

# Testing nickel tolerance of *Sorghastrum nutans* and its associated soil microbial community from serpentine and prairie soils

Jennifer H. Doherty\*, Baoming Ji, Brenda B. Casper

Department of Biology, University of Pennsylvania, 415 S. University Avenue, Philadelphia, PA 19104-6018, USA

Received 20 September 2006; received in revised form 19 March 2007; accepted 8 April 2007

*Ni tolerance of Sorghastrum nutans differs slightly between serpentine and prairie populations and is negatively affected by serpentine soil and root inoculation.*

## Abstract

Ecotypes of *Sorghastrum nutans* from a naturally metalliferous serpentine grassland and the tallgrass prairie were assessed for Ni tolerance and their utility in remediation of Ni-polluted soils. Plants were inoculated with serpentine arbuscular mycorrhizal (AM) root inoculum or whole soil microbial communities, originating from either prairie or serpentine, to test their effects on plant performance in the presence of Ni. Serpentine plants had marginally higher Ni tolerance as indicated by higher survival. Ni reduced plant biomass and AM root colonization for both ecotypes. The serpentine AM fungi and whole microbial community treatments decreased plant biomass relative to uninoculated plants, while the prairie microbial community had no effect. Differences in how the soil communities affect plant performance were not reflected in patterns of root colonization by AM fungi. Thus, serpentine plants may be suited for reclamation of Ni-polluted soils, but AM fungi that occur on serpentine do not improve Ni tolerance.

© 2007 Elsevier Ltd. All rights reserved.

**Keywords:** Arbuscular mycorrhizal fungi; Nickel; Serpentine grassland; Soil microbial community; *Sorghastrum nutans*; Tallgrass prairie

## 1. Introduction

Midwestern prairies, like many other natural systems, have been severely impacted by pollution of soil and water from toxic metals such as lead, zinc, and nickel due to strip mining and other anthropogenic activities such as landfills or industrial manufacturing (United States Environmental Protection Agency Superfund Website). Attempts at reclamation and remediation of polluted areas have been made using agricultural plant species and native plants found on polluted soil (Giordani et al., 2005; Remon et al., 2005), which have usually evolved metal tolerance (Antonovics and Bradshaw, 1970; Frérot et al., 2006). It has been suggested that native plants

are better for reclamation efforts than agricultural species (Bonfert and Ashby, 1984) as native species can also offer superior tolerance to drought and low soil nutrients.

Another potential pool of metal tolerant populations of prairie species that could be used to remediate prairie sites has gone uninvestigated. These are populations growing on naturally metalliferous soils such as serpentine outcrops. Serpentine soils are characterized by low soil depth, potentially toxic levels of metals such as nickel, magnesium, and chromium, and often low levels of essential plant nutrients, including low calcium to magnesium ratios (Brooks, 1987). Metal polluted sites are often sites of similar abiotic conditions to serpentine sites with high levels of metals and low levels of essential soil nutrients (Bradshaw and Chadwick, 1980).

Eastern serpentine grasslands are dominated by C<sub>4</sub> grasses that are also very common in the Midwestern prairies. It has not been determined whether these grasses display local adaptation to the metals of serpentine, although

\* Corresponding author. Tel.: +1 215 898 8608; fax: +1 215 898 8780.

E-mail addresses: [dohertyj@sas.upenn.edu](mailto:dohertyj@sas.upenn.edu) (J.H. Doherty), [baoming@sas.upenn.edu](mailto:baoming@sas.upenn.edu) (B. Ji), [bcasper@sas.upenn.edu](mailto:bcasper@sas.upenn.edu) (B.B. Casper).

serpentine ecotypes have been identified in other wide ranging plant species (Johnston and Proctor, 1981; O'Dell and Claassen, 2006; O'Dell et al., 2006; Wright et al., 2006; but see Miller and Cumming, 2000). For example, a serpentine ecotype of *Festuca rubra* was found to be more tolerant to both Mg and Ni than a non-serpentine ecotype (Johnston and Proctor, 1981).

Because microbes, including arbuscular mycorrhizal (AM) fungi, can affect metal tolerance of the plants with which they associate, it is important to view plant response to heavy metals in the context of the soil microbial community (Adriaensen et al., 2004; Carrasco et al., 2006; Chen et al., 2006; Ma et al., 2006; Vivas et al., 2006). AM fungi from both naturally metalliferous and polluted sites have been shown to increase plant growth under metal polluted conditions (Vivas et al., 2005; Vogel-Mikus et al., 2006; but see Enkhtuya et al., 2000).

