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SUCCESSION OF ARBUSCULAR MYCORRHIZAL FUNGI: CAUSES,
CONSEQUENCES, AND CONSIDERATIONS

By

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Succession of arbuscular mycorrhizal fungi: causes, consequences, and considerations

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Arbuscular mycorrhizal fungi (AMF) are soil fungi associations with the majority of terrestrial plants. These fungi may affect a wide range of ecosystem processes from primary productivity to soil stabilization. Understanding the patterns and controls of AMF abundance during succession soil will be critical to predicting and managing ecosystem development. The work presented in chapter 2 is the first to describe changes in the abundance of mycorrhizal types during development of an unregulated floodplain. These findings are important to floodplain regulation and management strategies as they suggest that flow regulation that limits early site formation will limit the ecosystem contributions of AMF on dammed rivers. Chapter 3 builds on the findings from chapter 2 to determine the mechanisms of how litter may affect the abundance of a native AMF community. We test the hypothesis that litter from cottonwoods (*Populus sp.*) is inhibitory to AMF colonization. Our works shows that even small amounts of litter and realistic concentrations of litter leachates can strongly inhibit AMF colonization of two plant species, and that this inhibition is not solely a result of changes in the soil nutrient status. Chapter 3 also demonstrates that common soluble phenolics found in cottonwood litter are inhibitory to AMF at ecologically realistic concentrations, whereas other root colonizing fungi are unaffected. These findings offer another explanatory mechanism for the shift we and others have observed between AMF and ECMF during succession. Chapter 4 addresses the potential consequences of a changing AMF community with respect to the key AMF mediated process of soil stabilization. This work is the first to document a significant interaction between co-occurring species of plants and AMF in soil aggregate formation. Chapter 5 synthesizes our findings with others to understand the potential consequences and management options to control AMF succession and species composition in agroecosystems. Chapter 6 is a product of my work with the ECOS program, and is a lesson on the importance of soil organic matter that is designed for the youngest of students. The significance of this body of work is discussed in chapter 7.

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Chapter 1

Introduction

Conceptual Overview

Arbuscular mycorrhizal fungi (AMF) are widely distributed symbionts that have significant roles ranging from enhancing plant growth to affecting ecosystem level processes (Rillig 2004). While the importance of mycorrhizae to plant establishment during succession has been documented, few studies have studied the successional changes of the fungi themselves during site development, and very few studies have looked at the changes in mycorrhizal abundance during floodplain development. Given that riparian zones are often the most productive and diverse systems in a region as well as critically threatened, an understanding of the ecologically significant mycorrhizal fungi is necessary for better understanding of how to manage floodplains. Following disturbance in temperate and boreal ecosystems, arbuscular mycorrhizae are often the first mycorrhizal association formed and are followed in succession by ECM as the dominant association. This “switch” has been frequently observed (Bellei et al. 1992, Boerner et al. 1996, Treseder et al. 2004), but the causes remain nebulous. The change is likely a product of changes in soil nutrient status and available plant hosts as hypothesized by Read (1991), however, other successional changes (e.g. increase in polyphenolic inputs from litter) may significantly affect AMF populations. Given that AMF species may differ in physiology and function, changes in AMF species composition of a system may affect plants as well as ecosystems (e.g. plant competitive relationships, NPP, soil aggregation, nutrient cycling).

Herein, I investigate mycorrhizal succession in a floodplain ecosystem, determine how cottonwood litter may affect AMF succession within a riparian context, test how a key, AMF mediated, ecosystem process (soil aggregation) is affected by different AMF/host combinations, and finally synthesize our current knowledge on AMF succession and unique species physiologies highlighting the importance of understanding AMF succession to ecological applications of these fungi. This work will enhance current theories of the role of AMF and ECM in plant community succession and allow better predictions of how alteration in AMF community structure will affect ecosystem processes.

In addition to my research, my dissertation will include one chapter on my work during my ECOS fellowship. This chapter describes the development a soil ecology curricula for early elementary school students. These lessons incorporate concepts studied and employed during my dissertation work.

Background and derivation of research questions

Biology and Ecology of AMF

Arbuscular mycorrhizae are symbiotic root/ fungi associations of over 80% of all terrestrial plants (Smith and Read 1997). These associations have been described from plant fossils 460 million years old, and are thought to have aided plants in land colonization (Redecker et al. 2000). While nearly all phyla of plants have members that can form arbuscular mycorrhizae, the fungi forming these associations are all members of the phylum Glomeromycota (Formerly Zygomycota, Schüßler et al. 2001). To date, less than 200 species of AMF have been described.

The arbuscular mycorrhiza symbiosis is generally advantageous to the host plant. In exchange for carbon and protection, the fungi provide a suite of benefits. AMF are best documented as efficient phosphorus scavengers, but they may also access zinc, copper, potassium, and nitrogen. Additionally, AMF may aid their host in drought and pathogen tolerance (Smith and Read 1997; Azcon-Aguilar and Barea 1996). These benefits may significantly increase plant growth and competition.

Through their effects on host growth, AMF contribute to ecosystem level processes. By affecting host growth, AMF can influence net primary productivity (NPP). AMF have also been shown to function in structuring plant communities by affecting the competition abilities of their hosts (Gagne et al. 1993, van der Heijden et al. 1998, Klironomos et al. 2000). Thusly, a plant community with members forming arbuscular mycorrhizae will have different productivity and structure than one formed in the absence of AMF. These fungi may also influence the soil microbial community and processes. Finally, AMF can directly affect soil structure and carbon storage through hyphae-mediated soil aggregation (Miller & Jastrow 1990; Jastrow et al. 1998).

Succession and AMF

The study of ecological succession has been overwhelmingly focused on plant communities. Theories of succession and community development have changed significantly in the past decade as the role of belowground microbiota has become apparent. Clements initial view that a community is interrelated and the structure is a product of these interactions (Clements 1916), although popular early in the century, was largely abandoned for Gleason's individualistic view of community development.

Gleason argues that communities were simply an assemblage generated by the abiotic conditions and individual plant genetics (Gleason 1926). Currently, theories accept that mechanisms of community development are not as simple as the view of either Clements or Gleason (Callaway 1997). When the roles of soil microbiota, especially mycorrhizae, are considered it becomes obvious that community development is more than a function of the abiotic environment. Regardless of the abiotic environment, highly mycotrophic plants (plants that rely heavily on mycorrhizae) will likely not establish in soil void of mycorrhizae. Hence, the importance of mycorrhizae to plant community development has garnered considerable attention.

Many studies have described correlations between mycorrhizal presence and plant community changes, and that AMF are crucial to establishment of some plants in early succession (Johnson et al. 1991, Miller and Jastrow 1992, Gange et al. 1993, Boerner et al. 1996, Corkidi and Rincon 1997, Koske and Gemma 1997, Gange et al. 1999, Barni and Siniscalco 2000, Oehl et al. 2003, Allen et al. . 2003), which has led to widely held models of the role of AMF in plant community succession. Janos (1980) proposed an early model for how AMF affect plant succession based on a tropical system. He states that following disturbance the mycorrhizal soil inoculum is reduced and pioneer plant species will be largely non-mycotrophic on fertile soils. As soil quality changes and the mycorrhizal inoculum increases, the shift is to facultative mycotrophes. Finally, in late successional stages with greater nutrient limitations, there will be a proliferation of obligate mycotrophic plants. While this pattern has been observed (Reeves et al. 1979, Miller 1987), exceptions are not uncommon (Pendleton and Smith 1983, Schmidt and Scow 1986), suggesting that following disturbance pioneer plants may be either

mycorrhizal or non-mycorrhizal. For instance, in western floodplains the earliest colonizer following flood disturbance is cottonwood (Bradley and Smith 1986). Cottonwood trees are mycotrophic, and host not only AMF but ectomycorrhizae as well. Nevertheless, it is generally agreed and well supported that late successional plant communities will have a large percentage of mycorrhizal plants.

While the presence of AMF can influence plant succession and community development, the role of these fungi becomes more complex when changes AMF community composition or even changes in abundance are considered. Some of the most influential papers in current mycorrhizal ecology were published only recently. Klironomos et al. (2000) and van der Heijden et al. (1998) showed experimentally that AMF diversity of a system affect plant diversity and productivity. The mechanisms for these observations are less clear, but are likely a function of AMF community physiologies and specific plant/ fungus interactions.

AMF community succession

As with plant succession, the species composition of the AMF community may change through time. Using an old-field successional system (secondary succession) Johnson et al. (1991) is one of the few studies that exclusively observed AMF succession. Her work demonstrated certain AMF species could be identified as “early successional species” and others as “late successional species.” Some species however, were cosmopolitan. Koske and Gemma (1997) found similar trends using a primary succession gradient along sand dune system on the Eastern coast of the US. Again, while some AMF species were found at all sites, others were found only in either early or late successional

soils. Conflicting results have also been observed, with no significant difference in AMF composition between early and late succession sites (Benjamin et al. 1989; Johnson and Wedin 1997; van der Heijden and Vosatka 1999).

AMF functionality

Studies have established that AMF differ in function. Some species elicit a greater growth response from the host than others, and the response can be dependent on the specific host/ fungus interactions, running the gamut from highly beneficial to nearly parasitic (Klironomos 2003). Jakobsen et al. (1992) determined AMF differ in nutrient absorption. Other studies indicate that species differ in spore production (Bever et al. 1996), colonization rates (Wilson 1984), and extraradical hyphae (Hart and Reader 2002).

Using a tropical system, Allen et al. (2003) found that late successional species of AMF were a greater carbon drain than early successional species, and thus were less beneficial to seedling establishment. The differences between the early and late species were resolved to the family level. Early sites were dominated by small-spored members of the Glomaceae, late sites had considerably more large-spored Gigasporaceae. This study suggests that as AMF community composition changes with succession, so too does AMF functionality with respect to aiding seedling establishment and increasing productivity. .

Succession of mycorrhizal groups

Mycologists currently recognize seven distinct types of mycorrhizal associations: arbuscular, ectomycorrhizae, ericoid, ectendo-mycorrhizae, arbutoid, monotropoid, and

orchidoid (Smith and Read 1997). Of these, arbuscular and ectomycorrhizae are the most widely distributed and well studied. AMF form associations with over 80% of all plants and typically form symbioses with grasses, forbs, shrubs, and some trees.

Ectomycorrhizae only colonize 3% of land plants, but are the association of most gymnosperms and hardwood trees. Each of these symbiotic root relationships aid their plant hosts in nutrient acquisition, but differ in their abilities to access certain key nutrients. AM are considered most adept at phosphorus uptake, whereas ECM may best scavenge nitrogen (Read 1991, Read and Perez-Moreno 2002). These two groups of mycorrhizae often coexist in soil systems; but, because of their different functions, many systems are considered exclusively AM or ECM (Kovachich 1984, Read 1991). AM are the primary mycorrhizal association in P limited grasslands and tropical forests and ECM dominate N limited temperate and boreal forests (Read 1991).

During plant succession in temperate and boreal systems, communities often begin as grasses and forbs and develop into mixed or conifer forests. Hence, during this succession the dominant mycorrhizal association changes from AM to ECM (Johnson et al. 1991, Bellei et al. 1992, Boerner et al. 1996, Barni and Siniscalco 2000, Treseder et al. 2004). Read's (1991) hypothesis offers an explanation for the distribution of mycorrhizal groups, which may be applied to succession of mycorrhizal types in temperate and boreal forests. Following disturbance the initial association is AM as P is most limiting, but as an organic layer develops so do more ECM associations. While this succession of mycorrhizal groups has been frequently observed, the impetus of the shift have only been speculated.

What are the potential consequences of AMF succession?

AMF can occupy many points along the parasite-mutualist continuum. While some species of AMF greatly enhance plant growth, others are inhibitory (Klironomos 2003). If AMF species composition changes, so too could the NPP of the system. Similarly, as plant community structure is in part a function of AMF community diversity, then succession of AMF could affect the plant community structure and subsequent function.

Additional consequences of AMF succession would be evident belowground. AMF function in nutrient cycling, with particular respect to phosphorus. As AMF species can differ in their phosphorus scavenging abilities, soil phosphorus cycling would be affected by changes in the AMF species composition. Hence, soil phosphorus levels could be depleted at greater rates if the dominant AMF species changed from a poor phosphorus scavenger to an efficient one.

Soil structure is a state ecosystem variable that would be affected by changes in both AMF abundance and species composition. AMF are one of the greatest biotic mediators of soil stabilization (Miller & Jastrow 1990; Jastrow et al. 1998) by ensnaring soil particles in their extraradical hyphae. Soil stabilization is strongly correlated with soil hyphal lengths (Jastrow et al. 1998; Rillig et al. 2002), thus changes in AMF soil abundance would affect stabilization. Furthermore, as AMF species differ in function and physiology, they may also differ in their ability to stabilize soil particles. Succession of AMF may result in either faster or slower soil stabilization depending on the dominant species present at the successional ages.

Questions addressed within each chapter

How do mycorrhizal groups change during floodplain development and what variables are most closely correlated with this change?

Chapter 2 is the first study to document changes in both arbuscular mycorrhizal fungi and ectomycorrhizal fungi during primary succession along the floodplain of an unregulated river. I test the hypothesis that AMF abundance will increase rapidly in early site development then decrease as abiotic and biotic changes select for greater abundance of ECMF. This hypothesis was derived from the predictions of Read (1991). We further attempt to determine a mechanism for the decline in AMF and discuss the interesting relationship between soil organic matter and litter accumulation and the declining AMF abundance.

Does cottonwood litter, and litter leachates negatively affect AMF ?

In chapter 3, I test the hypothesis, derived from the observations presented in chapter 2, that litter and litter leachates derived from *Populus trichocarpa* can suppress infectivity the AMF community from the Nyack floodplain chronosequence. This study is the first of its kind to test the effects of litter, litter leachates, and pure phenolic compounds that occur in cottonwood litter on an entire native community of AMF. I demonstrate that litter and leachates derived from the dominant cottonwood species of the Nyack floodplain is inhibitory to AMF inoculum in the soil and may contribute to the succession between AMF and ECMF. Within this chapter I attempt to tease apart the mechanism of the observed inhibition and determine if it is a result of increased nutrient availability or if the leachates are directly toxic to AMF.

What are the consequences of changes in AMF species to soil aggregation?

Within chapter 4, I address one potential consequence of a changing AMF community. From the work presented in chapters 2 and 3, and appendix A, we know that AMF change in both abundance and species composition during site development; however, one of the most pressing questions in AMF research today is, “how will changes in AMF species composition affect AMF mediated processes?” This chapter documents a strong interaction between AMF species and host with respect to the ecosystem process of soil stabilization. The extent of macroaggregate stabilization is a function of the specific plant/ fungus combination. This is relevant to AMF succession in that the contribution of AMF to soil stabilization during site development will not be constant, and could be greatly affected by the plant community composition.

What is the significance of AMF succession to agroecosystems?

Chapter 5 builds up not only the research from previous chapters, but also from the larger body of AMF successional literature to review what we know about the causes and consequences of AMF succession, but to also consider how AMF succession may affect agroecosystems that choose to manage and apply these symbiotic fungi for more sustainable agriculture. This chapter identifies gaps in AMF research that are necessary if we are to manage persistence of beneficial AMF species and maximize the utility of these fungi.

Chapter 6 is a departure from the research chapters and is the product of my work with the ECOS program. For two years I served as a ecologist in residence in local schools and helped develop more complete ecology curricula. This chapter is a simple

lesson on the importance of soil organic matter that can be taught to the youngest of students. Soil education in elementary and even higher levels of education is limited at best despite the relevance of the soil ecosystem to all of society. This lesson is designed to demonstrate that soil organic matter not only provides nutrients to plants but also helps hold water. The final chapter is a brief synthesis of the significance of this dissertation to terrestrial ecology with insights into necessary direction and research. I have also included an appendix that describes the considerable efforts involved in a molecular analysis of the AMF community from the Nyack chronosequence.

Broader significance of this work

In the past decade the role of AMF in structuring plant communities has been established, as well as their role in ecosystem processes; however, very little is known about these fungi in free flowing floodplain ecosystems. Read's (1991) hypothesis regarding the distribution of mycorrhizal types has been supported; still relatively little is known about the causes of shifting mycorrhizal groups, we simply know the numerous associated changes in biotic and abiotic properties. The role of polyphenolics in ecosystems is an emerging field (Hattenschwiler and Vitosek 2000). While their effect on other fungal groups has been investigated, very few studies have looked at the inhibitory effects of these secondary metabolites on AMF. This work elucidates the role litter derived polyphenolics play in shaping AMF abundance and in influencing succession.

If AMF communities do change through time as described by Johnson et al. (1991), how will changes in species composition affect the ecosystem role of AMF? AMF differ in physiology and function, thus early successional species may be adept at

soil aggregation while late successional species are not. My research will increase our understanding of the causes of shifts in AMF abundance. More importantly, I am seeking to understand how changes in AMF species composition may affect key ecosystem processes (in this case soil aggregation). Ultimately, this work will aid mycorrhizal and plant successional theory, and serve as groundwork in linking AMF successional theory with application to restoration ecology and sustainable agriculture.

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Chapter 2

Dynamics of mycorrhizae during development of riparian forests along an unregulated river

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Abstract

In this study, we explore two mycorrhizal groups during development of riparian soils along a freely-flowing river. We provide the first documentation of a shift in abundance between arbuscular mycorrhizae and ectomycorrhizae during floodplain succession. We used a chronosequence spanning 0-70 years along a river in northwestern Montana, USA, to test the hypothesis that abundance of arbuscular mycorrhizal fungi (AMF) is greatest in early stages of soil development, and abundance of ectomycorrhizal fungi (ECMF) is greatest later in floodplain succession. We also measured the AMF-mediated process of formation of soil aggregates during site development. AMF colonization of the dominant tree (black cottonwood, *Populus trichocarpa*) remained low (<5%), while AMF colonization of understory species was high (45-90%), across the chronosequence. Mycorrhizal inoculum potential (MIP) and hyphal length of AMF in soil peaked within the first 13 years of succession and then declined. No single variable significantly correlated with AMF abundance, but AMF tended to decline as litter and soil organic matter increased. Density of ectomycorrhizal root tips in soil increased linearly throughout the chronosequence, and ectomycorrhizal colonization of cottonwood

roots increased rapidly in early stages of succession. These patterns suggest that ECMF are not limited by dispersal, but rather influenced by abundance of host plants. Formation of water stable aggregates increased rapidly during the first third of the chronosequence, which was during the period of greatest AMF abundance in the soil. The observed peak in AMF infectivity and hyphal length during early succession suggests that regular flooding and establishment of new sites maximizes AMF abundance in this ecosystem. Regulation of rivers that eliminates deposition of new sites may reduce contributions of AMF to riparian ecosystems.

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Introduction

Globally, floodplains are some of the most threatened ecosystems (Tockner and Stanford 2002, Naiman et al. 2005). Although riparian areas often host high regional biodiversity, regulation of rivers changes fluvial dynamics that are required to maintain this diversity (Tockner and Stanford 2002, Naiman et al. 2005, Poole et al. 2006). High habitat diversity is maintained on floodplains through time as surfaces are recycled by the river through cut and fill alluviation (Ward et al. 2002). This process creates a shifting habitat mosaic of floodplain surfaces in different stages of plant succession (Stanford et al. 2005). Without regular flooding of different intensities, riparian vegetation may mature into relatively homogenous stands or be replaced by non-native species (Howe and Knopf 1991). For example, cottonwood trees (*Populus* spp.) dominate early-successional sites along many rivers in the northern hemisphere. Cottonwoods specialize in establishing on new surfaces created by seasonal floods (Karrenberg et al. 2002), and without floods these trees often senesce without replacement (Howe and Knopf 1991, Braatne et al. 1996, Poiani et al. 2001). As this displacement is documented for cottonwoods, the same may occur with other taxa, both above and below ground. A better understanding of both the above- and belowground component of riparian areas during succession will be critical in preserving floodplain biodiversity and function (Naiman et al. 1993).

Mature floodplain soils are often nutrient rich and highly productive compared to surrounding upland soil because of constant nutrient inputs from headwater and lateral drainages (Gregory et al. 1991, Tockner and Stanford 2002). Soil development and diversity are important aspects of the shifting habitat mosaic, but they have not been

widely studied in this context. Mycorrhizal fungi and other soil organisms affect development of soil as well as the plant community directly and through their effect on plant productivity (Rillig 2004, Rillig and Mummy 2006). Mycorrhizal associations are ecologically significant symbioses between soil fungi and over 80% of all terrestrial vegetation. Mycorrhizal fungi often confer benefits to their plant hosts, such as increased access to immobile nutrients greater tolerance to drought, and protection from pathogens (Smith and Read 1997). However, very few studies to date have examined the fungal component of developing floodplain soils (Jacobson 2004, Beauchamp et al. 2007).

During development of riparian forests, patches of vegetation within the habitat mosaic undergo succession. As the aboveground community changes in abundance and composition, so too may the soil community. In other temperate and boreal successional systems arbuscular mycorrhizal fungi (AMF) are the primary mycorrhizal associate in early succession, whereas in older soils the main associate is ectomycorrhizal fungi (ECMF) (Johnson et al. 1991, Boerner et al. 1996, Barni and Siniscalco 2000, Treseder et al. 2004). The mechanism of this shift is proposed to be related to soil nutrient status (Read 1991), but concurrent changes in other soil properties and plant community composition make it difficult to isolate a single causal agent. For instance, the effect could be driven by an increase in the abundance of conifer roots over successional time. Nevertheless, such a change in the dominant mycorrhizal association could have a number of ecosystem consequences as these fungi differ in their functions. AMF affect phosphorus cycling, aid seedling establishment of many plant groups, help maintain plant diversity, and strongly contribute to soil stabilization and carbon storage through soil aggregate formation (Smith and Read 1997, Rillig 2004, van der Heijden et al. 1998,

2004, Rillig and Mummey 2006). Conversely, ECMF contribute to decomposition, organic nitrogen cycling, and conifer establishment (Smith and Read 1997, Read and Perez-Moreno 2003, Ashkannejhad and Horton 2006). If AMF abundance follows the same pattern during floodplain succession as has been shown in other studies of temperate succession, then river regulation that limits creation of young sites would be expected to affect AMF abundance, and thus plant diversity, soil stabilization, and soil carbon storage.

The Nyack floodplain at the southern boundary of Glacier National Park, Montana, USA, offers a model system to study mycorrhizae during floodplain development. It is one of the longest, freely flowing segments of river in the continental U.S., and it also has protected headwaters. This floodplain has a mosaic of habitat patches of known age since flooding deposited the foundation material, all within several kilometers of each other (Stanford et al. 2005, Whited et al. 2007). The main objective of this study was to test the hypothesis that AMF are most abundant in early successional soils and ECMF are most abundant in late successional soils. Additionally, we characterized changes in abiotic and biotic site variables through time that may affect AMF abundance. Lastly, we documented the change of a key AMF mediated process, soil stabilization, during floodplain development to understand if soil stabilization is related to AMF abundance in floodplain development. Results of this study will serve as a reference for studies of mycorrhizal dynamics along rivers with altered flow regimes and provide insight into soil processes that may aid in river restoration.

Methods

Site description

The Nyack floodplain is located in northwestern Montana (48° 27' 30" N, 113° 50' W), on the Middle Fork of the Flathead River, a 5th order, free-flowing river with protected headwaters (catchment area = 2300 km²). The Nyack floodplain is approximately 2 km wide and 10 km in length and is comprised of active and abandoned channels, spring brooks, ponds and stands of regenerating and mature riparian vegetation. Actively scoured areas of the floodplain consist of gravel bars with shallow ponds, debris, and vegetation patches (Stanford et al. 2005).

