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# Effects of Carbon Quantity and Plant Diversity on Pathogen Suppression by Soil-borne Streptomyces

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**Abstract**

Members of the genus *Streptomyces* are some of the best candidates for biological control of soil-borne pathogenic bacteria. Antibiotic production by *Streptomyces* is thought to be crucial for this strain's ability to compete with other closely related soil microorganisms. Furthermore, *Streptomyces* is able to not only inhibit the growth of its competitors, but also that of many soil-borne plant pathogens. My thesis assessed *Streptomyces* performance under varying levels of soil resources (carbon componds that serve as energy sources) and in plots with varying degrees of plant diversity that, in turn, affects soil resources. This project aims to deconvolute the confounding effects of these environmental variables on plant pathogen suppression by *Streptomyces*. I characterized soils from low-diversity plant communities (monoculture) and high-diversity plant communities (polyculture (16-species)), while providing fungicide treatment to the leaves of the plant, which has been found to increase plant productivity, to half of the plots. I found that *Streptomyces* associated with plant monoculture versus plant polyculture is more likely to be subject to resource competition. In contrast, *Streptomyces* communities in polyculture are much more complex than those associated with plant monoculture. Therefore, in monoculture agricultural system, *Streptomyces* strains are likely to help suppress plant disease via antibiotics production, and attempts to control soil-borne plant diseases via antibiotics-producing *Streptomyces* strains should work well in plant monoculture (but perhaps not in plant polyculture). My findings also suggest that the inhibitory phenotypes of *Streptomyces* (that differ genetically from non-inhibitory strains, as indicated by our 16S sequencing data) are induced by low soil carbon quantity. Finally, *Streptomyces* responds to low plant diversity and low carbon levels in the soil by not only producing more antibiotics but also increasing their own resistance to antibiotics.

## Introduction

Soil contains diverse microorganisms that are crucial to plant and soil health in natural and agricultural systems. Soil microorganisms with multiple functions, including pathogens, pathogen antagonists, symbionts (organisms in a symbiotic relationship), and saprophytic microorganisms (living on dead or decaying matter), affect plant health (Schlatter et al., 2009). One important function of soil microbes is the suppression of soil-borne plant diseases. Currently, approaches to using soil microbes to inhibit plant pathogens have focused on developing biological controls agents for inoculation. Biological control is more favorable than pesticides both economically and environmentally because soil-borne microorganisms are able to selectively suppress soil-borne plant diseases (Gilbert et al., 1993, Howart, 1991). To date, the best candidates for biological control of soil-borne pathogenic bacteria are from the genera *Streptomyces* and *Bacillus* (Ezziyyani et al., 2007, Korsten et al, 1995, Crawford et al., 1993).

*Streptomyces* are filamentous, Gram-positive bacteria that are important components of soil communities as saprophytes and producers of a wide variety of antibiotics (Schlatter et al., 2009). *Streptomyces* have been used as biological control agents to suppress potato scab (Huber and Schneider, 1982). The mechanism of pathogen suppression may involve antibiotic production by *Streptomyces* since *Streptomyces* can produce a variety of antibiotics (Emmert et al., 1999). As is the case for *Bacillus*, antibiotics from *Streptomyces* prevent the growth of soil-borne pathogens, which results in disease suppression (Watve et al., 2001). Moreover, *Streptomyces* communities vary widely in antibiotic production, which affects their potential to suppress plant disease.

Antibiotic production is also believed to be crucial for *Streptomyces* to compete with other closely related microorganisms in soil (Williams et al., 1986). As a result of antibiotic-

mediated interactions, *Streptomyces* are able to inhibit the growth of not only their own competitors, but also many soil-borne plant pathogens (Kinkel et al., 2012). In agricultural systems, both plant and soil health may be improved by soil-borne-disease suppression via antibiotic production (Janvier et al., 2007). However, soil *Streptomyces* communities vary widely in densities, frequencies, and intensities of antibiotic production (Watve et al., 2001) and thus in their potential to suppress plant diseases. More studies are needed to explore the factors that select for soil *Streptomyces* communities that are efficient antagonists of plant pathogens.

Resource competition and coevolution are thought to be crucial to selection for antibiotic phenotypes of soil-borne *Streptomyces* (Samac and Kinkel, 2001). *Streptomyces* suppression of plant pathogenic bacteria and fungi was shown in an agricultural system to occur in the context of resource competition (Samac and Kinkel, 2001). In previous work, *Streptomyces* were shown to be locally adapted to produce antibiotics that effectively inhibit coexisting strains competing for the same resources (Kinkel et al., 2014). Moreover, frequencies of inhibitory interactions can vary widely among communities from different locations, suggesting that there may be alternatives to antibiotic-mediated resource competition, which led to a conceptual coevolutionary model for the development of highly antagonistic disease-suppressive *Streptomyces* communities in soil (Kinkel et al., 2011).

*Streptomyces* species are thus apparently involved in a coevolutionary antibiotic arms race that should favor antagonistic (antibiotics-producing) isolates of *Streptomyces* to prevent resource competition. To avoid resources competition, *Streptomyces* may also undergo coevolutionary niche differentiation, where strain 1 specializes on food A and strain 2 specializes on food B, which would reduce niche overlap and make costly antibiotic production unnecessary (i.e., reduce selection for antibiotics-producing *Streptomyces* phenotypes).

Antagonistic coevolution (based on antibiotics production) has been proposed to depend on resource quantity and resource diversity (Kinkel et al., 2014). *Streptomyces* exposed to greater resource levels are expected to rapidly undergo coevolution based on niche differentiation due to greater population densities and encounter rates. Conversely, in environments with a low diversity of resources, *Streptomyces* are likely to undergo antagonistic coevolution (if only a few food types are available, antibiotic production is critical to making sure that an isolate gets the food). Moreover, antibiotic-inhibitory and resource-use phenotypes of *Streptomyces* can be changed with specific carbon input (soil organic matters) into *Streptomyces* communities (Schlatter et al., 2009).

