Plant community richness and foliar fungicides impact antibiotic inhibition, resistance, and resource use traits among soil *Streptomyces*.

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Abstract

Introduction

Plants interact simultaneously with diverse microbes, nematodes, insects, and vertebrates both above and below the soil surface. As a result, plants are critical links between above- and below-ground ecosystems (Masters, Brown, and Gange 1993; Bardgett, Wardle, and Yeates 1998; Van der Putten et al. 2001; Bezemer and Dam 2005), allowing biota in above-ground compartments to indirectly impact soil communities by modifying plant productivity and behavior (Foley et al. 2021; Bagchi et al. 2017; Casas et al. 2011; Bardgett, Wardle, and Yeates 1998).

The phyllosphere represents a vast microbial habitat, with an estimated 6.4×10^8 km^2 of leaf surface area available for microbial colonization (Morris, Kinkel, et al. 2002), that harbors diverse communities of bacteria, yeasts, and filamentous fungi (Jumpponen and Jones 2009; Rastogi, Coaker, and Leveau 2013; Agler et al. 2016). Phyllosphere communities are functionally diverse and may have positive, neutral, or negative effects on plant health and productivity. Plant-beneficial phyllosphere inhabitants can provide defense from pathogens or herbivores (H. Liu, Brettell, and Singh 2020; Ritpitakphong et al. 2016), induce resistance (Bezemer and Dam 2005), produce plant hormones (Abadi et al. 2020; Lu et al. 2018), fix nitrogen (Madhaiyan et al. 2015), or confer resistance to abiotic stressors (Qu et al. 2020; Xu et al. 2022). However, foliar pathogens, especially fungi, often have strong negative effects on plant health and productivity and result in major crop losses worldwide (Teng, Shane, and MacKenzie 1984).

Plant responses to foliar microbes are likely to have consequences for below-ground communities. For example, Rudrappa et al. (2008) demonstrated that foliar infection of *Arabidopsis* by *Pseudomonas syringae* increased root exudation of D-malic acid and promoted root colonization by beneficial *Bacillus subtilis*. Over multiple growing seasons, changes in plant phenotypes or productivity as a result of plant-microbe interactions in the phyllosphere may modify soil resource concentrations (e.g. carbon, nitrogen, phosphorous, and potassium) in ways that can also impact soil microbial communities.

Likewise, research on plant herbivory suggests that alterations in plant physiology or resource allocation are important mechanisms mediating interactions across below- and above-ground ecosystems. For example, alterations in the quantity or quality of plant-produced compounds (root exudates, toxins), under moderate herbivory can stimulate decomposition and nutrient mineralization (Bardgett, Wardle, and Yeates 1998; Shariff, Biondini, and Grygiel 1994). In other cases, activation of plant defense pathways or induced production of phenolic compounds may have negative effects on soil processes (Siqueira et al. 1991; Bezemer and Dam 2005). Over prolonged periods, shifts in plant productivity, allocation of plant resources to above- or below-ground compartments, synthesis of defense compounds, and stimulation or repression of soil decomposers are expected to alter soil carbon sequestration and resource availability. However, despite multiple studies focusing on herbivory by relatively large animals (insects, vertebrates), there are few data on linkages between foliar and soil microbial communities.

Plant community richness can also have broad effects on the physical environment both above- and below-ground, which has direct consequences for foliar and soil communities. Specifically, diverse plant communities are often substantially more productive (Tilman et al. 2001) and experience reduced levels of foliar disease (Mitchell, Tilman, and Groth 2002) than monocultures. Moreover, plant richness is hypothesized to be a crucial driver of soil bacterial community composition and function through the provision of greater quantities and diversities of plant-derived resources (Schlatter et al. 2015; Meier and Bowman 2008; Bakker et al. 2013; Ng et al. 2014). For example, greater quantities of carbon compounds support higher soil microbial densities, alter microbial community structure and diversity, and impact soil enzyme activities (Griffiths et al. 1998; Hernández and Hobbie 2010). Further, greater diversity of carbon compounds in soil is hypothesized to increase the number of ecological niches available to soil microbes, and has been found to increase microbial diversity and shift microbial carbon use capacities (Orwin, Wardle, and Greenfield 2006; Essarioui, Kistler, and Kinkel 2016).

Despite the importance of plant-derived resources as a mechanistic link between plants and soil microbial communities, our understanding of the specific impacts of the quantity and diversity of plant-derived resources on traits of soil microbes in field settings remains limited. In addition to direct impacts on soil microbial community composition and activities, the quantity and diversity of resources is also hypothesized to modulate the significance of competitive interactions to the fitness of specific microbial populations (Pekkonen, Ketola, and Laakso 2013; Fiegna et al. 2015). As a result, variation in microbial interaction traits in soil are likely to reflect the quantity and diversity of soil resources, with potential consequences for foliar community diversity and function (Schlatter et al. 2009).

Streptomyces are filamentous, Gram-positive bacteria that are found ubiquitously in soils as saprotrophs and are often closely associated with plant roots in the rhizosphere or endosphere (Viaene et al. 2016). As tremendous producers of antibiotic compounds, *Streptomyces* are important in clinical medicine and in suppressing soil-borne plant pathogens in natural and agricultural systems (Viaene et al. 2016; Barka et al. 2016) **Linda to add self-citations here**. In addition to their antibiotic-producing capacities, *Streptomyces* are a substantial natural reservoir of antibiotic resistance genes (D'Costa, Griffiths, and Wright 2007) and are highly diverse in their abilities to utilize resources for growth (Schlatter et al. 2013). Together, *Streptomyces* antibiotic inhibition, resistance, and resource use traits are all hypothesized to be critical for their interactions with other microbes, and their capacities to respond to variation in resource inputs and impact plant health (Kinkel et al. 2014; Schlatter et al. 2009, 2013).

In this study we probe the relationship between soil nutrient composition and soil ecosystem func-

tion. We consider a 2x2 factorial experiment evaluating the consequences of plant richness (monoculture vs. sixteen species polyculture) and chronic disruption of foliar fungi (by fungicide applications) on soil resource levels and *Streptomyces* (inhibition, resistance, and nutrient-use) phenotype. We hypothesize that 1) disruption of foliar fungi will impact *Streptomyces* community composition, as well as antibiotic inhibitory/resistance and resource use phenotypes in soil; 2) the effects of foliar fungi disruption will depend on the plant richness context; and 3) changes in *Streptomyces* communities will be related to differences in soil resources.

Methods

Experimental design and soil sampling

Soils were collected from 3 m x 3 m plots in a long-term grassland biodiversity experiment at the University of Minnesota Cedar Creek Ecosystem Science Reserve (CCESR), part of the U.S Long Term Ecological Research (LTER) Network (45.4°N, 93.2°W) (Tilman et al. 1997). In particular, the plots from which our data were sampled were established in 1994 (19 years prior to sampling), and either maintained as monocultures or polycultures of 16 native perennial species. The plots used in this study included those in which a 2 L m^{-2} of foliar fungicide (Quilt (Syngenta Crop Protection, Inc.), a combination of Azoxystrobin (7.5%) and Propiconazole (12.5%)) was applied biweekly and control plots. Each plot was further divided into two subplots, one of which received nutrient amendment in the form of 10 g N m^{-2} $year^{-1}$ as timed-release urea $[(NH_2)_2CO]$, 10 g P m^{-2} $year^{-1}$ as triple-super phosphate $[Ca(H_2PO_4)_2]$, 10 g K m^{-2} $year^{-1}$ as potassium sulphate $[K_2SO_4]$ and 100 g m^{-2} of a micronutrient mix of Ca (6%), Fe (17%), S (12%), Mg (3%), Mn (2.5%), Cu (1%), Zn (1%), B (0.1%) and Mo (0.05%). Nutrients were applied once a year every spring except the micronutrient mix which was only applied in the first year to avoid toxicity. The entire experimental field is burned each spring to remove any litter remaining from the previous year (Zaret et al. 2022). A more detailed description can be found in Zaret et al. (2023) and at the CCESR website (http://www.cedarcreek.umn.edu/research/exper/e120).

Samples were collected from each subplot, at the base of individual *Andropogon gerardii* (Ag) plants. Triplicate (1 cm diameter) soil cores were collected from the base of n = 6 plants from fungicide and non-fungicide treatments in each plot in November 2013. Soil cores from the same individual plant were bulked in the field (n = 24 composite samples), transported in a cooler to the lab, sieved (using a 2 mm mesh), and stored at -20°C until processing.