This floodplain has high regional plant diversity, hosting over 200 plant species (Mouw 2001, Mouw and Alaback 2003). Common vegetation at our study sites (Table 1) is similar to other high latitude cottonwood-dominated riparian systems (Helm and Collins 1997). Following floods on Nyack, dense patches of cottonwood seedlings establish on top of freshly deposited sediment. Forbs and grasses that host AMF also recruit within the first couple of years. By ten years, cottonwoods establish a dense thicket with a grass and herbaceous understory. The earliest conifer seedlings occur between 10-15 years, and are very sparse (J. Piotrowski, pers. observation). By 28 years post disturbance, cottonwood density has decreased, and a dense, grass dominated understory exists with occasional conifers. This structure eventually yields to a mixed cottonwood and conifer forest and diverse grass, herbaceous, and woody understory (Mouw 2001, Mouw and Alaback 2003). Thus, both AMF and ECMF hosting plants are abundant at all sites.

Aging of sites along the Nyack floodplain is based on the average age of cottonwood trees at each site. Because cottonwoods colonize sites shortly after

disturbance and often recruit as even-aged stands, their age often reflects the time since disturbance (Everitt 1968). All sites on Nyack were initially aged in the summer of 2000 by coring cottonwoods (Harner and Stanford 2003).

Sample collection

We sampled along the Nyack chronosequence during October of 2003, October 2004, and June-August of 2005. We sampled again from the same older sites (7-69) in 2004-2005, but we aged new sites for collections < 5 years post disturbance as these sites may be lost to flooding yearly prohibiting return to all original young sites. While we collected over a three year period, we present the age of the sites we returned to (7-69) as their age at the first collection for consistency within graphs and tables. During 2003 we were able to collect replicates from three one year sites, thus hyphal length, fine root colonization, litter, and herbaceous biomass measurements of this age represent nine replicates. In 2005 we collected freshly deposited sediment from three sites, which we considered zero years old.

For soil analysis and arbuscular mycorrhizal measurements we collected approximately 4 L of soil from the top 10 cm beneath the litter layer from three randomly selected locations (five during 2005) within each of the different aged sites. We collected cottonwood roots for percent ectomycorrhizal colonization determination in October 2004 from five random cottonwood trees within each aged site. For ECMF tip density measurements we collected whole soil samples (including soil and total roots) from three randomly selected areas per aged site using a corer (5 cm in diameter) to a depth of 10 cm. We did not collect soil from the 12 and 37 year old site for the MIP bioassay because

high water limited access to the site. We were able to access the sites later in summer to collect for ECMF tip density measurements later in the year.

Site and soil characterization

We measured abiotic characteristics of soil on three replicate samples from each site that we collected in 2003. We selected one replicate from each one year old site for analysis, thus the means of soil variables at one year is of three samples. Samples were analyzed at South Dakota University soil testing laboratory for pH, Olsen phosphorus, potassium, nitrate, soil organic matter, and soil texture. Soil pH was analyzed in 1:1 soil:water (w/v). Soil organic matter was measured using the loss on ignition (LOI) method described in Combs and Nathan (1998).

We measured changes in the AMF-hosting herbaceous understory by clipping, drying, and weighing aboveground herbaceous material from three randomly selected 900 cm² plots per site. We used the same area to estimate litter accumulation at each site. We collected litter during a single sampling event rather than over a season; however, collection was after cottonwoods had lost the majority of their leaves and represents near maximum litter accumulation for a season. We dried litter and understory biomass for 2 days at 80 °C, and weighed. We converted these values into grams understory biomass or litter per square meter. We unfortunately lost one litter replicate from the four year old site and one biomass replicate each from the 7, 13, and 15 year sites, thus these site averages represent the mean of two samples.

AMF measurements

To determine how AMF change in abundance across the Nyack chronosequence, we assessed AMF colonization of random fine roots from the soil, AMF colonization of the cottonwoods, AMF potential (MIP) across the chronosequence, and AMF soil hyphal lengths. We collected fine cottonwood roots attached to three cottonwood trees at each site in August 2005. We collected fine roots from the soil by sieving the soil and picking out roots with forceps from the 2003 soil samples. We stained the community fine roots with trypan blue as described by Brundrett et al. (1994). We stained cottonwood roots the same way with the addition of a 5 minute 20% bleaching step after roots were cleared with KOH. Arbuscular mycorrhizal colonization (including presence of hyphae, vesicles and arbuscules) was assessed at 200X on a Nikon Eclipse E600 microscope by the gridline intersect method (McGonigle et al. 1990) at approximately 50 randomly selected locations per slide.

Mycorrhizal inoculum potential is directly related to the abundance of infectious AMF propagules (spores, hyphae, infected root fragments) present in a soil (Johnson et al. 1993). To determine AMF inoculum potential across the chronosequence we modified the MIP method described by Boerner et al. (1996). Fresh field soil (100g) was collected in July 2005 and transferred into 115 ml Cone-Tainerstm (Stuwe and Sons Inc., Canby, OR). We used replicates from four random samples from each aged site in the bioassay. Each pot received 3 seeds of sudan grass (*Sorghum sudanese*) that were thinned to two plants per Cone-Tainer after germination. Sudan grass is routinely used for MIP measurements as it is a good host for AMF (Johnson et al. 1993). We grew the plants under ambient greenhouse conditions for 30 days, and plants were watered with tap water as needed. We lost three plants during growth from the one year site, thus the MIP data

from this age represents only one replicate. Roots were stained and AM colonization was estimated as described above.

We estimated soil abundance of AMF by measuring hyphal lengths in bulk soil. External hyphae were extracted from 4.0 g portions of soil and lengths were measured by a gridline intersect method at 200X (Jakobsen et al. 1992, Rillig et al. 1999). We distinguished hyphae of non-AMF fungi from AMF by observing characters normally missing in the latter: melanization, clamp connections or regularly septate hyphae, non-dichotomous branching (Rillig et al. 1999).

ECMF measurements

We estimated percent ectomycorrhizal colonization ($[\text{number of ectomycorrhizal root tips}/\text{total number of root assessed}] \times 100$) by screening a gently rinsed sub-sample of cottonwood roots collected in October 2004 under a dissecting scope. We randomly screened 100 root tips for each of the five samples collected from each site. We considered any root tips with visible mantle development and morphology and color differing from the long, narrow, orange appearance of non-infected cottonwood roots to be colonized by ECMF.

We estimated ECMF abundance by collecting whole soil samples as described above in August 2005. Between 10-80 mL of homogenized whole soil was immersed in water over a 1 mm sieve to remove most of the soil and rinsed gently to avoid damaging the mycorrhizae. The content on the sieve was collected and examined under a dissecting scope. We counted the total number of ectomycorrhizal tips in each sample. We never

assessed hyphal lengths of ECMF because ECMF cannot be distinguished from non-mycorrhizal fungal hyphae (e.g. saprobes and pathogens; Wallander et al. 2001).

Water stable aggregate measurements

We measured the percent of water stable aggregates of the 1-2 mm diameter size class (% WSA_{1-2mm}) as a measure of physical soil structure (Kemper and Rosenau, 1986). We sieved air dried soils and collected the 1-2 mm fraction from three replicates within each aged site. We used 4 grams of the fraction for the analysis and moistened replicate samples of soil aggregates by capillary action for 10 min before measuring stability. We measured water-stability of aggregates with a wet-sieving method using the apparatus and procedure described in Kemper and Rosenau (1986). We calculated percentage of water-stable aggregates (% WSA_{1-2mm}) using the mass of aggregated soil remaining after wet sieving (5 min) and the total mass of aggregates at the beginning, correcting the initial and final weights of aggregates for the weight of coarse particles (> 0.25 mm) included in the soil samples.

Data analysis

We analyzed change of soil properties, litter, and herbaceous biomass through time with Spearman's rank correlation on the means from each site and site age using NCSS 2000 (NCSS, Kaysville, Utah, USA). We used regression analysis, after testing that the assumptions of normality and homoscedascity were met, to determine how mycorrhizal variables and water stable aggregate formation change with time using only the means (not individual samples, which would constitute pseudo-replication) of

response variables from each aged site with SigmaPlot 7.101 (SPSS Chicago, IL). Changes in AMF, ECMF, and aggregate formation across the chronosequence followed a distinctly nonlinear pattern, and because we had no *a priori* ecological basis on which to select a model for over this period of time we chose the model that best described the data. We verified the appropriateness of the nonlinear models by calculating Akaike's information criterion (AIC) values for the model compared to a linear model. All nonlinear models selected had a lower AIC than linear models. To test if any soil or site variables, including percent water stable aggregates, were correlated with AMF hyphal length we conducted Spearman's rank correlations using NCSS 2000 (NCSS, Kaysville, Utah, USA).

Results

Abiotic and biotic changes through time

Changes in abiotic variables along the chronosequence are presented in Table 2. While soil pH did not change dramatically across the chronosequence, it was negatively correlated with site age ($P < 0.05$). Additionally, nitrate was negatively correlated with site age ($P < 0.05$), whereas soil phosphorus and potassium were positively correlated with age ($P < 0.05$). Soil organic matter correlated positively with site age, displaying close to a ten fold increase between 4 and 31 years ($P < 0.05$). Percent sand was negatively correlated with age, while percent silt and clay were both positively correlated with age ($P < 0.05$). Changes in surface litter, understory biomass are presented in Table 3. Herbaceous understory biomass and litter were both positively correlated with site age ($P < 0.05$).

Changes of mycorrhizae across the chronosequence

AMF colonization of cottonwood roots was low across the entire chronosequence, averaging <2% and ranging from 0% at most sites to 4.38% at the youngest site (data not shown). Occasional vesicles were present, but very few arbuscules were visible in the cottonwood roots. Cottonwood roots also hosted non-AMF in roots. We observed regular septa and clamp connections in some hyphae, indicative of fungi other than AMF, when examined at 400X. AMF colonization of understory, non-cottonwood, fine roots displayed a peak early in site development (Figure 1). AMF colonization of fine roots ranged between 45 to 90%, increasing rapidly early in site development (0-5 years) then steadily declining to 30 years post disturbance after which colonization increased slightly.

AMF inoculum potential (Figure 2) and soil hyphal length of AMF (Figure 3) changed significantly during succession, and both fit a lognormal 4-parameter nonlinear model (adj. $R^2 = 0.58$ and adj. $R^2=0.68$ respectively, $P<0.05$, equation presented figure legend), which describes a rapid increase to a peak followed by a decline phase. The peak in inoculum potential occurred earlier (9 years post disturbance in 2005, presented as 7 years in graph for consistency) than the peak hyphal lengths (13 years post disturbance); however, hyphal lengths were near maximum by this age as well. We extracted AMF hyphal lengths from 2005 soil samples, and these had a similar trend, with a peak in hyphal lengths the same site age as inoculum potential (data not presented). No site variables measured were significantly correlated with AMF hyphal lengths across the chronosequence.

Ectomycorrhizal colonization and tip density in soil increased across the chronosequence. ECMF colonization of cottonwood roots increased rapidly early in site

development (Figure 4) and significantly fit a single rectangular two-parameter hyperbolic model (adj. $R^2=0.95$, $P<0.05$, equation presented in figure legend), which describes a rapid increase to a stable level. The soil density of ectomycorrhizal roots tips increased linearly across the chronosequence (Figure 5; adj. $R^2=0.98$, $P<0.05$), with the greatest density at the oldest site.

Changes in % WSA_{1-2mm}

Percent WSA_{1-2mm} increased (Figure 6) during the first half of the chronosequence and significantly fit a single rectangular two-parameter hyperbolic model (adj. $R^2=0.70$, $P<0.05$, equation presented in figure legend). Again, this model describes a rapid increase to a stable level. The greatest increase in the percent of WSA_{1-2mm} occurred within the first 30 years of site development, after which it remained relatively stable with a slight decline towards the oldest sites. Percent WSA_{1-2mm} did not have a significant correlation with AMF soil hyphal length across the entire chronosequence, but did increase rapidly during the period where AMF were most abundant.

Discussion

This is the first documentation of change in abundance of two ecologically important mycorrhizal groups during development of floodplain soil along an unregulated river. Our study supports our prediction that abundance of AMF in soil is greatest during early site development (1-13 years) and then declines. We also found a steady increase in ECMF abundance throughout the chronosequence as predicted. This is similar to the pattern of AMF and ECMF in other temperate and boreal systems (Johnson et al. 1991,

Boerner et al. 1996, Barni and Siniscalco 2000, Treseder et al. 2004), with this study the first to measure fine root colonization, mycorrhizal inoculum potential, and AMF hyphal length across successional time. ECMF colonization of cottonwood roots increased much more rapidly in early succession than expected. Early proliferation of AMF and subsequent decline suggests that some ecosystem contributions of AMF may be diminished if river regulation reduces early site deposition and forests progress to host ECMF dominated soils.

Potential consequences of a decline in AMF abundance during succession

The ecosystem contributions of AMF, insofar as they are a function of inoculum potential and soil hyphal length, might be attenuated if deposition of new sediment is reduced through river regulation. AMF facilitate seedling establishment by allowing them greater access to limiting nutrients during recruitment (van der Heijden 2004). Even though lack of open sites created by disturbance is often cited as a factor limiting recruitment of cottonwoods (Karrenberg et al. 2002), a reduced AMF inoculum potential as sites age may also affect cottonwood recruitment as well as other plant species. Additionally, the presence of AMF can strongly affect plant community composition and productivity (van der Heijden et al. 1998, Rillig 2004), which could ultimately affect floodplain biodiversity and primary productivity. Transport of mycorrhizal inoculum downstream during floods may be an important mechanism for dispersal of fungi (Veenendaal et al. 1992). Reduction in flooding could diminish the delivery of upstream sources of inoculum, thus also affecting plant communities downstream. Finally, AMF hyphae are significant contributing factors to soil stabilization and subsequent carbon

storage (reviewed by Rillig and Mummey, 2006). Despite a lack of correlation between AMF and %WSA_{1-2mm} across the whole chronosequence, our data show a rapid increase in this aggregate size class during early site development, which could be a product of AMF abundance in young soils; however, changes in organic matter content and clay accumulation during succession would also contribute to aggregate formation. Yet, soil stabilization (and hence potentially river bank stabilization) and carbon storage could be slowed with reduced AMF abundance in riparian systems.

Possible mechanisms contributing to the change between AMF and ECMF

AMF are not lost from the system in late succession as evidenced by the moderate to high colonization of fine roots and increase in biomass of AMF hosting herbaceous plants. Nevertheless, the abundance of these fungi in soil decreases in mid to late site development. This suggests that factors other than host availability may regulate soil AMF abundance and infectivity. There are several possible mechanisms. While no variable measured was significantly correlated with AMF hyphal length, an interesting trend was apparent. The lowest mean hyphal length, fine root AMF colonization, and near lowest inoculum potential occurred at the 31 year old site. This site also has the greatest percent soil organic matter and surface litter. While other studies have shown additions of organic matter to stimulate AMF (Nan et al. 2006, Cavender et al. 2003), the trend we observed suggests that litter quality may be at least as important as quantity to AMF. The increased organic matter and litter could have stimulated organisms that compete with AMF. Another explanation may be that the chemistry of cottonwood litter may suppress AMF. *Populus* foliage contains soluble phenolic compounds, some of

which can inhibit fungal spore germination and hyphal growth (Wacker et al. 1990, Schimel et al. 1998, Isidorov and Vinogorova 2003). Other fungi including ECMF have more complex extracellular enzyme systems capable of degrading these compounds and may be less affected (Münzenberger et al. 2003). Nevertheless, other factors also change concomitantly with time (Tables 2, 3), making it difficult to isolate any one main cause.

Ectomycorrhizal fungi do not decline at any point across this chronosequence. While the abundance of ECMF (as indirectly measured through the soil density of colonized cottonwood root tips) steadily increased throughout the chronosequence, percentage colonization of cottonwood roots by ECMF increased rapidly to near maximum within the first five years. This suggests that ECMF disperse quickly to new sites and that their abundance is strongly influenced by the presence of ectomycorrhizae hosting root tips. Increasing soil organic matter and litter accumulation may contribute to ECMF proliferation, which supports Read's (1991) hypothesis when applied to successional systems.

These data increase our sparse knowledge of the belowground component of a threatened type of ecosystem and offer an important factor to consider in managing and restoring riparian ecosystems. Our examination of the Nyack riparian chronosequence represents the first documentation of a change in mycorrhizal groups within a floodplain system and reveals a pattern that largely adheres to other observations of changes between AMF and ECMF abundance during plant community succession in temperate and boreal systems, but on a faster time scale. River management is an enterprise of increasing global significance (Bernhardt et al. 2005). River regulation may not always affect AMF community composition (Beauchamp et al. 2007), but the overall abundance

of these fungi may be strongly affected. In this riparian system, regular flooding events appear to be critical for maintaining AMF, without which soils may progress to dominance by ECMF within a relatively short period of time.

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Tables

Table 1. Common plant species along the Nyack chronosequence (Adapted from Mouw 2001, Mouw and Alaback 2003) and their occurrence across site ages.

Plant types	Sites present
Herbaceous	
<i>Agrostis gigantea</i>	All sites
<i>Arnica cordifolia</i>	34, 50
<i>Melilotus officinale</i>	1, 4, 7, 10, 12, 34
<i>Smilacina racemosa</i>	34, 69
<i>Centaurea maculosa</i>	All sites
<i>Verbascum thapsus</i>	4, 7
<i>Achillea millefolium</i>	28, 34
Woody Shrubs	
<i>Rosa woodsii</i>	53, 69
<i>Symphoricarpos albus</i>	31, 37, 53, 69
<i>Crataegus sp.</i>	50, 69
<i>Cornus stolonifera</i>	13, 34, 53, 69
<i>Rubus parviflorus</i>	66
<i>Salix spp.</i>	16, 50, 69
<i>Alnus tenuifolia</i>	34, 53, 69
Deciduous Trees	
<i>Amelanchier alnifolia</i>	53, 69
<i>Populus trichocarpa</i>	All sites
<i>Acer glabrum</i>	34, 53, 69
<i>Prunus virginiana</i>	69
Coniferous trees	
<i>Abies spp.</i>	34, 50, 69
<i>Picea spp.</i>	28, 34, 50, 69
<i>Pseudotsuga menziesii</i>	34, 50, 69

Table 2. Abiotic soil parameters of aged sites along the Nyack chronosequence (mean \pm standard error) and Spearman's correlation values of the variables correlated with site age. (“*” indicates significance at $P < 0.05$)

Site age	pH	P mg kg ⁻¹	N0 ₃ ⁻ mg kg ⁻¹	K mg kg ⁻¹	%OM	% Sand	% Silt	% Clay
1	8.0 (0.0)	2.7 (0.3)	5.0 (2.6)	39.0 (3.5)	0.7 (0.8)	69.3 (2.19)	16.7 (1.8)	14.0 (0.6)
4	8.1 (0.0)	2.0 (0.0)	1.5 (0.3)	37.0 (1.5)	0.4 (0.0)	79.7 (1.45)	8.3 (1.2)	12.3 (0.3)
7	8.1 (0.1)	2.0 (0.0)	1.8 (0.6)	59.0 (8.7)	0.6 (0.2)	78.0 (5.77)	9.7 (4.4)	13.0 (1.5)
13	8.1 (0.0)	2.0 (0.0)	1.0 (0.5)	54.0 (6.0)	0.7 (0.0)	71.0 (2.08)	16.7 (1.3)	12.3 (0.9)
15	8.1 (0.0)	1.7 (0.3)	1.7 (0.2)	52.0 (5.7)	0.7 (0.0)	71.3 (0.67)	16.7 (0.7)	12.0 (0.0)
19	7.8 (0.0)	3.3 (0.3)	1.0 (0.0)	76.7 (5.5)	1.7 (0.2)	51.7 (3.18)	31.3 (2.7)	17.3 (0.9)
31	7.7 (0.0)	3.7 (0.3)	0.8 (0.2)	89.7 (5.4)	3.7 (0.2)	26.7 (2.67)	54.7 (8.2)	19.3 (6.2)
37	7.6 (0.0)	4.0 (0.0)	1.2 (0.2)	98.3 (7.1)	2.7 (0.4)	42.7 (5.21)	38.7 (5.2)	19.3 (0.9)
53	7.8 (0.0)	3.0 (0.0)	1.0 (0.0)	90.3 (2.7)	2.0 (0.4)	38.7 (3.71)	42.0 (3.1)	19.3 (0.7)
69	7.7 (0.1)	3.3 (0.3)	0.8 (0.2)	96.0 (6.7)	2.4 (0.1)	50.3 (8.09)	34.0 (7.2)	15.7 (0.9)
r_s	-0.75*	0.63*	-0.76*	0.90*	0.82*	-0.76*	0.82*	0.65*

Table 3. Biotic parameters of aged sites along the Nyack chronosequence (mean \pm standard error) and Spearman's correlation values of the variables correlated with site age. (“*” indicates significance at $P < 0.05$)

Site age	Herbaceous Understory biomass (g m⁻²)	Litter biomass (g m⁻²)
1	15 (3)	0 (0)
4	39 (5)	24 (9)
7	48 (16)	110 (37)
13	42 (1)	479 (61)
15	20 (7)	415 (114)
19	118 (18)	488 (98)
31	79 (22)	916 (101)
37	124 (10)	529 (89)
53	156 (26)	600 (137)
69	64 (18)	423 (24)
r_s	0.78*	0.76*

Figures

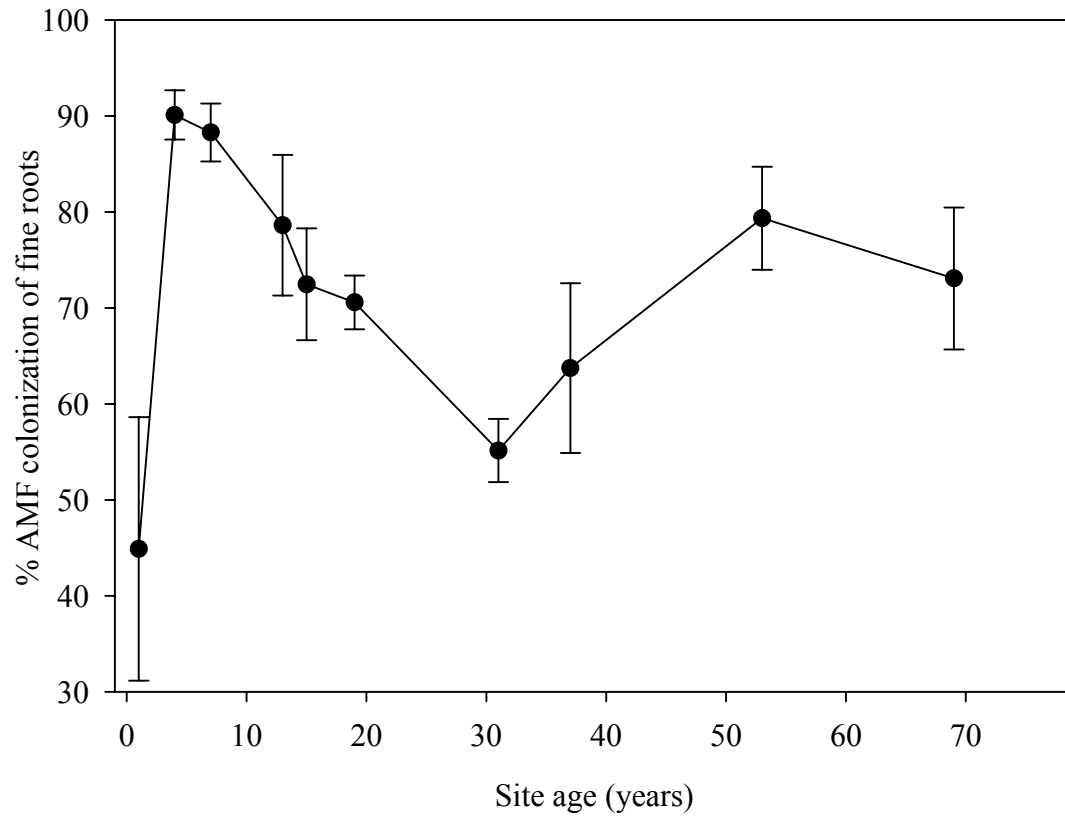


Figure 1. AMF Colonization of understory fine roots in October 2003 from bulk soil across the Nyack chronosequence (mean \pm standard error).

