

# Resource Use of Soilborne *Streptomyces* Varies with Location, Phylogeny, and Nitrogen Amendment

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**Abstract** In this study, we explore variation in resource use among *Streptomyces* in prairie soils. Resource use patterns were highly variable among *Streptomyces* isolates and were significantly related to location, phylogeny, and nitrogen (N) amendment history. *Streptomyces* populations from soils less than 1 m apart differed significantly in their ability to use resources, indicating that drivers of resource use phenotypes in soil are highly localized. Variation in resource use within *Streptomyces* genetic groups was significantly associated with the location from which *Streptomyces* were isolated, suggesting that resource use is adapted to local environments. *Streptomyces* from soils under long-term N amendment used fewer resources and grew less efficiently than those from non-amended soils, demonstrating that N amendment selects for *Streptomyces* with more limited catabolic capacities. Finally, resource use among *Streptomyces* populations was correlated with soil carbon content and *Streptomyces* population densities. We hypothesize that variation in resource use among *Streptomyces* reflects adaptation to local resource availability and competitive species interactions in soil and that N amendments alter selection for resource use phenotypes.

## Introduction

Recent advances in microbial ecology have greatly expanded our knowledge of microbial species distributions, community

composition, and diversity. However, the ecological niches of microbial species within soil communities remain poorly characterized. Resource use is a critical aspect of an organism's ecological niche that can influence competitive interactions among species and the assembly, diversity, and functioning of communities [1–3]. Variation in resource use among soil bacteria is hypothesized to reflect adaptation to resource availability [4, 5], microbial or plant–microbe interactions [6–8], or general life history strategies (e.g., generalist versus specialist) [9, 10]. However, few studies have explored variation in resource use within natural bacterial populations in soil. Consequently, we have limited insight into the natural history of microbial resource use patterns and the environmental or management factors that influence variation in resource use among specific groups of soil microbes.

*Streptomyces* (phylum Actinobacteria) are filamentous, Gram-positive bacteria that are of great interest in agricultural systems for plant disease suppression [11, 12] and in clinical settings as major producers of antibiotic compounds [13]. Additionally, *Streptomyces* employ an array of extracellular enzymes in order to break down complex resources, including cellulose, lignin, and chitin [14–17]. Because of their tremendous metabolic capacities, *Streptomyces* can occupy a wide variety of ecological niches in nature [18, 19] and are an ideal taxon for exploring variation and adaptation of resource use among soil bacteria. However, despite the long history of using resource use patterns for bacterial taxonomy, there are few systematic data on variation in resource use phenotypes among *Streptomyces* populations from an ecological perspective or correlates of resource use among natural *Streptomyces* communities.

*Streptomyces* are well known for their prolific production of antibiotics, which are thought to mediate species interactions [20–22]. Antibiotic production by *Streptomyces* is often tied to the amount and identity of available resources [23, 24], consistent with the hypothesis that antagonistic interactions occur during resource competition. Though spatial variation in antibiotic phenotypes among *Streptomyces* populations has

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been documented [22], little is known about how resource use varies among *Streptomyces* communities from different locations. Resource inputs and management practices have been implicated in the selection for resource use and antibiotic inhibitory phenotypes among soilborne *Streptomyces* [5, 11, 12]. Nitrogen (N) inputs from agricultural fertilizers or atmospheric deposition can significantly alter the composition of soil microbial communities and reduce soil enzyme activities, respiration rates, and decomposition [25–30]. In this manner, N additions may alter the capacity of soils to serve as sinks for carbon storage [25, 31]. However, the impact of N amendments on *Streptomyces* resource use has not been explored, and it remains unknown if changes in soil bacterial community function associated with N amendment result from shifts in community phylogenetic composition, adaptation of resource use preferences of resident communities to high N environments, or direct effects of N amendment on bacterial activities in vivo [29, 30, 32, 33]. More detailed information on *Streptomyces* resource use patterns and how they respond to long-term N amendment will offer unique insight into the potential for resource competition within and among communities and enhance our ability to predict the effects of N amendment on soil communities.

In this work, we characterize *Streptomyces* collected from prairie soils to ask the following questions: (1) how does resource use vary among *Streptomyces* isolates?; (2) is variation in resource use among *Streptomyces* associated with space, phylogeny, or N amendment history?; and (3) do soil edaphic characteristics or *Streptomyces* population densities correlate with resource use phenotypes among soil *Streptomyces* populations? These data provide important insight into variation in resource use niches among *Streptomyces* populations in soil and suggest that local adaptation, resource competition, and N amendment impact *Streptomyces* resource use.

## Materials and Methods

### Soil Sampling and Processing

Soil samples were collected from long-term N-amended and non-amended plots (experiment E001) at the University of Minnesota Cedar Creek Ecosystem Science Reserve ([www.cedarcreek.umn.edu](http://www.cedarcreek.umn.edu)), a NSF Long-Term Ecological Research site in East Bethel, MN. In this experiment, N fertilization ( $\text{NH}_4\text{NO}_3$ ) treatments have been applied to field plots twice a year in early May and late June since 1982 (18 years prior to sampling). Six of these plots ( $4 \times 4$  m) were chosen for sampling so that three N-amended plots receiving the same  $\text{NH}_4\text{NO}_3$  treatment were paired in space with three non-amended plots. Non-amended plots (E001 Field C plots 08-A, 26-A, and 47-A, referred to here as plots 1, 3, and 5,

respectively) received a base nutrient treatment that lacks a source of N ( $10 \text{ g/m}^2 \text{ P}_2\text{O}_5$ ,  $10 \text{ g/m}^2 \text{ K}_2\text{O}$ ,  $20 \text{ g/m}^2 \text{ CaCO}_3$ ,  $15 \text{ g/m}^2 \text{ MgSO}_4$ , and  $0.0625 \text{ ml/m}^2$  trace mineral solution). N-amended plots (E001 Field C plots 10-D, 19-D, and 46-D, referred to here as 2N, 4N, and 6N, respectively) received the base nutrient treatment plus an additional nitrogen treatment ( $\text{NH}_4\text{NO}_3$  at  $5 \text{ g/m}^2$ ) that has been found to impact plant diversity [34].

