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PLANT–SOIL FEEDBACK: TESTING THE GENERALITY WITH THE SAME GRASSES IN SERPENTINE AND PRAIRIE SOILS

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Abstract. Plants can alter soil properties in ways that feed back to affect plant performance. The extent that plant–soil feedback affects co-occurring plant species differentially will determine its impact on plant community structure. Whether feedback operates consistently across similar plant communities is little studied. Here, the same grasses from two eastern U.S. serpentine grasslands and two midwestern tallgrass prairie remnants were examined for plant–soil feedback in parallel greenhouse experiments. Native soils were homogenized and cultured (trained) for a year with each of the four grasses. Feedback was evaluated by examining biomass variation in a second generation of (tester) plants grown in the trained soils. Biomass was lower in soils trained by conspecifics compared to soils trained by heterospecifics in seven of 15 possible cases; biomass was greater in conspecific soils in one other. *Sorghastrum nutans* exhibited lower biomass in conspecific soils for all four grasslands, so feedback may be characteristic of this species. Three cases from the Hayden prairie site were explained by trainer species having similar effects across all tester species so the relative performance of the different species was little affected; plants were generally larger in soils trained by *Andropogon gerardii* and smaller in soils trained by *S. nutans*. Differences among sites in the incidence of feedback were independent of serpentine or prairie soils. To explore the causes of the feedback, several soil factors were measured as a function of trainer species: nutrients and pH, arbuscular mycorrhizal (AM) spore communities, root colonization by AM fungi and putative pathogens, and functional diversity in bacterial communities as indicated by carbon substrate utilization. Only variation in nutrients was consistent with any patterns of feedback, and this could explain the greater biomass in soils trained by *A. gerardii* at Hayden. Feedback at Nottingham (one of the serpentine sites) differed, most notably for *A. gerardii*, from that of similar past studies that used different experimental protocols. To understand the consequences of feedback for plant community structure, it is important to consider how multiple species respond to the same plant-induced soil variation as well as differences in the feedback detected between greenhouse and field settings.

Key words: Andropogon gerardii; *arbuscular mycorrhizae; root pathogens;* Schizachyrium scoparium; *serpentine grassland;* Sorghastrum nutans; Sporobolus heterolepis; *tallgrass prairie*.

Introduction

Plants alter soil characteristics in ways that feed back to affect the performance of that same or other plant species. This plant–soil feedback can involve changes in physical and chemical soil properties or various components of the soil biota (Reynolds et al. 2003, Ehrenfeld et al. 2005) including nematodes (Brinkman et al. 2005) and microbes such as soil-borne pathogens (Van der Putten et al. 1993, Packer and Clay 2000), mycorrhizal fungi (Bever 2002), and bacteria involved in biogeochemical processes (Ehrenfeld et al. 2005, Hawkes et al. 2005, Bezemer et al. 2006).

How plant-induced soil alterations affect the structure of the plant community depends on whether the changes improve or impair plant performance and also the specificity of plant responses. Feedback is considered negative if the performance of the species inducing the changes is reduced relative to other plants (Bever et al. 1997, Reynolds et al. 2003), as happens, for example, with species-specific pathogens (Packer and Clay 2000), and positive if the reverse is true. However, some changes, particularly those involving nutrients, may not be specific to the focal species but have more widespread effects across the plant community (Bonanomi and Mazzoleni 2005, Ehrenfeld et al. 2005, Bezemer et al. 2006, Kardol et al. 2007), but the occurrence of these general effects is little explored.

More studies are needed to examine the generality of feedback as a phenomenon and to determine whether there are patterns to the incidence and direction of the

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feedback in different ecological settings. Limited data suggest that exotic species are more likely than native species to engage in positive plant–soil feedback (Klironomos 2002, Reinhart and Callaway 2006) and that positive feedback may be more important where abiotic stress is severe (Reynolds et al. 2003, Ehrenfeld et al. 2005). Little is known about how the overall characteristics of the soil substrate might influence feedback, although variation as a function of substrate is expected (Ehrenfeld et al. 2005, Bezemer et al. 2006), given that both soil communities and plant performance are affected by nutrient availability and stressors such as heavy metals in soils (Brady et al. 2005, Oline 2006).

Additionally, there is a need for feedback studies that employ similar methodologies so that results can be directly compared. Greenhouse experiments often measure differential plant performance in soils either collected from the rhizosphere of different species in the field (Van der Putten et al. 1993, Callaway et al. 2004) or in soils with soil inocula cultured experimentally with different plant species (Bever 1994, Klironomos 2002, Bezemer et al. 2006). Less often, evidence for plant–soil feedback is sought through field experiments that include the previous soil occupant as a factor in the experimental design (Bonanomi et al. 2005, Casper and Castelli 2007). These different protocols are likely to reveal changes that take place over different time scales (Bardgett et al. 2005), which should influence the interpretation of results. In nature, changes to soils may occur over the lifespan of long-lived plants, while in soils cultured experimentally, changes are constrained by the length of the experiment, often a matter of months.

The overall purpose of the current study was to examine two eastern U.S. serpentine grasslands, with soils naturally high in heavy metals, and two midwestern, tallgrass prairie remnants, all with the same dominant grasses, for evidence of plant–soil feedback. We conducted parallel experiments with the four grasslands, homogenizing field-collected soils, culturing (training) them with the different grass species, and then examining how the cultured soils affected the performance of a second generation of grasses. Our investigation was governed by the hypothesis that plant species-specific plant–soil feedback is similar in occurrence and direction (negative or positive) at all four sites. Specifically, this factorial design enabled us to examine whether (1) plant growth generally differed between soils cultured by conspecifics vs. heterospecifics, (2) plant–soil feedback within a site affected all species similarly or some differentially, (3) across all sites, particular plant species were more likely to engage in plant–soil feedback than others, and (4) the incidence or direction of feedback differed between serpentine and prairie soils.