The objective of our study was to evaluate *Sorghastrum nutans*, a perennial C<sub>4</sub> grass, for nickel tolerance. Plants from one prairie and one serpentine population were compared in order to determine whether the serpentine population is more nickel tolerant. Nickel occurs in much higher concentrations in serpentine soil ( $1361 \pm 143$  mg total Ni/kg dry soil obtained by boiling aqua regia digestion) than in prairie soil ( $13 \pm 0.6$ , Casper et al., unpublished) and can be extremely toxic to plants (Seregin and Kozhevnikova, 2006). We also investigated the effects of soil microbial communities from both serpentine and prairie and AM fungi in root inoculum from serpentine on plant nickel tolerance.

## 2. Materials and methods

Two greenhouse experiments with slightly different goals were conducted. In the first experiment, *S. nutans* was grown under varying concentrations of Ni with or without AM root inoculum from the same serpentine site where *S. nutans* was collected. The second varied the timing of nickel application and used whole soil inoculum taken from trap cultures used to propagate AM fungi. Trap cultures were originally established using inoculum collected from the same serpentine site and a prairie site, so the source of inoculum was also a factor in the experiment.

### 2.1. Species and site description

*S. nutans* (L.) Nash is a community dominant in both the U.S. Midwestern tallgrass prairie (Sims and Risser, 2000) and grasslands on Eastern serpentine outcrops (Brooks, 1987), despite vastly different soils. Tallgrass prairie soils are among the most fertile in world. Serpentine soils, in contrast, have low levels of several macronutrients including P, K, and Ca, but excessively high, potentially toxic levels of other minerals such as Cr, Fe, Ni, and Mg (Brooks, 1987). Serpentine plant seed and microbial samples were collected from grasslands within the Nottingham County Park located in Chester County, Pennsylvania. Prairie plant seed was purchased from Ion Exchange (Harper's Ferry, Iowa) and microbial samples were collected from a tallgrass prairie remnant within the Anderson Prairie State Preserve located in Emmet County, Iowa. The AM fungal communities of both grasslands consist of several *Glomus* spp.: *Glomus aggregatum*, *Glomus claroideum*, *Glomus constrictum*, *Glomus etunicatum*, *Glomus mosseae*, *Glomus rubiforme*, and *Glomus tortuosum*, as well as *Scutellospora calospora*, *Entrophospora infrequens*, and *Archaeospora leptoticha*. In addition, *Gigaspora gigantea* and *Acaulospora spinosa* are found only at Nottingham while *Glomus geosporum* is found at Anderson (Casper et al., unpublished data).

### 2.2. AM fungi experiment

This experiment varied seed source (serpentine or prairie), AM fungi treatment (presence or absence of AM root inoculum), and level of Ni in a complete factorial design with 10 replicates of each treatment combination. The roots of *S. nutans* infected with AM fungi were collected from the same serpentine site as the seeds.

Roots were taken from 10 plants at Nottingham County Park, each separated by more than 30 m, in September 2005; roots from a different individual were used in each treatment replicate. Roots were removed from soil, washed, surface sterilized by soaking in ampicillin (500 mg/L) and streptomycin (500 mg/L) for 3 h, and chopped into 1 cm pieces. Cone-tainers (Stuewe and Sons, Inc.; 160 mL tapered cylindrical pots 3.8 cm in diameter and 21 cm in depth) for the AM root inoculum treatment were filled with a 100-mL layer of sterilized MetroMix 200 (MM) followed by a 50-mL layer of MM mixed with 0.5 g root inoculum. The no inoculum treatment cone-tainers were filled with 150 mL of MM. Seeds were stored dry at 4 °C then were surfaced sterilized with 75% ethanol for 5 min before being sown in sterilized vermiculite to germinate. When seedlings were three weeks old, one seedling was planted in each cone-tainer.

Three nickel treatments were applied five weeks after planting, in the form of an aqueous solution of NiCl<sub>2</sub>·6H<sub>2</sub>O: (1) high nickel application (700 mg Ni/kg soil), (2) low nickel application (350 mg Ni/kg soil), and (3) deionized water with no nickel.

The plants were grown in temperature controlled greenhouses at the University of Pennsylvania that averaged 25 °C between 0600 and 1800 h and 21 °C otherwise. They received a minimum PAR of 430 μmol/m<sup>2</sup>/s supplied by either active greenhouse lighting or ambient sunlight for 12 h each day. The experiment was maintained for 35 weeks.

