MECHANISMS OF PLANT SPECIES COEXISTENCE: ROLES OF RHIZOSPHERE BACTERIA AND ROOT FUNGAL PATHOGENS

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Abstract. Two independent experiments were conducted to investigate the influence of rhizosphere bacteria on the growth of Anthoxanthum odoratum and Panicum sphaerocarpon. We tested whether host-specific populations of Bacillus mycoides affected the growth of their Anthoxanthum and Panicum hosts and whether host-specific differences in Bacillus populations modified the strong detrimental effect of the root fungal pathogen, Pythium macrosporum. Our results showed both positive and negative effects of Bacillus inoculation and that Anthoxanthum and Panicum plants responded differently to Bacillus isolates that originated from different host plants. Anthoxanthum grew relatively better with isolates from Panicum, while Panicum grew relatively better with isolates from Anthoxanthum, consistent with a negative feedback. In both experiments Pythium infection was detrimental to plant growth, and Panicum was more negatively affected by inoculation with Pythium. Overall, Bacillus ameliorated the pathogenic effect of Pythium. However, there was no evidence that host-specific Bacillus populations had different effects on the interaction between these plant species and Pythium. Both host-specific differences in rhizosphere bacteria and host-specific accumulation of a fungal pathogen can generate negative feedback between these two plant species.

Key words: Anthoxanthum odoratum; Bacillus mycoides; host specificity; negative feedback; Panicum sphaerocarpon; perennial grasses; plant community diversity; Pythium macrosporum; rhizosphere bacteria; root fungal pathogens.

INTRODUCTION

A growing body of work indicates that soil organisms are key to coexistence among plants (e.g., Bever 1994, Mills and Bever 1998, Van der Heijden et al. 1998, Packer and Clay 2000). Fungal root pathogens (Mills and Bever 1998, Packer and Clay 2000) and fungal root symbionts (Grime et al. 1987, Van der Heijden et al. 1998) have been shown to make contributions to plant species coexistence. However, other components of the soil community may also exert strong effects on plant growth. These include rhizosphere bacteria, which have been shown to have positive and negative effects on plant growth (Albrecht et al. 1981, Neitko and Frankenburg 1989, Handman et al. 1991, Derylo and Skorupska 1992). Rhizosphere bacteria have also been shown to modify or eliminate the effects of other components of the soil community (e.g., Harris et al. 1997, Kim et al. 1997).

In experiments with soil organisms from a North Carolina grassland we found strong negative feedbacks on plant growth following changes in the composition of the soil community (Bever 1994, Bever et al. 1997). Negative feedback between two plant species occurs when both plant species change their respective soil communities in ways that are detrimental to themselves relative to the other species. Negative feedback can therefore contribute to the coexistence of competing plant species (Bever et al. 1997). For example, Anthoxanthum odoratum and Panicum sphaerocarpon change soil communities and these changes are detrimental for the respective host plant species (Bever 1994, Bever et al. 1997; K. Westover and J. Bever, unpublished data). Accumulation of Pythium, a fungal root pathogen, in the soil may contribute to the negative feedback between Anthoxanthum and Panicum (Bever 1994, Mills and Bever 1998). Additionally, the composition of bacterial (Westover et al. 1997) and arbuscular mycorrhizal fungal (Bever et al. 1996) communities in the rhizosphere can change due to influence of the host plant species, and differentiation of these communities may also generate negative feedback (Bever et al. 1997, Bever 1999). Moreover, the deleterious effect of Pythium may be ameliorated by interactions with rhizosphere bacteria (Harris et al. 1997).

