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SOIL MICROBIOLOGY

Plant Community Richness Mediates Inhibitory Interactions and Resource Competition between *Streptomyces* and *Fusarium* Populations in the Rhizosphere

Adil Essarioui[1,2] · **Nicholas LeBlanc**[1] · **Harold C. Kistler**[1,3] · **Linda L. Kinkel**[1]

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Abstract Plant community characteristics impact rhizosphere *Streptomyces* nutrient competition and antagonistic capacities. However, the effects of *Streptomyces* on, and their responses to, coexisting microorganisms as a function of plant host or plant species richness have received little attention. In this work, we characterized antagonistic activities and nutrient use among *Streptomyces* and *Fusarium* from the rhizosphere of *Andropogon gerardii* (*Ag*) and *Lespedeza capitata* (*Lc*) plants growing in communities of 1 (monoculture) or 16 (polyculture) plant species. *Streptomyces* from monoculture were more antagonistic against *Fusarium* than those from polyculture. In contrast, *Fusarium* isolates from polyculture had greater inhibitory capacities against *Streptomyces* than isolates from monoculture. Although *Fusarium* isolates had on average greater niche widths, the collection of *Streptomyces* isolates in total used a greater diversity of nutrients for growth. Plant richness, but not plant host, influenced the potential for resource competition between the two taxa. *Fusarium* isolates had greater niche overlap with *Streptomyces* in monoculture than polyculture, suggesting greater potential for *Fusarium* to competitively challenge *Streptomyces* in monoculture plant communities. In contrast, *Streptomyces* had greater niche overlap with *Fusarium* in polyculture than monoculture, suggesting that *Fusarium* experiences greater resource competition with *Streptomyces* in polyculture than monoculture. These patterns of competitive and inhibitory phenotypes among *Streptomyces* and *Fusarium* populations are consistent with selection for *Fusarium*-antagonistic *Streptomyces* populations in the presence of strong *Fusarium* resource competition in plant monocultures. Similarly, these results suggest selection for *Streptomyces*-inhibitory *Fusarium* populations in the presence of strong *Streptomyces* resource competition in more diverse plant communities. Thus, landscape-scale variation in plant species richness may be critical to mediating the coevolutionary dynamics and selective trajectories for inhibitory and nutrient use phenotypes among *Streptomyces* and *Fusarium* populations in soil, with significant implications for microbial community functional characteristics.

Keywords *Streptomyces* · *Fusarium* · Plant richness · Antibiotics · Niche overlap

Introduction

The battle for resources is a key challenge that faces microorganisms in natural habitats [39, 46]. Soil microorganisms in particular are perceived as active competitors that have evolved diverse strategies to facilitate resource acquisition [19]. Activities including motility, coordinated behavior, and antibiotic production can tip the competitive balance, resulting in numerical dominance or local extinction of competing populations. Functional consequences of microbial social behaviors have been studied extensively [19, 46] and demonstrate the importance of factors such as relatedness, kin discrimination, enforcement of cooperation, and competition among relatives

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✉ Adil Essarioui
essar002@umn.edu

[1] Department of Plant Pathology, University of Minnesota, Minneapolis, MN, USA

[2] National Institute of Agronomic Research, Regional Center of Errachidia, Errachidia, Morocco

[3] Cereal Disease Lab, USDA-ARS, 1551 Lindig Street, St Paul, MN, USA

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to microbial survival and fitness in the environment. However, most investigations have focused on interactions among closely related taxa, often among species or genera [24, 25, 34]. Yet, in natural environments, coexistence of individuals from diverse microbial phyla and kingdoms is common. In particular, bacteria and fungi commonly coexist in soil, but there has been relatively little study of the role of species interactions in mediating the dynamics of specific bacterial and fungal populations in soil. Moreover, we have little understanding of the extent to which the ecological context, and specifically plant host or plant community diversity, may influence bacterial-fungal interactions in soil. Such studies are needed to begin to establish a more complete understanding of the ecological and coevolutionary dynamics of soil microbial populations.

Streptomyces are Gram-positive filamentous bacteria in the phylum Actinobacteria. Members of the genus *Streptomyces* have been credited for their significant capacity to produce an extraordinary quantity [10] and diversity [43] of antibiotics. This feature has important therapeutic applications in human medicine [11, 21, 44] and is of paramount interest for disease suppression in agricultural systems [49, 50]. Additionally, *Streptomyces* possess diverse metabolic capacities that allow them to occupy wide-ranging ecological niches in nature as symbionts with animals, fungi and plants in terrestrial, marine, and freshwater habitats [23, 37]. *Streptomyces* are especially abundant in the soil and the plant rhizosphere. Because the rhizosphere is a biologically and chemically complex environment that supports diverse microbial communities [17], there is a great potential for resource competition and antagonistic interactions between *Streptomyces* and other microbial taxa, with significant implications for microbial population and community composition and functional characteristics.

Plant host and plant community richness have been shown to significantly influence *Streptomyces* competitive phenotypes [13, 14]. *Streptomyces* in monoculture were less niche-differentiated than *Streptomyces* in polyculture. Additionally, plant monocultures have more antagonistic *Streptomyces* than polycultures [3]. Together, these findings are consistent with the hypotheses that polyculture plant communities, producing a greater diversity of soil nutrients, select for niche-differentiated populations, while monoculture plant communities induce a coevolutionary antagonistic arms race, resulting in highly antagonistic soil populations [22, 23]. Although this framework provides a conceptual foundation for predicting the effects of resource competition among *Streptomyces* on their competitive phenotypes, and specifically how plant community richness may alter selective trajectories among competing populations, existing data do not address the potential for other microbial taxa to play significant roles in the selection for diverse resource use and inhibitory phenotypes in complex microbial communities. Here we consider the roles of plant host and plant species diversity in determining the resource competitive and antagonistic phenotypes between *Streptomyces* and coexisting *Fusarium* populations in soil.