In an effort to discern causes of the feedback, we measured soil nutrients and several indicators of the soil microbial community: arbuscular mycorrhizal (AM) spore communities, bacterial community profiles based on carbon substrate utilization, and root colonization by arbuscular mycorrhizal (AM) fungi and putative pathogens. Finally, we compared our findings with results of two prior feedback studies of one of the same serpentine grasslands we investigated here (Gustafson and Casper 2004, Casper and Castelli 2007). Those studies used alternative experimental protocols, allowing us to assess whether different approaches to investigating feedback lead to the same conclusions about its occurrence.

Methods

The study systems

We conducted four parallel greenhouse experiments using soils and plants from two serpentine grasslands, Nottingham County Park (Ser_{NOT}), Chester County, Pennsylvania (39°44′ N, 76°02′ W) and Soldiers Delight Natural Environment Area (Ser_{SOL}), Montgomery County, Maryland (39°37′ N, 76°50′ W), and two midwestern tallgrass prairie remnants, Hayden Prairie (Pra_{HAY}), Howard County, Iowa (43°26′ N, 92°23′ W) and Anderson Prairie (Pra_{AND}), Emmet County, Iowa (43°26′ N, 94°52′ W). We included the same four native C_4 grasses, with similar phenologies, from each of the four sites: *Andropogon gerardii*, *Sorghastrum nutans*, *Schizachyrium scoparium*, and *Sporobolus heterolepis*, except that *Sporobolus* is absent from Ser_{SOL}. Among the dominant species at all sites, the grasses grow as distinct bunch grasses on serpentine soils but intermingle more with other species in the prairie.

To characterize substrates, we analyzed soil from under each of the grasses at 10 of the 15 points of soil collection used in the plant growth experiment for pH and bioaccessible amounts of P, K, NH_4^+, NO_3^-, Ca, Mg, Ni, and Cr (Ni and Cr are heavy metals normally found in serpentine soils). Soil from under a given plant was first homogenized and then subsampled. For the purpose of comparing each soil factor among sites, we conducted a one-way ANOVA on mean values calculated for each collection point. Nutrients and pH were measured by the Analytical Services Lab at Pennsylvania State University (State College, Pennsylvania, USA). The metals were measured by the National Energy Technology Laboratory (U.S. Department of Energy, Pittsburgh, Pennsylvania, USA) after using a modified physiologically based extraction technique (Yang et al. 2001).

The plant growth experiment consisted of two generations of plants. The first generation was used to train (culture) homogenized field-collected soil; the second (tester) generation, consisting of the same plant species, tested whether the identity of the training species (soils) affected the performance of the tester species differentially. Soil for the plant growth experiment was collected in June and early July 2004 from 15 arbitrarily selected, widely separated (>30 m apart) collection points per site. At each point location, soil was collected from the root zone of the nearest large monospecific clump of each grass species, usually to a depth of 15 cm but sometimes less in the rocky serpentine soils. Soils

were transported in coolers, stored under refrigeration, and used to set up the trainer pots within three weeks. Collection points were treated as separate replicates in every aspect of this study.

To construct the training portion of the plant growth experiment, we thoroughly mixed together an equal quantity of soil taken from under each grass associated with a particular collection point and diluted the mixture with sterilized sand in a 6:1 (soil : sand) ratio to improve drainage. We then divided the mixed soil among four (or three, for Ser_{SOL}) 2-L pots. We planted a different grass species in each of the trainer pots associated with a single replicate collection point by transplanting into each pot six two-week-old seedlings. These were grown from seeds sterilized in 70% ethanol for approximately 3 minutes and germinated in a steam-sterilized mixture of sand and vermiculite. We maintained these training plants in temperature-controlled greenhouses at the University of Pennsylvania for one year until their soils were used to set up the feedback-testing portion of the experiment. Greenhouse temperatures averaged 25°C between 06:00 and 18:00 hours and 21°C otherwise. Plants received a minimum photosynthetically active radiation (PAR) of 430 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from supplemental lighting or ambient sunlight for 16-h days. Trainer pots were segregated by replicate point on greenhouse benches.

The seeds for both the Ser_{NOT} and Ser_{SOL} trainer pots were collected at Ser_{NOT} the preceding autumn. For the prairie soils, we used Iowa prairie ecotypes of *Andropogon*, *Sorghastrum*, and *Schizachyrium* (Ion Exchange, Harpers Ferry, Iowa, USA). The seeds of *Sporobolus* used for the Pra_{HAY} trainer pots were obtained from the Iowa Ecotype Group at Northern Iowa University, but due to an insufficient quantity, some *Sporobolus* in Pra_{AND} soils were obtained from Ernst Conservation Seeds (Meadville, Pennsylvania, USA) or (a few) taken from our Ser_{NOT} collection.

The trainer pots were weeded regularly and checked for mortality, which mostly occurred in the first month (July), except that considerable numbers of *Andropogon* died during December and January. We continued to replace plants through January with the goal of maintaining at least four individuals per pot, initially using seedlings of the same age, but growing new seedlings to replace dead *Andropogon* in the winter months. All plants essentially stopped growing during January and February despite supplemental lighting that extended day length but resumed growth in March, largely through the production of new shoots. The plants were fertilized once every two weeks from October to January with dilute Peters $N_{(15\%)}$-$P_{(0)}$-$K_{(15\%)}$ fertilizer and from February on with fish emulsion $N_{(5\%)}$-$P_{(1\%)}$-$K_{(1\%)}$.

To set up the feedback-testing pots in June 2005, the trainer pots were not watered for two to three days to facilitate working with the soil, aboveground portions of the trainer plants were clipped off, and the root crowns and rhizomes discarded. The soil and roots from a single trainer pot were divided equally into four (or three for Ser_{SOL}) 10 cm deep, 500-mL pots with the roots placed at the bottom. A different grass species was planted in each of the four (three for Ser_{SOL}) smaller pots generated by dividing a single training pot. These methods yielded a complete factorial design with the four (three for Ser_{SOL}) trainer soils and four (three for Ser_{SOL}) tester species and 15 replicates per site. There were 855 feedback tester pots in total.

