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# Plant community effects on the diversity and pathogen suppressive activity of soil streptomycetes

Matthew G. Bakker[a,*], Jerry D. Glover[b], John G. Mai[b], Linda L. Kinkel[a]

[a] Department of Plant Pathology, University of Minnesota, 1991 Upper Buford Circle, 495 Borlaug Hall, St. Paul, MN 55108, United States
[b] The Land Institute, 2440 E. Water Well Road, Salina, KS 67401, United States

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ABSTRACT

Ecological factors that promote pathogen suppressive microbial communities remain poorly understood. However, plants have profound impacts on the structure and functional activities of soil microbial communities, and land-use changes which alter plant diversity or community composition may indirectly affect the structure and function of microbial communities. Previous research has suggested that the streptomycetes are significant contributors to pathogen suppression in soils. We compared soil streptomycete communities from high and low plant diversity treatments using an experimental manipulation that altered plant diversity while controlling for soil structure and disturbance. Specifically, we characterized an isolate collection for inhibition of plant pathogens as a measure of functional activity, and for 16S rDNA sequence to measure community structure. In this system, high and low diversity plant communities supported streptomycete communities with similar diversity, phylogenetic composition, and pathogen suppressive activity. However, inhibitory phenotypes differed among treatments for several phylogenetic groups, indicating that local selection is leading to divergence between streptomycetes from high and low plant diversity communities. Although the ability to inhibit plant pathogens was common among soil streptomycetes, pathogen-inhibitory activity differed widely among phylogenetic groups. The breadth and intensity of pathogen inhibition by soil streptomycetes were positively related.

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## 1. Introduction

There has been a long-standing interest in the manipulation of microbial communities to enhance beneficial ecosystem services (Ducklow, 2008; Shennan, 2008). Using natural or manipulated microbial communities to perform useful functions such as control of plant disease holds promise for reducing environmental impacts relative to existing resource- or chemical-intensive methods. For example, the suppression of plant pathogens by indigenous soil microbes can enhance agricultural productivity and reduce the need for chemical inputs such as fungicides (Emmert and Handelsman, 1999). Many attempts have been made to emulate natural pathogen suppression through augmentative and inoculative biocontrol. Resource manipulation has also been used in attempts to alter microbial densities and community structure in ways that may limit pathogen activity (Schlatter et al., 2008; Perez et al., 2008). To date, such attempts have had mixed results in achieving adequate and reliable control of plant pathogens. Additional study of natural microbial communities is needed to shed light on the factors that influence pathogen suppression (natural biocontrol) and advance our efforts toward safe and sustainable plant disease management.

Many soils have been characterized as possessing pathogen suppressive activities (Ghini and Morandi, 2006; Hjort et al., 2007). Among such systems that have been well studied, the production of antimicrobial secondary metabolites has been identified as a significant factor in effective pathogen suppression (Raaijmakers and Weller, 1998; Weller et al., 2002). Enrichment of antibiotic producing bacteria in plant rhizospheres has been demonstrated (Mazzola et al., 2004), as has the ability of plants to differentially promote antibiotic production by associated bacteria (Bergsma-Vlami et al., 2005; de Werra et al., 2008; Okubara and Bonsall, 2008). However, all such studies that we are aware of relate to 2,4-diacetylphloroglucinol-producing pseudomonads. Plant impacts on other antibiotic producing bacteria are under explored.

The streptomycetes are ubiquitous members of soil microbial communities and are well known as prodigious producers of antimicrobial secondary metabolites (Bentley et al., 2002). There is a great deal of evidence that free-living *Streptomyces* can protect plants by inhibiting the causal organisms of plant disease, and members of this genus have been studied extensively as biological control agents. For example, *Streptomyces* isolates have been shown to reduce the severity of seedling diseases of alfalfa (Jones and Samac, 1996), Phytophthora root rot of soybean (Xiao et al.,

* Corresponding author. Tel.: +1 612 624 2253; fax: +1 612 625 9728.
*E-mail address:* matt.g.bakker@gmail.com (M.G. Bakker).

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2002), potato scab (Liu et al., 1995; Ryan et al., 2004), Pythium seed and root rots (Yuan and Crawford, 1995), spring black stem and leaf spot on alfalfa (Samac et al., 2003), pathogenic turf grass fungi (Chamberlain and Crawford, 1999), root lesion nematodes (Samac and Kinkel, 2001), and cavity spot disease of carrots (El-Tarabily et al., 1997). Moreover, the frequency, intensity, and diversity of antibiotic inhibitory interactions among streptomycetes have all been shown to be important to disease suppression in agricultural soils (Perez et al., 2008; Wiggins and Kinkel, 2005a,b). This suggests that strategies to enhance the frequency, intensity, or diversity of such competitive interactions may promote disease control.

