



## Plant monocultures produce more antagonistic soil *Streptomyces* communities than high-diversity plant communities



Matthew G. Bakker, Lindsey Otto-Hanson, A.J. Lange, James M. Bradeen, Linda L. Kinkel\*

Department of Plant Pathology, University of Minnesota, 491 Borlaug Hall, 1991 Upper Buford Circle, Saint Paul, MN 55108, USA

### ARTICLE INFO

#### Article history:

Received 13 October 2012

Received in revised form

11 June 2013

Accepted 12 June 2013

Available online 27 June 2013

#### Keywords:

Plant soil feedbacks

*Streptomyces*

Antagonism

Diversity

Antibiosis

Resource diversity

### ABSTRACT

Plant–soil feedbacks are important to productivity and plant community dynamics in both natural and managed ecosystems. Among soil bacteria, the *Streptomyces* possess particularly strong antagonistic activities and inhibit diverse plant pathogens, offering a clear pathway to involvement in plant–soil feedbacks. We hypothesized that feedback effects and the ability of individual host plant species to foster antagonistic *Streptomyces* populations may be modified by the richness of the surrounding plant community. To test this, we collected soil associated with four different plant species (two C4 grasses: *Andropogon gerardii*, *Schizachyrium scoparium*; and two legumes: *Lespedeza capitata*, *Lupinus perennis*), grown in communities that spanned a gradient of plant species richness (1, 4, 8, 16, or 32 species). For each of these soils, we characterized the potential of soil *Streptomyces* to antagonize plant pathogens, using an *in vitro* plate assay with indicator strains to reveal inhibition. We cultivated each plant species in each conditioned soil to assess feedback effects on subsequent plant growth performance. Surrounding plant richness modified the impacts of particular plant species on *Streptomyces* antagonistic activity; *A. gerardii* supported a higher proportion of antagonistic *Streptomyces* when grown in monoculture than when grown in 32-spp plant communities, and *L. capitata* supported more strongly antagonistic *Streptomyces* when grown in 4- or 32-spp plant communities than in 8-spp plant communities. Similarly, the feedback effects of particular plant species sometimes varied with surrounding plant richness; above-ground biomass production varied with plant species richness for *A. gerardii* in *L. perennis*-trained soil, for *L. capitata* in *A. gerardii*-trained soil, and for *L. perennis* in *L. capitata*-trained soil. *Streptomyces* antagonist density increased with overall *Streptomyces* density under low but not under high plant richness, suggesting that plant diversity modifies selection for antagonistic phenotypes among soil *Streptomyces*. This work highlights the complexity of feedback dynamics among plant species, and of plant–microbiome interactions in soil.

© 2013 Elsevier Ltd. All rights reserved.

### 1. Introduction

Plant–soil feedbacks occur when changes to the soil environment imposed by a particular plant alter the subsequent performance of other plants, belonging to either the same or different species (Callaway et al., 2008). Feedbacks may be positive, enhancing subsequent performance, or negative, reducing subsequent performance (Kulmatiski et al., 2008). Plant–soil feedbacks have been studied extensively because of their potential impacts on plant community dynamics (Klironomos, 2002; Eppinga et al., 2006; Petermann et al., 2008). For instance, negative feedbacks have been suggested to play a significant role in maintaining plant diversity across the landscape by preventing competitive exclusion

by dominant species (Janzen, 1970; Connell, 1971; Bever et al., 1997, 2010). On the other hand, positive feedbacks may play a role both in maintaining dominant species and in facilitating invasion by exotic species (Inderjit and Van der Putten, 2010). Plant–soil feedbacks also have important implications for agricultural systems, where the same species is often grown repeatedly or in a short rotation with a limited number of other species. A better understanding of the mechanistic bases that attenuate negative or promote positive feedbacks may suggest methods of managing agroecosystems to limit plant disease and improve plant health.

Plant–soil feedbacks can be mediated through soil microbial communities (Olf et al., 2000; McCarthy-Neumann and Kobe, 2010a), although mechanisms involving soil chemistry or nutrient levels can also be important (Ehrenfeld et al., 2005; Casper et al., 2008; McCarthy-Neumann and Kobe, 2010b). Pathogens and beneficial symbionts such as mycorrhizal fungi have been the primary foci for studies of microbially-mediated plant–soil feedbacks.

\* Corresponding author.

E-mail address: [kinkel@umn.edu](mailto:kinkel@umn.edu) (L.L. Kinkel).

Less attention has been given to suppression of pathogens as a mechanism for attenuating negative feedbacks. Negative feedback through pathogen accumulation has long been appreciated in agriculture and is a primary rationale for crop rotation (Hwang et al., 2009). At the same time, however, long-term monoculture has also been associated with the development of disease-suppressive soils (Menzies, 1959; Weller et al., 2002), in which little disease develops even when conditions are conducive for disease development. Such disease suppression often results from competition- or antibiotic-mediated antagonism of pathogens by other microbes (Haas and Keel, 2003; Anukool et al., 2004; Kinkel et al., 2011). This suggests that plant–soil feedbacks may incorporate more complex microbial interactions, including antagonism of plant pathogens.

Among soil bacteria, the *Streptomyces* possess particularly strong and diverse antagonistic activities against diverse plant pathogens (Doubou et al., 2001; Wiggins and Kinkel, 2005a, 2005b). Because most resources available to saprophytic *Streptomyces* are ultimately of plant origin, there is a strong potential for plant identity and community characteristics to impact *Streptomyces*-driven antagonism of plant pathogens (Wiggins and Kinkel, 2005b). Recent work has demonstrated that host plant species enrich different *Streptomyces* taxa and that host-specific impacts on *Streptomyces* community structure can be modulated by surrounding plant richness (Bakker et al., 2013). However, we do not have a predictive understanding of how changes in *Streptomyces* community structure relate to changes in pathogen-suppression. Aspects of resource availability might influence the development and maintenance of antagonistic phenotypes among *Streptomyces* (Kinkel et al., 2012). In particular, density-dependent selection may be important to the development of highly antagonistic communities capable of effectively suppressing pathogens; maintaining high *Streptomyces* densities may be important in selecting for more strongly competitive phenotypes (Kinkel et al., 2012). To explore specifically how plants may shape such selective forces that lead to pathogen antagonism, we assessed *Streptomyces* antagonistic phenotypes from communities associated with different host plant species and across a gradient of plant richness.