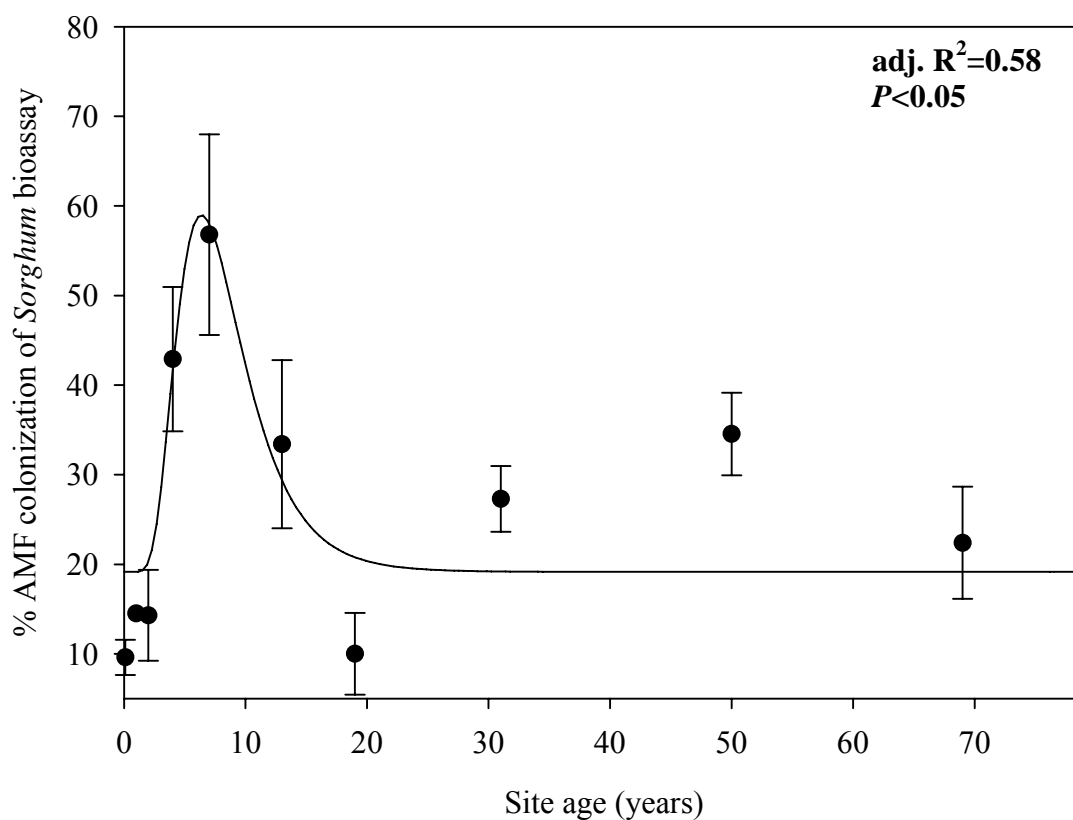


Figure 2. Mycorrhizal inoculum potential across the chronosequence in July 2005 as measured by percent colonization of *Sorghum* bioassay fitted along the lognormal 4 parameter nonlinear model ($(y=y_0+a^{-0.5}[\ln(x/x_0)/b]^2)$), where $a= 39.83$ $b=0.43$, $x_0= 6.36$, and $y_0= 19.14$ (mean \pm standard error).

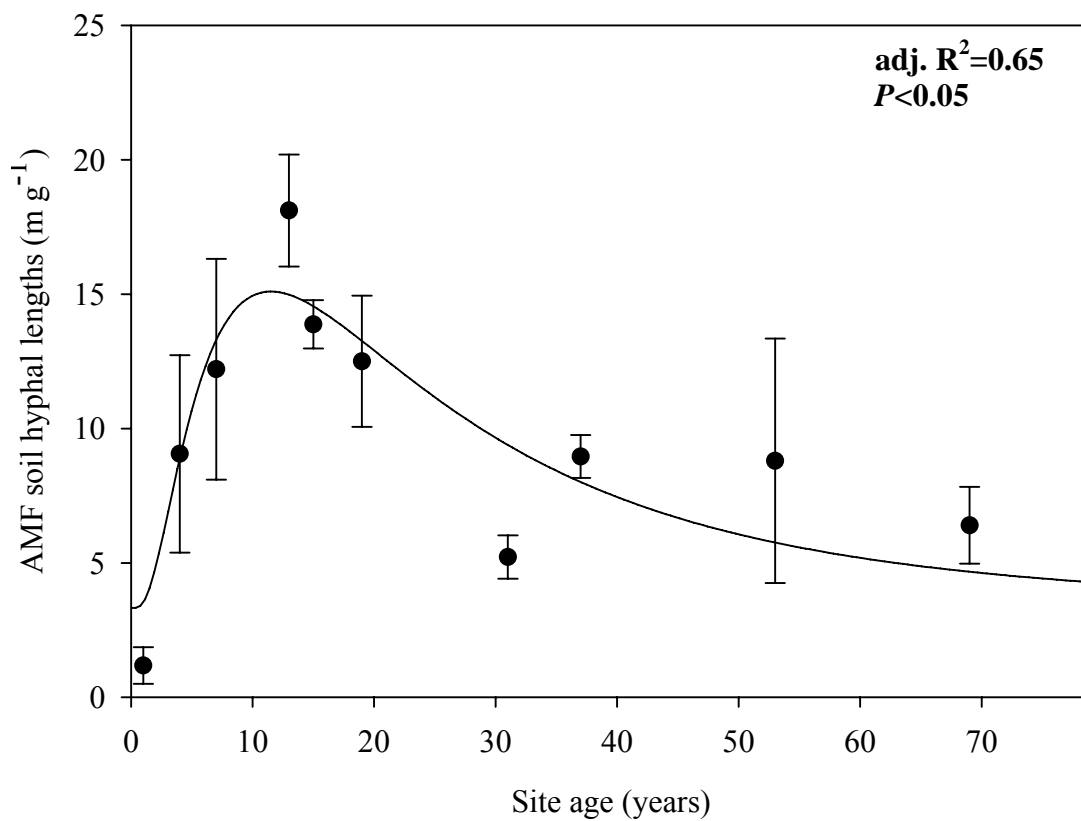


Figure 3. Changes in AMF biomass as measured by soil hyphal lengths (m g^{-1} soil) across the Nyack chronosequence fitted along the lognormal 4 parameter nonlinear model, where $a=11.7$, $b=0.86$, $x_0=11.5$, $y_0=3.31$ (mean \pm standard error).

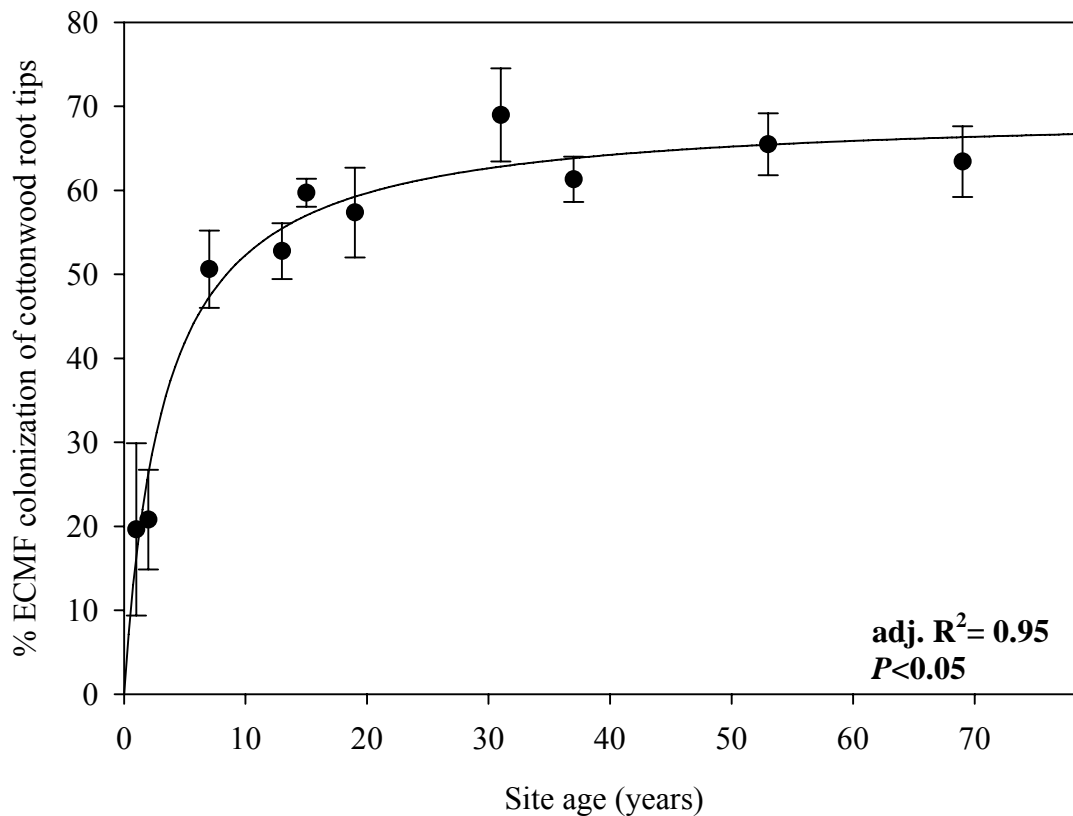


Figure 4. Changes in percent ectomycorrhizal colonization of cottonwood root tips in October 2004 across the Nyack chronosequence fitted along a single rectangular two parameter hyperbolic model ($y = ax / (b+x)$), where $a = 64.49$ and $b = 3.29$ (mean \pm standard error).

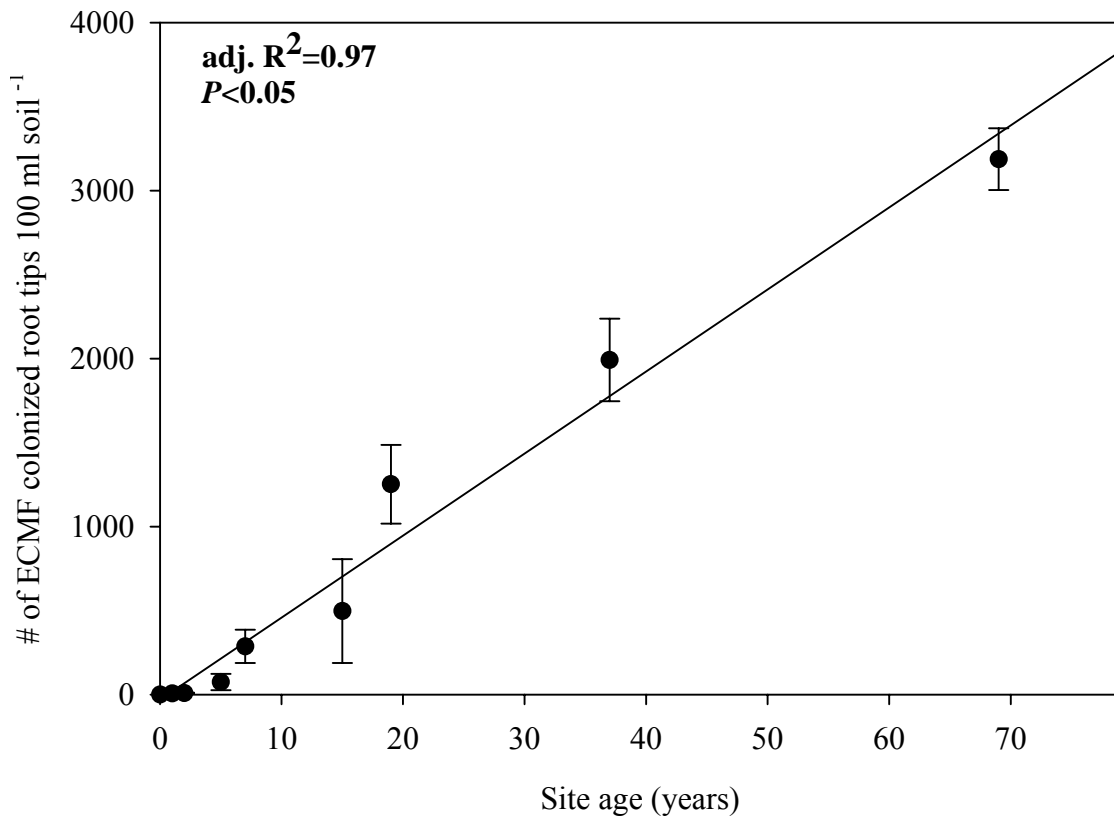


Figure 5. Changes in abundance of ectomycorrhizae in soil as determined by the number of ECMF colonized root tips in 100 ml bulk soil at sites in August 2005 across the Nyack chronosequence fitted with a linear model ($y=y_0 + ax$) where $y_0 = -62.35$ and $a = 59.4$ (mean \pm standard error).

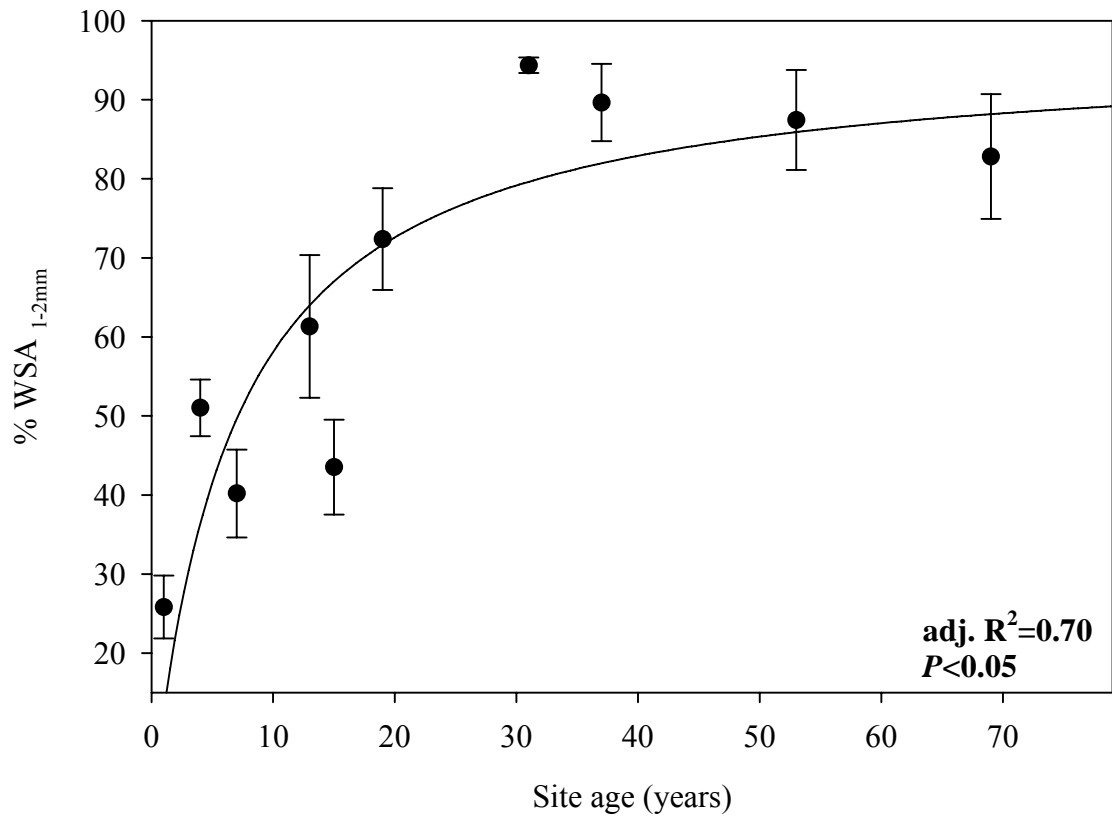


Figure 6. Change in percent water stability of the 1-2mm aggregate size class across the Nyack chronosequence fitted along a single rectangular two parameter hyperbolic model ($y = ax / (b+x)$), where $a = 96.67$ and $b = 6.63$ (mean \pm standard error).

Chapter 3

Inhibition of colonization by a native arbuscular mycorrhizal fungi community via *Populus trichocarpa* litter, litter extract, and soluble phenolic compounds.

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Abstract

Controls on the colonization and abundance of AMF in ecosystems are little understood and may be related to host factors, the fungal community, and soil physiochemical properties, and changes in these variables during soil development may affect succession between mycorrhizal groups. Here we investigated the effects of litter, litter leachates, and common soluble phenolic compounds on AMF colonization of roots. In previous studies, we observed a negative correlation between increases in cottonwood (*Populus trichocarpa*) litter and AMF abundance and inoculum potential along a riparian chronosequence in northwest Montana. From this we hypothesized that litter inputs negatively affect the native AMF community and may contribute to the shift between AMF and ectomycorrhizas. We tested the effects of cottonwood foliage and litter extract additions on the colonization of AMF of both cottonwood and Sudan grass (*Sorghum sudanese*) seedlings. Addition of 5% (v/v) dried cottonwood leaves completely inhibited AMF colonization of *S. sudanese*. AMF colonization of *S. sudanese* was significantly reduced by litter extract of *P. trichocarpa* foliage, and colonization was negatively correlated with litter extract concentrations. Additions of aqueous litter extract significantly reduced AMF colonization of cottonwood seedlings as well. The effect of

the litter extract on AMF colonization of *S. sudanese* did not appear to be mediated by changes in soil pH or plant biomass. Available phosphorus was higher in soil receiving highest concentration of litter extract, but not at a level expected to be inhibitory to AMF colonization. Litter additions significantly increased total soil phenolics, but with a range similar to natural soils of the Nyack floodplain. Pure soluble phenolic compounds common to *Populus* were tested for their effect on AMF colonization by native fungi from the Nyack floodplain. All tested compounds significantly reduced AMF colonization but did not affect colonization by non-AMF root colonizing fungi. This suggests secondary compounds present in cottonwood litter can affect colonization ability of a native AMF community. The potential mechanisms of inhibition and the relevance of these findings to AMF succession both within a single host and soil ecosystem are discussed.

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Introduction

Arbuscular mycorrhizal fungi (AMF) are a group of soil fungi that form symbiotic associations with over 80% of all terrestrial vegetation (Smith and Read, 1997). These fungi have been considered keystone species in that they can increase ecosystem productivity and have the potential to affect plant diversity by providing increased access to immobile soil nutrients, water, and by increasing root pathogen resistance (O'Neill et al., 1991; Rillig et al., 2004). Through these benefits, AMF may also shape plant successional trajectories (Gange et al., 1993; Gange and Brown, 2002; Hart et al., 2001). Hence, factors that affect the abundance and infectivity of these fungi could affect both plant community and soil ecosystem development.

During plant community succession in temperate and boreal systems the dominant mycorrhizal associate often changes from AMF to ectomycorrhizal fungi (ECMF) (Johnson et al., 1991; Read, 1991; Treseder et al., 2004; Jeff Piotrowski unpublished observation). The mechanism of this change is proposed to be a result of changing soil nutrient status; however, other soil and plant changes occur over successional time, making it difficult to identify a single driver (Lodge and Wentworth, 1997; Beauchamp et al., 2006). Previously we observed a relationship between litter accumulation and AMF during floodplain succession: AMF hyphal length, inoculum potential, and colonization of roots were all suppressed at sites of greatest litter and soil organic matter accumulation despite an abundance of AMF hosting plants (Jeff Piotrowski unpublished observation). While other studies have shown that certain sources of organic matter are stimulatory to

AMF (Cavender et al., 2003; Nan et al., 2006), the observed inhibition of AMF suggests that litter chemistry may be an additional driver in the successional shift between AMF and ECMF during ecosystem development.

Aboveground inputs such as litter fall, litter leachates, and canopy leachates can significantly alter the function and abundance of many soil organisms (Schimel et al., 1998; Wardle, 2002; Castells et al., 2005). While carbon and nutrients derived from aboveground materials are often stimulatory to saprobic organisms and detritivores, many plants produce secondary compounds that can inhibit the growth and function of soil microbes, affecting soil processes such as decomposition and nutrient cycling (Hättenschwiler and Vitousek, 2000). Symbionts, such as mycorrhizal fungi, may also be affected by litter inputs. To date, few studies have investigated the effect of litter leachates and plant secondary compounds on arbuscular mycorrhizal fungi within an ecosystem context.

The reported effects of plant secondary compounds on AMF growth are mixed. One class of phenolic compounds, flavonoids, has demonstrated both stimulatory and inhibitory effects on AMF depending on source, and certain flavonoids have been implicated as chemical signals that induce AM colonization (Morandi, 1996; Scervino et al., 2005; Bais et al., 2006). Yet, other phenolic compounds have an inhibitory effect on AMF. Wacker et al. (1990) found that ferulic acid, a common soluble phenolic found in high concentration in asparagus roots, inhibited germ tube elongation of germinating *Glomus fasciculatum* (Thaxter) spores *in vitro*. Fries et al. (1997) found additions of three phenolic compounds (*p*-coumaric acid, *p*-hydroxybenzoic acid, and quercin) to be stimulatory to colonization by *Glomus intraradices* (Schenck & Smith) at low

concentrations, yet inhibitory at higher concentrations. All these studies focused primarily on the effects of exogenously applied, pure phenolic compounds on single AMF species. Much less is known about how litter and leachate chemistry may affect AMF or entire natural communities of AMF. Yan and Choi (2002) recently demonstrated that extracts from *Artemisia princeps var. orientalis* (Pamp.) foliage applied to soil inhibited AMF colonization. This study suggests that litter leachates can affect AMF; however, the mechanism of inhibition remains unclear. Do litter and litter leachates suppress AMF by increasing soil phosphorus availability, altering soil pH, affecting host growth, stimulating antagonistic organisms, or is there direct toxicity? Irrespective of the mechanism, inhibition of AMF by litter leachates could be a contributing agent to the decline of AMF community observed during succession, and this phenomenon is thus clearly ripe for further investigation.

Black cottonwood (*Populus trichocarpa* Torr. & Gray) is the dominant tree species on the Nyack floodplain, located in northwestern Montana (48° 27' 30" N, 113° 50' W) on the Middle Fork of the Flathead River, a 5th order, free-flowing river with protected headwaters (Harner and Stanford, 2003). Members of the genus *Populus* have been well studied for their foliar chemistry and its effect on soil microbes (Olsen et al., 1971; Schimel et al., 1998; Madritch et al., 2006). These trees produce abundant secondary metabolites and trees may vary in productivity across genotype, age, and environmental gradients (Mansfield et al., 1999; Donaldson et al., 2006). Early studies have described foliage from members of this genus as inhibitory to some ectomycorrhizae species (Olsen et al., 1971); however, no studies have investigated the effects of litter and litter leachates from *P. trichocarpa* on a native community of AMF.

The aim of these studies was to test the effects of cottonwood litter on the AMF community of the Nyack floodplain. We hypothesized that organic matter and leachates derived from cottonwood litter could reduce AMF infectivity. Furthermore, we sought to gain a better understanding of the mechanisms of AMF inhibition by litter leachates and phenolics in natural soils by determining if this inhibition is a result of changes in plant growth, soil pH, phosphorus availability, or specific phenolic compounds. We test this hypothesis with three complementary experiments. The first experiment is designed to test the effects of whole *P. trichocarpa* litter on AMF colonization; the second tests a range of dilutions of litter leachates on colonization; and the last tests if pure, soluble phenolic compounds known to be in abundance in *Populus* litter are sufficient to inhibit colonization of a native AMF community.

Methods

Experiment 1: The effect of cottonwood leaves on AMF colonization

We conducted this experiment to determine if cottonwood leaves would affect AMF colonization. We collected whole cottonwood leaves from *P. trichocarpa* in Greenough Park Missoula, MT in June of 2005 and dried the leaves at 80° C for two days; then the leaves were pulverized using a Waring blender. We pulverized vermiculite to use as an inert control because it has a neutral pH and is commonly used in growth media with AMF.