Soil cores (three tightly bundled  $30 \times 1$  cm microcores) were taken from three randomly chosen locations within a  $1 \times 1$ -m section at the center of each plot. Soil samples from each microcore were dried overnight under a double layer of sterile cheesecloth, dilution-plated onto oatmeal agar amended with antibiotics ( $300 \text{ }\mu\text{g/l}$  nystatin,  $50 \text{ }\mu\text{g/l}$  cycloheximide,  $2.5 \text{ }\mu\text{g/l}$  polymyxin B,  $0.2 \text{ }\mu\text{g/l}$  penicillin), and incubated at  $28 \text{ }^\circ\text{C}$  for 7 days. Detailed description of microcore sampling and culturing conditions can be found in [22]. For each microcore ( $n=54$  cores), total culturable streptomycete densities were estimated (CFU per gram soil) and 20 randomly selected *Streptomyces* colonies were isolated based on characteristic *Streptomyces* morphology for a total of  $\sim 1,080$  isolates. Streptomycete densities were averaged across microcores for each location and plot for subsequent analyses. From this collection, 459 *Streptomyces* isolates were characterized for resource utilization phenotypes. Most isolates characterized were from plots 1 and 2N ( $n=152$  and  $128$ , respectively); fewer isolates were characterized from plots 3, 4N, 5, and 6N (plot 3,  $n=47$ ; plot 4N,  $n=47$ ; plot 5,  $n=46$ ; plot 6N,  $n=39$ ). Isolates for each location within plots were chosen randomly. This approach allows for the characterization of resource use phenotypes among *Streptomyces* within and among plots. Subsamples of microcores from each sampling location within each plot were bulked and submitted to the University of Minnesota Soil Testing Laboratory for determination of soil characteristics (pH,  $\text{NO}_3\text{-N}$ , Bray-P, K, and total C; <http://ral.cfans.umn.edu>).

### Resource Use Characterization

Resource use phenotypes were determined for *Streptomyces* isolates on 95 sole carbon sources using Biolog SF-P2 plates (Biolog, Inc. Hayward, CA) as described previously [5]. Briefly, fresh spore suspensions of each *Streptomyces* isolate were adjusted to an optical density of 0.22 at 590 nm, diluted according to the manufacturer's instructions ( $1.5 \text{ ml}$  spore suspension in  $13.5 \text{ ml}$   $0.2 \%$  carrageenan), and inoculated into 96-well Biolog plates. The absorbance (au) of each well was determined after 3 days of incubation at  $28 \text{ }^\circ\text{C}$  using a Multiskan EX microplate reader (Labsystems, Helsinki, Finland) at 590 nm. For each plate, the absorbance of the water control well was subtracted from the absorbance of all other wells before analysis.

## 16S rRNA Gene Sequencing

The 16S rRNA gene was sequenced for a random subset of 323 of the 459 isolates as described previously [35] (plot 1,  $n=146$ ; plot 2N,  $n=51$ , plot 3,  $n=39$ ; plot 4N,  $n=27$ ; plot 5,  $n=42$ ; plot 6N,  $n=18$ ). Briefly, genomic DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions with minor modification, and 16S rRNA genes were amplified using the universal bacterial primers 27F (5'-AGAGTTTGAT CCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGAT CCAGCCGCA-3') in a 50- $\mu$ l reaction volume using PCR Supermix High Fidelity master mix (Invitrogen, Carlsbad, CA) with 10 pM of each primer and 100 ng template DNA following the thermocycling protocol of [36]. Amplicons were sequenced using the forward primer (27F) at the University of Minnesota Biomedical Genomics Center (Saint Paul, MN). Sequences were edited manually, aligned, and trimmed to 703 bp of good quality alignment using BioEdit [37] for further analyses. Sequence accessions are available in GenBank (Supplemental Table 1).

## Analyses

### Resource Use

We considered used resources to be those on which a *Streptomyces* isolate grew to an absorbance greater than 0.005 above the water control well. Using this definition, niche width, resource use efficiency, and efficiency on preferred resources were determined for each isolate. We defined the niche width of an isolate as the number of used resources, the resource use efficiency of each isolate was defined as the mean absorbance value for used resources, and the efficiency on preferred resources was defined by calculating the mean absorbance on the five resources on which each isolate grew best (largest absorbance values). Similarity in resource use profiles among *Streptomyces* isolates was calculated using the Bray–Curtis index in the vegan package for R [38]. All subsequent statistical analyses were conducted in R version 2.14 [39].

### Phylogenetic Analyses

16S rRNA gene sequences were used to compute pairwise distances between isolates and construct a neighbor-joining (NJ) tree using 1,000 bootstraps. The NJ tree was analyzed using the unweighted unifracs metric and  $P$  test using 1,000 bootstraps to test for significant differences in *Streptomyces* community structure among different plots and locations and among nitrogen treatments. Nonmetric multidimensional scaling (NMDS) was conducted on the distance matrix to

visualize similarity among isolates. Further, isolates were binned into operational taxonomic units (OTUs) at 99 % sequence similarity using mothur version 1.22 [40].