Plant–soil feedbacks are often studied using monocultures grown singly in pots. However, most plants in natural habitats grow in complex communities, interacting with neighboring plants. The identity of neighboring individuals has been shown to impact various aspects of plant phenotype (Bartelt-Ryser et al., 2005; Broz et al., 2010), which suggests that interactions of plants with soil microbial communities may be similarly altered by the presence or identity of neighboring individuals (Bakker et al., 2013). Plant–plant interactions may also alter incentives for interactions with soil microbes. For instance, the development and maintenance of a beneficial microbial community may be energetically costly, while benefits may accrue to adjacent competing plants. In this case, a disincentive for investing in beneficial microbes may be experienced in higher diversity plant communities. Plant community diversity is also relevant from the perspective of plant diversity. Because disease pressure is likely to be strongest in low diversity plant communities (Garrett and Mundt, 1999; Keesing et al., 2006), the benefits for investing in pathogen-antagonistic microbial partners may be greatest in low-diversity plant communities. Finally, the identity and diversity of resource inputs to saprophytic microbial foodwebs will also vary with plant identity and diversity, with likely implications for the selective environment experienced by soil microbes. For instance, diverse resource inputs (i.e. exudates and litter from a diverse plant community) may encourage niche differentiation among soil saprophytes. By providing an alternative to strong antagonistic competition for resources, such specialization may reduce selection for antagonistic phenotypes (Kinkel et al.,

2011). Thus there is a broad theoretical basis for the expectation that pathogen-inhibitory soil microbes may be differentially impacted by plant species identity and diversity.

In this work, we investigated the feedback effects of four dominant prairie plant species (two C4 grasses, two legumes), with each conditioning species grown across a gradient of plant richness (communities planted with 1–32 plant species). We characterized the antagonistic activity of soil *Streptomyces* populations associated with each conditioning treatment and tested the same four species as feedback response species in a full factorial greenhouse experiment. In this way, we investigated whether surrounding plant richness modulated the ability of these host plant species to foster antagonistic soil *Streptomyces* populations or altered the feedback effects of each plant species on subsequent plant productivity.

## 2. Materials and methods

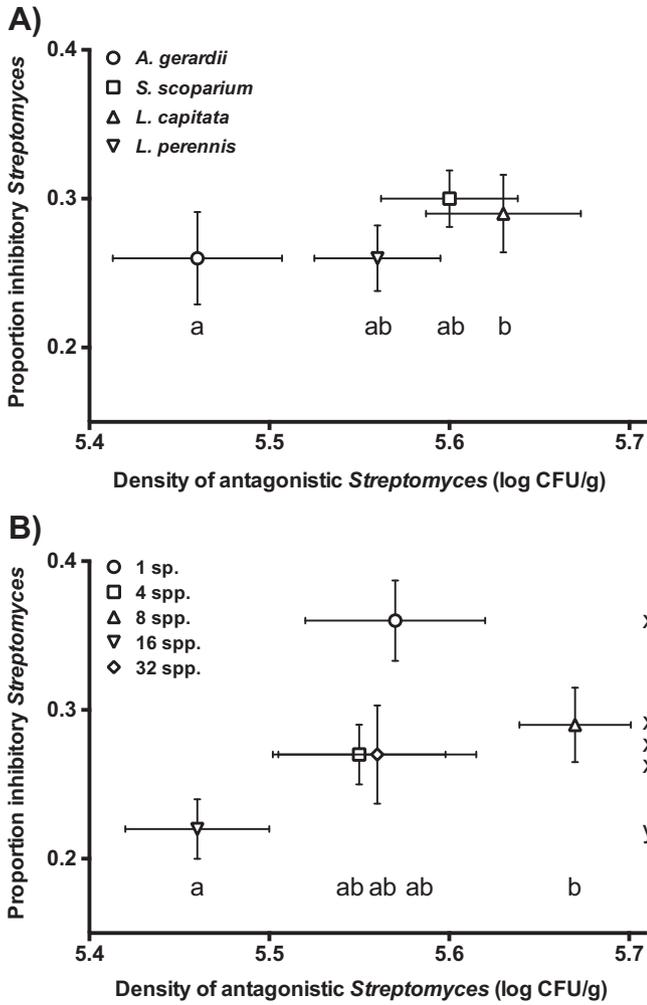
### 2.1. Soil collection and processing

Soil was collected from the Cedar Creek Ecosystem Science Reserve (CCESR; part of the National Science Foundation Long-Term Ecological Research network) in July of 2009, from experimental plots that have been maintained in a long-term plant richness manipulation (Tilman et al., 2001). These experimental plots were established in 1994 with defined levels of plant richness. For plant richness treatments of up to 16 species, plant species were drawn from a pool of 16 core native prairie plant species. Additional plant species were included for 32-species plots (Tilman et al., 1997). While other colonizing plant species are removed from each plot annually, the relative abundances of the planted species are allowed to fluctuate and so the experimental manipulation controls plant richness but not plant diversity per se. We targeted soil under the dominant influence of each of four different plant species (two C4 grasses: *Andropogon gerardii*, *Schizachyrium scoparium*; two legumes: *Lespedeza capitata*, *Lupinus perennis*) by collecting soil cores (5 cm × 30 cm) from the base of individual plants. Each sample consisted of four bulked and homogenized soil cores, one collected from each of four different individuals within a given plot. Each plant species was sampled in five different plant richness treatments (monoculture and assemblages planted with 4, 8, 16 or 32 species). There were three plot-level replicates per host-plant richness combination, except for monocultures of *A. gerardii* and *L. perennis*, for which only two plot-level replicates were available. Two different soil samples were processed from one of the plots in these cases. Thus we had a total of 60 soil samples (4 plant hosts × 5 plant community diversity levels × 3 replicates). Soil pH, potassium (K) and organic matter (OM) were measured at the University of Minnesota soil-testing lab using standard procedures (<http://soiltest.cfans.umn.edu/>).

