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REGULAR ARTICLE

Nutrient use preferences among soil *Streptomyces* suggest greater resource competition in monoculture than polyculture plant communities

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Abstract
Background an aims Nutrient use overlap among sympatric *Streptomyces* populations is correlated with pathogen inhibitory capacity, yet there is little information on either the factors that influence nutrient use overlap among coexisting populations or the diversity of nutrient use among soil *Streptomyces*.
Methods We examined the effects of plant host and plant species richness on nutrient use of *Streptomyces* isolated from the rhizosphere of *Andropogon gerardii* (*Ag*) and *Lespedeza capitata* (*Lc*) growing in communities of 1 (monoculture) or 16 (polyculture) plant species. Growth on 95 carbon sources was assessed over 5d.
Results Cumulative growth was significantly greater for polyculture vs. monoculture isolates, and for *Lc* vs. *Ag* isolates. Isolates from monocultures, but not polycultures, exhibited a drop in growth rates between 24 h and 72 h post-inoculation, suggesting resource allocation to non-growth functions. Isolates from high-carbon (polyculture) or high-nitrogen (*Lc*) soils had larger niche widths than isolates from low-C (monocultures) or low-N (*Ag*) soils. Sympatric isolates from polycultures were significantly more differentiated from one another in preferred nutrients for growth than sympatric isolates from monocultures.
Conclusions These results suggest that *Streptomyces* populations respond to selection imposed by plant host and plant community richness and that populations from polyculture but not from monoculture, mediate resource competition via niche differentiation.

Keywords *Streptomyces* · *Andropogon gerardii* · *Lespedeza capitata* · Plant richness

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Introduction

Bulk soil in natural and agricultural systems is often resource-limited and can impose major constraints to the growth of heterotrophic microbes (Hibbing et al. 2009). On the other hand, the rhizosphere is a biologically and chemically highly diverse environment where complex and dynamic interactions occur among plant roots, microbes and the soil (Hartmann et al. 2008). Within this habitat, plants and microbes have evolved intimate relationships that enable them to coexist (Hartmann et al. 2008). Specifically, plants provide nutrient resources to the rhizosphere that support microbial activities. In turn,

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microbes have strong effects on plants via pathogenicity, pathogen suppression, plant growth promotion, and nitrogen fixation (Nihorimbere et al. 2011). There is compelling evidence that, through the quantity and diversity of root exudates, plants influence the growth conditions experienced by soil microbial communities, with potential consequences for microbial functional activities (Dini-Andreote and van Elsas 2013). Though plant-driven selection on microbes has been the focus of a substantial number of studies on plant-microbe interactions (Broeckling et al. 2007; Thonart et al. 2011; Lamb et al. 2010), the specific mechanisms by which plant host and plant species richness mediate functional changes in rhizosphere communities remain poorly understood.

Streptomyces are Gram-positive bacteria that live in close association with plants in natural and agricultural habitats (Seipke et al. 2012). Members of the genus *Streptomyces* are well-known for their prolific production of antibiotics (Omura et al. 2001), and possess particularly potent and diverse antagonistic effects against plant pathogens (Doumbou et al. 2001; Wiggins and Kinkel 2005a, 2005b). Recent work suggests that competition for nutrients and nutrient use overlap among coexisting *Streptomyces* are correlated with antibiotic production and pathogen inhibitory capacity of soil *Streptomyces* populations (Kinkel et al. 2014; Schlatter and Kinkel 2014). Because the genus *Streptomyces* is frequently a major component of the complex biota that live in plant rhizospheres, and resources available to *Streptomyces* in the rhizosphere are predominantly of plant origin, there is a strong potential for plant host and community richness to shape *Streptomyces* community nutrient use profiles and dynamics. In particular, plant species and plant community characteristics are likely to influence nutrient use among *Streptomyces* by means of variation in the amount and chemical diversity of rhizosphere carbon inputs into the soil. Since the quantity and quality of root exudates are determined in part by plant species (Badri and Vivanco 2009) one would expect the composition of root exudates to be less diverse in the surroundings of the roots of plants growing in monoculture than polyculture. Such variation in the nutritional environment has been hypothesized to have implications for *Streptomyces* community structure and functions (Kinkel et al. 2011, 2012). Specifically, it has been hypothesized that polyculture, by increasing resource diversity, will foster niche differentiation among *Streptomyces*. That is, with chemically diverse nutrients in the rhizosphere of plants in high plant species richness communities, different *Streptomyces* isolates may prefer distinct substrates as a mechanism of avoiding potentially intense competition for nutrients. In contrast, increased inhibitory capacity in monocultures is hypothesized to reflect intense competition among sympatric *Streptomyces* for limited and low diversity resources, resulting in a co-evolutionary arms race and the accumulation of antagonistic phenotypes. That is, as nutrient composition in the soil is likely more homogeneous in monoculture than polyculture, plants in monoculture will likely select for *Streptomyces* with similar nutrient preferences. Thus, isolate nutrient preferences are expected to be more similar among isolates from plants growing in monoculture than in polyculture. However, despite the significant potential for plant identity and richness to modulate microbial functional traits, we have limited knowledge of how plant host or plant community richness may influence the dynamics of nutrient use among soil-borne *Streptomyces*.

This study examines the effects of plant host and plant species richness on nutrient use by *Streptomyces* populations from the rhizosphere of *Andropogon gerardii* (*Ag*) and *Lespedeza capitata* (*Lc*), each growing in communities of 1 (monoculture) or 16 (polyculture) plant species. The specific objectives of this work were to (1) characterize resource use phenotypes and nutrient preferences of *Streptomyces* from the rhizospheres of *Ag* and *Lc* in monoculture and polyculture; (2) assess the impact of plant host and species richness on the potential for nutrient competition among sympatric populations; and (3) explore variation in temporal dynamics of resource use and niche differentiation among *Streptomyces* from monoculture and polyculture. This study contributes significant insights into the effects of plant species and plant species richness on *Streptomyces* nutrient use dynamics, and expands our understanding of the potential for nutrient competition to influence coevolutionary dynamics among soil microbes.

Materials and methods

Soil sampling

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

network) in July of 2012, from plots in a long-term plant richness manipulation experiment (Tilman 2001). These plots were established in 1994 with defined levels of plant richness. Soil samples were collected from as close as possible to the center of the base of *Ag* and *Lc* plants growing in communities of monoculture or polyculture plant species. Plants to be sampled were selected randomly from individual plots at a distance of at least 20 cm from plot margins to avoid border effects. For each plant, (x, y) coordinates were determined using a random number table, and the closest plant of the targeted species to the coordinates was sampled. Each soil sample consisted of two bulked cores collected with a 2.5 cm-diameter corer to a depth of 10 cm; cores from the same plant were homogenized in plastic sample bags. For each plant species in each plant richness treatment, samples were taken from 3 plants, each growing in a different plot. Thus we had a total of 12 soil samples (2 plant hosts × 2 plant community diversity levels × 3 plot-level replicates). The soil samples were stored at −20 °C prior to processing.