### Comparing Resource Use and Phylogeny

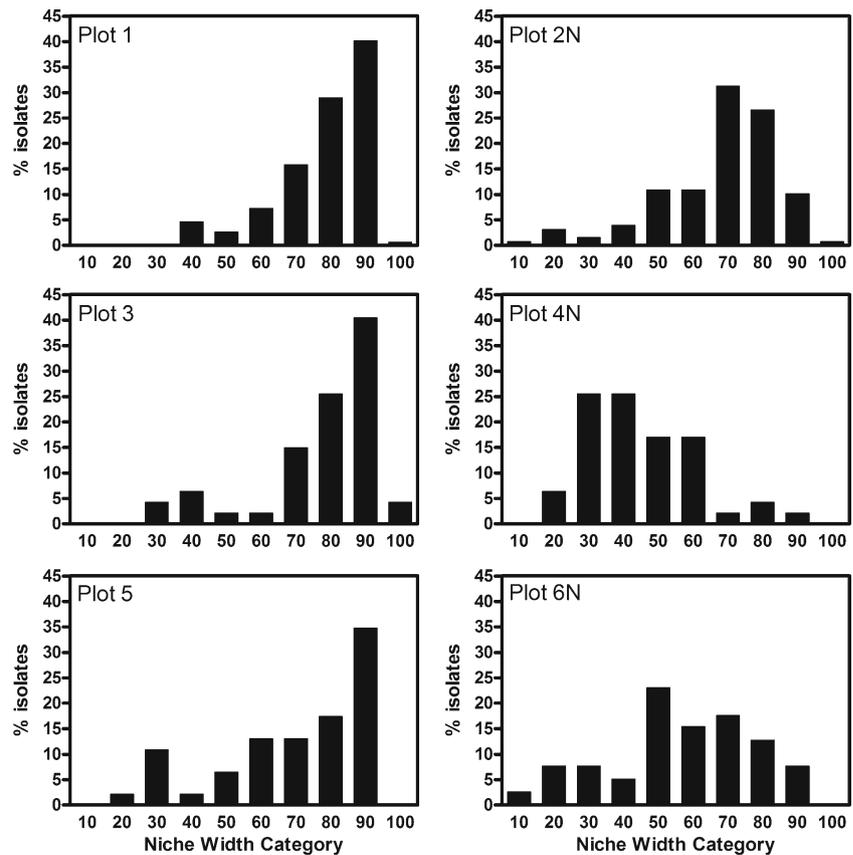
A Mantel test was performed to test for a relationship between genetic distance and resource use phenotypes. The distance matrix of 16S sequences and the Bray–Curtis dissimilarity of resource use profiles of *Streptomyces* isolates were correlated using 999 permutations in the vegan software package of R. Also, niche width, resource use efficiency, and efficiency on preferred resources were compared among large OTUs ( $n \geq 16$ ) and among isolates from the same OTU but from different plots and locations.

## Results

### Resource Use Among Soilborne *Streptomyces*

*Streptomyces* grew on a wide variety of carbohydrates, carboxylic acids, polymers, amines/amides, and amino acids as sole carbon sources. Glycerol was most frequently used as a sole carbon source overall (used by 98 % of isolates), followed by alpha-D-glucose and adenosine (each used by 96 % of isolates; Supplemental Table 2). However, the best growth on average occurred on Tween 40 and L-malic acid, followed more distantly by glycerol (Supplemental Table 3). On average, *Streptomyces* isolates used 68.7 of 95 possible resources, though niche widths (the number of resources used for growth) among individual *Streptomyces* demonstrated substantial variability and ranged from 11 to 95 resources. Frequency distributions of niche widths for each plot (Fig. 1) tend to be skewed left: *Streptomyces* communities were generally composed of many generalists with relatively large niche widths and fewer specialists with narrow niche widths. Across all isolates, resource use efficiency (the mean growth achieved across all used resources) ranged from 0.06 to 0.11 au with an average growth efficiency of  $0.08 \pm 0.02$  au. Growth efficiency on preferred resources (the mean growth achieved on the top 5 resources for each isolate) ranged from 0.93 to 1.91 au with an average of  $1.34 \pm 0.33$  au. Thus, the catabolic potential of *Streptomyces* varies substantially among individual isolates. Moreover, resource use patterns for individual *Streptomyces* were very diverse. Considering used resources using a discrete (+/-) approach, there were 453 unique patterns of resource utilization among 459 isolates. These data highlight the wide diversity of *Streptomyces* and their capacity to metabolize a range of resource sources and suggest that *Streptomyces* have substantial potential to adapt to different resource conditions in soil.

**Fig. 1** Frequency distributions of *Streptomyces* niche widths among plots. Isolates from non-amended plots (plots 1, 3, and 5) tended to have larger niche widths than isolates from N-amended plots (plots 2N, 4N, and 6N)

