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Inhibitory and nutrient use phenotypes among coexisting *Fusarium* and *Streptomyces* populations suggest local coevolutionary interactions in soil

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Summary

Bacteria and fungi are key components of virtually all natural habitats, yet the significance of fungal-bacterial inhibitory interactions for the ecological and evolutionary dynamics of specific bacterial and fungal populations in natural habitats have been overlooked. More specifically, despite the broad consensus that antibiotics play a key role in providing a fitness advantage to competing microbes, the significance of antibiotic production in mediating cross-kingdom coevolutionary interactions has received relatively little attention. Here, we characterize reciprocal inhibition among *Streptomyces* and *Fusarium* populations from prairie soil, and explore antibiotic inhibition in relation to niche overlap among sympatric and allopatric populations. We found evidence for local adaptation between *Fusarium* and *Streptomyces* populations as indicated by significantly greater inhibition among sympatric than allopatric populations. Additionally, for both taxa, there was a significant positive correlation between the strength of inhibition against the other taxon and the intensity of resource competition from that taxon among sympatric but not allopatric populations. These data suggest that coevolutionary antagonistic interactions between *Fusarium* and *Streptomyces* are driven by resource competition, and support the hypothesis that antibiotics act as weapons in mediating bacterial–fungal interactions in soil.

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Introduction

Interactions within microbial communities, including mutualism (win-win relationship), commensalism (one partner benefits without helping or harming the other), amensalism (one partner is harmed without any advantage to the other), and antagonism (loss-loss relationship), can influence the dynamics and functional capacities of soil microbes (Kawaguchi and Minamisawa, 2010; Liu *et al*., 2012; O'Brien *et al*., 2013). Microbial interactions are frequently mediated by bioactive compounds, including antibiotics. Antibiotics play important roles in signalling and antagonistic species interactions, conferring fitness benefits to producers in competitive habitats (Williams *et al*., 1989; Riley and Gordon, 1999; Martinez *et al*., 2009). However, most of our knowledge of the roles of antibiotics in mediating microbial interactions comes from studies on the bacterial kingdom. For example, numerous bacteria within the genera *Bacillus*, *Pseudomonas*, and *Streptomyces* are known for their prolific antibiotic production (Raaijmakers and Mazzola, 2012). In addition, recent work suggests that antibiotic production among soil *Streptomyces* is under significant local selection and mediates nutrient competition among sympatric populations (Kinkel *et al*., 2014). However, our understanding of the role of antibiotics in mediating cross-kingdom nutrient competition and coevolutionary antagonistic interactions remains limited. Here, we focus on adaptive antagonistic interactions between populations of a bacterial and fungal genus to begin to construct a more comprehensive knowledge of the role of antibiotics in mediating cross-kingdom microbial interactions and structuring functional characteristics of microbial communities.

Although specific microbial interactions are difficult to discern in highly complex environment, and their

understanding requires the incorporation of broader influences on microbiome composition and functioning, such as those emanating from soil edaphics and plant–microbe and microbe–microbe interactions; some microbes possess mechanisms and molecules that enable identifiable mutual interplays in complex habitats (Braga *et al*., 2016). Recently, members of the fungal genus *Fusarium* and the bacterial genus *Streptomyces*, notable for their capacities to produce a diverse array of antimicrobial compounds (Challis and Hopwood, 2003; Joshi, 2012), were shown to possess significant inhibitory activities against each other (Essarioui *et al*., 2017). Plant community richness was hypothesized to play a central role in mediating these antagonistic interactions through the alteration of the dynamics of nutrient competition across the landscape. While *Fusarium–Streptomyces* inhibitory interactions suggest significant potential for antibiotic-mediated coevolutionary dynamics between these microorganisms, there is little empirical data to support this hypothesis for indigenous soil populations.

The dynamics of competitive and coevolutionary antagonistic interactions among microbes can result in reciprocal accumulation of specific inhibitory and resistance phenotypes in interacting populations over time (Czaran *et al*., 2002; Laskaris *et al*., 2010; Kinkel *et al*., 2014). Such outcomes can also be inferred by characterizing variation in inhibition and/or resistance in different locations, or across space (Kinkel *et al*., 2014). For example, for interacting *Fusarium–Streptomyces* populations, if the production of antimicrobial compounds that are effective against sympatric (from the same location) competitors confers a specific fitness benefit to the producer, and is thus under local positive selection, then the frequency and/or the intensity of inhibitory interactions will be greater among sympatric than allopatric (from different locations) *Fusarium* and *Streptomyces* isolates. Moreover, if antibiotic inhibitory phenotypes mediate nutrient competition between coexisting competitors, inhibition should be stronger against sympatric isolates that have greater nutrient use similarity than against isolates with distinct nutrient preferences. Finally, if such interactions are under local selection, or locally adapted, then relationships between nutrient competition and inhibition should be observed among sympatric but not allopatric *Fusarium* and *Streptomyces* populations.