We grew plants in 250ml Cone-tainertm (Stewe and Sons Canby OR, USA) pots containing a homogenized mixture of 90% air dried field soil from a 9 year old sites on the Nyack floodplain, 5% (v/v) whole vermiculite for aeration, and 5% of either pulverized cottonwood leaves or vermiculite as an inert control. To enhance ecological realism, we selected the Nyack soil because it represented a soil with low organic matter (0.6%), low phosphorus ($\sim 2 \text{ mg kg}^{-1}$), and high native inoculum potential (Jeff Piotrowski unpublished observation). Because the soil was air dried prior to use, the infectious AMF propagules were mostly spores, as active hyphae and root fragments may have been desiccated and infectivity reduced. Each treatment had 7 replicates ($n=14$). We germinated surface sterilized *Sorghum sudanese* (Stapf .) seeds (5% H₂O₂ for 5 minutes) prior to sowing, then planted three per pot. *S. sudanese* was chosen for all experiments because it is readily colonized by AMF with little effect on its biomass. Plants were thinned to two per pot within the first week and grown for two months in greenhouse conditions with watering every 2-3 days.

Experiment 2: The effect of cottonwood litter extract on AMF colonization of S. sudanese and P. trichocarpa seedlings

For these experiments we used an extract of cottonwood litter to simulate cottonwood litter leachate. In October 2005 we collected 500 g of freshly fallen cottonwood leaves from around *P. trichocarpa* in Greenough Park Missoula, MT. We produced the extract by soaking the leaf litter in 12 liters of dH₂O for three days. The extract was filtered through a 53 μm sieve to remove particulate matter. We chose not to

sterilize the litter leachate before addition because we felt it was important to also add the microbes associated with this substrate and their products, as would be the case in the field. A portion of the litter extract was diluted with deionized water to concentrations of 1X, 0.5 X, 0.25X, 0.1X, 0.01X, 0.001X, and 0.0001X,0.00001 X. All dilutions were adjusted to pH 7.0 using 1 M NaOH.

We grew plants in 125ml Cone-tainertm (Stewe and Sons Canby OR, USA) pots containing a homogenized mixture of 100% air dried field soil from a 9 year old sites on the Nyack floodplain. We germinated surface sterilized *S. sudanese* seeds prior to sowing, then planted three per pot. Plants were thinned to two per pot within the first week. The plants were grown for two months in growth chamber conditions (25°C, 60% R.H., 320 $\mu\text{mol sec}^{-1}\text{P.A.R}$) and watered every 2-3 days with 20 ml of the prepared litter extract dilutions or dH₂O as a control. Each treatment had 5 replicates ($n=45$). We harvested and stained plant roots for assessment of AMF colonization as described above.

We also tested the effect of the litter extract versus water on *P. trichocarpa* seedlings grown from seeds collected from the Nyack floodplain. Seeds were surface sterilized as above and pre-germinated prior to planting. Cottonwood seedlings were grown in the same soil as above in the growth chamber. Half the seedlings were watered with 20 ml 1X litter extract every 2-3 days, the others with water for two months ($n=12$). Root growth of the cottonwood seedlings was very low and not assessed for biomass.

Experiment 3: The effects of individual soluble phenolics on AMF colonization

To test if soluble phenolic compounds could alone inhibit AMF colonization by fungi from the Nyack floodplain, we used a modified AMF inoculum potential bioassay. We exposed *S. sudanese* seedlings to common soluble phenolic compounds. We divided the seedlings into five treatments (water control, ferulic acid, caffeic acid, vanillic acid, and coumarin) with eight replicates each ($n=40$). Ferulic, caffeic, and vanillic acids were chosen as they are present in *Populus* foliage, coumarin was selected as a known antifungal secondary compound found in plant tissues previously untested on AMF (Greenaway et al., 1987; Isidorov and Vinogorova, 2003). To determine the treatment concentration, we determined the total phenolic concentration of the cottonwood litter extract we prepared prior using the Folin-Cioteau assay described Singleton and Rossi (1965) with ferulic acid as the standard. Total phenolic concentration of the litter extract was determined to be 497 mg kg^{-1} of ferulic acid equivalents. This concentration is comparable to total phenolic measurements from leachates found in natural systems (Castells et al., 2003; Suominen et al., 2003). Based on our prior experiment, 0.5X dilution of our litter extract was most inhibitory to *S. sudanese* colonization. Hence we decided to test a concentration of 250 mg kg^{-1} of each soluble phenolic.

We grew *S. sudanese* seedlings in 100ml of 50:50 mixture of field soil and trap culture medium to ensure a high colonization potential and because snow prevented fresh soil collection. *S. sudanese* seeds were surface sterilized as above and pre-germinated prior to planting. We planted 3 seeds in each pot, and thinned to one seedling per pot after 1 week. We treated the plants with 20 ml of either the phenolic solution or water every 2-3 days for a month, and grew the seedlings in environmental growth chambers as described above for one month.

Plant and mycorrhizal analysis

Upon harvest, we clipped, dried, and weighed shoot and root biomass. Roots were then separated from the shoots, washed, and stained. We stained the roots with trypan blue as described by Brundrett et al. (1994). We assessed mycorrhizal colonization at 200X on a Nikon Eclipse E600 microscope by the gridline intersect method (McGonigle et al., 1990) at ~50 randomly selected locations covering the entire slide, scoring any AMF structures as positive for colonization (hyphae, vesicles, arbuscules). We distinguished AMF in roots from other root colonizing fungi which have characters absent in AMF: melanization, clamp connections or regularly septate hyphae, non-dichotomous branching (Rillig et al., 1999). We did not assess AMF soil hyphal length because the short duration of these experiments would not allow sufficient hyphal production above background hyphae in these field soils.

Soil analysis

We determined soil pH using a 1:1 (soil: 0.01M CaCl₂) slurry. Available soil orthophosphate was estimated using the ascorbic acid method described by Murphy and Riley (1962). Total water soluble soil phenolics were determined by the method described by DeForest et al. (2005) with ferulic acid used to generate the standard curve.

Statistical analysis

We used Student's two sample T-test to compare the results of experiment one and the cottonwood seedling experiment; if data could not be transformed to meet assumptions of parametric statistics we used the Mann-Whitney *U* test. We used ANOVA to compare the effects of litter extract and pure phenolic compounds on AMF colonization, plant growth, and soil parameters with Tukey's HSD analysis where appropriate, if data fulfilled the assumptions of homoscedascity and normality of residuals. We log transformed the data if these assumptions were not met. If transformation did not allow data to fill assumptions, we used a Kruskal-Wallis one-way ANOVA with a Bonferroni corrected multiple comparison Z-test to determine differences between treatments. To test the effect of litter extract concentration on plant and soil parameters we calculated Spearman's rank correlation values between on the mean AMF colonization, litter extract concentration, plant root and shoot biomass, available soil phosphorus, and total soil phenolics. We used NCSS (NCSS, Kaysville, Utah, USA) for all statistical analyses after testing for assumptions of normality and equal variances using JMP (JMP, Version 6. SAS Institute Inc., Cary, NC, 1989-2005).

Results

The effects of cottonwood derived organic matter on AMF colonization

The first experiment indicated that additions of dried cottonwood leaves inhibited root colonization of *S. sudanese* by AMF indigenous to the floodplain. The roots of

seedlings receiving the cottonwood additions had no evidence of AMF colonization, whereas the vermiculite control had 37.5% colonization (Table 1). Roots with the litter added were intermittently colonized by other non-AMF fungi with regular septation, melanization and/ or microsclerotia. The pH of the soils was not statistically different (Table 1). The available phosphorus content of the soils was significantly different ($P<0.001$), with the soil receiving the leaf addition having 13.2 mg kg^{-1} compared to 2 mg kg^{-1} in the control (Table 1).

The effects of aqueous extract of cottonwood litter on AMF colonization

Aqueous extract of cottonwood litter significantly reduced AMF root colonization of *S. sudanese* (Figure 1, $F=2.74$, $P<0.05$). Colonization ranged from 51% with the water control to 6% when treated with 0.5X dilution of the extract. Seedlings receiving 0.5X and 1X dilution treatments had produced significantly less colonization than those receiving the water control. There was no detectable difference in colonization between other treatments. The addition of the cottonwood extract did not significantly alter root biomass of the *S. sudanese* seedlings (Table 2, $F= 1.6$, $P=0.17$). Shoot biomass was significantly lower in the treatment receiving 0.25X than treatments receiving 1X and 0X, while all others were not statistically different (Table 2, $F=2.79$, $P<0.05$). Final soil pH was increased by the extract addition (Table 2, $H=29.8$, $P<0.0001$). Treatments receiving 0.5X and 1X dilutions had higher soil pH than treatments receiving 0.001X and 0.1X, all other comparisons had not detectable differences. Available soil phosphorus differed across the litter extract treatments (Table 2, $F=2.7$, $P<0.05$), but Tukey's HSD test did not

resolve any differences between individual treatments. Total soil phenolics were significantly increased with litter extract additions (Table 2, $F=21.9$, $P<0.0001$). Multiple comparisons are presented in Table 2.

Colonization was significantly negatively correlated with increasing concentration of the litter extract (Table 3). Additionally, total soil phenolics increased with extract concentration. Final soil pH was positively correlated with available phosphorus (Table 3). No other correlations were statistically significant.

The litter extract significantly reduced AMF colonization of cottonwood seedlings (Table 4, $P<0.001$). Litter extract treatment also significantly reduced aboveground biomass (Table 4, $P<0.001$). Soil pH was not affected by the treatment ($P=0.07$). Both available soil phosphorus ($P<0.01$) and total soil phenolics ($P<0.01$) were significantly increased by the litter treatments.

The effects of pure phenolic compounds on the AMF community

Additions of each tested phenolic significantly reduced AMF root colonization of *S. sudanese* compared to the water control (Figure 2, $F=5.98$, $P<0.01$). These compounds did not significantly alter plant biomass, soil pH, or available soil phosphorus compared to the water control (Table 5). All plants in all treatments had evidence of AMF colonization (arbuscules, vesicles, hyphae) as well as colonization by non-AMF root colonization fungi (sporangia, regularly septate hyphae, melanization). The lowest mean AMF colonization occurred with the coumarin treatment, but this was not significant.

Colonization by non-AMF fungi was unaffected by the phenolic compounds (Figure 2, $F=0.47$, $P=0.75$).

Discussion

Results from these experiments support our hypothesis that leaves and litter extracts of *P. trichocarpa* as well as specific phenolic compounds found in *Populus* foliage are inhibitory to AMF colonization by fungi native to the Nyack floodplain. These data significantly increase our understanding of the effects of litter and soluble phenolics on communities of AMF in ecosystems. Results suggest an interesting host/ symbiont feedback within plants capable of hosting two mycorrhizal groups. Finally, this work indicates that these compounds alone are a potentially powerful control over the succession between AMF and ECMF during ecosystem development.

Mechanisms of AMF inhibition by Populus leaf litter

The mechanisms of AMF inhibition by litter are becoming clearer. Suppression of AMF root colonization was not likely a result of altered soil pH. While a lowering of soil pH can affect inorganic P mobility and AMF abundance (reviewed in Entry et al., 2002), this does not appear to be the way litter reduced AMF. Soil pH was not significantly changed by addition of cottonwood foliage, litter extract to cottonwood seedlings, or pure phenolics. Soil pH did increase with 0.5-1X dilutions and this is likely a result of decomposition of the extract by saprophytic organisms as decarboxylation of organic

compounds can increase soil pH (Yan et al., 1996), but the observed change is within a range not expected to negatively affect AMF colonization (Olivera et al., 2005).

Furthermore, the change in soil pH seen in the *S. sudanese* leachate experiment (Table 2) remains within the domain of Ca-P controlled fixation and would not dramatically alter the solubility of inorganic P species (Stevenson and Cole, 1999).

Additionally, with the exception of plants receiving the 0.25X dilution of the litter extract, the additions of both litter extracts and specific soluble phenolics did not significantly alter plant growth of *S. sudanese*. Hence, reduced colonization is not a product of reduced plant productivity. The extract additions did however reduce cottonwood seedling shoot biomass (perhaps as an indirect consequence of reduced mycorrhizal activity). Nevertheless, the treatment reduced both growth and AMF colonization of seedlings despite a significant increase in soil phosphorus. Increases in soil phosphorus provided by the litter treatments did not increase the growth of any plants, thus all were likely co-limited by other nutrients. The increase in available soil phosphorus we observed with the litter treatments was not at a level expected to be inhibitory to AMF colonization. The highest measured soil phosphorus level at the end of all experiments was 13 mg kg^{-1} , much lower than what has proven inhibitory to AMF colonization (Graham et al., 1981; Blanke et al., 2005). Moreover, at the 0.5X extract dilution, the treatment with the lowest AMF colonization, phosphorus concentration (7.9 mg kg^{-1}) was not significantly higher than in the weaker dilutions.

Our results do show that soluble phenolics compounds known to occur in *Populus* foliage alone can reduce colonization by an entire AMF community (Figure 2). It is likely these compounds in cottonwood litter are responsible for the reduced colonization in our

experiments, and that inhibition of AMF could occur in ecosystems as a result of their presence. Total soil phenolics present after litter extract additions were within a biologically realistic range (Muscolo and Sidari, 2006). Total soil phenolics at the 31 year old site on the Nyack floodplain was 22 mg kg^{-1} (Jeff Piotrowski unpublished observation), a level equivalent to the soils receiving the 0.5X treatment (24 mg kg^{-1}). Secondly, total phenolic concentration of the litter extract was 497 mg kg^{-1} , lower but within a realistic range of phenolic concentrations of leachates in other systems (Castells et al., 2003; Suominen et al., 2003). Moreover, as we adjusted our extract to a pH of 7.0 to reduce pH effects, this could have reduced the phenolic toxicity as well by reducing concentrations of the dissociated forms of the phenolic compounds, leading to an underestimation of their biological effects on mycorrhizal fungi.

There are several mechanisms by which these leachates and soluble phenolics derived from cottonwood litter could reduce AMF colonization. Soluble phenolic compounds like ferulic acid are inhibitory to AMF hyphal elongation following spore germination (Wacker et al., 1990). As we used air dried soils, the inoculum was expected to be largely in the form of spores. While phenolics inhibit hyphal elongation following spore germination, it is still uncertain how these compounds would affect colonization by other inoculum sources like hyphal networks and colonized root fragments. If only spores are affected, accumulation of phenolics could result in a shift to a AMF community with fewer species dependent on spores for colonization (e.g. fewer Gigasporaceae).

Increased inputs of phenolic compounds and other labile carbon sources in litter leachates may also stimulate organisms antagonistic to AMF. Some of our observations support this mechanism. In our first experiment we noted sporadic non-AMF root

colonizing fungi in roots receiving the litter treatment but no AMF. In our second experiment, while there were no detectable non-AM root colonizing fungi, the increase in soil pH is likely the result of increased decomposer activity. When we added only pure phenolic compounds, AMF colonization was reduced whereas other root colonizing fungi found in the trap culture soils were not. Inhibition of colonization was much greater with the litter leachate than with the pure phenolics, suggesting a combined effect of phenolic toxicity and stimulation of antagonistic organisms. Even so, AMF do not produce any documented extracellular enzymes capable of detoxifying phenolic compounds, whereas other fungi, including ECMF, do (Münzberger et al., 2003; Zeng and Mallik, 2006). Thus, AMF may not only be more susceptible to phenolic toxicity, but may also be at a competitive disadvantage to fungi and bacteria that can detoxify these compounds or use them or other compounds from litter leachates as carbon sources.

Implications for mycorrhizal succession

Many floodplains, including the one studied here, are dominated by members of the Salicaceae, a plant family capable of simultaneously hosting both AMF and ECMF (Vozzo and Hacskaylo, 1974; Chilvers et al., 1987; Khasa et al., 2002). Mycorrhizal symbionts can exert a significant carbon drain on their host. So far, no biochemical mechanism has been identified by which members of the Salicaceae can “select” which symbiont it associates with, but the displacement of AMF by ECMF in these roots is considered to be a product of the physical exclusion of AMF colonization when the ECMF mantel forms (Last et al., 1983; Santos et al., 2001). Our data suggest that

phenolics present in leaf litter could be inhibitory to AMF colonizing roots but not other fungi capable of detoxifying them, providing another potential mechanism by which a host can control its own symbiont community.

From a successional standpoint, whereas some ECMF can degrade phenolics and AMF cannot, increasing soil phenolic concentrations over time and host shifts in mycorrhizal associates could contribute to the frequently observed shift from AMF dominated soils to ECMF soils. Further field based studies are necessary to determine if these compounds are affecting AMF to ECMF succession *in situ*. Additionally, isolation, quantification, and assessment of the soluble phenolics of black cottonwoods will be necessary to determine if they alone inhibit AMF. Yet, these data strongly suggest a potentially powerful biochemical mechanism contributing to successional shifts of mycorrhizal groups.

Conclusions

Together, these results suggest that soluble phenolic compounds from litter are a significant and largely unexplored control on AMF abundance and potentially community composition in natural ecosystems. The mechanisms of inhibition may be a product of phenolic toxicity to AMF, stimulation of antagonistic fungi that compete for space and nutrients, or a combination of both where AMF are suppressed and other fungi capable of phenolic detoxification are able to proliferate. Nevertheless, soluble phenolic in ecologically realistic concentrations in both soil and litter leachates could be a driving force in succession of AMF to ECMF in both roots and soils.

Acknowledgements

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Tables

Table 1. Comparison of AMF colonization of *Sorghum* seedlings, soil pH, and soil phosphorus and following treatment with either 5% addition of ground cottonwood leaves or ground vermiculite (mean± standard error).

Treatment	% AMF colonization^b	Soil pH^a	Soil phosphorus (mg kg⁻¹)^b
Cottonwood litter	0.0 ± 0.0	7.64 ± 0.06	13.2 ± 0.8
Vermiculite	37.5 ± 7.5	7.80 ± 0.09	2.0 ± 1.1
	Z=2.99** N= 14	T=-1.55 N=14	Z=2.61* N= 8

* $P < 0.05$, ** $P < 0.001$

^a Comparisons were made using Student's two sample T-test.

^b Comparisons were made using the Mann-Whitney U test.

Table 2. Comparison of *S. sudanese* seedlings and soil parameters across the dilution of cottonwood extract (mean± standard error). Lettering indicates significant difference between treatments as determined by Tukey's multiple comparison test.

Leachate Dilution	Shoot biomass (g)	Root biomass (g)	Soil pH^a	Soil Phosphorus (mg kg⁻¹)^c	Total soil phenolics (mg kg⁻¹)^b
Control	0.06 ± 0.01 a	0.08 ± 0.02	7.65 ± 0.01 ab	2.8 ± 1.0	10.4 ± 1.6 ab
0.00001	0.07 ± 0.01 ab	0.08 ± 0.01	7.61 ± 0.01 ab	3.8 ± 2.0	6.3 ± 0.9 a
0.0001	0.09 ± 0.01 ab	0.07 ± 0.01	7.61 ± 0.03 ab	2.1 ± 1.2	12.2 ± 1.8 ab
0.001	0.07 ± 0.01 ab	0.07 ± 0.02	7.58 ± 0.01 a	1.2 ± 0.5	11.2 ± 2.1 ab
0.01	0.06 ± 0.01 ab	0.08 ± 0.02	7.62 ± 0.01 ab	2.4 ± 1.2	8.2 ± 1.2 ab
0.1	0.07 ± 0.01 ab	0.11 ± 0.01	7.59 ± 0.01 a	2.6 ± 0.6	8.6 ± 1.0 ab
0.25	0.05 ± 0.01 b	0.07 ± 0.01	7.76 ± 0.02 ab	4.8 ± 1.3	15.4 ± 1.3 bc
0.5	0.07 ± 0.01 ab	0.08 ± 0.01	8.04 ± 0.02 b	7.9 ± 2.8	24.2 ± 2.0 c
1 (full strength)	0.10 ± 0.01 a	0.10 ± 0.05	8.04 ± 0.05 b	13.0 ± 3.4	59.5 ± 7.4 d
	F=2.79* N= 45	F=1.6 N= 45	H=29.83*** N= 36	F=2.7* N= 36	F=21.91*** N= 36

* $P < 0.05$, *** $P < 0.0001$.

^a Comparisons were made using the Kruskal-Wallis test with the Z-test for multiple comparisons.

^b Indicates these data were log transformed prior to ANOVA.

^c Indicates Tukey's test did not resolve differences despite significance of the ANOVA.

Table 3. Spearman's Rank correlation matrix of *S. sudanese* AMF colonization, litter extract concentration and soil variables.

Variable	% AMF col.	Extract conc.	Available soil P	Total soil phenolics	Shoot biomass	Root biomass	Final soil pH
% AMF col.	1.00						
Extract conc.	-0.95*	1.00					
Available soil P	-0.60	0.58	1.00				
Total soil phenolics	-0.63	0.68*	0.52	1.00			
Shoot biomass	-0.16	0.21	0.13	0.34	1.00		
Root biomass	-0.25	0.30	0.42	-0.13	0.25	1.00	
Final soil pH	-0.48	0.55	0.84*	0.63	-0.06	0.18	1.00

* $P < 0.05$.

Table 4. Comparison of *P. trichocarpa* shoot biomass, soil pH, and soil phosphorus, and total soil phenolics following treatment with either 1X cottonwood litter extract or water (mean \pm standard error).

Cottonwood Extract treatment	% AMF colonization^b	Shoot biomass (g)^b	Soil pH^b	Soil Phosphorus (mg kg⁻¹)^a	Total phenolics (mg kg⁻¹)^b
Control	5.8 \pm 1.3	0.05 \pm 0.01	7.80 \pm 0.03	2.7 \pm 0.8	11.0 \pm 0.5
Full strength	37.6 \pm 7.3	0.02 \pm 0.01	7.88 \pm 0.01	8.9 \pm 0.9	36.0 \pm 2.6
	Z=-2.74** N=12	Z=3.13** N= 12	Z=1.6 N= 10	T= -5.08** N= 10	Z=2.89* N= 10

* $P < 0.05$, ** $P < 0.001$.

^a Comparisons were made using Student's two sample T-test.

^b Comparisons were made using the Mann-Whitney *U* test.

Table 5. Comparison of *S. sudanese* and soil parameters when treated with specific phenolic compounds of deionized water (mean± standard error).

Treatment	Shoot biomass (g)^c	Root biomass (g)^c	Soil pH^a	Soil P (mg kg⁻¹)
Ferulic Acid	0.019 ± 0.004	0.034 ± 0.006	7.61 ± 0.01	2.7 ± 0.6
Caffeic Acid	0.017 ± 0.002	0.029 ± 0.004	7.58 ± 0.02	2.1 ± 0.2
Vanillic Acid	0.018 ± 0.004	0.038 ± 0.008	7.63 ± 0.01	1.9 ± 0.9
Coumarin	0.014 ± 0.001	0.023 ± 0.002	7.61 ± 0.01	2.1 ± 0.6
Water	0.018 ± 0.002	0.026 ± 0.003	7.61 ± 0.01	2.7 ± 0.2
	F=0.60	F=1.18	H=6.6	F=0.43
	N= 40	N= 40	N= 25	N= 25

^a Comparisons were made using the Kruskal-Wallis test.

