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Short communication

Adaptation of *Liquidambar styraciflua* to coal tailings is mediated by arbuscular mycorrhizal fungi

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ABSTRACT

We performed a full factorial greenhouse experiment in order to determine if utilizing seedlings of *Liquidambar styraciflua* or communities of arbuscular mycorrhizal (AM) fungi, originating from coal mine conditions, could improve plant survivorship or accelerate revegetation of abandoned coal sites. Trees from the mine grew significantly more slowly than trees from natural areas. Both plant populations grew relatively better in their own soil type. Moreover, an AM symbiosis appeared to mediate local plant adaptation. Mine-soil adapted seedlings were more responsive to AM fungal colonization when grown in mine soil whereas the seedlings from natural areas were more responsive to AM fungal colonization when grown in Indiana's native low nutrient clay soil. AM fungal communities originating from the mined area showed significantly greater colonization levels in mine soil than in native soil, suggesting adaptation of AM fungi to mine-soil conditions. These results suggest that reclamation efforts could be improved by starting with plants and AM fungal communities which are already adapted to the target site.

1. Introduction

Studies have demonstrated plant adaptation to harsh environments, including exposure to toxins such as metals, contaminants from mining operations and other anthropological disturbances (Rincon and Gonzales, 1992; Samuels et al., 1997). AM fungi are impacted by disturbance (Stahl et al., 1988); yet are known to exist in these harsh environments (Khan, 1978; Selvam and Mahadevan, 2002; Selvaraj et al., 2005). Many studies have examined their potential for remediating sites contaminated with heavy metals, as reviewed by (Gaur and Adholeya, 2004). Yet few studies have examined their contribution to plant adaptation, particularly in the context of AM fungal adaptation to harsh edaphic conditions at the community level.

AM fungi associate with a broad range of plant species and might be expected to contribute to plant adaptation by mediating plant uptake of essential soil nutrients and heavy metals (Dosskey and Adriano, 1993; Harrison and Van Buuren, 1995; Lin et al., 2007; Zhang et al., 2009). Cumming and Ning (2003) showed that AM fungi enhanced the aluminum resistance of *Andropogon virginicus*, a possible survival mechanism on coal tailings, where pH is low and aluminum content is commonly high. Moreover, some AM fungi isolated from heavy metal contaminated soils have been shown to be more tolerant to heavy metals than fungi from natural sites (Gildon and Tinker, 1981) although their responses varied (Kelly et al., 2005). Still, this raises the possibility that mine-adapted fungi can influence the successful adaptation of plants to mine soils.

Heavy metal toxicity is not the only aspect of mine-tailings that challenge plants' ability to persist in mine soils. Unamended coal mine tailings can have low levels of N and P, and low water holding capacity. Ning and Cumming (2001) demonstrated that AM fungi helped *Andropogon virginicus* maintain nutrient homeostasis and improved phosphorus uptake, suggesting that these traits are important adaptations to survival on abandoned coal mines. AM fungi also have been shown to alleviate drought stress (Beltrano and Ronco, 2008; Ruizlozano et al., 1995), an additional risk in mine soils. AM fungi also may benefit plants suffering compaction stress (Miransari et al., 2007), which can be a problem in both amended and unamended soils. Taheri and Bever (in press) found that *A. virginicus* from coal mines grew slower than plants collected from natural sites, and that *P. lanceolata* from coal mines allocated more biomass to roots, both physiological traits being potential adaptations to drought. They also found that these plant species were more responsive to the fungal community originating from the coal mine. We follow-up the study by Taheri and Bever (in press) by utilizing *L. styraciflua* (sweet gum), a tree commonly found colonizing abandoned coal mines in Indiana. The goal of this study was to determine if using plants or AM fungi derived from the harsh

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edaphic conditions of coal mines could facilitate reforestation of these highly disturbed sites.

2. Materials and methods

We performed a full factorial, greenhouse experiment using plant seeds and fungal communities from mined and natural areas in Indiana. The experiment was replicated ten times for the natural population of plants and six times for the mine population of plants. Unequal replication was due to fewer seeds being collected from mines and lower germination rates for those seeds. A total of 96 trees (48 trees in each soil type) were grown under greenhouse conditions.

