.1. Introduction

The relative importance of host plant identity and soil characteristics as factors affecting AM community structure is unclear. Recent work has shown AM fungi to be more specific to particular host plant species than was previously thought (Eom et al. 2000, Johnson et al. 2005, King-Salter et al. 2007) and the composition of the plant community can alter AM fungal community composition (Mummey et al. 2005, Hawkes et al. 2006). But there is increasing evidence that abiotic soil characteristics also impact the AM community (Lekberg et al. 2007, An et al. 2008, Appoloni et al. 2008, West et al. 2009). With this in mind, we examined the AM communities associated with the same two grass species across a long-standing gradient in heavy metal contamination and evaluated how AM fungi taken from different parts of the gradient affected host plant performance in soils with different levels of contamination. The system enabled us to examine, for the same host species, the direct effects of soils on fungal community and to evaluate the utility of arbuscular mycorrhizae in helping plants cope with heavy metals. Although AM fungi can make plants more tolerant to heavy metals (Entry et al. 2002), it is not clear whether the AM fungal species that occur in such soils are more effective in this role or if the AM fungal community is simply limited to the species that can themselves tolerate heavy metal.

The contamination gradient is located along the north facing slope of Blue Mountain in east-central PA. The highest levels of contamination occur closest to two zinc smelters operated by the New Jersey Zinc Company for more than 80 years

(Buchauer 1973). High levels of zinc, cadmium, copper, and lead contamination has reduced the diversity and abundance of a wide variety of organisms, including vertebrates, arthropods, trees, shrubs, bacteria, and fungi (Jordan and Lechevalier 1975), but the AM fungi have never been investigated.

We used field spore collections, traditional root colonization measures, and a controlled greenhouse experiment to evaluate the influence of extreme heavy metal contamination on AM fungal community structure along the Blue Mountain contamination gradient. In the greenhouse experiment, we also measured several plant performance variables to investigate how AM fungal communities taken from different parts of the gradient affected their hosts in combination with different levels of contamination.

3.2. Methods

3.2.1. Site Description

Blue Mountain is located near the town of Palmerton, Carbon County, in east-central Pennsylvania, where over 2,000 acres have been contaminated with zinc, cadmium, copper, and lead from the metal-oxide aerosol emissions of two nearby zinc smelting plants that closed in 1980 (Buchauer 1973, Roberts et al. 2002). The first was opened in 1898 by the New Jersey Zinc Company in order to process zinc from franklinite and willemite ores, which contained no sulfur or trace heavy metals (Jordan 1975). Beginning in 1915 sulfide ores containing approximately 55% zinc, 31% sulfur, 0.15% cadmium, 0.30% lead, and 0.40% copper were also smelted (Buchauer 1973, Jordan 1975). With sulfide ores were smelted, acid rain left the areas near the smelters either sparsely vegetated or barren (Edenborn, personal communication, Jordan 1975). In

1982 the U.S. EPA placed the area on its National Priorities List as a Superfund Site because of the severe environmental degradation (Roberts et al. 2002). Soil contamination on Blue Mountain exists as a gradient with the highest levels closest to the smelters. Soil heavy metal content then decreases exponentially for about 20 km (40 km for lead) until leveling off (Buchauer 1973).

The vegetation on the ridge and north-facing slope of Blue Mountain is a mosaic of hairgrass–lowbush blueberry savanna, birch (blackgum) rocky slope woodland, exposed rock rubble with moderately sparse and scattered vegetation, exposed rock rubble nearly devoid of vegetation, and dry oak–heath forest (Latham et al. 2007). Our study focused on the hairgrass–lowbush blueberry savanna where *Deschampsia flexuosa*, common hairgrass, *Danthonia spicata*, poverty oatgrass, and *Vaccinium angustifolium*, early low blueberry are dominant, sometimes in single-species patches and sometimes in mixture (Latham et al. 2007). In the more highly contaminated soil closer to the smelters where vegetation is sparse, we sampled vegetation patches within the exposed rock rubble.

The soils on Blue Mountain are Dekalb and Laidig series stony loams derived from shale, sandstone, and conglomerate (Fisher et al. 1962). The topsoil in hairgrass-lowbush blueberry savanna is a thick layer of dark organic debris (Roberts et al. 2002). The large amount of organic matter, which does not exist in surrounding forest soils, is thought to indicate drastically reduced biodegradation (Roberts et al. 2002). Unvegetated soil is missing much of this bulky organic matter due to erosion (Buchauer 1973).

3.2.2. Field survey

We collected soil under the C_3 perennial grasses *Deschampsia flexuosa* (L.) Trin. and *Danthonia spicata* (L.) P. Beauv. ex Roem. & Schult to characterize the AM fungal community and quantify root colonization by AM and other fungi. Soil collections were made along the Appalachian Trail, which follows the ridge of Blue Mountain (Fig. 3.1), in late June and October of 2008. We collected soil at three locations along the ridge: an area of high contamination (HC) directly above smelter II, an area of lower contamination (LC) farthest from the smelters, and an area of medium contamination located in between (MC). Both grasses grow in well-defined clumps, so it was not difficult to collect soil and roots under the target plant exclusively. In June at each location, we collected soil from under 10 arbitrary individuals of *D. flexuosa* separated by at least 5 m (30 samples). In October, we also sampled under *Da. spicata* individuals and collected from under seven individuals of each species per location. At the MC point, we only sampled under four individuals of *Da. spicata* because there were not seven individuals at least 5 m apart (39 samples). Approximately 150 ml of soil was collected per sample and thoroughly mixed by hand before sub-sampling for AM fungal spores or roots (described below). Samples were transported in coolers and stored at 4 °C.

We analyzed all samples collected in October for Cd, Cu, Zn, Pb, P, K, NH_4^+, $NO_3^- + NO_2^+$, and pH. We measured pH after mixing 10 g soil with 10 ml strontium nitrate (Wolf and Beegle 1995, Chaney et al. 2007). We extracted Cd, Cu, Zn, Pb, P, and K from soil in two ways: using strontium nitrate and Mehlich-3, both weak acids. We used these extractants instead of strong acids to obtain total element concentrations because we were most interested in obtaining a proxy for bioavailability (Mehlich 1984,

Chaney et al. 2007). In fact, variation in AM colonization and spore numbers were found to be slightly better related to available Zn and Pb than total Zn and Pb by Zarei, et al. (2008b). Extractions were filtered using P8 coarse paper filter (Fisher Scientific) and frozen until analysis. When using strontium nitrate we added 20 ml of extractant to 10 g of soil and shook for 120 min at 200 rpm (Chaney et al. 2007). With Mehlich-3, we added 25 ml of extractant to 2.5 g of soil and shook for 5 min at 200 rpm (Wolf and Beegle 1995). Samples were analyzed on a Spectro Genesis ICP-OES according to EPA Method 6010C and data were reported as ppm. We extracted nitrate-N and ammonia-N by adding 20 ml of 2 M KCl to 4 g of soil (Keeney and Nelson 1982, Griffin 1995). Each sample was then shaken for 60 min at 100 rpm; these were analyzed by the Rutgers University Pinelands Field Station using a Technicon autoanalyzer and Methods 350.1 and 353.2 for NH_4^+ and $NO_3^- + NO_2^+$ respectively (EPA 1983b, a).

We examined the morphospecies making up the AM fungal community in each sample. Spores were extracted from a 50-mL soil sample within 45 days of collection using the wet sieve method (McKenney and Lindsey 1987) and identified based on morphological characters such as size and color, cell wall structure and texture, and differential staining with Melzer's reagent (Morton 1986). The spore communities, as defined by the numbers of spores of the various morphospecies, were compared.

A small amount of roots (approximately 0.3 g wet weight) was removed from each sample within 7 days of collection to quantify root colonization by AM and other fungi. Samples were cleared for 24 hours in 10% KOH at room temperature, acidified for 10 min in 5% HCl, and stained with 0.1% Trypan blue/lactoglycerol for 3 hours (Phillips and Hayman 1970). Roots were stored in tap water at 4 °C until mounted on

microscope slides in polyvinyl lactic acid glycerol. Each sample was represented by 10

root pieces of at least 1 cm length, and colonization was scored at 200X using the

modified line-intersect method with 100 intersections per sample (McGonigle et al.

