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

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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.

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_4 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

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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_4 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 ± 143 mg total Ni/kg dry soil obtained by boiling aqua regia digestion) than in prairie soil (13 ± 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 $NiCl_2 \cdot 6H_2O$: (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 $\mu mol/m^2/s$ 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