Plant nutrient inputs into the soil microbial community, including root exudates, senescent tissues, leaf litter, and leachates, are likely critical to mediating microbial competitive interactions through their impacts on resource availability. In particular, total nutrient inputs will constrain microbial densities and biomass in soil, and the variety of niches available to soil microbes will depend upon the diversity of the resource base available to soil food webs, in terms of chemical composition, spatial distribution, and availability over time. A simplified plant community may be expected to provide a concomitantly simplified suite of microbial niches as compared with a high diversity community. Diverse plant cover may also provide opportunities for more diverse species interactions (including plant–microbe interactions), which are vital to generating microbial diversity (Hansen et al., 2007; Thompson, 1999). Finally, more diverse plant communities are generally more productive than less diverse communities (Tilman et al., 2001), suggesting greater potential resource inputs into soils, correspondingly higher microbial population densities, and thus more frequent competitive interactions among soil microbes. Consequently, we hypothesize that plant community density, productivity, and diversity are all critical to generating the high density, frequency, and diversity of competitive interactions that may contribute to disease suppression by soil streptomycetes. This study characterizes the diversity and pathogen suppressive activity of streptomycete communities from a diverse prairie meadow soil and a simplified agricultural monoculture soil.

## 2. Methods

### 2.1. Site history

Soil samples were collected from a study site in Ottawa County, Kansas (N′38.58.145, W′97.28.616) that has been described in detail elsewhere (Culman et al., 2010; DuPont et al., 2010; Glover et al., 2010). Prior to the onset of experimental manipulation, this site was virgin prairie and had never been plowed. The site had been burned periodically, and hayed annually in June or July for approximately the previous 75 years, with the hay removed from the site. In 2004, three 20 m × 20 m research blocks were established on the site. Two treatments (prairie meadow or no-till annual cropping) were randomly assigned to the two halves of each block. Management of the prairie plots remained consistent with pre-experiment practices; no agricultural inputs were applied and once-annual removal of hay constituted the only nutrient or biomass removal. Plots assigned to the no-till annual cropping treatment received two applications of glyphosate in the fall of 2004 and were subsequently planted into a rotation of soybean, sorghum, and winter wheat from 2005 to 2007. Zero-tillage techniques were used exclusively, in order to minimize the confounding factors of soil disturbance and degradation. However, chemical fertilizer and herbicide have been used on the monoculture plots according to standard agronomic practices.

### 2.2. Isolate collection

In June of 2007, five soil cores were collected from random locations at least 2 m away from the edge of each plot (two treatments × three blocks). The monoculture plant community consisted of winter wheat, which was beginning to senesce. The prairie community consisted of a mixture of forbs, grasses, and legumes. The surface litter layer was removed and soil was collected to a depth of 10 cm. Soil edaphic characteristics and further site details for these plots at the time of sampling have been reported by DuPont et al. (2010). Cores were packed on ice and transported to the lab for refrigerated storage until processing. For colony counts and isolations, soil samples were dried overnight in a fume hood under 4 ply sterile cheese cloth. A 10% (w/v) soil solution in $K_2HPO_4/KH_2PO_4$ buffer was shaken for 1 h at 200 rpm, at 4 °C. Samples were serially diluted prior to plating on water agar (WA) and starch–casein agar (SCA) (Kuster and Williams, 1964) for determination of culturable community density and selection of isolates. Plates were incubated for 3 days at 28 °C.

Ten isolates showing typical streptomycete morphology were collected from each soil core (five from each of two growth media), for a total of 50 isolates per plot. Selection of colonies for isolation was performed randomly, according to proximity to predetermined points on a Petri dish. Isolates were purified by repetitively streaking and culturing until no contaminants were visible. Cultures were stored in 20% glycerol at −80 °C.

### 2.3. Pathogen antagonism

The ability of each of the 300 isolates to inhibit a set of four plant pathogens was assayed *in vitro*. The plant pathogens tested were *Fusarium graminearum* (isolate Butte86 ADA-11, obtained from R. Dill-Macky), *Rhizoctonia solani* (isolate 43, AG1, obtained from N. Anderson), *Verticillium dahliae* (strain VA33A, VCG 4A, obtained from N. Anderson), and *Streptomyces scabies* (Strain RB4, obtained from N. Anderson). Pathogen overlays followed the method of Wiggins and Kinkel (2005b) with minor modifications. Briefly, a dense spore suspension of each streptomycete isolates was spotted (7 μL/spot, four or five isolates per plate) onto 1.5% WA (18 mL/plate) and incubated at 28 °C for 2 days. At this point, streptomycete isolates were not yet differentiating to form aerial mycelium and spores, which could have complicated the inhibition assay by dispersing the test isolate into the overlay medium. However, this approach may not detect the full potential for antagonism, as antibiotic production is regulated in coordination with tissue differentiation in some cases (Horinouchi and Beppu, 1994).

A second layer of medium (14 mL) was poured over the plates for the pathogen overlays. Plates were filled with an automatic pipetter to ensure consistent medium depth. For *Fusarium*, the entire contents of a fully-colonized Petri dish (oatmeal agar, OA, incubated at room temperature for 7 days) were homogenized in a sterile Waring blender with 100 mL $H_2O$ (low speed, 2 s × 5 s, 1 s× 10 s). The resulting slurry was used to inoculate molten potato dextrose agar (PDA, cooled to 45 °C) at a rate of 20 mL inoculum per 500 mL PDA. For *Rhizoctonia*, liquid cultures in Czapek-Dox (CD) broth (incubated at room temperature for 7 days) were homogenized in a sterile Waring blender (low speed, 2 s × 5 s, 1 s × 10 s) and added to molten CD agar (1% final concentration agar, cooled to 45 °C) at a rate of 100 mL inoculum per 500 mL CD agar. For *Verticillium*, 10 mL of sterile $H_2O$ were added to a sporulating culture (OA, incubated at room temperature for 7 days). Spores were scraped loose with a sterile loop and decanted into molten PDA (cooled to 45 °C) at a rate of spores from two fully-colonized plates per 500 mL PDA. For *Streptomyces*, plates were covered with a layer of yeast malt-extract agar (YME; per litre: 4 g yeast extract, 10 g malt-extract, 4 g glucose, 10 g Bacto agar). After solidifying, a dense pathogen spore suspension