^b Indicates these data were log transformed prior to ANOVA

Figures

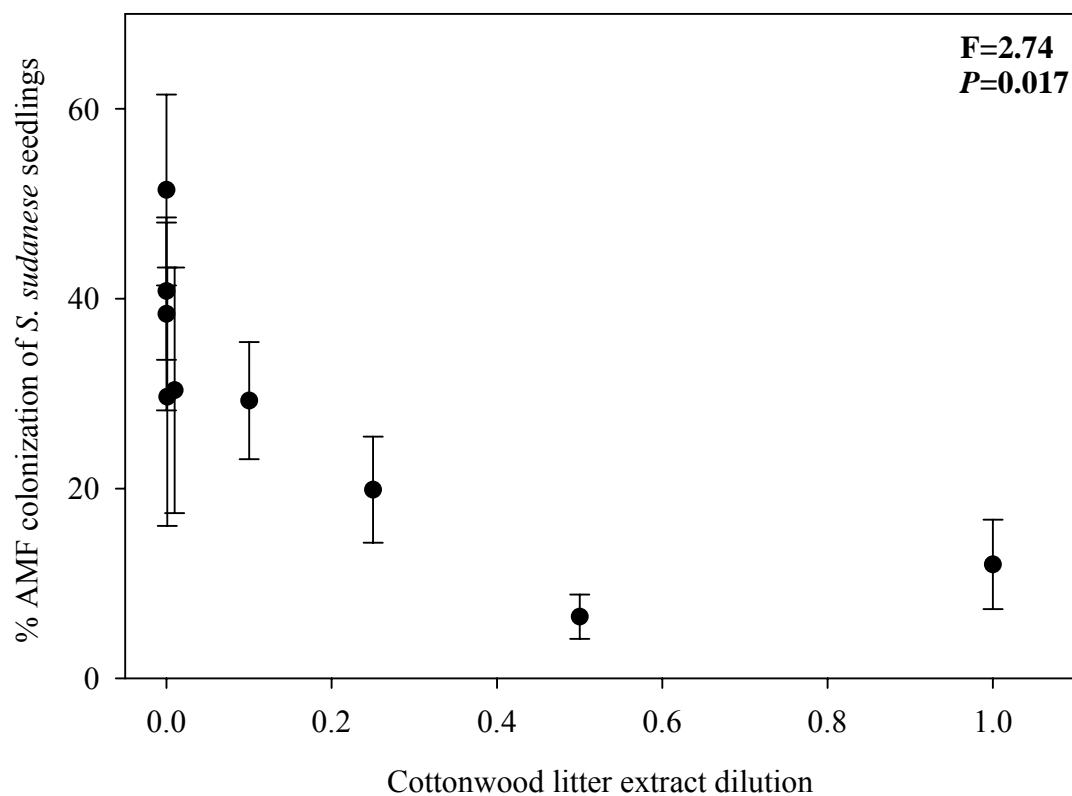


Figure 1. Percent AMF colonization of *S. sudanese* when treated across a range of cottonwood litter extract dilutions (mean \pm standard error). ANOVA F statistic and p-value are presented. Multiple comparison using Tukey's HSD test indicated AMF colonization of seedlings receiving 1X and 0.5X dilutions of the litter extract was significantly lower than colonization of the 0X treatment ($n=45$).

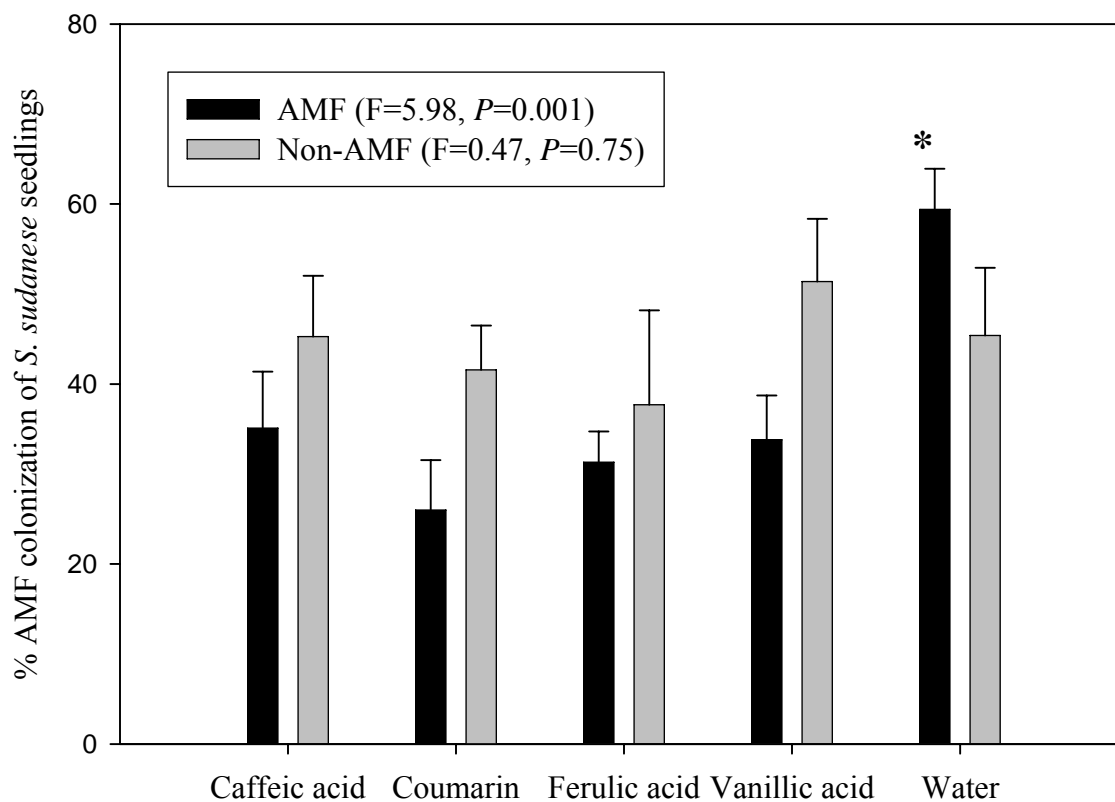


Figure 2. Percent root colonization of *S. sudanese* by AMF and non-AMF fungi when treated with either specific phenolic compounds or water (mean \pm standard error). Black bars indicate AMF colonization of roots and white bars indicate other root colonizing non-AM fungi. ANOVA F statistic and p-value are presented. “*” indicated the water treatment had significantly greater AMF colonization compared to the phenolic treatments as determined by Tukey’s HSD test. There was no significant difference in colonization by non-AM fungi ($n=40$).

Chapter 4

The effects of arbuscular mycorrhizae on soil aggregation depend on the interaction between plant and fungal species.

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Abstract

Arbuscular mycorrhizal fungi (AMF) and roots mediate soil stabilization, although the mechanisms and how their interactions affect soil stabilization are not known. We tested the effects of specific plant/ fungus combinations on aggregate stabilization, and whether hyphal length and root biomass determine stabilization, predicting that fungi with longer hyphae and plants with higher root biomasses would better stabilize soils. The percentage of water stable aggregates (%WSA_{1-2mm}), hyphal lengths, and root biomass were measured from a 5 AMF x 9 plant factorial experiment. AMF with longer hyphae were represented by the Gigasporaceae and plants of high root biomass by grasses. Other taxa represented lower hyphal lengths and root biomass. An interaction between symbionts with respect to %WSA_{1-2mm} was observed ($p < .0001$). Root biomass and total hyphal lengths were not positively correlated with %WSA. Combinations of grasses with Gigasporaceae fungi had the lowest %WSA. Mechanisms underlying aggregation were not captured by measuring root biomass and total hyphal lengths alone; suggesting other physiological or architectural mechanisms may be responsible.

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Introduction

Arbuscular mycorrhizal fungi (AMF) are functional components of terrestrial ecosystems worldwide. These fungi in the phylum Glomeromycota (formerly Zygomycota, Schüssler et al., 2001) form symbiotic relationships with the majority of land plants. Among the benefits AMF confer to their plant hosts are enhanced mineral nutrition (Smith & Read, 1997) and greater root-pathogen resistance (Newsham et al., 1995); in exchange, the obligate biotrophic fungi receive carbon. These effects at the scale of the individual plant can influence processes at the scale of the ecosystem, such as ecosystem engineering through their ability to aggregate soils.

Soil aggregation is a complex, hierarchical process mediated by both biotic and abiotic factors (Tisdall & Oades, 1982). Aggregation is essential to maintaining soil porosity, allowing gas exchange, water infiltration, and facilitating biogeochemical cycling (Diaz-Zorita *et al.*, 2002). Additionally, soil structure is crucial to the success of sustainable agriculture and erosion resistance. Over one third of the world's arable land was lost to erosion over the last 40 years (Pimentel et al., 1995), and much of the focus of sustainable agriculture has shifted towards managing for well-aggregated soils.

Hyphae of AMF are considered to be the primary soil aggregators for several reasons: the extraradical hyphae of AMF have a significant biomass in most soils (Rillig & Allen 1999). As obligate biotrophs these fungi do not need to compete with saprobes for soil carbon, and AMF hyphae are more resistant to fungivory than saprobic fungi (Klironomos & Kendrick, 1996). AMF may stabilize soils up to five months after their host's death (Tisdall & Oades, 1980). A positive correlation between AMF hyphae and

aggregate stabilization in natural systems is described by Miller & Jastrow (1990) and Jastrow et al. (1998). Rillig et al. (2002) described significant indirect effects of AMF hyphal length on WSA stabilization via the production of glomalin-related soil protein (GRSP) in a natural grassland system. AMF showed similar results on the five plant hosts used, but like the other grassland studies no AMF species involved were described (Rillig et al., 2002).

Little is known about the effects of different AMF taxa on aggregate stabilization. Schreiner et al. (1997) tested the WSA forming ability of three AMF species on soybean (*Glycine max*). The authors found *Glomus mosseae* stabilized aggregates in the 2-4mm size class significantly more than *G. etunicatum* and *Gigaspora rosea*, but there were no differences between species in the 1-2mm or 0.25- 1mm size classes. In natural grasslands, Miller & Jastrow (1992) correlated spore densities of *Gi. gigantea* with %WSA, but not densities of *G. etunicatum*.

Plants with dense, fibrous root systems (such as grasses) assist aggregate formation (Amézketa 1999, Oades 1993). Similarly, hyphal characteristics may contribute to aggregation ability. AMF with dense hyphal clusters may hold soil particles together better than diffuse hyphae. These mechanisms of aggregation may be species dependent like other AMF characters, and this has given rise to the hypothesis that AMF species particularly adept at soil aggregation exist. The idea of an aggregation “specialist” is attractive to agribusiness as well as to applications in ecosystem restoration. If a species of AMF promoted WSA stabilization independent of plant host or soil type, it could be used to inoculate crops or other soils with poor water aggregate stability.

Evidence exists that many AMF species, despite their broad host range, are host specific with regards to beneficial effects for the plant. AMF effects can run the gamut from mutualist to parasite depending on its host plant (Klironomos, 2003). In this study the effects of a single AMF on many hosts ranged from greatly stimulating shoot biomass to drastically reducing it. These types of interactions may also manifest in differential amounts of soil aggregation. If the effects of an AMF species vary widely from host to host, then the search for an applicable aggregation “specialist” may be complicated. If a certain AMF interacts with specific hosts to strongly promote aggregation, AMF-host combinations could be customized to yield the highest aggregate formation.

Overall, little is known about the effects of species of AMF on soil aggregation in natural ecosystems or agroecosystems. We know of no study in which the ability to promote stable soil aggregation has been compared among several hosts and fungal species combinations. This is the focus of the present study. Importantly, many studies have used biological material that was not derived from the same soil and ecosystem. The existence of intraspecific variation in AMF with respect to their ecosystem origin casts doubts on the degree of ecological realism of such studies (Klironomos, 2003). This experiment takes advantage of native inhabitants of a long-term mycorrhizal research site to study possible effects of plant/fungi interactions on WSA formation.

This experiment is one of the first designed to test for interactions between AMF and plant host from a natural community with regards to soil aggregate stabilization. To help explain the mechanism of aggregate stabilization we measured root biomass and hyphal lengths. As these variables are thought to be major determinants of stabilization,

correlation between these and aggregate percentage could predict which fungi/ plant combinations are most suited to form water stable aggregates.

We tested the following hypotheses:

- 1) Fungi of the family Gigasporaceae will be better soil aggregators independent of their plant host compared to non- Gigasporaceae members, as this family produces greater hyphal lengths and denser hyphal clusters (Hart & Reader 2002).
- 2) Plants in the Poaceae (grasses) will be better soil aggregators independent of their AMF partner, as grasses have more fibrous root biomass to ensnare soil particles (Amézketa 1999).
- 3) There will be three levels of aggregation based on the above assumptions. The combinations of grasses with Gigasporaceae fungi will yield the most water stable aggregates, followed by the combinations of Gigasporaceae fungi and non-grasses and Grasses and non-Gigasporaceae fungi, finally the lowest percent of water stable aggregates will be found in pots with the combination of non-grasses and non-Gigasporaceae fungi.

Material and Methods

Experimental design and Materials

The plant and AMF species used in this experiment are listed in Figure 1. All organisms were collected from the Long-Term Mycorrhiza Research Site (LTMRs) at the University of Guelph, Ontario, Canada (43°32'30"N, 80°13'00"W). Plant seeds were collected from May-September 2000 and stored under dry conditions at 4°C prior to the experiment. The five AMF species were isolated from the soil by first allowing them to

sporulate in trap cultures containing *Allium porrum* L. cv. Giant Musselburgh, and then using single AMF spores to start single species cultures (Brundrett, 1996). For a period of approximately four years prior to setting up the experiments described below, all the AMF were grown in dual pot culture with the host *Allium porrum* under similar greenhouse conditions. During that time, AMF were subcultured at three month intervals to keep the cultures clean and viable.

This experiment was set up using a (5 AMF x 9 plants) factorial design. The AMF factor consisted of one of five AMF species (Table 1). The plant factor consisted of one of nine plant species (Table 1). Each treatment combination consisted of ten replicated units, for a grand total of 450 experimental units. Each unit was positioned in a completely randomized design on benches in a greenhouse. The experiment ran from May 2001 – April 2002 under ambient light conditions, 22.1: 16.7°C average day:night temperatures, and 48.5 : 74.2 % average day:night relative humidity.

Each experimental unit consisted of a single pot (15cm D x 60cm L) containing sterile field soil, AMF inoculum, and an individual plant. The sandy-loam soil was collected from the LTMRS (total N = 80.5 mmol kg⁻¹; total P = 6.9 mmol kg⁻¹; percentage organic matter = 5.7). It was autoclaved and then added to individual pots. At a depth of 2 cm below the surface of the soil, we added a band of AMF inoculum with a mass of approximately 1g. This inoculum was composed of sheared *Allium porrum* roots (pre-colonized by one of the AMF isolates) and approximately 100 spores. To correct for possible differences in microbial communities, each experimental unit received a 50-mL filtered washing comprised of microbial extract from every AMF isolate used (Koide & Li, 1989). Plant seeds were germinated in a growth chamber at 20°C on moist filter

paper. Individual seedlings were then transferred to the pots. We initially added two seedlings, but after 1 week we removed one plant. The remaining plant in each pot was left to grow for a period of one year. In some experimental units, the second plant died during the course of the experiment, so these units were removed from the final analysis. Removal of these dead plants from this analysis is valid because the treatments (plant x fungus combo) themselves did not cause the plants to die. All plants were watered every 2 days or as needed with deionized water. They were also fertilized once per week with a modified Long-Ashton Nutrient solution (half strength P; Hewitt, 1966).

Plant and fungal measurements

At the end of the experiment, plant shoots and roots were harvested. Plant material was then dried at 60°C for 48 hours and then weighed to determine biomass. Only root biomass is reported here. Prior to drying, a subsample of roots was taken from each pot and stored in 50% ethanol. This subsample of roots was then cleared in 10% potassium hydroxide, and stained with Chlorazol Black E (Brundrett, et al., 1984) to confirm the presence of AMF structures. In all experimental units, plants were colonized by AMF (data not presented). Extraradical hyphal lengths were estimated by extracting hyphae from two 5-g portions of soil (Miller et al., 1995) and measuring lengths by a gridline-intersect method. Hyphal length (m g^{-1} dry soil) was calculated as in Newman (1966). The hyphae of non-mycorrhizal fungi were distinguished from those of arbuscular mycorrhizal fungi by careful observation of characters normally missing in the latter (melanization, clamp connections or regularly septate hyphae).

Percentage of water stable aggregates (%WSA_{1-2mm}) measurement

All soils had been stored as air-dried samples >4 months. We concentrated on macro-aggregates of 1-2 mm diameter, since the amounts of these aggregates are sensitive to short term (< 2 yr) management and treatment of soils (Kemper & Rosenau 1986). Replicate samples of soil aggregates were moistened by capillary action for 10 min. Water-stability of aggregates was then measured with a wet-sieving method using the apparatus and procedure described in Kemper & Rosenau (1986). Percentage of water-stable aggregates (%WSA) is calculated using the mass of aggregated soil remaining after wet sieving (5 min) and the total mass of aggregates at the beginning. The initial and final weights of aggregates were corrected for the weight of coarse particles (> 0.25 mm) included in the soil samples.

Statistical analysis

All analyses were conducted with SPSS version 10.0 (SPSS, Chicago, IL) or Number Cruncher Statistical Software (NCSS, Kaysville, UT). Univariate analyses of variance (ANOVA) were used to examine the effect of the two factors plant species and fungus type on soil aggregates (WSA), root biomass, and hyphal length. Analysis was undertaken separately for these three responses as there was no significant correlation between them. Three *Bromus inermis* treatments had unusually high root biomass and were pot bound. Due to these potentially confounding pot size effects, models were run with and without the three *Bromus inermis* plant treatments grown with the fungi *G. intraradices*, *G. etunicatum*, and *A. denticulata* (BI3). In this way, a total of six ANOVAs were performed as summarized below. Tukey-Kramer HSD or Kruskal-Wallis Z-test was used for post-hoc comparisons, where applicable.

Additionally, for each of the three responses, the three sets of contrasts outlined earlier on the plant/fungus treatment combinations were estimated. These contrasts were chosen to represent the following comparisons:

1. Grasses vs. Non-grass plant types
2. Gigasporaceae vs. Non-Gigasporaceae fungus types
3. An ordering of treatments as:

$$(\text{Grass-Giga}) > (\text{NonGrass-Giga}, \text{Grass-NonGiga}) > (\text{NonGrass-NonGiga})$$

This third contrast was examined in two parts, by first comparing the (Grass-Giga) treatments to the middle treatments, and then the middle treatments to (NonGrass-NonGiga).

Univariate ANOVAs of WSA, root biomass, and hyphal lengths on the factors plant species and fungus type both with and without the *Bromus inermis* (BI3) treatments were performed. To correct the variance heterogeneity in WSA percentages and root biomass, the log-transformed variables $\log(100\text{-WSA})$ and $\log(\text{root biomass})$ respectively were used in all analyses, and a square root transformation was used for hyphal lengths. Root biomass and hyphal length were initially considered as covariates in the WSA ANOVA but added nothing significant to the model ($p=0.819, 0.768$ respectively).

Results

Soil aggregate water stability (%WSA_{1-2mm})

Factor effects and contrast effects are summarized in the first two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on WSA ($p<0.0001$), and between plants ($p<0.0001$), but no significant differences among fungus species ($p=0.253$) whether the interaction is included in the

model or not. There are significant differences in soil aggregation between grasses and non-grasses ($p=0.018$) where the median percentage of water unstable aggregates is 1.12 times higher for grasses than non-grasses (95% CI: 1.02 – 1.23). The primary contribution to this difference occurs within the fungal type *A. denticulata* (type 3) where the median percentage of water-unstable aggregates is 1.38 times higher for grasses than non-grasses (95% CI: 1.10 – 1.73). There are mild differences in soil aggregation between the families Gigasporaceae and non-Gigasporaceae ($p=0.039$) where the median percentage of water unstable aggregates is 1.09 times higher for Gigasporaceae than non-Gigasporaceae (95% CI: 1.00 – 1.17). The contrasts identifying the proposed ordering of treatments indicate that the ordering (Grass-Giga) > (NonGrass-Giga, Grass-NonGiga) > (NonGrass-NonGiga) is not present in WSA ($p=0.967$, $p=0.091$ respectively). In fact, as indicated in Table 3, mean WSA is highest for the middle group and lowest for the Grass-Gigasporaceae treatments.

All fungal species except *S. calospora* had significant differences in %WSA_{1-2mm} across the plant hosts. *G. etunicatum* had the lowest mean %WSA_{1-2mm} when grown with *Plantago*, *Daucus*, *Crysanthemum*, and *Rudbeckia*; but, had the highest with *Fragaria* (Figure 1). In contrast, *Gi. gigantea* had the highest mean % WSA_{1-2mm} when associated with *Plantain*, *Daucus*, *Crysanthemum*, and *Rudbeckia*, and had the lowest with *Fragaria*. Both species of *Glomus* had lowest mean %WSA_{1-2mm} with *Plantago*, and the Gigasporaceae species had the highest mean % WSA_{1-2mm} with *Daucus*. The species of *Plantago*, *Bromus*, *Daucus*, *Fragaria*, and *Rudbeckia* had significant differences in %WSA_{1-2mm} depending on the AMF associate; the others did not.

With the three *Bromus inermis* treatments (B13) removed, the plants factor and plant by fungus interaction remain highly significant ($p < 0.0001$) with no differences among fungus types ($p = 0.257$), as with the full data. However, removal of these treatments resulted in no differences in WSA between grasses and non-grasses ($p = 0.926$). This lack of significance stems from the relatively small WSA values for the *Bromus inermis* treatments. Significant differences between Gigasporaceae and non-Gigasporaceae treatments remain with the median percentage of water unstable aggregates 1.10 times higher for Gigasporaceae (95% CI: 1.01 – 1.19). Finally, based on the p-values in Table 3, there is no evidence of the proposed ordering, with the smallest WSA values again residing in the (Grass-Giga) treatments (Table 4).

Root biomass

The variability in root biomasses across treatments is presented in Figure 2. Factor effects and contrast effects are summarized in the middle two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on root biomass ($p = 0.001$), between plants ($p < 0.0001$), and among fungus species ($p < 0.0001$). There are significant differences in root biomass between grasses and non-grasses ($p < 0.0001$) where the median root biomass is 1.29 times higher for grasses than non-grasses (95% CI: 1.19 – 1.40). There are significant differences in root biomass between fungi of types Gigasporaceae and non-Gigasporaceae ($p < 0.0001$) where the median root biomass is 1.26 times lower for Gigasporaceae than non-Gigasporaceae (95% CI: 1.18 – 1.35). The main contributors to this difference were *P. lanceolata* and *B. inermis*.

With the three *Bromus inermis* treatments removed, the plant and fungus factors ($p < 0.0001$) as well as the plant by fungus interaction ($p = 0.002$) remain highly significant, as with the full data. However, removal of these treatments completely reversed the direction of the difference in root biomass between grasses and non-grasses. With these treatments removed, the median root biomass is now 1.20 times lower for grasses than non-grasses (95% CI: 1.07 – 1.34, $p = 0.003$). This reversal of effect direction is due to the very large biomass values for the *Bromus inermis* treatments. Significant differences between Gigasporaceae and non-Gigasporaceae treatments remain with the median root biomass 1.23 times lower for Gigasporaceae (95% CI: 1.15 – 1.32). Finally, based on the p-values in Table 3, there is again no evidence of the proposed ordering, with the smallest root biomass values residing in the middle treatment group (Table 4).

Hyphal lengths

S. calospora and *Gi. gigantea* had the highest mean hyphal lengths on seven of the nine plants tested (Figure 3). Factor effects and contrast effects are summarized in the middle two columns of Tables 2 and 3. There are significant interactions between plant and fungus species in their effects on root biomass ($p = 0.0004$), between plants ($p = 0.004$), and among fungus species ($p < 0.0001$). There is no difference in hyphal lengths between grasses and non-grasses ($p = 0.775$); however, there is a significant difference between fungi of types Gigasporaceae and non-Gigasporaceae ($p < 0.0001$) with the mean square root hyphal length (m/g dry soil) of Gigasporaceae being 0.688 larger than that for non-Gigaspora treatments (95% CI: .580 - .795). This difference in hyphal lengths for the two groups was highly significant within all but the *A. novae-angliae*. The contrasts identifying the proposed ordering of treatments indicate that the ordering (Grass-Giga) >

(NonGrass-Giga, Grass-NonGiga) > (NonGrass-NonGiga) is present in the hyphal lengths ($p=.0003$, $p<.0001$ respectively). This ordering can be seen through comparison of the mean hyphal lengths in column 5 of Table 2.

With the three *Bromus inermis* treatments removed, the plants and fungus factors ($p=0.004$, $p<0.0001$) as well as the plant by fungus interaction ($p=0.0004$) remain highly significant, as with the full data. There is still no difference in hyphal lengths between grasses and non-grasses ($p=0.498$), and the mean square root hyphal length (m/g dry soil) remains significantly larger (0.651) for Gigaspora treatments than for non-Gigaspora treatments (95% CI: .535 - .768). Finally, based on the p-values in column 6 of Table 2, although the middle group of treatments (Grass/Non-Gig, Non-Grass/Gig) has a significantly higher square root mean hyphal length than the (Non-Grass/Non-Gig) group ($p<0.0001$), the (Grass/Gig) treatment group is no longer significantly larger in terms of hyphal length than the middle group. This loss of ordering is due to the small hyphal lengths in the (Grass/Non-Gig) removed treatments (see columns 5 & 6 of Table 3).