In natural ecosystems, plant communities play a pivotal role as nutrient sources for soil communities. Specifically, plant community characteristics (species diversity, productivity) are likely to impact the quantity and diversity of resources supplied to soil (Hooper et al., 1998). As a result, plant communities may serve as the context in which coevolution among *Streptomyces* strains occurs. *Streptomyces* are found to exhibit distinct coevolution patterns (antagonistic coevolution versus niche differentiation) depending on the plant communities present, with *Streptomyces* communities in plant monocultures favoring an antagonistic trajectory (Kinkel et al., 2014). In contrast, in diverse plant communities (polycultures), *Streptomyces* are likely to undergo a niche differentiation trajectory (Bakker et al., 2013). Based on the latter studies, I hypothesized that plant monoculture would produce low resource diversity in the soil, and that diverse plant communities would favor a wider variety of resource types. Although these latter studies are compelling, a limitation from Bakker's work is that plant diversity (monoculture versus polyculture) and resource amounts (high and low quantities of soil carbon) are highly

inter-correlated, thus confounding resource quantity and plant diversity. My thesis aims to deconvolute these factors.

In order to differentiate the effects of resource quantity from those of resource diversity, I characterized soils from low-diversity plant communities (monoculture) and high-diversity plant communities (16-species), while providing fungicide treatment to the leaves of the plant, which has been found to increase plant productivity, to half of the plots (unpublished data) . This plan results in a design (see Table 1), where plant monocultures with low or high soil resource quantities are compared with diverse plant communities (polycultures) with low or high soil resource quantities, with each of these treatments done with or without foliar fungicide treatment. Because I expect *Streptomyces* to follow different coevolutionary trajectories in plant monocultures (antibiotics-based arms race coevolution) compared to diverse plant communities (coevolutionary niche differentiation), I predicted that higher resource quantities should enhance the rate of coevolution via niche differentiation.

Table 1: Experimental Outline

| **Experimental outline** | **Plant monoculture** | **Plant Polyculture,16-Species** |
|---|---|---|
| **Fungicide** | **Low plant diversity**<br>**High resource quantity** | **High plant diversity**<br>**High resource quantity** |
| **No Fungicide** | **Low plant diversity**<br>**Low resource quantity** | **High plant diversity**<br>**Low resource quantity** |

Using these treatments (Table 1), I tested the following hypotheses:

1) *Streptomyces* communities in plant monocultures harbor more inhibitory *Streptomyces* than in plant polyculture.

2) Carbon quantity in plant polyculture is greater than in plant monoculture.

3) High carbon quantity in the soil is negatively correlated with inhibitory intensity in *Streptomyces* communities.

4) *Streptomyces* communities in plant monocultures have a greater niche overlap (more similar resources used) than in polyculture, which will be provided with the comparison of resources used characterization of each isolate.

5) *Streptomyces* community composition differs as a result of the level of plant diversity. Furthermore, we explored the role of plant communities in determining the antibiotics-resistance phenotypes of *Streptomyces* communities.

## Materials and Methods

### Assessing inhibitor densities (number of *Streptomyces* that inhibit pathogens), and inhibition intensity (proportion of inhibitors *Streptomyces* to total *Streptomyces*) of *Streptomyces* communities.

Soil samples were collected from the base of 6 individual plants (*Andropogon gerardii*, Big Bluestem) from each treatment within a long-term plant diversity experiment (experiment E120) at the University of Minnesota Cedar Creek Ecosystem Science Reserve. Treatments were: 1) plant monoculture with fungicide treatment, 2) plant monoculture without fungicide treatment, 3) plant polyculture (16 plant species) with fungicide treatment, and 4) plant polyculture (16 species) without fungicide treatment. From these four treatments, a total of 24 samples, with 6 replicates from each treatment, were collected. Approximately 5 g of each soil sample were covered by sterile cheesecloth dried overnight. Dry soil samples were finely ground and 5 g of dry soil were added to 25 milliliters of sterile deionized (DI) water and placed on orbital shaker at 175 rpm at 4$^{o}$C for 60 minutes.

Soil mixtures were serially diluted in sterile DI water. 100 µl of diluted soil suspensions were spread onto 1% water agar (WA) (5 g of agar in 500 ml $H_2O$). Plates were air dried for about 5 minutes or until mixtures were absorbed. Five ml of 1% starch casein agar (SCA) was pipetted to cover the entire plate. SCA was cooled to the touch to prevent media from killing the microorganisms. SCA was used as semi-selective medium for *Streptomyces* in this procedure. When plates had solidified, they were incubated for 3 days at 28$^{o}$C. After 3 days of incubation, *Streptomyces* density was evaluated by counting the number of colonies on the plates. Then, 10 ml of 1% starch casein agar (SCA) was pipetted to cover the entire plate (all colonies). 150 µl of spore suspension of an indicator strain (*Streptomyces* strain 1324.2, or *Streptomyces scabies*

DL87 (Potato scabies pathogen)) was spread on the plates to indicate pathogen suppression of *Streptomyces*. When the plates had solidified, they were incubated for 3 days at 28$^{o}$C. After 3 days, the number of inhibitory colonies was counted and inhibition zone sizes (growth of pathogen inhibited by *Streptomyces* appeared as the inhibition zone [no pathogen growth zone] were measured.

**Isolation and purification of *Streptomyces* strains.**

Isolations of *Streptomyces* were done on Starch Casein Agar (SCA) plates. For each soil sample mixture from above, 100 µl of diluted soil mixture was spread onto SCA. *Streptomyces* colonies were isolated by dividing each plate into a grid and selecting colonies from a random grid cell. For each soil sample, 5 *Streptomyces* colonies per plate were selected from 3 replicate plates. In total, 180 *Streptomyces* were isolated from all four treatments combined (forty-five *Streptomyces* strains per treatment). Then, *Streptomyces* isolates were purified on Oatmeal Agar (OA) plates. After purification, each *Streptomyces* isolate was grown on another OA plate to produce a spore lawn. Spore suspensions for each isolate were kept in 4 ml of 20% glycerol and stored at -20°C.