### 2.2. *Streptomyces* antagonistic potential

Soil samples were stored at 4 °C and were processed in random order over a 6 month period. For each sample, a single 5 g sub-sample was dried overnight under sterile cheesecloth as the first enrichment for *Streptomyces*, which are tolerant of desiccation. Dried soil samples were dispersed in 50 mL of sterile deionized water on a reciprocal shaker (175 rpm, 60 min, 4 °C). Soil dilutions were spread onto 15 mL water agar plates and then covered with 5 mL of cooled, molten starch-casein agar (SCA). This method suppresses the growth of many unicellular bacteria, while allowing filamentous *Streptomyces* to grow up through the SCA (Wiggins and Kinkel, 2005b). After three days of incubation (28 °C), *Streptomyces* colonies were enumerated based on morphology. These plates were also used to assess *Streptomyces* inhibitory activities using a



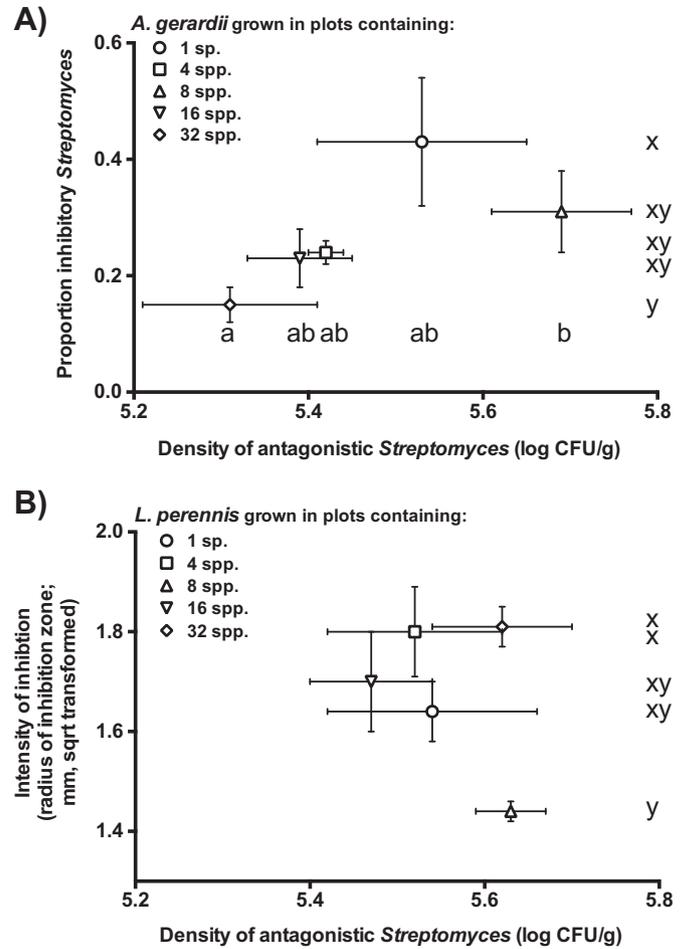


**Fig. 1.** The density (horizontal axis) and frequency (vertical axis) of antagonistic *Streptomyces* associated with **A)** different host plant species, or **B)** plant species richness treatments. Means + SE are shown; treatments marked with different letters (*a* vs. *b* for differences along the horizontal axis; *x* vs. *y* for differences along the vertical axis) differed significantly from each other ( $p < 0.05$ , ANOVA with Tukey contrasts).

significantly lower when grown in 8-spp assemblages than in 4- or 32-spp assemblages (Fig. 2B;  $p < 0.05$ ). Antagonistic characteristics of *Streptomyces* populations associated with *L. capitata* and *S. scoparium* did not differ significantly with surrounding plant richness (data not shown).

Across all treatments, *Streptomyces* density was negatively correlated with the proportion of inhibitory colonies ( $r = -0.62$ ,  $p < 0.001$ ). This could suggest that greater carbon availability reduces competitive pressure, and thus the benefits of antibiotics to fitness, even while supporting greater *Streptomyces* densities. Using soil OM as a proxy for carbon availability, the proportion of inhibitory colonies declined more rapidly with increasing *Streptomyces* density for sites with high carbon availability (upper third of samples ranked by organic matter content; range 1.1–2.2% OM) than for sites with low carbon availability (lower third of samples; range 0.7–1.0% OM; Fig. 3A).

Resource diversity may be as important as absolute carbon availability in determining the selective pressures experienced by saprophytic soil microbes. Since plants supply the majority of resources available to soil saprophytes, resource diversity should increase with plant species richness or diversity. Among *Streptomyces* communities explored here, both the strength and the slope of the