### 2.3. Whole soil microbial community experiment

This experiment varied three factors, seed source (serpentine or prairie), soil microbial community source (serpentine soil inoculum, prairie soil inoculum, or no inoculum), and timing of nickel application in a complete factorial design with 14 replicates of each treatment combination. The nickel treatments consisted of a low concentration aqueous application (350 mg Ni/kg soil) five weeks after planting (early), at seven weeks after planting (late), or no application. We omitted the high Ni application due to the high mortality observed in the previous experiment.

The whole soil inoculum used in this experiment was taken from sorghum–sudan grass hybrid trap culture pots used to propagate the soil community with the objective of cultivating AM fungi. The pots were established in July 2004 in a greenhouse at the University of Wisconsin, Oshkosh. The original inoculum used to start trap cultures was rhizosphere soil and roots from *S. nutans* collected from Nottingham County Park or from Anderson Prairie State Preserve. Cone-tainers were filled with 115 mL of sterilized MM and either 35 mL of inoculum or sterilized sand (no inoculum). In the inoculum from both sites, the number of spores per 100 mL ranged from fewer than 100 to more than 400, with slightly higher spore densities in Anderson inoculum than in Nottingham. Again, three-week-old seedlings germinated from sterilized seeds were transplanted to cone-tainers and grown in the University of Pennsylvania greenhouse. The experiment was maintained for 12 weeks.

### 2.4. Plant sampling

Plants in both experiments were harvested by clipping the aboveground biomass which was then dried at 60 °C (Precision, Winchester, VA) for 48 h and weighed. Mortality, which all occurred within four weeks, was recorded in the AM fungal experiment. There was no mortality in the whole soil community experiment.

### 2.5. Nickel content

The nickel content of the dried aboveground biomass was determined for a sub-sample of the replicates of each treatment ( $n = 3$ ) of the AM fungal

experiment except for the plants of serpentine origin grown with AM inoculum and treated with high nickel. Only three plants of this treatment survived and none were large enough to analyze for nickel content. The dry aboveground biomass of each plant was ground in a coffee grinder and passed through a 1 mm sieve. Ground samples were then digested by boiling in a solution consisting of 15 mL trace metal grade concentrated HCl and 5 mL HNO<sub>3</sub>. When half of the solution had evaporated (approximately 1 h) 10–20 mL of 30% H<sub>2</sub>O<sub>2</sub> was added. The samples were then heated until almost evaporated, filtered through Whatman 540 paper, and brought up to 50 mL in deionized water prior to ICP analysis on a Perkin Elmer Optima 3000 ICP-OES according to EPA Method 6010C. Controls consisting of the reagents but not plant tissue were also analyzed; all values were below analytical detection limits of 0.5 mg/kg Ni.

2.6. Mycorrhizal colonization

A small amount of roots (0.5 g wet weight) was harvested from a sub-sample of the replicates in each treatment (*n* = 5) of the whole soil community experiment to measure percent AM root colonization. Samples were cleared for 1 h in 10% KOH at 80 °C, stained following the method of Phillips and Hayman (1970) then scored for mycorrhizal colonization using the line intersect method (Giovannetti and Mosse, 1980). A small sub-sample was also collected in the AM fungal experiment to check for colonization; though mycorrhizal colonization was found, it was not quantified.

2.7. Statistical analysis

Biomass and root colonization data were analyzed using separate three-way (soil microbial treatment × nickel treatment × seed source) ANOVA models. Post hoc analyses were conducted using Tukey’s HSD method. Due to the missing AM fungi × high nickel × serpentine seed source treatment samples, nickel content data were analyzed across all treatment combinations by treating each combination as a separate treatment in a one-way ANOVA. Planned contrasts were used to compare among seed source, AM fungal, and nickel treatments. Analyses were performed in JMP IN Version 5.1 (SAS Institute Inc. 2005). Survival data were analyzed for independence using G tests (RxC) (Sokal and Rohlf, 1994).

3. Results

3.1. AM fungal experiment

3.1.1. Survival

Mortality was higher for the prairie seed source and in the high nickel treatment (700 mg Ni/kg soil; Table 1) but did not differ between AM fungal treatments. There was a seed source × nickel × AM fungal interaction; under high nickel, there was no mortality among plants of serpentine origin grown without AM fungi while all other treatment combinations with high nickel experienced high mortality (Table 1).