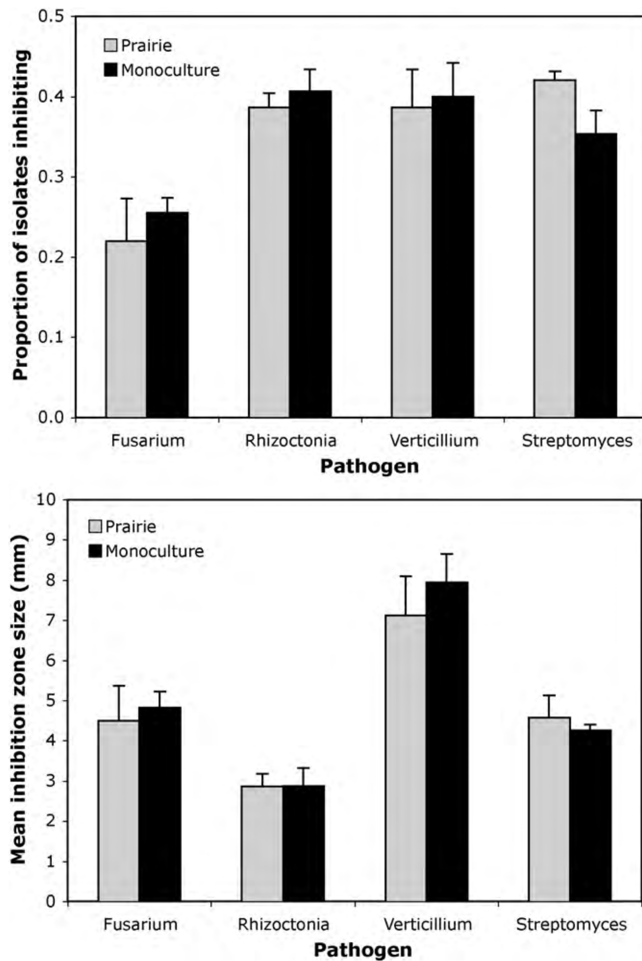


**Fig. 1.** Pathogen antagonism by *Streptomyces* isolates from virgin prairie meadow and never tilled no-till monoculture plots, based on average values by block. (A) The proportion of isolates showing inhibitory activity against each of four plant pathogens. (B) The intensity of inhibition (measured as inhibition zone size) against each of four plant pathogens. Values shown are means and standard errors. No significant differences were found between treatments ($p > 0.1$, by $t$-test).

was spread over the surface. *Streptomyces* overlays were inverted and incubated at 28 °C, while the other overlays were inverted and incubated at room temperature for 2 days.

Each isolate–pathogen combination was assayed for inhibitory activity on three separate plates. Pathogen antagonism was measured as the radius of the zone of inhibition, starting from the edge of the inhibiting colony; average values were determined for each isolate over the three plates. In cases where inhibition was evident but did not extend past the edges of the inhibiting colony, a small non-zero zone size was assigned (0.01 mm).

Inhibitory activity against the four test pathogens was used to assign each streptomycete isolate to one of 16 possible inhibitory phenotypes; each isolate was given a dichotomous rating of 'inhibitory' or 'non-inhibitory' for each of the four test pathogens ($4^2 = 16$, Fig. 1). Four-character labels were used to denote these phenotypes, with each character of the label corresponding to one the four test pathogens. A lower case letter indicates no inhibition, while an uppercase letter indicates inhibition of that pathogen. For example, isolates with the phenotype 'FRVS' inhibited all four of the pathogens tested, while isolates with the phenotype 'frvs' did not inhibit any of the pathogens.

### 2.4. Community composition and diversity

Streptomycete isolates were cultured in yeast dextrose (YD) broth with 0.5% glycine (Kieser et al., 2000) on a reciprocal shaker (175 rpm, 28 °C) for 2–4 days. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit from Promega, following the manufacturer's directions. Primers pA and pH (Edwards et al., 1989) were used to amplify the 16S ribosomal RNA gene by PCR, with the use of PCR Supermix High Fidelity (Invitrogen). The following thermocycle program was used: 94 °C for 30 s, 35 cycles of (94 °C for 30 s, 55 °C for 30 s, 70 °C for 1 min 40 s), final extension step of 72 °C for 7 min. PCR products were visualized on an agarose gel. Products of successful PCR reactions were purified with the QIAquick PCR Purification Kit (Qiagen) prior to sequencing. Sequencing was performed with the ABI PRISM 3130xl Genetic Analyzer, using ABI BigDye version 3.1 Terminator chemistry. Sequences were edited manually based on the chromatographs using Chromas 2 (http://www.technelysium.com.au/). Sequencing reads of fewer than 500 base pairs were not included in the analysis. The Classifier function of the Ribosomal Database Project (Wang et al., 2007) was used to verify the identity of each sequenced isolate. Sequences can be found in GenBank under accession numbers EU699478–EU699737. Sequences were aligned with Clustal W (Larkin et al., 2007) and trimmed to the same length (600 nucleotides). The resulting partial 16S ribosomal RNA gene sequence included the variable gamma region (Stackebrandt et al., 1991), which has been used previously for the study of streptomycete diversity (Kataoka et al., 1997; Anderson and Wellington, 2001). The gamma region corresponds to the V2 variable region of the 16S rDNA, and the V3 region (Neefs et al., 1990) was also included in the sequence fragment considered here. Generation of a pairwise distance matrix, designation of operation taxonomic units (OTUs) by the furthest neighbor method, and diversity analyses were performed with the program mothur (Schloss et al., 2009).