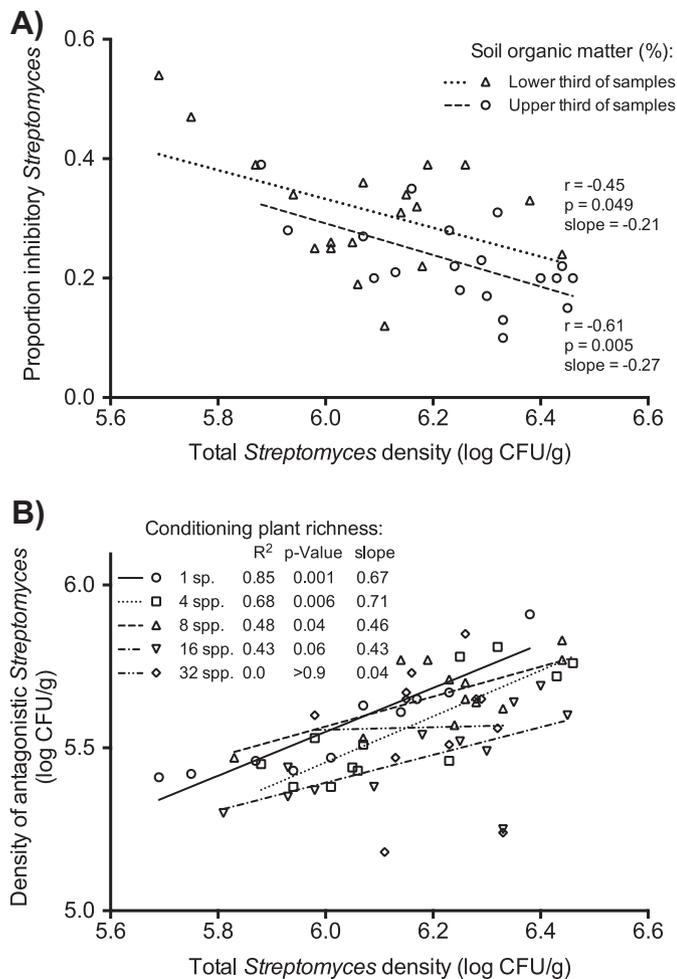
**Fig. 2.** **A)** The density (horizontal axis) and proportion (vertical axis) of antagonistic *Streptomyces* associated with *A. gerardii* when grown in communities of varying plant species richness. **B)** The density (horizontal axis) and inhibition intensity (vertical axis) of antagonistic *Streptomyces* associated with *L. perennis* when grown in communities of varying plant species richness. Means + SE are shown; treatments marked with different letters (*a* vs. *b* for differences along the horizontal axis; *x* vs. *y* for differences along the vertical axis) differed significantly from each other ( $p < 0.05$ , ANOVA with Tukey contrasts).

relationship between total *Streptomyces* density and antagonistic *Streptomyces* density declined consistently as plant diversity increased (Fig. 3B; see legend for statistics). In high diversity plant communities, antagonist densities increased more slowly with increasing *Streptomyces* densities than in low diversity plant communities. These results suggest that *Streptomyces* density may be a more significant factor in selection for antagonistic phenotypes within low-richness plant communities than in high-richness communities.

### 3.2. Impacts of soil conditioning on plant performance

Growth of the four target plant species varied significantly among soil conditioning treatments, demonstrating variable plant–soil feedback dynamics among conditioning plant species (species from which soil was collected), conditioning plant community richness, and response plant species (species for which growth performance was assessed). All four plant species showed sensitivity to soil conditioning treatments, though responses varied among treatments and measures of growth response.

Conditioning species identity impacted *S. scoparium* above-ground biomass production, with significantly more aboveground



**Fig. 3.** A) Frequency of antagonistic *Streptomyces* colonies, as a function of soil organic matter content. B) Relationships between total *Streptomyces* densities and densities of antagonist *Streptomyces*, by plant richness treatment (across host plant species). Pearson correlations and trend line slopes are listed on the figure.

biomass produced in soils conditioned by the legume *L. perennis* compared to soils conditioned by either of the two grasses (Fig. 4;  $p \leq 0.05$ , ANOVA with Tukey contrasts). *L. capitata* produced significantly more belowground biomass in soils conditioned by *A. gerardii* than in conspecific soils (Fig. 4;  $p = 0.05$ ).

Plant community richness during soil conditioning also impacted subsequent plant growth, although to a lesser extent overall than conditioning species. Significant differences in growth among plant richness treatments were found only for root length. Roots were significantly longer for both *L. perennis* and *S. scoparium* in soil conditioned by the richest plant communities, and there was a similar trend for *A. gerardii* (Fig. S1). Root length in response plants could be related to soil physical properties, nutrient status, or to microbial effects.

These results highlight the complexity and specificity of feedback dynamics. Adding further complexity, we found that plant community richness modulated feedback dynamics within pairs of conditioning- and response-species. That is to say, the same conditioning host had distinct effects on subsequent plant performance as a function of surrounding plant species richness. *A. gerardii* produced more aboveground biomass in *L. perennis*-conditioned soils from 8- or 16-spp plots than from 32-spp plots (Fig. 5A;  $p < 0.05$ , ANOVA with Tukey contrasts). *L. capitata* produced more aboveground biomass in soils conditioned by monocultures of *A. gerardii* compared to soils conditioned by *A. gerardii*

growing in 8- or 32-species assemblages (Fig. 5B;  $p < 0.05$ ). *L. perennis* produced more aboveground biomass in *L. capitata*-conditioned soil from 8-spp plots than from 32-spp plots (Fig. 5C;  $p = 0.05$ ).

Little disease was evident at the time of harvest, as revealed by visual inspection of washed roots (data not shown), indicating that feedback effects occur even in the absence of significant disease. Nodulation rates were low, and no significant treatment effects on nodulation were observed.