Table 1  
Percent mortality of plants from prairie or serpentine seeds grown with and without AM fungi in three levels of nickel

	Prairie seed		Serpentine seed	
	+AM fungi (%)	–AM fungi (%)	+AM fungi (%)	–AM fungi (%)
No nickel	0	0	0	0
Low nickel	12	10	0	0
High nickel	70	70	70	0

3.1.2. Plant biomass

Aboveground biomass differed between AM fungal treatments ( $F_{1,81} = 54.609, p < 0.001$ ) and nickel treatments ( $F_{2,81} = 3.841, p = 0.026$ ) but not between seed sources. Plants with AM root inoculum were smaller than plants that were not inoculated (Fig. 1). There was a significant treatment interaction between AM fungal treatment and nickel ( $F_{2,81} = 3.146, p = 0.048$ ). This is explained by there being no difference among nickel treatments without AM fungi, but with AM fungal inoculum plants exposed to high levels of nickel were smaller (Fig. 1).

3.1.3. Nickel content

Nickel content in aboveground biomass varied among the 11 treatment combinations ( $F_{1,23} = 5.441, p < 0.001$ ). Planned comparisons showed no differences between prairie and serpentine seed sources or AM fungal treatment. However, plants that were treated with a high level of nickel (700 mg Ni/kg soil) had more nickel in their tissues than those treated with a low level of nickel (350 mg Ni/kg soil) ( $F_{1,23} = 6.687, p = 0.017$ ) (Fig. 2), and plants treated with a low level of nickel had more nickel than those treated with deionized water ( $F_{1,23} = 22.836, p < 0.001$ ).

3.2. Whole soil microbial community experiment

3.2.1. Plant biomass

Aboveground biomass differed among soil microbial community treatments ( $F_{2,232} = 29.894, p < 0.001$ ) but not between seed sources. Biomass was lower when inoculated with the serpentine soil microbial community compared to the prairie soil microbial community or no microbial community, which did not differ from each other (Fig. 3). There was also a main effect of nickel treatment ( $F_{2,81} = 18.397, p < 0.001$ ). While both applications of nickel decreased aboveground biomass compared to the control, the early

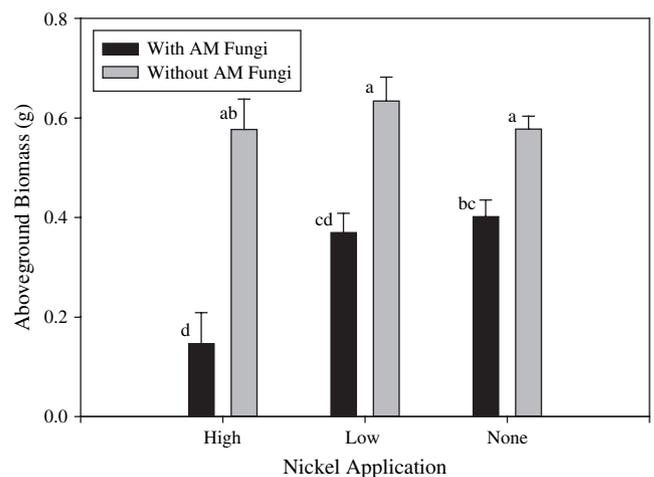


Fig. 1. Mean aboveground biomass (±SE) of *S. nutans* grown with and without AM fungi under three levels of nickel application (high: 700 mg Ni/kg soil, low: 350 mg Ni/kg, or none). Significant differences in mean aboveground biomass are indicated by different letters (*p* < 0.05).

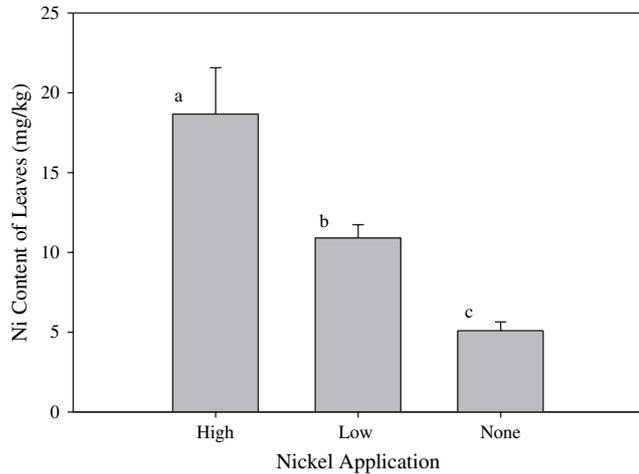


Fig. 2. Mean nickel content of aboveground biomass (mg/kg  $\pm$  SE) of *S. nutans* grown with and without AM fungi under three levels of nickel application (high: 700 mg Ni/kg soil, low: 350 mg Ni/kg, or none). Significant differences as in Fig. 1.