The genus *Fusarium* (teleomorph *Gibberella*) comprises a large number of species that are cosmopolitan, widely distributed in the soil as saprophytes, and found commonly in association with belowground and aerial plant parts as both endophytic and exophytic symbionts [2, 6, 8, 32]. The economic importance of many *Fusarium* species as plant pathogens has been demonstrated on a wide range of agricultural, horticultural and silvicultural crops worldwide. Among their most notable features is their capacity to produce diverse bioactive secondary metabolites or toxins with inhibitory activities against many higher organisms as well as fungi and bacteria [20, 41]. Given their ubiquity, diverse life history strategies, and capacity to produce antimicrobial compounds, *Fusarium* species are hypothesized to play important roles in nutrient cycling and in structuring soil microbial communities. Despite their potential significance, interactions of *Fusarium* with coexisting soil microbes have been little explored. Studying competitive interactions and their outcomes among *Fusarium* and other microbial taxa will shed light on forces that shape the structure and function of soil microbial communities.

Although *Fusarium* and *Streptomyces* are common symbionts of plants, little attention has been given to studying *Fusarium-Streptomyces* antagonistic and competitive interactions in relation to plant host or plant community characteristics. Here, we explored the effects of plant community richness and plant species on the inhibitory interactions and nutrient niche overlap between *Fusarium* and *Streptomyces* populations from the rhizosphere of *Andropogon gerardii* (*Ag*) and *Lespedeza capitata* (*Lc*) plants, each growing in communities of 1 (monoculture) or 16 (polyculture) plant species. The specific objectives of this work were to (1) characterize inhibitory interactions between *Fusarium* and *Streptomyces* isolates from the rhizosphere of *Ag* and *Lc* in monoculture and polyculture and (2) determine the effects of plant host and plant community richness on the potential for nutrient competition between *Fusarium* and *Streptomyces*. This investigation provides valuable insight into variation in resource use among *Streptomyces* and *Fusarium* and sheds light on the effects of plant community characteristics, as determinants of soil nutrient characteristics on the potential for nutrient competition and antagonistic interactions between these two common soil microbial taxa.

Materials and Methods

Soil Sampling

Soil samples were collected from the Cedar Creek Ecosystem Science Reserve (CCESR; part of the National Science Foundation Long Term Ecological Research Network) in July

of 2012, from plots in a long-term plant richness manipulation experiment [40]. These plots were established in 1994 with defined levels of plant richness. Soil samples were collected from as close as possible to the center of the base of individual *Ag* and *Lc* plants growing in communities of monoculture or polyculture plant species. Plants to be sampled were selected randomly from individual plots at a distance of at least 20 cm from the plot margins to avoid border effects. For each plant, (*x*, *y*) coordinates were determined using a random number table, and the closest plant of the targeted species to the coordinates was sampled. Each soil sample consisted of two bulked cores collected with a 2.5-cm-diameter corer to a depth of 10 cm; cores from the same plant were homogenized in plastic sample bags. For each plant species in each plant richness treatment, the samples were taken from 3 plants, each growing in a different plot. Thus, we had a total of 12 soil samples (2 plant hosts × 2 plant community diversity levels × 3 plot-level replicates). The soil samples were stored at −20 °C prior to processing.

Streptomyces and *Fusarium* Isolates

Streptomyces isolates were randomly selected from each composite soil sample following dilution plating onto a dual-layer water agar-Starch Casein Agar medium (SCA; 10 g starch, 0.3 g casein, 0.02 g $CaCO_3$, 2.0 g KNO_3, 2.0 g NaCl, 2.0 g K_2HPO_4, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnCl_2$, and 15 g agar per liter of deionized H_2O) [5]. Briefly, 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 suspensions were serially diluted to 10^{-5}, a dilution previously determined to provide 40–70 isolates per plate from this soil. Single 100 μl aliquots of the diluents were spread onto plates of 15 ml of water agar (WA) and subsequently 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 [50]. After 3 days of incubation (28 °C), 10–15 colonies were randomly chosen, using a random sampling grid, from each of the 12 soil samples based on characteristic *Streptomyces* morphology, with a goal of 120 purified isolates.

The 10^{-3} soil diluents of each soil sample were also used to isolate *Fusarium* spp. using peptone pentachloronitrobenzene (PCNB) agar medium (PPA; peptone 15 g, KH_2PO_4 1 g, $MgSO_4.7H_2O$ 0.5 g, PCNB (75%) 750 mg, agar 20 g, H_2O 1 L), a medium that is highly inhibitory to most other fungi and bacteria but allows slow growth of *Fusarium* [33]. Following autoclaving, streptomycin and neomycin were added to the cooled medium to inhibit the growth of Gram-negative and Gram-positive bacteria, respectively, and the pH was adjusted to 5.5–6.5. The streptomycin stock solution was 5 g of streptomycin in 100 ml distilled H_2O and was used at the rate of 20 ml/L of medium. The neomycin stock solution was 1 g of neomycin sulfate in 100 ml distilled H_2O and was used at the rate of 12 ml/L. For each soil suspension, individual aliquots of 100 μl were spread onto 15 ml plates of PPA and incubated at 28 °C for 3–4 days. Subsequently, 10 to 15 randomly chosen single fungal colonies from each plate were transferred to plates containing 15 ml of potato dextrose agar medium (PDA) and allowed to grow for 3 days, and *Fusarium* isolates were identified based on the morphology and pigmentation of the colonies and the shape of macroconidia [28]. The numbers of *Fusarium* isolated from each of the 12 soil samples are presented in Table 1.