Here, we evaluated reciprocal inhibitory interactions and nutrient (niche) overlap among sympatric and allopatric *Fusarium* and *Streptomyces* populations from multiple locations in prairie soils. Using these data, we explore two major questions for each genus: (i) Are *Streptomyces–Fusarium* and *Fusarium–Streptomyces* inhibitory phenotypes locally adapted? (ii) Is antibiotic inhibition specifically targeted toward nutrient competitors? Collectively our data show greater sympatric than allopatric inhibitory interactions, which support the conclusions that *Streptomyces–Fusarium* coevolve in soil and that antibiotics play a key role in mediating nutrient competition between these genera, and highlight the significance of resource competition and antibiotics in mediating cross-kingdom species interactions within complex soil communities.

Results

Inhibitory interactions and reciprocal adaptation among Streptomyces *and* Fusarium *populations*

Overall, *Fusarium* isolates were significantly more inhibitory against *Streptomyces* isolates (*F–S*) than *Streptomyces* isolates were against *Fusarium* isolates (*S–F*). The mean proportion of sympatric *F–S* inhibitory interactions was significantly greater than the mean frequency of sympatric *S–F* inhibition (58% F–S vs. 11% S–F) (paired *t*-test; $p < 0.001$). The consistent occurrence of antagonistic phenotypes between *Fusarium* and *Streptomyces* in the rhizosphere suggests significant potential for selection imposed by each taxon on the other.

Inhibitory interactions between sympatric and allopatric *Fusarium* and *Streptomyces* populations were compared to evaluate evidence for local selection of inhibitory phenotypes for either taxon. We found that *Streptomyces* isolates were better at inhibiting *Fusarium* isolates from the same than from different locations (Fig. 1). Specifically, the mean proportion of inhibitory *Streptomyces* isolates was significantly higher for sympatric than allopatric *Fusarium* isolates. Moreover, the intensities of *Fusarium* inhibition by *Streptomyces* differed significantly for sympatric versus allopatric *Fusarium*. Mean inhibition zones were significantly greater against sympatric than allopatric *Fusarium* isolates (Fig. 1). These results suggest that both *Streptomyces* and *Fusarium* populations vary in their inhibitory and resistance phenotypes among locations, and that *Streptomyces* populations have evolved to preferentially inhibit locally coexisting *Fusarium* populations.

In contrast to *Streptomyces* inhibition of *Fusarium*, there was a smaller effect of *Streptomyces* sympatry on *Fusarium* inhibitory activities (Fig. 2). Specifically, comparing the frequency and intensity of *Fusarium* inhibition of *Streptomyces*, there was no significant difference in the frequency of inhibition between the sympatric and allopatric isolate pairs. However, there was a small but statistically significant increase in the intensity of *Fusarium* antagonistic activity against *Streptomyces* from the same location when compared with those from different locations (Fig. 2).