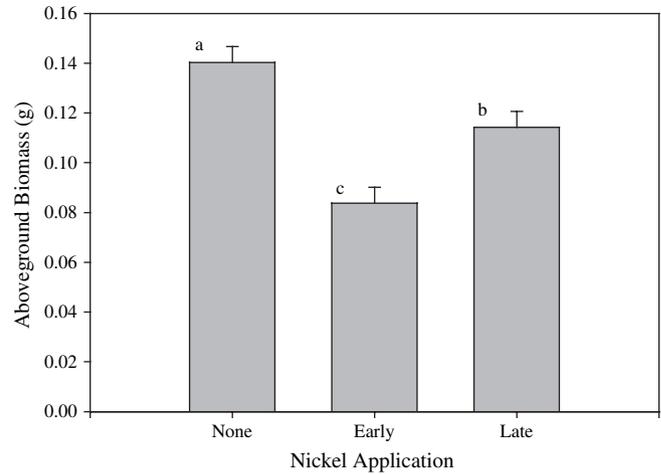


Fig. 4. Mean aboveground biomass ( $\pm$ SE) of *S. nutans* in three nickel treatments (no nickel, 350 mg Ni/kg applied two weeks after planting, or 350 mg Ni/kg applied four weeks after planting). Significant differences as in Fig. 1.

application (two weeks after planting) had the largest effect (Fig. 4). There were no significant treatment interactions.

### 3.2.2. Mycorrhizal root colonization

Mycorrhizal root colonization differed between nickel treatments ( $F_{2,69} = 3.679$ ,  $p = 0.030$ ) but not between seed or soil community origins, and there were no significant interactions. Colonization was significantly lower in the late nickel treatment when compared to the no nickel treatment (Fig. 5), while colonization in the early nickel treatment was intermediate and not significantly different from either. There was some root colonization in the no soil community treatment group ( $3.2\% \pm 0.008\%$ ), but it was significantly and substantially lower than when the prairie or serpentine soil community was added ( $26.4\% \pm 0.03\%$  or  $31.3\% \pm 0.04\%$ ;  $F_{2,69} = 25.672$ ,  $p < 0.0001$ ).

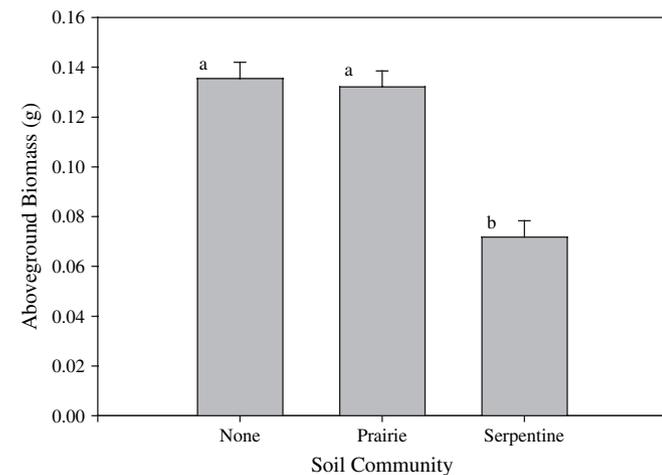


Fig. 3. Mean aboveground biomass ( $\pm$ SE) of *S. nutans* grown with three microbial soil community treatments (no microbial community, a prairie microbial community, or a serpentine microbial community). Significant differences as in Fig. 1.

## 4. Discussion

There are four main findings from these experiments: (1) the serpentine plant ecotype has marginally greater nickel tolerance, (2) neither microbial soil community improves plant performance compared to uninoculated plants; the serpentine community decreases plant performance, especially in the presence of nickel, (3) differences in how the soil communities affect plant performance are not reflected in patterns of root colonization by AM fungi, and (4) the application of nickel reduces AM fungal colonization.

Evidence for differences between plant populations in nickel tolerance comes from the first experiment in which serpentine plants without AM fungi experienced higher survival with high nickel than did serpentine plants grown with AM fungi and all prairie plants, regardless of AM fungal presence.

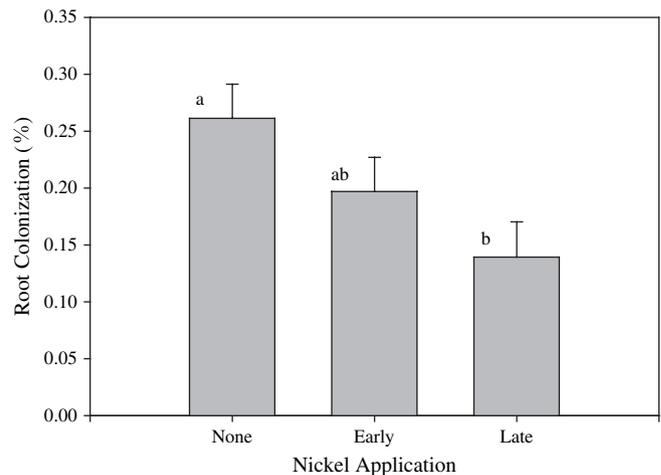


Fig. 5. Mean AM fungal root colonization percentage ( $\pm$ SE) of plants in three of nickel treatments (no nickel, 350 mg Ni/kg applied two weeks after planting, or 350 mg Ni/kg applied four weeks after planting). Significant differences as in Fig. 1.