Streptomyces isolates

Streptomyces isolates were randomly selected from each composite soil sample following dilution plating onto a dual-layer water agar-Starch Casein agar medium (SCA; 10 g starch, 0.3 g casein, 0.02 g $CaCO_3$, 2.0 g KNO_3, 2.0 g NaCl, 2.0 g K_2HPO_4, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.001 g $ZnCl_2$, and 15 g *Ag*ar per liter of deionized H_2O) (Becker and Kinkel 1999). For each sample, a single 5 g sub-sample was dried overnight under sterile cheesecloth as the first enrichment for *Streptomyces*, which are tolerant of desiccation. Dried soil samples were dispersed in 50 mL of sterile deionized water on a reciprocal shaker (175 rpm, 60 min, 4 °C). Soil suspensions were serially diluted to 10^{-5}, a dilution previously determined to provide 40–70 isolates per plate in this soil. Single 100 μl-aliquots of the diluents were spread onto plates of 15 ml of water agar (WA) and subsequently covered with 5 mL of cooled, molten starch-casein agar (SCA). This method suppresses the growth of many unicellular bacteria, while allowing filamentous *Streptomyces* to grow up through the SCA (Wiggins and Kinkel 2005b). After three days of incubation (28 °C), 10–15 colonies were randomly chosen, using a random sampling grid, from each of the 12 soil samples based on characteristic *Streptomyces* morphology, with a goal of 120 purified isolates.

Edaphic soil properties

Subsamples of each of the 12 soil samples were submitted to the University of Minnesota Soil Testing Laboratory for determination of soil characteristics (pH, NO_3^--N, Bray-P, K and total C; (http://ral.cfans.umn.edu).

Streptomyces resource use characterization

Utilization of carbon sources by every individual *Streptomyces* isolate (n = 120) was determined using Biolog SF-P2 plates (Biolog, Inc. Hayward, CA). Biolog SF-P2 microplates measure the growth of an isolate on a single source of carbon by comparing the turbidity of each well to a water control. Spore suspensions of each isolate were made by swabbing spores from a pure culture grown on SCA for 10 days into 1.5 ml of 0.2 % carrageenan. Suspensions were adjusted to an optical density of 0.20–0.24 at 590 nm, and then diluted in 13.5 ml of 0.2 % carrageenan. One hundred microliters of the new suspension were pipetted into each well of the Biolog plate. Plates were incubated at 28 °C. Utilization of each of the 95 sole carbon compounds on the Biolog SF-P2 plate was assessed over time by recording the absorbance of each well at 590 nm at 3 time points (24, 72 and 120 h) post-inoculation. The absorbance of the well containing only water was subtracted from the absorbance of every other well to standardize absorbance values.

Analyses

Streptomyces nutrient use

Used nutrients were defined as those on which a *Streptomyces* isolate grew to an absorbance value greater than 0.005 (Vaz Jauri et al. 2013). Using this definition, total growth and niche width were determined for each isolate at each time point. Total growth was defined as the mean absorbance value over all used nutrients, and niche width as the number of used nutrients for an isolate. Mean growth and mean niche width of sympatric isolates were determined for each community; and mean growth overall used nutrients and mean niche width among isolates were determined for every treatment at each time point. Finally, growth

rate per hour within early (0–24 h), middle (24–72 h), and late (72–120 h) time periods were determined for every treatment.

In addition to overall nutrient use, we considered nutrient use based on carbon substrate characteristics. Specifically, the 95 carbon substrates in the SF-P2 panel belong to 11 carbon groups: alcohols, amides, amines, amino acids, aromatic compounds, carbohydrates, carboxylic acids, esters, phosphorylated compounds and polymers (http://www.biolog.com/pdf/milit/00A_008rA_SFN2_SFP2.pdf). For each community, mean growth per carbon group was calculated across all used substrates (OD > 0.005) in that carbon group, and mean growth among isolates on each carbon group for each time period was determined for every treatment.

To index the potential for resource competition within communities, pairwise niche overlap (PNO) and pairwise niche escape (PNE) (Fig. 1) were determined for every pairwise of isolate combinations within each communities using the formulas:

$$\mathrm{PNO} = \left[\left(\frac{\begin{array}{c}\text{Number of shared nutrients}\\ \text{x}\\ \text{Total growth of isolate1 on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of isolate1}\\ \text{x}\\ \text{Total growth of isolate1 on all used nutrients}\end{array}} + \frac{\begin{array}{c}\text{Number of shared nutrients}\\ \text{x}\\ \text{Total growth of isolate2 on the shared nutrients}\end{array}}{\begin{array}{c}\text{Niche width of isolate2}\\ \text{x}\\ \text{Total growth of isolate2 on all used nutrients}\end{array}}\right) \Big/ 2\right] \text{x } 100$$

$$\mathrm{PNE} = \left[\begin{array}{l}\left(\begin{array}{l}\text{Niche width of isolate1 x Total growth of isolate1} -\\ \text{Number of shared nutrients x Total growth of isolate1 on the shared nutrients}\end{array}\right) +\\ \left(\begin{array}{l}\text{Niche width of isolate2 x Total growth of isolate2} -\\ \text{Number of shared nutrients x Total growth of isolate2 on the shared nutrients}\end{array}\right)\end{array}\right] \Big/ 2$$

Community niche overlap and community niche escape were computed for sympatric isolates by averaging pairwise niche overlap and niche escape values among all isolate pairs within each community, and mean niche overlap and mean niche escape were also determined for every treatment.

Streptomyces preferred nutrients

As the differences in treatment effects on *Streptomyces* were more pronounced at 72 h than any other time point, which is consistent with previous work (Schlatter et al. 2013), analyses on preferred nutrients considered only data from 72 h time point.

We defined preferred nutrients for each isolate as the five carbon sources on which that isolate grew most (largest absorbance values). For each individual community, the total number of distinct preferred nutrients across isolates within that community was determined, resulting in a community-wide preferred niche width. For each treatment, mean community-wide preferred niche width and the total number of nutrients preferred by at least one *Streptomyces* isolate from every individual plant were also determined. Finally the mean proportions of isolates using each of the preferred nutrients were determined for each treatment (preferred use frequency).

Statistical analysis

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/). Analysis of variance and Turkey's HSD tests with $p < 0.05$ as the significance level were carried out to determine the significance of effects of plant host and diversity on mean growth, niche width, niche overlap, niche escape, and community-wide preferred niche width at 72 h growth. In addition, simple linear regression was applied to determine the relationships among soil edaphic characteristics, and between each of the soil edaphic parameters and *Streptomyces* nutrient use characteristics at 72 h growth. Finally, stepwise multiple regression was conducted to determine significant predictors of *Streptomyces* growth efficiency and niche width among soil parameters.