Seeds used for the feedback-testing part of the experiment were collected in autumn 2004 in the same four grasslands as the soils. Two seedlings, grown from sterilized seeds and germinated in sterile sand and vermiculite as before, were transplanted into each of the feedback-testing pots, except only one *Schizachyrium* seedling was available per pot for Ser_{SOL}. These plants were grown for three months in the same greenhouse as the training plants. The pots were regularly weeded and mortality noted. Seedlings dying during the first month were replaced with seedlings of the same age, but none was replaced subsequently. Mortality for the first month was 2–3% for Ser_{SOL}, Pra_{AND}, and Pra_{HAY}, and 7% for Ser_{NOT} soils.

Harvests

The shoots of the tester plants, including any reproductive tissues, were harvested after three months in late September 2005, oven dried, and weighed. Mean aboveground biomass of the two plants per pot was used as the dependent variable in analyses, except for *Schizachyrium* at Ser_{SOL} for which there was only a single value per pot. We did not include belowground biomass because separating the root systems from the soil and from the dead roots remaining from the trainer pots was not feasible. At harvest, most of the 2.5% mortality in Ser_{NOT} plants that happened after the first month involved *Sporobolus*; otherwise, mortality was $<2.0\%$ for the other three sites. Recognizing that mortality could be part of the response to different soils and that the death of one plant per pot released the other from competition, we represented the dead plant by a value of zero in obtaining mean aboveground biomass. Results did not change qualitatively if we omitted those pots.

We examined the effect of the trained soils on the biomass of tester plants using two approaches: (1) With respect to each tester species, we grouped soils as trained by conspecifics or trained by heterospecies and used a single mixed model ANOVA to examine plant biomass (log-transformed) as a function of conspecific vs. heterospecific soil, tester species, and site, all fixed effects with the (random) effect of replicate nested within site. (2) To help interpret the results of the first analysis, we also examined the biomass data separately by site. First, we compared the biomass of each species in conspecific vs. heterospecific soils. Next, we applied a GLM ANOVA model that included trainer soil (from

the four distinct trainer species) and tester species as fixed effects and replicate as a random factor. A significant main effect of trainer soil would indicate that the soil trained by a particular species had similar effects across all tester species while a significant trainer soil × tester species interaction would indicate that the trainer soils affected the tester species differentially. We further inspected the data to understand whether a significant main effect of trainer soil might explain cases where there were significant differences in biomass between conspecific vs. heterospecific soils. For Ser_{NOT}, this involved repeating analyses after omitting a species that contributed significantly to the trainer soil main effect. These and other analyses applying ANOVA or MANOVA were conducted in JMP version 6 (SAS Institute, Cary, North Carolina, USA).

Soil properties and root colonization

To explore variation in abiotic and biotic soil characteristics that developed in trainer soils, we measured the following parameters for at least one serpentine site and one prairie site: soil nutrients, AM fungal spore communities, bacterial community-level physiological profiles, root colonization by AM fungi, and root colonization by putative pathogenic fungi and fungus-like organisms. For soil bacteria profiles, about 20 mL of soil was collected aseptically from the middle of the pot one week before harvest. Roots and soils for other analyses were obtained when the trainer pots were dismantled to start the feedback portion of the experiment.

To determine if the identity of the trainer species differentially affected soil nutrient levels, trainer soils from one serpentine (Ser_{NOT}) and one prairie (Pra_{HAY}) site were analyzed for Mg, Ca, K, P, NH_4^+, NO_3^- and pH as for the field soils described in *The study systems*. The level of each was examined as a function of the species training the soil using ANOVA.

The composition of the AM fungal community in the trainer soils was examined as a function of trainer species for each of the four sites. AM spores were extracted from a 50-mL soil sample from each trainer pot using the wet sieve method (McKenney and Lindsey 1987) and identified to species based on morphological characters. The spore communities, defined as the numbers of spores of the various AM fungal species (log-transformed), were compared among training species using two different statistical methods, MANOVA and canonical analysis of principal coordinates (CAP) as a discriminant analysis, based on 999 permutations (Anderson and Robinson 2003, Anderson and Willis 2003, Anderson 2004). Where trainer plant-specific AM spore community differences were detected, comparisons were made among trainer species in the spore abundance of particular AM fungal species and in the total number of spores using ANOVA.

Bacterial communities in the 20 pots of training soils associated with five collection points for one serpentine (Ser_{NOT}) and for one prairie site (Pra_{HAY}), were characterized using Biolog ECO microplates (BIOLOG, Hayward, California, USA) and examined as a function of the species training the soil. Each plate contains triplicate sets of 31 environmentally relevant substrates, including nine constituents of root exudates (Campbell et al. 1997). Oxidation of a substrate produces a color change via the reduction of a tetrazolium dye. Microplates were inoculated with 100 μL per well of a 1×10^{-3} dilution of soil suspended in sterile phosphate buffer and incubated in the dark at 24°C. Absorbance values at 595 nm were monitored daily for seven days using a multiwell spectrophotometer (MTX Lab Systems, Vienna, Virginia, USA). Values on day 5 were used for analyses after discarding values below 0.2 and dividing all values by the average well color development per plate. The average absorbance value was calculated for the three replicates of a particular substrate on each plate. For each site, the average values for all substrates were compared among the training soils using CAP (Anderson 2004).

To compare the AM fungal infection potential of roots from the different trainer species, a 0.5-g sample (wet mass) of roots was scored for plants associated with five collection points at each site. Roots were examined microscopically for percentage total colonization by AM fungal hyphae, vesicles or arbuscles using the gridline intersect method (Giovannetti and Mosse 1980) after clearing in KOH and staining in 0.1% Trypan blue (Koske and Gemma 1989). Percentage colonization was compared among these trainer species using ANOVA. Roots of the tester plants for five points, for Ser_{NOT} only, were likewise examined as a function of both trainer species (soils) and tester species.