1990). We distinguished AM hyphae from nonmycorrhizal hyphae by morphological

structures, as AM hyphae are usually non-septate, have irregularly shaped walls and

display angular, unilateral branching (Bonfante-Fasolo 1984).

3.2.3. Greenhouse experiment

We set up a reciprocal transplant experiment in the greenhouse to investigate if

the AM fungi found in the LC and HC soils at Blue Mountain are adapted to their

respective soils and to determine if soil environment influences community composition.

Additionally, we included a non-AM fungi treatment in each soil type so we could

quantify what benefit, if any, AM fungi confer to the host plant. Each treatment was

replicated 10 times.

Soils to be used as the growth media were collected in June 2008. We thoroughly

mixed each type of soil by hand and added white bar sand in a 4:1 ratio to improve

drainage. The mixture was autoclaved twice for 60 min at 100 °C, 1 atm and used to fill

500 ml pots. One three-week-old seedling of *Da. spicata,* grown from seed collected in

October 2006 from an HC area and germinated in sterilized vermiculite, was transplanted

into each pot. Seeds were collected in October of 2006 from a HC area. After

autoclaving, we analyzed five samples of each soil type for pH, Cd, Zn, P, and K using

Mechlich-3 described above, in order to discern effects of autoclaving on edaphic factors.

AM fungal spores extracted from the HC and LC collection points in June were

stored at 4 °C after quantification and used as inocula in the two fungal treatments of this

experiment. Spores were not isolated individually but contained in a soil extraction fraction with a density less than that of 60% sucrose and a particle size between 45 μm and 250 μm. The majority of AM fungal spores are larger than 45 μm, and no AM fungal species at Palmerton has spores larger than 250 μm. After counting the spores of each morphotype, the spore communities were pooled by soil type and suspended in water at a concentration of 1600 spores per 5 ml; thus, the two fungal treatments had the same number of spores per pot. The mixture was shaken to suspend spores, and 5 ml quantities were removed to serve as inocula. One 5 ml quantity was applied to the bare roots of each seedling as it was transplanted. We also added non-AM fungal microbes from the corresponding soil type back to each pot. These microbes were isolated by creating a slurry of fresh field soil (500 ml) and water (1000 ml) and passing it through a series of sieves, the smallest of 20-μm. 20 ml of filtrate was added to each pot.

The plants were grown in a temperature controlled greenhouse at the University of Pennsylvania that averaged 25 °C between 0600 and 1800 h and 21 °C otherwise. They received a minimum PAR of 430 μmol/m^2/s supplied by either active greenhouse lighting or ambient sunlight for 14 h each day. All mortality occurred within the first four weeks.

Plants were harvested twelve weeks after transplanting. Aboveground biomass was obtained by clipping the plants at the soil surface, drying at 60 °C for 48 h and then weighing. Roots were removed from the soil and washed; 0.5 g (wet mass) of roots was collected from each pot to quantify root colonization by AMF fungi and putative root pathogens. We weighed the remaining root mass wet and dry and converted the 0.5 g wet mass to dry mass using each samples' wet mass: dry mass ratio. Soil from each pot was

thoroughly homogenized and stored at 4 °C until spores were extracted and counted. It was not always possible to distinguish between spores produced during the experiment and spores that had been present in the autoclaved field soil at the start of the experiment. To control for this, we also quantified the number of spores in the treatments to which no AM fungi had been added.

3.2.4. Statistical Analysis

All data were log transformed before analysis. Separate one-way analyses of variance (ANOVA) were used to compare edaphic factors along the gradient and in soil after autoclaving. One-way multivariate analysis of variance (MANOVA) was used to compare spore communities and the various root pathogens among the field collections. Roy's Maximum Root test was reported for the MANOVA as eigenvalues differed widely but all MANOVA statistics were in agreement (Seber 1984). When differences among collections were detected, comparisons were made in the abundance of particular root pathogens using ANOVA. Linear discriminant function analysis (LDFA) was also used to compare spore communities. Because of the incomplete sampling design of the field survey (samples were only collected under *Da. spicata* in October), planned contrasts were used to compare among spores communities and the various root pathogens when the one-way analyses were significant. For collections under *D. flexuosa*, comparisons were made between June and October, among the three collection locations, and the interaction. For all collections in October, comparisons were made between *D. flexuosa* and *Da. spicata*, among the three collection locations, and the interaction. ANOVA was used to compare root colonization by AM fungi.

Two-way MANOVA, with soil type and AM fungal origin as factors, was used to analyze spore communities and root pathogen measures in the greenhouse experiment. When treatment differences in root pathogen measures were detected, comparisons of individual root pathogen measures were made using ANOVA. Two-way ANOVA was used to analyze above and belowground plant biomass and percent root colonization by AM fungi. Spore communities and AM fungal root colonization were compared between LC and HC AM fungi treatments using a planned contrast. LDFA was also used to compare spore communities. Within each soil type, planned contrasts were used to determine if any AM fungi affected plant growth compared to no AM fungi and if plant growth differed between LC and HC AM fungi treatments. JMP 7.0.1 (SAS Institute Inc. 2007) was used for all analyses.

3.3. Results

3.3.1. Field Survey

3.3.1.1. Soil Analyses

Both collection location and plant species affected the bioavailabilty of heavy metals and nutrients in the soil. Generally, metal availability was higher in HC soils and especially under *D. flexuosa* (Table 3.1). K was also higher in HC *D. flexuosa* soil. P was equally high in LC and HC soil, but lower in MC soil. The pH was very low (3.8 ± 0.07) along the entire gradient.

After autoclaving, the availability of P increased sharply. HC autoclaved soil had both higher amounts of Zn and Cd and a lower pH.

3.3.1.2. Field AM fungal community

While every 50 ml soil sample collected along the soil contamination gradient contained large numbers of spores, only four fungal morphospecies were detected by our sampling efforts. Those spore communities collected in June consisted, on average, of 3634 ± 236 spores (mean $\pm$ SE) and were four times more than those collected in October (904 ± 71). The four morphospecies were: *Acaulospora mellea*, an unidentified *Glomus* species (brown, between 80-140 μm in diameter, *Glomus* Brown), *Glomus rubiforme,* an unidentified *Acaulospora* species (light gold, between 60-100 μm in diameter with an obvious cicatrix, *Acaulospora* Cicatrix). The overwhelming majority of *Acaulospora* and *Glomus* Brown spores were heavily parasitized. *A. mellea* was by far the most common morphotype, but its proportionate contribution to the community varied (Fig. 3.2). *A. Cicatrix* was found in low abundance in June and was almost undetectable in October.

Collection date and location were important determinants of spore community while host plant species was not (Table 3.2). Canonical variable one (x-axis) from the LDFA easily separated June from October collections and June LC from June HC collections (Fig. 3.3). Communities in October were not as well separated between sampling locations or host plant species. The x-axis accounted for 85% of the variation and was defined by differences in the numbers of *A. Cicatrix* and *G. rubiforme*. Canonical variable two (y-axis) accounts for another 10% of the variation and was defined by differences in the amount of *A. mellea* and *G.* Brown. Spore communities from LC and MC soil are more diverse than those found in HC soil. LC and MC collections, especially in June, have relatively more of each every except *A. mellea* (Fig. 3.2).

3.3.1.3. Field root colonization

Root colonization by AM fungi in field collected roots was generally low and widely variable (2.60% ± 0.33%, mean ± SE; 0-20%, min-max). There were no statistically significant patterns with regards to collection date, location, or host plant species (Table 3.2). All together, root infection by other fungi was generally higher than AM fungal colonization (4.65% ± 0.61%). Four morphological categories were observed: blue staining septate hyphae, an brown septate fungus with hyphae and microsclerotia which appears similar to *Gaeumannomyces*, a grass root pathogen (Bentivenga, personal communication) or dark septate endophytic fungus (DSE), light blue structures, and smaller brown structures (Fig. 3.9). DSE are a miscellaneous group of ascomycetous anamorphic fungi that colonize root tissues and can act as a parasitic or mutualistic partner to the host plant (Jumpponen 2001). The MANOVA analysis showed significant differences in percent colonization by other fungi among collections. There was an interaction between collection date and location (Table 3.2). ANOVA revealed only blue staining septate fungi had significant differences in percent colonization among the collections. Specifically, percent root colonization by blue staining septate fungi was lowest in HC sites in June and MC sites in October (Table 3.3, Fig. 3.4). In October collections the MANOVA showed there were root pathogen differences by host plant species (Table 3.2). ANOVA again demonstrated these differences were explained by blue septate fungi colonization; there was much less colonization by the blue fungi in the roots of *Da. spicata* (Table 3.3, Fig. 3.4). Additionally, though not a statistically significant pattern, the light blue structures were only observed in roots collected in June (Fig. 3.4).