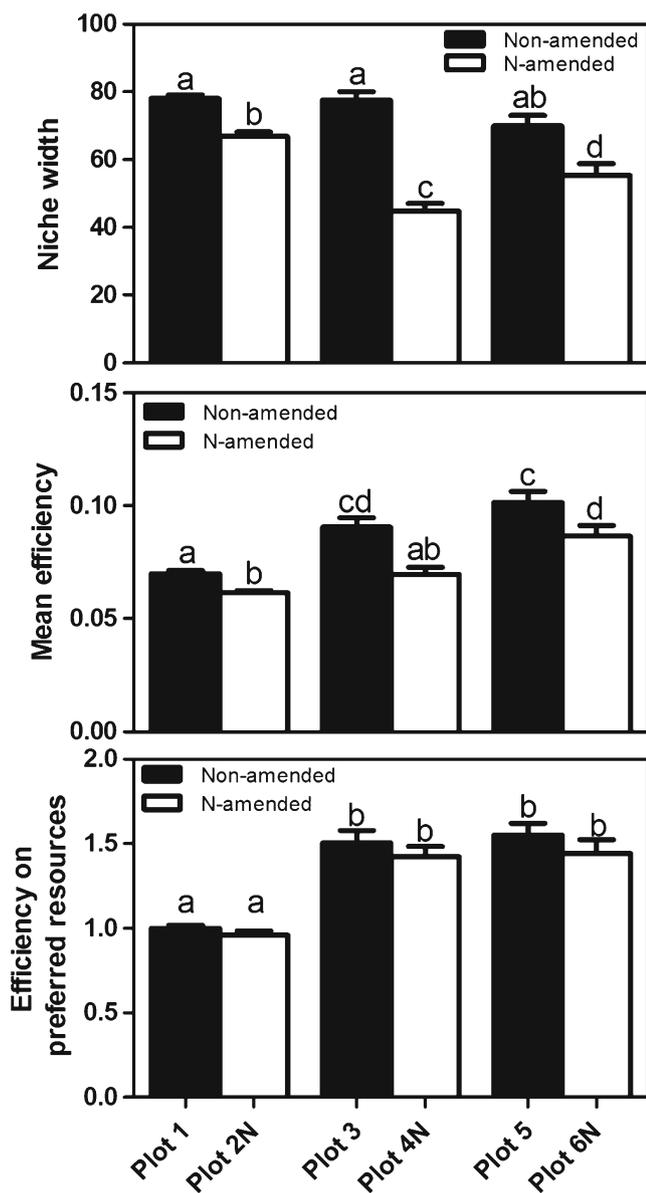


### Spatial Variation in *Streptomyces* Resource Use

Among individual *Streptomyces*, isolates from the same plots had more similar resource use phenotypes than *Streptomyces* from different plots (mean Bray–Curtis dissimilarity=0.43 and 0.52 for same versus different plots, respectively; Welch's *t* test,  $p < 0.0001$ ,  $t = 81.22$ ). Within plots (sampling locations  $< 1 \text{ m}^2$  apart), *Streptomyces* from the same location consistently had more similar resource use phenotypes than those from different locations in that plot (Welch's *t* test of mean Bray–Curtis dissimilarity among locations versus within location: plot 1,  $p < 0.0001$ ,  $t = 5.20$ ; plot 2N,  $p < 0.0001$ ,  $t = 4.53$ ; plot 3,  $p < 0.0001$ ,  $t = 8.76$ ; plot 4N,  $p = 0.058$ ,  $t = 1.90$ ; plot 5,  $p < 0.0001$ ,  $t = 4.50$ ), though differences were not statistically significant for *Streptomyces* from plot 6N ( $p = 0.61$ ,  $t = 0.51$ ). Thus, isolates from the same locations in soil were more likely to be able to metabolize the same resources and use them at similar growth efficiencies when compared to isolates from different locations.

Niche width, resource use efficiency, and growth efficiency on preferred resources varied among spatially distinct *Streptomyces* communities. Niche width differed significantly among *Streptomyces* from different plots (Fig. 2; ANOVA:  $F = 36.8$ ,  $p < 0.0001$ ). *Streptomyces* from non-amended plots

1 and 3 had the largest niche widths on average, while those from N-amended plots 4N and 6N used the fewest resources. There were also significant differences in mean niche widths among *Streptomyces* communities from different locations within the same plot for four of the six plots (plots 1, 2N, 3, and 4N, but not 5 or 6N; ANOVA,  $p \leq 0.03$ ,  $F \geq 3.57$ ;  $p \geq 0.09$ ,  $F \leq 2.57$ , respectively). Similarly, mean resource use efficiency varied among *Streptomyces* from different plots (Fig. 2; ANOVA:  $F = 35.0$ ,  $p < 0.0001$ ). *Streptomyces* from plots 3, 5, and 6N grew more efficiently than those from plots 1, 2N, and 4N (Tukey HSD,  $p \leq 0.05$ ). However, there were no significant differences in mean growth efficiency of *Streptomyces* among locations within plots (data not shown). When considering only the five most preferred resources for each isolate, *Streptomyces* from plots 1 and 2N were less efficient than *Streptomyces* from all other plots (Fig. 2; ANOVA,  $F = 45.85$ ,  $p < 0.0001$ ; Tukey HSD,  $p < 0.0001$ ). Further, among locations within plots, there were significant differences in growth efficiency on preferred resources for plots 3 (ANOVA,  $F = 10.27$ ,  $p = 0.0002$ ) and 5 (ANOVA,  $F = 11.77$ ,  $p < 0.0001$ ), but not plots 1, 2N, 4N, or 6N (ANOVA,  $p \geq 0.14$ ,  $F \leq 2.12$  for each plot). Together these data demonstrate variation in *Streptomyces* resource use phenotypes at spatial scales ranging from  $< 1$  to  $50 \text{ m}^2$ .



**Fig. 2** Mean niche width (*top row*), mean growth efficiency (*middle row*), and efficiency on preferred resources (*bottom row*) of *Streptomyces* from different plots in soil. Non-amended plots (*black bars*) are grouped with their paired N-amended plot (*white bars*). Significant differences ( $p < 0.05$ ) among plots are indicated by *different letters above bars* and *letters found in common above bars* indicate no significant difference ( $p > 0.05$ )

#### Long-term Nitrogen Amendment and *Streptomyces* Resource Use

Long-term N-amended plots had *Streptomyces* communities that differed significantly in resource use from communities from non-amended plots. Among paired plots (1 and 2N; 3 and 4N; 5 and 6N), *Streptomyces* from N-amended plots had consistently smaller average niche widths than those from non-amended plots (Fig. 2; Welch's  $t$  test,  $p \leq 0.002$  and