2.1. Site characteristics

The mine-soil inoculum (FM) and the mine soil (MS) originated from an abandoned coal mine designated Site 289 by the Indiana Department of Natural Resources, and located in Greene County, Indiana, which is two miles east of Midland, Indiana. This was an abandoned strip mine that had been mined for three years then left fallow for ten years. It was dominated by a large sludge pond filled with cattails (*Typha* spp.). Sassafras (*Sassafras* spp.), oak (*Quercus* spp.), maple (*Acer* spp.), gum (*Liquidambar* spp.), pine trees (*Pinus* spp.), as well as herbaceous plants including broom sedge (*Andropogon* spp.), true sedges (*Cyperus* spp.), golden rod (*Solidago* spp.), black-eyed Susan (*Rudbeckia* spp.), and other forbs were the dominant plant species in the area. The water in the sludge pond was the bright orange color typically associated with acid mine runoff, commonly called yellow boy.

Our natural site was located in Greene County, Indiana, five miles west of Site 289. We designated this as Site 600. It had been mined and restored by the DNR ten years previous. There was a lake nearby and many native species, including the species found at Site 289. Pine trees were far fewer at Site 600, and overall diversity was greater, particularly among the forbs and woody shrubs. Given that the surrounding area was planted in remedial monocultures and lacking trees, we suspected this was an undisturbed remnant or small ridge area that was missed while the surrounding area was mined.

2.2. The fungal communities

Plants and soil were collected from an area around the sludge pond at Site 289 and around the lake at site 600. Spores were extracted using the sucrose centrifuge method (Bever et al., 1996). The AM fungal community from Site 289 was dominated by *Paraglomus occultum*, but also harbored *Glomus mosseae* and an undescribed species of *Acaulospora*. The AM fungal community from Site 600 had a higher diversity, based on morphotypes, but also contained a large fraction of *P. occultum* and *G. mosseae* with fewer spores from *Acaulospora* species.

2.3. Preparation of inocula

Direct extractions from the two sites were used to inoculate a previous experiment (Taheri and Bever, in press). Negative controls (F0 treatments) for that experiment were a filtered microbial rinse that excluded fungal hyphae and spores (Taheri and Bever, in press). Pot cultures of *Andropogon virginicus* and *Plantago lanceolata* were reserved from that experiment, which were grown in the same soils used in the current experiment, and under similar greenhouse conditions. Plants were watered daily, given 16 h of light and fertilized weekly with 50 ml of Peterson's 15–0–15 fertilizer, diluted to 5.5 g/L. After 120 days, pot contents were removed and roots from plants that received a microbial rinse were sampled, cleared, stained and verified microscopically to be free of mycorrhizal colonization. Roots then were chopped to roughly 2 cm in length and mixed with soil for the designated treatment, in a 10-fold dilution. This became the FM (fungi from the mine), FN (fungi from the natural site) and F0 (no AM fungi) inocula.

Extractions were performed on the FM and FN inocula. Both were found to be dominated by *Glomus mosseae* spores, but probably contained hyphal propagules from other AM fungi as well. Inoculum potential was measured by growing ten replicates of sorghum in a mixture of 1/10 inocula with sterile soil in a glasshouse and scoring root colonization after 30 days. The density of colonization resulting from the two inocula did not differ significantly ($T_7 = 1.67$, p-value = ns).

Table 1
Soil characteristics for the mine soil and low nutrient clay soil used in the experiment.

Measurement	Mine soil	Low nutrient clay soil
Al	16,333	20,193
N	*	1200
P	287	500
K	3033	1500
Ca	1907	4900
Mg	807	2200
Na	433	100
S	1077	300
Fe	17,166	19,723
Zn	58	82
Mn	35	1156
Cu	31	18
B	127	8

* Chemical analysis of the coal tailings was based on data collected by the Indiana Geological Survey for coal seams in the area and did not include nitrogen. All measurements are in ppm.

2.4. Soils

The mine soil (MS) was composed of coal tailings from Site 289 in Greene County, Indiana. The native soil (NS) was a low nutrient, Indiana clay soil from a road cut near Bloomington, Indiana, in Monroe County. Both soils were passed through a one inch screen then mixed in equal parts with sand, prior to being steam pasteurized for two hours each day for three successive days. The sterilized media was then mixed in a 1/10 ratio with their respective inocula. Organic matter was less than 3% in both soils. The coal tailings had a much lower pH (3.8) than the low nutrient soil (7.7). Macronutrients were below levels considered necessary for plant growth with the exception of calcium and magnesium in the low nutrient soil, and sulfur in the mine soil. Iron and aluminum levels were high in both soil types. Manganese was high in the low nutrient soil and boron occurred in the mine soil at potentially toxic levels. Cation exchange (CEC) was estimated from milliequivalents of the bases K, Mg, Ca and Na, revealing the low nutrient soil to have a higher CEC (23.55) than the mine soil (12.95). This was driven by the higher calcium and magnesium levels in the low nutrient soil. The mine soil was coarser in texture than the low nutrient soil and water filtered through it more quickly than it did through the low nutrient soil. Table 1 provides a chemical analysis of both soils.