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3.3.2. Greenhouse experiment

3.3.2.1. Spore communities

Only three AM fungal species sporulated during the 12-week greenhouse experiment; *Acaulospora* Cicatrix did not sporulate. As in the field soils, *A. mellea* was by far the most abundant species present (Fig. 3.5). Soil type, not AM fungal origin, determined spore community composition at the end of the 12 week period (Table 3.4). Treatments that received the same spore community at the beginning of the experiment were not similar to one another at the end; instead spore communities were more similar to those in soils with the same level of soil contamination (Fig. 3.5). Canonical variable one (x-axis) accounted for 69% of the variation and easily separated communities with added AM fungi from those with none added (Fig. 3.6). The x-axis is defined mostly by the amount of *A. mellea* in the community. Canonical axis two (y-axis) accounted for another 30% of the variation and separated communities growing in LC soil from those growing in HC soil. The y-axis is defined by *G. rubiforme* and *G.* Brown. Communities growing in LC soils had proportionally more of each.

3.3.2.2. Root Colonization

AM fungal root colonization of greenhouse grown *Da. spicata* inoculated with either LC or HC spores was similar to *Da. spicata* field roots collected in October (greenhouse 2.53 ± 0.20, field 2.69 ± 0.67). In greenhouse roots, we detected the blue and brown structures and blue staining septate fungi that we observed in field roots. We also observed a structure unique to greenhouse roots, large staining spores that were not attached to hyphae. We did not observe the brown hyphae or microsclerotia. Colonization by other fungus (4.22 ± 0.72) was again greater than AM fungal

colonization. The MANOVA indicated an interaction between soil type and AM fungal treatment (Table 3.4, Fig. 3.7). This pattern was explained by significant interactions between soil and AM fungal origin in the amount of brown structure and blue staining septate fungi colonization. Brown structures were only observed in the LC AM fungal treatment of HC soil and the no fungal treatment of LC soil (Fig. 3.7). Blue septate fungal colonization was the lowest in treatments where AM fungi were added to soil of a different origin (i.e. HC fungi in LC soil).

3.3.2.3. Plant Biomass

Soil type and AM fungal treatment both affected the size of *Da. spicata* in the greenhouse (Table 3.6). Plants grown in LC soil were larger aboveground than in HC soil, but belowground biomass did not differ between soil types (Fig. 3.8). AM fungal inoculation increased aboveground plant biomass in LC soil but not in HC soil (Table 3.6, Fig. 3.8). Without an AM fungal treatment, plants growing in the LC soil were similar in size aboveground as plants growing in HC soil (Fig. 3.8). A different pattern was observed for belowground biomass; plants inoculated with AM fungi had greater belowground biomass in HC soils but not in LC soils. With respect to belowground biomass, any AM fungal treatment increased plant size equal to that of plants grown in LC soil.

3.4. Discussion

We draw the following four conclusions from this study: 1) Although at a low diversity, the AM and other fungi are flourishing in the heavily contaminated soils along the ridge of Blue Mountain previously thought to be depauperate of microbial life. Although we measured fungi colonizing roots, not soil fungal communities in general, as

Jordan and L did in 1975, our data suggest there may have been some recovery of the fungal community in the last three decades. 2) Edaphic factors determine AM spore community structure while the type and abundance of other fungi colonizing roots are influenced by soil type, host plant species, and, in the greenhouse, AM fungal origin. 3) The AM spore and putative root pathogen communities, but not AM root colonization, are influenced by time of year. 4) AM fungi, or some other unidentified factor in the AMF inoculum, improve plant growth differently based on soil type.

We provide indirect and direct evidence that the soils associated with grasses along Blue Mountain determine the community structure of the AM community: 1) Indirectly, we observed no differences between the AM spore communities associated with *D. flexuosa* and *Da. spicata* in October. This evidence may be strengthened by surveying soil from under plants of different growth forms along the gradient and adding multiple survey dates under each species. 2) Directly, when an AM spore community from either LC or HC soil was transplanted into the opposite soil it became completely indistinguishable from a community from that soil type. HC soil seems to act as a sieve, removing the *Glomus* species from the community.

AM fungal species diversity at Palmerton is very low compared to other natural, uncontaminated grasslands (Bentivenga and Hetrick 1992, Bever et al. 1996, Eom et al. 2001, Casper et al. 2008). Yet spore densities, such as those found in June, are higher than those typically found in generally undisturbed sites (Johnson and Wedin 1997, Lugo and Cabello 2002, Pringle and Bever 2002, Castelli and Casper 2003, Ji 2007). Spore densities are usually much lower in metal contained soils (Del Val et al. 1999, Zarei et al. 2008b). Cd, Cu, Pb, and Zn soil contamination often decreases both AM spore

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community diversity and density (Del Val et al. 1999, Zarei et al. 2008b). Soil pH can also affect the number of species in a habitat, but soils with comparable pH to these generally have more species than we found (O'Connor et al. 2001, Zhang et al. 2004, Oliveira and Oliveira 2005). This suggests that metal contamination is the cause of the low species diversity found in these soils. The decrease in species diversity with increasing metal contamination in both the field observations and greenhouse experiment strongly support this idea.

It is not surprising the bulk of the AM spores in the community were *Acaulospora* species. *Acaulospora* species are commonly found and often dominant in stressful soils, such as HM contaminated soils or soils with a low pH (review in Clark 1997, Zarei et al. 2008b). In one study of the AM community of east-southern China, *Acaulospora mellea*, was found be restricted to soils with a pH below 5.0 (Zhang et al. 1999, Casper et al. 2008).

It is possible that more AMF diversity would be revealed by molecular analysis of roots. Few studies have examined such diversity as a function of metal contamination. Whitfield et al. (2004) found that AM fungal diversity within roots of *Thymus polytrichus* did not decrease over a multiple-metal contamination gradient along the River South Tyne in the UK. Instead the community composition changed with some fungi only found at each end of the gradient. Zarei et al. (2008a) identified only three morphospecies in Zn and Pb contaminated soils using spore collections but found an additional four species using sequencing analysis on roots. It is possible that there is genetic variation that we could not detect without using molecular tools on roots and the identified morphospecies.

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The temporal pattern we observed in other fungal colonization of roots is not unusual. Root colonization by AM and other fungi is frequently found to vary over the course of the year (Beena et al. 2000, Ruotsalainen et al. 2002, Whitfield et al. 2004, Li et al. 2005, Garcia and Mendoza 2007, Fuzy et al. 2008), be influenced by host plant species (Ruotsalainen et al. 2002, Li et al. 2005), soil pH (Clark 1997), and, for AM fungi, soil HM content (Deram et al. 2008). AM root colonization is often negatively affected by HM contamination in both in field and greenhouse studies at HM levels comparable to this study (Griffioen et al. 1994, Leyval et al. 1995, Whitfield et al. 2004, Gucwa-Przepiora et al. 2007, Deram et al. 2008, Zarei et al. 2008b). AM fungal root colonization is also depressed in low pH soils when compared to higher pH soils (see review in Clark 1997). Postma et al. (2007) found that AM colonization decreased while DSE colonization increased with decreasing pH in Swedish acidic beech forests leading them to suggest that DSE might replace AM fungi in extremely low pH environments. DSE have also been found to be more frequent than AM fungi in other high stress environments, such as high altitude and semi-arid conditions (Barrow 2003, Olsson et al. 2004, Addy et al. 2005).

Our results can be compared to other studies of fungal root colonization in *D. flexuosa.* Many populations have higher levels of AM colonization than DSE (Read and Haselwandter 1981, Ruotsalainen et al. 2002, Zijlstra et al. 2005, Ruotsalainen et al. 2007), and AM colonization is usually an order of magnitude greater than what we observed. *D. flexuosa* populations growing in areas of the Netherlands experiencing high rates of atmospheric N deposition have colonization values around 73% for AM fungi and 10% for DSE (around 73% AM and 10% DSE) (Zijlstra et al. 2005). However, a

Swedish oak forest population of *D. flexuosa*, growing in acidic, Al-rich soils was found to have AM and other fungal colonization values very similar to our values (Goransson et al. 2008). There are no reports of *Da. spicata* colonized with DSE, but this species is reportedly obligately mycorrhizal (Darbyshire and Cayouette 1989) and vulnerable to root pathogens (Bever 1994).