Inhibitory Activity of *Streptomyces* Against *Fusarium*

Streptomyces inhibitory activity against *Fusarium* isolates was studied using a modification of a method described by Herr [18] [49, 50]. Soil samples were dilution plated as described above. After 5 days of growth, individual cultures of a single *Fusarium* isolate were grown on PDA for 7 days and were subsequently cut into approximately 1 cm^2 pieces with a sterile knife. The resulting pieces were blended with 50 ml sterile water in a sterile flask three times for 5 s each, and once for 10 s. Four milligrams of this suspension was added to 100 ml of molten potato dextrose water agar (PDWA; 2.4 g potato dextrose broth, 10 g agar, and 1 L deionized water), mixed thoroughly, and 10 ml of the resulting suspension for each isolate was overlaid separately onto the top of three plates containing *Streptomyces* (40–70 CFU each) from the soil sample of origin of that isolate. For each soil sample, the proportions of *Streptomyces* that exhibit antagonistic activities against each sympatric *Fusarium* isolate were calculated by dividing the density of antagonistic *Streptomyces* by their total density, and the size of the inhibition zone caused by every inhibitory *Streptomyces* in each plate was determined as the average diameter of the inhibition zone measured at two right angles. For each replicate plant (soil sample), the proportions of inhibitory *Streptomyces* and the average size of the inhibition zones caused by these were averaged across all sympatric *Fusarium* isolates. Finally, the mean proportion of inhibitory *Streptomyces* and the mean size of inhibition zone were determined for each treatment.

Inhibitory Activity of *Fusarium* Against *Streptomyces*

The agar-disk method was used to evaluate the inhibitory activity of *Fusarium* against *Streptomyces* in all possible sympatric *Fusarium-Streptomyces* isolate pairwise combinations. Briefly, for each *Fusarium* isolate, 1-cm-diameter disks of

Table 1 Number of *Streptomyces* and *Fusarium* isolates per soil sample

Sample number	Plant species	Diversity	Number of *Streptomyces*	Number of *Fusarium*
1	*Lc*	Monoculture	10	10
2	*Lc*	Monoculture	10	7
3	*Lc*	Monoculture	10	9
4	*Lc*	Polyculture	10	10
5	*Lc*	Polyculture	10	10
6	*Lc*	Polyculture	10	10
7	*Ag*	Monoculture	10	3
8	*Ag*	Monoculture	10	4
9	*Ag*	Monoculture	10	4
10	*Ag*	Polyculture	10	4
11	*Ag*	Polyculture	10	2
12	*Ag*	Polyculture	10	10
Total isolate number			120	83

Lc Lespedeza capitata, Ag Andopogon geradii

mycelium, collected from the outer actively growing mycelium from a 7- to 10-day-old PDA culture, were transferred onto SCA plates with three replicate disks of a single *Fusarium* isolate on each plate. Plates had been previously inoculated with 100 μl of a high concentration spore suspension of a single *Streptomyces* isolate. Plates were kept at 4–5 °C overnight to allow for *Fusarium* antimicrobial compounds to diffuse. Plates were then incubated for 3–4 days at 28 °C. For each soil sample, the proportion of *Fusarium* isolates that were inhibitory against each sympatric *Streptomyces* isolate was determined by dividing the number of inhibitory *Fusarium* by their total number, and the size of the inhibition zone caused by every antagonistic *Fusarium* against each *Streptomyces* isolate was defined as the mean radius of the inhibition zone around the mycelium-agar disk measured at two right angles were determined. For each replicate plant (soil sample), the average proportion of inhibitory *Fusarium* and the average intensity of their inhibition were averaged across all sympatric *Streptomyces* isolates. Finally, the mean proportion of inhibitors and the mean intensity of inhibition were determined for each treatment.

Streptomyces and *Fusarium* Resource Use Characterization

Utilization of carbon sources by every individual *Streptomyces* (n = 120) and *Fusarium* (n = 83) isolate was determined using Biolog SF-P2 plates (Biolog, Inc., Hayward, CA). A portion of these results have been reported in previous work [13, 14, 27]. Biolog SF-P2 microplates measure the growth of an isolate on 95 individual sources of carbon by comparing the turbidity of each well to a water control. The 95 carbon substrates in the SF-P2 panel belong to 11 carbon groups (the numbers of substrates for each group are indicated between parentheses): alcohols (3), amides (3), amines (1), amino acids (9), aromatic compounds (4), carbohydrates (41), carboxylic acids (15), esters (3), phosphorylated compounds (8), and polymers (8) (http://www.biolog.com/pdf/milit/00A_008rA_SFN2_SFP2.pdf). Microbial suspensions were made by swabbing spores/mycelium from 10-day-old pure cultures of *Streptomyces* (grown on SCA) or *Fusarium* (grown on PDA) into 1.5 ml of 0.2% carrageenan. Suspensions were adjusted to an optical density of 0.20–0.24 at 590 nm and then diluted in 13.5 ml of 0.2% carrageenan. One hundred microliters of the resulting microbial suspensions were pipetted into each well of a Biolog plate. Plates were incubated at 28 °C for 3 days. Utilization of each of the 95 carbon compounds on the Biolog SF-P2 plate was assessed by recording the absorbance of each well at 590 nm. The absorbance of the well containing only water was subtracted from the absorbance of every other well to standardize absorbance values; resulting negative values were transformed to zeros. For each *Fusarium* and *Streptomyces* isolate, the absorbance in each well within every plate was transformed to a percentage of the maximum OD in that plate to standardize relative growth on distinct nutrients among *Fusarium* and *Streptomyces* in subsequent analyses.