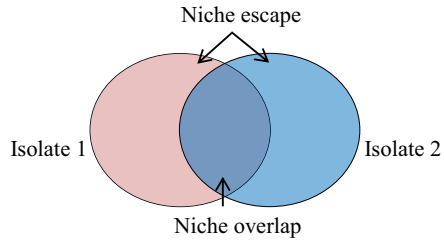

Fig. 1 Niche overlap and niche escape illustrated for a pair of competing *Streptomyces*. The niche of each isolate is denoted by a shaded circle, representing the total growth of that isolate on all its used nutrients. Niche overlap is the area of intersection between the two circles and quantifies the total growth of each isolate on all of the nutrients shared by the two isolates. Niche escape is the non-intersecting area of each circle and measures the fraction of the total growth for which an isolate does not compete with the paired isolate

Results

Streptomyces nutrient use

Soil-borne *Streptomyces* grew on a broad range of carbon sources. Each individual nutrient was used by at least 28 of the 120 isolates, with a core set of 10 substrates used by all isolates. These core substrates belong to five carbon groups and include glycerol (alcohols); L-glutamic acid (amino acid); D-gluconic acid (carboxylic acid); N-acetyl-D-glucosamine, D-cellobiose, α-D-glucose, and maltotriose (carbohydrates); and dextrin, glycogen, and Tween 40 (polymers). Other nutrients such as tween 80 (polymer), D-arabitol, and D-fructose (carbohydrates) were used by more than 95 % of isolates. However, the most frequently used nutrients were not necessarily those on which most growth occurred. At 120 h, the greatest growth on average was recorded on D-mannose, maltotriose, tween 40, and tween 80. Overall, isolates used all 11 carbon groups, ranging from labile carbon substrates such as carbohydrates, amino acids, and amines to recalcitrant carbon sources including esters, phosphorylated compounds, and polymers (Supplemental Table 1), illustrating the diverse metabolic capacities among *Streptomyces* populations.

Niche widths among individual *Streptomyces* isolates varied substantially and ranged from 33 to 95 carbon substrates (average of 81 per isolate). This suggests that *Streptomyces* isolates are often generalists (Fig. 2). Despite their large niche widths, specific resource use profiles varied considerably among individual isolates. Indeed, there were 96 unique patterns of resource utilization among the 120 isolates. These findings indicate a rich array of nutrient use profiles among *Streptomyces* and highlight their potential capacities to adapt to diverse soil trophic conditions.

Streptomyces mean growth, niche width, and growth rates

Mean growth and niche width

Streptomyces mean growth over all nutrients varied significantly among isolates from different plant hosts and richness treatments at all incubation times (Fig. 3). Specifically, mean growth of *Streptomyces* from *Lc* in polyculture was at least 30 % higher, at all times, than that of isolates from the other plant communities. Similarly, there were significant differences among treatments in *Streptomyces* niche widths (Fig. 4). Mean niche width for communities from *Lc* in polyculture was also significantly higher than that used by *Streptomyces* from the other communities. On average, *Streptomyces* from *Lc* polyculture used 93 nutrients whereas the mean number of carbon sources used by *Streptomyces* from the other treatments varied between 75 and 78. Thus, *Streptomyces* from *Lc* in polyculture plots had an average 19 % or greater increase in niche width compared with other plant host and richness treatments.

The effects of plant richness on nutrient use among *Streptomyces* varied between plant species and over time. Isolates from *Lc* in polyculture had significantly greater mean growth per nutrient than those from *Lc* in monoculture at all time points (t-test, $p < 0.001$). Similarly, mean growth per nutrient among *Streptomyces* from *Ag* in polyculture was significantly greater than in monoculture at 72 h (t-test, $t = 2.2$, $p = 0.046$), though differences were not significant at the end of the incubation period (120 h). In contrast, mean growth of *Streptomyces* from *Ag* in monoculture was significantly greater than that of isolates from *Ag* in polyculture at 24 h post-inoculation, (t-test, $t = 2.5$, $p = 0.03$).

At the end of the incubation period (120 h), *Streptomyces* from *Lc* had greater mean growth than those from *Ag* in both plant richness treatments. In monoculture, isolates from *Lc* had grown 47 % more than isolates from *Ag* (t-test, $t = 2.2$, $p = 0.046$); and in polyculture, *Streptomyces* from *Lc* had an average 71 % increase in

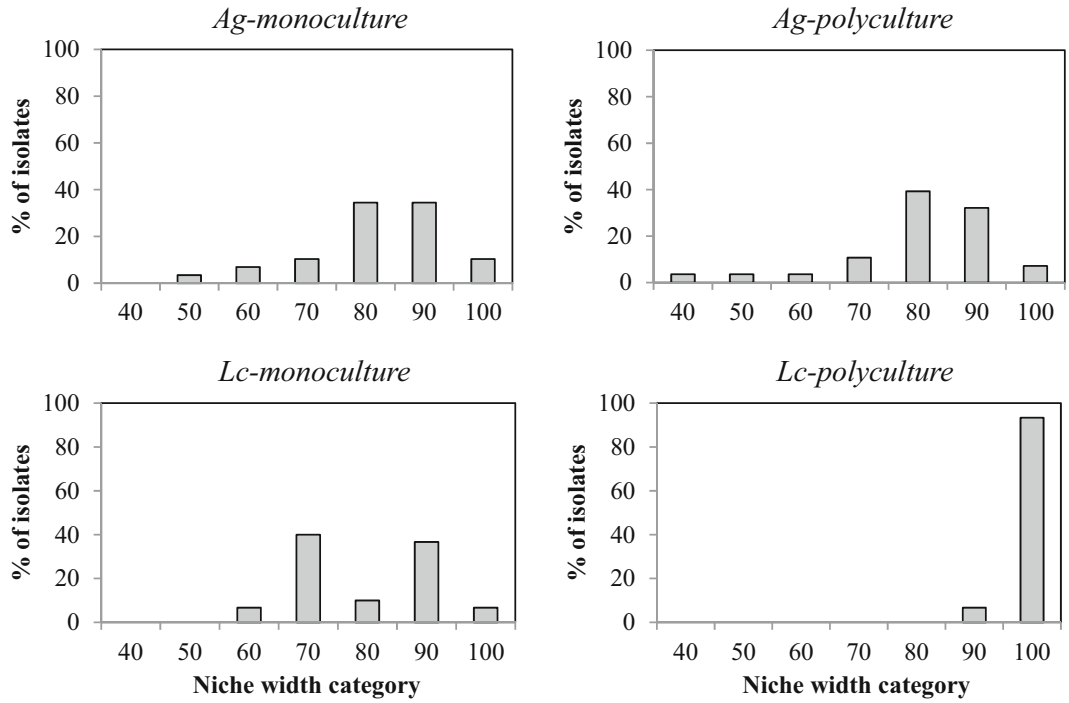

Fig. 2 Frequency distribution of *Streptomyces* niche widths among isolates (n = 30 per panel) for populations collected from *Ag* and *Lc* in monoculture or polyculture (n = 16 plant species) plots at 120 h growth. Niche width is defined as the total number of used nutrients (OD > 0.005) for an isolate

their mean growth per nutrient relative to *Streptomyces* from *Ag* (t-test, $t = 4.5$, $p = 0.005$).