Under low levels of nickel, plants from the serpentine population and the prairie population did not differ in survival rate. There is no evidence that using plants from serpentine populations would allow greater phytoextraction of nickel from impacted soils as neither aboveground biomass nor nickel content as a proportion of biomass differed with seed source.

This experiment shows a strong difference in the ecological function of microbial communities from the prairie and serpentine, but apparently neither community ameliorated the toxic effects of high levels of nickel found in serpentine soil. Both the serpentine derived whole soil community and serpentine root inoculum negatively affected plant growth, especially in the presence of nickel, whereas the prairie derived soil community had no effect. An increase in aboveground nickel content with serpentine root inoculum was not responsible for this finding, however, as plants grown with root inoculum were not found to have more nickel in their tissues than those grown without root inoculum. One possible explanation for these results is that even in the first experiment in which root inoculum was treated with antibiotics, other microbes besides AM fungi, such as fungal pathogens, contributed to the negative plant growth responses observed with serpentine inocula.

Alternatively, the serpentine AM fungal community could simply act parasitically (Johnson et al., 1997). It has been suggested that microbes adapted to live in environments with poor plant growth and less carbon need to be more parasitic than mutualistic in order to survive (Johnson, 1993) but other research in our lab has not shown the serpentine AM fungal community to act parasitically in native soil (Casper et al., unpublished data). It is also true that the amount of benefit or detriment provided by AM fungi can depend on soil type (Auge, 2004; Lee and George, 2005). Therefore it is possible that the serpentine AM fungal community in this experiment acted as a carbon drain when their ability to take up nutrients was superfluous to the plant. If the negative growth effect is, in fact, due to AM fungi it is due to the summation of the effects of the whole AM fungal community and not simply individual “cheater” species acting parasitically as found by Klironomos (2003).

Other studies have shown that mycorrhizal infection by native fungi from prairie or serpentine populations have a positive or neutral effect on growth of *S. nutans*, respectively. In a mixed species garden plot of fully grown plants from prairie populations, *S. nutans* produced less plant biomass in a fungicide-treated microcosm that decreased root colonization by arbuscular mycorrhizal fungi (Wilson and Hartnett, 1997). Wilson and Hartnett (1998) also performed a greenhouse study using autoclaved soil to examine the mycorrhizal responsiveness of many tallgrass prairie species ( $((\text{dry mass mycorrhizal plant} - \text{dry mass nonmycorrhizal plant})/\text{dry mass mycorrhizal plant}) \times 100$ ), and found that *S. nutans* had a significant positive responsiveness of 99.5%. In greenhouse pots with autoclaved serpentine soil, serpentine mycorrhizal fungi were found to have no effect on the biomass of *S. nutans* (Castelli, 2001).

Effects of soil communities on plant growth appear to be independent of the level of AM fungal root colonization. Although the two microbial communities had different

effects on plant performance, the level of AM fungal colonization was not different. While the amount of benefit, such as phosphorus uptake, provided by AM fungi is often correlated with percent root colonization, it is not always the case (Smith and Read, 1997). Species-specific differences have been implicated in the lack of correlation observed. Species of AM fungi have different intra-radical to extra-radical hyphae ratios, in other words, different amounts of root colonization hyphae per unit nutrient foraging hyphae, and therefore may have differing amounts of root colonization per unit benefit (Smith and Read, 1997).

There are several possible explanations for why the late nickel application negatively affected the percentage of AM fungal root colonization in the whole soil community experiment. Nickel could reduce actual AM fungal colonization or hyphal growth, increase root growth but not hyphal growth, or selectively kill the roots that are colonized. Early nickel application decreased root colonization to a lesser degree. It is unlikely that colonization rates are less affected by nickel when the plants were young. More likely colonization was depressed by nickel at first but then rebounded with time (Koo-men et al., 1990).