"Used nutrients" were defined to be those on which an individual *Streptomyces* or *Fusarium* isolate grew to more than 50% of its growth potential (maximum growth capacity). Using this definition, total growth, average growth, and niche width

were determined for each isolate. Total and average growth were defined as the total and average percentage growth over all used nutrients, respectively, and niche width as the number of used nutrients for an isolate. For both *Fusarium* and *Streptomyces,* mean total growth, mean growth, and mean niche width of sympatric isolates were determined for each community, and mean growth and mean niche width were determined for every treatment by averaging community mean growth and mean niche width among replicate communities.

For each treatment, the potential for resource competition between sympatric *Fusarium* and *Streptomyces* was indexed. As the significance of the shared niche to each taxon's growth might differ between *Fusarium* and *Streptomyces*, asymmetrical pairwise niche overlap values were determined for each *Fusarium* isolate and each *Streptomyces* isolate in every pairwise *Fusarium*-*Streptomyces* combination. Pairwise niche overlap of *Streptomyces* with *Fusarium* was defined as

$$\mathrm{PNO_{S->F}} = \left(\frac{\begin{array}{c}\text{Number of shared nutrients between Fusarium and Streptomyces}\\ \times \\ \text{Total growth of Fusarium isolate on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of Fusarium isolate}\\ \times \\ \text{Total growth of Fusarium isolate on all used nutrients}\end{array}} \right) \times 100,$$

and pairwise niche overlap of *Fusarium* with *Streptomyces* as

$$\mathrm{PNO_{F->S}} = \left(\frac{\begin{array}{c}\text{Number of shared nutrients between Fusarium and Streptomyces}\\ \times \\ \text{Total growth of Streptomyces isolate on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of Streptomyces isolate}\\ \times \\ \text{Total growth of Streptomyces isolate on all used nutrients}\end{array}} \right) \times 100.$$

Finally, the mean sympatric niche overlap was determined for each treatment by averaging mean sympatric pairwise niche overlaps among replicate communities.

Statistical Analysis

All statistical analyses were performed using R statistical softwares version 3.1.1 (http://cran.r-project.org/bin/windows/base/old/3.1.1/). Analysis of variance (ANOVA) and least significant difference tests (LSD) with $p < 0.05$ as the significance level were carried out to determine the significance of plant host and richness effects on mutual inhibitory activity between sympatric *Streptomyces* and *Fusarium*, and on *Streptomyces* and *Fusarium* mean total growth, mean growth, mean niche width, and sympatric niche overlap. In addition, non-metric multidimensional scaling (NMDS) analysis was performed using the vegan package (version 2.2-1) [35] to explore variation among *Streptomyces* and *Fusarium* communities based on their nutrient use profiles.

Results

Effects of Plant Host and Community Richness on the Inhibitory Interactions between *Streptomyces* and *Fusarium*

Plant community richness, but not host, had significant effects on the inhibitory interactions among *Streptomyces* and *Fusarium* isolates (2-way ANOVA, Supplemental Tables 1–4). Specifically, significantly greater proportions of inhibitory *Streptomyces* were present in soil from monoculture than from polyculture (Fig. 1a; 2-way ANOVA, $p < 0.001$). However, there were no significant differences in the proportion of inhibitory *Streptomyces* between *Ag* and *Lc*. Additionally, the intensities of *Streptomyces* inhibition against *Fusarium* were significantly higher in monoculture than polyculture (Fig. 1c;