Collectively, these findings show that plant host and species richness are significant determinants of *Streptomyces* nutrient use phenotypes and important selective factors in structuring *Streptomyces* community functional diversity.

Streptomyces growth rate

Streptomyces communities used carbon sources at different rates (OD/h) over time. Isolate mean growth rate varied significantly among treatments within early (ANOVA, $F = 7.32$, $p = 0.03$), middle (ANOVA, $F = 7.12$, $p = 0.03$) and late (ANOVA,

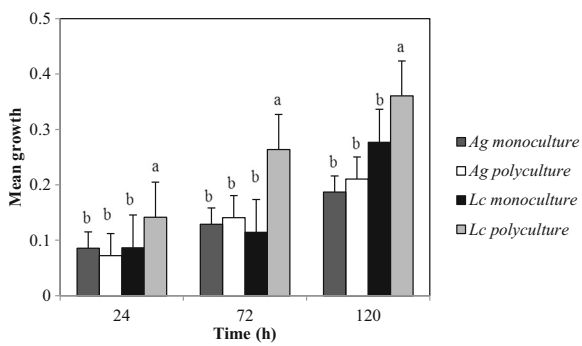

Fig. 3 Mean growth on used substrates of *Streptomyces* from *Ag* and *Lc* in monoculture and polyculture. Within each time point, different letters above bars indicate significant differences among treatments (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

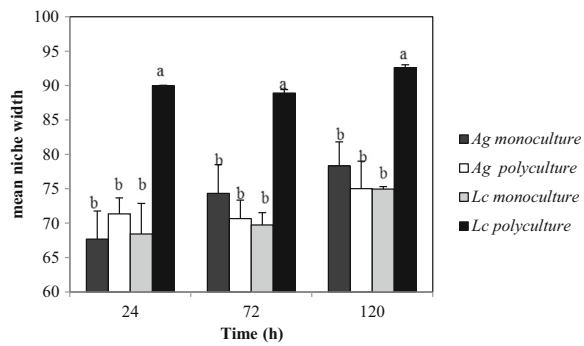

Fig. 4 Mean niche width of *Streptomyces* from *Ag* and *Lc* in monoculture and polyculture. Within each time point, different letters above bars indicate significant differences among treatments (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

F = 6.1, p = 0.04) intervals of the incubation period (Fig. 5). Early growth rates of isolates from *Lc* in polyculture were significantly higher than those of *Streptomyces* from all other treatments. Similarly, *Streptomyces* from *Lc* and *Ag* in polyculture grew significantly faster than their counterparts in low plant diversity in the middle phase of growth. However, *Streptomyces* from *Lc* in monoculture had significantly higher growth rates than *Streptomyces* from any other treatment in late growth phase. Thus, plant identity and species richness may have a significant effect on the growth dynamics of *Streptomyces* in natural systems.

Considering the temporal dynamics of nutrient use among different resource types sheds further light on the selective effects of plant host on *Streptomyces* nutrient use profiles. Indeed, though there were no significant differences in early growth rates between communities from *Ag* in monoculture and in polyculture, the breakdown of growth rate by carbon substrate group revealed significant differences between communities from the two treatments in their early use of carbohydrates and esters. Specifically, *Streptomyces* from *Ag* in monoculture access both labile and recalcitrant carbon sources more in early growth phase than isolates from polyculture (Supplemental Table 2). However, as time passed *Streptomyces* from *Ag* in polyculture grew relatively more rapidly on almost all carbon sources. Similarly, in monoculture, early and mid-phase growth rates of *Streptomyces* from *Ag* were greater than those of *Streptomyces* from *Lc* on carbon sources with amine functional group (NH2), amines and amino acids, but the difference was significant only in mid-phase growth rate. As amines and amino acids can be used by *Streptomyces* as source of nitrogen as well as carbon, and given the potential for nitrogen enrichment in the rhizosphere of the legume *Lc* plants, these data illustrate the prospect that plant functional group, through the modification of soil nutrient characteristics, might play an important role in the selection of *Streptomyces* with varying capacities to metabolize diverse nitrogen sources.

Regardless of host, the patterns of *Streptomyces* resource use dynamics over time varied between monoculture and polyculture (Fig. 5). For both plant diversities the rate of carbon utilization was high early in the incubation period and dropped significantly during the middle phase of growth. This significant decrease in mid-phase growth rate was more dramatic among *Streptomyces* in monoculture that in polyculture. Indeed, mid-phase growth rates of *Streptomyces* from *Ag* and *Lc* in monoculture were 75 % and 84 %, respectively, lower than their early growth rates. In contrast, the declines in growth rate between the early and the middle phase of growth for *Streptomyces* from *Ag* and *Lc* in polyculture were only 53 % and 57 %, respectively. Moreover, while *Streptomyces* in polyculture continued to use carbon substrates in the late phase of growth at an equal or slightly lower rate than the middle phase, the late growth rate of isolates from monoculture increased significantly with a dramatic spike of 480 % in growth rate among *Streptomyces* from *Lc* relative to mid-phase growth rate. Differences in temporal nutrient use dynamics among isolates from monoculture and polyculture may reflect differential effects of plant species richness on

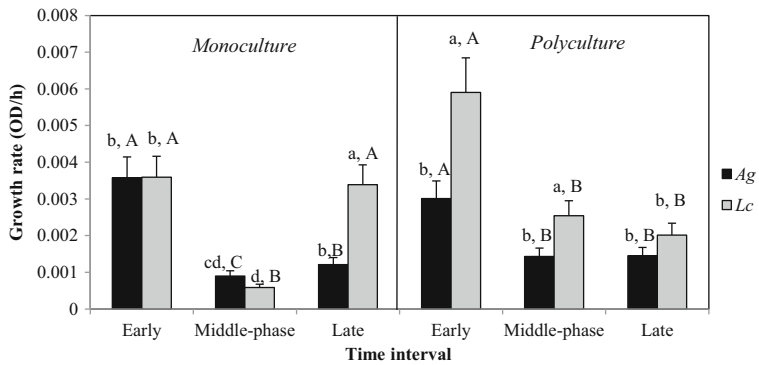

Fig. 5 Growth rate of *Streptomyces* from *Ag* and *Lc* in monoculture and polyculture during early (0–24 h), middle (24–72 h), and late-phase (72–120 h) growth periods. Within each time interval, differences in lowercase letters above bars indicate significant differences among treatments (HSD, $p < 0.05$); within each plant richness, differences in uppercase letters above bars indicate significant differences among time intervals (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

Streptomyces resource allocation over time, and specifically shifts in investments in growth versus secondary metabolic pathways.