## 3. Results

### 3.1. Pathogen antagonism

A majority of isolates showed inhibitory activity; 64% of isolates inhibited at least one of the four plant pathogens tested. Among our isolates, the frequency of inhibition against *Fusarium*, *Rhizoctonia*, *Verticillium*, and *Streptomyces* was 0.24, 0.40, 0.40, and 0.39, respectively. When all isolates were considered, antagonistic activity, including measures of both frequency and intensity, did not differ significantly by isolate origin (monoculture vs. prairie plant communities; Fig. 1).

The inhibition assay was able to distinguish among 16 different inhibitory phenotypes. All but 3 ('Frvs', 'FrvS', and 'FRvS') of the 16 possible phenotypes were observed. The most frequently observed phenotypes were 'frvs' (36% of isolates), 'frvS' (13% of isolates), and 'FRVS' (11% of isolates). The distribution of isolates among the phenotypic groups differed slightly between treatments (Fig. 2). A significantly higher proportion of the monoculture isolates showed no inhibitory activity compared to the prairie isolates (phenotype 'frvs'; $t = 2.98$, $p = 0.04$). There was a trend toward a higher proportion of prairie isolates inhibiting *Streptomyces* only compared to monoculture isolates (phenotype 'frvS'; $t = -2.53$, $p = 0.06$). Intensity of inhibition within phenotypes differed only in one case; among isolates with the 'fRVS' phenotype, intensity of inhibition against *Streptomyces* was significantly greater for prairie isolates than for monoculture isolates ($t = -3.54$, $p = 0.02$). Intensity of inhibition did not differ between the two treatments for any of the other phenotypes (data not shown).

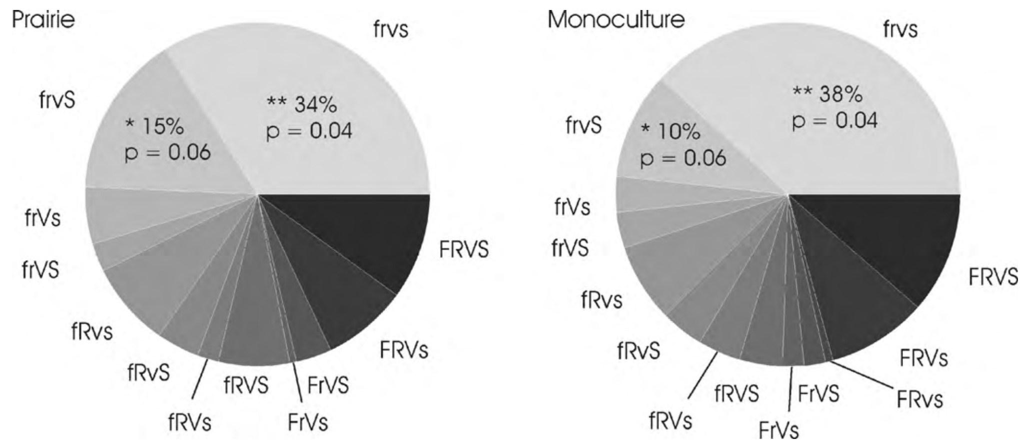


**Fig. 2.** Pathogen-inhibitory phenotypes of a *Streptomyces* isolate collection from diverse prairie (left) and from monoculture (right) plant communities, based on *in vitro* inhibition of four test pathogens. Each letter of the phenotype label corresponds to one of the four test pathogens. An upper case letter indicates inhibition, while a lower case letter indicates no inhibition of that pathogen. F, *Fusarium*; R, *Rhizoctonia*; V, *Verticillium*; S, *Streptomyces*. Differences in proportion of isolates belonging to each phenotype were determined by *t*-test, assuming equality of variances.

Although the monoculture community included one phenotype that was not observed among prairie isolates (‘FRvs’, Fig. 2), the prairie community had modestly greater phenotypic diversity (reciprocal Simpson diversity index (Zhou et al., 2002) of 6.13 vs. 5.48, $p = 0.11$). The 50 isolates from each plot divided into phenotypes as follows: prairie plot one (PP1), 11 phenotypes, including three singletons (phenotypes represented by a single isolate); PP2, 11 phenotypes, two singletons; PP3, 10 phenotypes, one singleton; monoculture plot one (MP1), 11 phenotypes, three singletons; MP2, 12 phenotypes, four singletons; MP3, 10 phenotypes, one singleton.