Streptomyces densities and inhibitor densities

Streptomyces densities and inhibitor densities were determined as described previously (Bakker et al. 2013). Briefly, soil samples were dried under two layers of sterile cheesecloth in a fume hood overnight. Dry soil samples were finely ground and 5 g of each soil was added to 25 ml of sterile deionized (DI) water and placed on orbital shaker at 175 rpm at 4°C for 60 minutes. Soil suspensions were serially diluted in sterile DI water and 100 l of each dilution was spread onto 1% water agar. After plates were allowed to dry, 5 ml of 1% starch casein agar (SCA; Küster and Williams (1964)) was pipetted to cover the entire plate. Prior to overlaying, SCA was allowed to cool to prevent the medium from killing microorganisms. SCA is a semi-selective medium for *Streptomyces* and allows filamentous microbes to grow through the medium while suppressing non-filamentous bacteria (Oskay et al. 2009). Plates were incubated for 3 days at 28°C and *Streptomyces* densities were evaluated by counting the number of colonies exhibiting characteristic *Streptomyces* morphology. After determining densities, a modified Herr's assay (B. E. Wiggins and Kinkel 2005;

B. Wiggins and Kinkel 2005) was used to assess inhibitor densities. Briefly, plates were overlaid with 10 ml of 1% SCA, dried for [[n]] minutes, and then overlaid with 150 l of spore suspension of an indicator strain (*Streptomyces* strains LK1324.2 or DL87). After 3 days of growth at 28°C, *Streptomyces* colonies inhibiting the indicator overlay were counted and inhibition zone sizes were measured twice from the edge of the colony to the edge of clear overlay inhibition at right angles to one another. Overlay DL87 is a *Streptomyces* scabies isolate, which causes common scab disease of potato (D. Liu, Anderson, and Kinkel 1996), and LK1324.2 is a non-pathogenic isolate previously obtained from CCESR soil (Davelos et al. 2004). For each soil, *Streptomyces* and inhibitor densities were averaged over three replicate plates for each overlay isolate (n = 6 plates total).

Streptomyces isolation

After processing, soils from two individual plants/treatment (n = 8 soils) were selected for *Streptomyces* isolation. Soils were serially diluted in sterile DI and 100 l was spread on SCA. After 5 d of growth, colonies exhibiting characteristic *Streptomyces* morphology were selected by dividing each plate into a grid and randomly picking grid cells from which to select colonies. Five colonies were collected from 3 separate plates for each soil sample. *Streptomyces* colonies were picked with a sterile toothpick and streaked onto oatmeal agar (OA; Kharel et al. (2010)). After 5 days of growth, single colonies were swabbed with a cotton applicator and spread as a lawn on OA. After ~7 days growth, spores were collected by gently swabbing *Streptomyces* lawns with 4 ml of a 20% glycerol solution. Spore stocks were stored at -20°C until further use. Eighty *Streptomyces* isolates (n = 10 isolates per individual plant) were selected for further characterization.

Streptomyces resource use characterization

For each *Streptomyces* isolate, resource use phenotypes on 95 distinct carbon sources were determined using Biolog SF-P2 plates (Biolog, Inc. Hayward, CA) as described previously (Schlatter et al. 2013). Briefly, fresh spore suspensions of *Streptomyces* isolates were adjusted to an OD590 of 0.22 and 100 l of a spore suspension was inoculated into each well of a Biolog SF-P2 plate. After 3 days of growth at 28°C, the absorbance in each well at 590nm (AU590) was measured using a BioTek Synergy H1 plate reader (BioTek Instruments, Inc. Winooski, VT, USA). The absorbance in the water control well was subtracted from all other wells prior to subsequent analyses. A carbon source was considered to be used by an isolate if the AU590 was greater than 0.01 above the water control well. Niche width and growth efficiency were determined for each isolate, where the niche width of an isolate is the number of used resources and resource use efficiency is the mean absorbance value for used resources. Niche overlap, a measure of shared resource use among isolates, was calculated for each pair of isolates a and b as the average pairwise niche overlap: $\bar{\omega}_{a\to b} = \frac{1}{n}\sum_i^n \frac{min(od_a^{(i)}, od_b^{(i)})}{od_a^{(i)}}$, where $od_x^{(i)}$ is the absorbance (i.e. growth) of isolate x on carbon source i. Notably, this metric is asymmetric, such that, in general, $\bar{\omega}_{a\to b} \neq \bar{\omega}_{b\to a}$.

16S rRNA gene sequencing

Partial 16S rRNA gene sequences were obtained for n = 77 isolates using previously described protocols (Davelos et al. 2004). Briefly, genomic DNA was extracted from cultures of each isolate grown in Yeast-Dextrose broth for 3 days (28°C, 175rpm) using the Wizard Genomic DNA Purification kit (Promega Corp., Madison, WI). PCR reactions consisted of 12.5 l HotStart Master Mix (Qiagen), 0.75 l of pA (10 pM), 0.75 l of pH (10 pM), 1.0 l of *Streptomyces* DNA (25 ng), and 10.0 l of PCR grade H_2O. Thermocycling conditions consisted of an initial denaturation of 95°C for 4 min,

followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 90 s, and a final extension at 72°C for 7 min. PCR products were checked for the expected product on a 1% agarose gel, purified with the Qiaquick PCR cleanup kit (Qiagen, Valencia, CA), and sequenced using the forward primer (pA) at ACGT, Inc (Wheeling, IL). Sequences were edited manually, classified using the RDP Classifier (Wang et al. 2007), and aligned using MUSCLE (v3.8.1551) (Edgar 2004). Gaps were removed using trimAl (v1.4.rev22) (Capella-Gutiérrez, Silla-Martínez, and Gabaldón 2009) and a maximum likelihood phylogenetic tree was constructed using RAxML (v8.2.12) (Stamatakis 2014) using a GTR+γ+I substitution model, *Embleya hyalina* strain NBRC 13850 as an outgroup, and 10,000 bootstraps to assess confidence.

Antibiotic resistance

Resistance profiles against 9 clinical antibiotics (Kanamycin, Streptomycin, Erythromycin, Vancomycin, Amoxicillin, Novobicin, Chloramphenicol, Rifampicin, and Tetracycline) were determined for each isolate using a disk-diffusion assay (Otto-Hanson et al. 2013). Each isolate-antibiotic combination was replicated twice.

Soil edaphic characteristics

Total soil carbon (C; %) and nitrogen (N; %) were determined for each soil at the University of Nebraska soil testing lab using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc.). Soil organic matter (%), Bray-1 extracted phosphorus (P; ppm), NH_4OAc extracted potassium (K; ppm), and pH were determined at the University of Minnesota Research Analytical Laboratory (www.ral.cfans.umn.edu) using standard protocols.

Statistical analysis

Clustering of treatments with phylogentic distance was identified with a permutational multivariate analysis of variance using the `adonis2` function in the `vegan` (v. 2.6-4) `R` package (Oksanen et al. 2022). Differences in *Streptomyces* populations (densities, inhibitor densities and proportions),resource use (niche width, mean efficiency, niche overlap), and soil characteristics with plant richness and fungicides were assessed with a nested ANOVA, where the fungicide factor was nested within plant richness. All statistical analyses were performed in `R` (v. 4.3.1) (R Core Team 2023) unless otherwise indicated.

Results

Streptomyces community composition

The composition of *Streptomyces* communities differed among treatments (Figure 1; $p = 0.038$). However, when comparing plant richness or fungicide treatments, communities were significantly phylogenetically clustered according to plant richness ($p = 0.013$), but not disruption of foliar communities with fungicide within monocultures or polycultures ($p = 0.517$).

Soil edaphic characteristics

Soil resources (total carbon (C; %), total nitrogen (N; %), and organic matter (%)), as well as soil pH varied significantly across foliar community disruption, but only in polyculture plots Figure 2. Specifically, in polycultures, untreated plots tended to have greater C, N, and organic matter than treated polyculture plots and both treated and untreated monoculture plots. Monoculture plot soil

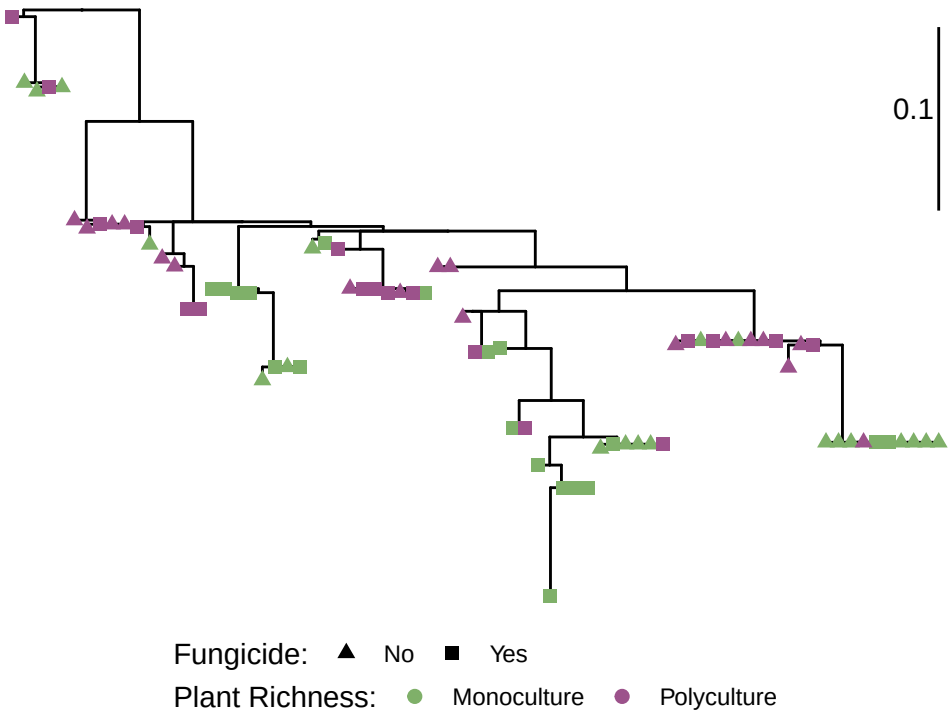

Figure 1: Phylogenetic relationships between isolates. Isolates are colored according to the plant richness of the sample plot and shaped according to foliar-community disruption via application of a foliar fungicide. Tree was generated with RAxML (v8.2.12) (Stamatakis 2014) using a GTR+γ+I substitution model, *Embleya hyalina* strain NBRC 13850 as an outgroup (not included in displayed tree), and 10,000 bootstraps to assess confidence.