Niche overlap and niche escape among sympatric *Streptomyces*

There were significant differences in niche overlap among *Streptomyces* isolates within communities from different plant host-species richness treatments (Fig. 6). Not surprisingly, communities from *Lc* in polyculture, which had the largest niche widths and mean growth per nutrient, had the largest mean sympatric niche overlap at all time points. Niche overlap among sympatric *Streptomyces* from *Ag* was significantly higher in monoculture than in polyculture at 24 h (t-test, $t = 2.92$, $p = 0.02$) and 72 h (t-test, $t = 4.45$, $p = 0.0006$) post-inoculation, but differences were not significant at any later time points. There were no significant differences in niche overlap among isolates from *Ag* and *Lc* growing in monoculture at any time.

Niche escape (competition-free growth for sympatric isolates) was greatest among sympatric isolates from *Ag* in polyculture at all times, followed by that of *Streptomyces* from *Lc* and *Ag* in monoculture (Fig. 7). Niche escape for communities from *Lc* in polyculture was significantly lower than for the other treatments at all times. Thus, *Streptomyces* isolates have significantly greater total growth on nutrients for which they need not compete with other isolates when in the rhizosphere of *Ag* in polyculture plots, suggesting that resource competition is less important to *Streptomyces* fitness in these plots. However, when only the 5 preferred nutrients for each isolate were considered, niche escape for sympatric isolates from *Ag* and *Lc* in polyculture was significantly greater than that for isolates from monoculture at 72 h (t-test; *Ag*: $t = 2.9$, $p = 0.02$, *Lc*: $t = 2.2$, $p = 0.046$) and 120 h (t-test; *Ag*: $t = 3.1$, $p = 0.02$, *Lc*: $t = 2.3$, $p = 0.04$). Thus, high plant richness appears to enhance niche differentiation among sympatric *Streptomyces*, and this effect is more pronounced on *Streptomyces* most-preferred nutrients.

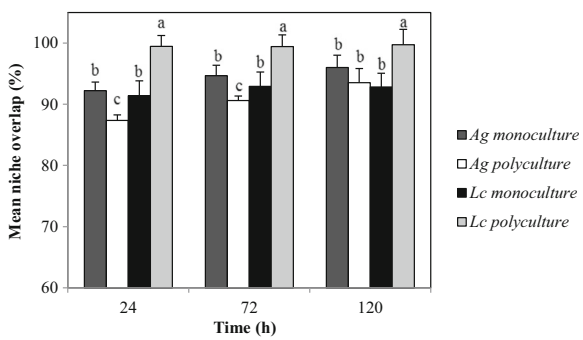

Fig. 6 Mean niche overlap among all possible pairwise sympatric *Streptomyces* isolate combinations from *Ag* and *Lc* in monoculture and polyculture. At each time point, different letters above bars indicate significant differences among treatments (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

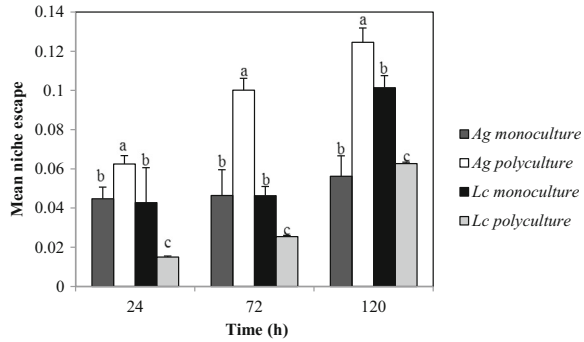

Fig. 7 Mean niche escape among all possible pairwise sympatric *Streptomyces* isolate combinations from *Ag* and *Lc* in monoculture and polyculture. At each time point, different letters above bars indicate significant differences among treatments (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

Plant impacts on *Streptomyces* preferred nutrients

Mean community-wide preferred niche width, representing the mean number of distinct nutrients preferred for growth (top five for growth for each isolate) among replicate communities of the same treatment, varied significantly among treatments (Fig. 8; ANOVA, $F = 32.44$, $p < 0.001$). Within plant richness treatments, communities from *Lc* had significantly greater mean-community-wide preferred niche widths than communities from *Ag*. For both plant hosts, polyculture *Streptomyces* communities preferred significantly greater numbers of nutrients than monoculture communities. These findings demonstrate that *Streptomyces* isolates from *Lc* and high plant richness plots utilize a greater diversity of nutrients for maximum growth, and are consistent with the observation that the isolates from plots are more niche-differentiated than isolates from *Ag* and low plant richness, respectively.

Preferred nutrients varied substantially among plant hosts and richness (Supplemental Table 3). For each treatment, nutrients that were used by at least one isolate from every individual plant are shown in Fig. 9. The

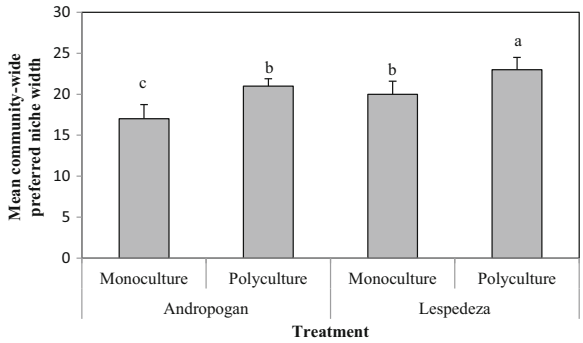

Fig. 8 Mean numbers of nutrients that rank within the top five for growth among all isolates for *Streptomyces* communities from *Ag* or *Lc* growing in monoculture or polyculture (mean community-wide preferred niche width). Different letters above bars indicate significant differences among treatments (ANOVA and HSD tests, $p < 0.05$). *Error bars* represent standard errors

mean proportion of isolates using each of these nutrients varied within and among treatments. For both plant hosts, the number of nutrients that were used by 50 % or more of isolates was greater for monoculture than polyculture. Specifically, there were 4 and 3 carbon substrates preferred by 50 % or more of the *Streptomyces* isolates in monoculture for *Ag* and *Lc*, respectively, versus only 1 and 2 carbon substrates for the same hosts in polyculture. This further suggests that plants growing in monoculture have more consistent impacts on *Streptomyces* nutrient preferences than the same plant hosts growing in polyculture plant communities.