*Streptomyces* isolates that were able to inhibit a greater number of pathogens were also better inhibitors. Inhibition of *Verticillium* was significantly more intense among isolates which also inhibited two or three other pathogens, compared to those that inhibited *Verticillium* alone or along with one other pathogen (Fig. 3). A similar, though not significant, trend existed for inhibition against the other pathogens (Fig. 3).

### 3.2. Community composition and phylogenetic diversity

Partial 16S ribosomal RNA gene sequences were obtained for 218 isolates (118 isolates from the prairie soil and 100 isolates from the monoculture soil) belonging to the family Streptomycetaceae. Eight isolates were placed in the genus *Kitasatospora* rather than *Streptomyces*; however inclusion of non-

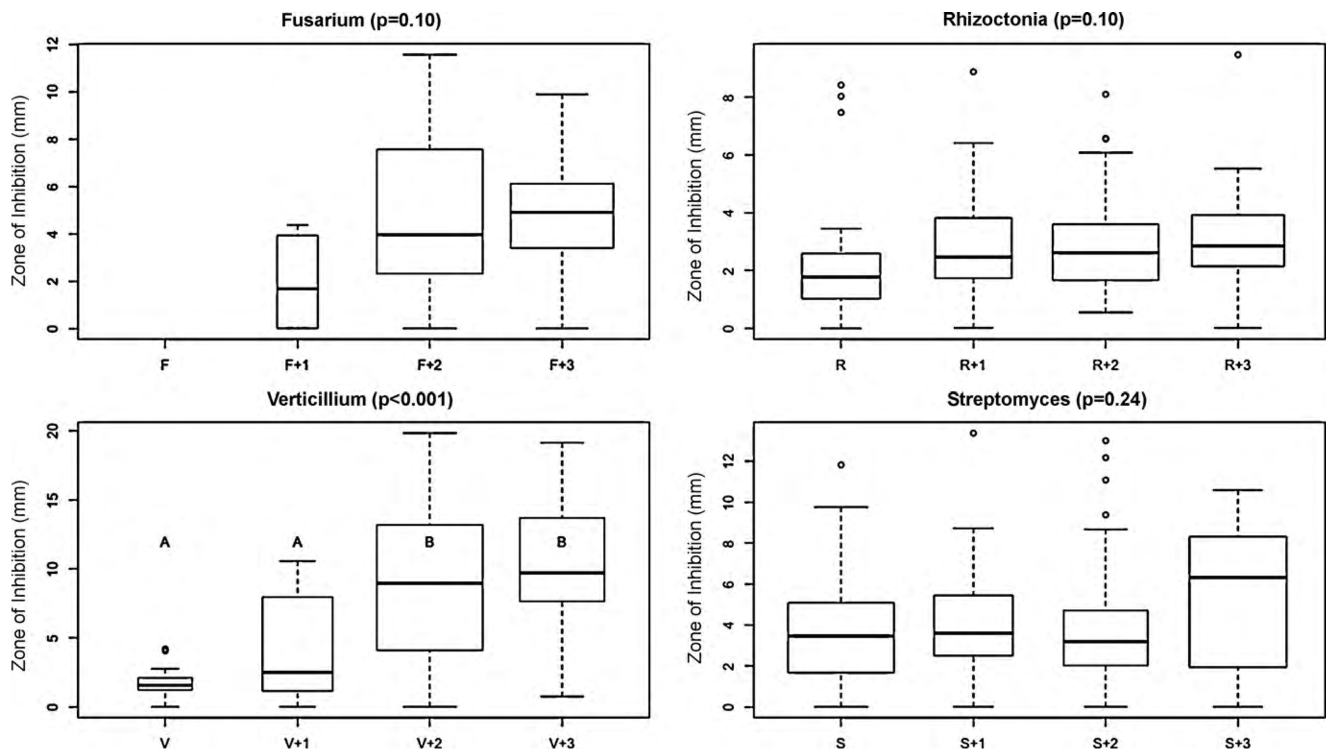


**Fig. 3.** Inhibition zone sizes created by streptomycete isolates against each of four target pathogens (F, *Fusarium*, R, *Rhizoctonia*; V, *Verticillium*; S, *Streptomyces*), according to the number of other pathogens inhibited. Within each panel, box width is proportional to the number of observations. Differences among means within each panel were tested with a non-parametric Kruskal–Wallis test, with a multiple comparison test performed where differences were detected. Significantly different means are indicated by different letters. No isolate in our collection inhibited *Fusarium* without also inhibiting one or more other pathogens.