Here we investigated the influence of rhizosphere bacteria on the growth of Anthoxanthum odoratum and Panicum sphaerocarpon. Specifically, we tested whether host plant mediated changes in populations of Bacillus mycoides affected growth of their respective Anthoxanthum and Panicum hosts. Bacillus mycoides was very common at our site (Westover et al. 1997)
and through characterization of strains using restriction fragment length polymorphisms, we have found that the composition of *Bacillus mycoides* populations was influenced by host plant species (K. Westover, unpublished data). Members of the genus *Bacillus* are generally thought to be beneficial to plants (Claus and Berkeley 1996). *Bacillus* has been shown to enhance plant growth by producing phytohormones (Srinivasan et al. 1996) as well as by protecting plants from insects (Hofte and Whiteley 1989) and fungal pathogens (Faull and Campbell 1979, Fiddaman and Rossall 1993, Silos-Suh et al. 1994). Therefore, we also tested whether host-specific differences in *Bacillus* populations modulated the strong effects of *Pythium*, an important crop pathogen (e.g., Abad et al. 1994, Pankhurst et al. 1995) which has been shown to change their respective soil communities in several independent laboratory and field experiments and these changes were detrimental for host-specific plant growth, consistent with negative feedback (Bever 1994, Bever et al. 1997; K. Westover and J. Bever, unpublished data).

**Experimental overview**

We used two parallel, but independent, experiments to test the joint effect of *Bacillus* and *Pythium* on the growth of *Anthoxanthum* and *Panicum*. In the first experiment, isolates of *Bacillus* were obtained from the rhizospheres of these two species growing in the field. We used isolates of *Bacillus* obtained from the rhizospheres of greenhouse grown *Anthoxanthum* and *Panicum* for the second experiment. Because plant species in close proximity to *Anthoxanthum* and *Panicum* in the field may also be influencing soil communities, we might expect *Anthoxanthum* and *Panicum* influence on field soil microbes to be less defined compared to the effect of only *Anthoxanthum* or *Panicum* on soil in pots. This effect, in turn, would be reflected by a larger difference in plant growth response to host-specific *Bacillus* isolates collected from the individual pots. To test this we evaluated the effect of both greenhouse and field-collected isolates of *Bacillus* on plant growth.

Both experiments shared a similar design. Specifically, the experiments were full factorial tests of the effects of five independent isolates of *Bacillus* (obtained from each of the two plant species; i.e., ten isolates total plus the sterile control) and *Pythium* on the growth of the two plant species. The treatments were replicated five times each, except for the un inoculated *Bacillus* combinations, which were replicated ten times (in order to increase the power of testing the net effects of *Bacillus*).

**Isolation and preparation of Bacillus for soil inoculation**

For the first experiment, isolates of *Bacillus* were collected directly from *Anthoxanthum* and *Panicum* in the field. These plants had been originally surface ster-
ilized (see Bever 1994) and planted into the field in a randomized block design as part of a larger experiment. Due to their random placement in the field, the differences in Bacillus isolates are attributed to host effects. After the plants had been established for 24 mo, isolates of Bacillus were obtained from all Anthoxanthum and Panicum plants (16 plants of each species). Isolates used for inoculation were obtained by assigning all a number and then randomly choosing five from each plant species. We allowed only one Bacillus isolate from any particular plant. All isolates were therefore considered to be independent.

Isolates for the second experiment were obtained from Anthoxanthum and Panicum plants potted individually in the greenhouse. Before potting, these plants were de-rooted and surface sterilized to remove microbes and then planted into pots containing a field soil mixture (as in Bever 1994). Therefore, the plants grew roots into an initially similar soil community. The soil communities were allowed to differentiate for six months and were then sampled for Bacillus mycoides. Isolates were again obtained from all plants (10 plants of each species). Five isolates from each plant species were randomly chosen as described for the first experiment.

Bacillus mycoides was isolated from soil as follows. Soil was collected from a plant by gently lifting and shaking ~2 g of rhizosphere soil from the roots (Tate 1995). Each soil sample was homogenized and serially diluted using 0.1% CaSO4. Small (25 μL) aliquots from dilutions (10^-3, 10^-4, 10^-5, 10^-6, and 10^-7) of each sample were plated onto tryptic soy agar (TSA) (Wollum 1982). Colonies were counted after 24 h at 24°C and were re-plated onto TSA. Bacillus inoculum was prepared by transferring isolates to 10-mL tryptic soy broth tubes. The liquid cultures were incubated for 48 h at 24°C before inoculation. Aliquots of extra liquid cultures were serially diluted and plated onto TSA to estimate initial inoculum densities (colony-forming units per mL) of Bacillus cultures. There were no significant differences between estimated inoculum densities of Bacillus cultures in either experiment. The mean inoculum density was 10^7 colony-forming units per mL tryptic soy broth.