Streptomyces resource use and soil properties

Soil edaphic characteristics were generally significantly correlated among plots (Table 1). There were no significant differences in soil pH, NO_3^--N, P, K, or total C among plant host and richness treatments (Supplemental Table 4). However, total organic carbon was 80 % greater in polyculture than in monoculture regardless of host (t-test; $t = 2.66$, $p = 0.01$), and soil nitrogen was 77 % greater in the rhizosphere of *Lc* than in *Ag* (t-test; $t = 2.01$; $p = 0.04$).

Both isolate total growth and niche width of *Streptomyces* were significantly correlated with soil N, K and total C of the soil (Table 2). Nitrate N was more strongly positively correlated with growth than with niche width, while both growth and niche width were negatively correlated with K. Finally, growth but not niche width was positively correlated with total C. Thus, soil edaphics are significantly related to *Streptomyces* growth potential and niche width in the soil.

Further analyses considered multiple linear regression models of soil characteristics and their interactions as a means of differentiating the significance of distinct factors in predicting *Streptomyces* growth or niche width (Table 2). The best model explained 72 % of the variation in *Streptomyces* growth; this model included K, N, the interaction between K and N, and the interaction between N and total C as significant terms. Slightly better than single factor models, the best multi-factor model that explains the greatest variation (40 %) in *Streptomyces* niche width included P, the interaction between N and K; and the interaction between N and total C as significant variables. Collectively these findings illustrate that mineral and organic components of the soil, and perhaps most importantly their interactions, may influence selection for distinct nutrient use profiles among *Streptomyces*.

Discussion

The rhizosphere is a biologically and chemically diverse environment in which complex and dynamic interactions occur among plant roots, microbes, and the soil physicochemical environment (Hartmann et al. 2008). Enormous strides have been made over the past years toward understanding these interactions (Hirsch et al. 2003), and the fields of plant biology and microbial ecology have recognized the critical role of plant driven-selection, through root exudates and direct plant-microbe interactions, in determining the structure and function of soil microbial communities (Broeckling et al. 2007; Bremer et al. 2009). However, despite advances in our understanding of soil microbes achieved through high-throughput sequencing techniques, comprehensive surveys of the phenotypic characteristics of soil microbes remain rare. Such phenotypic data are critical for identifying the selective forces that determine microbial growth strategies and ecosystem diversity and function. Here, we surveyed nutrient use phenotypes of a collection of *Streptomyces* isolated randomly from two different plant hosts growing in monoculture and polyculture plots in a long-term plant richness manipulation experiment, and showed significant effects of plant species richness and identity on the nutrient preferences and dynamics of resource use among soil-borne *Streptomyces* communities.

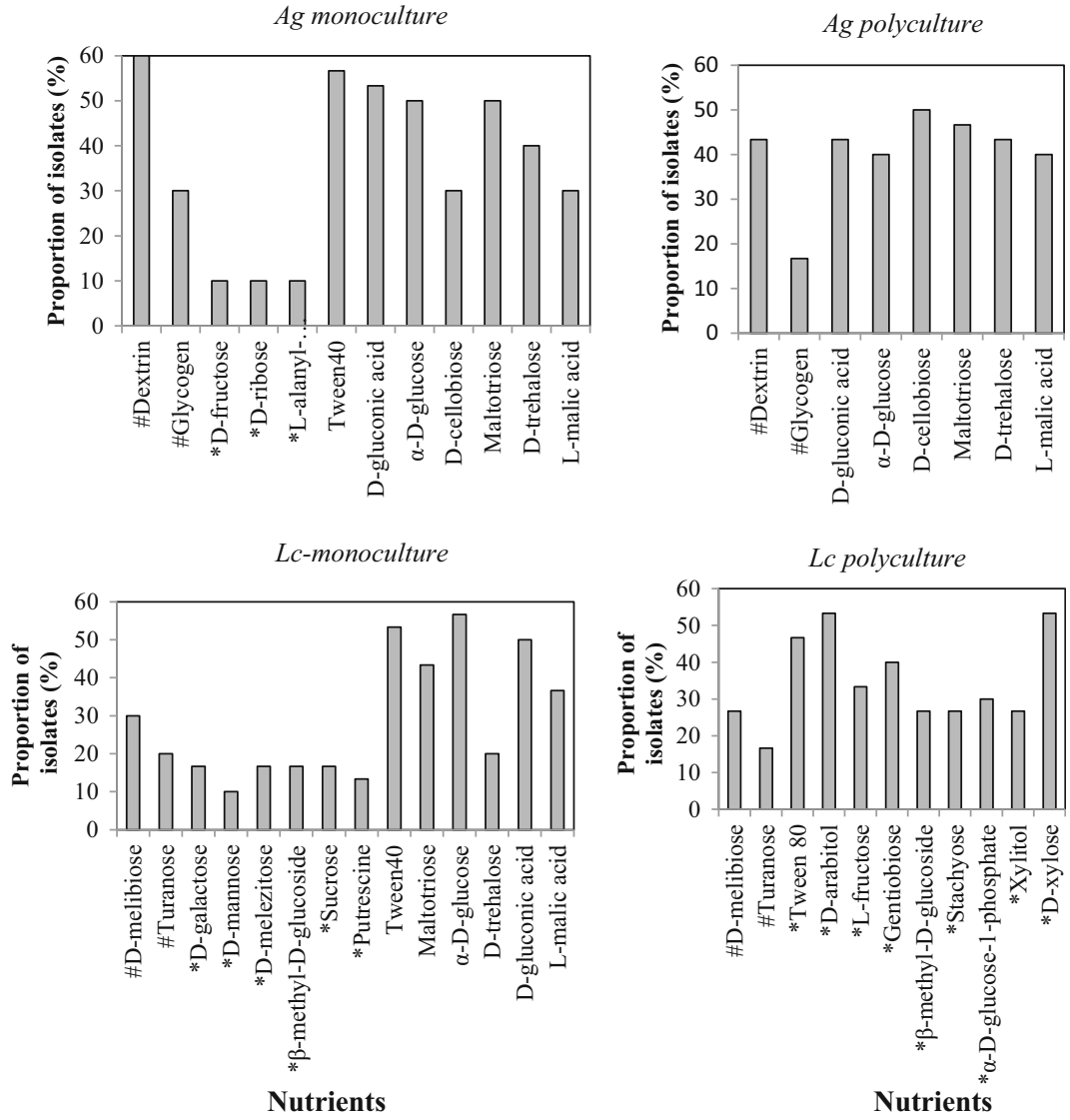

Fig. 9 Proportions of isolates using the preferred nutrients that were utilized by at least one *Streptomyces* isolate from every individual plant for each treatment. Host-specific preferred nutrients preferred only by *Streptomyces* communities on that plant host are indicated by #, and treatment-specific preferred nutrients preferred by only *Streptomyces* communities on that plant host in that plant richness treatment are indicated by *