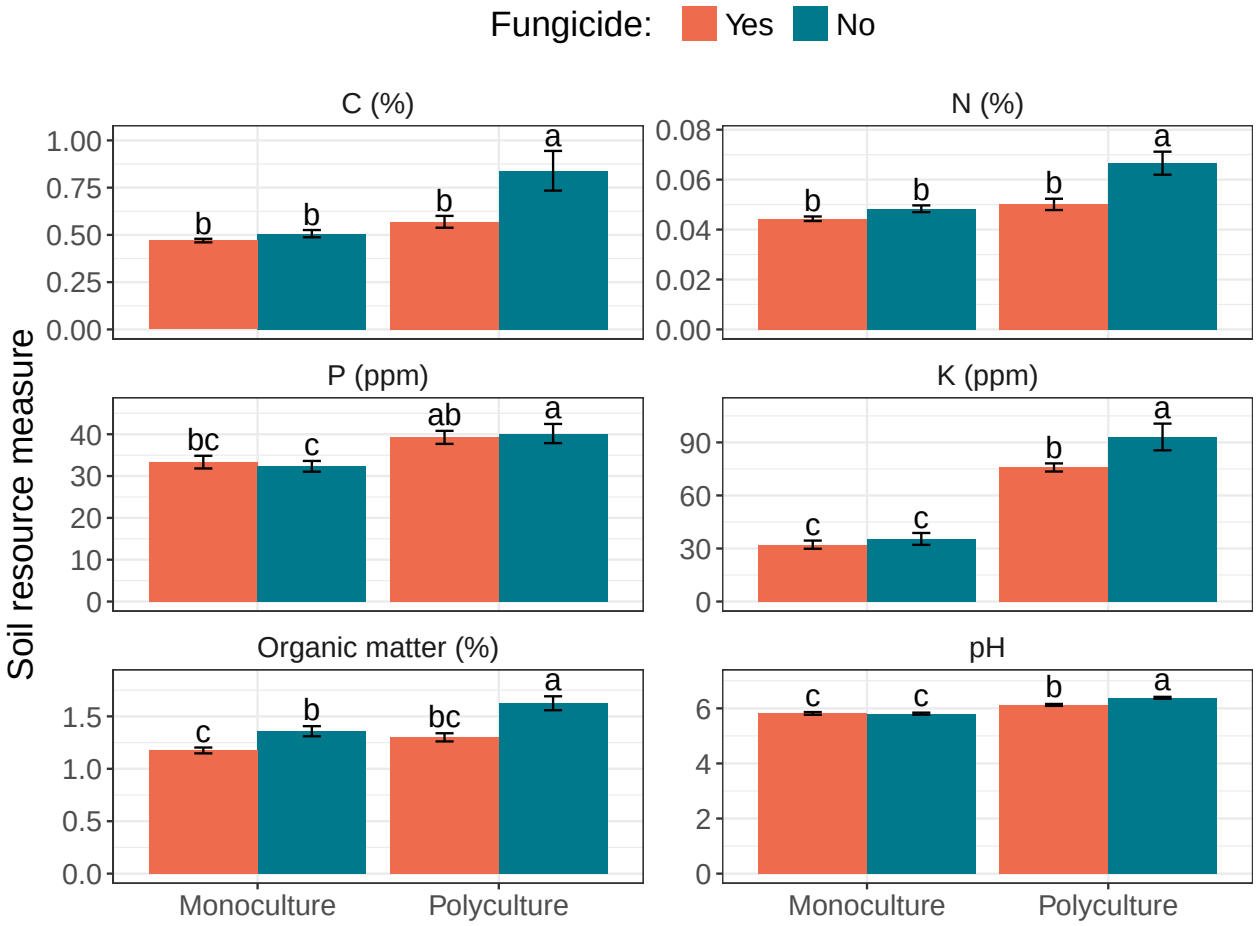

Figure 2: Soil edaphic characteristics among plant richness and fungicide treatments (n = 6 plots/treatment). Error bars represent standard errors and different letters above bars indicate statistically significant differences using Tukey's HSD post-hoc test ($p < 0.05$).

was found to be more acidic than polyculture soil, with foliar fungal community disruption having a significant effect in the latter. Specifically, plots in which the foliar fungal communities were disrupted had more acidic soil (that is, more similar to monocultures) than did untreated polyculture plots. Potassium (K; ppm) was found to be higher in polycultures than monocultures, but without differences across foliar fungicide treatment, and phosphorus (P; ppm) was unchanged across all treatments. In summary, resource quantity was found to vary significantly across treatments, especially considering the effect of foliar community disruption within polyculture plots.

Streptomyces densities and inhibitory activities and their relationships with soil edaphic characteristics

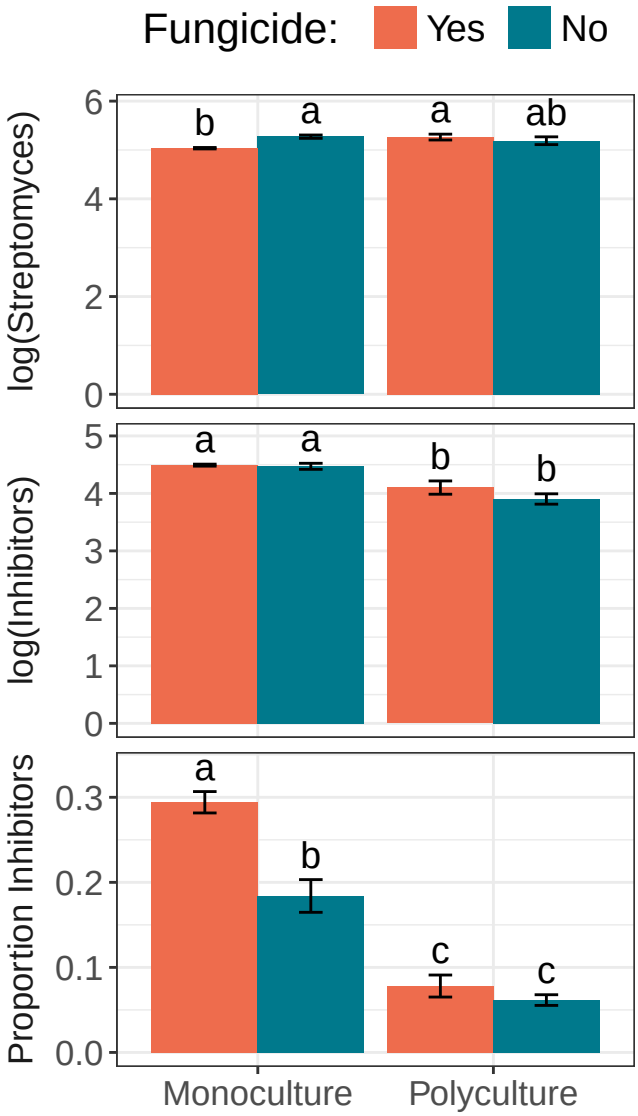

Figure 3: Densities of *Streptomyces* (top panel), inhibitory *Streptomyces* (middle panel), and proportions of *Streptomyces* that are inhibitory (bottom panel) in untreated (blue) and fungicide-treated (orange) monoculture and polyculture plots. Error bars signify standard errors from the mean, and differing letters signify significant (p0.05) differences according to a Tukey's Honestly Significant Difference post hoc test of an Analysis of Variance.

Streptomyces densities and densities of inhibitory *Streptomyces* differed significantly among plant richness and fungicide treatments (Figure 3). *Streptomyces* densities were lower in monocultures whose foliar fungal community was disrupted and higher in treated polycultures and untreated monoculture plots (Figure 3, top). Considering only *Streptomyces* exhibiting inhibitory phenotypes, plant monocultures supported significantly higher densities of inhibitors than polycultures, though there was no significant effect of foliar fungicide treatment on inhibitor densities in either plant richness treatment (Figure 3, middle). Proportions of *Streptomyces* exhibiting inhibitory phenotypes varied significantly with both plant richness and disruption of foliar fungal communities (Figure 3, bottom). Inhibitory *Streptomyces* composed ~10-20% more of the total *Streptomyces* community in monocultures versus polycultures. Further, disruption of foliar fungal communities further increased the relative frequency of inhibitory *Streptomyces* in monocultures, but not in polycultures. Together, these data suggest that antibiotic-producing *Streptomyces* are more fit in monocultures versus polyculture settings, and that disruption of foliar community structure increases the fitness benefit of antibiotic inhibition, but only in monocultures. In contrast with densities and proportions of inhibitory *Streptomyces*, mean inhibition zone sizes, or the intensity of *Streptomyces* inhibitory phenotypes, did not vary significantly among treatments (data not shown).