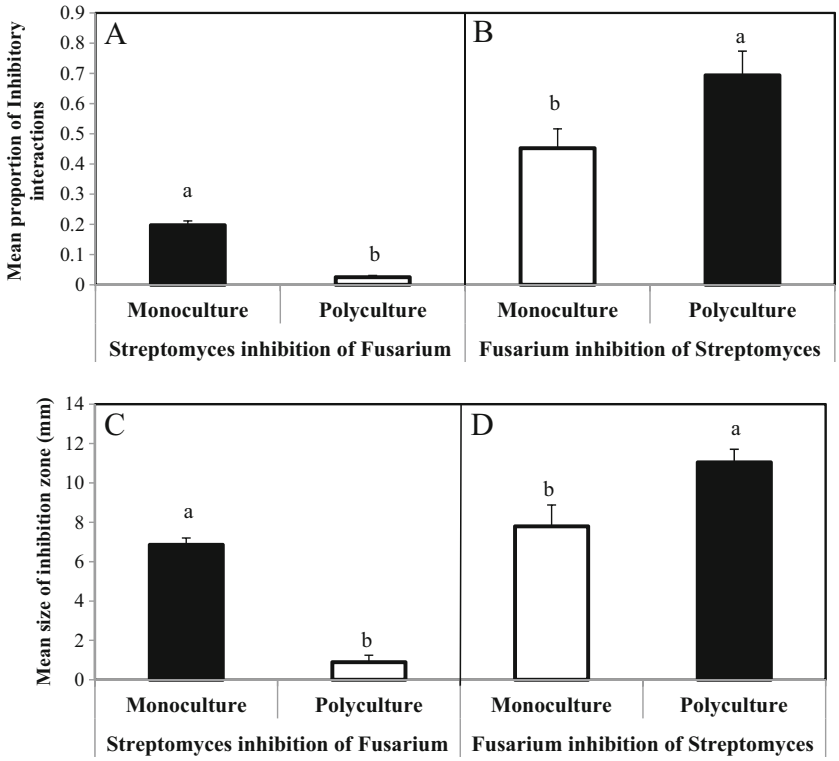

Fig. 1 Frequency (panels **a** and **b**) and intensity (panels **c** and **d**) of inhibitory interactions between sympatric *Streptomyces* and *Fusarium* from *Ag* and *Lc* in monoculture and polyculture. Plant richness, but not host, had significant effects on the frequency and intensity of inhibitory interactions among *Streptomyces* and *Fusarium* isolates (2-way ANOVA, $p < 0.05$). Each *bar* represents the mean of six replicate plants from each plant richness treatment regardless of host. For each plant, the proportions of *Streptomyces* isolates that were inhibitory to each sympatric *Fusarium* isolate (panel **a**) or the mean sizes of inhibition zones (panel **c**) were averaged, and the proportion of *Fusarium* isolates that were inhibitory to each sympatric *Streptomyces* isolate (panel **b**) or the mean sizes of inhibition zones (panel **d**) were averaged. Within each panel, *different letters above bars* indicate significant differences between plant richness treatments. *Error bars* represent standard errors

2-way ANOVA, $p < 0.001$), while no significant differences were found between the two plant species in inhibition intensity. Thus, plant monocultures have *Streptomyces* communities with greater antagonistic activities against *Fusarium* than do the same plant hosts grown in polycultures.

In contrast, the proportions of *Fusarium* antagonistic against *Streptomyces* were significantly greater in polyculture than in monoculture (Fig. 1b; 2-way ANOVA, $p = 0.012$). In a parallel manner, *Fusarium* isolates associated with plants in polyculture had higher inhibition intensities against *Streptomyces* than those from monoculture (Fig. 1d; 2-way ANOVA, $p = 0.007$), and no significant differences were observed between plant hosts. Therefore, plant polycultures produce *Fusarium* populations that are more antagonistic against sympatric *Streptomyces* than do monocultures.

The increase in *Streptomyces* antagonistic capacity in monoculture versus polyculture plant communities was greater than increases in *Fusarium* inhibition in polyculture versus monoculture. Indeed, the proportions of antagonistic *Streptomyces* from monoculture were 685.8% higher than those from polyculture, whereas the proportions of inhibitory *Fusarium* from polyculture were only 53.2% greater than those from monoculture. Likewise, inhibition intensities of *Streptomyces* from monoculture exceeded by 667.5% those from polyculture, while the intensities of inhibition by *Fusarium* from polyculture were only 41.6% greater than those from monoculture. Hence, in terms of inhibitory capacity, *Streptomyces* were more responsive than *Fusarium* to change in plant richness.

Collectively, these findings demonstrate that plant community richness, but not plant host, has significant and differential impacts on *Streptomyces-Fusarium* antagonistic interactions in the soil.

Fusarium and *Streptomyces* Resource Use

Nutrient Use Profiles of Fusarium and Streptomyces

Fusarium isolates were more similar to each other overall and less responsive to plant host or plant richness treatments in terms of nutrient use profiles than *Streptomyces* isolates (Fig. 2). Eighty-one of 83 (97.5%) *Fusarium* isolates had unique carbon use patterns, as compared with 109 of 120 (90.8%) of *Streptomyces* isolates. However, though individual

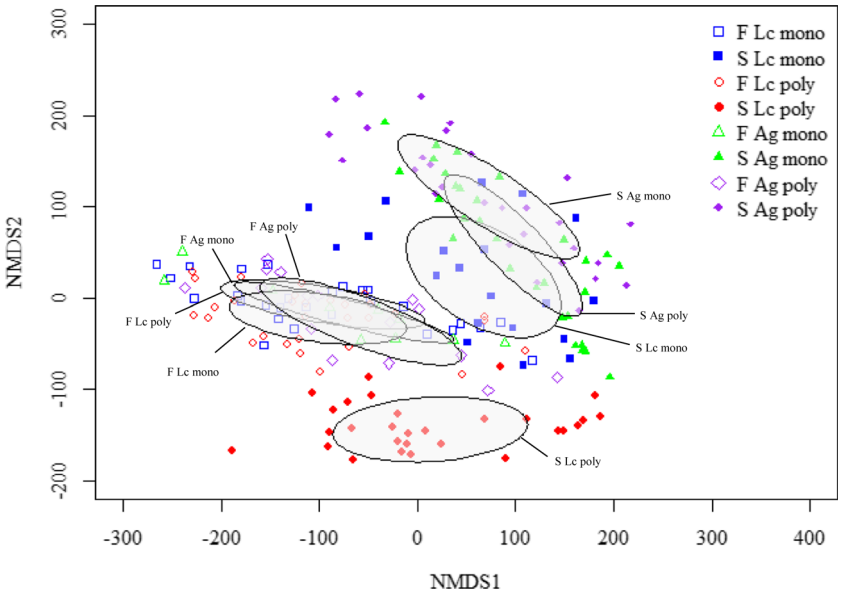

Fig. 2 Non-metric multidimensional scaling (NMDS) based on isolate nutrient profiles for *Fusarium* (*F*) and *Streptomyces* (*S*) from *Lc* and *Ag* in monoculture (*mono*) and polyculture (*poly*). *Ellipses* connect and define the centroid of each community from different plant host/diversity treatments. *Ellipses* were constructed using the function "ordiellipse" in R vegan package