Table 1 Matrix of Pearson (upper triangle) and Spearman (lower triangle) correlations among soil edaphic characteristics

Treatment	NO_3^--N (ppm)	K (ppm)	P (ppm)	Total C (%)
NO_3^--N (ppm)	1	−0.13	0.09	0.33***
K	−0.05	1	0.49***	−0.13
P	0.19*	0.55***	1	0.33***
Total C	0.58***	0.23*	0.48***	1

* $p < .05$, ** $p < .01$, ***$p < .001$

Table 2 Simple and multiple linear regression correlation coefficients (r) and weights from regression analysis for the relationship between *Streptomyces* isolate total growth and niche width at 72 h, and soil edaphic parameters

Variables	Simple linear regression correlation coefficients		Multiple linear regression weights	
	Isolate total growth	Isolate niche width	Isolate total growth; Multiple $r^2 = 0.72$***	Isolate niche width; Multiple $r^2 = 0.4$***
NO_3^--N K	0.57***	0.22**	0.1	20.86
P	−0.2**	−0.2*	0.04***	0.07
Total C	0.002	0.02	−0.05***	2.84*
NO_3^--N * K	0.3***	0.007	−0.2	−69.64
NO_3^--N -N*P	-	-	0.0001	0.37***
NO_3^--N *Total	-	-	−0.003	−0.96
C	-	-	0.05**	9.9*
K*P	-	-	−0.0007***	−0.02
K*Total C	-	-	−0.0007	-
P*Total C	-	-	-	−0.02

* $p < .05$, ** $p < .01$, ***$p < .001$

Streptomyces grew on a wide array of carbon sources ranging from simple molecules to complex biopolymers. The ability to degrade diverse carbon molecules with different levels of chemical complexity reflects significant metabolic capacities among *Streptomyces* (Kontchou and Blondeau 1992), and corresponds to their active role in biogeochemical nutrient-cycling processes. The highly diverse resource use patterns among *Streptomyces* isolates may be due to ecological phenotype screening in response to rhizosphere conditions (Langenheder and Székely 2011) created by different plant host species in disparate plant richness environments. *Streptomyces* possess large genomes (Kontchou and Blondeau 1992) rich in transposable and horizontally-transferred elements (Chen et al. 2002) allowing for widespread exchange of genes. This offers flexibility in fine-tuning of metabolic machinery, and is likely to foster *Streptomyces* adaptation to local conditions in the soil created by variation in plant community richness and plant host.

In this work, plant species richness was a significant experimental factor influencing *Streptomyces* nutrient use. Specifically, isolates from polycultures had greater mean growth than those from monocultures. This may reflect effects of greater total carbon inputs in the rhizosphere of host plants growing in polyculture, and specifically selection for more growth-efficient *Streptomyces* at the high population densities associated with greater soil carbon. Plant mixtures have increased rhizodeposition of organic carbon (Xu et al. 2014) and polyculture plots in this study have significantly more soil carbon than monoculture plots (Supplemental Table 4). Conversely, low carbon conditions in the rhizosphere of plants in monoculture may select for *Streptomyces* that balance their resource allocation among multiple life history traits to maximize fitness. For instance, antibiotic production to mediate microbial antagonistic competition, accumulation of resistance to secondary metabolites produced by competitors, and spore production to enhance survival or dissemination have all been reported to occur to the disadvantage of microbial growth in low-nutrient environments (Czàràn et al. 2002; Tilman 2000). These data offer insight into how plant diversity, through changes in the abundance of nutrients released into the soil, may have considerable impacts on *Streptomyces* life history strategies, and on the functional characteristics, species interactions, and, consequently, coevolutionary dynamics of *Streptomyces* communities in soil.

These results indicate that plant community richness also influenced *Streptomyces* niche widths beyond the effects of the individual plant host. *Streptomyces* from the rhizosphere of plants growing in polyculture communities possess greater niche widths than those growing in the rhizosphere of the same plant hosts in monoculture. We hypothesize that increasing plant richness will correspond to increased resource diversity in soil which, in turn, will select for populations with the capacity to use a broad array of available resources. Further support for the idea of nutrient diversity enrichment in polyculture and possible consequences for

rhizosphere microbial community structure lies in the findings of Micallef et al. (2009) who showed strong evidence of differences in exudate profiles among *Arabidopsis thaliana* accessions, and variation in bacterial communities associated with different accessions. These findings provide ecological insight into the potential mechanistic bases of plant community effects on soil microbial community functional characteristics beyond the influences of individual plant hosts. The potential for neighboring plants to modulate interactions of a given host species with associated soil microbes has been reported by many authors but the mechanisms underlying such influences remain speculative (LeBlanc et al. 2014; Bakker et al. 2013; Wardle et al. 1999). The prospect that plant community richness impacts *Streptomyces* niche widths through changes in the diversity of carbon substrates released into the soil suggests the significance of understanding not only plant host but also plant community effects on soil microbial community composition, structure, and function.

Variation in growth rates among *Streptomyces* in response to plant diversity sheds further light on the ways in which plant communities may influence microbial strategies for managing resource competition. Specifically, these data suggest tradeoffs in resource allocation among primary and secondary metabolic functions among *Streptomyces* populations from plant communities varying in plant richness (Schlatter and Kinkel 2015). In this work, following quite similar early growth rates, *Streptomyces* from monoculture underwent a more accentuated decrease in their mid-phase growth rate than isolates from polyculture. Faster growth among isolates from polyculture may reflect selection for isolates that maximize resource allocation to microbial biomass accumulation. In contrast, slower growth of monoculture *Streptomyces* may be due to investment of resources among alternative traits, including secondary metabolites (antibiotics) to facilitate antagonistic interactions. Our data are in line with findings from recent work showing that *Streptomyces* with very high inhibition capacities had less efficient growth and utilized a smaller number of resources for growth than those with low inhibition capacities (Schlatter and Kinkel 2015). Consistent with this claim, monocultures have been shown to produce more antagonistic soil *Streptomyces* communities than polycultures (Bakker et al. 2013). Globally, our data lend support to the concept that the presence of multiple distinct nutrients, as hypothesized in polyculture habitats, offers opportunities for coevolutionary niche differentiation among *Streptomyces* as an alternative to direct antagonistic interactions more likely to prevail in monoculture habitats (Schlatter et al. 2008; Kinkel et al. 2011). Plant richness, by means of changes in the quantity and diversity of nutrients, may thus play a critical role in determining *Streptomyces* coevolutionary trajectories towards either an antagonistic arms race in monoculture or niche differentiation in polyculture.