Correlations between *Streptomyces*/inhibitor densities and soil edaphic characteristics differed between polyculture and monoculture plots. In monocultures, soil C, N, K, and organic matter were all positively correlated with *Streptomyces* densities (Pearson's R^2 0.36, $p < 0.041$; Supplementary InformationTable S1). In contrast, the proportion of *Streptomyces* that exhibited inhibitory phenotypes were significantly negatively correlated with N and organic matter (R^2 0.42, $p < 0.022$; Supplementary InformationTable S1). In polycultures, however, there was only one significant correlation: a negative relationship between the density of inhibitors and soil P ($R^2 = 0.53$, $p = 0.017$; Supplementary InformationTable S2). This suggests that *Streptomyces* in polycultures are less responsive to soil resource levels than are those in monocultures.

Thus, overall densities of *Streptomyces* increased with greater soil organic matter, while abundances and proportions of inhibitory *Streptomyces* decreased with greater soil resources. Consistent with previous work (Bakker et al. 2013), these data suggest the significance of antibiotic production to *Streptomyces* fitness is lower in high resource environments, though resource quantity and diversity are confounded in these analyses.

Interestingly, the proportions of inhibitory *Streptomyces* was negatively correlated with overall *Streptomyces* densities in monocultures ($R^2 = 0.81$, $p < 0.001$), but not polycultures ($R^2 = 0.05$, $p = 0.490$), while raw densities of inhibitors showed the opposite trend, significantly increasing with *Streptomyces* density in polycultures ($R^2 = 0.64$, $p = 0.002$), but not in monocultures ($R^2 = 0.02$, $p = 0.691$). Taken together, these suggest that as *Streptomyces* abundance increases in monocultures, non-inhibitory *Streptomyces* increase faster than their inhibitory counterparts, resulting in non-inhibitory *Streptomyces* representing a larger proportion of the total *Streptomyces* community than they did at lower abundances. However, in polycultures, the relative frequency of inhibitory *Streptomyces* remains largely constant, with both inhibitory and non-inhibitory *Streptomyces* increasing in tandem.

Nutrient use and niche overlap among *Streptomyces* isolates

Niche width, or the number of resources that individual *Streptomyces* could grow on, varied significantly with disruption of the foliar fungal community, but not with plant richness. However, the effect of this disruption on *Streptomyces* niche widths differed between monocultures versus

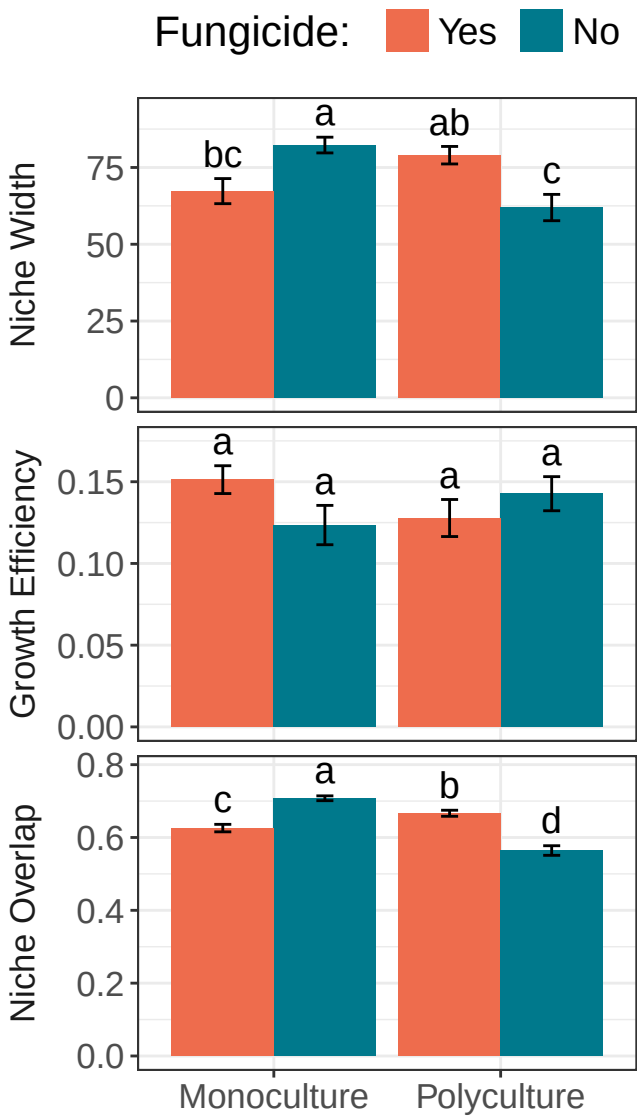

Figure 4: Niche width among *Streptomyces* from different plant richness and fungicide treatments. Error bars represent standard errors and different letters above bars indicate statistically significant differences using Tukey's HSD post-hoc test (p0.05).

polycultures (Figure 4). Specifically, in monoculture plots where foliar fungicides were applied, *Streptomyces* had reduced niche widths compared to plots with undisturbed foliar fungal communities, while in polyculture plots, niche widths were increased in plots where foliar fungicide was applied.

Considering this in combination with the inhibition results from Figure 3 suggests a trade-off associated with maintaining both broad niche widths and highly antagonistic phenotypes. In contrast, the polyculture plots did not exhibit different rates of inhibition (Figure 3), but did show differences in nutrient quantity (Figure 2), with higher resource quantity (untreated plots) corresponding to lower niche widths.

Growth efficiency, or the average growth of individual *Streptomyces* isolates across used resources, did not vary significantly with plant richness or fungicide treatment (Figure 4). Niche overlap, or the degree to which isolates from the same treatment grow on the same resources, varied significantly across both foliar fungicide and plant richness treatments (Figure 4), and were strongly positively correlated with Niche width ($R^2 = 0.76$, $p < 0.001$).

Antibiotic resistance

For six of the nine antibiotics tested, we found no significant difference in inhibition zone size across treatment (Supplemental Information Table S10). For Amoxicillan, Erythromycin, and Chloramphenicol, however, we found *Streptomyces* from monoculture were significantly more susceptible (i.e. had larger inhibition zones in response to antibiotic disks) than those from polyculture (N.b. that the difference in Chloramphenicol was significant in an analysis of variance despite no significant pairwise differences found in the post-hoc analysis; Figure 5, Table S10).

We found additional effects of foliar fungicide application for isolates' susceptibility to Amoxicillan, with isolates coming from plots in which the foliar fungal communities were disrupted were found to be even more susceptible to Amoxicillan, compounding the effect of plant richness (Figure 5). Yet, in all three cases, the richness treatment explained more of the variation than did disruption of the foliar fungal community. Aggregating across treatments, we note that *Streptomyces* isolates were most susceptible to Kanamycin and Streptomycin, while being least susceptible to Rifampcin and Tetracycline (Figure S1).

Differentially used nutrients among *Streptomyces* Include?

Streptomyces growth rates were found to differ significantly across richness treatments for nine carbon sources, while growth differed for sixteen resources across fungicide treatments within richnesses (Figure 6). Three carbon sources were significant for both model terms: Amygdalin, l-alaninamide, and N-actyl-b-D-mannosamine. Generalizing to molecule type, four of the nine significant for plant richness were polymers, while eight of the sixteen significant for foliar fungal disruption were carbohydrates. With respect to particular patterns, we do not note any trends across carbon sources, with all combinations of ranking and significant difference between treatments represented.

Discussion

With respect to our hypotheses, we found fairly idiosyncratic effects of disrupting the foliar fungal communities on soil *Streptomyces*. While effects were found across the composition of inhibitory, resistance, and resource use phenotypes, in no cases did we see consistent effects of the fungicide