Discussion

Other studies have demonstrated an interaction between AMF and host plant species with respect to host and/ or fungal growth and hence net primary productivity (Adjoud et al., 1996; Bever et al., 1996; Eom et al., 2000; Klironomos, 2003). Here we significantly extend these findings by showing that AMF/ host species combinations also differentially control the percentage of water stable soil aggregates, and thus another major ecosystem state variable (*i.e.* soil structure). Previous studies established that %WSA varied between fungi associated with a single host (Schreiner et al., 1997); but ours is the first study to use multiple co-occurring plant and fungal species combinations

from a single, natural ecosystem. Additionally, the soil used in our study was from the same field site from which the AMF isolates and plants were obtained, maximizing ecological relevance of this greenhouse experiment. Klironomos (2003) showed that exotic AMF species have far different effects on their plant host than co-occurring species, and vice versa. Previous pot experiments on aggregation have used soils or fungi that are exotic to the symbionts, adding further complications (Bearden & Petersen, 2000; Andrade et al. 1998, Schreiner et al., 1997).

This greenhouse study stands in contrast to other field studies showing positive correlation between %WSA and hyphal lengths/ root biomass (Jastrow et al., 1998; Rillig et al., 2002). Negative correlations have been observed however (Schreiner et al. 1997). A possible explanation for the decrease in %WSA_{1-2mm} with grasses may be a result of our experiment's duration and the extremely high root biomass of *B. inermis*. The plants were grown in pots for a year and some *B. inermis* were pot bound at harvest time. Such a high density of roots could have inhibited aggregate formation. The differences between the %WSA of grasses and non-grasses disappeared when the *Bromus* treatment combinations yielding extremely high root biomass were removed from the analysis. While root biomass is positively correlated with %WSA in field studies, greenhouse pot experiments must consider the deleterious effects of high root densities on %WSA formation.

In our study the AMF family with greater overall hyphal lengths (Gigasporaceae) produced significantly lower %WSA in contrast to our initial hypothesis. While members of the Gigasporaceae generally have more abundant and denser hyphal growth (Hart & Reader, 2002), the species used in our study yielded lower percentages of WSA than

members of the Glomaceae and Acaulosporaceae. Although *S. calospora* (Gigasporaceae) hyphal lengths were greater, Jakobsen et al. (1992) states they do not spread as far from the root as *A. lavis* (Acaulosporaceae), which could explain this difference. This study considered only one host (*Trifolium subterraneum*) however, and a potential interaction between *S. calospora* and *T. subterraneum* may have compromised *S. calospora*'s aggregation ability. Nevertheless, soil aggregate formation may depend more on hyphal spread from the host root rather than hyphal length alone. Hyphae that forage farther from the host root could form more %WSAs because a higher proportion of runner hyphal (Friese & Allen 1991) could “string” together more soil particles.

Our hypothesis that combinations of grasses and Gigasporaceae fungi would stabilize more aggregates than non-grasses and non-Gigasporaceae combinations was not supported. Actually, the grass-Gigaspora combination had the lowest mean %WSA. This again suggests that other mechanisms mediated by the symbiont's interaction may dictate WSA stabilization rather than root biomass and total hyphal length. Given that these obvious mechanisms may not function as strongly as first thought in WSA stabilization, we must consider that other aspects of extraradical hyphae and root development could be determined by host interaction and affect %WSA_{1-2mm}.

AMF hyphae, like plant roots, can vary widely in their branching patterns. More highly branched hyphae or roots may be more effective in binding soil particles. Moreover, AMF species can differentially affect root branching (Norman et al., 1996). Future studies should consider measurement of both root and hyphal branching.

Glomalin related soil protein (GRSP) is strongly positively correlated with %WSA_{1-2mm} (Rillig in press; Rillig et al., 2001; Wright & Anderson, 2000; Wright & Upadhyaya,

1998. Production of GRSP per fungal mycelium biomass may vary as a function of AMF species (Wright et al., 1996), although the AMF species used for that study did not come from the same ecosystem. However, the same pattern appears to hold up for AMF from the same ecosystem (Rosier and Rillig, unpublished.). Certain hosts might differentially stimulate GRSP production in their AMF symbionts, resulting in increased WSA formation. In this experiment we could not test for this mechanism because background levels of GRSP in the soils used were high and fluxes of GRSP are generally small (Rillig et al., unpublished observation). Further, beyond a level of WSA of ca. 80% (using the WSA measurement technique we used here), the relationship between glomalin concentration and water stability plateaus (Wright & Upadhyaya, 1998).

No evidence of an AMF aggregation “specialist” was apparent in this study; even so, species less affected by their host, which simultaneously provide overall high WSA (i.e. *S. calospora*) may be better candidates for applications in restoration. These could confer the benefit of higher WSA stabilization to a broad range of hosts in the field. While we do not suggest that field inoculation should only be carried out with one fungal species, our data lend support to the idea of using a cocktail of AMF species, a component of which could be an AMF isolate that is specifically included for promoting soil stabilization.

Our data also show that specific fungal host species combinations can maximize soil aggregate water stability (e.g., in our case *Daucus*/ *Gi. gigantea* and *Fragaria*/ *G. etunicatum*). In situations where a specific host plant is the target, such as in production agroecosystems or in certain restoration and revegetation applications, our data strongly suggest that AMF inoculum could be specifically tailored to maximize aggregate

formation. Alternatively, in restoration situations where the host plant is a variable, it is clear that host plant choice can co-determine soil stabilization together with AMF inoculum identity. We conclude soil aggregation is a function of both the fungi and its host, and exists as a spectrum from a poor interaction to strongly positive much like other AMF- host exchanges, and management of soils for aggregate stability must consider this.

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Tables

Table 1. Species and families of AM fungi and plants used in this study.

Plant species	Family
<i>Plantago lanceolata</i>	Plantaceae
<i>Bromus inermis</i>	Poaceae
<i>Daucus carota</i>	Apiaceae
<i>Chrysanthemum leucanthemum</i>	Asteraceae
<i>Aster novae-angliae</i>	Asteraceae
<i>Fragaria virginiana</i>	Rosaceae
<i>Rudbeckia hirta</i>	Asteraceae
<i>Solidago canadensis</i>	Asteraceae
<i>Agrostis gigantea</i>	Poaceae
Fungus species	
<i>Glomus intraradices</i>	Glomaceae
<i>Glomus etunicatum</i>	Glomaceae
<i>Acaulospora denticulata</i>	Acaulosporaceae
<i>Scutellospora calospora</i>	Gigasporaceae
<i>Gigaspora gigantea</i>	Gigasporaceae

Table 2: Main Effect p-values

Main Effects	Models					
	WSA (all data)	WSA (no BI3)	Root Biomass (all data)	Root Biomass (no BI3)	Hyphal Length (all data)	Hyphal Length (no BI3)
Plant Type	< .0001	< .0001	< .0001	< .0001	.011	.004
Fungus Type	.253	.257	< .0001	< .0001	< .0001	< .0001
Plant x Fungus	< .0001	< .0001	.001	.002	< .0001	.0004

Table 3: Contrast Effects Summary

Contrast	Summary	Models					
		WSA (all data)	WSA (no BI3)	Root Biomass (all data)	Root Biomass (no BI3)	Hyphal Length (all data)	Hyphal Length (no BI3)
Grass	Estimate ¹	.115	.007	.258	-.180	.019	-.066
vs.	SE ¹	.048	.071	.040	.059	.066	.097
Non-grass	p-value	.018	.926	<.0001	.003	.775	.498
Gigaspora	Estimate	.083	.092	.234	.210	-.688	.651
vs.	SE	.040	.043	.033	.036	.055	.059
Non-Giga.	p-value	.039	.034	<.0001	<.0001	<.0001	<.0001
GG >(GN,NG)	p-value ²	.967	.755	.212	.979	.0003	.269
(GN,NG) > NN	p-value ²	.091	.015	.958	>.9999	<.0001	<.0001

¹ Estimates and standard errors (SE) are on a log scale for 100-WSA and root biomass and a square root scale for hyphal length.

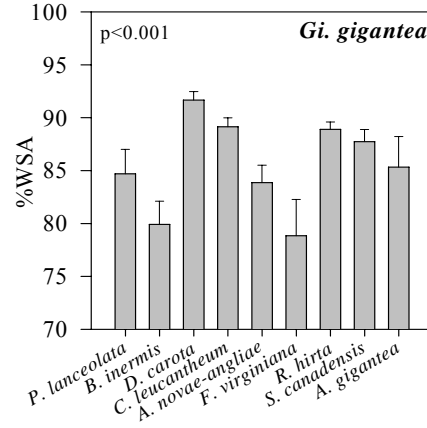
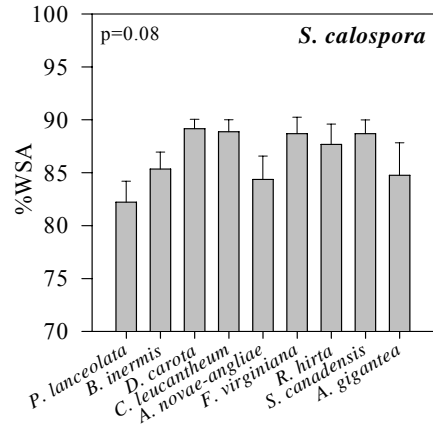
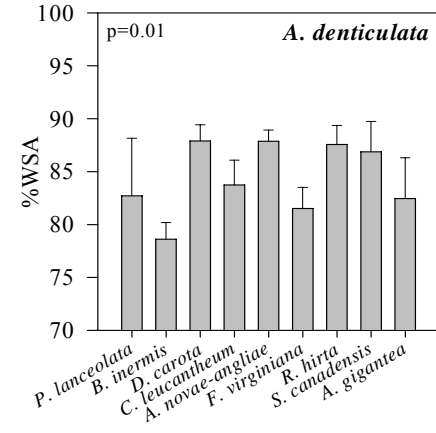
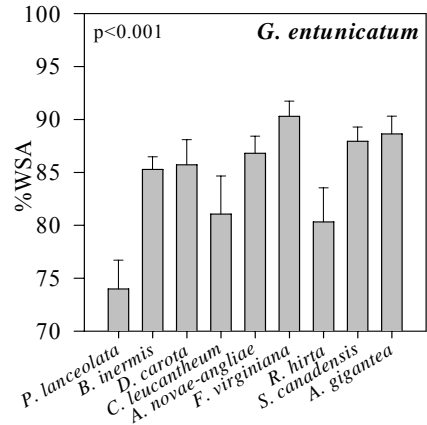
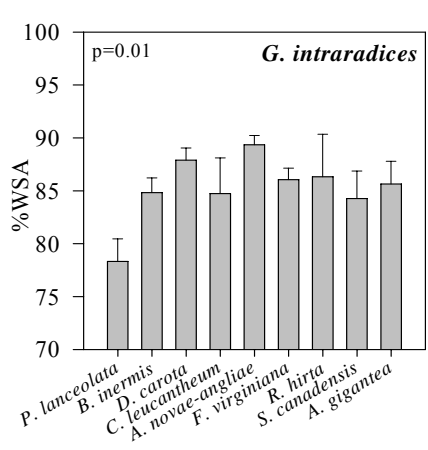
² P-values for the two contrasts testing the ordering of treatments are one-sided.

Table 4: Grass/Gigaspora Group Means

Treatment Groups	Models					
	WSA (all data)	WSA (no BI3)	Root Biomass (all data)	Root Biomass (no BI3)	Hyphal Length (all data)	Hyphal Length (no BI3)
Grass/Gigaspora	83.81	83.81	6.709	6.709	5.517	2.658
Grass/Non-Giga.	83.94	85.59	10.704	5.068	2.492	2.658
Non-Grass/Gigaspora	86.67	86.67	4.952	4.952	4.894	4.894
Non-Grass/Non-Giga.	84.68	84.68	6.240	6.240	2.534	2.534
Grass	83.89	84.43	9.039	6.131	3.752	4.511
Non-Grass	85.48	85.48	5.724	5.724	3.480	3.480
Gigaspora	86.05	86.05	5.336	5.336	5.031	5.031
Non-Gigaspora	84.53	84.76	7.167	6.132	2.525	2.546

Figures

Figure 1. Mean percentage plus standard error of 1-2 mm WSA containing one of 5 AMF isolates in combination with 9 plant species. P values are from Kruskal-Wallis tests. Standard errors shown are not computed on the log-transformed scale used in analysis of these data.



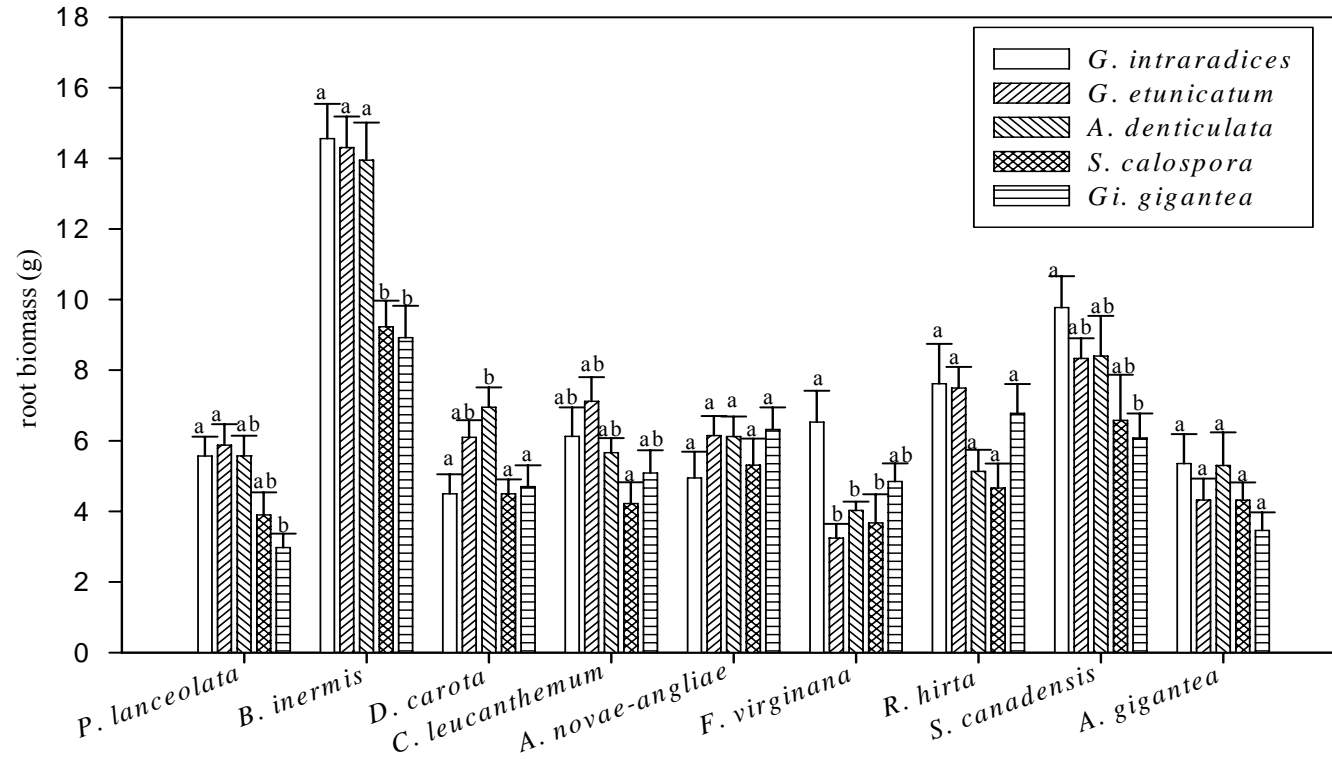


Figure 2. Mean root biomass plus standard error of the nine plant hosts associated with the five AMF species. Standard errors shown are not computed on the log-transformed scale used in analysis of these data.

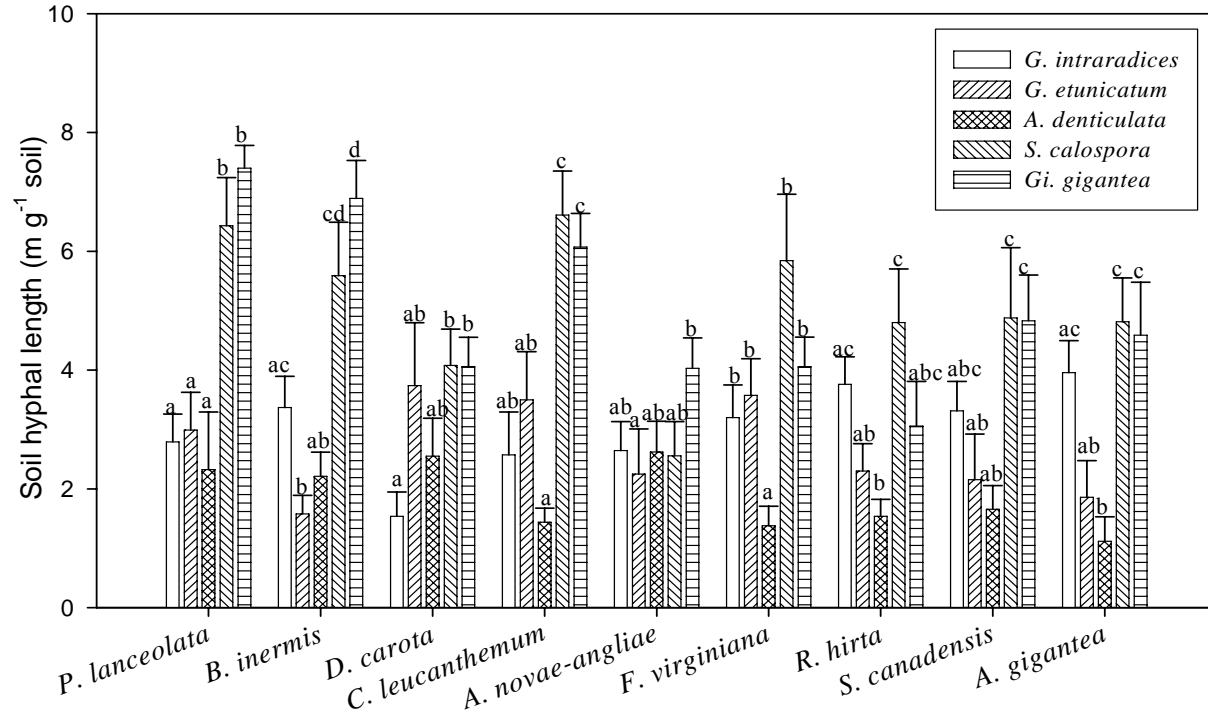


Figure 3. Mean hyphal length plus standard error of the five AMF species associated with the nine plant hosts. Standard errors shown are not computed on the square root-transformed scale used in analysis of these data.

Chapter 5

Succession of arbuscular mycorrhizal fungi: patterns, causes and considerations for organic agriculture

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Abstract

Arbuscular mycorrhizal fungi (AMF) have been promoted as a biofertilizer for sustainable agriculture and production of inoculum is a widespread and growing industry. The last decade of AMF research has revealed far greater host specificity and interactions of the fungi than previously imagined, with the effects of specific host/ AMF combinations ranging from beneficial to parasitic; hence, the next step in AMF application will be employing beneficial combinations. AMF communities and abundances may fluctuate throughout seasons and years because of changes in the abiotic and biotic environment. To date, we have little information on the persistence of applied AMF in systems, and how changes in the AMF community through time may affect plant growth. We must consider how to manage the soil environment to direct succession of AMF species and the displacement of applied or beneficial AMF. This review attempts to merge our current understanding of AMF succession from natural ecosystems with that of AMF application in agriculture. We discuss the patterns and causes of both change in both AMF abundance and species compositions through time, considering how common organic farming techniques may affect these fungi. We propose that management techniques could be employed to direct AMF succession and maintain specific, beneficial

species or species groups, with the potential to increase the sustainability and benefits derived from AMF in organic agriculture.

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Introduction

Sustainable agriculture and organic agriculture are broad terms that describe numerous crop and land management techniques that are designed to reduce anthropogenic inputs like chemical fertilizers, pesticides, and water while preserving the integrity of the soil for future farming (Altieri, 1995). While there are unique crop specific techniques, commonly employed practices include crop rotation, no till farming, integrated pest management, green manures, polyculture, and the use of so-called “biofertilizers” (Altieri, 1995). Biofertilizers are beneficial, often mutualistic soil organism that can promote plant growth and reduce inputs; these include nitrogen fixing bacteria in leguminous crops, ectomycorrhizal fungi like *Pisolithus* in timber plantations, *Azospirillum* in rice culture, and arbuscular mycorrhizal fungi (AMF) in many crop plants (Rai, 2006).

The past two decades have seen an exponentially increasing interest in the use of AMF as biofertilizers in sustainable agriculture, site amelioration, and renaturalization (Gianinazzi *et al.*, 1995; Cuenca *et al.*, 1998; Jeffries *et al.*, 2003; Hart and Trevors, 2006). Despite a lack of consistent and predictable benefits (Ryan and Graham 2002; Gosling *et al.* 2006), the commercial production of these symbiotic fungi has developed into a global industry with dozens of companies producing inoculum (Gianinazzi and Vosatka, 2004). The use of AMF in sustainable agriculture is particularly attractive in that through the symbiotic association with these fungi plants are able to increase nutrient uptake and reduce inputs of fertilizers, water, and pesticides. Even with their popularity,

widespread, prophylactic application, and the potential hazards of careless introductions of non-native mycorrhizal species there still is a paucity of information regarding the persistence of applied AMF (Schwartz *et al.*, 2006). AMF are susceptible to successional pressures resulting from changes in the abiotic and biotic environment; yet, little definitive information is available on the mechanisms behind AMF community changes. Displacement of applied AMF by indigenous AMF or other competitive soil microbes may occur very shortly after application, which has the potential to greatly attenuate the purported benefits of these fungi, resulting potentially in a considerable waste of resources

Over time, soils under certain organic practices may change in certain biotic and physiochemical properties that can affect AMF. Many sustainable practices are designed to increase soil nutrients and organic matter (low till, no till, green manures), and in many cases these can stimulate AMF inoculum potential (Oehl *et al.*, 2004; Gosling *et al.*, 2006). However, in order for the benefits of AMF to be maximized or to be even simply positive, agriculturalists must consider that abundance alone will not always translate to benefit, rather the specific fungi selected must be identified (Douds *et al.*, 2005). Moreover, AMF vary widely in their tolerances and requirements, and soil parameters that change under long term organic management techniques may select less beneficial AMF and displace applied species of known benefit.

Owing to the functional diversity of soil microbial groups, the importance of microbial species diversity to sustainable agriculture, not just abundance, has been realized (Kennedy and Smith, 1995). Studies of AMF have highlighted that it is not merely their presence, but rather the community composition and specific plant/ AMF

combinations that can determine the benefit of these fungi. AMF can vary tremendously with respect to promoting host plant growth (Klironomos, 2003), phosphorus uptake (Jakobsen *et al.*, 2002), conferring drought tolerance (Ruiz-Lozano *et al.*, 1995), soil aggregation (Piotrowski *et al.*, 2004), pathogen protection (Maherali and Klironomos, 2007), and seedling establishment (Van der Heijden, 2004). Hence, managing for abundance alone may not yield the greatest benefit these symbionts can offer, but promoting AMF diversity, or even the proliferation of particular species or families of AMF could be a more effective strategy. Furthermore, if specific AMF are applied, then understanding the physiological tolerances and requirements of the applied species will be necessary to promoting their persistence and reduce the need and costs of multiple applications. If specific AMF/ host combinations are to be employed to maximize a desired function, then displacement of inoculum by immigrant or indigenous fungi could have significant economic and production consequences. To protect the investment in AMF inoculum by stabilizing to the desired abundance and community structure, we must understand the drivers and patterns of AMF succession.