**Characterizing resource use of *Streptomyces* strains**.

A subset of 80 *Streptomyces* isolates (20 isolates/treatment) was tested for their ability to utilize 95 different carbon sources, such as glycogen, fructose, lactose etc.using Biolog SF-P2 plates (Biolog Inc., Heyward CA). Turbidity (optical density as absorbance at a wavelength of 590 nm) was used to follow growth of isolates relative to a water control. To prepare inoculations for Biolog SF-P2 plates, 100 µl of each isolates spore stock were plated on OA plates and incubated at 28°C for 7 days. After 7 days, fresh spores were suspended in 4 ml of

0.2% carragenan in a snap-cap tube using a cotton applicator. After thorough mixing, half of the suspension was transferred to another snap-cap tube for measuring optical density. The first snap-cab tube was used to adjust optical density to 0.20-0.24 at 590 nm. When the exact dilution was obtained from the first tube, the second snap cap tube was diluted the same as the first tube under sterile conditions. Then, 1.5 ml of spore suspension was added to 13.5 ml of 0.2% carragenan. The spore suspension was then mixed thoroughly and 100 µl of the spore suspension was pipetted into Biolog SF-P2 plates that were then incubated at 28$^{o}$C. After 1 day and 3 days of incubation, respectively plates were measured for optical density using a BioTech SynergyH1 microplate reader. To standardize absorbance in each plate, absorbance of the water-control well on each plate was subtracted from that of each well. Niche width (number of resources on SF-P2 plate that were used) for each *Streptomyces* isolates were evaluated for the number of resource used. Niche overlap (number of resources used by two *Streptomyces* strains) was calculated for each pair of *Streptomyces* isolates using the following formula.

$$\text{Niche overlap} = \frac{\left(\frac{\text{Nshare}}{\text{A}}\right) + \left(\frac{\text{Nshare}}{\text{B}}\right)}{2}$$

Nshare is the sum of minimum absorbance values of each nutrient as the measurement of shared growth across all 95 nutrients. A and B are the total growth of each isolate.

**16S rRNA gene sequencing**

16S rRNA gene sequences were obtained for a subset of 80 *Streptomyces* isolates. To extract DNA, 3 ml of Yeast Dextrose (YD) broth was inoculated with *Streptomyces* spores using a sterile toothpick. Then, *Streptomyces* cultures were incubated by setting up the tubes at a 45 degree angle in orbital shaker at 175 rpm and 28°C for 3 days. After 3 days of incubation, 600 µl of culture from each isolate was used for DNA extraction.

For each *Streptomyces* isolate, 600 µl of the culture was added to 1.5 ml microcentrifuge tubes. These tubes were centrifuged at 12000 rpm for 1 minute to form pellets of cells. The supernatant was removed by using a micro-pipette. 600 µl of distilled water was added to the pellet of cells and mixed thoroughly. Then, tubes were centrifuged at 12000 rpm for 1 minute and the supernatant was removed using a micro-pipette. 240 µl of 50 mM EDTA was added to resuspend the cells. 60 µl of 10 mg/ml lysozyme was added and mixed with the cells. These cells were incubated at 37°C overnight. After incubation, 600 µl of Nuclei Lysis Solution was added and gently mixed. Cells were incubated at 70°C for 20 minutes and cooled down at room temperature. After incubation, 200 µl of Protein Precipitation Solution was added and vortexed at high speed for 20 seconds. These samples were incubated in ice for 5 minutes and centrifuged at 12000 rpm for 3 minutes. 600 µl of the supernatant was transferred to clean 1.5 ml tubes with 600 µl of isopropanol. Tubes were gently mixed until a visible mass of DNA was obtained, after which tubes were centrifuged at 7500 rpm for 30 seconds. Then, tubes were drained and 600 µl of 70% ethanol was added to wash the pellet. These tubes were centrifuged at 7500 rpm for 30 seconds and drained with an air-dry procedure for 10-15 minutes. To assess DNA concentration, 100 µl of TE Buffer was added to the tube that were then incubated at 65 °C for 1 hour. DNA concentrations were determined by measuring absorbance at 260 nm and 280 nm.

For the Polymerase Chain Reaction (PCR) protocol, *Streptomyces* DNA concentration was adjusted to 25 ng/µl. PCR primers were diluted to be 10 µM. For each isolate, 25 µl of reaction solution containing 12.5 µl of Master Mix, 0.75 µl of Forward Primer, 0.75 of Reverse Primer, 1.0 µl of *Streptomyces* DNA, and 10.0 µl of $H_2O$ (PCR grade) was prepared. This reaction solution was mixed thoroughly using a pipette. PCR conditions were as described in table 2.

| **Step** | **Cycles** | **Target Temperature** | **Time** |
|---|---|---|---|
| Initial Denaturation | 1 | 95°C | 4 min |
| Denaturation | 30 | 95°C | 30s |
| Annealing | | 50°C | 30s |
| Elongation | | 72°C | 1.5 min |
| Final Extension | 1 | 72°C | 7 min |

Table 2: PCR temperature profile

The PCR was run for 30 cycles to obtain enough PCR products. For each isolate, amplified products were purified using the Qiaquick PCR cleanup kit (Qiagen, Valencia CA) and checked by gel electrophoresis. The gel contained 1% agarose and 2 µl of SYBR. The power supply was set at 70 Volt and 400 mA for 50 minutes. 2 µl of PCR DNA extracted was loaded with 2 µl of dye. To compare DNA size, 5 µl of DNA was loaded at the first well of the gel. After confirmation of DNA purification, DNA of *Streptomyces* isolates was submitted for sequencing to ACGT, inc. using the forward primer.

**Characterizing antibiotic resistance**

Antibiotic resistance of 80 *Streptomyces* isolates was examined on the antibiotics, amoxicillin, chloramphenicol, erythromycin, vancomycin, rifampin, novobiocin, tetracycline, streptomycin, and kanamycin, which will allow assessment of the correlations between antibiotic resistant and pathogen suppression characteristic of *Streptomyces*. For each *Streptomyces* isolate,

100 µl of spore collection was spread onto 3 SCA plates and allowed to dry. On each plate 3, different antibiotics were tested by placing the antibiotic disks onto the dried spore suspensions. Plates were incubated for 3 days and the inhibition zones were measured for each antibiotic disk against each isolate.

## Results

*Assessing densities and inhibition intensity of Streptomyces communities.* Competitive phenotypes of *Streptomyces* in soil were evaluated from four different treatments – plant monoculture with fungicide, plant monoculture without fungicide, plant polyculture (16 species) with fungicide, and plant polyculture (16 species) without fungicide. Proportions of inhibitory *Streptomyces* from each treatment were assessed by using Herr's assay. Herr's assay provides an indication of pathogen suppression characteristics of *Streptomyces*. The pathogen spread on top of *Streptomyces* will show a clear inhibition zone (no pathogen growth) if *Streptomyces* can suppress pathogen growth. The bigger the inhibition zone is, the stronger the pathogen suppression (Figure 1).