Fusarium isolates were more likely to be unique in the specific nutrients they could utilize for growth, *Streptomyces* had greater variability in nutrient use in the number and variety of nutrients used for growth. Indeed, the total number of nutrients that were used by at least one isolate was 75 for *Streptomyces* versus only 61 for *Fusarium*. Additionally, *Streptomyces* isolates used substrates from all carbon groups, whereas none of the *Fusarium* isolates used nutrients from either the 8 aromatic or the 4 phosphorylated compounds (Supplemental Table 5). Moreover, the number of nutrients used uniquely by *Streptomyces* isolates was 16 versus only 2 for *Fusarium* isolates. Two of the substrates solely utilized by *Streptomyces,* α-cyclodextrin (polymer) and p-hydroxyphenylacetic acid (carboxylic acid), were used by 22 and 17 *Streptomyces* isolates, respectively. The other *Streptomyces* unique substrates were used by 1 to 8 isolates. In contrast, 1 of the 2 substrates utilized exclusively by *Fusarium,* α-methyl-D-glucoside (carbohydrate), was used by 42 isolates, whereas the second carbon source, Lactamide (amide), was used only by 1 isolate. These results suggest that the core pool of nutrients utilized by *Streptomyces* is more diverse than that by *Fusarium* and that *Streptomyces* possess greater flexibility in accessing distinct carbon substrates, critical to adaptation to wide-ranging trophic conditions.

Niche Width, Mean Growth, and Total Growth of *Fusarium* and *Streptomyces*

The effects of isolate identity (*Streptomyces* or *Fusarium*), plant host (*Ag* or *Lc*) and plant community richness (monoculture or polyculture), and their interactions on isolate niche width, mean growth, and total growth were investigated using 3-way ANOVA. Isolate taxonomy had significant effects on isolate growth characteristics, but no plant host or plant community richness effect was found (Supplemental Tables 6–8). Indeed, *Fusarium* mean niche width was significantly broader than *Streptomyces* niche width (Fig. 3a; 3-way ANOVA, $p < 0.001$). However, the mean growth per used nutrient among *Streptomyces* was modestly, but significantly, greater than that of *Fusarium (*Fig. 3b, 3-way ANOVA, $p = 0.002$) suggesting positive trade-offs between niche width and growth efficiency for *Streptomyces* isolates. As a result of their greater niche widths, *Fusarium* isolates had significantly greater total growth than *Streptomyces* isolates (Fig. 3c, 3-way ANOVA, $p = 0.001$) regardless of plant host and plant community richness. Thus, *Fusarium* and *Streptomyces* have comparable resource use efficiency, but *Fusarium* isolates use larger numbers of carbon sources, suggesting the potential for greater total growth than *Streptomyces*.

Niche Overlap Between *Fusarium* and *Streptomyces*

Niche overlap between *Fusarium* and *Streptomyces* was asymmetrical. Specifically, *Fusarium* isolates had greater average niche overlap with *Streptomyces* than *Streptomyces* had with *Fusarium* (1-way ANOVA, $p < 0.001$). Over all isolates from each plant host and richness treatments, the niche overlap of *Fusarium* with *Streptomyces* ($NO_{F->S}$) was 75%, whereas the niche overlap of *Streptomyces* with *Fusarium* ($NO_{S->F}$) was only 57%. Thus, *Fusarium* have larger competition-free niche space than *Streptomyces*, with considerable potential implications for their species interactions.

Niche overlap between the two taxa varied significantly with plant richness and plant host (2-way ANOVA, Supplemental Tables 9–10). There was an interaction effect