Temporal variation in the standing spore community is not suprising given that AM spore or propagule production is seasonal and AM species-specific (Scheltema et al. 1987, Beena et al. 2000, Lugo and Cabello 2002, Pringle and Bever 2002, Escudero and Mendoza 2005). Peaks in sporulation tend to occur after the species of fungus is physiologically active (Douds and Schenck 1990, Gazey et al. 1992, Abbott and Gazey 1994) suggesting AM fungi might be active and perhaps with higher levels of root colonization before we began collections in June. Collections earlier in the growing season would reveal more detailed information on colonization and sporulation phenology.

The drastic decrease in AM fungal spore number between June and October without a concurrent increase in AM root colonization leads us to wonder, what happened to the spores during that time? Field spores collected in both June and October were heavily parasitized and it is possible that parasitized spores quickly decomposed. Alternatively, spores could have germinated between June and October and 1) not colonized the grasses 2) replaced June infection lost through root-turnover or 3) colonized grasses but the increase in colonization did not last until October due to root-turnover.

The AM size fraction had different effects on host plant biomass depending on the soil type. AM fungi improved aboveground biomass in LC soil and belowground biomass in HC soil. It could be that, in general, the particular benefits conferred by AM fungi are context dependent. An alternative explanation is that the AM species that grow in LC soil, that is, the more diverse community generally or the *Glomus* species specifically, could favor increased aboveground biomass, while *A. mellea* on its own might favor increased belowground biomass. AM fungi have been shown to help plants in metal contaminated soils by decreasing metal absorption (Kelly et al. 2005, Carrasco et al. 2006, Wang et al. 2007), immobilize metal in the soil and fungal tissue (Christie et al. 2004, Janouskova et al. 2006, Gonzalez-Guerrero et al. 2008), activating oxidative damage protection genes (Waschke et al. 2006, Hildebrandt et al. 2007), and increasing mineral nutrition (Christie et al. 2004). It is also clear that AM fungal species vary in their ability to provide these services. It is therefore plausible that different AM fungal species in our system may provide metal tolerance through different mechanisms. Strong soil effects on AM fungal community structure make it difficult to determine if the particular AM fungal species that occur in a soil with a particular level of contamination are more effective in promoting plant growth or if the AM fungal community is simply made up of fungal species that can grow best in that soil.

Table 3.1: Soil chemistry values (mean ± SE, expressed as µg g^{-1} except for pH) for the three October collection locations (LC, MC, HC) and for the autoclaved soil (LC, HC) used in the greenhouse experiment. Letters indicate significant differences (p < 0.05, based on Tukey HSD post-hoc tests for field soil and ANOVA for autoclaved soil).

| | *D. flexuosa* | | | Autoclaved | |
	LC	MC	HC	LC	HC
Cd	9.6 ± 1.34[b]	10.3 ± 1.58[b]	9.19 ± 0.85[b]	9.3 ± 0.4[B]	19.0 ± 0.2[A]
Cu	2.73 ± 0.27[bc]	3.23 ± 0.25[ab]	4.45 ± 0.31[a]	2.63 ± 0.29	3.78 ± 0.37
Zn	320 ± 39.9	350 ± 68.1	269 ± 27.5	249 ± 3.9[B]	424 ± 13.0[A]
Pb	56.6 ± 4.79[b]	68.3 ± 6.67[ab]	85.6 ± 6.64[a]	49.8 ± 7.34	62.7 ± 9.11
P	67.3 ± 10.0[a]	44.3 ± 12.1[b]	95.3 ± 15.4[a]	159 ± 4.3	173 ± 4.2
K	135 ± 8.19[b]	128 ± 5.14[b]	187 ± 19.1[a]	117 ± 0.9	119 ± 6.4
$NO_3^- + NO_2^+$	1.49 ± 0.26[b]	1.27 ± 0.32[b]	1.53 ± 0.33[b]	-	-
NH_4^+	1.86 ± 0.40	2.86 ± 0.81	2.77 ± 1.63	-	-
pH	3.8 ± 0.1	4.0 ± 0.3	3.8 ± 0.2	4.0 ± 0.02[A]	3.8 ± 0.01[B]

	Da. spicata		
Cd	10.9 ± 1.29[b]	15.3 ± 2.51[a]	17.9 ± 3.50[a]
Cu	2.18 ± 0.36[c]	2.13 ± 0.20[bc]	3.44 ± 0.47[ab]
Zn	387 ± 49.0	513 ± 114	467 ± 121
Pb	39.1 ± 2.77[b]	38.9 ± 1.15[b]	53.3 ± 8.94[b]
P	98.3 ± 24.4[a]	22.6 ± 5.50[b]	87.3 ± 18.6[a]
K	152 ± 12.5[ab]	120 ± 8.16[b]	124 ± 6.27[b]
$NO_3^- + NO_2^+$	5.49 ± 1.13[a]	4.84 ± 1.84[a]	4.25 ± 0.63[a]
NH_4^+	1.23 ± 0.10	1.30 ± 0.23	1.27 ± 0.08
pH	-	-	-

Table 3.2: (M)ANOVA for the effects of collection date, location, and host plant species on AM fungal spores communities, AM fungal root colonization, and various root pathogen measures in the greenhouse experiment. An * indicates that F is approximate based on the Roy's Maximum Root test.

Source	F	DF	p-value
MANOVA: AM fungal spore communities			
Collection	**39.3455***	**4, 60**	**<0.0001**
Planned contrasts			
Within *D. flexuosa* collections			
June v. October	**56.1678**	**4, 57**	**<0.0001**
LC v. MC v. HC	**12.7430***	**4, 58**	**<0.0001**
Interaction	**11.1526***	**4, 58**	**<0.0001**
Within October collections			
D. flexuosa v. *Da. spicata*	1.8014	4, 57	0.1412
LC v. MC v. HC	**7.8300***	**4, 58**	**<0.0001**
Interaction	1.1530*	4, 58	0.3410
ANOVA: AM fungal root colonization			
Collection	0.7553	8, 53	0.3448
MANOVA: root pathogen measures			
Collection	**4.8223 ***	**8, 53**	**0.0002**
Planned contrasts			
Within *D. flexuosa* collections			
June v. October	**6.6061**	**4, 50**	**0.0002**
LC v. MC v. HC	1.0360 *	4, 51	0.3978
Interaction	**4.4403 ***	**4, 51**	**0.0037**
Within October collections			
D. flexuosa v. *Da. spicata*	**3.6011**	**4, 50**	**0.0118**
LC v. MC v. HC	1.9696 *	4, 51	0.1132
Interaction	0.4257 *	4, 51	0.7894

Table 3.3: ANOVA for the effects of collection date, location, and host plant species on individual root pathogen measures in the greenhouse experiment.

Source	F	DF	p-value
Light blue structures	0.7713	8, 53	0.6294
Brown structures	0.7091	5, 53	0.6822
Blue staining septate fungi	**2.2397**	**8, 53**	**0.0386**
Planned contrasts			
Within *D. flexuosa* collections			
June v. October	**5.1958**	**1, 53**	**0.0267**
LC v. MC v. HC	1.5983	2, 53	0.2118
Interaction	**3.3307**	**2, 53**	**0.0434**
Within October collections			
D. flexuosa v. *Da. spicata*	**8.4425**	**1, 53**	**0.0053**
LC v. MC v. HC	2.6234	2, 53	0.0820
Interaction	1.9181	2, 53	0.1569
Brown septate fungi	0.9691	8, 53	0.4700

Table 3.4: (M)ANOVA for the effects of soil (LC, HC) and source of AM fungi (LC, HC, none) on AM fungal spores communities, AM fungal root colonization, and various root pathogen measures in the greenhouse experiment. An * indicates that F is approximate based on the Roy's Maximum Root test.