Therefore, the purpose of this paper is to merge our knowledge of patterns and causes of AMF succession from natural systems with frequently observed soil changes that occur during the course of long term organic agricultural techniques; the goal is to predict how these changes will affect AMF communities in agroecosystems and what techniques would allow management of specific AMF. We seek to highlight areas of collaboration between both AMF biologists and agriculturalists that desire to use the fungi in order to create more efficient application strategies. We will primarily focus on annually cropped systems, realizing that AMF management in woody production systems

may be considerably different. Herein we describe some predictable patterns of AMF succession, identify driving causes of these changes that may occur under long term organic management, and discuss if it is feasible to direct AMF species succession. Finally, we outline future research needed to properly address AMF succession to ensure more consistently effective application of these fungi.

Are there predictable patterns of AMF succession?

Succession in natural systems

Major studies reporting on patterns of successional changes in AMF abundance across decades are presented in Table 1. From these a consistent pattern has emerged in temperate and boreal systems. Following disturbance, the abundance of AMF increases rapidly with increases in AMF hosting species (~0-20 years post disturbance); however, the period of AMF dominance is brief as ectomycorrhizal fungi (ECMF) and ECMF-hosting plants replace AMF plants (Johnson *et al.*, 1991; Boerner *et al.*, 1996; van der Heijden and Vostaka, 1999; Barni and Siniscalco, 2000; Treseder *et al.*, 2004; Trowbridge and Jumpponen 2004; Piotrowski *et al.*, *in press*). Lowland tropical systems display a continual increase in AMF abundance following disturbance as ECMF hosting species are not generally as common in these systems (Janos, 1980). Additionally, assessment of AMF abundance from some dune ecosystems has shown steady increases in AMF abundance during succession, with the greatest abundances at the oldest site (Allen and Allen, 1980; Gemma and Koske, 1990; Koske and Gemma, 1997; Greipsson and El-Mayas, 2000).

Regarding changes in AMF species composition through many years, Johnson *et al.* (1991) provided the first and most complete documentation of AMF succession during old field development. This study suggests that “early succession” versus “late succession” species of AMF species could exist. Johnson *et al.* (1991) did not find an increase in AMF richness through time, but increasing species evenness through time as *Glomus aggregatum* spores became less abundant. Nevertheless, some species were much more abundant in late succession sites (*Acaulospora elegans*) and some in early sites (*Scutellospora persica*). Koske and Gemma (1997) presented a similar pattern based on spore data from a dune system of the eastern United States. This study of a five year chronosequence documented an increase in species richness across the artificially planted system. Like Johnson *et al.* (1991) they identified certain species characteristic of certain successional stages. For instance, one species of *Acaulospora* was only found in mid to late successional soils, and *Glomus* 7243 was only present in the oldest sites. Thus far, no consistent patterns of early vs. late AMF species have been defined, and some studies have documented no change in AMF during succession (Johnson and Wedin 1997), or a decline in species richness through time (Beauchamp *et al.*, 2007). Nevertheless, similar patterns have been documented in agroecosystems.

AMF succession in organically managed agricultural systems

A large body of literature supports the fact that conventional agriculture practices of tillage and high inputs of phosphorus fertilizers can drastically reduce AMF inoculum in agroecosystems over time (Douds and Millner, 1999; Jansa, 2002; Kabir, 2005). The scenario can be different under certain long-term organic management regimes. Many

studies have demonstrated that after decades of organic management AMF abundance, as measured by hyphal density or spore numbers, is significantly increased compared to conventionally managed plots (Kabir *et al.*, 1998; Mäder *et al.*, 2002; Oehl *et al.*, 2003). Long term sustainable practices may also increase AMF diversity compared to conventional agriculture (Jansa *et al.*, 2002; Oehl *et al.*, 2003, 2004). These studies have also revealed a change in species composition similar to succession in natural systems. Notably, over long term organic management (BiodynamicTM and no-till) species of *Acaulospora* increase in abundance, as well as other slow growing species like *Scutellospora* and *Entrophospora* (Jansa *et al.*, 2002; Oehl *et al.*, 2004). These data suggest that significant changes in AMF abundance and species composition occur over the years and decades since conversion to organic management, similar to natural systems (Johnson *et al.*, 1991; Koske and Gemma, 1997). What are the changes driving the succession of AMF? Over time abiotic changes similar to those in natural soil development (*e.g.* decreasing pH, increasing OM and litter leachates, and increasing saprophytic fungi) could affect AMF. A list of potential effects of long term organic management on AMF is presented in Table 2. Sustainable management practices in long term cropping may have to consider these pressures, as well as AMF host diversity, in order to maintain a beneficial AMF abundance and effective species composition.

Potential environmental drivers of AMF succession in organic management

In natural systems, many studies have correlated changes in AMF abundance with changes in abiotic properties in an attempt to define a specific cause of changes in AMF during succession. During soil development, components of the soil physio-chemical

environment known to influence AMF growth (*e.g.*, pH, soil moisture, phosphorus) can change dramatically over relatively short periods of time. AMF abundance and species composition can also change through time, even without changes in the plant community, indicating that exogenous forces are affecting proliferation of these fungi (Wacker, 1988; Koske and Gemma, 1997; Husband *et al.*, 2002).

Organic agricultural practices are designed to reduce seasonal inputs and tillage, and therefore soils in these systems may develop more similarly to natural soil systems following disturbance, compared to conventionally tilled and managed fields. During development of non-managed soils, predictable changes in soil texture, pH, organic matter, and nutrient content can occur (Walker and Moral, 2003); similar changes may occur in some organically managed systems. For instance, pH may be reduced (Pekrun *et al.*, 2003), organic matter increases (Marriott and Wander, 2006), soil nutrient status changes (Gosling and Shepherd, 2005), and phenolic compounds accumulate (Blum *et al.*, 1991). These variables all have the potential to affect AMF abundance and species composition. While these changes are correlated with increases in AMF abundance and diversity (Mäder *et al.*, 2002; Oehl *et al.*, 2004), selection for less beneficial AMF might also occur depending on the host/ AMF interaction. This section discusses what we know of controls of AMF abundance and species composition from natural systems and how we may use these to predict changes in agroecosystems.

Long term abiotic changes of organic management that can affect AMF

Soil pH

Decreases in soil pH have been correlated with reduction of AMF in soils (reviewed in Entry *et al.*, 2002). The mechanism may be a function of the pH tolerance of AMF, increasing metal toxicity, or alteration of phosphate availability. There is considerable variability in how pH responds to long term organic management practices. In general, organic management such as reduced tillage increases soil buffering capacity and reduces large shifts in pH; however, there are instances where long term no-till can significantly alter soil pH (Pekrun *et al.*, 2003). In a summary of studies, Pekrun *et al.* (2003) found that half of the soils under no till management for >5 years had significantly reduced soil pH, the other half had no significant change. Other practices like green manure, compost additions, and crop rotations can also affect soil pH (Astier *et al.*, 2006; Godsey *et al.*, 2007). No research to date has documented that such shifts in pH are correlated with reduced AMF abundance; however, these changes may affect species composition in a predictable manner. A few studies suggest that species of *Acaulospora* are more tolerant of low soil pH (Porter *et al.*, 1987, Johnson *et al.*, 1991). This could explain proliferation of these AMF in late succession or after long term no till management (Johnson *et al.*, 1991; Jansa *et al.*, 2002; Oehl *et al.*, 2004). Interestingly, this species is also more adept at phosphorus acquisition than *Glomus* or *Gigaspora* species (Jakobsen *et al.*, 1992). Perhaps the *Acaulospora* is a preferred associate in late succession soils where low pH limits phosphorus availability.

Soil nutrient status

Changes in soil nutrient status may have a strong effect on AMF colonization and abundance. The soil nitrogen to phosphorus ratio has been shown repeatedly to affect

AMF colonization (Liu *et al.*, 2000; Johnson *et al.*, 2003), but the exact mechanism is still unclear. Changes in available (mineralized) soil phosphorus and nitrogen are the basis of Read's (1991) hypothesis regarding the distribution of AMF across ecosystems, and this idea has since been applied to changes in AMF across successional time in temperate and boreal ecosystems (Treseder *et al.*, 2004; Piotrowski *et al.*, *in press*). While excess phosphorus inputs can reduce AMF abundance in agricultural settings, the role of P inhibition of AMF during soil development in natural ecosystems is not conclusive because concomitant changes in other variables make it difficult to isolate a single cause.

The effect of organic management on soil nutrient status again depends on the particular practice. As with conventional fertilizer amendments, long term use of organic fertilizers and green manures can affect soil phosphorus and nitrogen levels (Edmeades, 2003), potentially affecting AMF. To date no studies have described nutrient inhibition of AMF by organic fertilizers that is similar to long term reduction of AMF through conventional fertilizer regimes, and this may not be a strong selective force under organic regimes. Nevertheless, alteration of the nutrient status over time may result in similar shifts towards less beneficial AMF as described by Johnson *et al.* (1993).

Soil organic matter and crop residues

Following disturbance soil organic matter can increase rapidly in natural systems as well as under many organic regimes (*e.g.*, no till, green manure, compost additions). Read (1991) proposed that as soil organic matter increases, the preferred mycorrhizal associate will be one that can access organic nutrients, hence AMF will be replaced by

ECMF that have extracellular enzyme systems capable of accessing organic nutrients. While this phenomenon is frequently observed, it is unknown if organic matter is directly involved in the displacement of AMF. Experimental tests of organic matter additions on AMF are mixed. Experiments have shown that organic matter additions stimulate AMF colonization and soil abundance (Cavender *et al.*, 2003; Nan *et al.*, 2006). Other studies however, have shown that organic matter inputs in the form of litter and litter leachates may strongly inhibit AMF colonization (Yun and Choi, 2002, Piotrowski *et al.*, 2007). As with other microorganisms, the effect of organic matter inputs on AMF depend on the litter chemistry. Piotrowski *et al.* (2007) document that increases in soluble phenolic compounds over time may result in inhibition of AMF colonization. The mechanism may be a product of phenolic toxicity or competitive exclusion by organisms capable of detoxifying or tolerating these compounds. Of course, this may only occur in young soils with low humic substance content, as humic materials may bind phenolic substances (Cecchi *et al.*, 2004). Similarly, some no till systems have significantly higher soil phenolic concentrations than conventional till (Blum *et al.*, 1991). The effect of these substances from crop residues on AMF may be most significant in crops that have a high foliar phenolic concentration and less noticeable in other crops.

Phenolics might even contribute to succession of AMF species. A study by Wacker *et al.*, (1990) described inhibition of germ tube elongation of AMF spores by the common phenolic compound ferulic acid. This suggests that accumulation of soil phenolics could lead to selection of AMF species that can infect through other means besides spores alone. In this instance, accumulation of phenolics could potentially reduce root colonization by Gigasporaceae species in a soil (which colonize primarily from

spores), compared to members of the Acaulosporaceae and Glomaceae that have more infective soil hyphae (Hart and Reader, 2002).

Long term biotic changes affecting AMF

In addition to changes in the abiotic soil properties discussed above, many biotic parameters that can affect AMF abundance and/ or diversity can change through soil development. Some sustainable agriculture practices allow for greater changes in the biotic environment than conventionally managed fields. Green manures, polyculture, and crop rotation introduce greater plant diversity to a cropped system, and these practices have the potential to determine AMF abundance as well as species composition.

Host identity can affect sporulation and abundance of particular AMF species (*e.g.*, Bever *et al.*, 1996, Vandenkoornhuyse *et al.*, 2002). Here the choice of the cover crop or co-cultivated crop can strongly affect AMF. It is established that using non-AMF host species as cover crops may reduce soil inoculum (Miller, 2000; Arihara and Karasawa, 2000), but the AMF communities associated with the cover crop may not be optimal for conferring a benefit to the crop. If so, the cover crop has the potential to amplify a less beneficial AMF community and affect the performance of the production crop, similar to the negative feedback phenomena described in the work of Bever (2002). The same negative feedback phenomena could also hold for crop rotations. Rotating one crop species and its associated AMF community with another crop species that does not benefit from the previous AMF community could affect production.

Increases in organic matter inputs during soil development can stimulate populations of saprobic organisms. These could compete with AMF for resources within

the soil. Also, saprobic organisms parasitic to AMF may be stimulated with increased organic matter (Lozupone and Klein, 1994). Hyphal grazers like collembolans can significantly increase under reduced tillage (Titi, 2003). While collembolans are thought not to prefer AMF over other fungi (Klironomos and Kendrick, 1996), in high abundance they could still reduce AMF hyphal lengths enough to give other proliferating fungi a competitive advantage, or even selectively graze certain AMF species.

During succession in natural systems there typically is a large turn-over in plant species, presumably also resulting in larger changes in the AMF community. In most conventional agricultural settings, there are no large changes in the plant community outside of the crop; however, in less intensively managed systems, neighboring plants and weeds with their associated AMF could lead to changes in inoculum identity within a field. For instance Mummey and Rillig (2006) describe the decrease in diversity of an AMF community of a natural grassland after invasion by *Centaurea maculosa*; a similar phenomenon may occur as weeds invade cropped fields. Over seasons and in the post-harvest periods, inoculum of any applied fungi could quickly be displaced if they have lower sporulation with the established weeds.

Inoculum immigration

The initial AMF community is determined by the resident inoculum, which will be a function of site history. However, over time immigration of AMF inoculum from off-site has the potential to alter the community composition. AMF inoculum is available as spores, hyphal fragments, or colonized root fragments. While the amount of inoculum available will depend on disturbance intensity (*e.g.* tillage intensity) and time since

disturbance (*e.g.*, hyphal fragments and colonized root lengths do not persist as long as spores), the invading species composition of the inoculum will be almost entirely a product of vegetation surrounding the disturbed site. Unlike most fungi, wind is not the primary dispersal agent of AMF. While aeolian deposition of AMF spores is possible (Allen *et al.*, 1989), their spores are much larger than other fungi and produced entirely underground. AMF spores are transported into disturbed and denuded environments via a variety of vectors, including rodents (Allen *et al.*, 1984; Mangan and Alder, 2002), earthworms and microarthropods (Doube *et al.*, 1994; Klironomos and Moutoglis, 1999), and anthropogenic dispersal (Schwartz *et al.*, 2006). The extent of dispersal may depend on the specific AMF family and spore size (Klironomos and Moutoglis, 1999). Members of the Glomeraceae produce copious, small-volume spores compared to the Gigasporaceae which produce fewer spores of a significantly greater size (Hart and Reader, 2002). In early successional sites, members of Glomeraceae spores will likely be the primary immigrants; however, over longer time scales, species that have larger spores, produce fewer spores, or both will immigrate as well.

Consequences of AMF succession to production

The reduction of AMF abundance and colonization alone has the potential to reduce plant performance in highly mycorrhizal-responsive plants, but what about changing AMF community composition? While AMF diversity can affect plant diversity in natural plant communities (van der Heijden *et al.*, 1998, Hartnett and Wilson, 1999), in low diversity agricultural systems the composition of the AMF community might not always be beneficial. Increasing AMF diversity can lead to a greater likelihood of a

beneficial host/ plant combination and functionally complementary combinations of AMF; however, although not yet documented, the opposite might also be possible. During succession of agricultural fields employing AMF, a shift in community composition could potentially affect the benefits that the desired applied community confers. Our knowledge of how changes in AMF communities change in function through time is almost nonexistent, but we can make a few predictions.

During soil development the nutrient status, water holding capacity, and soil pathogen load can change dramatically. Early successional soils are often exposed and dry, whereas older soils may host a greater density of root pathogens. We hypothesize that as a whole, early successional AMF communities in arid and semi-arid environments have a greater capacity for drought tolerance whereas AMF in older soils as a whole may be more adept at conferring pathogen resistance to their hosts. If this predicted change in AMF function through time holds, then new crop rotation strategies may be designed. Plants that greatly benefit from AMF-assisted pathogen protection could be used late in rotation or following crops that increase populations of AMF that confer greater pathogen tolerance.

It is difficult to predict how the phosphorus acquisition abilities of an AMF community would change over time. In many natural systems, as sites age, soil pH decreases markedly, hence phosphorus becomes more limiting as it is bound in iron and aluminum complexes. Late successional species of AMF may be more adept at phosphorus scavenging, and this may not be a function of plant allocating carbon to the most beneficial associate. For instance, some species of *Acaulospora* are able to tolerate low pH soils and have a greater capacity to uptake phosphorus (Porter *et al.*, 1987;

Jakobsen *et al.*, 1992). These species are characteristic of late successional soils (Johnson *et al.*, 1991). Thus, as phosphorus becomes less available, AMF species that can tolerate low pH soil persist and maintain phosphorus scavenging for hosts. To the organic farmer, this suggests that plants that benefit from *Acaulospora* associations can be rotated into soils of decreased pH to enhance the occurrence of the association.

One known phenomenon that affects production is the “organic transition.” The organic transition is a recognized phenomenon that occurs as a conventionally managed agroecosystems is converted to a lower input, organically managed system (Liebhardt *et al.*, 1989; Delate and Cambardella, 2004). This period of approximately 3 years entails significantly lower yields before returning to higher production rates (Delate and Cambardella, 2004). This transition has been attributed in part to the microbial community of the soil (Tu *et al.*, 2006). If traditional agriculture selects for less beneficial, more parasitic AMF (Johnson *et al.*, 1993), then the lower yields of the organic transition could result from the succession of less beneficial AMF to ones of greater host specificity, as host controls on populations begin to outweigh edaphic factors. Through a better understanding of controls and succession of AMF, perhaps better management practices acting on the AMF community could be employed to shorten the organic transition period.

Can we manage AMF succession in organic agriculture?

Managing succession of AMF abundance

A number of studies have explored innovative practices that can stimulate the abundance of AMF in agricultural fields (*e.g.*, reduced tillage, liming, organic matter

additions, green manures) (reviewed in Gosling *et al.*, 2006). Moreover, additions of living top soils, organic matter, and commercially produced inoculum are all able to temporarily increase the mycorrhizal inoculum potential of a soil. But do the indigenous AMF communities offer a net benefit to the crop species? An exploration of this interaction prior to planting on the local scale may help maximize the benefits of native AMF, determine management strategies to promote proliferation of beneficial AMF groups, or highlight the need to apply specific fungi.

Maintaining AMF species composition

Our current understanding of patterns of AMF species succession relies on only a few studies, and the mechanisms behind the observed shifts between species or groups are even more obscure; however, some controls on AMF species are apparent and may serve as the basis for testing direct manipulation of AMF in field settings. For instance, members of the Acaulosporaceae have been found to be more abundant in mid to late succession (Johnson *et al.*, 1991; Koske and Gemma, 1997). While this could be a product of the family's slow-growing life history strategy (Hart and Reader, 2002), genera within this family have demonstrated a tolerance of low soil pH (Porter *et al.*, 1987). During soil development or across some long term organic management regimes, soil pH may decrease, leading to a greater abundance of *Acaulospora* species. If these species are beneficial to the crop, artificially reducing soil pH may help select for them in cases where the decrease in pH is not detrimental to the crop species. The opposite is possible as well, if *Acaulospora* associations are not as beneficial to the specific crop, then their abundance may be suppressed through maintaining a neutral to alkaline pH.

Another example of a successional change in the abiotic environment that could be managed is soil moisture content. During soil development in both natural and long term organic systems, the water holding capacity of a soil can increase with increases in organic matter and texture changes. Changes in soil moisture have been shown to alter the colonizing ability of AMF compared to other root colonizing fungi (Lodge, 1989). Also, certain AMF species are able to tolerate a drying environment and confer greater drought tolerance (Ruiz-Luzano *et al.*, 1995). If the goal of AMF management is to confer greater drought tolerance, then cover crops could be water starved to stimulate the abundance of drought tolerant AMF species like *Glomus deserticola*, or one could choose a cover crop that promotes significantly more sporulation of *Glomus deserticola* than another.

We know that AMF species sporulation can be host dependent (Bever *et al.*, 1996). Thus, a better understanding of crop/ AMF interactions with respect to sporulation as well as indigenous non-crop AMF hosting species is necessary. The abundance of certain beneficial species may be stimulated by cover crops and crops that maximize sporulation of a desired AMF species. Crop rotation may be developed to capitalize on positive feedbacks delivered by AMF communities beneficial to multiple plants. Finally, controlling the immigration of AMF undesirable AMF inoculum that could displace the applied or managed community will be critical to maintaining beneficial AMF communities in agroecosystems and reducing repeat inoculations. Preventing the establishment of weeds that are non-mycorrhizal or promote a change to a less beneficial AMF community will help slow succession towards potentially less optimal crop/ AMF interactions.

Future research needs to improve AMF application

Measures of persistence

For AMF to be most useful as biofertilizers, they should persist across seasons. If specific fungi are used to maximize benefits, they must remain when confronted by other edaphic or biotic changes. Our understanding of this is limited and presents a huge gap in the knowledge of application of these fungi. Molecular techniques have made identification of AMF species from roots and soil easier, and recent studies are beginning to apply these to monitoring the persistence of applied AMF (Farmer *et al.*, 2006).

Physiological studies and environmental match of AM species and ecotypes

There are approximately <200 described AMF morpho-species globally. AMF can vary dramatically in their physiology, even within genera (Munkvold *et al.*, 2004). To understand which AMF are most adept at certain desirable functions (*e.g.*, P uptake, biomass stimulation, pathogen protection) and manage for their persistence, the unique physiologies and environmental tolerances of these fungi must be more thoroughly documented. To that end, we must also gain a better understanding of host/ fungi interactions with respect to function and inoculum production. As the benefits of AMF colonization can range from positive to neutral to negative, it will be important to know that the applied species will not result in decreased biomass despite increased drought tolerance. Optimal combinations that minimize the parasitic aspects of the association will be important in effective application.

Characterization of native communities

The widespread application of non-native AMF inoculum has the potential to promote species invasions and negative associations (Schwartz *et al.*, 2006). Sustainable AMF application will likely depend on managing native AMF species. Applied AMF can vary in their ability to establish and persist; however, management that stimulates inoculum potential and abundance of native AMF species, and reduces the need to apply AMF would be a more cost-effective strategy devoid of the risks associated with non-native introductions. Additionally, we need greater understanding of AMF succession after conversion to organic management from a greater number of cropping systems. We have a rudimentary understanding of the end-point AMF communities between conventional and organic systems (Mäder *et al.*, 2002; Jansa *et al.*, 2002; Oehl *et al.*, 2003), yet almost no information about the time-course of changes in the AMF community.

Conclusions

Despite the potential of AMF to enhance organic agriculture, many unknowns regarding host/ AMF interactions and persistence in the face of soil changes remain to guarantee predictably positive results from application. Further studies of changes in AMF abundance, infectivity, and species composition over years of low input farming practices will be critical to identifying and maintaining beneficial AMF in croplands. It is apparent that AMF diversity alone may not be the absolute goal of mycorrhizal management and application for every crop, rather it should be to develop and maintain an AMF community that can provide the greatest benefit to the crop. Our sparse understanding of the control of the soil and plant environment does indicate a potential to

manage not only for AMF abundance but for community composition. To achieve effective use of these often beneficial symbionts we still need a great deal of information on the functioning of indigenous AMF communities, how to select for certain species, and how to maintain the most beneficial host/ AMF combinations in the face of changing abiotic and biotic environments.

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Table 1. Studies that directly measure long term changes in AMF abundance during soil development following disturbance using chronosequences. (MIP= Mycorrhizal inoculum potential, PLFA= phospholipid fatty acid analysis)

Disturbance	Chronosequence age	AMF Measure	Summary of AMF pattern
Tillage			
Barni & Sinicalo 2000	0- >60	MIP	MIP peaks at 10 years then declines
Johnson et al. 1991	0- >60	Spores, MIP	Spores increase across most of sequence but are very low at oldest sites. MIP peaks at 19 yr then declines
Boerner et al. 1996	5-30	MIP	Decrease through time
Dune formation			
Greipsson & El-Mayas 2000	0-245	Spore number	Spores increase across entire sequence 1 st sampling, peak in abundance at penultimate site following year
Koske and Gemma 1997	0- >5 (oldest not determined)	Spores, MIP	Rapid steady increase
Volcano			
Balser et al. 2005		Hyphal length, PLFA	Peak at middle site, lowest abundance at the oldest site
Fire			
Treseder et al. 2004	3-80	Hyphal lengths	Peak and decline
Flood			
Piotrowski <i>in review</i>	0-70	Hyphal length, MIP	Peak at 10-13 then decline in both measures

Table 2. Comparison of common sustainable agriculture practices and their long term effects on the soil environment and potential effects on AMF.