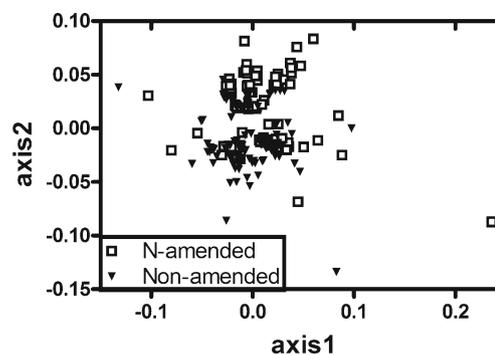
$t \geq 3.16$  for each plot pair). *Streptomyces* from N-amended plots used from 14 to 42 % fewer resources than *Streptomyces* from their paired non-amended plot. Moreover, *Streptomyces* communities from N-amended plots also had significantly reduced resource use efficiency compared to communities from non-amended plots (Fig. 2; Welch's  $t$  test,  $p \leq 0.03$  and  $t \geq 2.12$  for each pair), with mean growth efficiency over all used resources reduced 12–21 % in N-amended plots. *Streptomyces* from N-amended plots also grew consistently less efficiently on preferred resources than those from paired non-amended plots, though the differences were not statistically significant (Fig. 2; Welch's  $t$  test,  $p \geq 0.17$ ,  $t \geq 1.39$ ). Additionally, *Streptomyces* from the same nitrogen treatment (N-amended and non-amended) but different plots had significantly more similar resource use phenotypes than those from different treatments (Bray–Curtis dissimilarity=0.49 and 0.52, respectively,  $p < 0.0001$ ,  $t = 32.26$ ). These results indicate that N-amended soils support *Streptomyces* communities that use fewer resources, have distinct resource use profiles, and grow less efficiently than communities in non-amended soils.

#### *Streptomyces* Phylogeny

*Streptomyces* community composition varied among plots and among locations within plots (unweighted unifracs and  $P$  test,  $p < 0.001$ ). Moreover, N-amended and non-amended communities differed significantly in their phylogenetic composition (unweighted unifracs and  $P$  test,  $p < 0.001$ ; Fig. 3). Thus, *Streptomyces* community composition in soils varied across small spatial scales and soil communities under long-term N amendment supported *Streptomyces* that were phylogenetically distinct from those found among non-amended soils.

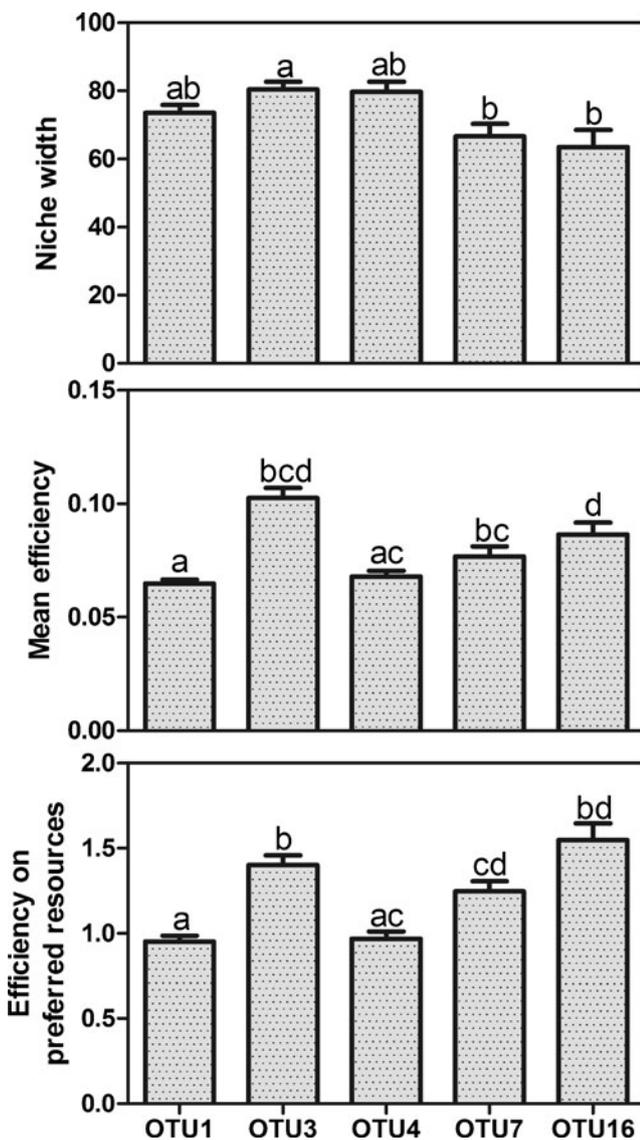
#### Relationships Between Resource Use and Phylogeny

Isolates that were more closely related had more similar resource use phenotypes (Supplemental Fig. 1). Among all



**Fig. 3** Nonmetric multidimensional scaling (NMDS) plot of 16S rRNA gene sequences (Bray–Curtis dissimilarity) among *Streptomyces* from N-amended (*open squares*,  $n = 96$ ) and non-amended (*closed triangles*,  $n = 227$ ) plots

isolate pairs ( $n=52,003$  pairwise combinations), there was a significant positive correlation between 16S sequence distance and dissimilarity in resource use patterns (Mantel  $r=0.24$ ,  $p=0.001$ ). The 323 *Streptomyces* isolates for which the 16S rRNA gene was sequenced formed 66 OTUs at a 99 % similarity cutoff. *Streptomyces* OTUs differed in their capacity to utilize resources. Among the five largest OTUs (Fig. 4,  $n \geq 16$  isolates per OTU), there were significant differences in mean niche width (ANOVA,  $p=0.002$ ,  $F=4.48$ ), mean resource use efficiency (ANOVA,  $p<0.001$ ,  $F=24.3$ ), and mean efficiency on preferred resources (ANOVA,  $p<0.001$ ,  $F=21.6$ ). Moreover, among all non-unique OTUs ( $n>1$  isolates per OTU),