**Table 1**
Inhibition data for isolates belonging to OTUs with at least 10 members.

| OTU | Treatment | Isolates | *Fusarium* | | | *Rhizoctonia* | | | *Verticillium* | | | *Streptomyces* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Zone (mm) | Zone (inhibitors only; mm) | Prop | Zone (mm) | Zone (inhibitors only; mm) | Prop | Zone (mm) | Zone (inhibitors only; mm) | Prop | Zone (mm) | Zone (inhibitors only; mm) | Prop |
| 4 | Prairie | 14 | 0.19 | 2.63 | 0.07 | 0.40 | 2.78 | 0.14 | 1.05 | 3.69 | 0.29 | 2.02 | 4.70 | 0.43 |
| | Monoculture | 9 | 0.17 | 0.51 | 0.33 | 0.13 | 0.56 | 0.22 | 1.14 | 2.04 | 0.56 | 2.91 | 4.37 | 0.67 |
| 6 | Prairie | 12 | 0.00 | | 0.00 | 0.74 | 4.47 | 0.17 | 0.12 | 1.42 | 0.08 | 0.32 | 3.83 | 0.08 |
| | Monoculture | 9 | 0.00 | | 0.00 | 0.89 | 4.02 | 0.22 | 0.00 | | 0.00 | 0.07 | 0.62 | 0.11 |
| 7 | Prairie | 11 | 4.17 | 6.55 | 0.64 | 2.51 | 2.76 | 0.91 | 12.13 | 13.34 | 0.91 | 1.39 | 3.05 | 0.45 |
| | Monoculture | 14 | 5.10 | 6.49 | 0.79 | 2.58 | 3.01 | 0.86 | 12.55 | 13.52 | 0.93 | 1.84 | 4.30 | 0.43 |
| 9 | Prairie | 6 | 1.75 | 10.52 | 0.17 | 3.79 | 3.79 | 1.00 | 3.97 | 7.94 | 0.50 | 2.56 | 3.84 | 0.67 |
| | Monoculture | 4 | 0.00 | | 0.00 | 2.89 | 3.85 | 0.75 | 0.87 | 3.47 | 0.25 | 2.47 | 3.29 | 0.75 |
| 11 | Prairie | 5 | 0.46 | 2.32 | 0.20 | 1.35 | 2.25 | 0.60 | 0.24 | 0.59 | 0.40 | 0.00 | | 0.00 |
| | Monoculture | 5 | 0.00 | | 0.00 | 1.66 | 2.08 | 0.80 | 1.03 | 2.58 | 0.40 | 0.00 | | 0.00 |
| 14 | Prairie | 8 | 3.84** | 4.39** | 0.88 | 2.74** | 2.74** | 1.00 | 9.23 | 9.23 | 1.00 | 8.58* | 8.58 | 1.00 |
| | Monoculture | 5 | 6.18** | 6.18** | 1.00 | 3.66** | 3.66** | 1.00 | 8.81 | 8.81 | 1.00 | 7.56* | 7.56 | 1.00 |
| 17 | Prairie | 14 | 0.00* | | 0.00* | 0.00* | | 0.00* | 0.00* | | 0.00* | 0.00 | 0.01 | 0.07 |
| | Monoculture | 13 | 1.33* | 5.76 | 0.23* | 0.39* | 1.70 | 0.23* | 1.89* | 8.20 | 0.23* | 1.47 | 6.39 | 0.23 |
| 19 | Prairie | 7 | 0.00 | | 0.00 | 2.36 | 2.36 | 1.00 | 0.00 | | 0.00 | 0.54 | 1.27 | 0.43 |
| | Monoculture | 7 | 0.00 | | 0.00 | 1.43 | 2.00 | 0.71 | 0.00 | | 0.00 | 0.49 | 1.14 | 0.43 |
| 21 | Prairie | 10 | 1.02 | 2.05* | 0.50 | 0.66 | 2.19 | 0.30 | 4.09 | 6.81 | 0.60 | 1.78* | 2.22 | 0.80** |
| | Monoculture | 7 | 1.20 | 4.19* | 0.29 | 1.21 | 4.23 | 0.29 | 2.29 | 5.34 | 0.43 | 0.00* | 0.01 | 0.14** |

Zone is the average inhibition zone size for all isolates. Zone (inhibitors only) is the average inhibition zone size for only those isolates which are positive for inhibition of the test pathogen. Prop is the proportion of isolates which are positive for inhibition of the test pathogen.

* The means for prairie and monoculture isolates differ at $p < 0.10$ ($t$-test, assuming equality of variances).

** Differences significant at $p < 0.05$.

**Table 2**
Inhibition data for isolates belonging to OTUs with at least 10 members.

| OTU | Isolates | *Fusarium* | | *Rhizoctonia* | | *Verticillium* | | *Streptomyces* | |
|---|---|---|---|---|---|---|---|---|---|
| | | Proportion of isolates inhibiting | Inhibition zone (mm) | Proportion of isolates inhibiting | Inhibition zone (mm) | Proportion of isolates inhibiting | Inhibition zone (mm) | Proportion of isolates inhibiting | Inhibition zone (mm) |
| 4 | 23 | 0.17 | 1.04 b | 0.17 | 1.67 a | 0.39 | 2.78 c | 0.52 | 4.54 b |
| 6 | 21 | 0.00 | | 0.19 | 4.24 a | 0.05 | 1.42 bc | 0.10 | 2.23 b |
| 7 | 25 | 0.72 | 6.51 a | 0.88 | 2.90 a | 0.92 | 13.44 a | 0.44 | 3.73 b |
| 9 | 10 | 0.10 | 10.52 a | 0.90 | 3.81 a | 0.40 | 6.82 bc | 0.70 | 3.60 b |
| 11 | 10 | 0.10 | 2.32 ab | 0.70 | 2.15 a | 0.40 | 1.58 c | 0.00 | |
| 14 | 13 | 0.92 | 5.13 ab | 1.00 | 3.09 a | 1.00 | 9.06 b | 1.00 | 8.18 a |
| 17 | 27 | 0.11 | 5.76 ab | 0.11 | 1.70 a | 0.11 | 8.20 abc | 0.15 | 4.79 ab |
| 19 | 14 | 0.00 | | 0.86 | 2.21 a | 0.00 | | 0.43 | 1.21 b |
| 21 | 17 | 0.41 | 2.66 b | 0.29 | 3.01 a | 0.53 | 6.32 bc | 0.53 | 1.98 b |

Shown are mean values for inhibition zone size, considering only those isolates which successfully inhibit the pathogen. Different letters indicate significant differences between mean values within the column ($p < 0.05$, ANOVA with Tukey multiple test correction).