There are three possible explanations for the smaller aboveground biomass observed in the early nickel application treatment when compared to the later nickel application treatment. (1) The plants in the early application treatment had to cope with nickel for two weeks longer than the late application treatment plants, and the longer exposure caused a more negative effect on biomass. (2) The earlier timing of the nickel application produced more damage to the younger plants. (3) The higher AM fungal colonization in the early application treatment and presumed higher carbon flux from plant to fungus could explain the differences in aboveground biomass between early and late nickel applications.

## 5. Conclusion

Serpentine populations may be suited for reclamation or remediation purposes due to their high survival in the presence of high nickel conditions. This may be especially true in highly disturbed areas where an AM fungal community might not be present or where AM fungal communities may occur at smaller sizes and lower diversity than non-disturbed areas (Degroot et al., 2005; Del Val et al., 1999; Jasper et al., 1987; Pfeleger et al., 1994). We found no evidence that AM fungi help plants cope with nickel specifically. In fact, our findings suggest that AM fungi decrease plant biomass in the presence of nickel.

## Acknowledgements

The authors thank Chester Country Parks & Recreation for access to Nottingham County Park, the Iowa Department of Natural Resources for access to Anderson Prairie State Preserve, S. Bentivenga for maintenance of the trap cultures, V. Shrivastava, L. Nickell, V. van Deurs, R. Skold, and T. Savage for their help in the experiments, L. Spindler and

B. Helliker for comments, and D. Burse and R. Thompson of the National Energy Technology Laboratory (U.S. DOE) for analysis of nickel in plant samples. The work was supported by an NSF grant DEB0620310 to Casper.