Roots were also examined for putative pathogens by staining a 0.5-g sample and examining them microscopically at 50–100×. We specifically looked for dark ectotrophic hyphae and perithecia characteristic of *Leptosphaeria* spp., *Magnaporthe poae*, and *Gaeumannomyces graminis*. We searched for resting spores of *Magnaporthe poae*, *Phialophora graminicola*, or *Microdochium bolleyi* and structures produced by fungus-like protists such as *Olpidium* spp. and *Polymyxa graminis*. Any other abnormalities, such as bacterial growth inside the root or apparent damage from stylets of pathogenic nematodes were also noted. Suspected pathogens were classified morphologically, and the percentage of root occupied by the putative pathogen was estimated by the gridline intercept method. Percentage colonization by the different types of putative pathogens was analyzed using MANOVA and total colonization by all types combined using ANOVA.

Results

Comparison of field soils

The two serpentine sites differed from the prairie sites in all soil factors except pH, which did not differ among sites, and NH_4^+ (Table 1). The serpentine sites had much

TABLE 1. Soil chemistry values for the two serpentine (Ser) and two prairie (Pra) sites (mean ± SE; $n = 10$ collection points per site).

Soil variables	Ser_{NOT}	Ser_{SOL}	Pra_{AND}	Pra_{HAY}	F	P
pH	6.42 ± 0.07	6.66 ± 0.04	6.63 ± 0.1	6.37 ± 0.1	1.92	0.14
P (mg P/kg soil)	4.9^{a} ± 0.5	2.7^{b} ± 0.2	9.3^{c} ± 0.5	21.6^{d} ± 1.3	150.30	<0.001
K (mg K/kg soil)	74.5^{a} ± 6.7	45.6^{b} ± 2.2	230.3^{c} ± 17.0	130.3^{d} ± 5.7	100.80	<0.001
NH_4^+ (mg NH_4^+/kg soil)	14.7^{a} ± 1.6	9.7^{bc} ± 0.5	8.6^{c} ± 0.3	11.4^{ab} ± 0.5	11.53	<0.001
NO_3^- (mg NO_3^-/kg soil)	11.4^{a} ± 1.3	10.2^{a} ± 0.7	3.6^{b} ± 0.4	3.9^{b} ± 0.4	44.23	<0.001
Mg (mg Mg/kg soil)	2471.6^{a} ± 156.7	1655.1^{b} ± 76.1	647.1^{c} ± 26.0	543.2^{c} ± 21.0	224.53	<0.001
Ca (mg Ca/kg soil)	570.5^{a} ± 41.0	430.8^{b} ± 23.4	3520.7^{c} ± 296.4	3330.1^{c} ± 157.0	297.49	<0.001
Ni (mg Ni/kg soil)	163.6^{a} ± 23.2	219.7^{b} ± 10.6	3.4^{c} ± 0.2	2.9^{c} ± 0.1	685.64	<0.001
Cr (mg Cr/kg soil)	0.25^{a} ± 0.03	0.38^{b} ± 0.05	0.14^{c} ± 0.01	0.15^{c} ± 0.01	17.60	<0.001

Notes: We conducted four greenhouse experiments using soils and plants from two serpentine grasslands, Nottingham County Park, Pennsylvania (Ser_{NOT}), and Soldiers Delight Natural Environment Area, Maryland (Ser_{SOL}), and two midwestern tallgrass prairie remnants, Hayden Prairie, Iowa (Pra_{HAY}), and Anderson Prairie, Iowa (Pra_{AND}). ANOVA results are for differences among four field sites (all df = 3, 36). For field site mean values, superscript letters indicate significant differences ($P < 0.05$) based on Tukey's hsd post hoc tests.

lower levels of P, K, and Ca, and much higher levels of NO_3^-, Mg, Ni, and Cr than the prairie sites. The two serpentine sites differed from each other in that Ser_{SOL} had lower levels of P, K, Mg, Ca, and NH_4^+ but higher levels of Ni and Cr than Ser_{NOT}. There were also differences between the two prairie soils; Pra_{AND} site had lower levels of P and NH_4^+ and higher levels of K than Pra_{HAY}.

Plant biomass

As a general trend, plants were smaller in conspecific soils than in heterospecific soils (Fig. 1; main effect in ANOVA, $F_{1,56} = 30.18$, $P < 0.001$) and this was similar across all sites (no soil × site interaction). However, the various plant species responded differently to conspecific vs. heterospecific soils (soil × tester species interaction; $F_{8,154} = 12.41$, $P < 0.001$). Only *Sorghastrum* was smaller in conspecific soils at all four sites (Table 2; Fig. 1). There were three other specific cases where plants were smaller in conspecific soils (*Sporobolus* and *Schizachyrium* at Ser_{NOT} and *Sporobolus* at Pra_{HAY}) and one case (*Andropogon* at Pra_{HAY}) where biomass was greater in conspecific soils. The soil × tester species × site interaction was not significant.

When plant performance as a function of the four distinct trainer soils was examined, all sites but Ser_{SOL} showed a similar effect of trainer soil across all tester species (main effect in each site-specific ANOVA; $P < 0.05$; Table 2; Fig. 2). In general, plants growing in soils trained by *Sorghastrum* were the smallest. For Pra_{HAY}, plants in soils trained by *Sorghastrum* differed significantly from those trained by *Andropogon* (Tukey hsd post hoc test; $P < 0.05$), while at Pra_{AND}, plants in soils trained by *Sorghastrum* were significantly smaller than those in soils trained by either *Andropogon* or *Schizachyrium*. At Ser_{NOT}, the main effects were more complicated, but the general result still held that plants grown in soils trained by *Sorghastrum* were the smallest, and those trained by *Andropogon* the largest.

For all sites but Pra_{HAY}, the species training the soils also altered the relative performance of the tester species. This was indicated because the trainer soil × tester species interaction was significant for Ser_{NOT} and Pra_{AND} ($P < 0.001$; Table 2) and nearly significant for Ser_{SOL} ($P < 0.06$).