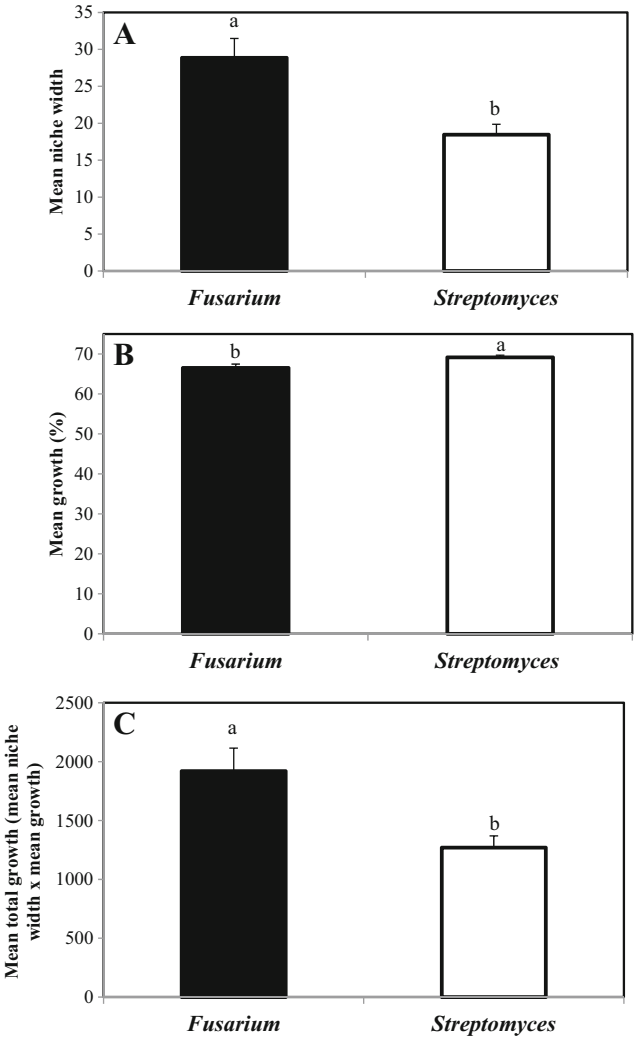

Fig. 3 Mean niche width (panel **a**), mean growth (panel **b**), and mean total growth (panel **c**) of *Fusarium* and *Streptomyces* from *Ag* and *Lc* in monoculture and polyculture. Isolate taxonomy, but not plant host or plant species, had significant effects on isolate growth characteristics (3-way ANOVA, $p < 0.05$). Each *bar* represents the mean of 12 replicate plants regardless of plant richness and host. For each plant, the average number of nutrients used (niche width) by each *Fusarium* or *Streptomyces* isolate for growth and their average growth on those nutrients were determined, and the average total growth for each *Streptomyces* or *Fusarium* isolate was calculated by multiplying their average niche width and average growth. Within each panel, *different letters above bars* indicate significant differences among isolates. *Error bars* represent standard errors

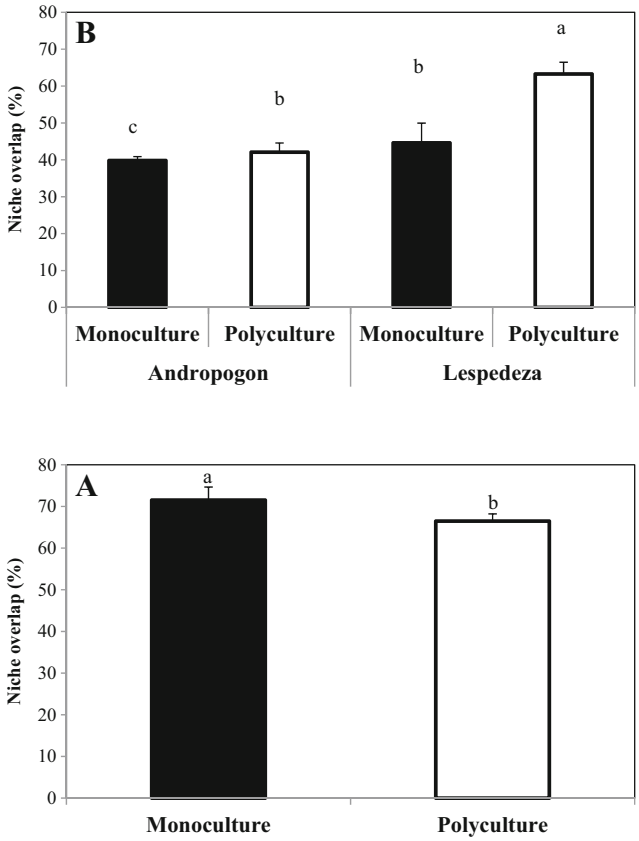

Fig. 4 Niche overlap (NO) between sympatric *Fusarium* and *Streptomyces* from *Ag* and *Lc* in monoculture and polyculture. NO was asymmetrical. Panel **a** shows the niche overlap of *Streptomyces* with *Fusarium* ($NO_{S->F}$); there was an interaction effect of plant host and plant community richness on ($NO_{S->F}$). Panel **b** shows the niche overlap of *Fusarium* with *Streptomyces* ($NO_{F->S}$); plant richness, but not host, had a significant effect on $NO_{F->S}$. Each *bar* represents the mean of three (panel **a**) or 6 (panel **b**) replicate plants. For each plant, $NO_{S->F}$ and $NO_{F->S}$ were determined for each *Fusarium* isolate and each *Streptomyces* isolate in every pairwise *Fusarium-Streptomyces* combination; NO was averaged across *Fusarium* or *Streptomyces* isolates. Within each panel, *different letters above bars* indicate significant differences among treatments in the niche overlap between *Fusarium* and *Streptomyces* (2-way ANOVA and LSD tests). *Error bars* represent standard errors

between plant host and plant community richness. Specifically, the niche overlap of *Streptomyces* with *Fusarium* ($NO_{S->F}$) was greater in polyculture than monoculture for both *Ag* and *Lc* (Fig. 4a; 2-way ANOVA, $p = 0.04$), while the niche overlap of *Fusarium* with *Streptomyces* ($NO_{F->S}$) was smaller in polyculture than monoculture (Fig. 4b; 2-way ANOVA, $p = 0.04$). These results indicate that, in terms of nutrient acquisition, *Fusarium* impose greater resource competition on *Streptomyces* in monoculture than polyculture, whereas *Streptomyces* challenge *Fusarium* more in polyculture than monoculture.

In addition, average $NO_{F->S}$ was significantly greater in *Lc* than in *Ag* (Supplemental Table 10; 2-way ANOVA, $p = 0.004$). Moreover, the mean niche overlap between *Fusarium* and *Streptomyces* (average of $NO_{F->S}$ and $NO_{S->F}$ among isolate pairs) in *Lc* exceeded significantly than that in *Ag* regardless of plant community richness (*t* test, $p = 0.007$). Specifically, the mean niche overlap between all pairwise *Fusarium-Streptomyces* combinations from *Lc* plants was 59%, but only 52% among *Fusarium-Streptomyces* combinations from *Ag* plants. This suggests that resource competition between the two taxa is likely to be greater in the rhizosphere of *Lc* than *Ag*.