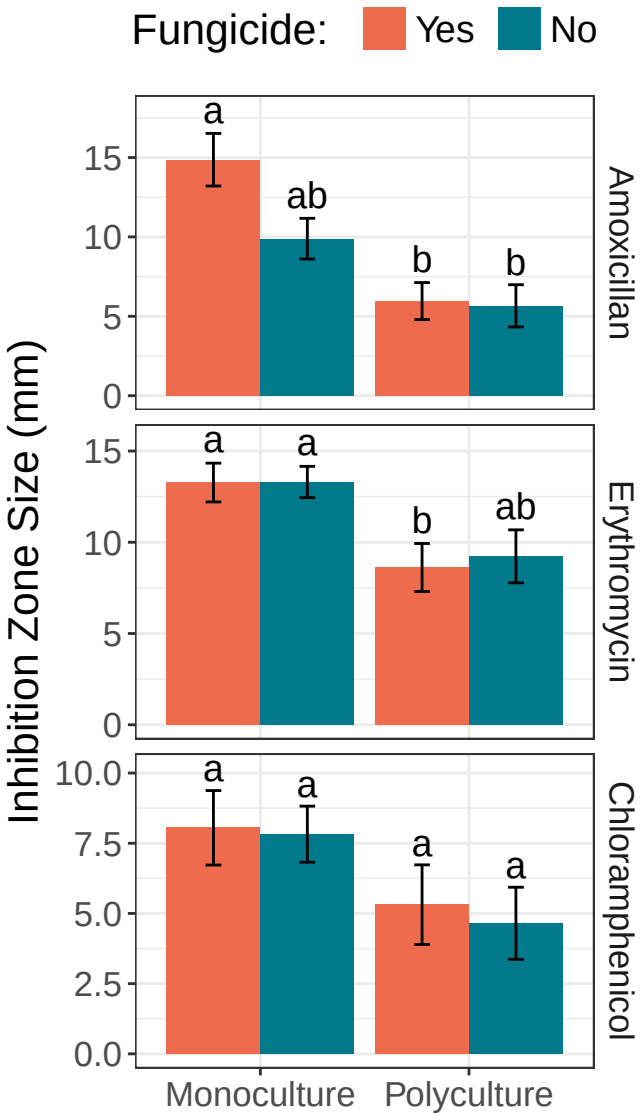

Figure 5: Resistance zone sizes of *Streptomyces* isolates from different plant richness and fungicide treatments (n = 18 - 20 isolates/treatment). Error bars represent standard errors and different letters above bars indicate statistically significant differences using Tukey's HSD post-hoc test ($p < 0.05$). Note that an analysis of variance reports a significant difference in Chloramphenicol across plant richness, despite no pairwise differences being detected in the post-hoc analysis (Table S10).

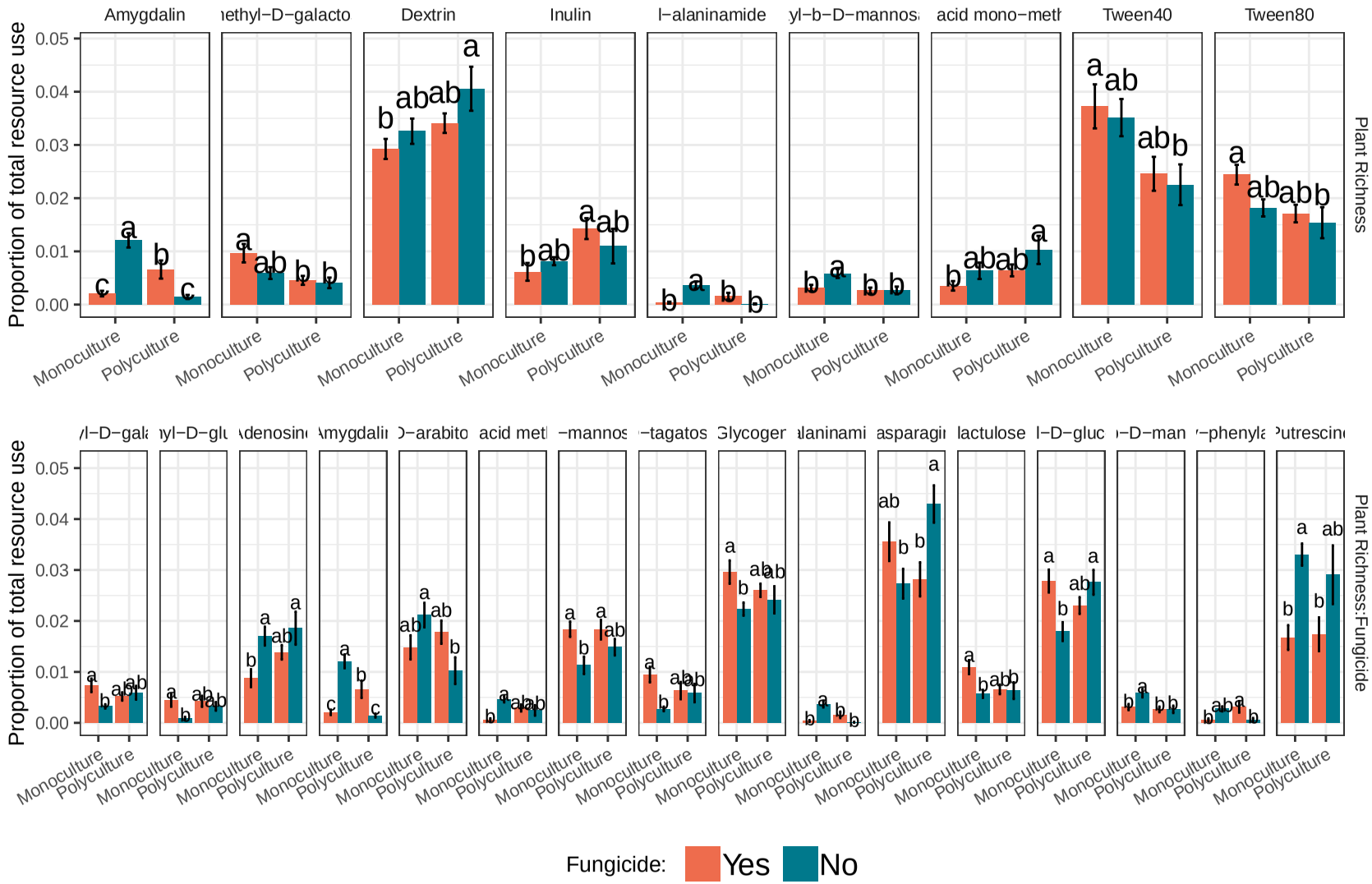

Figure 6: Proportion of total resource use by *Streptomyces* isolates from different plant richness and fungicide treatments (n = 18 - 20 isolates/treatment). Error bars represent standard errors and different letters above bars indicate statistically significant differences using Tukey's HSD post-hoc test ($p < 0.05$). Only the 23/95 resources with at least one significant difference across treatments are shown.

treatment across plant richness treatments. Similarly, while isolated *Streptomyces* did differ phylogenetically by the plant richness of their source plot, there was not a phylogenetic signature of foliar fungal community disruption.

In particular, we found that disruption of the foliar fungal community via application of a foliar fungicide in the context of a diverse plant community produced a soil chemistry that was more similar to that observed in monocultures (regardless of fungicide application). Despite the absence of chemistry differences across monoculture treatments, there were significantly fewer *Streptomyces* and proportionally more inhibitors in monocultures that had their foliar fungal communities disrupted. Fungicide treated monocultures also showed a significant reduction in resistance to Amoxicillan. Meanwhile, the effect of the fungicide treatment on nutrient-use profiles was opposite in monocultures vs. polycultures: reducing niche width and overlap in monocultures, while increasing niche width and overlap in polycultures. **TODO: individual nutrients**

Finally, we expected to find that observed changes in *Streptomyces* communities would be related to differences in soil resources. Yet, while we found some idiosyncratic correlations between soil resource levels and *Streptomyces* inhibition, resistance, and niche overlap phenotypes, none of these were significant following correction for multiple hypothesis testing.

The impact of soil edaphics on *Streptomyces* inhibition, resource-use, and resistance phenotypes

Resource environments can influence the co-evolutionary dynamics of microbial populations (Craig MacLean, Dickson, and Bell 2005; Lawrence et al. 2012). Specifically, a greater diversity of resources is hypothesized to promote adaptive radiation and niche-differentiation among microbial taxa, so that microbial populations minimize resource competition (Craig MacLean, Dickson, and Bell 2005; Kinkel et al. 2014). In contrast, low-diversity resource environments, where microbes must compete for resource pools, are predicted to favor high frequencies of antagonistic microbes (e.g. antibiotic producers) and may generate a co-evolutionary arms-race (Bakker et al. 2013; Kinkel et al. 2014). Finally, greater quantities of resources, by supporting higher microbial densities and encounter rates, may strengthen selection imposed by species interactions and speed the rate of co-evolution (Lopez Pascua et al. 2014).

In this experiment, we had two treatments that sought to alter soil resource levels and therefore elicit responses in soil microbial phenotypes: we disrupted foliar fungal communities through the application of a foliar fungicide, and we considered two levels of plant richness. the former was expected to *alter* soil resource levels by suppressing fungal endophytes and _____ [@]. With respect to the latter, previous research has shown that richness is generally correlated with resource quantity and diversity [@]. We found congruence with these expectations in the form of higher levels of carbon, nitrogen, organic matter, and potassium in soil from polyculture than monoculture plots. Interestingly, for carbon, nitrogen, and organic matter, the disruption of the foliar fungal communities was sufficient to offset this increase, producing levels consistent with those observed in the monoculture plots. (**mention acidity here too?**) Surprisingly, we did not find an effect of foliar fungal community disruption on any measure of soil edaphics across monoculture plots.