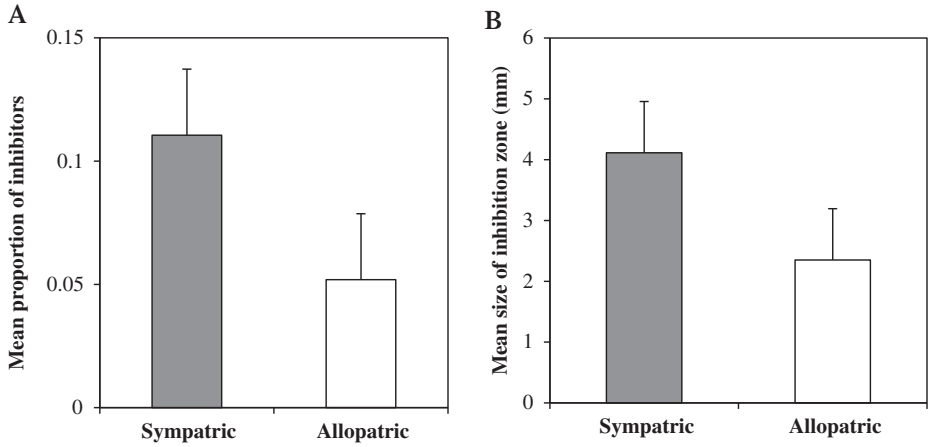

Fig. 1. Frequency (panel A, mean proportion of inhibitors) and intensity (panel B, mean inhibition zone) of *Streptomyces* inhibition of *Fusarium* from the same (sympatric) and different (allopatric) soil samples. For each soil sample (n = 12), the proportions of *Streptomyces* that were inhibitory against sympatric and allopatric *Fusarium* isolates were determined and averaged across sympatric or allopatric *Fusarium* isolates; mean sizes of inhibition zones caused by individual inhibitory *Streptomyces* isolates against each sympatric and allopatric *Fusarium* isolate were measured and averaged across all sympatric or allopatric *Fusarium* isolates from each sample. The mean proportions of inhibitors and mean zone sizes were then averaged across soil samples. Both the frequency of *Streptomyces* inhibition of *Fusarium*, and the intensity of inhibition were significantly greater against sympatric versus allopatric *Fusarium* (frequency, p = 0.002; intensity, p = 0.001; df = 11; paired t-test). Error bars represent standard errors.

Niche overlap and inhibition among Fusarium–Streptomyces *sympatric and allopatric isolates*

Antimicrobial compound production is hypothesized to confer fitness benefits to the producer through the inhibition of resource competitors (Williams and Vickers, 1986; Kinkel *et al*., 2014). We characterized the relationships between niche (nutrient use) overlap and inhibition among sympatric and allopatric *Fusarium* and *Streptomyces* isolates. *Fusarium* isolates were more susceptible to inhibition by sympatric *Streptomyces* isolates with which they had larger niche overlap than with individuals with which they exhibited little niche overlap (Fig. 3). Specifically, among *Fusarium* isolates that were susceptible to inhibition by *Streptomyces* (inhibition >0.1 mm), there was a significant positive correlation between *Fusarium* inhibition by *Streptomyces* and *Fusarium* niche overlap with *Streptomyces* from sympatric but not allopatric locations (Fig. 3). In a similar fashion, among *Streptomyces*

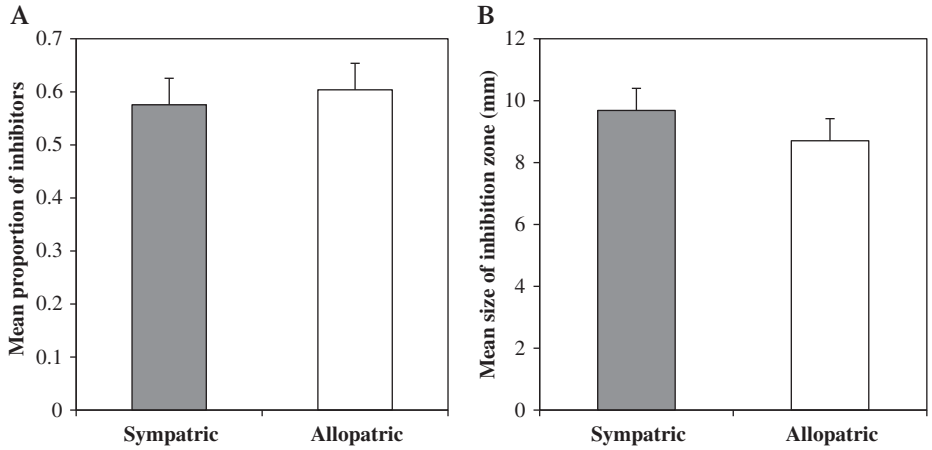

Fig. 2. Frequency (panel A, mean proportion of inhibitors) and intensity (panel B, mean inhibition zone) of *Fusarium* inhibition of *Streptomyces* from the same (sympatric) and different (allopatric) soil samples (n = 12). For each soil sample, the proportions of *Fusarium* isolates that were inhibitory against sympatric and allopatric *Streptomyces* isolates were determined and averaged across sympatric or allopatric *Streptomyces* isolates; mean sizes of inhibition zones caused by individual inhibitory *Fusarium* isolates against each sympatric and allopatric *Streptomyces* isolate were measured and averaged across all sympatric or allopatric *Streptomyces* isolates from each soil sample. The mean proportions of inhibitors and mean zone sizes were averaged across soil samples. The intensity, but not the frequency, of *Fusarium* inhibition of *Streptomyces* was significantly greater against sympatric versus allopatric *Fusarium* (frequency, p = 0.233; intensity, p = 0.038; df = 11; paired t-test). Error bars represent standard errors.