### 3.3. Correlations between antagonistic *Streptomyces* and plant–soil feedbacks

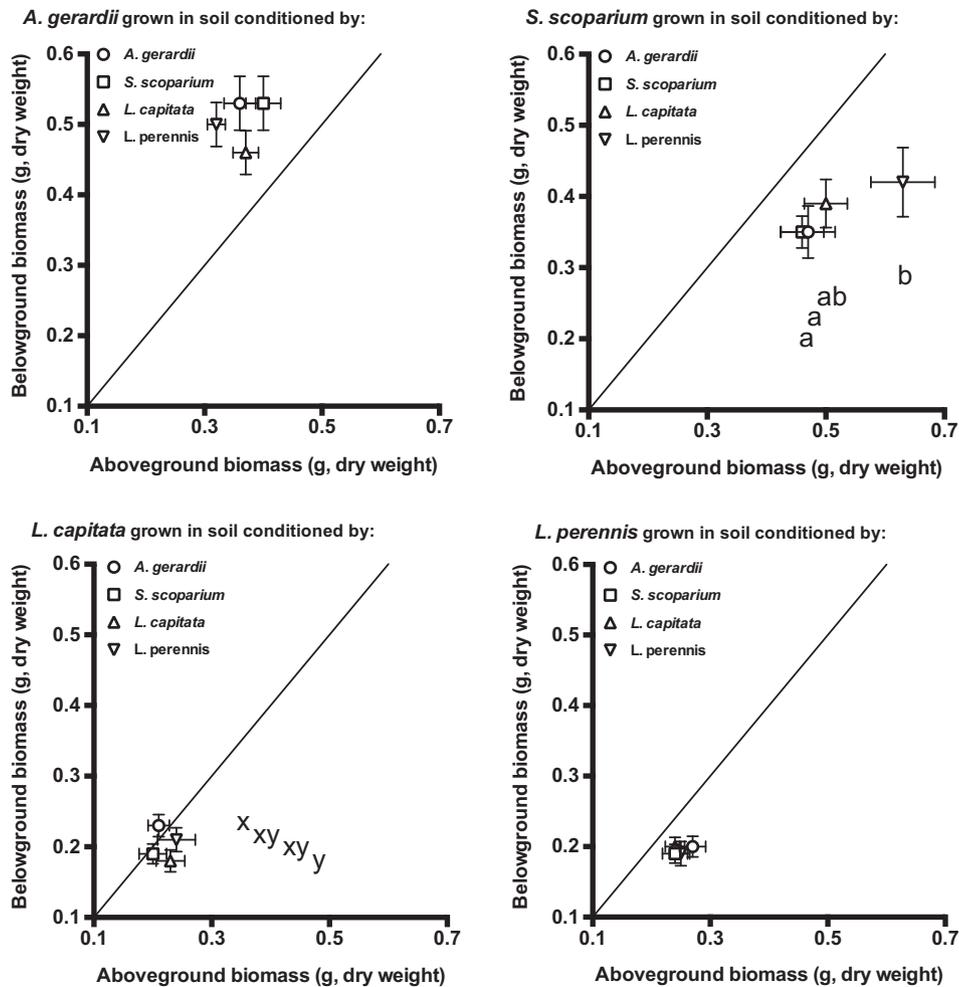
In order to gain insights into the selective forces acting on *Streptomyces* antagonistic phenotypes, we tested for relationships of *Streptomyces* antagonistic potential with plant growth performance in conditioned soil and with soil properties and plant community characteristics. Measures of *Streptomyces* density and antagonistic potential were related to growth performance only for *S. scoparium* (data not shown). As *Streptomyces* densities increased, so did root length and belowground biomass of *S. scoparium* ( $r = 0.39$ ;  $p = 0.02$  and  $r = 0.33$ ;  $p = 0.06$ , respectively). On the other hand, belowground biomass of *S. scoparium* decreased as the proportion of antagonistic *Streptomyces* increased ( $r = -0.38$ ;  $p = 0.02$ ).

In general, *Streptomyces* densities were positively correlated with measures of productivity and of fertility, while measures of *Streptomyces* antagonistic activity were negatively correlated with many of the same variables. *Streptomyces* density was significantly positively correlated with plant diversity and with soil pH, OM and K content (Table 2). Antagonist frequency was significantly negatively correlated with conditioning plant diversity and productivity (belowground biomass, percent cover, aboveground biomass) and with soil OM and K content (Table 2). Intensity of inhibition was similarly negatively correlated with conditioning plant productivity (belowground biomass, percent cover, aboveground biomass) and with soil OM and K content, though not with conditioning plant diversity (Table 2). Antagonist density was significantly positively correlated with soil pH (Table 2). Overall, the suggestion of these relationships is that in natural settings, selection for antagonistic phenotypes is strongest where nutrient availability is low, although the highest absolute densities of antagonists may still occur in sites with higher nutrient availability.

## 4. Discussion

Although many studies have addressed plant–soil feedbacks and the selective effects of plant host species on associated microbial communities, the impacts of plant diversity on these processes have received far less attention. Only very rarely have plant diversity and plant identity been considered concurrently. In this work, both plant species identity and plant community richness were shown to be important to plant–soil feedbacks and to plant-driven impacts on *Streptomyces* antagonistic potential. Furthermore, for two of the four host plant species, plant species identity and plant community richness were shown to have interactive effects on *Streptomyces* community antagonistic potential. This is consistent with previous work, which has demonstrated that effects of host plant species identity on *Streptomyces* community structure vary with surrounding plant richness (Bakker et al., 2013).

Plants grow in association with other plants within communities ranging from simple, low diversity monocultures to very high diversity assemblages. Because the presence of neighboring plants can alter aerial plant morphology (Bartelt-Ryser et al., 2005) and



**Fig. 4.** Aboveground and belowground biomass production by four prairie plant species, in soils conditioned by each of the same species. Means + SE are shown; treatments marked with different letters (*a* vs. *b* for differences along the horizontal axis; *x* vs. *y* for differences along the vertical axis) differed significantly from each other ( $p < 0.05$ , ANOVA with Tukey contrasts). The diagonal line reflects an even shoots:roots allocation of biomass.

secondary metabolism (Broz et al., 2010), it is also possible that root architecture, root exudation and litter chemistry may vary depending on the specific characteristics of the community in which a plant is grown. This suggests the possibility that plant-specific impacts on associated soil microbial communities and resulting plant–soil feedbacks may exhibit unique dynamics as a function of plant community characteristics. For instance, this work demonstrates that both the subsequent growth performance of a response plant, and the impacts of a given host plant species on the antagonistic potential of associated *Streptomyces* may vary with surrounding plant richness. Further work is needed to identify traits in these plants that show differential responsiveness to the neighboring plant community. For example, among the plant species tested here, impacts of *S. scoparium* and *L. capitata* on soil *Streptomyces* communities did not vary significantly with surrounding plant richness. This may suggest that *S. scoparium* and *L. capitata* root exudation or architecture are less impacted by neighboring plants than are root traits of *A. gerardii* or *L. perennis*.

The relevance of broader plant community characteristics to plant–soil feedbacks has been best appreciated in studies of temporal plant community dynamics. For example, feedback effects have been investigated extensively in relation to plant invasion (Klironomos, 2002) and have been shown to differ with plant community successional stage (Kardol et al., 2006). However, we are not aware of another study that considers the possibility of

variation in plant–soil feedbacks associated with a given plant species as a result of plant community context. A meta-analysis of plant–soil feedback studies found that most have used monocultures (Kulmatiski et al., 2008), and are thus unlikely to capture subtle interactions that may be important in nature. For instance, invasion by an exotic species is often accompanied by a reduction in plant richness. If species-specific feedback effects vary with surrounding plant richness, plant–soil feedbacks may vary significantly over the course of an invasion. Our results suggest that this may be true for some, but not all plant species.