Given some differences in soil edaphic characteristics, we expected to see soil microbial phenotypes changing as well. Indeed, the higher resource quantities seen in polyculture were found reflected in lower levels of inhibition (both absolutely and proportionally; Figure 3, Supplementary InformationTable S8) and niche overlap (but not niche width; Figure 4, Supplementary InformationTable S9). This is in agreement with prior literature suggesting that microbes are more likely

to invest in direct competition when resources are scarce [@]. In contrast, *Streptomyces* from polyculture plots were found to have greater resistance to some common antibiotics (Figure 5, Supplementary InformationTable S10). **explanation?**

Interestingly, however, we also saw significant changes in soil microbial phenotypes independent of changes in soil edaphics. For instance, while monoculture plots were not significantly different in any measure of soil chemistry, we nevertheless observed differences in *Streptomyces* densities, proportion of inhibitory *Streptomyces*, niche width, and niche overlap across foliar fungicide treatment (Figure 3, Figure 4). For *Streptomyces* densities and the proportion of *Streptomyces* that were inhibitory, the disruption of foliar fungal communities appeared to preferentially reduce the densities of non-inhibitory *Streptomyces*: leaving the total number of inhibitors unchanged, but substantially increasing their relative abundance (Figure 3). When looking at resource use, we saw contrasting effects across monocultures vs. polycultures. In monocultures, disruption of the foliar fungal community corresponded to a reduction in niche width and niche overlap (Figure 4), while in polycultures we observe the opposite. Together, these suggest a potential trade-off for *Streptomyces* from monocultures between being an inhibitor and being a generalist in nutrient use. Interestingly, this trade-off is not apparent in *Streptomyces* from polycultures.

Drivers of *Streptomyces* inhibition, resource-use, and resistance phenotypes beyond edaphics

If phenotypes were exclusively driven by soil resource levels, we would expect *Streptomyces* from polyculture plots in which the foliar fungal communities were disrupted to be more similar to monoculture plots than those without the fungicide treatment. For nutrient use and niche overlap, *Streptomyces* from plots with disrupted foliar fungal communities do tend to look like an average of the monoculture treatments, yet, we see no significant differences across polyculture plots with respect to inhibitory or resistance phenotypes. This could be because nutrient use phenotypes are more closely linked to nutrient availability than are more complex phenotypes of antibiotic production and resistance.

Rather than soil edaphics, the key driver to variation in *Streptomyces* inhibition, nutrient-use, and resistance in our experiment was the richness of the plant community. In particular, we found that *Streptomyces* clustered phylogenetically according to source plot plant richness and that monocultures consistently had higher rates (absolute and proportional) of inhibition, higher levels of pairwise niche-overlap (though this was nuanced by the effect of foliar fungicide application, as mentioned above), and less resistance to specific antibiotics than did *Streptomyces* from polycultures. **TODO: individual nutrients?**

This primary effect of plant richness is interesting and in agreement with results in the study of disease-suppressive soils, where sustained low-diversity plant communities dramatically impacts microbial interaction phenotypes and the functioning of soil communities. Suppressive soils develop in agricultural systems after long-term monoculture where high densities and/or frequencies of antagonistic microbes accumulate and limit plant disease development (typically species of *Streptomyces*, *Pseudomonas*, *Bacillus*, or *Trichoderma*; [@]). In contrast, introducing plant richness (temporally) with crop rotation disrupts the development of disease-suppressive soil communities [@]. Similarly, in experimental plant richness manipulations, long-term monocultures consistently contain higher frequencies of pathogen-antagonists than more diverse plant communities, which may feedback to contribute to plant fitness (Bakker et al. 2013; Essarioui, Kistler, and Kinkel 2016). Though the mechanisms of general pathogen suppression are largely unknown, both ecological and evolutionary

dynamics are likely to be crucial to the generation of disease-suppressive microbial communities.

Limitations

In this study, we considered several aspects of *Streptomyces* biology, including their ability to inhibit one another through the production of antibiotics, their ability to resist the antibiotics produced by their peers, and their nutrient-use, including its relationship with resource competition. While *Streptomyces* are abundant and ecologically important, they represent but a fraction of microbial diversity in soils, and the constraint of considering only culturable microbes is a clear limitation. Moreover, the question of appropriate spatial scale for microbial communities remains an area of active consideration [@], highlighting a potential question of whether our samples were within the range of influence from host plants that (for instance) underwent foliar fungicide treatment. The foliar fungicide application is applied strictly to the leaves of plants, with minimal soil contamination [@], yet it is possible that there are unintended consequences of this application for soil microbes. Finally, our polyculture plots contained sixteen species each, but the particular diversity around our focal plants was no prescribed. This likely contributed to the increased variance in soil resource measures among polyculture plots compared to monocultures.

Conclusion

As expected, *Streptomyces* phenotypes of inhibition, antibiotic resistance, and nutrient-use differed across our treatments. Yet, while we expected such differences would be driven by underlying changes in soil chemistry, our results show that most of the observed changes were unrelated to differences in soil resource levels. Instead, the predominant cause of phenotypic differences in our data was differing plant richness (in the absence of differences in soil edaphic properties).

Our treatment of foliar fungicide was intended to elucidate the connection between above- and below-ground microbial communities: disrupting the former to produce an effect on the latter. While we did see some effects of this treatment, they tended to be idiosyncratic, either compounding (as in the case of increasing the proportion of inhibitory *Streptomyces*) or negating (as in the case of making nutrient levels more akin to monocultures in polyculture plots) the effects of plant richness.

Grand Conclusion

Supplementary Information:

Table S1: Pearson's correlation R^2-values and p-values among soil edaphic characteristics and *Streptomyces* densities among monocultures only. Sign was preserved to indicate direction of relationship. Significant correlations are indicated by trailing *'s, with *** corresponding to a p-value < 0.001, ** to p-values < 0.01, and * to p-values < 0.05.

	C (%)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
log(Streptomyces)	0.36 *	0.53 **	-0.03	0.42 *	0.49 *	0.08
log(Inhibitors)	-0.01	-0.03	-0.10	0.00	-0.04	0.05
Mean Kill Zone (mm)	-0.01	-0.01	-0.18	-0.12	-0.03	0.04
Proportion Inhibitors	-0.27	-0.42 *	0.00	-0.32	-0.46 *	-0.06

Table S2: Pearson's correlation R^2-values and p-values among soil edaphic characteristics and *Streptomyces* densities among polycultures only. Sign was preserved to indicate direction of relationship. Significant correlations are indicated by trailing *'s, with *** corresponding to a p-value < 0.001, ** to p-values < 0.01, and * to p-values < 0.05.

	C (%)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
log(Streptomyces)	0.12	0.09	-0.32	0.01	0.07	0.00
log(Inhibitors)	-0.03	-0.05	-0.53 *	-0.03	-0.02	0.02
Mean Kill Zone (mm)	0.07	0.08	-0.40	-0.10	0.02	-0.01
Proportion Inhibitors	-0.25	-0.29	-0.17	-0.08	-0.20	0.04

Table S3: Pearson's correlations (R^2) among soil edaphic characteristics in monoculture plots. Significant correlations are indicated by trailing *'s, with *** corresponding to a p-value < 0.001, ** to p-values < 0.01, and * to p-values < 0.05.

	C (%)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
C (%)		0.72 ***	0.06	0.00	0.21 *	0.10
N (%)			0.06	0.00	0.16	0.00
P (ppm)				0.23 *	0.03	0.00
K (ppm)					0.01	0.01
Organic matter (%)						0.18 *

Table S4: Pearson's correlations (R^2) among soil edaphic characteristics in polyculture plots. Significant correlations are indicated by trailing *'s, with *** corresponding to a p-value < 0.001, ** to p-values < 0.01, and * to p-values < 0.05.

	C (%)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
C (%)		0.77 ***	0.01	0.16	0.31 **	0.10
N (%)			0.04	0.31 **	0.62 ***	0.22 *
P (ppm)				0.38 **	0.05	0.05
K (ppm)					0.49 ***	0.11
Organic matter (%)						0.27 **

Table S5: Mean (± standard deviation) resistance zone sizes of *Streptomyces* isolates from different plant richness and fungicide treatments (n = 18 - 20 isolates/treatment). Different letters following means indicate significant differences among treatments using Tukey's post-hoc test ($p < 0.05$).