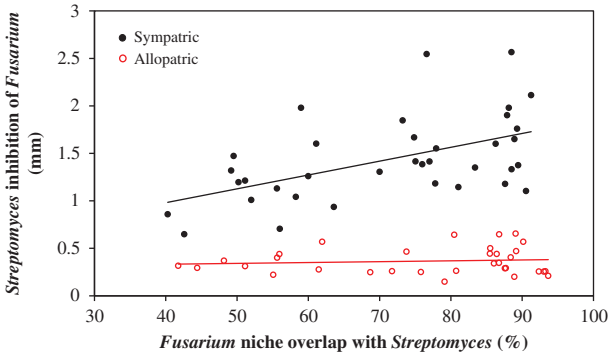

Fig. 3. Relationships between mean niche overlap of *Fusarium* with *Streptomyces* and *Streptomyces* inhibition of *Fusarium* among isolates from the same (n = 36, sympatric) and different (n = 33, allopatric) soil samples. Each data point represents the mean niche overlap with allopatric or sympatric *Streptomyces* and corresponding mean inhibition by allopatric or sympatric *Streptomyces* for an individual *Fusarium* isolate. *Streptomyces* inhibition of *Fusarium* was significantly positively correlated with *Fusarium* niche overlap with sympatric (R = 0.52; p = 0.001), but not allopatric (R = 0.1; p = 0.57), *Streptomyces*. [Color figure can be viewed at wileyonlinelibrary.com]

isolates that were inhibited by *Fusarium* isolates (inhibition >0.5 mm), we found a significant positive correlation between *Streptomyces* niche overlap with *Fusarium* and *Fusarium* inhibition of sympatric, but not allopatric, *Streptomyces* isolates (Fig. 4). Thus, *Fusarium* inhibition is directed more towards coexisting *Streptomyces* with similar nutrient use profiles. Overall, these important inhibitory interactions between *Streptomyces* and *Fusarium* are significantly related to resource competition.

Notably, *Fusarium* inhibition of sympatric *Streptomyces* increased more steeply (Slope = 11.32; p < 0.0001) with increasing niche overlap than did *Streptomyces* inhibition

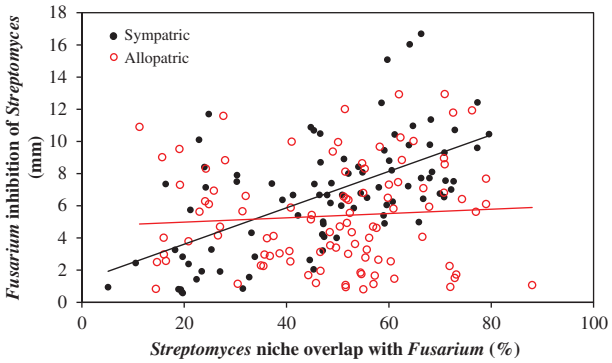

Fig. 4. Relationships between mean niche overlap of *Streptomyces* with *Fusarium* and *Fusarium* inhibition of *Streptomyces* among isolates from the same (n = 88, sympatric) and different (n = 100, allopatric) soil samples. Each data point represents the mean niche overlap with sympatric or allopatric *Fusarium* and corresponding mean inhibition by allopatric or sympatric *Fusarium* for an individual *Streptomyces* isolate. *Fusarium* inhibition of *Streptomyces* was significantly positively correlated with *Streptomyces* niche overlap with sympatric (R = 0.59; p < 0.0001), but not allopatric (R = 0.08; p = 0.441), *Fusarium*. [Color figure can be viewed at wileyonlinelibrary.com]

of sympatric *Fusarium* (Slope = 1.46; p = 0.001). This may suggest that *Fusarium* isolates are more responsive to resource competition than *Streptomyces*, or that *Fusarium* possess a greater range of capacities to inhibit *Streptomyces* than vice versa. Moreover, we found that *Fusarium* niche overlap with *Streptomyces* in relation to *Fusarium* total growth was a stronger correlate for allopatric versus sympatric *Streptomyces* (R = 0.83; p < 0.0001 allopatric vs. R = 0.67; p < 0.0001 sympatric populations). Thus, this may suggest that *Fusarium* isolates are specializing in growth on nutrients not preferred by sympatric *Streptomyces*, or active niche differentiation of *Fusarium* from sympatric *Streptomyces* competitors. In contrast, no evidence for *Streptomyces* niche differentiation from *Fusarium* was found regardless of whether *Fusarium* isolates were sympatric or allopatric (R = 0.86, p < 0.0001).

Overall, these data provide compelling evidence that, among *Streptomyces* and *Fusarium* populations in soil, functional characteristics including antagonism, niche preferences, and growth capacities are shaped by cross-kingdom coevolution.