Discussion

Microbes have coevolved to engage in complex networks of interactions with coexisting kin and non-kin individuals in the soil and in association with plants. Microbial antagonism and resource competition within complex communities have been the focus of many studies that have explored the effects of diverse factors on the ecological outcomes of these interactions [12, 15, 26, 47, 48]. However, the concurrent impacts on these processes of plant species richness and plant host, which jointly structure the environment within which rhizosphere microbes interact, have received far less attention. This is despite the fact that both plant community richness and plant species have been shown to impact microbial community composition [27] and function [3, 14, 42] in rhizosphere soil, with potential implications for microbial interactions and pathogen suppression in natural and agricultural habitats. In the present study, we investigated the consequences of long-term differences in plant community richness and plant host to nutrient use and inhibitory phenotypes of rhizosphere soil populations of the fungus *Fusarium* and the bacterium *Streptomyces.* Plant community richness, but not plant host, impacted *Fusarium* and *Streptomyces* resource niche overlap and inhibitory capacity in different ways. *Fusarium* had greater niche overlap with *Streptomyces* in monoculture than in polyculture, which we suggest is a significant factor in generating highly antagonistic *Streptomyces* communities in monoculture. Conversely, *Streptomyces* had greater niche overlap with *Fusarium* in polyculture than in monoculture, which we hypothesize results in selection for more inhibitory *Fusarium* populations in polyculture.

We showed significant effects of plant community richness on the inhibitory effects of *Streptomyces* communities against *Fusarium* populations. *Streptomyces* populations from monoculture were more frequently inhibitory against and were stronger inhibitors of coexisting *Fusarium* than *Streptomyces* from polycultures. The greater potential for monocultures than polyculture plant communities to produce more antagonistic *Streptomyces* against pathogenic and non-pathogenic *Streptomyces* isolates has been reported previously [3]. However, this is the first report of enhanced sympatric inhibition against another taxon (*Streptomyces* against *Fusarium*). In addition, prior work suggests that nutrient competition among *Streptomyces* is likely to select for highly inhibitory phenotypes [22, 36]. However, the previous work considered only the effects of single nutrient additions on inhibitory phenotypes. The present work hypothesizes that a high diversity of carbon substrates, for example in the rhizosphere of polyculture plant communities, offers the potential for niche differentiation as an alternative to a coevolutionary arms race. This premise is supported by the recent work [13, 14] showing that *Streptomyces* populations are more niche-differentiated in monoculture than in polyculture plant communities. Along the same lines, we hypothesize here that competition for nutrients in the rhizosphere of monoculture plant hosts imposes significant selection for *Streptomyces* that are antagonistic against *Fusarium*, while polyculture plant communities are more likely to select for *Streptomyces* that are relatively more niche-differentiated from sympatric *Fusarium* populations.

Our data provide evidence in support of these hypotheses. Indeed, the niche overlap of *Fusarium* with *Streptomyces* was greater in monoculture than in polyculture, and *Streptomyces* isolates from monoculture are more inhibitory against sympatric *Fusarium* populations than are *Streptomyces* from polyculture. In contrast, *Fusarium* resource competition with *Streptomyces* was relatively greater in monoculture than in polyculture. This is correlated with reduced investment in inhibition of *Fusarium* among *Streptomyces* in high richness plant communities. Thus, the variation in *Streptomyces* antagonistic and resource use phenotypes between monoculture and polyculture plant communities suggests that selection for *Streptomyces* inhibitory activities toward *Fusarium* may be mediated by competition for resources.

If resource competition were explicitly reciprocal, it would be logical to predict that *Fusarium* antagonistic phenotypes against *Streptomyces* would be similarly most frequent or of greater intensity among *Fusarium* populations in monoculture than polyculture plant communities. However, *Fusarium* isolates from monoculture were less potent in their inhibitory activities toward *Streptomyces* than *Fusarium* isolates from polyculture. Antibacterial properties of chemically diverse compounds from members of the genus *Fusarium* have been long reported [29, 38, 41], but, to our knowledge, the ecological forces influencing the production of such biologically active substances have not been addressed. Our results suggest that plant diversity fundamentally alters the selective trajectories for antagonistic and nutrient use phenotypes for *Fusarium* and *Streptomyces* populations, but in distinct ways. Thus, though the niche overlap of *Fusarium* with *Streptomyces* was greater in monoculture than polyculture, the niche overlap of *Streptomyces* with *Fusarium* was greater in polyculture than monoculture. This suggests stronger potential for *Streptomyces* to compete with *Fusarium* for nutrients in polyculture than in monoculture, which we suggest results in the greater antagonistic potential among *Fusarium* against *Streptomyces* in high versus low plant richness. Potential effects of plant communities [4] and litter chemistry [7] on resulting microbial functional diversity have been reported previously, and competition among microbes for space and nutrients in the soil has been suggested to lead to the evolution of distinct competitive strategies among microbes [9, 16]. Our results suggest that soil carbon resources, which are a function of plant community richness [31, 45], are a focal point of competition between *Fusarium* and *Streptomyces*

and are central to mediating selection for inhibitory phenotypes among both *Fusarium* and *Streptomyces* populations in soil.

A long-term goal for agricultural development is the ability to effectively manage microbial communities to control soil-dwelling plant pathogens without pesticides [30]. Our findings may have direct implications for sustainable and environmentally friendly management of soilborne *Fusarium* plant pathogens. Specifically, and contrary to standard management recommendations, the practice of monoculture-based production systems (avoiding intercropping, fostering weeding, etc.) in agricultural settings has the potential to improve *Streptomyces* antagonistic activities against *Fusarium* and other plant pathogens. Monoculture cropping systems may also facilitate selection for saprophytic *Fusarium* populations that are highly competitive for soil carbon resources, which may offer an additional pathway to pathogen suppression [1]. Further research is needed to uncover those plant hosts, plant community, and soil nutrient characteristics that govern complex microbial interactions in the soil to provide insights into better management of microbial communities and pathogen suppression in agricultural systems.

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