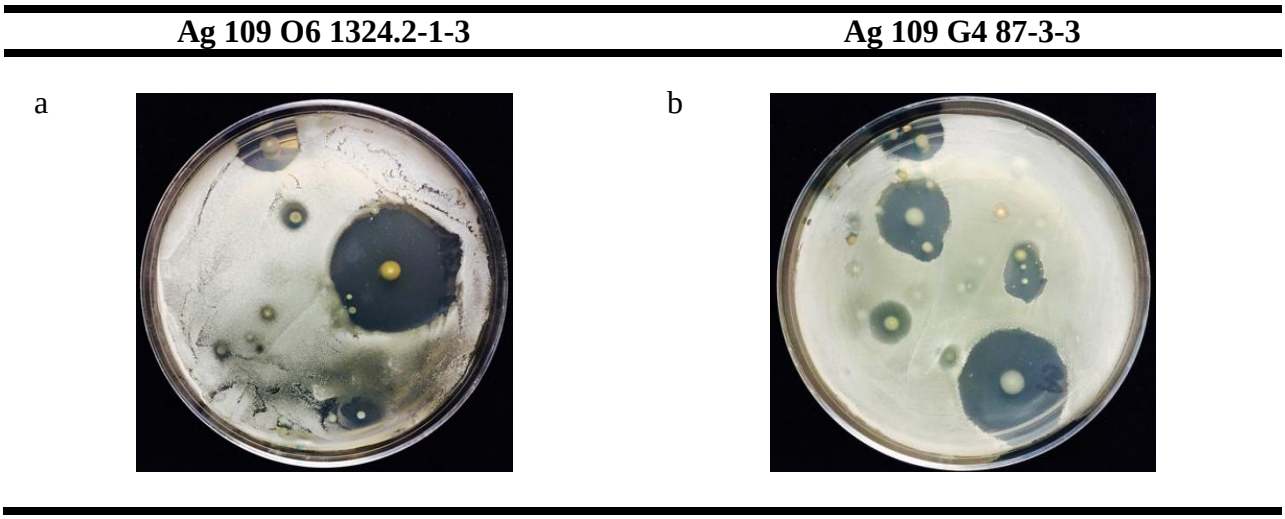



**Figure 1.** Examples of inhibition of soil-borne plant pathogen (*Streptomyces scabies*) by *Streptomyces* colonies using Herr's assay.

The proportion of (antibiotics-producing) inhibitors in *Streptomycin* communities was significantly influenced by plant community richness. Specifically, proportions of inhibitory *Streptomyces* strains in plant monocultures (either treated with fungicide or untreated) were significantly greater than those in plant polycultures ($p<0.001$) (Fig 2a). Within each plant diversity treatment, comparison between plots treated with fungicide and non-treated plots

indicates that *Streptomyces* in fungicide-treated monoculture plots include a greater proportion of inhibitor *Streptomyces* than plots not treated with fungicide (p=0.001; Fig 2b). In contrast, inhibitory proportions of *Streptomyces* in plant polyculture did not differ significantly as a function of fungicide treatments (Fig 2c). From these results, *Streptomyces* proportion of inhibitors is influenced mostly by plant diversity and partly by carbon quantity. Soil carbon quantity (fungicide treatment increases plant productivity which further expands carbon deposition) affects inhibitory proportion of *Streptomyces* only in monoculture plots but does not have significant effects in polyculture plots.

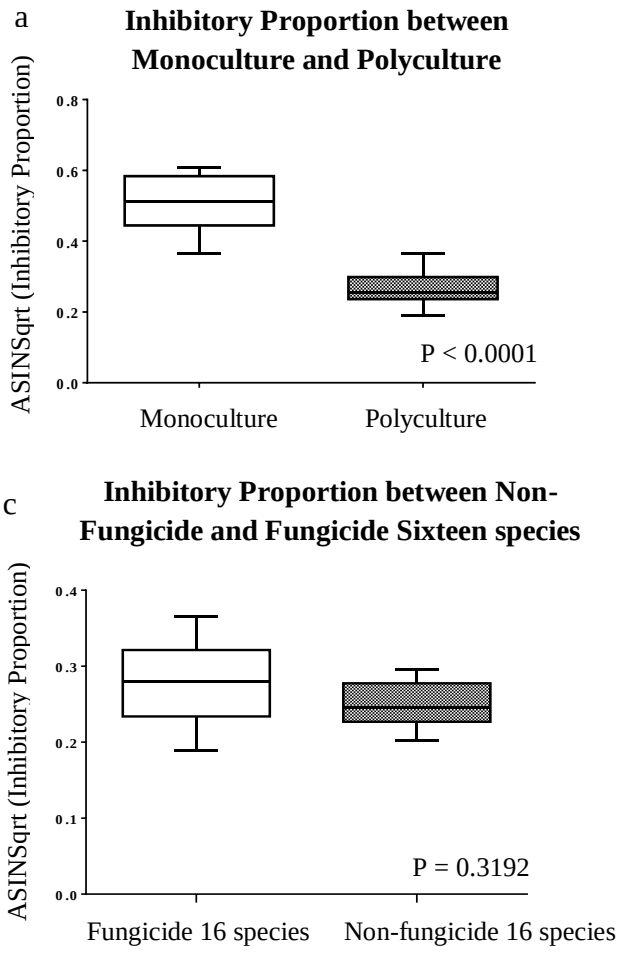



**Figure 2** Inhibitory proportion for *Streptomyces* communities from four sites with different plant richness (monoculture or polyculture) and fungicide treated or non-treated plots. a. Inhibitory proportion in monoculture is greater than in polyculture ($p<0.001$). b. Inhibitory proportion in monoculture with fungicide treatment is higher than non-fungicide treatment ($p=0.001$). c. Inhibitory proportion between fungicide and non-fungicide treatment are not significantly different ($p=0.3192$).

*Isolation and purification of Streptomyces strains.* Isolations of *Streptomyces* communities are represented by the total of 180 *Streptomyces* isolates (Fig 3).