Discussion

Local, within-community interspecies and intraspecies interactions stimulate a wide variety of ecological and coevolutionary dynamics that contribute to the generation of functional diversity in living organisms (Thompson and Cunningham, 2002; Forde *et al*., 2004). Although the significance of microbial species interactions to microbial adaptation is widely accepted, much of our current knowledge on microbial local adaptation and coevolutionary dynamics comes from studies of host-pathogen or bacterium-phage systems (Middelboe *et al*., 2009; Scanlan *et al*., 2011; Koskella, 2014; Koskella and Parr, 2015). However, non-parasitic or non-pathogenic adaptive interactions between bacteria and other microbes, especially fungi, remain poorly explored. This is unfortunate given the widespread coexistence of bacterial and fungal populations in terrestrial ecosystems. In this study, we provide support for the proposition that *Streptomyces* and *Fusarium* populations coevolve in soil, and that antibiotics mediate resource competition between members of these genera.

Both the intensities and frequencies of *Streptomyces* inhibition of *Fusarium* were greater in sympatric than in allopatric combinations suggesting that *Streptomyces* populations have evolved adaptations that are specific to inhibiting their local *Fusarium* populations. In a similar fashion, the mean intensity of inhibition of *Streptomyces* by *Fusarium* was greater in sympatric versus allopatric confrontations. This indicates local adaptation of *Fusarium* to better inhibit coexisting than spatially distant

Streptomyces. In contrast, there was no significant effect of sympatry on the frequency of *Fusarium* inhibition of *Streptomyces*. However, the substantial accumulation of inhibitory phenotypes among *Fusarium* in all locations, coupled with the enhanced inhibition of sympatric (but not allopatric) *Streptomyces* resource competitors, reinforce support for the hypothesis of ongoing coevolution between coexisting *Streptomyces* and *Fusarium* populations.

It could be argued that the results observed here would be due to species turnover among soil samples. Indeed, for both *Streptomyces* and *Fusarium*, subsets of sympatric isolates (from the rhizosphere of the same plant) used in this study tend to cluster phylogenetically (Fig. S1), suggesting plant-driven selection of particular phylogenetic groups. Specific plant–microbe associations were shown to reflect environmental filtering imposed by plant community richness, plant species, and even species genotypes (Lundberg *et al*., 2012; Bakker *et al*., 2014). Thus, because *Fusarium* and *Streptomyces* genera are classical plant symbionts, specific functional groups of these taxa may be associated with different sampled plants, with great potential for coevolutionary inhibitory interactions among sympatric populations. Indeed, when inhibition occurs (>0.1 for *Fusarium* and >0.5 for *Streptomyces*), its varying patterns among closely related *Fusarium* and *Streptomyces* coexisting isolates used in this study provide support of this hypothesis and indicates that phylogenetic relatedness does not predict isolates' resistance similarity. Overall, the consistent occurrence of reciprocal antagonistic interplays throughout all of the soil samples of this investigation indicates nested reciprocal selection that, by scaling up from species-level, may result in diffuse coevolution between *Fusarium* and *Streptomyces* genera. These dynamics may extend to other coexisting microbial groups under the influence of multiple local factors including soil edaphics, microbial taxon, and community characteristics. Further studies that factor in the complexity of inhibitory phenotype selection could add richness to our understanding of coevolution among populations in natural habitats.

We found significant positive correlations between inhibitory interactions and niche overlap among sympatric, but not allopatric, *Fusarium* and *Streptomyces* populations. These findings suggest that local selection for antibiotic inhibitory phenotypes among *Fusarium* and *Streptomyces* populations is mediated by nutrient competition in local soil communities. Though the use of Biolog plates provide an imperfect measurement of the potential for resource competition due to the arbitrary inclusion of carbon sources, and this limitation has been reported as a caveat in using Biolog for microbial community characterization (Glimm *et al*., 1997), Biolog-based analyses have been used reliably for comparing functional diversity of microbial communities from multiple sites (Stefanowicz, 2006). Moreover, regardless of the specific nutrient profile in soil, Biolog assays provide quantitative and reproducible data on the metabolic capacities and competitive potentials of *Streptomyces* and *Fusarium* populations, which are the focus of the analyses here.

Our findings show differences in the dynamics and potential role of antibiotics in the life histories of *Fusarium* and *Streptomyces*. For example, *Fusarium* inhibition of sympatric *Streptomyces* increased more with increasing niche overlap than did *Streptomyces* inhibition of sympatric *Fusarium*. This may suggest greater fitness incentives for inhibition of *Streptomyces* by *Fusarium* than vice versa, or perhaps a greater genetic capacity of *Fusarium* to respond to competition with *Streptomyces* as a consequence of *Fusarium's* larger genome size. Studies on host–pathogen interactions from across various *Fusarium* pathosystems, coupled with comparative genomics, have demonstrated that *Fusarium* species possess dynamic genomes with high plasticity that contribute directly to host specialization and adaptation to changing environments (Ma *et al*., 2013; 2010). Our work shed larger light on *Fusarium* adaptive capacity to respond to coexisting microbes in complex competitive communities.