2.5. Seeds

All seeds were cold stratified for ninety days at 3 °C then surface sterilized for ten minutes in ten percent chlorine bleach before being sown into germination trays of autoclaved Sun Gro Metro-Mix® 360. Seedlings were transplanted into their treatments as soon as they looked sturdy enough for handling (approximately sixty days). Plants were grown in 656 ml Deepots™ (Stuewe & Sons,

D40 type H), which were 25 cm. deep. Pots were randomized within blocks and blocks were randomized on the bench. All plants were watered daily and fertilized once per week with 50 ml. of Scotts Peters Professional 15–0–15 fertilizer, diluted to 5.5 g/L. Natural light was supplemented by high pressure sodium lamps to provide an 18 h day. Temperatures ranged roughly between 25 and 30 °C. Harvest of the entire experiment occurred at the first visible signs of roots penetrating the drainage holes of the pots, which was ten months after transplanting.

2.6. Statistical analysis

Plant biomass was analyzed using SAS software's GLM procedure (SAS version 9.1). Total biomass (TBM) was replaced by ranks to improve homogeneity of variance. Root–shoot ratios and percent root colonization were arc-sin-square root transformed (Ars) in order to improve homogeneity of variance. Plant population, soil type, inoculum, percent root colonization and all interactions were tested using analysis of variance, with initial size at time of transplanting as a covariate. The inoculum main effect and interactions were decomposed into two orthogonal *a priori* contrasts: one for the average effect of mycorrhizal inoculum and the second for differences between the two inoculum sources.

3. Results

Liquidambar styraciflua was highly responsive to colonization by AM fungi, showing little growth and poor survivorship among plants without the AM symbiosis, which averaged 0.21 g (std. dev. 0.15) compared to mycorrhizal plants, which averaged 10.86 g (std. dev. 5.39) (Table 2, p-value < 0.0001). Overall *L. styraciflua* was 16% larger when grown with fungi from a natural area (FN) than with mine fungi (FM) (Table 2, p-value = 0.03). Mycorrhizal *L. styraciflua* allocated more resources to roots based on a mean root–shoot ratio of 0.73 (std. dev. 0.08) compared to 0.65 (std. dev. 0.16) (Table 2) in non-mycorrhizal plants.

Plants derived from mines grew more slowly than plants from natural areas, supporting the hypothesis that these populations were genetically differentiated. The total biomass of mine plants averaged 7.2 g, whereas plants from natural areas averaged 9.3 g (Table 2, p-value = 0.0004). Significant two-way interactions support the hypothesis of local adaptation, as both mine and natural plant populations grew best in the soil type from which they were derived (Fig. 1, Table 2, p-value = 0.04). A significant three-way interaction in the growth response of *L. styraciflua* supports the hypothesis that local adaptation is mediated by mycorrhizal fungi, as both populations were most responsive to mycorrhizal fungi when grown in the soils from which the plants were collected (Fig. 2, Table 2, p-value = 0.04).

Liquidambar styraciflua did not grow differently with the two inocula types (Table 2), but the percent colonization data suggests that the two fungal communities differed in their response to soil type. Fungal communities originating from native soil established high colonization in both soil types. However, the fungal community originating from the coal mine colonized roots at a significantly higher rate in mine soil (Fig. 3 and Table 2, p = 0.0024).

4. Discussion

Liquidambar styraciflua from mines grew better in mine soil than in the native soil, while plants from natural areas performed better in the native soil suggesting that the populations were better adapted to their local soil type. *Andropogon virginicus* from coal mines in Indiana showed a similar reduction in growth rates (Taheri and Bever, in press) suggesting that the reduced growth rate may be a component of adaption to the mine soils. Slow growth rates may be correlated with higher survival rates in the nutrient deprived and drought-prone environment of the mine soil. In a comparative study of Amazonian rainforest trees, for example, Laurance et al. (2004) found that slower growing trees had significantly higher longevity.