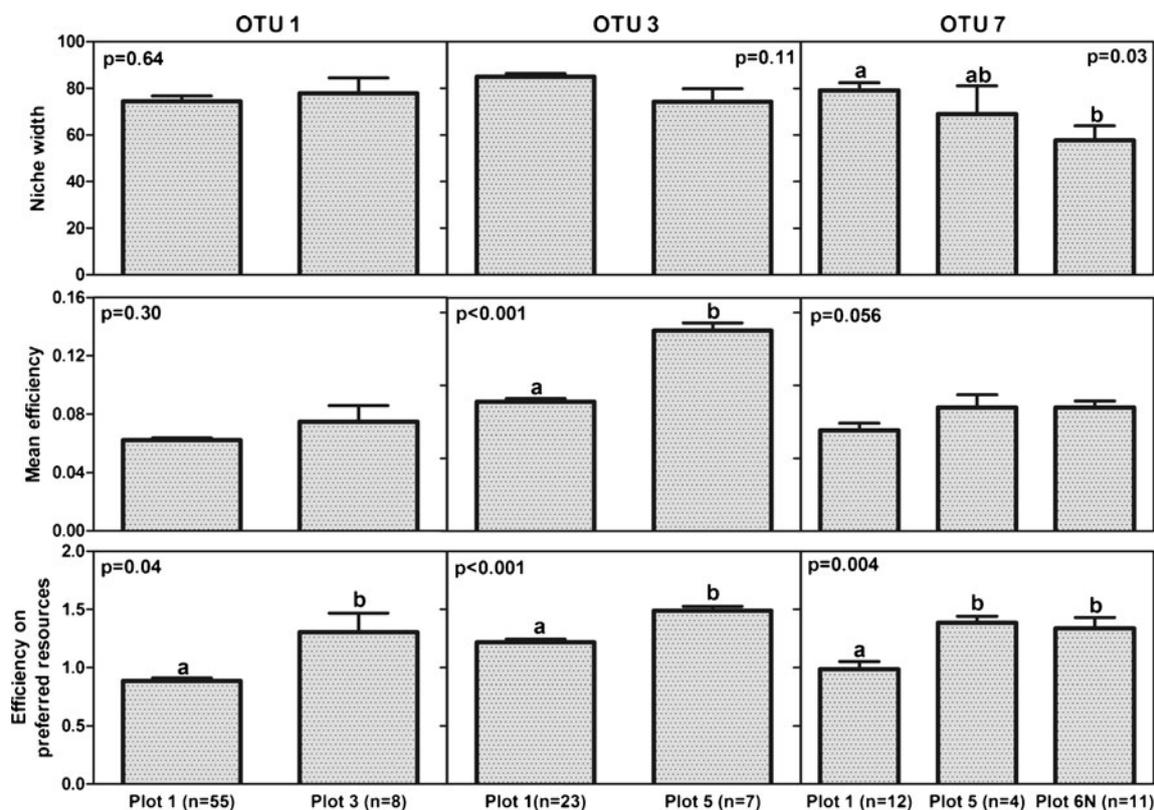


**Fig. 4** Niche width (top row), mean growth efficiency (middle row), and growth efficiency on preferred resources (bottom row) among the five largest *Streptomyces* OTUs. In each category, significant differences among OTUs are indicated by different letters above bars ( $p<0.05$ ) and letters found in common above bars indicate no significant difference ( $p>0.05$ )

*Streptomyces* from the same OTU had significantly more similar resource use phenotypes to each other than to isolates from different OTUs (Welch's  $t$  test,  $p<0.0001$ ,  $t=47.53$ ; Bray–Curtis index=0.37 versus 0.48, respectively). Thus, some *Streptomyces* OTUs could use a broader array of resources (e.g., OTUs 3 and 4) or grow more efficiently (e.g., OTUs 3 and 16) than others, suggesting that OTUs may have different life history strategies and be adapted to distinct ecological niches in the soil environment.

There was also extensive variation in resource use within individual OTUs. For example, niche widths ranged from 11 to 93 of 95 possible resources within OTU 3. *Streptomyces* belonging to the same OTU but originating from different plots differed significantly in resource use phenotypes. When resource use phenotypes among four OTUs with  $n \geq 4$  *Streptomyces* each from at least two plots were compared (OTUs 1, 3, 7, and 16), there were significant differences in resource use phenotypes among *Streptomyces* from different plots for three of the four OTUs (Fig. 5). Growth efficiency on preferred resources varied among plots for three *Streptomyces* OTUs (OTUs 1, 3, and 7), whereas mean growth efficiency and niche width differed among plots for a single OTU each (OTUs 3 and 7, respectively). Additionally, within each of the four OTUs, *Streptomyces* from the same plot had significantly more similar resource use patterns to each other than *Streptomyces* from different plots (Welch's  $t$  test of mean Bray–Curtis index;  $p<0.003$ ,  $t \geq 3.04$  for each OTU). This suggests that variation in resource use phenotypes within *Streptomyces* genetic groups is associated with space and that closely related *Streptomyces* are differentially adapted to local environments across the landscape.

Because N-amended plots differed in composition from non-amended plots, there were only two OTUs (OTU 7 and OTU 16) with sufficient numbers of *Streptomyces* isolates in both treatments to test for differences in resource use across nitrogen treatments among isolates from the same OTU (Fig. 6). Among *Streptomyces* belonging to OTU 7 (N-amended,  $n=14$ ; non-amended,  $n=17$ ), isolates from N-amended plots were significantly more efficient overall resources (Welch's  $t$  test,  $p=0.03$ ,  $t=2.30$ ) and on preferred resources (Welch's  $t$  test,  $p=0.03$ ,  $t=2.36$ ), but had smaller niche widths than those from non-amended plots (Welch's  $t$  test,  $p=0.002$ ,  $t=3.47$ ). Similarly, niche widths among *Streptomyces* from OTU 16 (N-amended,  $n=10$ ; non-amended,  $n=14$ ) were significantly smaller among isolates from N-amended plots than those from non-amended plots (Welch's  $t$  test,  $p=0.03$ ,  $t=2.41$ ). However, mean efficiency and efficiency on preferred resources did not differ significantly between treatments for isolates in OTU 16 (Welch's  $t$  test,  $p \geq 0.12$ ,  $t \leq 1.69$  in each case). Thus, N amendment consistently selected for more narrow niche widths among *Streptomyces* from the same 16S group but had variable effects on resource use efficiency. Further, when resource use patterns among isolates from the same OTU were compared between N