Interestingly, in addition to selection for more inhibitory phenotypes, our data also suggest selection for less-inhibitory sympatric than allopatric *Fusarium* phenotypes when *Streptomyces* are weak competitors for resources with *Fusarium* (Fig. 4). This may imply manipulation of *Streptomyces* by *Fusarium*, and specifically the potential that *Fusarium* populations exploit the antibiotic inhibitory capacity of sympatric *Streptomyces* against other competitors in the soil. In contrast, the apparently weaker inhibitory responses of *Streptomyces* versus *Fusarium* to resource competition may reflect alternative life history strategy options including enhanced production of stress-resistant spores and local dispersal by *Streptomyces* in response to *Fusarium* resource competition or inhibition. Regardless of mechanisms by which they adapt to one another, our results suggest that antibiotics play complex roles in the life history of *Streptomyces* versus *Fusarium* populations, and in their competitive and coevolutionary interactions.

This work is among the first reports to document the central role of antibiotic inhibition and nutrient competition in mediating *Streptomyces–Fusarium* interactions and determining their functional capacities and coevolutionary dynamics in soil. Such antagonistic interplays can offer novel opportunities for the fight against plant diseases caused by pathogenic *Fusarium* using antagonistic *Streptomyces* (e.g., *Fusarium* wilt and root rots) and vice versa (e.g., *Streptomyces* scabs). Further studies on the factors that dictate the potential for diverse

coevolutionary outcomes will contribute significantly to expanding our understanding of the long-term trajectories of antibiotics-producing microbes in soil (Kinkel *et al*., 2014) and improving pathogen suppression in agricultural habitats (Kinkel *et al*., 2011; Martinez *et al*., 2011; Kinkel *et al*., 2012).

Experimental procedures

Soil sample collection

Soil samples were collected from as close as possible to the centre of the base of individual *Andropogon gerardii* (*Ag*), C4 grass, and *Lespedeza capitata* (*Lc*), legume, plants growing in well-established (19 years post-establishment) communities of 1 (monoculture) or 16 (polyculture) plant species at the Cedar Creek Ecosystem Science Reserve (CCESR), a National Science Foundation Long-Term Ecological Research site in east-central Minnesota. Plants to be sampled were selected randomly from individual plots at a distance of at least 20 cm from plot margins to avoid border effects as described in Essarioui *et al*. (2016). 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, samples were taken from three plants from each plot. Thus, we had a total of three samples from every plant species in each plant community richness; *Ag* in monoculture, *Ag* in polyculture, *Lc* in monoculture, and *Lc* in polyculture; for a total of 12 soil samples. The soil samples were stored at −20°C prior to processing.

Soil from each sample was placed under two layers of sterile cheesecloth to dry overnight and 5 g subsamples from each plant were subsequently dispersed in 50 ml of sterile deionized water on a reciprocal shaker (175 rpm, 60 min, 4°C). Resulting soil suspensions were dilution (10^{-5}) plated onto molten starch-casein agar (SCA) as previously described (Wiggins and Kinkel, 2005b). This method suppresses the growth of many unicellular bacteria, while allowing filamentous *Streptomyces* to grow up through the SCA. Plates were incubated at 28°C for 3 days. Colonies exhibiting characteristic *Streptomyces* morphology were randomly selected, purified and spore suspensions of each isolate were maintained in 20% glycerol at −20°C. Ten *Streptomyces* isolates from each soil sample were selected for a total of 120 isolates (Table 1). Soil diluents (10^{-3}) of each soil sample were also used to isolate *Fusarium* spp. as in Essarioui *et al*. (2016). For each soil suspension, individual aliquots of 100 µl were spread onto 15 ml plates of peptone pentachloronitrobenzene, a medium that is highly inhibitory to most other fungi and bacteria but allows slow growth of *Fusarium* (Nelson *et al*., 1983), and incubated at 28°C for 3–4 days. Subsequently, 10–15 randomly chosen single fungal colonies from each plate were transferred to plates containing 15 ml of potato dextrose agar (PDA) medium 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 (Leslie and Summerel, 2006). From 2–10 *Fusarium* isolates were selected from each soil sample for a total of 84 isolates from 12 soil samples (Table 1). Cores (1-cm diameter) from 7 to 10 days old cultures of each isolate were maintained in 20% glycerol at −20°C.

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	10
3	*Lc*	Monoculture	10	8
4	*Lc*	Polyculture	10	10
5	*Lc*	Polyculture	10	10
6	*Lc*	Polyculture	10	10
7	*Ag*	Monoculture	10	2
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	84

Abbreviations: Lc, *Lespedeza capitata*; Ag, *Andropogon gerardii*.