Source	F	DF	p-value
MANOVA: AM fungal spore communities			
Soil type	**34.1785**	**3, 38**	**<0.0001**
AM fungal origin	**60.9525***	**3, 39**	**<0.0001**
Planned contrast: LC v. HC	0.5366	3, 38	0.6600
Interaction	1.0168*	3, 39	0.3956
ANOVA: AM fungal root colonization			
Soil type	1.8307	1, 26	0.1877
AM fungal origin: LC v. HC	0.3008	1, 26	0.5881
Interaction	0.4412	1, 26	0.5124
MANOVA: root pathogen measures			
Soil type	2.3677	7, 31	0.5041
AM fungal origin	2.6057 *	7, 32	0.0519
Interaction	**4.6413***	**7, 32**	**0.0040**

Table 3.5: ANOVA for the effects of soil (LC, HC) and source of AM fungi (LC, HC, none) on individual root pathogen measures in the greenhouse experiment.

		Light blue structures	Brown structures	Blue staining septate fungi	Spores
Soil	F	0.0009	0.001	1.6717	0.9606
	DF	1, 38	1, 38	1, 38	2, 38
	p-value	ns	ns	ns	ns
AM fungal origin	F	1.3265	2.5462	**3.7611**	1.2543
	DF	2, 38	2, 38	**2, 38**	2, 38
	p-value	ns	ns	ns	ns
Interaction	F	1.8305	**0.2733**	**8.7711**	0.7576
	DF	2, 38	**2, 38**	**2, 38**	2, 38
	p-value	ns	**0.7623**	**0.0007**	ns

Table 3.6: ANOVA for the effects of soil (LC, HC) and source of AM fungi (LC, HC, none) on *Da. spicata* biomass in the greenhouse experiment.

Source	F	DF	p-value
Aboveground biomass			
Soil type	**21.514**	**1, 40**	**<0.0001**
AM fungal origin	**4.6611**	**2, 40**	**0.0151**
Interaction	0.4109	2, 40	0.6658
Planned contrasts			
In LC soil:			
AM fungi v. no AM fungi	**7.2633**	**1, 40**	**0.0102**
LC AM fungi v. HC AM fungi	0.7710	1, 40	0.3852
In HC soil			
AM fungi v. no AM fungi	2.8749	1, 40	0.0977
LC AM fungi v. HC AM fungi	0.0719	1, 40	0.7899
Belowground biomass			
Soil type	0.0298	1, 40	0.8639
AM fungal origin	3.0579	2, 40	0.0581
Interaction	2.0719	2, 40	0.1393
Planned contrasts			
In LC soil:			
AM fungi v. no AM fungi	0.0340	1, 40	0.8546
LC AM fungi v. HC AM fungi	3.2195	1, 40	0.0803
In HC soil			
AM fungi v. no AM fungi	**5.4937**	**1, 40**	**0.0241**
LC AM fungi v. HC AM fungi	0.6776	1, 40	0.4153
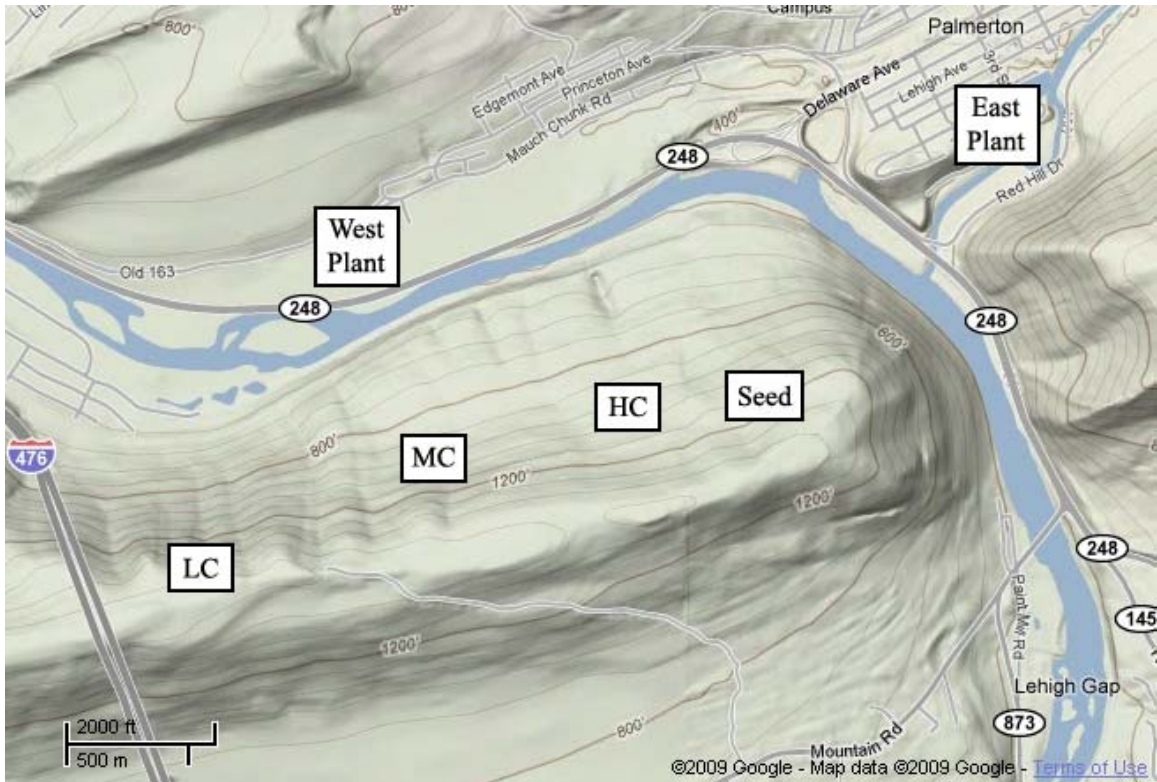
Figure 3.1: Map of Blue Mountain Ridge with the general location of the two New Jersey Zinc Company smelters, the three sample collections points (LC, MC, HC) and the seed collection point.
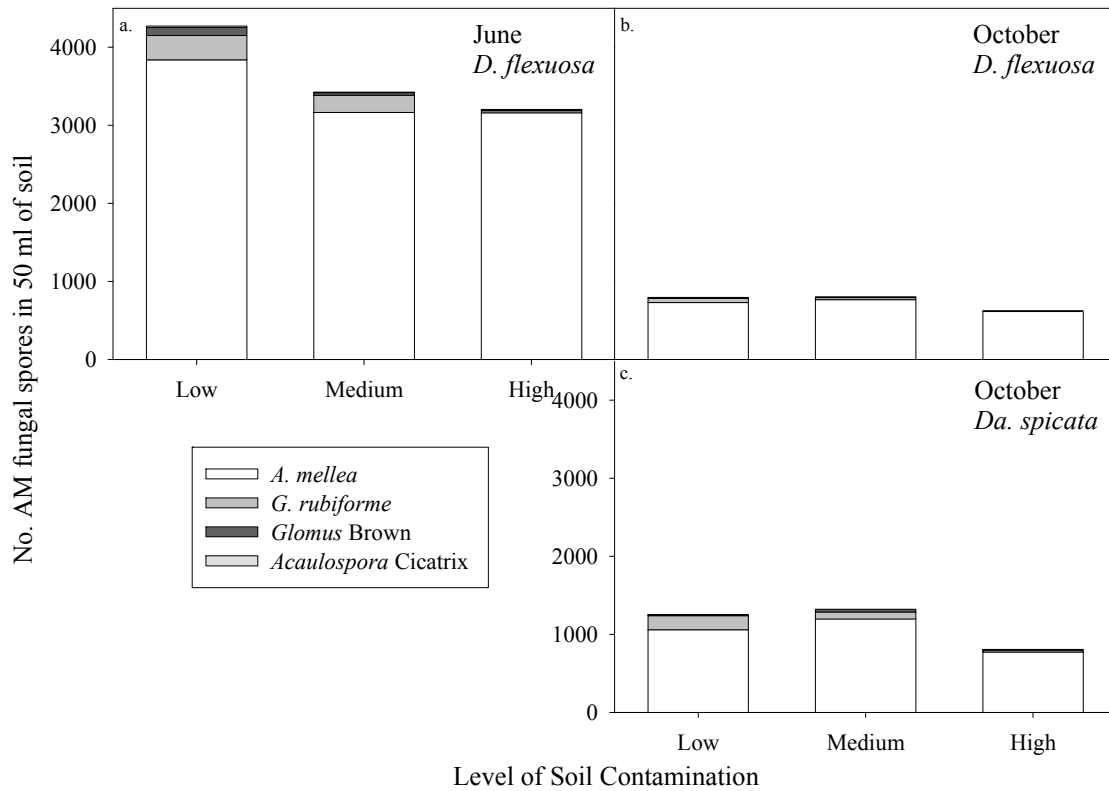
Figure 3.2: Mean abundance of AM fungal spores of the various morphospecies in 50 ml

of soil collected under *D. flexuosa* in a) June, b) October or under c) *Da. spicata* in

October.