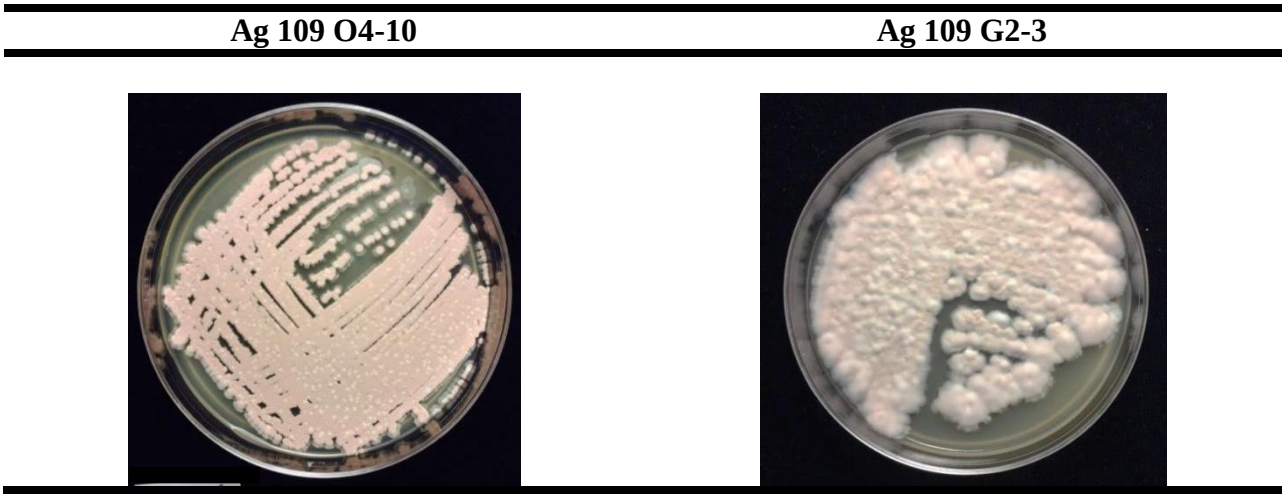



**Figure 3** Isolation and purification of *Streptomyces* isolates on Oatmeal Agar (OA) plates.

*Characterizing resource use of Streptomyces strains.* A subset of 80 *Streptomyces* isolates was tested on 95 different carbon sources. After one day of inoculation, *Streptomyces* communities in plant monoculture had a greater average niche overlap (number of resources which two *Streptomyces* used identically was calculated for each pair of *Streptomyces* isolates using the Niche overlap formula) than those from plant polyculture. *Streptomyces* from plant monocultures not treated with fungicide had significantly greater niche overlap than those from fungicide-treated monocultures after 1 day. In contrast, for the case of plant polycultures, *Streptomyces* from fungicide-treated plots had significantly higher niche overlap than those from plots not treated with fungicide (Fig 4a), which might reflect the effect of plant diversity. After 3 days of inoculation, there was no significant difference in niche overlap among *Streptomyces* from plant monocultures irrespective of fungicide treatment. However, niche overlap among *Streptomyces* from fungicide-treated polyculture remained significantly greater that among *Streptomyces* from plots not treated with fungicide (Fig 4b).

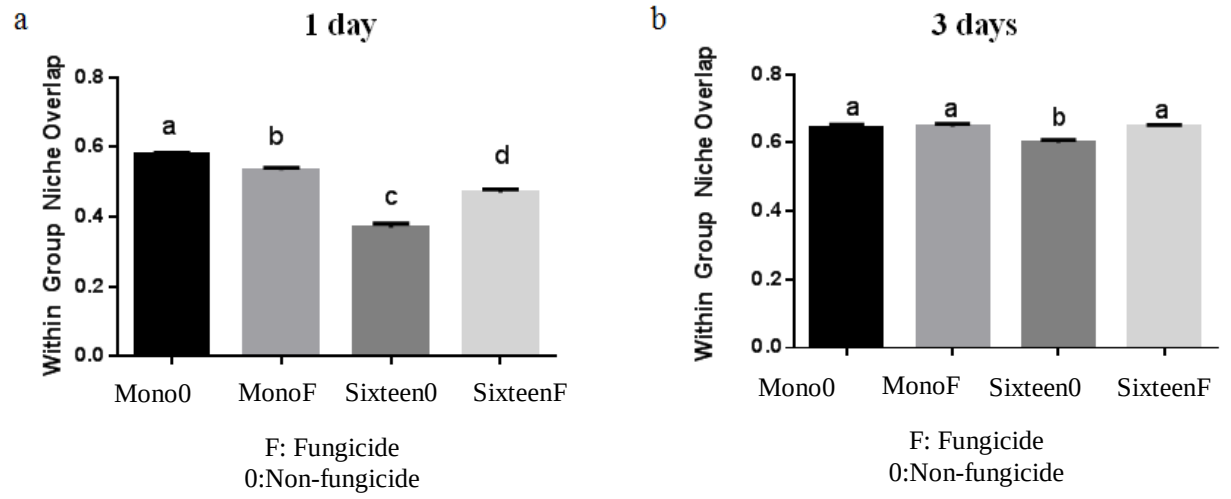



**Figure 4** Niche overlap among *Streptomyces* isolates from each treatment. a. Within group niche overlap after 1 day inoculation. b. Within group niche overlap after 3 days ioculation.

The response of *Streptomyces* niche width to fungicide treatment was affected by plant diversity (monocultures versus polyculture). Average niche width of *Streptomyces* from monocultures were significantly smaller than those from polycultures both after 1 day and 3 days of growth. *Streptomyces* from untreated monocultures had significantly greater niche widths than those from fungicide treated monocultures. In contrast, *Streptomyces* from fungicide-treated plant polycultures had higher niche widths than those from polycultures not treated with fungicide (Fig 5).