Because there was no interaction between trainer soil and tester species at Pra_{HAY}, it was likely that the strong main effect of trainer soil explained the significant differences in biomass between conspecific and heterospecific soils. That is, the fact that all plants were larger in soils trained by *Andropogon* could explain the significantly greater biomass of *Andropogon* in conspecific soils and help explain the significantly greater biomass of *Sorghastrum* and *Sporobolus* in heterospecific soils. To explore this possibility, we conducted the Pra_{HAY} site-specific ANOVA again after eliminating *Andropogon* from the model. Trainer soil was no longer significant, thereby indicating the overall importance of *Andropogon* to the original main effect, and biomass was not greater in heterospecific (compared to conspecific) soils for any species, although it was nearly so for *Sorghastrum* ($F_{1,54} = 3.85$, $P < 0.06$). For Ser_{NOT}, in contrast, the main effect of trainer soil cannot fully explain the result that three different species were smallest in conspecific soils.

Trainer soil properties

When soil nutrients in the training soils were examined as a function of the species training the soil, there were no differences for Ser_{NOT}. For Pra_{HAY}, soils trained by *Andropogon* had higher levels of Mg and Ca than all other soils and higher levels of K than soils trained by *Schizachyrium* or *Sorghastrum* (Fig. 3).

A total of 15 AM fungal species were identified across all four sites (Appendix A). The composition of the AM fungal spore community based on numbers of spores was remarkably similar across all trainer soils for Ser_{SOL}, Pra_{AND}, and Pra_{HAY} (MANOVA; Wilks' lambda > 0.148, $P < 0.89$) but differed among trainer species in Ser_{NOT} soils (Wilks' lambda = 0.016; $F_{48,36} = 2.28$, $P < 0.01$). The same results were obtained by the CAP analysis (for Ser_{NOT}, trace statistic = 0.781, $P <$

0.01; $\delta_1^2 = 0.597$, $P < 0.001$; where δ_1^2 is the first squared canonical correlation). The community composition in soils trained by *Sporobolus* is well separated on the first canonical axis (Fig. 4).

For Ser_{NOT}, examination of spore numbers for individual AM fungal species shows differences among trainer species for *Glomus rubiforme* ($F_{3,27} = 4.48$, $P < 0.01$), *Gigaspora gigantea* ($F_{3,27} = 6.27$, $P < 0.01$), *Scutellospora pellucida* ($F_{3,27} = 8.13$, $P < 0.001$), and *Glomus aggregatum* ($F_{3,27} = 5.22$, $P < 0.01$). The abundance of the fungal species in soils trained by *Sporobolus* always differed significantly from soils trained by one or more of the other training species: differing from soils trained by *Andropogon* in the case of *G. rubiforme*, differing from *Sorghastrum* soils for *Gi. gigantea*, differing from *Andropogon* and *Schizachyrium* soils for *S. pellucida*, and differing from soils trained by all other species for *G. aggregatum* (Tukey hsd; $P < 0.05$).

The total of all AM fungal spores (log-transformed) in a 50-mL soil sample similarly differed among trainer species only at Ser_{NOT} ($F_{3,27} = 5.89$, $P < 0.01$). Fewer spores were produced by *Sorghastrum* (373 ± 180 spores [mean ± SE]) than by *Sporobolus* (700 ± 45); spore numbers produced by *Andropogon* and *Schizachyrium* were intermediate and not significantly different from the other two.

The characterization of soil bacterial communities based on substrate utilization patterns revealed no differences among soils trained by the different plant species for either of the two sites for which this was measured (Pra_{HAY} and Ser_{NOT}). For Ser_{NOT}, the trace statistic in the CAP analysis was significant ($P < 0.05$) but δ_1^2 was not ($P < 0.20$), and there was no separation by plant species on the first two canonical axes (data not shown).

Root colonization

Root colonization by AM fungi was generally high and varied among plant species in the trainer pots at all sites (Fig. 5; $F_{3,12} = 4.12$, $P < 0.05$). The pattern of variation was similar for the two prairie sites (Pra_{HAY} and Pra_{AND}), where *Andropogon* was the most infected and *Sporobolus* the least. The pattern was also similar for the two serpentine sites (Ser_{NOT} and Pra_{AND}), where *Schizachyrium* was the most infected, differing from *Andropogon* at Ser_{NOT} and *Andropogon* and *Sorghastrum* at Ser_{SOL}.

There were differences among tester species in AM fungal colonization (main effect $F_{3,12} = 5.28$, $P < 0.02$), which was quantified only for Ser_{NOT}, but there were no significant differences as a function of the trainer soil. (Neither the trainer soil nor the trainer soil × tester species interaction was significant.)

In the trainer pots, root infection by putative pathogens was extremely low. Among the four morphological categories detected, no one category exhibited more than 3.3% colonization on any trainer species at any site. The four categories were (1) "brown round" (small clusters of two to five resting spores, 20 μm × 20–60 μm, that were melanized, (2) "*Phialophora*-like" (elongated clusters of four to 20 melanized resting spores, 20 μm × 40–150 μm), (3) "bacterial mass" (bacterial colonies of various sizes inside roots), and (4) "hyphal mass" (very large masses of melanized septate hyphae, up to 400 μm long). It is possible that the