*Streptomyces* isolates in the collection was expected because morphological screening for isolate selection was deliberately permissive in order to maximize the captured diversity of culturable streptomycetes.

*Streptomyces* isolates were grouped into 24 operational taxonomic units (OTUs) based on a cutoff of 2% sequence dissimilarity using the uncorrected *P* distance measure with gaps considered as insertions/deletions, which is the default distance measure in mothur (Schloss et al., 2009). Comparisons of the diversity and community composition (OTU richness and abundance) of prairie and monoculture streptomycete communities revealed a high degree of similarity. Isolates from the prairie treatment were included in 22 of the OTUs, while monoculture isolates were included in 20 OTUs. Four OTUs included only prairie isolates and two OTUs included only monoculture isolates. However, each of the OTUs that were exclusive to a single treatment contained only one or two isolates. Various diversity indices did not differ significantly between the two communities; for example, the reciprocal Simpson diversity index was 14.6 for the prairie treatment and 14.1 for the monoculture treatment ($p > 0.05$).

### 3.3. Phylogeny and pathogen inhibition

Among the larger OTUs (containing at least 10 isolates; $n = 9$ OTUs), three OTUs showed differences in inhibitory activities between prairie and monoculture isolates (Table 1). In OTU 14, monoculture isolates exhibited more intense inhibition against *Fusarium* and *Rhizoctonia* than prairie isolates. In OTU 17, monoculture isolates exhibited greater inhibitory activity against all four pathogens tested. In OTU 21, monoculture isolates showed more intense inhibition of *Fusarium*, while prairie isolates showed more frequent and intense inhibition of the pathogenic *Streptomyces* overlay.

Independent of isolate origin, phylogenetic groups (OTUs) differed in both overall inhibitory activity and inhibition of target pathogens. In some OTUs, the majority of isolates had very limited inhibitory activity against the pathogens tested, while other OTUs were characterized by isolates having broad inhibitory activity against multiple pathogens (Fig. 4). Intensity of inhibition also differed significantly among OTUs (Table 2), except against *Rhizoctonia*. Some OTUs, such as OTU17, tended toward more intense inhibition of the test pathogens, while other OTUs, such as OTU4, tended toward less intense inhibition. Thus, though OTU did not predict the specific inhibitory phenotype (such as ‘FrvS’ or ‘fRVs’), the intensity and breadth of inhibitory activities differed among phylogenetic groups.

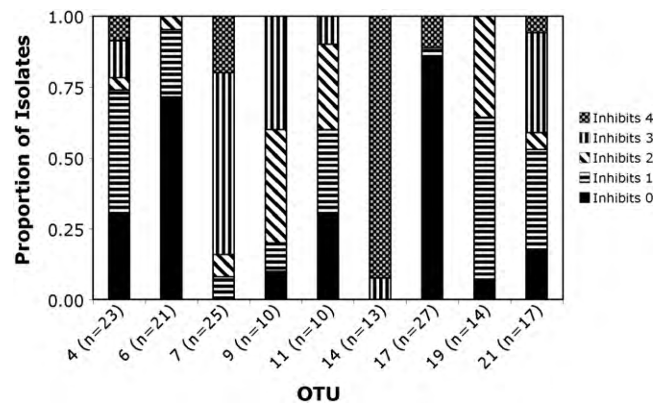


**Fig. 4.** Pathogen-inhibitory characteristics of *Streptomyes* operational taxonomic units (OTUs), showing proportion of isolates having inhibitory activity against 0, 1, 2, 3, or 4 of the pathogens tested.

## 4. Discussion

It is recognized that many different selective influences shape soil microbial communities. These include microbe–microbe interactions (Marshall and Alexander, 1960), chemical and physical aspects of the soil environment (Lauber et al., 2009), and the influence of particular host plants (Mazzola et al., 2004). We investigated host plant community as a selective force shaping streptomycete community structure and function. We found similar diversity, phylogenetic composition, and pathogen suppressive activity among streptomcyete communities from high and low plant diversity treatments, 3 years after the onset of experimental manipulation. However, inhibitory phenotypes differed among treatments for three taxonomic groups, indicating that local selection is leading to divergence between streptomycetes from high and low plant diversity communities. In these groups, inhibitory activity against the fungal pathogens was greater among monoculture streptomycetes, while prairie streptomycetes showed greater inhibitory activity against the pathogenic *Streptomyces* overlay. Our data do not address the abundance or activity of plant pathogens *in situ*, but it is tempting to speculate that greater fungal pathogen activity in the simplified agricultural community has led to selection for pathogen-inhibitory phenotypes among streptomycetes.