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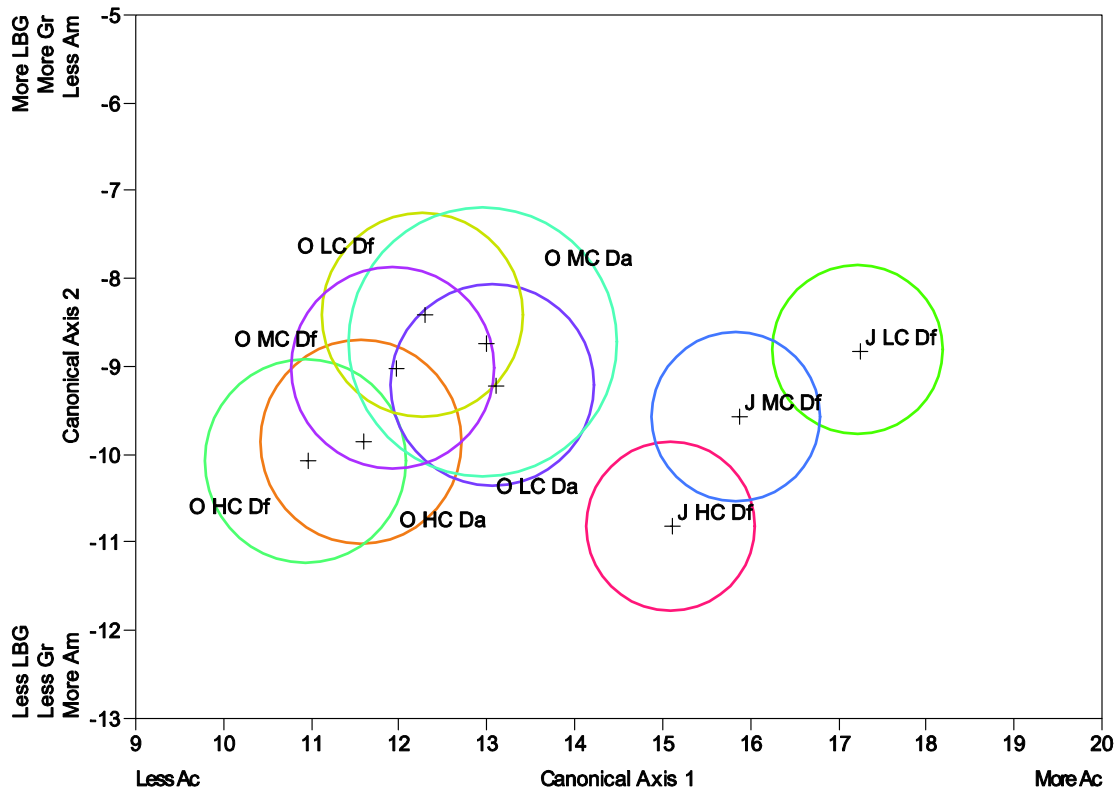
Figure 3.3: Canonical plot scores and 95% confidence ellipses from linear discriminant function analysis of field AM fungal spore communities. Ellipses are labeled with month of collection (J=June, O=October), sample collection point (LC, MC, HC), and grass species (Df = *D. flexuosa*, Da = *Da. spicata*). Canonical axis 1 accounts for 85% of variation, while axis 2 accounts for 10%. Axes are annotated with associated AM fungal species.
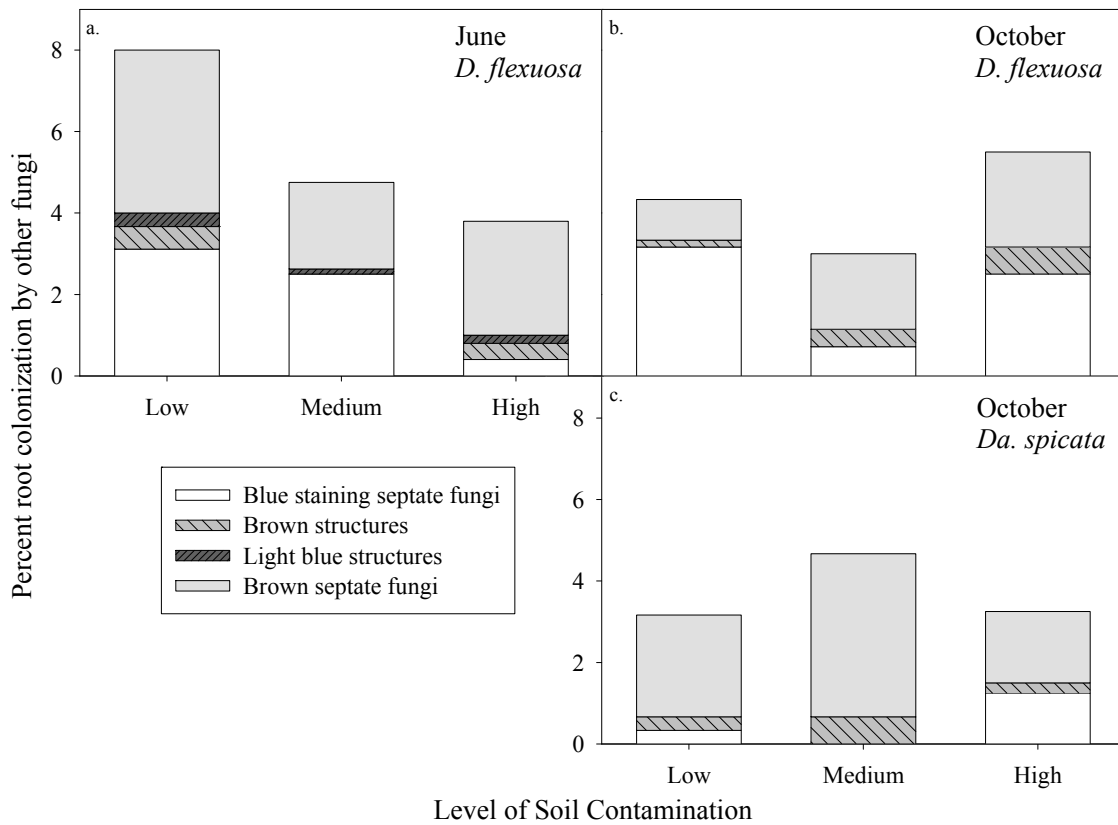
Figure 3.4: Mean abundance of the various putative pathogens in roots collected under *D. flexuosa* in a) June, b) October or under c) *Da. spicata* in October.
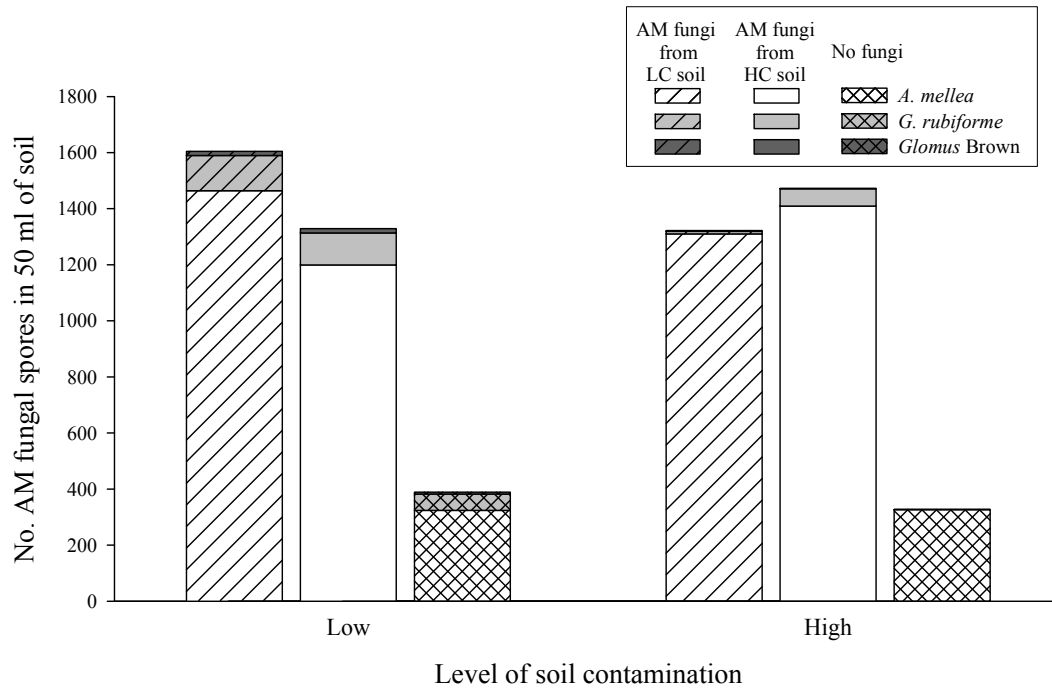
Figure 3.5: Mean abundance of spores of the various morphospecies grown with *Da. spicata* in LC or HC soil inoculated with AM fungi from LC or HC or none at all.
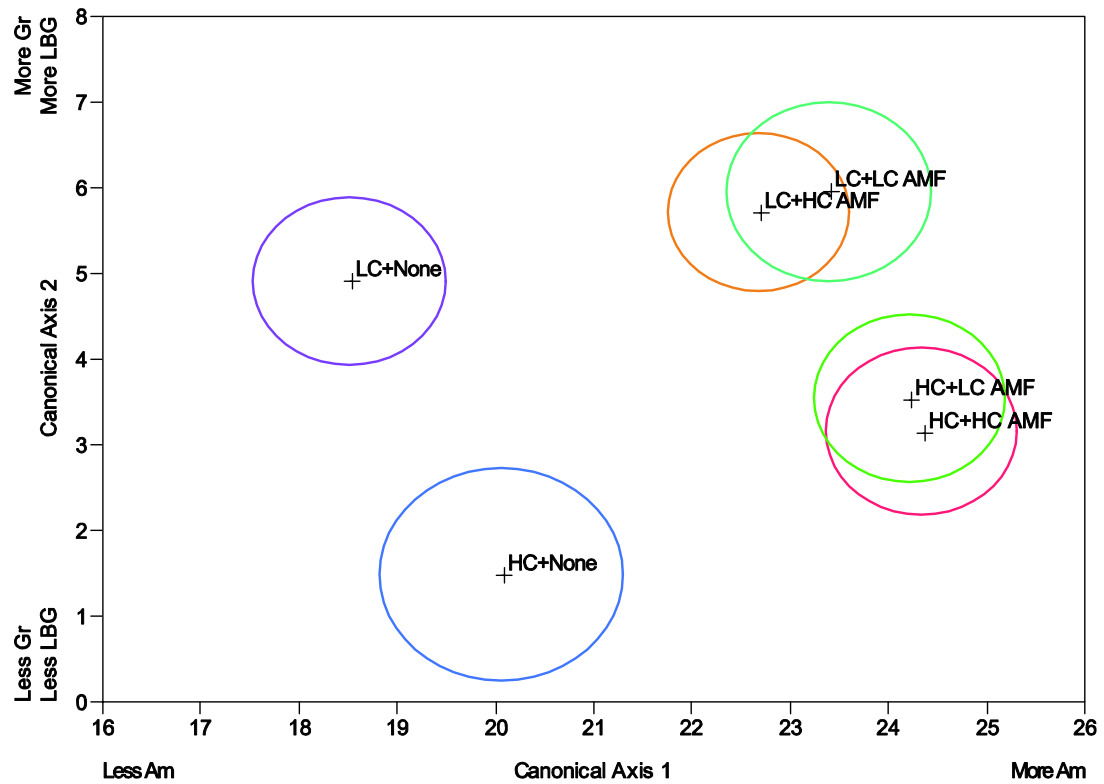