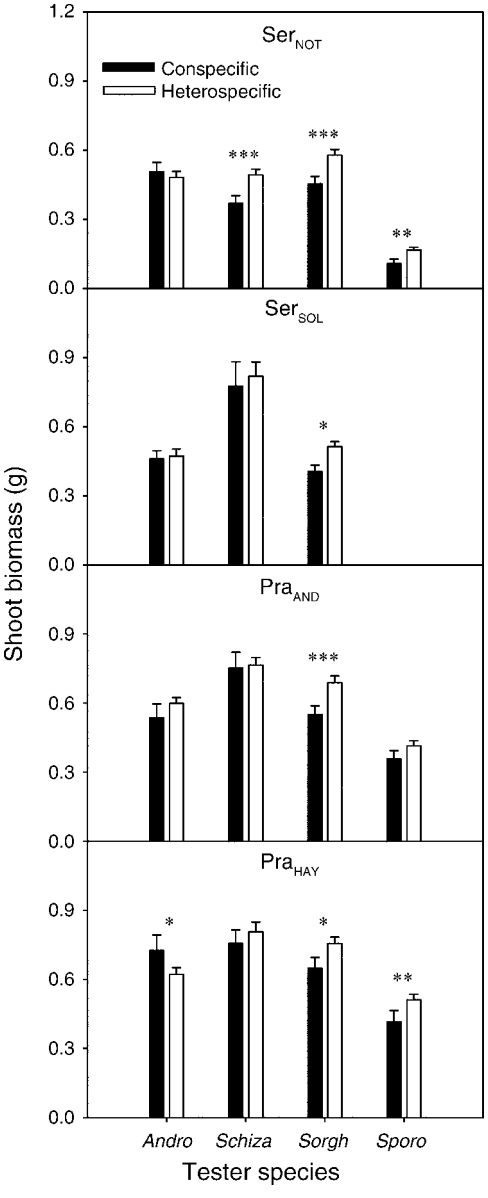

FIG. 1. Shoot biomass (mean and SE) for each tester species compared between soils trained by conspecifics and heterospecifics for each tester species. Significant differences are indicated with asterisks. There were two plants per pot except for *Schizachyrium* at Ser_{SOL}, where there was only one. Abbreviations are: Ser, serpentine; Pra, prairie; *Andro*, *Andropogon*; *Schiza*, *Schizachyrium*; *Sorgh*, *Sorghastrum*; *Sporo*, *Sporobolus*. Site abbreviations are as in Table 1.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Biomass of tester plants in soils trained by conspecifics compared to soils trained by heterospecifics and ANOVA results examining the effect of trainer soil, tester species, and their interaction.

Effect	Ser_{NOT}		Ser_{SOL}		Pra_{AND}		Pra_{HAY}	
	df	*F*	df	*F*	df	*F*	df	*F*
Andro vs. others	1, 126	0.8	1, 55	0.10	1, 126	3.81	1, 124	5.85*
Schiza vs. others	1, 126	20.71***	1, 55	1.32	1, 126	0.11	1, 124	1.15
Sorgh vs. others	1, 126	18.16***	1, 55	7.11*	1, 126	12.46***	1, 124	6.74*
Sporo vs. others	1, 126	9.49**	n/a	n/a	1, 126	0.08	1, 124	7.90**
Trainer soil	3, 42	5.92**	2, 28	2.03	3, 42	3.43*	3, 42	7.80***
Tester species	3, 42	106.48***	2, 28	37.78***	3, 42	50.99***	3, 42	31.02***
Soil × species	9, 126	5.18***	4, 55	2.49	9, 126	3.88***	9, 124	0.95

Note: Analyses are presented separately for each of the four sites. Abbreviations are as in Table 1.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

"brown round," "*Phialophora*-like," and "hyphal mass" are produced by the same species (or group of species), but we kept these data in separate categories. No other pathogen groups (e.g., *Olpidium*, *Polymyxa*, and others) were detected. An examination of their abundance (Appendix B) using MANOVA reveals differences among training species in the composition of these putative pathogens at every site (Wilks' lambda < 0.456, $P < 0.001$), but only Ser_{NOT} showed differences among training species in total percentage infection by all four categories combined (ANOVA; $F_{3,42} = 4.50$, $P < 0.01$); *Andropogon* had significantly higher infection than *Sorghastrum*, with *Sporobolus* and *Schizachyrium* intermediate and not significantly different from the other two. There was almost no evidence of damage by pathogenic nematodes on the root surface.

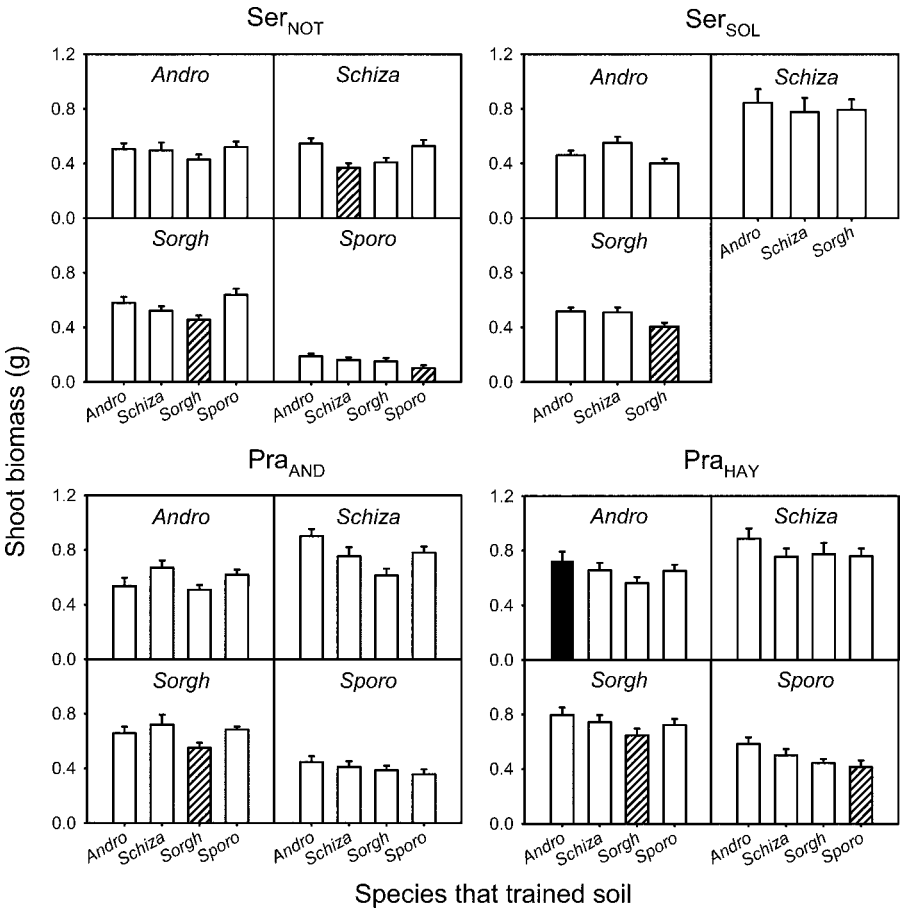

FIG. 2. Shoot biomass (mean and SE) for each (tester) species as a function of the species that trained the soil for each of the four sites (Ser_{NOT}, Ser_{SOL}, Pra_{HAY}, Pra_{AND}). Different patterns in bars indicate either significantly less biomass (hatched bars) or greater biomass (solid bars) in conspecific soils compared to heterospecific soils as in Fig. 1. Significance levels are shown in Fig. 1.