Preparation of Pythium for soil inoculation

The Pythium isolate was obtained from the roots of Panicum as described in Mills and Bever (1998). Pythium inoculum containing active mycelia and oospores that could survive in and infest the soil of treatment plants was prepared by growing the Pythium isolate in sterile grass blade culture (Martin 1992). Two plugs (each 0.5 cm^3) of corn meal agar with mycelium were placed in separate sterile tissue culture dishes (Falcon 3025, 150 X 25 mm style). Sterile deionized water was added to the dishes to a level where the water just covered the agar plugs, and 50 pieces (each 1.0–1.5 cm long) of autoclaved tall fescue grass leaves were placed in each dish. Five replicate dishes containing grass blade cultures were incubated at room temperature under continuous light for four days (Abad et al. 1994). Infection of grass blades by Pythium mycelia was monitored under the dissecting scope. Four days after the cultures were started, at which time most of the grass blades were infected, the grass blades were ready for inoculation of soil.

Planting, soil inoculation, and harvesting processes

Seedlings of Anthoxanthum and Panicum were started from surface-sterilized seeds of plants grown in the greenhouse. All seeds were planted in sterile seedling mix and allowed to grow until small seedlings were available. In June 1997, five replicate plants of each species were grown with each of the 10 field Bacillus isolates (five from Anthoxanthum and five from Panicum), alone, and with Pythium. All plants for this experiment were grown in 120-cc cone-tainers filled with sterile background soil. To prepare the background soil, field soil was first homogenized and passed through a 1-cm mesh. Equal parts of the sieved field soil and sand were then thoroughly mixed and autoclaved for 1.5 h. In January 1998, an experiment of similar design was planted using the greenhouse isolates of Bacillus.

Bacillus was added as 48-h 10-mL tryptic soy broth liquid cultures. A hole was made in the cone-tainer soil using a sterile glass rod and the liquid culture was well mixed and poured into the center. The plant was placed in this opening using sterile forceps. An additional set of control was planted in the same manner with sterile 10 mL of uninoculated media to serve as controls. Two Pythium infected grass blades from the grass blade cultures served as Pythium inocula. The infected grass blades were added to the pots using a sterile forceps and placed beneath the top 40 mL of soil in each pot. Another set of controls was planted in the same manner with sterile uninoculated grass blades instead of Pythium infected grass blades.

In both experiments, all cone-tainers were randomized and placed in a phytotron C chamber with 12-h day lengths and temperatures ranging from 10° to 25°C (Duke University Phytotron, Durham, North Carolina). The cone-tainers were spaced in racks with 5 cm between them to prevent cross contamination. All containers were kept well watered with distilled water during the first few days to allow initial establishment of the seedlings and Pythium. After the first week, plants were watered once daily with distilled water without application of nutrients. To prevent cross contamination, each cone-tainer was watered individually without splashing.

The experiments were harvested after ~8 wk and before the plants became “root bound.” Plants were removed from the soil and washed. Roots and leaves were separated, dried, and weighed. Estimates of plant size (e.g., leaf and tiller number) and fitness (e.g., number of reproductive inflorescences and seed set) are
correlated with plant biomass for Anthoxanthum and Panicum (A. Pringle, unpublished data). Therefore, dry mass of plant leaves and roots from each pot were summed for the subsequent analysis. Samples of soil and roots were also taken from select plots to ensure the presence of Bacillus and Pythium using the same procedures. There was no evidence of Pythium or significant Bacillus populations in the controls at harvest and as at the beginning of experiments, there were no significant differences between estimated densities of Bacillus in the Bacillus treatments. Mean Bacillus density was $10^7$ colony-forming units per gram of soil.