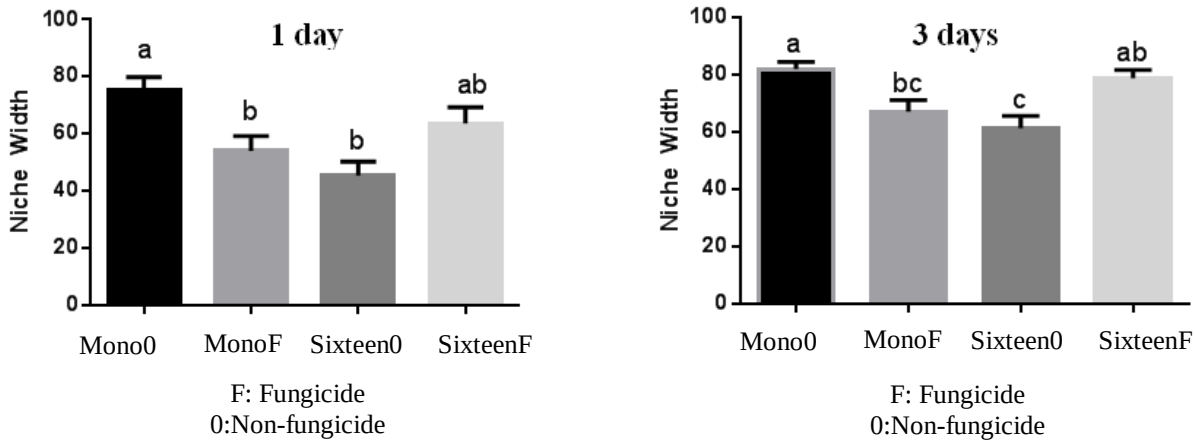



**Figure 5** Average niche widths of *Streptomyces* isolates from each treatment after 1 day and 3 days growth

*Soil Analysis.* Soil samples were submitted to the University of Minnesota Soil Research and Analytical lab for determination of soil organic matter, pH, P, and K. Carbon quantity in soil samples was determined by measuring the percentage of organic matter. Carbon quantity between fungicide-treated monoculture and monoculture not treated with fungicide was not significantly different (Fig 6a). In contrast, carbon quantity in plant polyculture not treated with fungicide was significantly greater than in fungicide-treated polycultures (Fig 6a). Moreover, in plant monoculture, soil carbon quantity was significantly negatively correlated with the proportion of inhibitors in *Streptomyces* communities ($p=0.01$). There was no significant correlation between carbon quantity and the proportion of inhibitory *Streptomyces* among plant polycultures (Fig 6b).

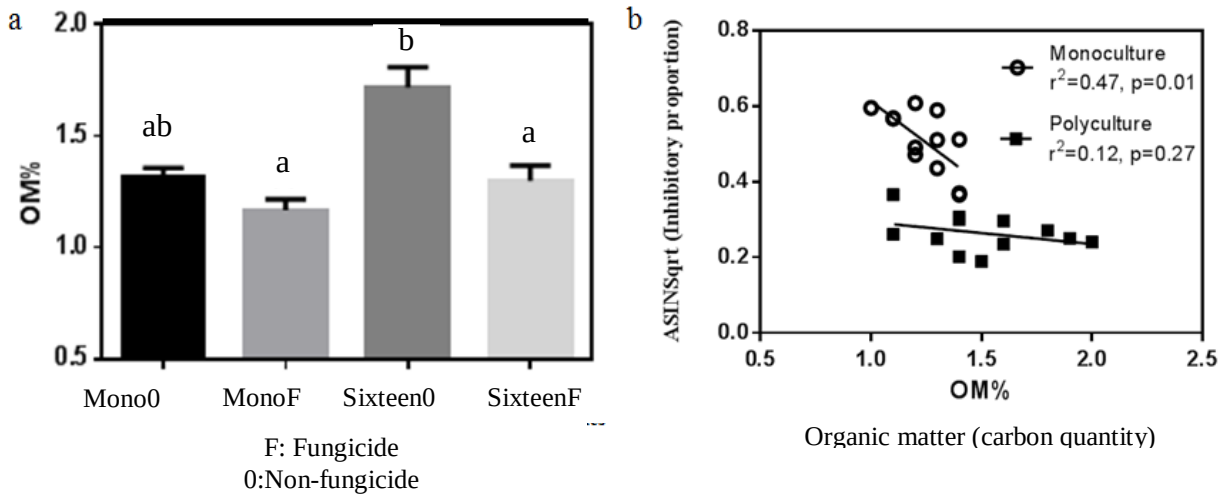
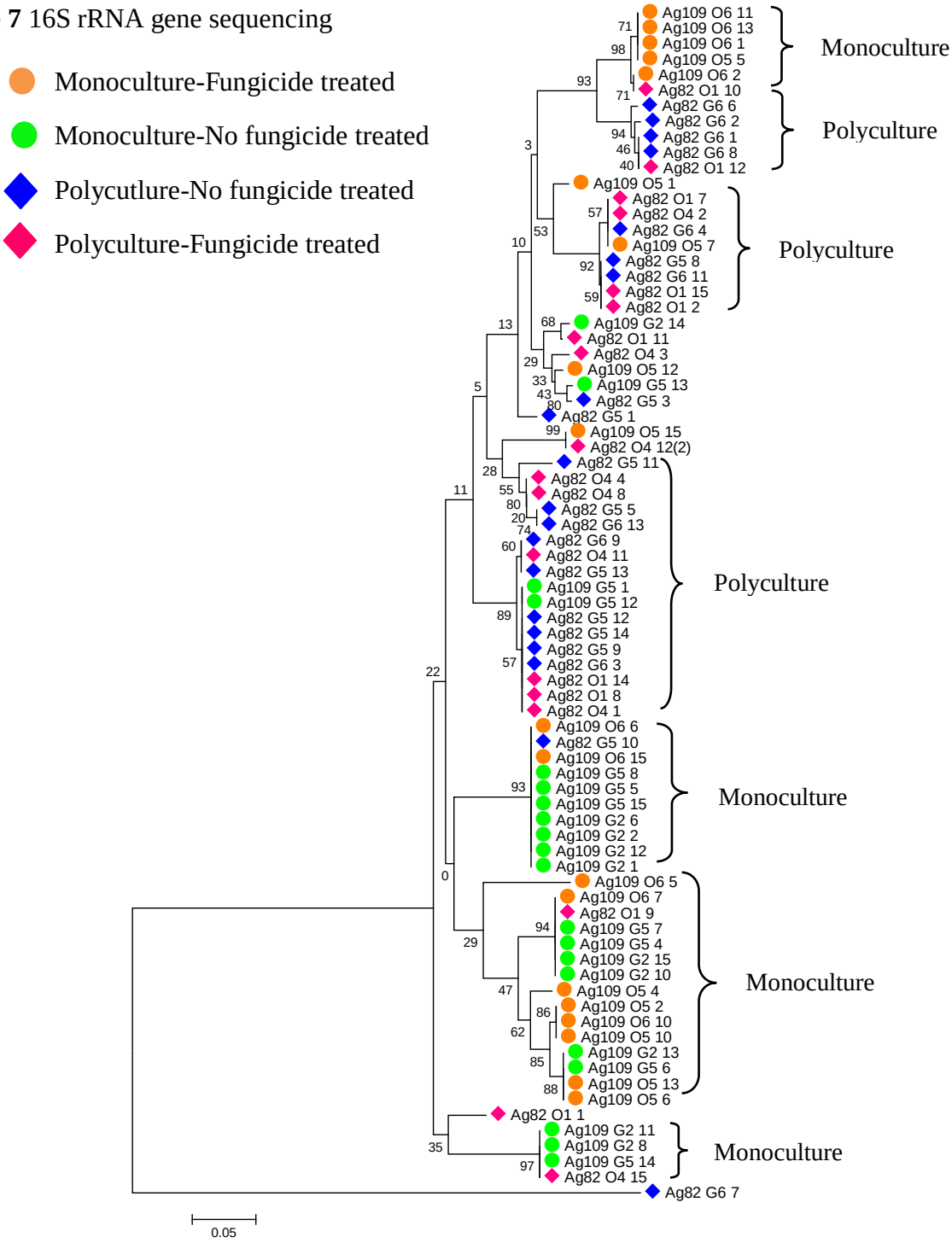