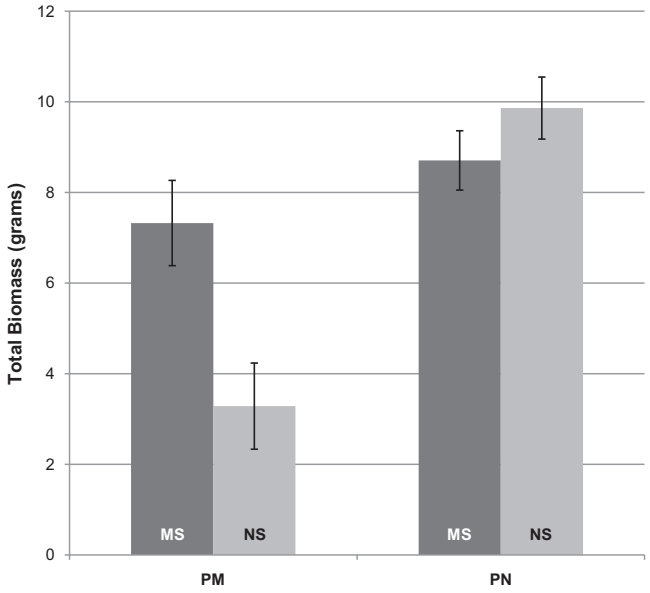

Fig. 1. Two-way interactions between soil type and plant ecotype indicate local adaptation. Plants from the mine (PM) produce more biomass in mine soil (MS) than in native soil (NS), while plants from natural areas (PN) performed best in the native soil (p = 0.04).

Adaptation of *L. styraciflua* to the low nutrient soil appeared to be mediated by plant-AM fungal interactions. *Liquidambar styraciflua* was highly responsive as evidenced by higher root–shoot ratios and a 50-fold increase in biomass. The greatest benefits of inoculation with mycorrhizal fungi occurred when plant ecotypes were grown in soils from which they were collected. Of published examples of microbial mediation of plant adaptation, most involve shifts in responsiveness during adaptation (Schultz et al., 2001; Seifert et al., 2009; Taheri and Bever, in press; Thrall et al., 2008). Only Johnson et al. (2009) provided evidence of greater responsiveness in their own soil. This study provides the second example of such a pattern.

We saw differential adaptation of fungal communities to the mine soil, but despite this plant performance was not improved.

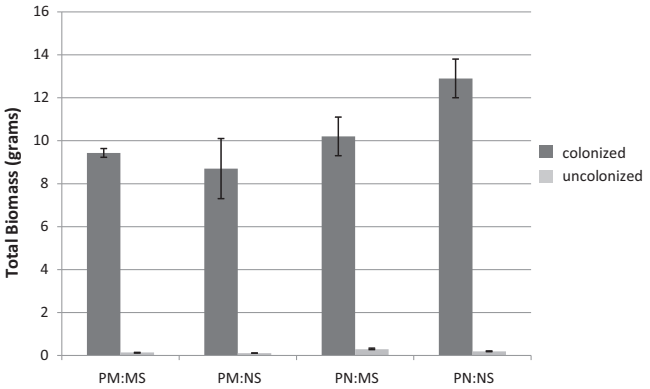

Fig. 2. Three-way interactions show evidence of local adaptation. Mycorrhizal colonization confers greater benefits to mine plants (PM) when they are in mine soil (MS), and to plants from natural areas (PN) when they are in native soil (NS), (p = 0.04). Colonized plant groups include fungal treatments FM and FN combined. Uncolonized is fungal treatment F0.

Table 2
Statistical results for plant biomass, root–shoot ratios and percent root colonization. Feco represents fungal ecotypes: FM, FN and F0 treatments. FM is inoculum originating from a mined area (Site 289). FN is inoculum derived from a more natural area, Site 600. F0 lacks AM fungi. Live includes both FM and FN treatments. Av inoc includes both FM and FN treatments. Peco is plant ecotype. TBM is total biomass measured in grams. Ars indicates data was arcsin-square root transformed. R/S provides root/shoot ratios in grams.