**Statistical analysis**

Total plant dry mass values were log$(x + 1)$-transformed to improve the homogeneity of variance. The effect of plant species, Bacillus inoculation treatment (including the presence of Bacillus and source of Bacillus isolate; i.e., three levels), Pythium inoculation treatment (two levels), Bacillus isolate nested within Bacillus inoculation treatment, and the appropriate interaction terms were analyzed using the General Linear Models Procedure of SAS (SAS Institute 1986). We analyzed the results in two ways. First, we treated Bacillus isolates as fixed effects. Second, we treated Bacillus isolates as random effects. The fixed-effects model gave us our most powerful test of differences among the ten particular Bacillus isolates used in each experiment. The mixed-model analysis (treating the Bacillus isolates as random effects) assumes that the particular Bacillus isolates are independent representatives of the host-specific soil communities. Testing effects with the mixed model allowed us to test for robust patterns, patterns that were likely to hold up even if we went back and randomly chose another five independent isolates from each plant species and repeated the experiment. For example, we could test whether the overall effect of Pythium would be negative even in association with ten different Bacillus isolates.

Within our design, the Bacillus inoculation treatment included the control along with the isolates from the two sources. This treatment factor was decomposed into two orthogonal components to test both the average effect of Bacillus (inoculated vs. controls), and the difference between the two sources of Bacillus (Anthoxanthum vs. Panicum). These effects were separated in all higher level interaction terms.

We were particularly interested in evaluating three sets of hypotheses relating to our prior observations of negative feedback between these two plant species. First, we wanted to test whether host-specific differences in Bacillus populations affected plant growth and therefore feedback between Anthoxanthum and Panicum (e.g., in Bever 1994). We tested this by comparing Anthoxanthum and Panicum growth with their own Bacillus populations compared to growth with each other’s Bacillus populations (a “home” vs. “away” contrast as derived in Bever et al. [1997]).

Our second specific interest was to test whether the presence of Bacillus modified our interpretation that accumulation of Pythium on Panicum contributed to the negative feedback between these two plant species (from Mills and Bever 1998). We evaluated this by testing whether the presence of Bacillus altered plant response to Pythium, explicitly testing the two-way interaction of Pythium by presence of Bacillus and the three-way interaction of test plant species by Pythium by presence of Bacillus.

Our final specific interest was to evaluate the more subtle possibility that differences in Bacillus populations modify or ameliorate the deleterious effect of Pythium, and therefore modify negative feedback through accumulation of host-specific fungal pathogens. We evaluated this possibility by testing the two-way interaction of Pythium × Anthoxanthum vs. Panicum source of Bacillus contrast and the three-way interaction of Test plant species × Pythium × Anthoxanthum vs. Panicum source of Bacillus contrast.

**RESULTS**

**Plant response to Bacillus inoculation**

The mass of plants grown in soil inoculated with field and greenhouse isolates of Bacillus was significantly different compared to plants grown in uninoculated soil (Uninoculated vs. Bacillus contrast, Tables 1 and 2). There was evidence for both positive and negative effects of Bacillus inoculation. Plants grown in soil inoculated with field isolates of Bacillus were smaller than those grown in uninoculated soil, while plants grown in soil inoculated with greenhouse isolates of Bacillus were larger (Fig. 1a, 1b, negative and positive effects, respectively). The negative effect of the particular field isolates used in this experiment may not be representative of all Bacillus isolates from the field, as this effect was not significant in the mixed model (Table 1). However, the growth promotion by the greenhouse isolates was representative of the population of Bacillus that accumulated in the greenhouse (Table 2).

There was no significant difference between the overall effect of field-collected Bacillus isolates from Anthoxanthum vs. those from Panicum on plant mass (AN vs. PA isolate contrast, Table 1, Fig. 1a). But there were significant differences among individual Anthoxanthum isolates and among individual Panicum isolates collected from both the field and the greenhouse (Rhzosphere × Isolate interaction, Tables 1 and 2). Plant mass varied depending upon the particular Anthoxanthum or Panicum isolate used to inoculate the soil in both experiments (Fig. 1a, 1b). There were differences between the overall effect of greenhouse cultured Bacillus isolates from Anthoxanthum vs. those from Panicum on plant mass (AN vs. PA isolate contrast, Table 2, Fig. 1b). Plants inoculated with isolates of Bacillus from Anthoxanthum were larger than plants inoculated...
with Bacillus isolates from Panicum (Fig. 1b). This difference, however, was not significant when tested over the substantial variation among greenhouse Anthoxanthum and Panicum in the mixed-model analysis (Table 2), indicating that the difference observed between these five particular isolates from Anthoxanthum and Panicum may not be representative of the populations of Bacillus associated with these species in the greenhouse.