As a rebuttal to this conjecture, it might be argued that greater niche widths in polyculture will increase the likelihood of niche overlap among *Streptomyces*, which we indeed observed among isolates from *Lc*. In this case, antagonistic interactions may be expected to be more intense among *Streptomyces* with large niche widths in polyculture. However, a closer look at the isolates from polyculture communities, having larger average niche widths, shows these isolates are also more differentiated in their preferred nutrients than isolates from monocultures possessing more narrow niche widths. Overall, this yields communities with more complex nutritional capacities in polyculture than in monoculture. Therefore, for a *Streptomyces* isolate in the rhizosphere of a plant in polyculture, access to a broad spectrum of substrates offers more opportunities to specialize on a subset of nutrients for which competition is minimized to support maximum growth and avoid costly direct antagonistic competition. These results provide further support for the hypothesis that plant polyculture offers more favorable trophic conditions for *Streptomyces* to become differentiated from one another in their nutrient use capacities than does monoculture.

Plant host also influenced *Streptomyces* mean growth, as isolates from *Lc* consistently exhibited more growth than isolates from *Ag*. Higher nitrogen content in the rhizosphere of the legume *Lc* (Supplemental Table 4), which establishes mutualistic symbioses with nitrogen-fixing *Rhizobium spp.* (Becker and Crockett 1976), may underlie the effects of plant host species on soil-borne *Streptomyces* growth in this work. This view is consistent with the finding that increased soil nitrogen content following legume planting has positive effects on microbial growth in rhizosphere soil (Li et al. 2012). In contrast, competition for limited nitrogen in the rhizosphere of *Ag* may impose selection for *Streptomyces* populations that invest more in nitrogen acquisition at the price of efficient growth. In addition to mean growth, *Streptomyces* from *Lc* plants

growing in polyculture had greater niche widths than did those from *Ag* plants in polyculture. Efficient nourishment on several carbon sources could be a characteristic of successful *Streptomyces* but requires advanced capacities to produce a high quantity and diversity of hydrolytic enzymes. Because of high nitrogen requirements for enzyme production (Allison 2005), we hypothesize that greater soil nitrogen content in the rhizosphere of *Lc* coupled with increases in the amount and diversity of carbon sources in polyculture were significant drivers for the selection of *Streptomyces* having greater competence to use a wider range of carbon nutrients with greater efficiency.

Accumulating evidence suggests that plant host and plant species richness are both critical determinants of the ecological niches for microbes in the rhizosphere. Consistent with previous work (Schlatter and Kinkel 2014), the data generated in this work provide evidence that *Streptomyces* possess diverse metabolic capacities, suggesting key roles in organic matter decomposition and nutrient cycling. Additionally, our data show that niche width and mean growth are greater among *Streptomyces* populations in the rhizosphere of the same plant hosts growing in polyculture than in monoculture, and in *Lc* than *Ag*, suggesting the significant roles of both plant host and plant community richness in imposing selection on soil populations. Moreover, *Streptomyces* from different plant species richness displayed distinct temporal growth dynamics, suggesting differences in resource allocation to distinct primary and secondary metabolic functions among *Streptomyces* from monoculture vs. polyculture. Finally, *Streptomyces* from high-richness plant communities were more niche-differentiated than those from low-richness communities, and this differentiation was more pronounced for *Streptomyces* growth on preferred nutrients. This implies more intense competition among *Streptomyces* for preferred resources in monoculture than polyculture plant communities. Overall, these findings suggest that *Streptomyces* populations respond in diverse ways to selection imposed by both individual plant hosts and by plant community characteristics (richness) (Fig. 10), and support the view that nutrient competition among *Streptomyces* in carbon-limited monocultures versus carbon-rich polycultures may result in distinct coevolutionary dynamics among soil populations, with significant consequences for community functional characteristics. Further work on the dynamics of nutrient use and competitive characteristics

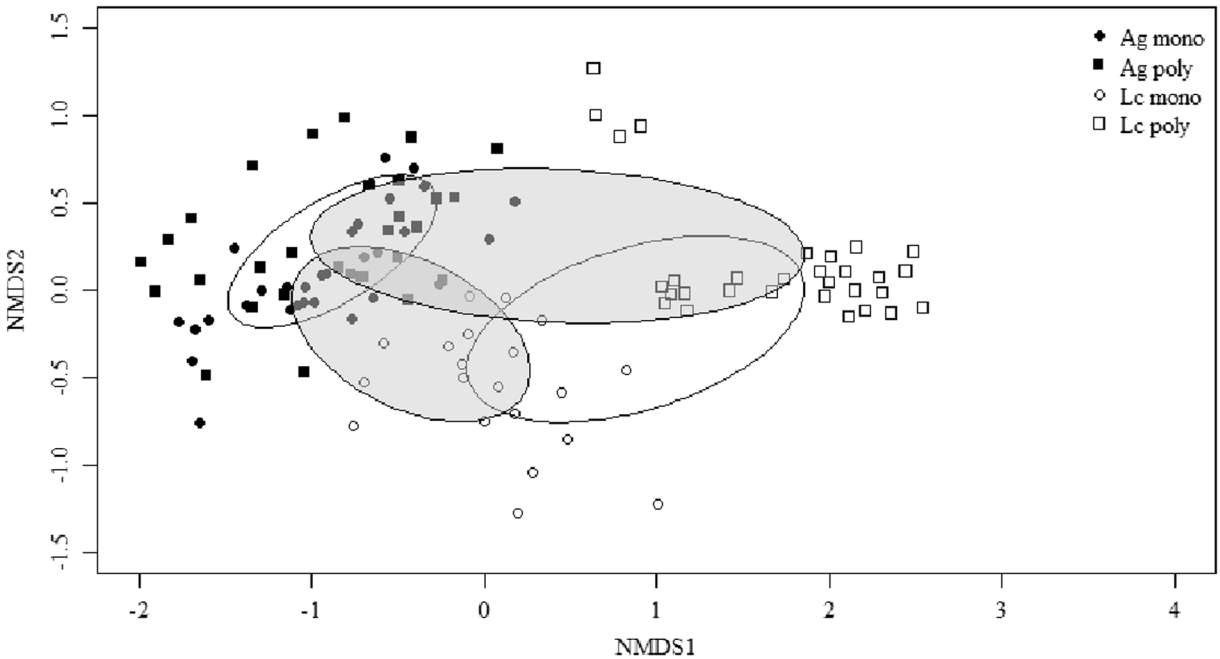

Fig. 10 Non metric multidimensional scaling (NMDS) based on nutrient use profiles for *Streptomyces* isolates from *Lc* and *Ag* in monoculture (mono) and polyculture (poly) at the end of the incubation period (time point 120 h). Ellipses connect and define the centroids of communities from different plant host regardless of plant community richness (unshaded ellipses) or different plant community richness regardless of plant host (shaded ellipses). Ellipses were constructed using the function "ordiellipse" in R vegan package

among sympatric *Streptomyces* and their implications for microbial fitness and ecosystem functioning is needed.

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