## References

- Adriaensen, K., van der Lelie, D., Van Laere, A., Vangronsveld, J., Colpaert, J.V., 2004. A zinc-adapted fungus protects pines from zinc stress. *New Phytologist* 161, 549–555.
- Antonovics, J., Bradshaw, A.D., 1970. Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity* 25, 349–362.
- Auge, R.M., 2004. Arbuscular mycorrhizae and soil/plant water relations. *Canadian Journal of Soil Science* 84, 373–381.
- Bonfert, G.A., Ashby, W.C., 1984. Native prairie grasses for reclaiming ungraded mine spoil in southern Illinois. In: Graves, D.H. (Ed.), *Proceedings of the Symposium on Surface Mining Hydrology, Sedimentology, and Reclamation*. University of Kentucky, Lexington, pp. 19–24.
- Bradshaw, A.D., Chadwick, M.J., 1980. *The Restoration of Land: The Ecology and Reclamation of Derelict and Degraded Land*. University of California Press, Berkeley, California.
- Brooks, R.R., 1987. *Serpentine and Its Vegetation*. Dioscorides, Portland, Oregon.
- Carrasco, L., Caravaca, F., Alvarez-Rogel, J., Roldan, A., 2006. Microbial processes in the rhizosphere soil of a heavy metals-contaminated Mediterranean salt marsh: a facilitating role of AM fungi. *Chemosphere* 64, 104–111.
- Castelli, J.P., 2001. *Negative Mycorrhizal Feedback and Plant Species Coexistence in a Serpentine Grassland*. Ph.D. thesis, University of Pennsylvania, Philadelphia, Ann Arbor, UMI (Publication Number: AAT 3015302).
- Chen, B.D., Zhu, Y.G., Smith, F.A., 2006. Effects of arbuscular mycorrhizal inoculation on uranium and arsenic accumulation by Chinese brake fern (*Pteris vittata* L.) from a uranium mining-impacted soil. *Chemosphere* 62, 1464–1473.
- DeGrood, S.H., Claassen, V.P., Scow, K.M., 2005. Microbial community composition on native and drastically disturbed serpentine soils. *Soil Biology & Biochemistry* 37, 1427–1435.
- Del Val, C., Barea, J.M., Azcon-Aguilar, C., 1999. Diversity of arbuscular mycorrhizal fungus populations in heavy-metal-contaminated soils. *Applied Environmental Microbiology* 65, 718–723.
- Enkhtuya, B., Rýdlova, J., Vosatka, M., 2000. Effectiveness of indigenous and non-indigenous isolates of arbuscular mycorrhizal fungi in soils from degraded ecosystems and man-made habitats. *Applied Soil Ecology* 14, 201–211.
- Frérot, H., Lefèbvre, C., Gruber, W., Collin, C., Dos Santos, A., Escarré, J., 2006. Species interactions between local metallicolous plants improve the phytostabilization of mine soils. *Plant and Soil* 282, 53–65.
- Giordani, C., Cecchi, S., Zanchi, C., 2005. Phytoremediation of soil polluted by nickel using agricultural crops. *Environmental Management* 36 (5), 675–681.
- Giovannetti, M., Mosse, B., 1980. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84, 489–500.
- Jasper, D.A., Robson, A.D., Abbott, L.K., 1987. The effect of surface mining on the infectivity of vesicular–arbuscular mycorrhizal fungi. *Australian Journal of Botany* 35, 641–652.
- Johnson, N.C., 1993. Can fertilization of soil select less mutualistic mycorrhizae? *Ecological Applications* 3, 749–757.
- Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist* 135, 575–586.
- Johnston, W.R., Proctor, J., 1981. Growth of serpentine and non-serpentine races of *Festuca-Rubra* in solutions simulating the chemical conditions in a toxic serpentine soil. *Journal of Ecology* 69, 855–869.
- Klironomos, J.N., 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84, 2292–2301.
- Koomen, I., McGrath, S.P., Giller, K.E., 1990. Mycorrhizal infection of clover is delayed in soils contaminated with heavy-metals from past sewage-sludge applications. *Soil Biology & Biochemistry* 22, 871–873.
- Lee, Y.-J., George, E., 2005. Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. *Plant and Soil* 278, 361–370.
- Ma, Y., Dickinson, N.M., Wong, M.H., 2006. Beneficial effects of earthworms and arbuscular mycorrhizal fungi on establishment of leguminous trees on Pb/Zn mine tailings. *Soil Biology & Biochemistry* 38, 1403–1412.
- Miller, S.P., Cumming, J.R., 2000. Effects of serpentine soil factors on Virginia pine (*Pinus virginiana*) seedlings. *Tree Physiology* 20, 1129–1135.
- O'Dell, R.E., Claassen, V.P., 2006. Serpentine and nonserpentine *Achillea millefolium* accessions differ in serpentine substrate tolerance and response to organic and inorganic amendments. *Plant and Soil* 279, 253–269.
- O'Dell, R.E., James, J.J., Richards, J.H., 2006. Congeneric serpentine and non-serpentine shrubs differ more in leaf Ca:Mg than in tolerance of low N, low P, or heavy metals. *Plant and Soil* 280, 49–64.
- Pfeger, F.L., Stewart, E.L., Noyd, R.K., 1994. Role of VAM and mine land revegetation. In: Pfeger, F.L., Linderman, R. (Eds.), *Mycorrhizae and Plant Health*. American Phytopathological Society Press, St. Paul, MN, pp. 47–81.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55, 158–161.
- Remon, E., Bouchardon, J.L., Cornier, B., Guy, B., Leclerc, J.C., Faure, O., 2005. Soil characteristics, heavy metal availability and vegetation recovery at a former metallurgical landfill: implications in risk assessment and site restoration. *Environmental Pollution* 137, 316–323.
- Seregin, I., Kozhevnikova, A., 2006. Physiological role of nickel and its toxic effects on higher plants. *Russian Journal of Plant Physiology* 53, 257–277.
- Sims, P.L., Risser, P.G., 2000. Grasslands. In: Barbour, M.G., Billings, W.D. (Eds.), *North American Terrestrial Vegetation*. Cambridge University Press, Cambridge, pp. 323–356.
- Smith, S.E., Read, D.J., 1997. *Mycorrhizal Symbiosis*, second ed. Academic Press, San Diego, CA.
- Sokal, R.R., Rohlf, F.J., 1994. *Biometry: The Principles and Practice of Statistics in Biological Research*, third ed. W.H. Freeman and Co., New York.
- United States Environmental Protection Agency Superfund Website. <http://www.epa.gov/superfund/>.
- Vivas, A., Barea, J.M., Azcon, R., 2005. *Brevibacillus brevis* isolated from cadmium- or zinc-contaminated soils improves in vitro spore germination and growth of *Glomus mosseae* under high Cd or Zn concentrations. *Microbial Ecology* 49, 416–424.
- Vivas, A., Biro, B., Ruiz-Lozano, J.M., Barea, J.M., Azcon, R., 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity. *Chemosphere* 62, 1523–1533.
- Vogel-Mikus, K., Pongrac, P., Kump, P., Necemer, M., Regvar, M., 2006. Colonisation of a Zn, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *Environmental Pollution* 139, 362–371.
- Wilson, G.W.T., Hartnett, D.C., 1997. Effects of mycorrhizae on plant growth and dynamics in experimental tallgrass prairie microcosms. *American Journal of Botany* 84, 478–482.
- Wilson, G.W.T., Hartnett, D.C., 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. *American Journal of Botany* 85, 1732–1738.
- Wright, J.W., Stanton, M.L., Scherson, R., 2006. Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*. *Evolutionary Ecology Research* 8, 1–21.