Plant Richness	Fungicide	Amoxicillan	Chloramphenicol	Erythromycin	Kanamycin	Novobiocin	Rifampicin	Streptomycin	Tetracycline	Vancomycin
Monoculture	Yes	14.86 ± 7.2 a	8.05 ± 5.8 a	13.27 ± 4.6 a	17.75 ± 5.7 a	8.35 ± 2.8 a	2.93 ± 4.5 a	15.29 ± 4.4 a	2.14 ± 2.0 a	12.31 ± 2.5 a
	No	9.89 ± 5.7 ab	7.82 ± 4.5 a	13.30 ± 3.8 a	16.07 ± 6.7 a	6.54 ± 4.1 a	1.64 ± 2.0 a	15.50 ± 3.8 a	1.76 ± 1.8 a	10.69 ± 3.0 a
Polyculture	Yes	5.96 ± 4.9 b	5.31 ± 6.0 a	8.62 ± 5.6 b	17.28 ± 7.4 a	8.96 ± 3.7 a	2.90 ± 2.5 a	13.46 ± 4.2 a	3.35 ± 3.4 a	11.67 ± 2.9 a
	No	5.66 ± 5.6 b	4.65 ± 5.4 a	9.23 ± 6.2 ab	15.95 ± 6.2 a	6.94 ± 3.0 a	1.85 ± 2.8 a	14.94 ± 4.0 a	1.56 ± 3.4 a	9.99 ± 4.3 a

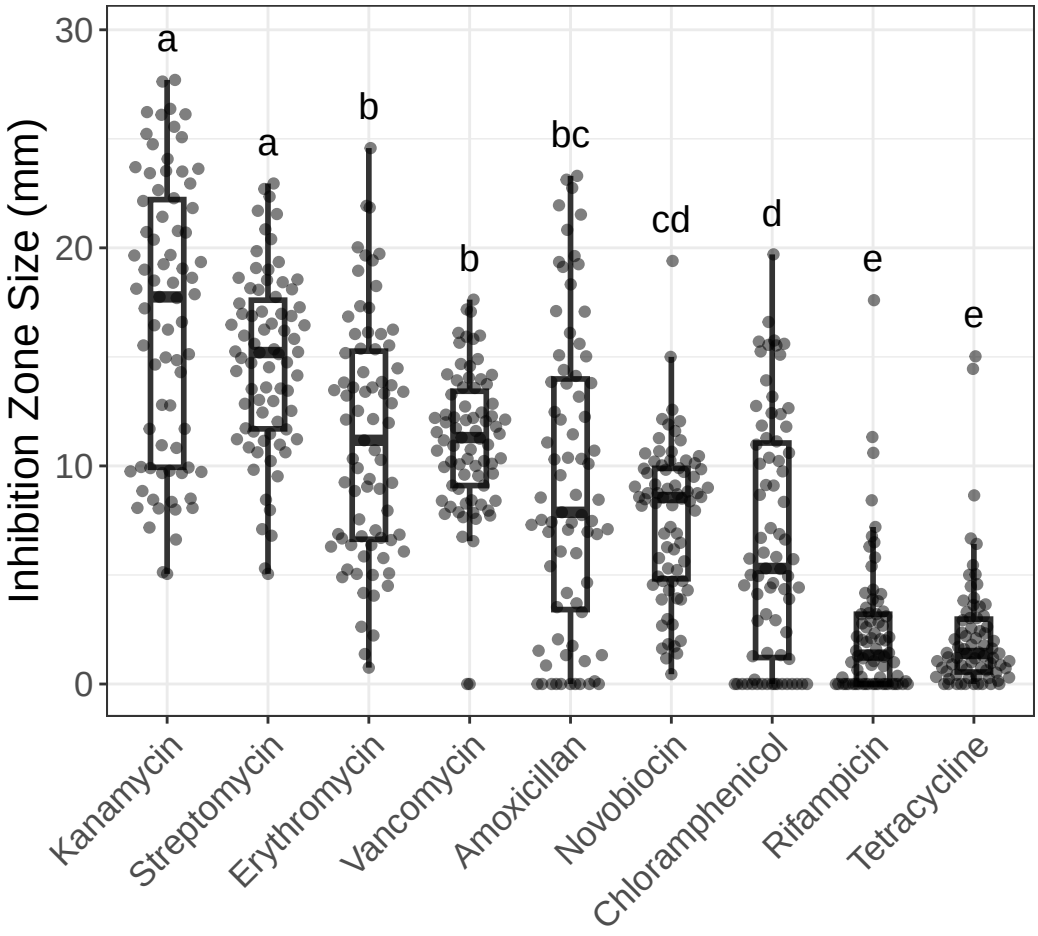

Figure S1: Individual *Streptomyces* isolate inhibition zone sizes (mm) when exposed to nine standard antibiotics. Each point is a single isolate-antibiotic combination. Overlaid boxes indicate 25th, 50th, and 75th percentiles, with whiskers extending no more than 1.5 times the interquartile range beyond the box. Letters above the data correspond to sgnicidant differences as identified by a Tukey's post-hoc test ($p < 0.05$).

Table S6: Mean (± standard deviation) soil edaphic characteristics among plant richness and fungicide treatments (n = 6 per treatment). Differing letters signify significant (p<0.05) differences according to a Tukey's Honestly Significant Difference post hoc test of an Analysis of Variance.

Plant Richness	Fungicide	C (%)	N (%)	P (ppm)	K (ppm)	Organic matter (%)	pH
Monoculture	No	0.51 ± 0.07 b	0.05 ± 0.005 b	32.33 ± 4.5 c	35.42 ± 11.7 c	1.36 ± 0.2 b	5.81 ± 0.1 c
	Yes	0.47 ± 0.03 b	0.04 ± 0.003 b	33.33 ± 5.2 bc	32.17 ± 8.0 c	1.18 ± 0.1 c	5.82 ± 0.2 c
Polyculture	No	0.84 ± 0.4 a	0.07 ± 0.02 a	40.17 ± 7.9 a	93.08 ± 26.2 a	1.62 ± 0.2 a	6.38 ± 0.1 a
	Yes	0.57 ± 0.1 b	0.05 ± 0.008 b	39.25 ± 5.4 ab	75.83 ± 8.0 b	1.30 ± 0.1 bc	6.12 ± 0.1 b