**Fig. 5** Niche width (*top row*), mean growth efficiency (*middle row*), and growth efficiency on preferred resources (*bottom row*) among *Streptomyces* from the same OTU but isolated from different plots. *p* values for OTUs 1 and 3 are from Welch's *t* test between *Streptomyces* from different plots. *p* values for OTU 7 represent an ANOVA comparing

*Streptomyces* among plots 1, 5, and 6N. Within each category, different letters above bars represent significant differences ( $p < 0.05$ ) among plots for each OTU and letters found in common above bars indicate no significant difference ( $p > 0.05$ )

treatments, resource use patterns were significantly more similar among isolates from the same versus different treatments (Welch's *t* test,  $p < 0.0001$ ,  $t = 6.20$  and  $p = 0.0015$ ,  $t = 3.21$  for OTU 7 and OTU 16, respectively). These data suggest that even when the same OTU is considered, resource use patterns among *Streptomyces* shift significantly in soils under long-term N amendment.

#### Soil Characteristics and *Streptomyces* Resource Utilization

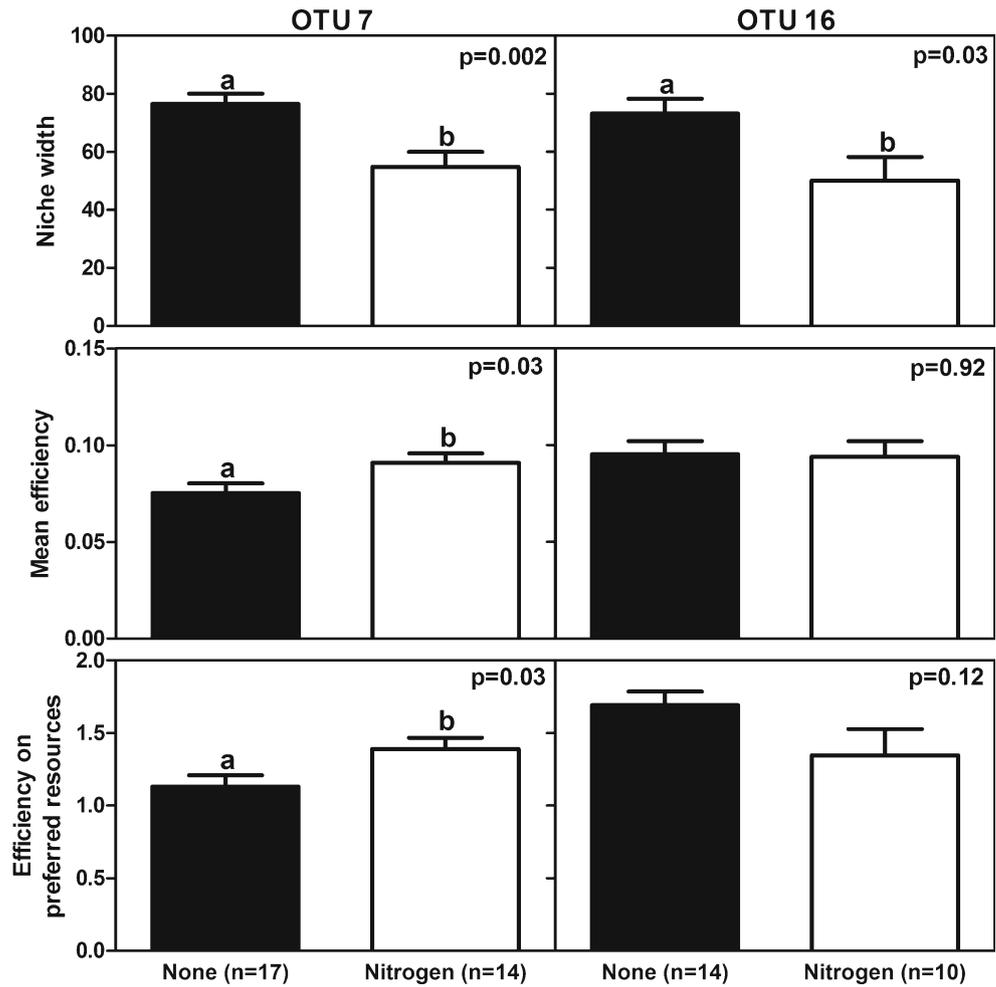
Plots differed significantly in soil pH, N, and C but not P or K (Supplemental Table 4). Among measured soil resources, only C and K were correlated significantly with resource use phenotypes among *Streptomyces* communities. Specifically, total C was negatively correlated with mean niche width (Fig. 7;  $r = -0.51$ ,  $p = 0.03$ ) and mean resource use efficiency (Fig. 7;  $r = -0.52$ ,  $p = 0.03$ ). Thus, *Streptomyces* from soils with more C used fewer resources and grew less efficiently than soils with less C, suggesting that C limitation may select for *Streptomyces* with more efficient growth and the capacity to use a broader range of resources. In contrast, soil K was positively correlated with efficiency on preferred resources ( $r = 0.47$ ,  $p = 0.05$ ). *Streptomyces* from high potassium soils grew more

efficiently on preferred resources than *Streptomyces* from low potassium soils.