The potential for neighboring plants to modulate interactions of a given host species with associated soil bacteria offers a new perspective on the importance of plant diversity to ecosystem properties and functions. Previous work has considered multiple hypotheses for the importance of diversity to plant community functioning. For instance, the sampling effect hypothesis (Wardle et al., 1999) suggests that diversity may be important primarily for increasing the likelihood of the presence of particular plant species having disproportionate impact on ecosystems properties and functioning. Other studies have emphasized that diversity enhances temporal stability through cumulative slight variations in functioning (Tilman and Downing, 1994). Our work suggests that plant diversity may carry additional important implications for ecosystem functions through modification of the traits and interactions of individual species. While such interactive effects of

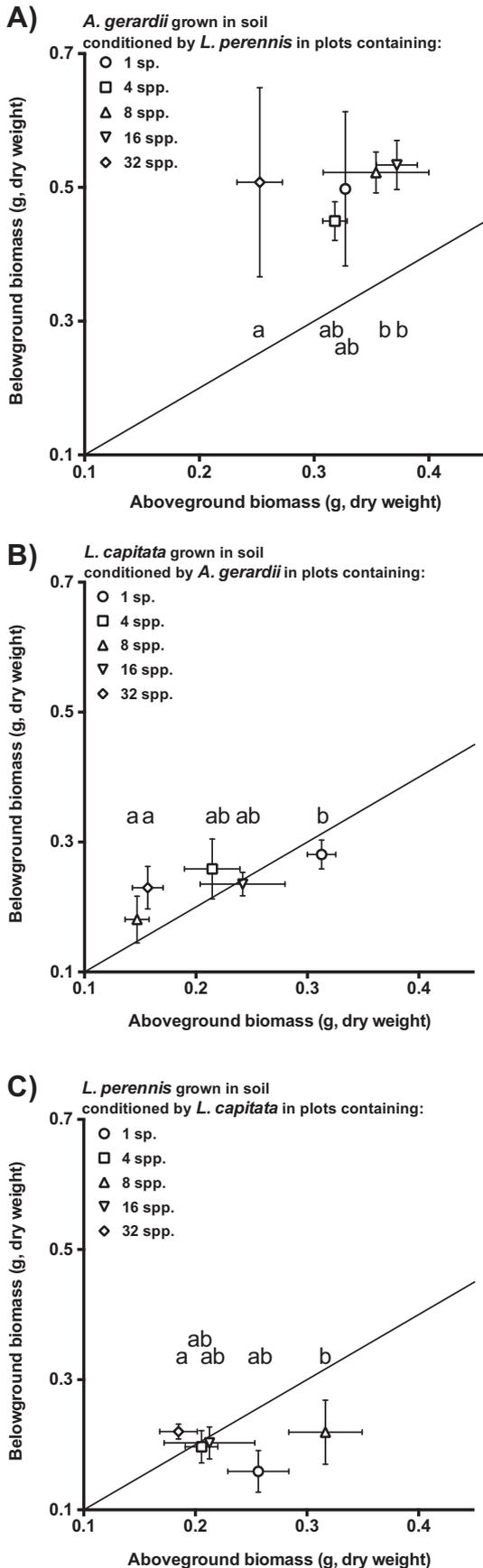


Fig. 5. Aboveground (horizontal axis) and belowground (vertical axis) biomass production by A) *A. gerardii* in soils conditioned by *L. perennis* grown in assemblages of

plant diversity may be important to the provision of ecosystem services, it should also be noted that these effects do not appear to be universal. For instance, only for two of the four plant species tested here did surrounding plant richness significantly alter the antagonistic activity of associated *Streptomyces* populations.

A long-term goal for agricultural management is the ability to use cultivar selection, green manures and crop rotations to manage microbial communities in ways that promote plant health and productivity (Mazzola, 2004; Bakker et al., 2012). An ideal situation would be agricultural management that creates positive plant–soil feedbacks. For such application, it is important to elucidate the mechanistic basis for plant-driven effects on beneficial microbial activities. We observed strong relationships between soil edaphic characteristics and measures of *Streptomyces* antagonistic potential, suggesting that plant-driven effects on associated bacterial populations may be partially mediated through changes to the chemical environment in soil. We found that *Streptomyces* densities increased along with indicators of soil fertility such as organic matter and potassium content (Table 2), although the causal mechanism for this increase in diversity may be related to other factors. Importantly, however, plant diversity dampened the rate at which densities of antagonistic *Streptomyces* increased with overall *Streptomyces* densities (Fig. 3). In the highest diversity plant communities, antagonist densities were independent of total *Streptomyces* densities, suggesting that antagonistic competition was less important to fitness in high- vs. low-diversity plant communities. Because higher antagonist densities are related to lower disease severity (Wiggins and Kinkel, 2005b), this suggests that it may be more difficult to manage *Streptomyces* communities for disease suppression in highly diverse plant communities.

From an ecological and evolutionary perspective, the decoupling of *Streptomyces* density from antagonistic phenotypes in high diversity plant communities may suggest that diverse plant cover facilitates alternatives to chemical antagonism as a strategy for coping with microbial competition. An explanation may be found in the chemical diversity of the resource base available for saprophytic microbes under different plant communities. As plant diversity increases, a corresponding increase in resource diversity may foster niche differentiation, or specialization on a subset of available resources, among soil saprophytes. By reducing the intensity of direct antagonistic resource competition, such niche differentiation may reduce the intensity of selection for antagonistic phenotypes among *Streptomyces* (Kinkel et al., 2011). This prospect has significant implications for pathogen suppression and attempts to manage soil *Streptomyces* communities for enhanced pathogen suppression. For instance, complex rotations or frequent variation in cover crop species may preclude the development of highly antagonistic *Streptomyces* populations by encouraging niche differentiation in response to a highly diverse resource base.