Anthoxanthum and Panicum test plants responded similarly to being grown in soil inoculated with Bacillus compared to uninoculated soil in both experiments (Test plant species × Uninoculated vs. Bacillus contrast, Tables 1 and 2). Anthoxanthum and Panicum also responded similarly to the presence of isolates of Bacillus collected from greenhouse pots of Anthoxanthum and Panicum isolates (Test plant species × AN vs. PA isolate contrast, Table 2). However, Anthoxanthum and Panicum responded differently to the presence of field-collected Anthoxanthum vs. Panicum isolates (Test plant species × AN vs. PA isolate contrast, Table 1). This contrast remained significant ($P < 0.04$) when the variation among Anthoxanthum and Panicum isolates was taken into consideration by using the mean square for the Test × Rhizosphere × Isolate interaction as the error term in the mixed-model analysis (Table 1). This again indicates that these differences are representative of the populations of Bacillus associating with these two species in the field. Anthoxanthum test plants were significantly larger when grown in soil inoculated with Panicum isolates of Bacillus, and Panicum test plants were significantly larger when grown in soil inoculated with Anthoxanthum isolates of Bacillus (Fig. 2), suggesting that the differentiation of Bacillus on different host plant species can contribute to negative feedback between Panicum and Anthoxanthum.

**Plant response to Pythium inoculation**

The presence of Pythium resulted in significant reductions in plant mass in both experiments (Pythium effect, Tables 1 and 2). Plant mass was reduced from a mean of $0.26 \pm 0.01$ g to $0.20 \pm 0.01$ g in the experiment where field isolates of Bacillus were used and from a mean of $0.23 \pm 0.01$ g to $0.15 \pm 0.01$ g in the experiment where greenhouse isolates of Bacillus were used. In both experiments, this deleterious effect of Pythium was consistent across multiple Bacillus isolates, as confirmed in the mixed-model ANOVA (Tables 1 and 2).

Panicum was more susceptible to Pythium than Anthoxanthum in both experiments, although this was only significant in the experiment where greenhouse
isolates of Bacillus were used (Test plant species × Pythium interaction, Tables 1 and 2). Although not significant, in the first experiment (field isolates of Bacillus), Panicum mass was reduced by 52% and Anthoxanthum mass reduced by 23%. In the second experiment (greenhouse isolates of Bacillus), Panicum mass was reduced by 71% while Anthoxanthum mass was reduced by only 4% (Fig. 3). Again, this effect was also significant in the mixed-model ANOVA (Table 2).

Plant response to co-inoculation with Bacillus and Pythium

Plants grown in soil inoculated with Pythium and greenhouse Bacillus isolates were larger than those grown in soil inoculated with Pythium alone (Pythium × Unoinoculated vs. Bacillus contrast, Table 2, Fig. 4). This contrast remained significant (P < 0.01) when the variation among Anthoxanthum and Panicum isolates was taken into consideration by using the mean square for the Pythium × Rhizosphere × Isolate interaction as the error term in the mixed-model analysis (Table 2). However, this effect was not significant in the experiment where field isolates of Bacillus were used (Table 1).

There was no evidence that plants responded any differently to being grown in soil inoculated with Pythium and isolates of Bacillus associated with Anthoxanthum or Panicum in either experiment (Pythium × AN vs. PA isolate contrast, Tables 1 and 2). There was also no indication from either experiment that Anthoxanthum and Panicum test plants responded differently to soil inoculated with Pythium and Bacillus (Test plant species × Pythium × Unoinoculated vs. Bacillus contrast, Tables 1 and 2). Furthermore, Anthoxanthum and Panicum test plants did not respond differently to soil co-inoculated with Pythium and Bacillus isolates associated with Anthoxanthum or Panicum (Test plant species × Pythium × AN vs. PA isolate contrast, Tables 1 and 2). However, the ten individual isolates of Bacillus isolated from the field differed in their ability to alleviate the deleterious effect of Pythium, as confirmed by the significance of the Pythium × Rhizosphere × Isolate interaction in the fixed model (Table 1).