**Figure 6** Soil analyses from all sites. a. Percentage of organic matter (OM) as a measure of average carbon quantity in each plot. Significant differences are indicated by different letters above the bars. b. Correlation between carbon quantity and inhibitory intensity has been presented by the trend line in both monoculture and polyculture.

*16S rRNA gene sequencing.* A subset of 80 *Streptomyces* isolates were randomly selected to be sequenced. When using the sequences to construct a phylogenetic tree, *Streptomyces* isolates differed by plant diversity rather than by fungicide treatment (resource quantity) (Fig 7). As a result, plant richness affects the composition of *Streptomyces* communities more than resource quantity does.

**Figure 7** 16S rRNA gene sequencing



*Antibiotic Resistance*. A subset of 80 *Streptomyces* isolates were tested for resistance to nine different antibiotics in order to find correlations between antibiotic resistant and inhibitory proportion of *Streptomyces*. This was done to determine whether inhibitory *Streptomyces,* that can suppress plant pathogens, are susceptible to their own antibiotics. Inhibitory proportions (the number of *Streptomyces* inhibitors over the total number of *Streptomyces*) of *Streptomyces* isolates were significantly positively correlated with antibiotic resistance to amoxicillin and erythromycin. For chloramphenicol and vancomycin, a positive, albeit not significant trend was with inhibitory proportion was observed (Fig. 8). In contrast, there was no correlation between inhibitory proportion and antibiotic resistance to rifampin, novobiocin, tetracycline, streptomycin, and kanamycin (data not shown).

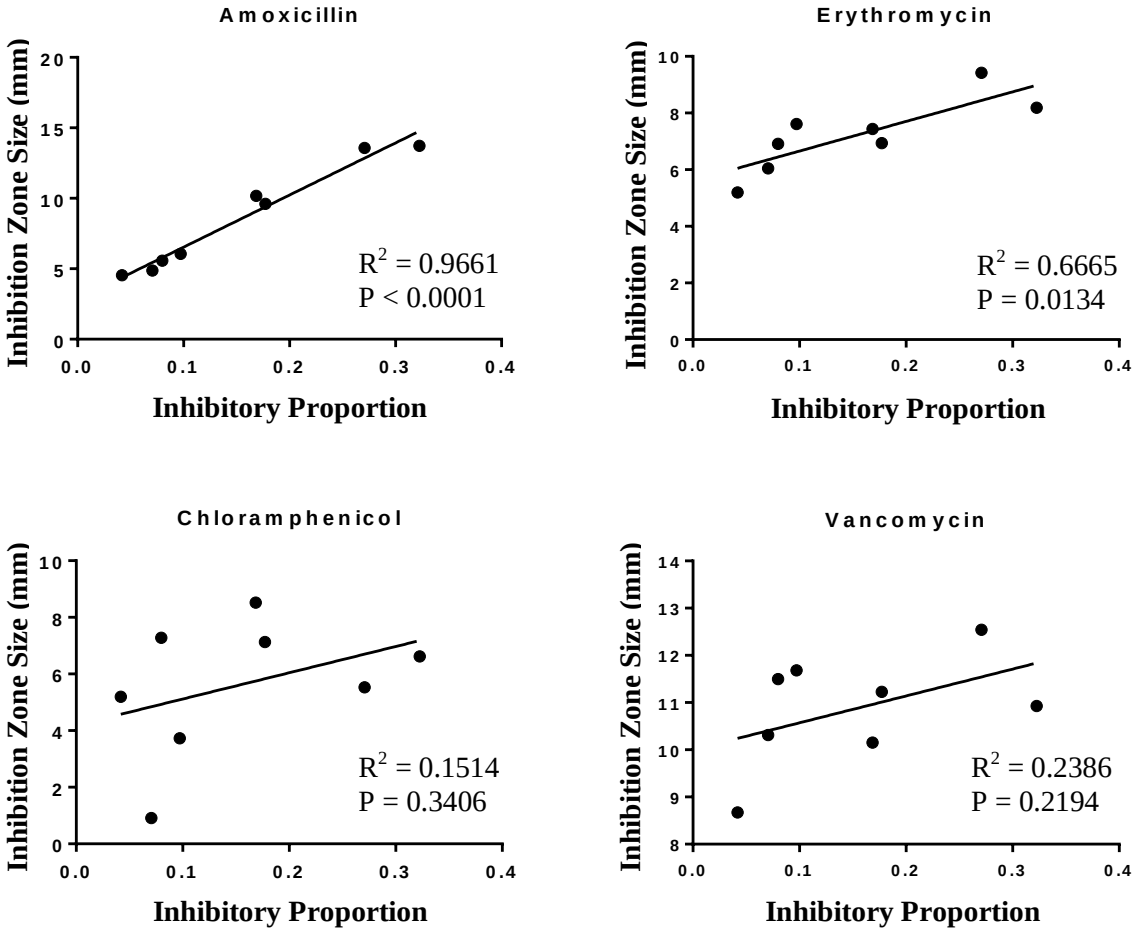



**Figure 8** Correlation of inhibitory proportion and antibiotic resistance of *Streptomyces* isolates. Amoxicillin, chloramphenicol, erythromycin,and vancomycin resistance positively correlate with inhibitory proportion. In contrast, rifampin, novobiocin, tetracycline, streptomycin, and kanamycin resistance does not.

