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

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Abstract

Controls on the colonization and abundance of arbuscular mycorrhizal fungi (AMF) in ecosystems are little understood and may be related to host factors, the fungal community, and soil physio-chemical 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 black 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. We tested pure soluble phenolic compounds common to *Populus* 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 within both a single host and soil are discussed.

Keywords: Arbuscular mycorrhizae; Litter leachates; Soil phenolics; Mycorrhizal succession; *Populus*

1. Introduction

Arbuscular mycorrhizal fungi (AMF) are a group of soil fungi that form mutualistic 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, 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

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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, 1990; 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; 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). Mutualists, 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 AMF 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. Yun and Choi (2002) recently demonstrated that extracts from *Artemisia princes* 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 (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 production across genotype, age, and environmental gradients (Mansfield et al., 1999; Donaldson et al., 2006). Early studies have described foliage from members of this genus, and other tree species, as inhibitory to some ectomycorrhizal fungal species (Olsen et al., 1971; Conn and Dighton, 2000; Jonsson et al., 2006); 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* leaves on AMF colonization, the second tests a range of dilutions of litter leachate 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.

2. Methods

2.1. 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 2 days; then the leaves were pulverized using a Waring blender. We pulverized vermiculite to use as an inert control; it is commonly incorporated into growth media with AMF-colonized plants without strongly altering the soil physio-chemical environment.