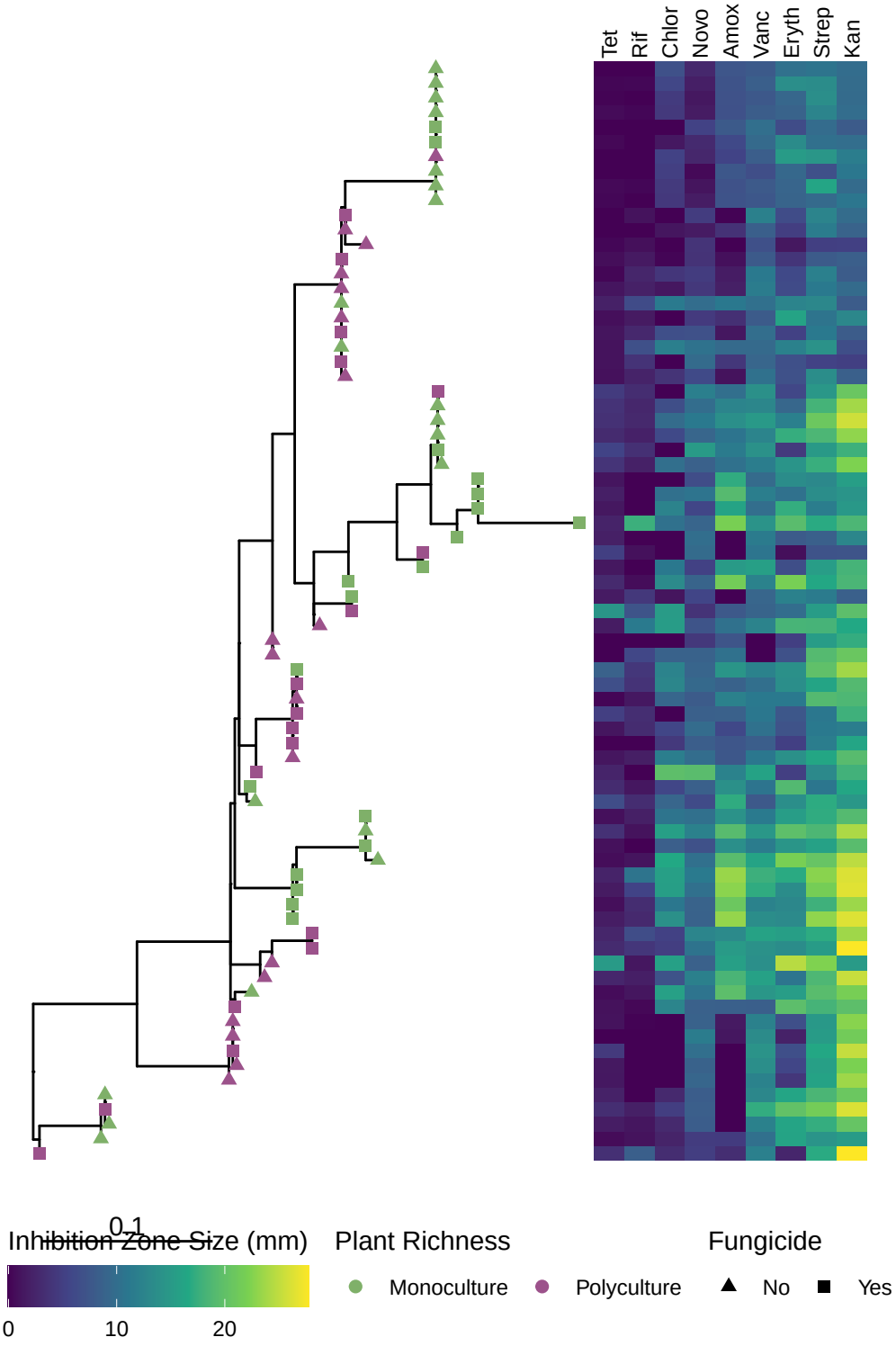

Figure S2: TBD

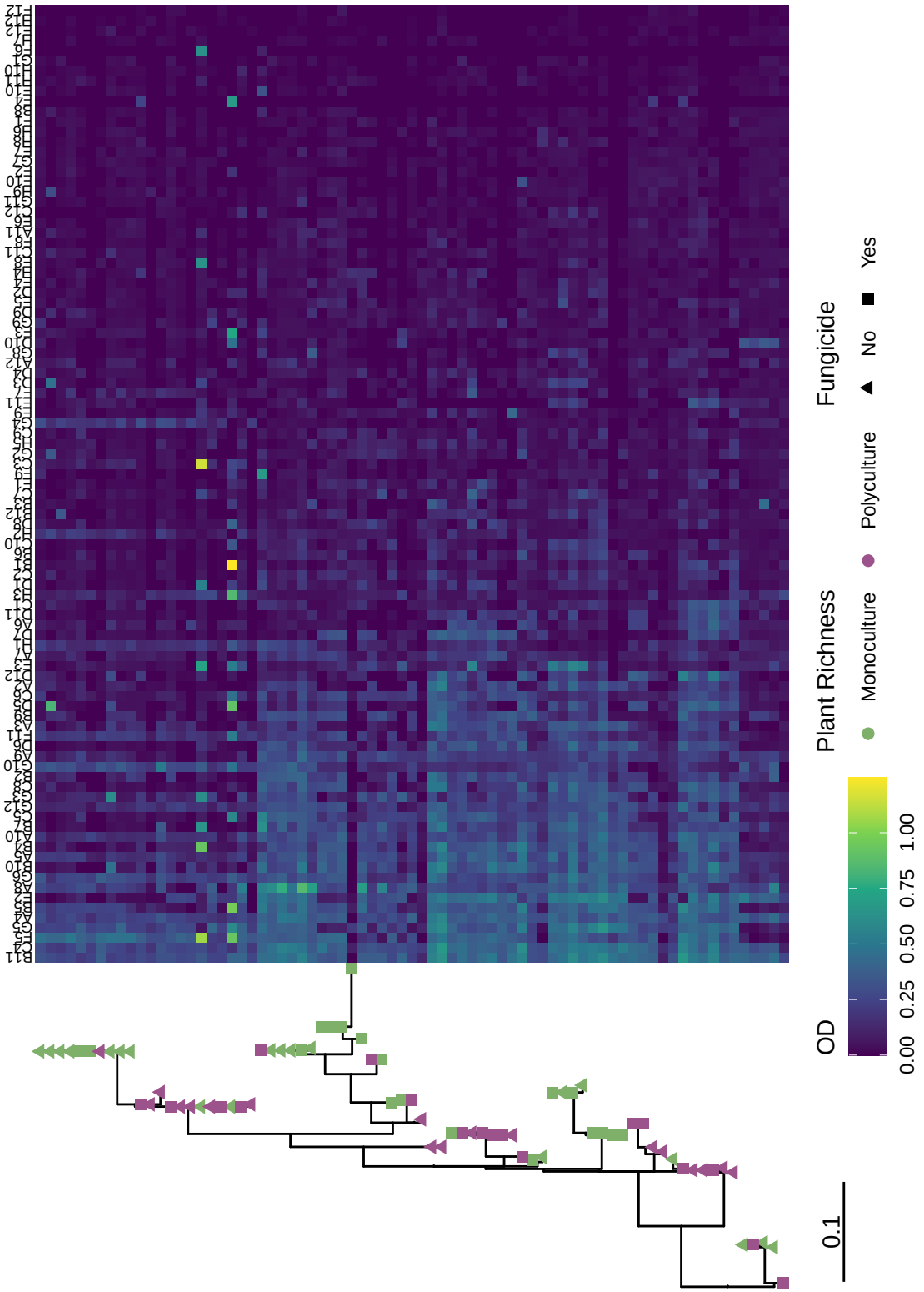

Figure S3: TBD

Table S7: Raw analysis of variance results for soil edaphic measures, considering the effect of plant richness and foliar fungicide (nested within the richness treatment).

	term	df	sumsq	meansq	statistic	p	
C (%)	plant richness	1	0.56	0.56	14.99	3.544e-04	***
	plant richness : fungicide	2	0.45	0.22	5.97	0.005076	**
	Residuals	44	1.64	0.04	NA	NA	
K (ppm)	plant richness	1	30805.33	30805.33	130.07	9.958e-15	***
	plant richness : fungicide	2	1848.75	924.37	3.90	0.02752	*
	Residuals	44	10421.17	236.84	NA	NA	
N (%)	plant richness	1	0.00	0.00	19.81	5.761e-05	***
	plant richness : fungicide	2	0.00	0.00	9.92	2.787e-04	***
	Residuals	44	0.00	0.00	NA	NA	
Organic matter (%)	plant richness	1	0.46	0.46	16.96	1.657e-04	***
	plant richness : fungicide	2	0.84	0.42	15.39	8.56e-06	***
	Residuals	44	1.19	0.03	NA	NA	
P (ppm)	plant richness	1	567.19	567.19	16.26	2.166e-04	***
	plant richness : fungicide	2	11.04	5.52	0.16	0.8541	
	Residuals	44	1535.25	34.89	NA	NA	
pH	plant richness	1	2.34	2.34	127.95	1.306e-14	***
	plant richness : fungicide	2	0.40	0.20	10.95	1.378e-04	***
	Residuals	44	0.81	0.02	NA	NA	

Table S8: Raw analysis of variance results for *Streptomyces* community inhibition phenotypes, considering the effect of plant richness and foliar fungicide (nested within the richness treatment).

Soil edaphic measure	term	df	sumsq	meansq	statistic	p	
Proportion Inhibitors	plant richness	1	0.17	0.17	155.82	6.75e-11	***
	plant richness : fungicide	2	0.04	0.02	16.88	5.08e-05	***
	Residuals	20	0.02	0.00	NA	NA	
log(Inhibitors)	plant richness	1	1.38	1.38	37.49	5.541e-06	***
	plant richness : fungicide	2	0.12	0.06	1.62	0.2219	
	Residuals	20	0.74	0.04	NA	NA	
log(Streptomyces)	plant richness	1	0.03	0.03	1.87	0.1872	
	plant richness : fungicide	2	0.18	0.09	5.43	0.01304	*
	Residuals	20	0.34	0.02	NA	NA	

Table S9: Raw analysis of variance results for *Streptomyces* nutrient-use metrics, considering the effect of plant richness and foliar fungicide (nested within the richness treatment).

Nutrient-use metric	term	df	sumsq	meansq	statistic	p	
Growth Efficiency	plant richness	1	0.00	0.00	0.04	0.8411	
	plant richness : fungicide	2	0.01	0.00	2.19	0.1194	
	Residuals	76	0.17	0.00	NA	NA	
Niche Overlap	plant richness	1	0.90	0.90	27.75	1.605e-07	***
	plant richness : fungicide	2	2.88	1.44	44.57	1.769e-19	***
	Residuals	1368	44.15	0.03	NA	NA	
Niche Width	plant richness	1	374.11	374.11	1.51	0.2237	
	plant richness : fungicide	2	5157.02	2578.51	10.37	1.038e-04	***
	Residuals	76	18889.35	248.54	NA	NA	

Table S10: Raw analysis of variance results for *Streptomyces* resistance to nine antibiotics, considering the effect of plant richness and foliar fungicide (nested within the richness treatment).

Antibiotic	term	df	sumsq	meansq	statistic	p	
Amoxicillan	plant richness	1	792.03	792.03	22.31	1.135e-05	***
	plant richness : fungicide	2	241.91	120.95	3.41	0.03863	*
	Residuals	71	2520.20	35.50	NA	NA	
Chloramphenicol	plant richness	1	163.22	163.22	5.52	0.02158	*
	plant richness : fungicide	2	4.47	2.24	0.08	0.9272	
	Residuals	71	2099.13	29.57	NA	NA	
Erythromycin	plant richness	1	356.67	356.67	13.76	4.085e-04	***
	plant richness : fungicide	2	3.34	1.67	0.06	0.9377	
	Residuals	71	1839.94	25.91	NA	NA	
Kanamycin	plant richness	1	1.41	1.41	0.03	0.8559	
	plant richness : fungicide	2	43.26	21.63	0.51	0.603	
	Residuals	71	3013.84	42.45	NA	NA	
Novobiocin	plant richness	1	5.26	5.26	0.45	0.5057	
	plant richness : fungicide	2	68.86	34.43	2.93	0.06	
	Residuals	71	834.93	11.76	NA	NA	
Rifampicin	plant richness	1	0.21	0.21	0.02	0.8809	
	plant richness : fungicide	2	26.08	13.04	1.37	0.2598	
	Residuals	71	673.81	9.49	NA	NA	
Streptomycin	plant richness	1	26.87	26.87	1.61	0.2081	
	plant richness : fungicide	2	19.90	9.95	0.60	0.5528	
	Residuals	71	1182.05	16.65	NA	NA	
Tetracycline	plant richness	1	4.83	4.83	0.65	0.4221	
	plant richness : fungicide	2	30.08	15.04	2.03	0.1389	
	Residuals	71	526.04	7.41	NA	NA	
Vancomycin	plant richness	1	7.95	7.95	0.77	0.384	
	plant richness : fungicide	2	50.84	25.42	2.45	0.09321	
	Residuals	71	735.43	10.36	NA	NA	

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