**Discussion and Conclusions**

A significant amount of soil organic matter (carbon resources) is derived from plants, which, in turn, could influence the community characteristics of soil-borne microorganisms (Kuzyakov, 2000). In this project, I examined the impacts of plant diversity and productivity (carbon resources) on antagonistic *Streptomyces* communities in soil. It was hypothesized that dissimilar plant diversity and productivity might differently affect soil-borne microorganisms, especially *Streptomyces*. It was hypothesized that competition for soil (carbon) resources has an important effect on the potential of *Streptomyces* to suppress plant-pathogenic bacteria and fungi in agricultural systems due to differences in plant carbon input (Samac and Kinkel, 2001). Competition among microbes is thought to be important in soil and to be able to select for antibiotic producers, while plants can influence this competition through soil carbon quantity and diversity (Schlatter, 2009). Therefore, antibiotics-producing phenotypes of soil-borne *Streptomyces* are thought to be selected by plant diversity and resources competition.

My results show that plant diversity and productivity impact *Streptomyces* densities, inhibitor densities, and the intensity of inhibition. The inhibitory proportion, which is number of *Streptomyces* inhibitors relative to the total number of *Streptomyces*, is significantly greater in monoculture than in polyculture plant communities (Figure 2a). Inhibitory *Streptomyces* species are influenced by plant diversity because plant richness impacts soil carbon resource diversity and quantity. The inhibitory proportion results also show that low resource diversities or quantities support higher frequencies of antibiotic producing *Streptomyces*, which is consistent with the idea that stronger resource competition in these environments selects for antibiotic inhibitory phenotypes (Samac and Kinkel, 2001). Therefore, my results support conclusions from previous studies that *Streptomyces* exhibit an antagonistic relationship to compete for resources in low-resource / low plant diversity environments (monoculture) (Kinkel et al., 2014). My

results also suggest that plants can significantly impact *Streptomyces* competitive dynamics and inhibitory phenotypes.

In a previous study, it was shown that antibiotic inhibitory and resource-use phenotypes of *Streptomyces* in response to changed specific carbon inputs (Schlatter et al., 2009), which, in turn, correlated with plant diversity and productivity. My results show that inhibitory proportions are related to soil carbon quantity (amount of soil organic matter) in monoculture, but that they are not related to soil carbon quantity in polycultures. These results suggest that the impacts of carbon quantity depend on plant diversity. The negative correlation between soil carbon and proportions of antibiotic producing *Streptomyces* in monocultures, but not in polycultures, suggests that soil carbon quantity (as affected by fungicide treatment of the leaves) influences proportions of inhibitory *Streptomyces* in monoculture plots, but does not have a significant effect in plant polyculture plots. Thus, in soils with high resource diversity (plant polycultures), further increases in the presumably already high carbon quantity do not significantly affect selection for antibiotic producing *Streptomyces.* In contrast, in soils with low resource diversity (such as those associated with plant monoculture), carbon quantity modification greatly changes *Streptomyces* responses. Thus, *Streptomyces* employ antibiotics to compete for resources (high inhibitory proportion) when they experience low soil carbon quantity in monocultures, but not in polycultures. By competing with other strains for the same soil resources (as occurs in plant monoculture), *Streptomyces* increase their inhibitory phenotype and responded with antibiotics production (VazJauri et al., 2014). The coevolutionary arms race thus appears to affect *Streptomyces* communities' phenotype. Specifically, high soil-carbon quantity in plant monoculture was significantly negatively correlated with the proportion of inhibitory strains in *Streptomyces* communities. This negative correlation suggests that the

inhibitory phenotypes of *Streptomyces* (that differ genetically from the non-inhibitory strains, as indicated by our 16S sequencing data) are induced by low soil carbon quantity. Finally, the fact that antibiotic resistance was significantly positively correlated with inhibitory proportions of *Streptomyces* means that *Streptomyces* respond by simultaneously producing more antibiotics and increasing their own resistance to antibiotics.

Characterization of the resource use of *Streptomyces* strains showed that, under low plant richness (plant monoculture), *Streptomyces* exhibit a greater niche overlap (involving use of the same resources and increasing the need for mutual growth inhibition by different strains) than under plant polyculture. Moreover, *Streptomyces* niche width (as a measure of the number of carbon resources used) in plant monoculture is significantly narrower than in plant polyculture. Our data thus support the coevolutionary arms race model, whereby *Streptomyces* competing for the same resources will share the same type and number of resources and will increase the proportion of inhibitory strains to minimize resource competition.

In conclusion, the results presented here support the following hypotheses:

1) *Streptomyces* communities in plant monocultures harbor more inhibitory *Streptomyces* than in plant polyculture, with the inhibitory proportion of *Streptomyces* in monoculture being significantly greater than in polyculture (Figure 2a);

2) Carbon quantity in plant polyculture is greater than in plant monoculture based on percentage of organic matter (Figure 6a);

3) High carbon quantity in the soil is negatively correlated with inhibitory intensity in *Streptomyces* communities (Figure 6b);

4) *Streptomyces* communities in plant monocultures have a greater niche overlap and niche width (more similar resources used) than in polyculture (Figure 4 and 5);

5) *Streptomyces* community composition differs as a result of the level of plant diversity, as shown via 16s rRNA sequencing (Figure 7). Furthermore, inhibitory proportions of *Streptomyces* isolates were significantly positively correlated with antibiotic resistance to amoxicillin and erythromycin (Figure 8).

In conclusion, the phenotypic and genetic composition of *Streptomyces* in soil is influenced by the environment both in terms of plant richness and soil carbon quantity. The overall data suggest that plant species influence competitive interactions among *Streptomyces*, and thus may impact selection for antibiotic and resource use phenotypes. *Streptomyces* associated with plant monoculture are likely to be subject to more resource competition than those associated with plant polyculture. In contrast, *Streptomyces* communities in polyculture are much more complex than those associated with plant monoculture. Therefore, in monoculture agricultural system, *Streptomyces* strains are likely to help suppress plant disease because of antibiotics production, and attempts to control soil-borne plant diseases via antibiotics-producing *Streptomyces* strains should work well in plant monoculture (but perhaps not in plant polyculture). Since *Streptomyces* also promotes plant growth by root colonization (Schrey et al., 2008), *Streptomyces* could be considered as a strong candidate for biological control in agricultural system.

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