Agricultural practice	Some known long term effects abiotic and biotic environment	Hypothesized effects on AMF
No till	<ul style="list-style-type: none"> • Increased soil organic matter • Alteration of soil pH • Increased soil moisture • Increased soil phenolics • Increase saprophytic fungi/ hyphal grazers 	<ul style="list-style-type: none"> • Mixed effects on abundance, potential selection for non-Gigasporaceae depending on organic matter chemistry • Mixed effects on abundance, potential selection of <i>Acaulospora</i> species with pH decrease • Potential decrease in abundance at high moisture levels, unknown selection • Decrease in abundance, potential selection for non-Gigasporaceae • Potential decrease in abundance, unknown selection
Crop rotation	<ul style="list-style-type: none"> • Alteration of soil nutrient status • Alteration of soil pH • Alteration of AMF host interactions 	<ul style="list-style-type: none"> • Mixed effects on abundance, potential selection towards more parasitic <i>Glomus</i> species at high nutrient levels • Mixed effects on abundance, potential selection of <i>Acaulospora</i> • Mixed effects of abundance and species selection depending on the specific AMF/ host combination
Green manures/ ley crops	<ul style="list-style-type: none"> • Soil organic matter • Alteration of soil nutrient status • Increase in soil moisture • Increased soil phenolics • Increase saprophytic fungi • Alteration of AMF host interactions 	<ul style="list-style-type: none"> • Mixed effects on abundance, potential selection for non-Gigasporaceae depending on organic matter chemistry • Mixed effects on abundance, potential selection towards more parasitic <i>Glomus</i> species at high nutrient levels • Potential decrease in abundance at high moisture levels, unknown selection • Decrease in abundance, potential selection for non-Gigasporaceae • Potential decrease in abundance, unknown selection • Mixed effects of abundance and species selection depending on the specific AMF/ host combination

Chapter 6

The radish party: an exciting exploration of soil organic matter for K-2 students

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Abstract

Healthy soils are crucial to society, and they depend on soil organic matter. This inquiry teaches about soil organic matter to the youngest of students.

Its not dirt, its soil!

Young children like soil (even though they may refer to it as dirt). Soil is a part of their daily lives: they play on it, dig in it, and are often covered with it. Hence, soil can be a highly visible and relevant ecosystem to children. Classroom inquiries that incorporate soil explorations are an excellent way to teach basic biological and ecological lessons that are applicable to their lives, even at very young ages.

In addition to being an appealing medium for making mud pies, soil is the basis of all terrestrial life (Hillel 1991). Healthy soils sustain ecosystems, agriculture, and human societies. However, because of inefficient agricultural, the earth is losing large amounts of cultivatable soil at alarming rates. For instance, Pimentel et al. (1995) estimate that over 40% of the world's farmable soil has been lost to erosion by water and wind. One crucial element of healthy soil is soil organic matter (SOM). SOM is decomposed and decomposing remains of organisms, which give soil a dark color and spongy texture. In

addition to greater nutrient content, soil rich in organic matter forms water stable soil aggregates, which are more resistant to the forces of erosion (Six et al. 2002).

This inquiry is designed to teach the importance and relevance of soil organic matter to first K-2 grade students through a fun and rewarding experiment. “The radish party” (originally named by Kathy Dungan’s combined first and second grade class at Lewis and Clark elementary) requires few materials (see Table 1), minimal set up and addresses many of the National Science Education Standards for this grade band. This experiment can also be easily extended into a series of integrated lessons (Table 2) on soil biology and ecology applicable to the daily lives of even the youngest elementary students (see <http://www.bioed.org/ecos/inquiries.aspx> to download additional lessons).

Getting Started

Before beginning this investigation, three types of soil (sand, sand plus nutrients, and potting soil) must be prepared (see Table 1 for ingredients). First place half the sand in one bucket and label it “sand.” Next place the potting soil in a bucket labeled “soil.” Finally, thoroughly mix the Osmocote™ (a common, widely available fertilizer) with the remaining half bag of sand in a third bucket labeled “sand + nutrients.” Osmocote™ was chosen as the nutrient addition because they are large capsules that children can see and know nutrients were added. Now you are ready to have a radish party. Radishes were chosen because they germinate and grow quickly, alleviating impatience of some students. The experiment requires either fluorescent grow lights or a sunny, warm window. The other materials are readily available and inexpensive.

Getting to know soil

There are many informative websites that teach the basics of soil ecology (see additional resources at the end of this article). Young kids will certainly know what soil is and reasons plants need it to survive, but some more thought provoking questions to get them excited about the investigation are:

- What is soil made of?
- Where does soil come from?
- Why do plants need soil to grow?
- What do plants get from soil?
- What are roots for?
- What are the differences between a good soil for plants and a bad soil?

The first key lesson of this investigation is that soil comes from both the weathering of rocks (inorganic portion) and the decomposition of plants and animals (organic portion). Students need to understand that plants get their nutrients and water from the soil. Without these nutrients, plants would not be able to grow.

Introduce the students to three types of soil that you have prepared ahead of time: sandy soil, sandy soil plus nutrients, and potting soil. Osmocotetm is not edible, so the children should be warned not to taste any of the soils. As the different soils are passed around, ask the students to feel them and smell them. Explain to the students that they are about to be both farmers and scientists. The story line for the investigation is that we want to grow radishes for our food but are not sure which soil is best. The challenge is to test which of these three soil types is best for growing radishes. Be sure to have a few radishes or pictures of radishes for the children to see and touch. After students have seen

the soils and radishes, and understand the question, ask the students to predict which soil will grow the biggest radish. They will do this by drawing a picture of the radishes growing in each different soil type (Figure 1). The students should draw a big radish in the soil they think will be the best and a small radish in poor soil. If they think soil type will not make a difference, they should draw all radishes the same size. Ask them to make sure they carefully label their drawings with both the type of soil each radish is growing in and also label the basic parts of the radish: stem, leaves, roots, radish tuber. This may take 10-15 minutes. Afterwards, gather all the students in a circle and have them describe their pictures, predictions, and rationale for predictions.

Time to start growing radishes

The radish party investigation worked best for our class when the students worked in groups of two during the experimental set up.

1. Supply each group with a box containing one 6-chambered seed starting pot, 6 popsicle sticks, a spoon, and 20 radish seeds.
2. Have the students fill 2 chambers of the seedling tray with each of the three soil types for a total of six filled chambers.
3. Next have the groups design popsicle sticks that indicate the soil types and the date of planting: (e.g. Sand+ 8/30/06). Each chamber should have one clearly labeled stick.
4. Now ask the students to plant three radish seeds into each chamber, leaving a little space between the seeds to avoid crowding. Plant the seeds at least 1/2" deep and

make sure they are covered with soil. Ideally, if no seeds are lost, each team will have two extra.

5. Place all the pots into a large garden tray that does not leak, and put this tray under the grow lights or in the sunny window.
6. Ask the children to water each pot carefully with an exact volume of water (50-100mL). They can use a cup with a “fill-to” mark on the side or a graduated cylinder if they are available. Why use the exact amount of water for each plant? In addition to providing nutrients, soil organic matter holds more moisture than sand, thereby prevents plants from wilting and dying. Consequently, to have a “fair test,” each pot should receive the same amount of water.
7. Allow the plant to grow for several weeks to a month (if you want to try to harvest a mature radish) watering every 2-3 days with the same amount of water. Have the students make observations every week: when do seedlings first emerge, which ones are growing faster, taller, slower?

Harvest time and assessment questions

Finally it is time to collect data and compare the results with the students’ predictions. Gather all the plants together on a table in front of the students. Ask the students to carefully remove all the plants from the all the different treatment pots and place them in a pile on the table in three separate piles (sand, sand + nutrients, potting soil). Ask the students to make observations and look for differences (advanced students may even make a few measurements of plant height). Some questions for discussion are

listed below. These questions can also form the basis of an assessment of learning from the inquiry.

- What differences do you see?
- Which soil yielded the largest plants? The most healthy looking plants?
- Is the tallest plant always the healthiest?
- Where your predictions met? Why or why not?
- What if we grew cacti instead of radishes?

Once they have determined which soils produced the “best” plants, you can explore the following questions:

- Why didn’t the plants with added nutrients grow better than the plant in the dark soil?

The answer is likely to be because the plants in sand dry out more quickly than plants in soil, which holds more water.

- How can you increase the organic matter in your soil at home in the garden or houseplants?

Radishes, soil, and the National Science Standards

Science as inquiry

The relevance of this inquiry to the National Science Standards is presented in Table 3. This investigation introduces students to asking questions and helps them focus their questions during the initial discussion. Students are asked to make predictions based on their current knowledge of plants and soil and communicate their predictions to the entire class as pictures. Children also employ simple skills in setting up the experiment

(planting seeds, labeling pots, measuring and watering). While the radishes grow, the students can be encouraged to observe plant growth using magnifying glasses and other tools. At the end of the experiment students can measure radish growth. Finally, the children must come up with a conclusion about which soil type was best for radish growth based on their observations, and then explain their conclusion in a group discussion

Life Science

This is an excellent inquiry to introduce young students to plant form and function, life cycles, and environmental requirements. Children become familiar with the basic parts of a plant (e.g. seed, stem, leaves, roots) and their function, as well as part of a radish's life cycle from seed to plant. If time allows, the radishes could be grown to the flowering stage. To keep the plants alive, students learn about the plant's requirements for growth (e.g. water, light, soil, warmth) and the consequences if these requirements are not properly met (i.e. when the soil is poor).

Science in personal and social perspectives

Through this experiment students learn about the conditions that plants, specifically an agricultural crop, need to grow. From this inquiry, teachers can help students understand more about their own food. For example, if food crops don't thrive, humans will not have an abundance of fruits and vegetables. An excellent extension is to explore the resources good soils provide to plants, and this includes not only nutrients, but also water. They might discuss what would happen if good soil was lost, and how human-caused environmental changes that reduce soil quality can affect food production, and ultimately, them. A key learning outcome is that organic matter makes soil healthy,

and that by adding organic matter to soil in the form of compost plant growth increases. Students may wish to interview a local farmer about soils and the value of soil organic matter.

The radish party was enjoyed by all

The students of Kathy Dungan's combined first and second grade class were engaged and excited during the entire experiment. Every week they were eager to share new stories about what the seedlings were doing, when they emerged, which were growing better, and even if their predictions were correct after just a few weeks of growth. This proved a very exciting inquiry for these grade levels. Why? The students loved being farmers and actually growing vegetables and watching the stages of growth. Second they were excited to see how their predictions would turn out and they kept an ever-vigilant eye on their crops, in addition to very detailed pictures of their predictions and lucid explanations of why they made to their predictions. Finally, students were enthralled when their predictions were correct (often the case). Through the concluding discussions the children gave evidence of a greater understanding of soil organic matter. They understood how organic matter adds nutrients, as well as retaining water. More importantly, they seemed to understand how this inquiry and its results could apply to their lives and home gardens, making it relevant to them personally.

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Additional resources

<http://soil.gsfc.nasa.gov/>

<http://www.bioed.org/ecos/inquiries.aspx>

<http://soils.usda.gov/education/>

<http://www.wtamu.edu/~crobinson/DrDirt.htm>

<http://www.bioed.org/ecos>

Tables

Table. 1 Supplies necessary to host a radish party with your class. Almost every item is always available at a local hardware or garden supply store, with the exception of radish seeds that are seasonally available in some areas.

Supply	Quantity
Radish seeds	3-4 packs
Potting soil	1 medium bag
Sand	1 medium bag
Osmocote™ granules	1 small bottle
Popsicle sticks	6 per group
Seedling pots (6 packs)	1 6 pack per group
Seedling trays that don't leak	2-3
Fluorescent lights*	Enough to cover the seedlings
Measuring cups (mL)	2-3
Rulers	1 per group

* If fluorescent lights are unavailable, a warm sunny window could be an alternative. Be aware though that the effects of too little light could outweigh the effects of the soil types leading to different results.

Table 2. Companion exercises to “The radish party” on soil ecology appropriate for K-2 students available for download and comments at

<http://www.bioed.org/ecos/inquiries.aspx>.

A tour of soils

This is a simple and fun outdoor activity that is a perfect introduction to soils, especially good to use before “The radish party.” Children are on a scavenger hunt to find and describe five different soils from around the schoolyard. With only the help of a shovel, magnifying glasses and their senses the students explore the diversity of soil types and organic matter content from areas around the schoolyard or anywhere (Figures 2 and 3). Teachers and students alike will be surprised at the diversity of soil characters in even the most homogenous schoolyard.

Composting 101: It’s the microbes

This is a long term experiment where students actually create soil organic matter from fallen leaves they collect in the fall. Here the influence of soil microbes on decomposition take center stage as students test if “living soil” speeds up decomposition compared to “dead soil.” This is another activity that can precede “The radish party.” Students can even use some of the compost they make in this experiment in the radish experiment for an integration of concepts.

Soil erosion: causes and cures

Here students learn how soil organic matter and litter help prevent soil erosion. This is an advanced experiment that takes a little set up and time, but is very informative, dramatic, and relevant to their daily lives. The teacher with the help of the students create an erosion machine and test how different soil types with varying organic matter and litter layer respond to the erosive force of water. Students can see how organic matter and litter mulch can greatly reduce soil loss from erosion. They are encouraged to think of ways to prevent erosion in their own gardens at home.

Table 3. Specific National Science Content Standards addressed with “The Radish Party” investigation.

National science standards	How addressed
<p><i>Science as inquiry</i></p> <ul style="list-style-type: none"> - Abilities necessary to do scientific inquiry - Understanding about scientific inquiry 	<p>Students ask questions, make predictions, set up and maintain an experiment, make observations and conclusions, and present their conclusions to a group.</p>
<p><i>Life Science</i></p> <ul style="list-style-type: none"> - Characteristics of organisms - Life cycles of organisms - Organisms and environments 	<p>Students learn plant form and functions, life cycles, and environmental requirements for growth.</p>
<p><i>Science in personal and social perspectives</i></p> <ul style="list-style-type: none"> - Types of resources - Changes in environments - Science and technology in local challenges 	<p>Students learn how agriculture crops depends on environmental conditions and soil, and thus so do human societies, how soil quality is changing from human activity, and they learn how they can improve growing conditions for plants of their own.</p>

Figures

Figure 1. Examples of student predictions of how well radishes will grow in the three different soil treatments. A note on the bottom two drawings: students weren't explicitly told that radishes grow underground.



Figure 2. A student at Lewis and Clarke elementary gets down and dirty during a “Tour of soils” (Table 1, available at <http://www.bioed.org/ecos/inquiries.aspx>) field trip to explore soil biology and ecology.



Figure 3. Students on the “Tour of soils” experience many aspects of soil: feel, smell, texture, and appearance.



Chapter 7

Synthesis

Owing to their ubiquity in nearly all ecosystems and consistently demonstrated benefits to many plant hosts and plant diversity, research of arbuscular mycorrhizal fungi (AMF) is at the forefront of terrestrial ecology as well as agroecology. Understanding these fungi has given ecologists a measure of predictive power of many ecosystem phenomena, from plant community development to soil genesis. AMF research and the desire for application of these symbionts have developed very rapidly and gaps in our understanding of the basic biology and life history of them is becoming more apparent. For instance, how do changing soil variables like pH affect abundance, community composition, and even functioning of AMF through time? The work presented here as a whole will hopefully aid our current understanding of the dynamics and controls of AMF during site development in natural systems, increase our knowledge of plant AMF interactions with respect to the ecosystem process of soil stabilization, and describe necessary considerations for effective application of AMF in the agriculture and restoration industries.

Mycorrhizal abundance can vary considerable during site development, potentially affecting plant establishment, community development, and soil aggregation among other things. The work presented in chapter 2 describes changes in two mycorrhizal groups, AMF and ectomycorrhizae (ECMF), during soil development of an unregulated floodplain. Floodplains of free-flowing rivers are some of the most critically threatened ecosystems. My work describes the brief “window” of AMF proliferation in

this system. These data are very significant to both understanding the development of floodplain plant communities, often representing the greatest diversity within a region, as well as the effect of river regulation. As flow regulation reduced flood intensity and the creation of young successional sites, intense regulation has the potential to reduce AMF abundance in the floodplain and potentially to downstream soils. From a floodplain management perspective, these data are valuable to effort to maintain floodplain plant diversity and soil carbon storage.

Exogenous controls on AMF abundance have been well explored since the early days of the discipline. When AMF were thought to be uniform in benefit, the goal of AMF management was to maximize soil inoculum available to the host. To date several edaphic variables have shown strong correlation with AMF abundance: soil phosphorus, soil pH, soil moisture, soil N:P ratio. My work describes another largely unexplored control on AMF inoculum, soil phenolics. I have shown that these powerful, litter derived biochemicals can strongly inhibit AMF colonization by an entire native AMF community. Moreover, these effects do not seem to result from alteration of soil nutrient status via these litter leachates. This is the first to describe the effects of the litter of a plant capable of hosting both AMF and ECMF on these largely beneficial symbionts, and hints at a novel mechanism by which plants that can host both mycorrhizal types may select their symbionts and potentially reduce competition from AMF dependent species.

As AMF communities can change in abundance over time, so to can AMF community composition, regardless of host changes. These fungi can differ in their physiologies and host benefits, and this may depend on the specific host fungi combination. This work is the first to describe the interaction between host and AMF of

naturally co-occurring species with respect to soil stabilization. These data are very relevant to both ecosystems and AMF applications. This is a valuable finding in that it suggests that specific AMF/ host combinations might be selected to maximize soil stabilization. Additionally, with increased knowledge of native AMF and the effects of time and exogenous factors, fields may simply be managed to promote species of AMF that are most adept at a desired function.

Despite the known benefits of the arbuscular mycorrhizae condition, consistently benefits of AMF application are yet to be achieved. These inconsistencies, and even negative growth responses of crops could be a result of displacement of beneficial AMF resulting from changing soil conditions over time. This work attempts to identify significant yet unexplored causes for lack of applied AMF benefits. Researchers must shift focus from simply applying AMF, to applying the most beneficial AMF/ host combinations and/ or managing for their persistence. Moreover, I suggest that indigenous AMF communities can be stimulated and even their composition selected by managing the successional pressures of the abiotic and biotic environment.

My work with the ECOS program at the University of Montana provided insight into the appalling state of soils education not only at the elementary level, but also at the undergraduate level. The soil ecosystem is the basis of societies, and preservation of a countries soil health will ultimately rely in part on the knowledge of the voting populous. I have spent considerable time designing a series of lessons on soil ecology that are accessible to all elementary teachers of even the youngest students, one of which is presented here. This work will be accessible to all teachers via publication and the ECOS website and was designed with the goal of demonstrating that many principles of biology

and ecology, from organisms to ecosystem function, can be taught through exploring the soil environment.

In conclusion, the importance of AMF on the ecosystem scale is apparent; however, to explain the patterns and significance of AMF diversity, more research is necessary at the physiological level of these fungi. With less than 200 species of AMF worldwide, we still have a striking lack of information on how these species differ with respect to their known functions. To fully predict how a change in AMF species composition will affect ecosystem processes, either seasonally, yearly, or longer, there needs to be greater information of the unique contributions of individual AMF species and ecotypes. This research will not only aid our understanding of ecosystem development, but also yield more effective applications of AMF employing the most beneficial plant/ fungus combinations.

Appendix

Exaction of total DNA from floodplain soil

Introduction

I initially proposed to characterize changes in the arbuscular mycorrhizal fungi (AMF) community composition across the Nyack chronosequence. To date molecular characterization of AMF succession during floodplain development has not been documented. Beauchamp et al. (2007) characterized floodplain AMF based on spore morphology; however, as not all species of AMF sporulate (Helgason et al. 1999) total community assessment cannot be achieved through spores alone. Based on my data of AMF abundance across the chronosequence and observations of AMF host diversity, I predicted that AMF diversity would follow a pattern similar to abundance, diversity would be greatest in early to middle aged sites and decline at older sites as more ectomycorrhizal host establish. To test this hypothesis I choose to characterize changes in the AMF community based on terminal restriction fragment length polymorphisms (T-RFLP) analysis using the large subunit (LSU) of the ribosomal repeat.

The extraction of total soil DNA has been widely employed for nearly a decade and many methodologies have been developed. One of the most common method of extraction is through the use of commercially produced extraction kits. While these kits have documented shortcomings when used with soils of high clay, organic matter, or humic material (Whitehouse and Hottel 2006), they have proven an efficient method for many soil types. Therefore, I initially choose to use a “kit based” methods for DNA extraction of soils from the Nyack floodplain.

Methods, results, and conclusions

I collected soils from aged sites along the floodplain describes in Chapter 2 (5 replicates per site). We collected 25 ml of soil from the top 10 cm of soil beneath the litter layer and immediately placed the samples on dry ice for transport until storage at -20 C. To extract DNA I initially used a the MoBio UltraSoil extraction protocol . Previous work had indicated that 0.25-0.4 g of soil was optimal for extraction (Mummey and Rillig 2006). Following extraction we attempted to amplify AMF DNA using the nested protocol described by Gollotte et al. (2004), employing the LR1 and FLR2 primer set for the first reaction and the FLR3 and FLR4 set for the second reaction. The reaction is nested to achieve a high specificity and amplification of AMF DNA. We tested dilutions of 1X, 1/10X and 1/100X to optimize PCR product. Additionally, we used labeled FLR3/ FLR4 primers to generate product for T-RFLP analysis. While we had weak amplification with 1/10X dilutions using the unlabelled primers, the FAM and HEX labeled primers did not allow for successful amplification of our samples despite amplification of the positive control (genomic *Glomus intraradices* DNA). These extractions and amplifications did not provide a sufficient amount of DNA for T-RFLP analysis. We also tested if using only one labeled primer (FLR3-FAM) would aid amplification as these flours can reduce PCR efficiency. Nevertheless, no product was detectable at any of our tested dilutions.

I decided that the extraction trouble may be a result of low amounts of soil DNA. AMF hyphal lengths are very low across the chronosequence compared to grassland soils, especially at the earliest sites. I decided to increase the amount of soil extracted to 1

g, by combining 2 separate extractions of 0.5 g of a replicate soil using silicone capture after a phenol chloroform extraction. This method also proved fruitless.

I further decided that I needed to extract a much larger volume of soil. I had the option of testing the MoBio MegaSoil kit, designed to extract total DNA from up to 10 g of soil. These kits are considerable expensive given my number of samples ($N=45$), and that they have questionable extraction efficiency because of decreased ability to achieve high cell disruption because of the high volume of soil (Dan Mummey, pers. obser.). I decided to use a method described in Lord et al. (2002). This paper describes DNA extraction from soils similar to the floodplain soils, sandy with low microbial biomass. This is a modified kit based method that allows for the extraction of DNA from many grams of soils through a final silicon capture step of the pooled extracts. We extracted three samples (1 g each) from each of the replicate soils using the MoBio Ultraclean kit. These were pooled together with previously extracted samples from each replicate sample for a total of 3.8 g of soil extracted from each replicate site. I added binding solution to the pooled extracts and these were filtered through a silicon membrane, washed with ethanol, and eluted with 50 μ l of TE buffer.

Following extraction we amplified the extracted DNA using the nested reaction as described above with the single label second reaction (FLR3-FAM/ FLR4) across a range of dilutions (1X, 1/10X, 1/100X, 1/250X, 1/500X). I detected significant amounts of product in all of the tested samples, with some variation in the dilution that gave the strongest banding. The youngest sites had the greatest product using a 1X dilution, supporting our hypothesis that the soils have exceedingly low amounts of AMF DNA. The older soils yielded good amplification at dilutions of 1/100X and higher. This

method of extraction, while labor intensive compared to other kit based methods, offers a simply way to extract DNA from large amounts of soils when required, and may be used for future studies of AMF along floodplain.

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