Streptomyces and Fusarium phylogeny

Identification to genus level of the isolates and their phylogenetic relatedness were determined by sequencing *Streptomyces* 16S rRNA (ribosomal RNA) and a portion of *Fusarium* RPB2 (DNA directed RNA polymerase second largest subunit) genes based on methods described in Schlatter and Kinkel (2014) and LebLanc *et al*. (2017) respectively. Briefly, *Streptomyces* genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions with minor modification; and 16S rRNA genes were amplified using the universal bacterial primers 16S-27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 16S-1541R (5′-AAGGAGGTGATCCAGCCGCA-3′) in a 50 µl reaction volume using PCR Supermix High Fidelity master mix (Invitrogen, Carlsbad, CA), 10 pM of each primer and, 100 ng template DNA with the following protocol: initial denaturation at 95°C for 2.5 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, and an additional extension step consisting of 72°C for 10 min. *Fusarium* DNA was extracted using a

cetrimonium bromide/chloroform-based protocol as described in Gale *et al*. (2011) and diluted to 10 ng ml^{-1}. A portion of the RPB2 gene was amplified from the extracted DNA using the RPB2-5F2 (5′-GGGGWGAYCAGAAGAAGGC-3′) and RPB2-7cR (5′-CCCATRGCTTGYTTRCCCAT-3′) primers. Reactions were performed in 20 ml volume with 2 ml of Ex-Taqbuffer, 1.6 ml of dNTPs (2.5 mM of each), 2 ml of each forward and reverse primers (10 mM), 11.3 ml of water, 0.1 ml of Takara Ex-Taq (Takara Bio, Shiga, Japan) polymerase (5 units ml^{-1}), and 1 ml template (10 ng of DNA/ml). The PCR reactions were performed with an initial denaturing step at 95°C (5 min), followed by 24 cycles at 95°C (1 min), 57°C (1 min), 72°C (1 min), and ended with a final extension of 72°C (10 min). *Streptomyces* and *Fusarium* amplicons were sequenced using the forward primers (16S-27F and RPB2-5F2) at the University of Minnesota Biomedical Genomics Centre (Saint Paul, MN). *Streptomyces* 16S sequences were aligned with the 16S sequences of three *Streptomyces* reference strains retrieved from the NCBI GenBank; *Streptomyces griseoviridis* (accession number AB184210.1), *Streptomyces vinaceus* (accession number KY753358.1), and *Streptomyces mirabilis* (accession number EF371431.1). Similarly, *Fusarium* RPB2 sequences were aligned with the RPB2 sequences of three *Fusarium* reference strains from the NCBI sequence database; *Fusarium commune* (accession number MH582180.1), *Fusarium solani* (accession number MF276958.1), and *Fusarium oxysporum* (accession number LN828101.1). *Fusarium* and *Streptomyces* phylogenetic trees, including reference strains (Fig. S1), were constructed using the neighbour-joining method with 1000 bootstraps implemented in MEGA6 (Tamura *et al*., 2013).

Assaying Streptomyces–Fusarium *inhibitory interactions*

Inhibitory activity of *Streptomyces* communities in each soil sample was evaluated against every single sympatric (from that soil sample) and allopatric (from samples taken from different individuals of the same plant species and plant community richness). To test for *Streptomyces* inhibition of a *Fusarium* isolate, a method described by Herr (1959) (Wiggins and Kinkel, 2005a, 2005b) was used. Soil samples were dilution-plated to allow for selective growth of *Streptomyces* as described above. After 5 days of incubation, *Streptomyces* were enumerated based on morphology. Plates with 40–70 colony-forming units (CFU) were used to assess *Streptomyces* inhibitory activity against *Fusarium* isolates. In order to achieve this, 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. Four millilitre of this suspension was added to 100 ml of molten potato dextrose water agar, mixed thoroughly, and 10 ml of the resulting suspension was overlaid onto the top of the plates containing *Streptomyces*. Three replicate plates were made for every soil sample-*Fusarium* isolate combination. Plates were incubated at 28°C for 3 days. For each soil sample, the proportion of *Streptomyces* that exhibit antagonistic activities against each sympatric or allopatric *Fusarium* isolate was calculated, and the size of the inhibition zone surrounding every inhibitory *Streptomyces* was determined as the average diameter (mm) of the inhibition zone measured at 2 right angles; the proportions of inhibitory *Streptomyces* and the average size of the inhibition zones were averaged across all sympatric or allopatric *Fusarium* isolates. Finally, inhibition of each *Fusarium* isolate by sympatric or allopatric *Streptomyces* community was determined by multiplying the proportion of inhibitory *Streptomyces* against that isolate by the mean size of the inhibition zones. A *Fusarium* isolate was considered to be inhibited by *Streptomyces* when the inhibition of that isolate is >0.1 mm.