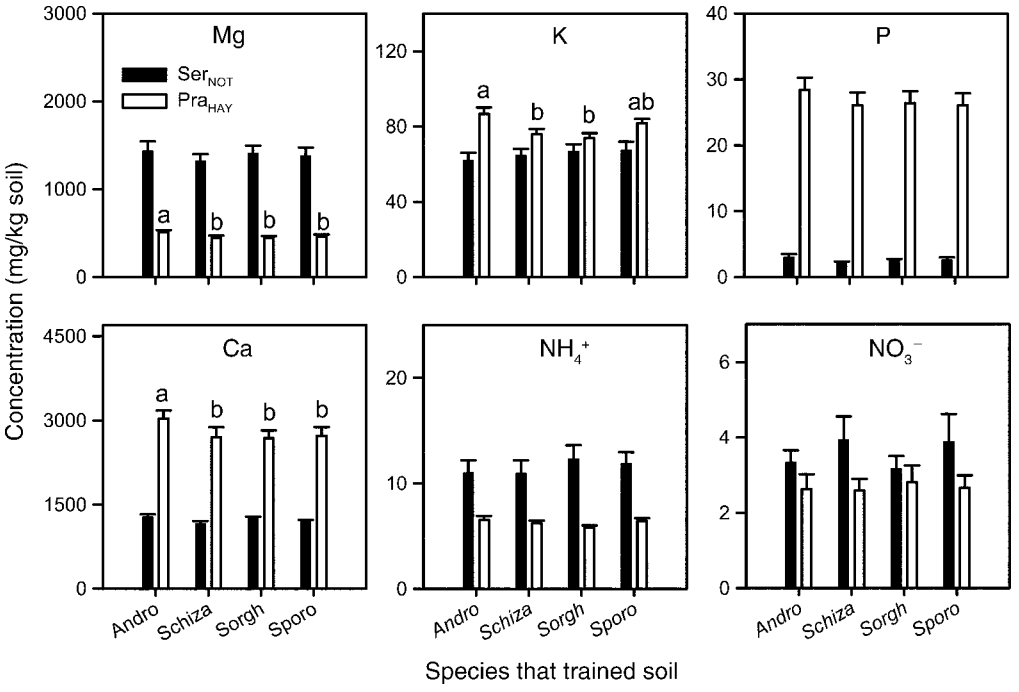

FIG. 3. Soil nutrients (mean and SE) for one serpentine (Ser_{NOT}) and one prairie (Pra_{HAY}) site as a function of the species that trained the soil. For soil factors differing among trainer species, bars with the same letter are not significantly different ($P > 0.05$). Not shown is pH, which was not different among training species for either site.

DISCUSSION

Feedback patterns

With respect to our original objectives, we draw the following conclusions from this study: (1) Across all four sites, plant–soil feedback was generally expressed as plants performing worse in soils previously occupied by the same species than in soils previously occupied by other species. *Andropogon* at Ser_{NOT} was the single case in which the opposite was observed. (2) Plant–soil feedback sometimes affected the species differentially thereby changing their relative performance, but there were also nonspecific, more general effects of plant–soil feedback where relative performance was unaffected. This general effect was particularly obvious at Pra_{HAY}, where all species were larger (greater biomass) on soils trained by *Andropogon*; this general effect could by itself explain why *Andropogon* was larger in conspecific soils and *Sorghastrum* and *Sporobolus* were larger in heterospecific soils. (3) The fact that *Sorghastrum* was smaller in conspecific soils at all four sites suggests that negative feedback may be characteristic of this particular species. (4) For the remaining three species, the occurrence of feedback differed among sites. (5) There were no obvious differences in the frequency or direction of feedback between the prairie soils and the more metalliferous serpentine soils, so variation among sites is probably driven by other abiotic or biotic factors.

The occurrence of generalized feedback that affects several species uniformly has been detected in at least three other studies (Bonanomi and Mazzoleni 2005, Bezemer et al. 2006, Kardol et al. 2007), but its implications have been little discussed. When experimental designs only permit comparisons of plant performance in conspecific vs. heterospecific soils, the general effect of a species grouped among the heterospecifics can be overlooked, especially if its performance is not evaluated in its own soils. General effects that impact all (or several species) uniformly should have very different consequences for plant community struc-

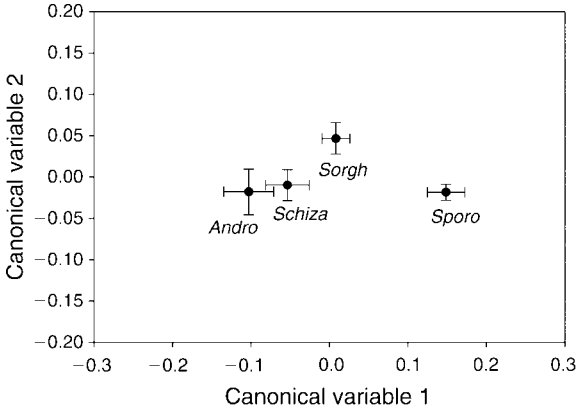

FIG. 4. Canonical discriminant plot (mean ± SE) showing the separation of arbuscular mycorrhizal (AM) fungal communities in Ser_{NOT} training soils as a function of trainer species. The first and second canonical axes explain 34.4% and 19.8% of the variation, respectively.