This work also sheds light on the feedback dynamics of four important prairie plant species. A legacy effect of the prior plant community, mediated through the soil, had an impact on subsequent growth performance of these species. By extension, competitive dynamics among plant species may differ depending on the history of plant species and plant community richness at a given site. Feedback effects were observed despite the absence of visible disease symptoms, suggesting the possibility of chemical or nutrient effects, the presence of asymptomatic infections, or

increasing plant richness; B) *L. capitata* in soils conditioned by *A. gerardii* grown in assemblages of increasing plant richness; C) *L. perennis* in soils conditioned by *L. capitata* grown in assemblages of increasing plant richness. Means + SE are shown; treatments marked with different letters differed significantly from each other ( $p < 0.05$ , ANOVA with Tukey contrasts). The diagonal line reflects an even shoots:roots allocation of biomass.

**Table 2**Pearson correlation coefficients (*p*-values) for relationships between *Streptomyces* density or antagonistic potential, and characteristics of the soil or resident plant community.

		<i>Streptomyces</i> density (log CFU/g)	Antagonist density (log CFU/g)	Antagonist frequency (proportion of isolates)	Inhibition zone (mm; sqrt transformed)
Properties of conditioned soil	Soil organic matter (%)	0.33 (0.06)	0.41	(ns)	–0.50 (<0.01)
	Soil pH	0.33 (0.06)		(0.01)	(ns)
	Soil potassium (ppm)	0.33 (0.06)		(ns)	–0.65 (<0.01)
Plant community characteristics	Belowground biomass (g/m <sup>2</sup> )	(ns)	(ns)	–0.45 (0.02)	–0.37 (0.06)
	Total plant cover (%)	(ns)	(ns)	–0.54 (<0.01)	–0.35 (0.04)
	Plant diversity (Shannon index)	0.44 (0.02)	(ns)	–0.39 (0.04)	(ns)
	Aboveground biomass (g/m <sup>2</sup> )	(ns)	(ns)	–0.50 (<0.01)	–0.46 (0.01)

changes to communities of plant-growth promoting microbes. It remains possible that antagonistic *Streptomyces* could be significant to plant–soil feedbacks under conditions of greater pathogen pressure. There are also a variety of other mechanisms whereby plant-associated *Streptomyces* can influence plant growth. For instance, *Streptomyces* have been shown to promote plant growth (Nassar et al., 2003; Verma et al., 2011), to synthesize plant hormones (Tuomi et al., 1994; Joshi and Loria, 2007), to prime the plant innate immune response system (Conn et al., 2008), and to modify interactions between plants and other symbiotic microorganisms (Tokala et al., 2002; Lehr et al., 2007; Schrey et al., 2007).

This work investigated relationships between plant growth performance and *Streptomyces* community antagonistic potential. We found that both plant species identity and plant community richness impact soil *Streptomyces* communities, highlighting the previously unstudied significance of plant community richness in mediating plant–microbe feedbacks. This work also adds complexity to the simple concept of density-dependent selection for antibiotic phenotypes, and suggests that density is more important to selection for antagonistic phenotypes in low diversity than in high diversity plant communities. Further attention should be given to exploring the implications of nutrient availability and diversity for the selection and maintenance of antagonistic *Streptomyces* phenotypes.

### Acknowledgments

We thank D. Tilman and the Cedar Creek Ecosystem Science Reserve for permission to collect soil samples from an on-going experiment. MGB received support from the National Science Foundation (Graduate Research Fellowship). Research funding was provided by the National Science Foundation Long-Term Ecological Research Network Grant 0620652 and the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, through the Microbial Observatories program (2006-04464).

### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2013.06.007>.