Figure 3.6: Canonical plot scores and 95% confidence ellipses from linear discriminant function analysis of greenhouse AM fungal spore communities grown with *Da. spicata*. Ellipses are labeled with soil type (LC, HC) + AM fungal treatment (LC AMF, HC AMF, None). Canonical axis 1 accounts for 69% of variation, while axis 2 accounts for 30%. Axes are annotated with associated AM fungal species.
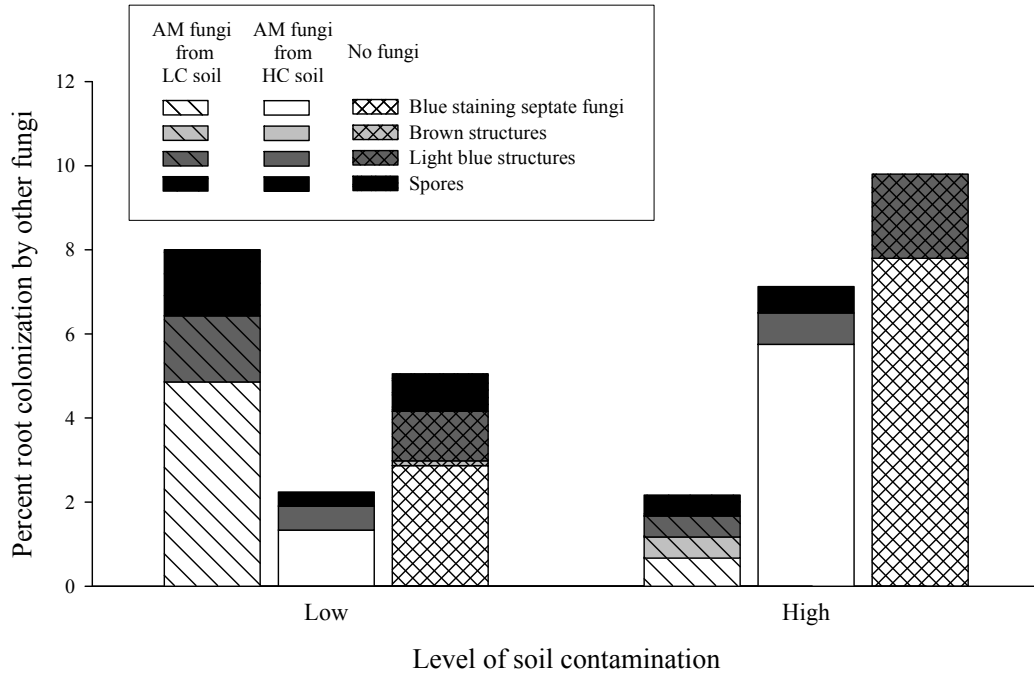
Figure 3.7: Mean abundance of the various putative pathogens in *Da. spicata* roots grown in LC or HC soil inoculated with AM fungi from LC or HC or none at all.
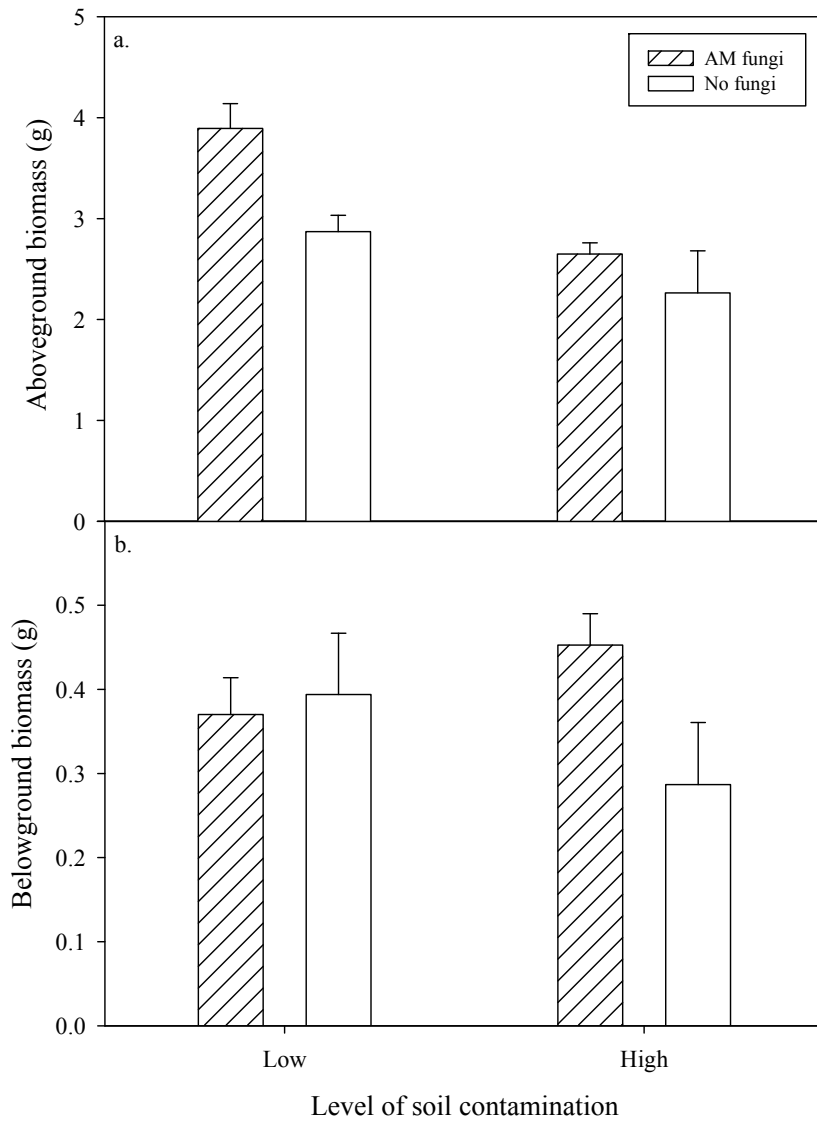
Figure 3.8: Mean + SE aboveground (a) and belowground (b) biomass of *Da. spicata* grown in LC or HC soil inoculated with the AM fungi from LC or HC or none at all.
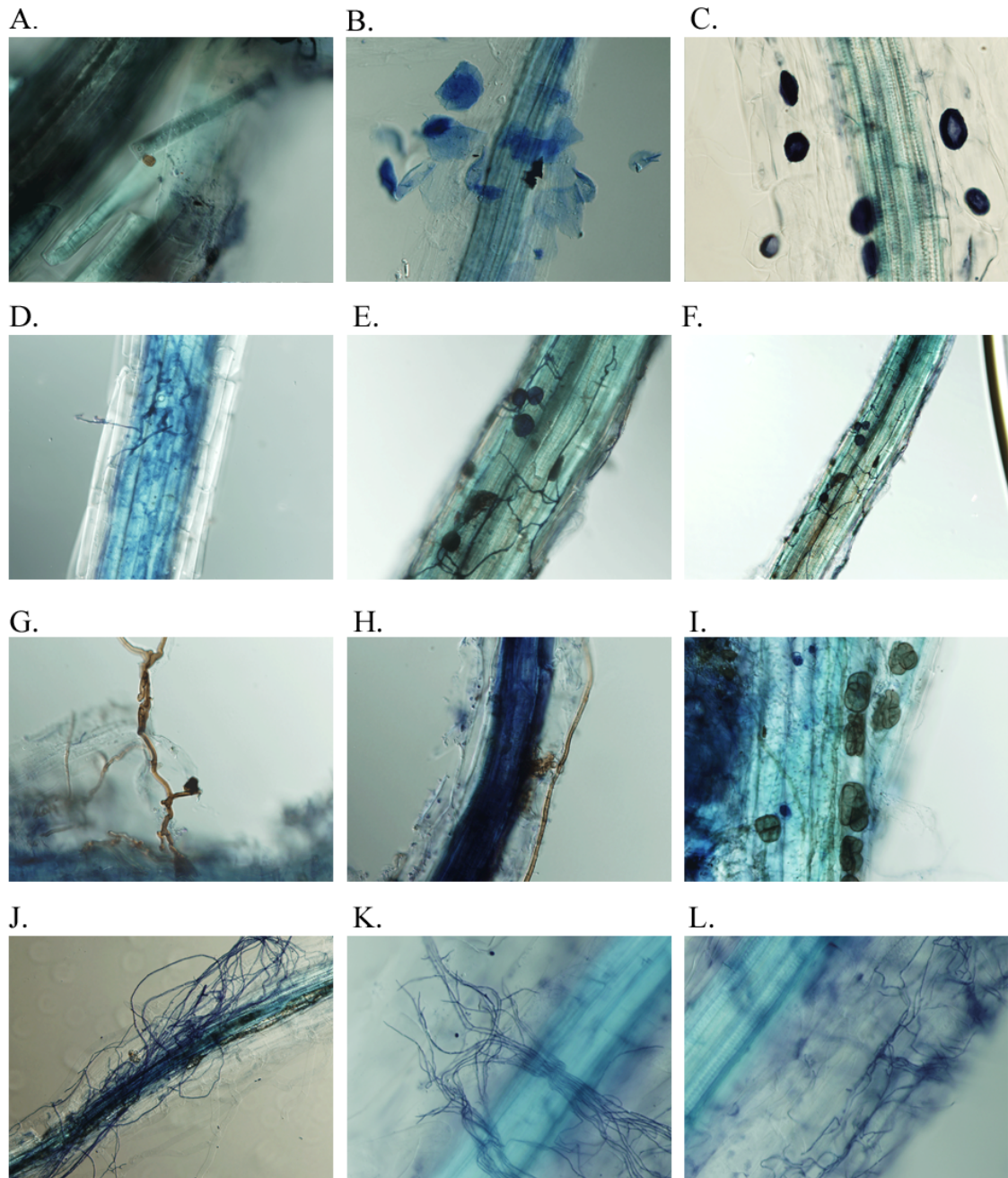
Figure 3.9: Pictures the various fungi observed in the field and greenhouse roots. A) Brown structure in the middle of the photo, B) Light blue structures, C) Spores not attached to hyphae, D) AM hyphae, E-F) AM hyphae with spores, G-I) brown septate hyphae and microsclerotia, J-L) Blue staining septate hyphae.