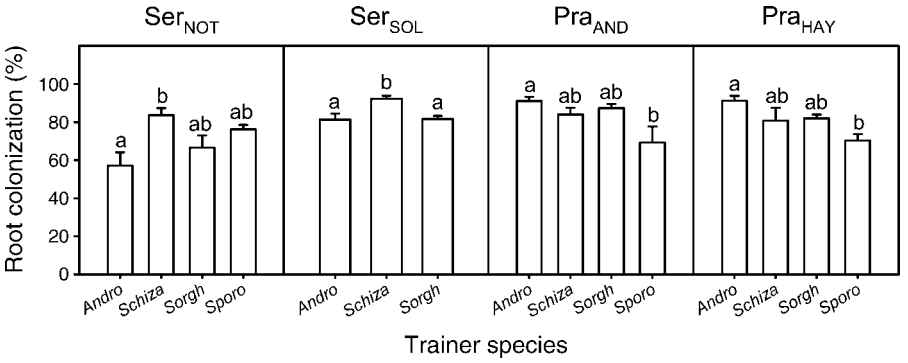

FIG. 5. Percentage root colonization (mean and SE) of the trainer plants by AM fungi for each of the four sites. Within a site, bars with the same letter are not significantly different ($P < 0.05$).

ture than feedback that changes the relative performance of the constituent species.

Possible causes of feedback

For Pra_{HAY}, the measured variation in soil nutrients as a function of training plant species parallels the main effect that all tester species had greater biomass when grown in soils trained by *Andropogon*; Pra_{HAY} soils trained by *Andropogon* had the highest levels of K, Mg, and Ca. However, the pattern of nutrient variation cannot explain why plants were generally smallest in soils trained by *Sorghastrum* at Pra_{HAY} since nutrients were not lowest in *Sorghastrum* soils. Thus, plant induced changes in nutrients may play a role in feedback at Pra_{HAY}, just as found in another grassland study (Bezemer et al. 2006), but it cannot be the sole explanation.

Other studies have shown feedback mediated through AM fungi (Castelli and Casper 2003, Sigüenza et al. 2006), pathogenic fungi (Van der Putten et al. 2007), and bacteria (Westover and Bever 2001), but none of the variation in the biotic factors we measured suggests a causal link to the feedback. It is possible that for Ser_{NOT}, the unique AM fungal community in soils trained by *Sporobolus* or the trainer soil differences in overall AM spore abundance contributed to plant–soil feedback, but the AM root colonization of tester plants does not show a difference in infection rate. Infection by putative root pathogens was low compared to accounts of non-mycorrhizal fungi in roots of non-native grasses (Rillig et al. 1999), and there were no striking patterns of abundance for the different pathogen morphotypes that correlated with plant size variation. Likewise, we identified no functional differences in soil bacterial communities among plant species. Similar to our results, Bezemer et al. (2006) documented nutrient variation consistent with plant–soil feedback in a sandy grassland but were unable to assign a cause to feedback for a grassland on chalk soils.

There are reasons why some causes of plant–soil feedback may go undetected. Our list of possible feedback agents was not exhaustive, and we may have simply overlooked responsible factors. We did not, for example, quantify root-feeding nematodes, which mediate feedback in a foredune system (Brinkman et al. 2005), and we might not have detected the damage they cause. The ability to detect community shifts in microbial metabolism using the Biolog method may be limited by a number of factors (Garland et al. 2007). Additionally, feedback could be mediated by hidden functional variation within AM fungi (Koch et al. 2006), and, in fact, some evidence exists that intraspecific AM fungal variation is linked to feedback at Ser_{NOT} (Castelli and Casper 2003). Also, standard soil nutrient analyses do not determine the actual availability of nutrients, which can be affected by root exudates (Bais et al. 2006) or rates of microbial activity (Hawkes et al. 2005). Finally, it is possible that the cumulative effect of many individual factors ultimately add up to affect plant biomass even if individual factors do not differ statistically.

Comparisons to other studies of Nottingham

We can compare our results for Ser_{NOT} with two prior feedback studies of the same grassland that used different methodologies. One study assessed plant growth in the greenhouse in non-sterilized soils collected under the grasses in the field (Gustafson and Casper 2004). The second was a field study in which seedlings were planted into gaps created within existing clumps of the same grasses and included competition with the clump (or not) as an additional factor (Casper and Castelli 2007). Both studies omitted *Sporobolus*. In all three studies, biomass was lower in conspecific soils for *Sorghastrum* just as it was here, although that difference was erased in the field when the seedlings were allowed to compete with the established clumps. On the other hand, conspecific soils reduced biomass in *Schizachyrium* in the present study, but showed no feedback at all in the other two.

The most striking difference in results for the Ser_{NOT} site applies to *Andropogon*, which showed strong negative feedback in the earlier studies, but none here. *Andropogon* exhibited exceptionally high mortality and

reduced biomass in conspecific soils in the field (Casper and Castelli 2007), regardless of competition treatment, and reduced biomass in conspecific soils in the greenhouse when fertilizer was added (Gustafson and Casper 2004). Those results, especially the high seedling mortality, which could be indicative of pathogens (Freckleton and Lewis 2006), suggests the action of biotic feedback agents, which we did not detect. There could be several explanations for different feedback results between field and greenhouse studies. (1) Cultivation biases in the AM fungal communities (Sýkorová et al. 2007) or other microbial groups may result in inherent differences between the soil communities between the two settings. (2) The length of greenhouse studies may be insufficient for the development of the same plant-related variation in soil characteristics that occurs over time in the field. (3) Plant decomposition and nutrient cycling undoubtedly differ from nature when shoots from the first (trainer) generation of plants are discarded (Bezemer et al. 2006, Kardol et al. 2006).

Regardless of the cause, the fact that different results were obtained for the same grassland using different protocols means that it is not always possible to infer the occurrence of plant–soil feedback in natural systems from greenhouse experiments, which is the most common investigatory approach. Plant–soil feedback is recognized as a potentially important factor contributing to the species composition and temporal dynamics of plant communities (Bever et al. 1997, Reynolds et al. 2003, Wardle et al. 2004, Ehrenfeld et al. 2005, Kardol et al. 2006), but greater validation of experimental results is needed. Experiments also need to separate species-specific feedback from more general feedback that affects all plants equally because these should have very different consequences for the composition of the plant community.

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APPENDIX A

Relative abundance of AM fungal species within each of the four sites (*Ecological Archives* E089-122-A1).

APPENDIX B

Relative abundance of putative root pathogens graphed for trainer plants at each site (*Ecological Archives* E089-122-A2).