Total biomass (TBM replaced by ranks)				Root–shoot ratios (Ars)		
Source	DF	SS	*p*-value	DF	SS	*p*-value
Block	9	3524	N/S	9	0.1322	N/S
Soil	1	100	N/S	1	0.0045	N/S
Feco	2	34,813	<.0001	2	0.1751	0.0007
Live vs. Sterile	1	32,469	<.0001	1	0.1683	0.0002
FM vs. FN	1	1230	0.03	1	0.0027	N/S
Soil * Feco	2	852	N/S	2	0.0317	N/S
Live vs. Soil	1	819	0.07	1	0.0171	N/S
(FM vs. FN) * soil	1	28	N/S	1	0.0151	N/S
Peco	1	3374	0.0004	1	0.3509	<.0001
Soil * Peco	1	1072	0.04	1	0.0003	N/S
Peco * Feco	2	274	N/S	2	0.1685	0.0009
Live vs. Peco	1	256	N/S	1	0.1561	0.0003
(FM vs. FN) * Peco	1	22	N/S	1	0.0096	N/S
Soil * Peco * Feco	2	1473	0.05	2	0.0103	N/S
Av inoc three way	1	1040	0.04	1	0.0087	N/S
(FM vs. FN) 3-way	1	466	N/S	1	0.0018	N/S
Error	73	17,568		73	0.7932	

Percent root colonization (Ars)

Source	DF	SS	*p*-value
Block	9	0.3491	0.6205
Soil	1	0.4230	0.0047
Feco	1	3.0562	<0.0001
Soil * Feco	2	0.6578	0.0024
Total Biomass	1	0.3088	0.0148

Percent root colonization (Ars)

Source	TBM	Source	R/S
FM	9.5	Inoc	0.73
FN	11.1	Uninoc	0.65
F0	0.2		
PM	7.2		
PN	9.3		

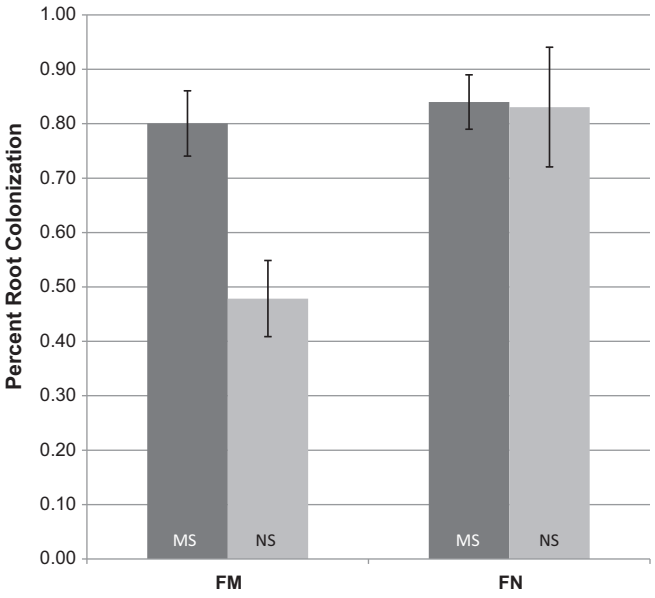

Fig. 3. The AM fungal community originating from the mine (FM) has a significantly higher percentage of root colonization when in mine soil (MS), while the community from a natural area (FN) seems indifferent to soil type ($p = 0.0024$). NS is native soil (Indiana low nutrient clay soil).

Rather, we found that the natural fungal community from native soil better promoted growth of *L. styraciflua* regardless of the soil in which they were grown. In contrast, AM fungal communities originating from mined areas were better growth promoters of *Andropogon virginicus* and *Plantago lanceolata* in both mine soil and low nutrient soil (Taheri and Bever, in press). This difference could be caused by a change in composition or reduction in diversity of the AM fungal community. A year of cultivation under greenhouse conditions may have attenuated their beneficial properties, as many of the stresses to which they were adapted were alleviated under greenhouse conditions. Other studies have shown that the benefits of the symbiosis depend upon plant responsiveness and fungal composition (Bever, 2002). These benefits can increase with greater diversification of the AM fungal community (van der Heijden et al., 1998; Vogelsang et al., 2006). Alternatively, the difference could reflect a longer lag time to full expression of growth benefits in trees compared to grasses and herbaceous plant species. Our observation that plants do not do best when fungal communities are matched with their soil of origin is in contrast to other studies of local adaptation (Johnson et al., 2009; Stahl et al., 1988). It is possible, however, that mine-adapted fungi would better promote plants under other environmental conditions, such as drought or soil compaction. Overall, our study indicates complex interactions may be involved in adaptation given the range of stresses presented in a coal mine, and that community composition can change quickly in response to alteration of these environmental stresses, such as

being removed to greenhouse conditions. Yet in spite of this, we still saw local adaptation in the three-way interactions between plant ecotype, soil and plant responsiveness to AM colonization as well as local adaptation of AM fungi to soil type. This emphasizes the importance of matching both plants and fungi to soil conditions.

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