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Soil Biology & Biochemistry 40 (2008) 709–717

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

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

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Received 26 June 2007; received in revised form 10 October 2007; accepted 11 October 2007
Available online 8 November 2007

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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doi:10.1016/j.soilbio.2007.10.005

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.

We grew plants in 250 ml Cone-tainer^{tm} (Stewe and Sons Canby OR, USA) pots containing a homogenized mixture of 90% air-dried field soil from a 9-year-old site on the Nyack floodplain, 5% (v/v) whole vermiculite for aeration, and 5% of either pulverized cottonwood leaves or vermiculite as an inert control. The Nyack floodplain is located in northwestern Montana (48°27′30″N, 113°50′W) on the Middle Fork of the Flathead River, and is a fifth order, free-flowing river with protected headwaters. To enhance ecological realism, we selected the Nyack soil because it represented a soil with low organic matter (0.6%), low phosphorus ($\sim$2 mg kg^{-1}), and high native inoculum potential (Jeff Piotrowski unpublished observation). Because the soil was air-dried prior to use, the infectious AMF propagules were mostly spores, as active hyphae and root fragments may have been desiccated and infectivity reduced. Each treatment had 7 replicates ($n = 14$). We germinated surface-sterilized *Sorghum sudanese* (Stapf.) seeds (5% H_2O_2 for 5 min) prior to sowing, and 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 2 months in greenhouse conditions with watering every 2–3 days.

2.2. 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 dH_2O for 3 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 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 1×, 0.5×, 0.25×, 0.1×, 0.01×, 0.001×, and 0.0001×, 0.00001×. All dilutions were adjusted to pH 7.0 using 1 M NaOH.

We grew plants in 125 ml Cone-tainer^{tm} (Stewe and Sons Canby OR, USA) pots containing a homogenized mixture of 100% air-dried field soil from a 9-year-old site 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 2 months in growth chamber conditions (25 °C, 60% R.H., 320 μmol s^{-1} P.A.R) and watered every 2–3 days with 20 ml of the prepared litter extract dilutions or dH_2O as a control. Each treatment had 5 replicates ($n = 45$). We harvested and stained plant roots for assessment of AMF colonization as described below.

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 1× litter extract every 2–3 days, the others with water for 2 months ($n = 12$). Root growth of the cottonwood seedlings was very low and not assessed for biomass.

2.3. 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 estimated the total phenolic concentration of the cottonwood litter extract we prepared prior using the Folin–Cioteau assay described by 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., 2005; Suominen et al., 2003). Based on our prior experiment, 0.5× 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 100 ml of 50:50 mixture of field soil and trap culture medium to ensure a high colonization potential. We made the trap culture medium by growing *Sorghum* in a 50:50 mixture of sand and fresh field soil for 3 months to amplify the AMF community from the field soil, after which we separated the roots from the soil and used the soil alone our experiments *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 1 month.

2.4. 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 Brundett (1994). We assessed mycorrhizal colonization at 200 × 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 that 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.

2.5. Soil analysis

We determined soil pH using a 1:1 (soil: 0.01 M $CaCl_2$) 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.

2.6. 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 normality. We log transformed the data if these assumptions were not met. If transformation did not allow data to fulfill 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 for 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).

3. Results

3.1. The effects of cottonwood derived organic matter on AMF colonization

The first experiment indicated that an addition 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). Other non-AMF fungi with regular septation, melanization, and/ or microsclerotia intermittently colonized roots in the presence of the litter addition. 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).

3.2. The effects of aqueous extract of cottonwood litter on AMF colonization

Aqueous extract of cottonwood litter significantly reduced AMF root colonization of *S. sudanese* (Fig. 1, $F = 2.74$, $P<0.05$). Colonization ranged from 51% with the water control to 6% when treated with 0.5 × dilution of the extract. Seedlings receiving 0.5 × and 1 × dilution

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[a]	Soil pH[b]	Soil phosphorus (mg kg^{-1})[a]
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 the Mann–Whitney *U* test.
[b]Comparisons were made using Student's two sample *T*-test.

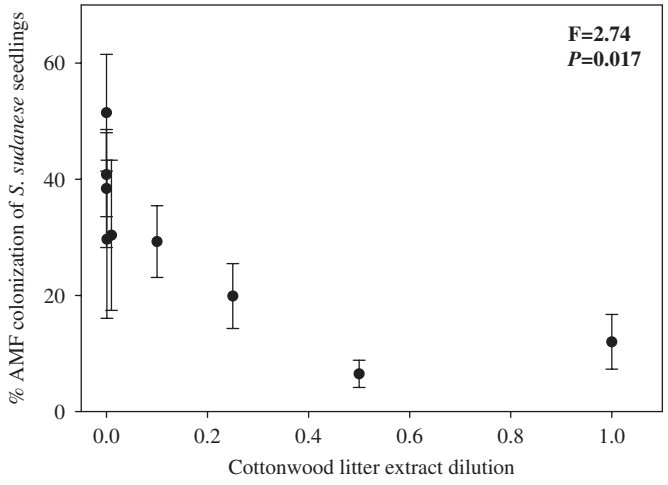

Fig. 1. Percent AMF colonization of *S. sudanese* when treated across a range of cottonwood litter extract dilutions (mean ± standard error). ANOVA *F* statistic and *p*-value are presented. Multiple comparison using Tukey's HSD test indicated AMF colonization of seedlings receiving 1 × and 0.5 × dilutions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

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 $0.25\times$ than treatment receiving $1\times$, 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 $0.5\times$ and $1\times$ dilutions had higher soil pH than treatments receiving $0.001\times$ and $0.1\times$, all other comparisons had no detectable differences. Available soil phosphorus differed across the litter extract treatments (Table 2, $F = 3.8$, $P < 0.05$), with all treatments except the $0.25\times$ and $0.5\times$ significantly lower in soil phosphorus than the $1\times$ addition. Total soil phenolics significantly increased with litter extract additions (Table 2, $F = 21.9$, $P < 0.0001$). Multiple comparisons are presented in Table 2.