Conclusions

As in most mutualistic guilds, we understand little about the factors that determine and maintain local species diversity of arbuscular mycorrhizal (AM) fungi, the reasons why a single plant has multiple AM fungal partners, and how that diversity influences host plant performance. The extent to which co-occurring AM fungal species occupy different niche space, based on their ability to tolerate different soil conditions or differentially promote host plant growth in those differing conditions, offers possible explanations for the maintenance of diversity. The ultimate goal of my dissertation was to improve our understanding of how AM fungal diversity is maintained in a heterogeneous soil environment and to explore the consequences of that diversity on host plant performance.

In Chapter 1 field survey results from a natural serpentine grassland show that AM fungal community composition is strongly influenced by soil factors. Chapter 2 represents the first study to demonstrate that the amount of host plant growth promotion provided by naturally co-occurring AM fungal species changes with soil condition. Additionally, the chapter shows that, in some cases, the rank order of growth promotion by different species changes with soil condition. These greenhouse studies are also the first to evaluate the function of a particular AM fungal species in a given environment by examining the consequences of deleting the species from the community. This deletion method revealed that functional redundancy, with regards to host plant growth promotion, was the most common consequence of multiple species infecting one root. Functional complementarity and functional synergy, which may help explain why plants

support multiple partners, were also demonstrated. Each of these interactions was found to be soil context dependent for most fungal species.

Chapter 3 provides a direct demonstration of the effects of soils on the AM fungal community. Additionally, the data suggest that the particular benefits conferred by AM fungi are context dependent. However, the strong soil effects on AM fungal community structure made it difficult to determine if the particular AM fungal species that occur in a soil with a particular level of contamination were more effective in promoting plant growth or if the AM fungal community is simply made up of fungal species that can grow best in that soil.

In this dissertation, I provide evidence that local diversity of AM fungal communities is at least partially maintained by environmental niche partitioning among fungal species. I also demonstrate that the composition of the fungal community colonizing a host plant is important for plant performance and the consequences of colonization change with soil condition. Monitoring fungal species-specific root colonization in different environments is one of the next big challenges in mycorrhizal ecology. How can the functional redundancy be explained? Is it actual functional redundancy with no additional benefit from each species that colonizes a plant or do only a few species dominate the mixed community? To better understand the role of each fungal species in a mixed community we need to know their relative abundance within the extraradical hyphae and on the root system of a shared host. We also need to evaluate what services each fungal species is providing to the host plant and if a plant can select the best suite of fungal partners for the circumstance.

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Introduction

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