**Discussion**

Our results suggest that plant species-specific Bacillus isolates contribute to the maintenance of plant diversity in a North Carolina old field community. Differentiation of Bacillus isolates on plant hosts is consistent with an analysis of restriction fragment length polymorphisms using these Bacillus isolates (K. West-
over, unpublished data) and with host-specific patterns of substrate utilization for entire rhizosphere bacterial communities (Westover et al. 1997). In the present study, we also found evidence of this host-specific differentiation among the field isolates as demonstrated by the significant interaction between test plant species and contrast of AN vs. PA isolates (Table 1). Such differentiation was suggested in the greenhouse isolate experiment as well by the significance of the AN vs. PA isolate effect within the fixed model (Table 2, Fig. 1b). *Anthoxanthum* and *Panicum* plants then responded differently to *Bacillus* isolates depending on their host of origin (Table 1). Specifically, *Anthoxanthum* grew relatively better with isolates from *Panicum*, while *Panicum* grew relatively better with isolates from *Anthoxanthum* (Fig. 2). This pattern held true for both experiments, though only statistically significant for field-collected isolates. This result demonstrates that host-specific differences in *Bacillus* populations can generate negative feedback on host growth. This is consonant with repeated observations of negative feedback between these same plant species due to changes in soil community composition (both in the lab, Bever 1994,
Bever et al. 1997; and in the field, K. Westover and J. Bever, unpublished data). Together, these works suggest that the dynamics within Bacillus populations can contribute to the coexistence of these two plant species.

Previous results suggested that accumulation of Pythium on Panicum also contributed to the negative feedback observed between these two species (Mills and Bever 1998). We have previously found that Pythium accumulates in greater abundance on Panicum and that growth of Panicum was more sensitive to Pythium compared to Anthoxanthum. The present work also reinforces this hypothesis. In both experiments, Pythium infection was detrimental to plant growth and Panicum was more negatively affected by inoculation with Pythium than was Anthoxanthum. The difference in plant response (Test plant species × Pythium interaction) was highly significant in the greenhouse isolate experiment, but not in the field isolate experiment (Tables 1 and 2, Fig. 3).

Bacillus ameliorated the pathogenic effect of Pythium. Bacillus protects plants from Pythium infection in agricultural contexts and has been used in biological control efforts (Kim et al. 1997). Members of the Bacillus genus are known to produce antifungal compounds (Faull and Campbell 1979, Fiddaman and Ros-sall 1993, Silo-Suh 1994). Individual Bacillus isolates altered the response of plants to Pythium in the experiment with field-derived isolates where there was greater heterogeneity among the ten Bacillus isolates tested (Table 1). Such variation in the ability of Bacillus isolates to protect plants from fungal pathogens has been demonstrated in agricultural contexts as well (Landa et al. 1997).

Host-specific populations of Bacillus were important for plant growth, but there was no evidence that this had a net effect on the interaction between these plant species and Pythium (Test plant species × Pythium × AN vs. PA isolate term, Tables 1 and 2). Therefore we reject the hypothesis that the negative feedback that occurs through changes in the composition of the Bacillus populations is mediated by Bacillus protection from Pythium. However, given the level of variability in the protection from Pythium provided by different Bacillus isolates, this hypothesis warrants further exploration. In addition, some interactions among pathogens and mutualists can depend on life stage (Smith and Read 1997). Given that the plants used in the current experiment were seedlings, a potential temporal aspect to the interaction of these plant species with Bacillus and Pythium might also be expected.