Fusarium inhibition of *Streptomyces* was evaluated for all possible sympatric and allopatric *Fusarium–Streptomyces* isolate pairwise combinations using the agar-disc method as described in Essarioui *et al*. (2016). Briefly, 1-cm diameter discs of *Fusarium* mycelium, collected from the outer actively growing mycelium from a 7 to 10 days old PDA culture, were transferred onto SCA plates with three replicate discs 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 to give approximately 10^6–10^7 CFU/plate. Plates were kept at 4–5°C overnight to allow for *Fusarium* antimicrobial compounds to diffuse. Plates were subsequently incubated for 3–4 days at 28°C. The size of any zone of growth inhibition of the *Streptomyces* isolate surrounding any *Fusarium* agar-disc was measured in millimetre from the edge of the disc to the edge of the cleared zone; each inhibition zone was measured in two locations, at right angles to one another. For each soil sample, the proportion of *Fusarium* that was inhibitory against each sympatric or allopatric *Streptomyces* isolate and the size (mm) of the inhibition zone surrounding every inhibitory *Fusarium* were determined; the proportions of inhibitory *Fusarium* and the average size of the inhibition zones were averaged across all sympatric or allopatric *Streptomyces–Fusarium* isolate pairs. Finally, inhibition of each *Streptomyces* isolate by sympatric or allopatric *Fusarium* was determined as the proportion of sympatric or allopatric *Fusarium* isolates that was inhibitory to that *Streptomyces* isolate multiplied by the mean size of the inhibition zones. A *Streptomyces* isolate was considered

to be inhibited by *Fusarium* when the inhibition of that isolate is >0.5 mm.

Characterizing nutrient utilization

Nutrient use profiles were evaluated for each *Streptomyces* (n = 120) and *Fusarium* (n = 84) isolate for 95 single carbon sources using Biolog SF-P2 plates (Biolog, Hayward, CA) as described previously. A portion of these results has been reported in previous work (LeBlanc *et al*., 2014; Essarioui *et al*., 2016; Essarioui *et al*., 2017). Briefly, fresh spore suspensions taken from previously prepared stock spore solutions (10^7–10^8 spore/ml) were quantified to an absorbance of 0.22 at 590, diluted according to the manufacturer's instructions, and inoculated into Biolog plates. Plates were incubated at 28°C for 3 days and the growth on each carbon source was evaluated by measuring the absorbance of each well at 590 nm using a microplate reader. The absorbance of the water control was subtracted from the absorbance of every other well. The absorbance in each well within every plate was transformed to a percentage of the maximum optical density 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 (total percentage growth) and niche (the number of used nutrients) were determined for each isolate. The potential for resource competition between sympatric and allopatric *Fusarium* and *Streptomyces* was indexed for each soil sample. 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* ($PNO_{S->F}$) was defined as:

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

and pairwise niche overlap of *Fusarium* with *Streptomyces* ($PNO_{F->S}$) as:

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

Finally, for each *Fusarium* isolate, niche overlap with *Streptomyces* isolates was defined as the average niche overlap of all possible pairwise combinations of that *Fusarium* isolate with sympatric or allopatric *Streptomyces* isolates. Similarly, for each *Streptomyces* isolate, niche overlap with *Fusarium* isolates was defined as the average niche overlap of all possible pairwise combinations of that *Streptomyces* isolate with sympatric or allopatric *Fusarium* isolates.

Analyses

Paired t-test with $p < 0.05$ as the significance level was carried out to test for differences in inhibitory capacity between *Fusarium* and *Streptomyces* and for differences in the intensity and frequency of sympatric versus allopatric reciprocal inhibitory interactions. Linear regression analysis was performed to examine the relationship between niche overlap and inhibition. All proportions were arcsin transformed to meet test assumptions for statistical analyses. All statistical analyses were performed using R statistical software version 3.1.1 (http://cran.r-project.org/bin/windows/base/old/3.1.1/).

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Supplemental Table 1 Number of *Fusarium* and *Streptomyces* isolates using each of the carbon sources in the SF-P2 microplates.

Fig. S1: Neighbour-joining phylogenetic tree based on the sequences of *Streptomyces* (S) 16S (left) and *Fusarium* (F) RPB2 (right) genes. For each $S_{x\text{-}y}$ and $F_{x\text{-}y}$, x indicates sample code and y denotes isolate number. Reference strains are denoted by arrows. Adjacent to each taxon tree is a heatmap displaying mean inhibition of each isolate of that taxon by isolates of the other taxon.