The ability to inhibit plant pathogens *in vitro* was common among our collection of streptomycete isolates. However, the suite of pathogens that each isolate inhibited varied widely, probably as a function of the quantity and variety of toxic secondary metabolites produced. The applicability of our *in vitro* assay for antibiotic production to actual disease development has been demonstrated

previously (Wiggins and Kinkel, 2005b) by the finding that the density of antagonistic streptomycetes is negatively correlated with the number of potato scab lesions in field plots. Approaches have been developed to assess antagonism toward pathogens *in situ* or under more realistic conditions in mesocosms (van Elsas et al., 2002). However, these approaches are better suited to assessments of community inhibitory potential, rather than the antagonistic activity of isolates, which is required for linking pathogen-inhibitory activity to taxonomy.

Pathogen-inhibitory activity differed widely among OTUs, with taxonomic groups below the level of genus contributing unequally to plant pathogen suppression in both prairie and monoculture soils. This suggests that screening by taxonomy could facilitate the identification of superior isolates for biocontrol. The preferred taxa in which to search for maximum antagonistic potential may vary according to the target pathogen. Particular phylogenetic groups appear to have characteristic life history strategies, with some groups employing broad and intense chemical inhibition against competitors, while other groups displayed very little inhibitory activity. However, the ability to inhibit a particular combination of pathogens or competitors (inhibitory phenotype) varied widely among isolates within phylogenetic groups. In this regard, our research supports previous observations about the variability of inhibitory interactions among soil microbes (Davelos Baines et al., 2007). The dominant inhibition phenotypes recovered among our isolates also suggested the possibility of differing life history strategies among soil streptomycetes; most isolates either inhibited none of the pathogens tested, inhibited all four of the test pathogens, or inhibited only the pathogenic *Streptomyces* isolate. These phenotypes may correspond to a non-antagonistic strategy (perhaps relying instead on an alternative strategy such as niche differentiation), a broadly antagonistic strategy, or a strategy of targeted competition against other streptomycetes. Future work should explore this concept of differing life history strategies among soil streptomycetes.

Our observation that intensity of inhibition against *Verticillium* (with similar trends for the other pathogens) increases with breadth of inhibitory activity indicates that isolates with broader inhibitory capacities may be producing either superior (more effective) antibiotics, or multiple compounds with additive or synergistic inhibitory effects on pathogens. Screening against multiple pathogens is thus likely to be beneficial when prospecting for pathogen antagonists for biocontrol applications because of the potential for discovery of both the most effective inhibitory antibiotics and for maximizing the probability of uncovering additive or synergistic antibiotic activities (Challis and Hopwood, 2003) by isolates that can inhibit multiple plant pathogens. Although not explored in this study, the importance of species interactions to antibiotic production (Angell et al., 2006) and successful antagonism of pathogens (Guetsky et al., 2002) are well known. Future work should explore the effectiveness of mixtures and consortia of streptomycetes in limiting plant disease.

It is recognized that culture-dependent studies of microbial communities miss the majority of microbes present, since only a small fraction of microbial cells are readily culturable (Joseph et al., 2003). While culture-independent techniques allow for more comprehensive sampling of microbial communities, such techniques are not yet able to address many complex microbial functions such as pathogen suppression. Isolate-based studies continue to be necessary for providing phenotypic information on microbes, and will illuminate the results of subsequent culture-independent studies. Furthermore, it is not clear that resistance to cultivation is equally prevalent among microbial taxa (da Rocha et al., 2009), and bias due to cultivation of isolates may be reduced in this study by the emphasis on a specific group of actinobacteria that appear to be readily culturable. Our work addresses the impacts of plant diversity on a narrow range of the organisms present in soil microbial communities, and it is clear that additional work is needed to examine the impacts of plant communities on microbial community composition, diversity, and functional activity.

Because of the historical absence of tillage at this study site, intact soil structure and high organic matter content may have buffered the soil microbial community from the effects of a massive change in plant cover. A legacy effect may exist in the form of labile soil organic matter, maintaining high resource availability for saprophytic microbial food webs in the manipulated plots. The *Streptomyces* are spore-forming bacteria, and although little information is available regarding the longevity of spores within an active soil microbial community (Ruddick and Williams, 1972), it is possible that our collection included isolates that had remained quiescent (not subject to selective forces) since prior to the onset of experimental manipulation. The rate of change of community composition would be slowed by the re-entry of community members from a dormant state. Additionally, temporal plant diversity continues to be a feature of the monoculture treatment, since the plots are planted in a crop rotation system. Nitrogen inputs were not equal among the prairie and monoculture treatments. However, total soil nitrogen did not differ significantly between treatments (DuPont et al., 2010). While chronic nitrogen additions have been shown to change microbial community composition in some cases (Compton et al., 2004; Nemergut et al., 2008), other studies have found no effect (Sarathchandra et al., 2001; DeForest et al., 2004). Because our data are derived from an isolate collection, several possible impacts of unequal nitrogen application, such as changes in the frequency of *Streptomyces* within the broader soil community or changes in total microbial biomass (Wang et al., 2008) or respiration (Bowden et al., 2004) should not impact our results. Altered community composition within the streptomycetes was not observed in our study.

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