The effect of individual Bacillus isolates on plant growth varied both within and between the two experiments, with evidence for both positive and negative effects of Bacillus inoculation. There was also slight variation in the growth of uninoculated plants between experiments. Uninoculated plants in the experiment where field isolates were used were ~0.1 g larger than uninoculated plants in the experiment where greenhouse isolated were used (Fig. 1a, 1b). In the field isolate experiment, Bacillus generally had negative impacts on plant growth, while the isolates in the greenhouse experiment had significantly positive effects (Fig. 1a, 1b, Tables 1 and 2). Moreover, the variation among isolates within the experiments was lower in the greenhouse experiment than in the field experiment (Fig. 1a, 1b, Tables 1 and 2). This suggests that while growing with plants in the greenhouse prior to collection of isolates for the current study (6 mo), the composition of the Bacillus populations narrowed and shifted from a generally pathogenic relationship towards a generally mutualistic one. Perhaps this indicated some level of adaptation to the greenhouse conditions, which benefited the plant. Or we might postulate that the positive greenhouse results are unrealistic. However, results from the mixed-model analysis indicated that the negative effect of the field isolates may not be representative (Table 1). It is also possible that strong host-specific mediated responses are moderated by diffuse interactions in the field or that Bacillus may have positive and negative effects depending upon physical conditions or the presence of diverse microbial communities.

A similar shift in variability and benefit was observed with respect to the ability of Bacillus to protect plants against Pythium (Tables 1 and 2). This result is surprising given our observation that host-specific shifts in Bacillus populations result in decreased performance of the host with their own Bacillus isolates. However, as we have previously demonstrated (Bever et al. 1997, Bever 1999), the direction of feedback through community differentiation (i.e., positive or negative) can be independent of the direction of direct ecological effect (i.e., mutualistic or pathogenic). For example, a mutualistic organism can still act as an agent of negative feedback if it provides a relatively greater benefit to a neighboring species.

In this paper, we demonstrated different effects in populations of Bacillus mycoides associated with Anthoxanthum and Panicum suggesting that previous observations of differentiation of rhizosphere bacterial communities associated with these and other plant species in the North Carolina old field (Westover et al. 1997) were likely an underestimation. Not only did previous methods count only culturable bacteria, but also the method used could not have detected host-specific “genotype” or “strain” differences among bacterial populations of the same species. In the present work, we were able to demonstrate the importance of this host-specific differentiation for negative feedback between Anthoxanthum and Panicum. Clearly, a complete understanding of how feedback through the soil organisms can contribute to the maintenance of diversity within plant communities must include not only evaluation of the role of community differentiation, but the role of population genetic differentiation within species as well. Conversely, the negative feedback ob-
served through differentiation of Bacillus mycoides populations is likely itself to be a subset of the larger negative feedbacks generated by community level differentiation on rhizospheres (as observed in Westover et al. 1997) and differentiation within other populations.

Together with the previous work of Mills and Bever (1998), a summary of current research indicates that two mechanisms generate the negative feedback observed between Anthoxanthum and Panicum, namely the accumulation of Pythium in Panicum soil and the differentiation of host-specific Bacillus populations. Note, however, that while the accumulation of Pythium can explain the reduced growth of Panicum when grown in Panicum vs. Anthoxanthum soil, this mechanism cannot explain the reduced growth of Anthoxanthum in Anthoxanthum vs. Panicum soil (also observed in Bever 1994; and J. Bever and K. Westover, unpublished results). The host-specific differentiation within Bacillus mycoides populations, however, can contribute to the reduction in growth of both species in their respective soil communities. By identifying the complementary roles of Pythium and Bacillus, we would not wish to claim to have identified all agents for the observation of negative soil community feedback between Anthoxanthum and Panicum. Rather this body of work provides illustration of the multidimensionality of the interaction between plants and their soil community. That is, we have tested two potential agents and found them both capable of generating negative feedback. There are numerous other potential agents of negative feedback between Anthoxanthum and Panicum. For example, other members of the rhizospheric bacterial (Westover et al. 1997) and mycorrhizal fungal communities (Bever et al. 1996) also differentiate on these host plants, and may contribute to the negative feedback we observed. Other potential root pathogens have been observed in this system as well, including Fusarium and root-feeding nematodes (J. Bever, personal observation). It therefore seems likely that there are multiple complementary mechanisms within the soil community that can contribute to the coexistence of competing plant species.

In summary, both the host-specific differentiation of rhizosphere bacteria and the host-specific accumulation of a fungal pathogen can generate negative feedback between these two plant species. Moreover, while Bacillus may ameliorate the detrimental effect Pythium on plant growth, this does not appear to alter the Bacillus role in negative feedback and therefore in plant species coexistence. Both beneficial and pathogenic members of the soil community can play an important role in the maintenance of plant species diversity.

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