Colonization was significantly and 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.

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

Table 2
Comparison of *S. sudanese* seedlings and soil parameters across the dilution of cottonwood extract (mean ± standard error)

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

Lettering indicates significant difference between treatments as determined by Tukey's test or the Kruskal–Wallis Z-test.*$P < 0.05$, ***$P < 0.0001$.
[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.

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 1 × cottonwood litter extract or water (mean ± standard error)

Cottonwood extract treatment	% AMF colonization[a]	Shoot biomass (g)[a]	Soil pH[a]	Soil phosphorus ($mg\,kg^{-1}$)[b]	Total phenolics ($mg\,kg^{-1}$)[a]
Control	37.6 ± 7.3	0.05 ± 0.01	7.80 ± 0.03	2.7 ± 0.8	11.0 ± 0.5
Full strength	5.8 ± 1.3	0.02 ± 0.01	7.88 ± 0.01	8.9 ± 0.9	36.0 ± 2.6
	$Z = -2.74$**	$Z = 3.13$**	$Z = 1.6$	$T = -5.08$**	$Z = 2.89$*
	$n = 12$	$n = 12$	$n = 10$	$n = 10$	$n = 10$

*$P<0.05$, **$P<0.001$.
[a]Comparisons were made using the Mann–Whitney U test.
[b]Comparisons were made using Student's two sample T-test.

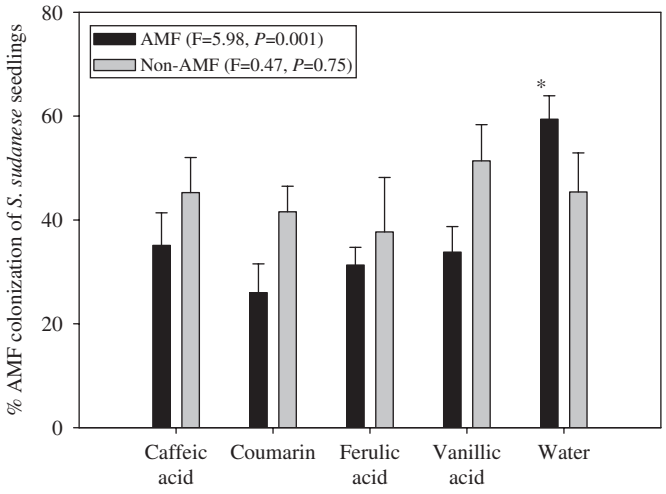

Fig. 2. Percent root colonization of *S. sudanese* by AMF and non-AMF fungi when treated with either specific phenolic compounds or water (mean ± standard error). Black bars indicate AMF colonization of roots and white bars indicate other root-colonizing non-AMF. ANOVA F statistic and p-value are presented. "*" 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-AMF ($n = 40$).

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)[a]	Root biomass (g)[a]	Soil pH[b]	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]Indicates these data were log transformed prior to ANOVA.
[b]Comparisons were made using the Kruskal–Wallis test.

4. 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.

4.1. 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 phosphorus 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 0.5–1 × dilutions of litter extract added to *S. sudanese* 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).

Additionally, with the exception of plants receiving the 0.25 × 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}, lower than what has proven inhibitory to AMF colonization (Graham et al., 1981; Blanke et al., 2005). Moreover, at the 0.5× 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 (Fig. 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 because 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 0.5× 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., 2005; Suominen et al., 2003).

These leachates and soluble phenolics derived from cottonwood litter could reduce AMF colonization through several mechanisms. Soluble phenolic compounds, such as 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, such as hyphal networks and colonized root fragments. If only spores are affected, accumulation of phenolics could result in a shift to an 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. 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 extract than with the pure phenolics, suggesting increased overall toxicity of the extract containing a number of different phenolics that may have additive effect on AMF inhibition, stimulation of antagonistic organisms, or a combined effect of both. Even so, AMF do not produce any documented extracellular enzymes capable of detoxifying phenolic compounds, whereas other fungi do (Münzenberger et al. (2004); 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.

4.2. 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 Hackskaylo, 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 mantle 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 mutualist community. The studies presented explored an AMF response on *Populus*; further research is necessary on this mechanism with regard to ECMF, taking into account the variable response these fungi to litter chemistry and phenolics (Koide et al., 1998; Jonsson et al., 2006).

From a succession 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.

5. 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

phenolics in ecologically realistic concentrations within both soil and litter leachates could be another driving force in AMF succession. Future studies that test a variety of litter and litter extract from AMF hosting, ECMF hosting, and tree species that host both mycorrhizal groups have the potential to yield greater insight into the controls and changes in these symbiont communities during soil and plant community development.

Acknowledgments

MCR acknowledges financial support from the National Science Foundation (DEB 0613943). JSP was supported on an NSF GK-12 ECOS fellowship.

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