### References

- Anukool, U., Gaze, W.H., Wellington, E.M.H., 2004. *In situ* monitoring of streptomycin production by *Streptomyces rochei* F20 in soil and rhizosphere. *Applied and Environmental Microbiology* 70, 5222–5228.
- Bakker, M., Manter, D., Sheflin, A., Weir, T., Vivanco, J., 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant & Soil* 360, 1–13.
- Bakker, M.G., Bradeen, J.M., Kinkel, L.L., 2013. Effects of plant host species and plant community richness on streptomycete community structure. *FEMS Microbiology Ecology* 83, 596–606.
- Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balsler, T., 2005. Soil feedbacks of plant diversity on soil microbial communities and subsequent plant growth. *Perspectives in Plant Ecology Evolution and Systematics* 7, 27–49.
- Bever, J., Westover, K., Antonovics, J., 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* 85, 561–573.
- Bever, J.D., Dickie, I.A., Facelli, E., Facelli, J.M., Klironomos, J., Moora, M., Rillig, M.C., Stock, W.D., Tibbett, M., Zobel, M., 2010. Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution* 25, 468–478.
- Broz, A., Broeckling, C.D., De-la-Peña, C., Lewis, M.R., Greene, E., Callaway, R.M., Sumner, L.W., Vivanco, J.M., 2010. Plant neighbor identity influences plant biochemistry and physiology related to defense. *BMC Plant Biology* 10, 115.
- Callaway, R.M., Cipollini, D., Barto, K., Thelen, G.C., Hallett, S.G., Prati, D., Stinson, K., Klironomos, J., 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology* 89, 1043–1055.
- Casper, B.B., Bentivenga, S.P., Ji, B., Doherty, J.H., Edenborn, H.M., Gustafson, D.J., 2008. Plant-soil feedback: testing the generality with the same grasses in serpentine and prairie soils. *Ecology* 89, 2154–2164.
- Conn, V.M., Walker, A.R., Franco, C.M.M., 2008. Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions* 21, 208–218.
- Connell, J.H., 1971. On the Role of Natural Enemies in Preventing Competitive Exclusion in Some Marine Animals and in Rain Forest Trees. *Dynamics of Populations*. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands, pp. 298–312.
- Davelos, A.L., Xiao, K., Flor, J.M., Kinkel, L.L., 2004. Genetic and phenotypic traits of streptomycetes used to characterize antibiotic activities of field-collected microbes. *Canadian Journal of Microbiology* 50, 79–89.
- Doumbou, C.L., Hamby Salove, M.K., Crawford, D.L., Beaulieu, C., 2001. Actinomycetes, promising tools to control plant diseases and to promote plant growth. *Phytoprotection* 82, 85–102.
- Ehrenfeld, J.G., Ravit, B., Elgersma, K., 2005. Feedback in the plant-soil system. *Annual Review of Environment and Resources* 30, 75–115.
- Eppinga, M.B., Rietkerk, M., Dekker, S.C., De Ruiter, P.C., Van der Putten, W.H., 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. *Oikos* 114, 168–176.
- Garrett, K.A., Mundt, C.C., 1999. Epidemiology in mixed host populations. *Phytopathology* 89, 984–990.
- Haas, D., Keel, C., 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annual Review of Phytopathology* 41, 117–153.
- Hwang, S.F., Ahmed, H.U., Gossen, B.D., Kutcher, H.R., Brandt, S.A., Strelkov, S.E., Chang, K.F., Turnbull, G.D., 2009. Effect of crop rotation on the soil pathogen population dynamics and canola seedling establishment. *Plant Pathology Journal* 8, 106–112.
- Inderjit, Van der Putten, W.H., 2010. Impacts of soil microbial communities on exotic plant invasions. *Trends in Ecology & Evolution* 25, 512–519.
- Janzen, D.H., 1970. Herbivores and the number of tree species in tropical forests. *The American Naturalist* 104, 501–528.
- Joshi, M.V., Loria, R., 2007. *Streptomyces turgidiscabies* possesses a functional cytokinin biosynthetic pathway and produces leafy galls. *Molecular Plant-Microbe Interactions* 20, 751–758.
- Kardol, P., Bezemer, T.M., van der Putten, W.H., 2006. Temporal variation in plant-soil feedback controls succession. *Ecology Letters* 9, 1080–1088.
- Keesing, F., Holt, R.D., Ostfeld, R.S., 2006. Effects of species diversity on disease risk. *Ecology Letters* 9, 485–498.
- Kinkel, L.L., Bakker, M.G., Schlatter, D.S., 2011. A coevolutionary framework for managing disease-suppressive soils. *Annual Review of Phytopathology* 49, 47–67.
- Kinkel, L.L., Schlatter, D.C., Bakker, M.G., Arenz, B.E., 2012. *Streptomyces* competition and co-evolution in relation to plant disease suppression. *Research in Microbiology* 163, 490–499.
- Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417, 67–70.
- Kulmatiski, A., Beard, K.H., Stevens, J.R., Cobbold, S.M., 2008. Plant-soil feedbacks: a meta-analytical review. *Ecology Letters* 11, 980–992.

- Lehr, N.A., Schrey, S.D., Bauer, R., Hampp, R., Tarkka, M.T., 2007. Suppression of plant defense response by a mycorrhiza helper bacterium. *New Phytologist* 174, 892–903.
- Mazzola, M., 2004. Assessment and management of soil microbial community structure for disease suppression. *Annual Review of Phytopathology* 42, 35–59.
- McCarthy-Neumann, S., Kobe, R.K., 2010a. Conspecific plant-soil feedbacks reduce survivorship and growth of tropical tree seedlings. *Journal of Ecology* 98, 396–407.
- McCarthy-Neumann, S., Kobe, R.K., 2010b. Conspecific and heterospecific plant-soil feedbacks influence survivorship and growth of temperate tree seedlings. *Journal of Ecology* 98, 408–418.
- Menzies, J.D., 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. *Phytopathology* 49, 648–652.
- Nassar, A.H., El-Tarabily, K.A., Sivasithamparam, K., 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine-producing isolate of *Streptomyces griseoluteus*. *Plant Growth Regulation* 40, 97–106.
- Olf, H., Hoorens, B., de Goede, R.G.M., van der Putten, W.H., Gleichman, J.M., 2000. Small-scale shifting mosaics of two dominant grassland species: the possible role of soil-borne pathogens. *Oecologia* 125, 45–54.
- Petermann, J.S., Fergus, A.J.F., Turnbull, L.A., Schmid, B., 2008. Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology* 89, 2399–2406.
- R Development Core Team, 2011. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rizopoulos, D., 2006. LTM: an R package for latent variable modelling and item response theory analyses. *Journal of Statistical Software* 17, 1–25.
- Schrey, S.D., Salo, V., Raudaskoski, M., Hampp, R., Nehls, U., Tarkka, M.T., 2007. Interaction with mycorrhiza helper bacterium *Streptomyces* sp Ach 505 modifies organization of actin cytoskeleton in the ectomycorrhizal fungus *Amanita muscaria* (Fly Agaric). *Current Genetics* 52, 77–85.
- Tilman, D., Downing, J.A., 1994. Biodiversity and stability in grasslands. *Nature* 367, 363–365.
- Tilman, D., Knops, J., Wedlin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277, 1300–1302.
- Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and productivity in a long-term grassland experiment. *Science* 294, 843–845.
- Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F., Morra, M.J., 2002. Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). *Applied and Environmental Microbiology* 68, 2161–2171.
- Tuomi, T., Laakso, S., Rosenqvist, H., 1994. Indole-3-acetic acid (IAA) production by a biofungicide *Streptomyces griseoviridis* strain. *Annales Botanici Fennici* 31, 59–63.
- Verma, V.C., Singh, S.K., Prakash, S., 2011. Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica* A. Juss. *Journal of Basic Microbiology* 51, 550–556.
- Wardle, D.A., Bonner, K.I., Barker, G.M., Yeates, G.W., Nicholson, K.S., Bardgett, R.D., Watson, R.N., Ghani, A., 1999. Plant removals in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. *Ecological Monographs* 69, 535–568.
- Weller, D.M., Raaijmakers, J.M., Gardener, B.B.M., Thomashow, L.S., 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of Phytopathology* 40, 309–348.
- Wiggins, B.E., Kinkel, L.L., 2005a. Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. *Plant and Soil* 268, 271–283.
- Wiggins, B.E., Kinkel, L.L., 2005b. Green manures and crop sequences influence potato diseases and pathogen inhibitory activity of indigenous streptomycetes. *Phytopathology* 95, 178–185.