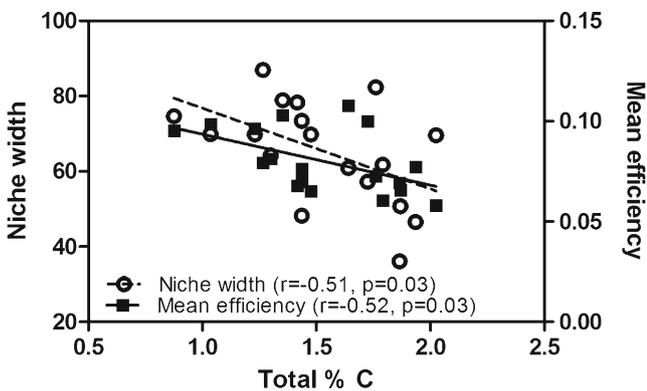
#### Relationships Between *Streptomyces* Densities and Resource Use

Relationships between *Streptomyces* densities and resource use phenotypes differed among treatments. Among N-amended plots, there were no significant correlations between *Streptomyces* densities and niche width ( $r = 0.22$ ,  $p = 0.57$ ), mean efficiency ( $r = -0.02$ ,  $p = 0.96$ ), or efficiency on preferred resources ( $r = -0.38$ ,  $p = 0.32$ ). In contrast, among non-amended plots, *Streptomyces* densities were negatively correlated with mean efficiency ( $r = -0.68$ ,  $p = 0.05$ ) and efficiency on preferred resources ( $r = -0.66$ ,  $p = 0.05$ ), but not niche width ( $r = 0.21$ ,  $p = 0.58$ ). Thus, higher density communities supported *Streptomyces* with less efficient growth than lower density communities in non-amended soils but not in N-amended soils. Among all locations, there were no significant correlations between *Streptomyces* densities and niche width ( $r = 0.16$ ,  $p = 0.53$ ) or mean growth efficiency ( $r = -0.24$ ,  $p = 0.24$ ), though there was a marginally significant correlation between *Streptomyces* densities and mean growth

**Fig. 6** Niche width (*top row*), mean growth efficiency (*middle row*), and efficiency on preferred resources (*bottom row*) among *Streptomyces* from the same OTU but different nitrogen treatments. *p* values from Welch's *t* tests comparing *Streptomyces* from non-amended plots (*black bars*) and N-amended plots (*white bars*) are presented. In each category, significant differences among OTUs are indicated by different letters above bars ( $p < 0.05$ )



efficiency on preferred resources ( $r = -0.46, p = 0.057$ ). *Streptomyces* from high-density communities tended to be less efficient on their preferred resources than those from low-density communities.



**Fig. 7** Relationship between average niche widths (*open circles, dashed line*) and mean growth efficiency (*closed squares, solid line*) of *Streptomyces* from different locations with soil total carbon content (total % C)

**Discussion**

Resource use patterns among soilborne *Streptomyces* are highly diverse. Almost every isolate (453 of 459) grew on a unique suite of resources. The ability of *Streptomyces* isolates to use a wide range of resources and their extensive variation in growth efficiencies suggests great potential for *Streptomyces* to adapt to local environments in soil. Indeed, the greater similarity in resource use phenotypes among *Streptomyces* from the same versus different soil locations less than 1 m apart suggests that adaptation of resource use phenotypes among *Streptomyces* populations is highly localized across the landscape. Localized patterns in resource use phenotypes may result from ecological species sorting, where *Streptomyces* best adapted to exploit local resource pools are able to colonize and survive [41], or ongoing selection for indigenous *Streptomyces* to use available resources [42]. Significant variation in community phylogenetic composition among locations in soil suggests that species sorting may contribute to local patterns in resource use. However, *Streptomyces* belonging to the same OTU but isolated from different locations in

soil also differed in resource use phenotypes, providing evidence that *Streptomyces* within an OTU are locally adapted. This study provides empirical data to support the hypothesis that adaptation of resource use phenotypes contributes to the ability of *Streptomyces* to occupy a wide variety of soil niches [43] and that resource use patterns among *Streptomyces* are shaped in part by local selection pressures among locations in soil. Local adaptation of *Streptomyces* OTUs is likely to contribute to the large diversity of resource use phenotypes found within and among phylogenetic groups.

Adaptation of resource use patterns in natural communities is hypothesized to reflect the quantity and types of available resources [5, 8]. The negative relationship of soil C and *Streptomyces* niche width and mean growth efficiency suggests that the availability of C has a significant selective effect on resource use phenotypes among *Streptomyces* in soil. Specifically, *Streptomyces* in high-C environments tended to be niche specialists and on average grew less efficiently than *Streptomyces* from low-C environments. In contrast, since C availability in soil is often considered to limit microbial growth [44], low-C environments may select for generalist *Streptomyces* that are better able to exploit a broad range of carbon sources for nutrition (large niche widths) and use them more efficiently for growth. These data seem contrary to the predictions of r- and K-selection theory that niche specialists should be able to grow more efficiently than generalists [45]. However, tradeoffs between niche width and resource use efficiency potentially ignore fitness costs incurred by microbial investment in species interaction phenotypes, such as antibiotic production or resistance [46–48]. For example, high-C environments, which are likely to support higher microbial population densities and more intense competition, may favor *Streptomyces* that grow less efficiently but invest more energy in antagonistic antibiotic production or resistance phenotypes [49–51]. In contrast, low-C environments may favor *Streptomyces* that are able to use a wide variety of resources and grow efficiently but invest little in species interaction phenotypes. However, while total carbon may be one predictor of resource use or inhibitory phenotypes among *Streptomyces* [11, 12, 52–54], recent work suggests that resource diversity may also play a significant role in local selection [19, 55].

Plants are a major source of carbon for heterotrophic soil microbes and changes in plant community diversity and productivity can impact soil microbial communities [56–58]. At Cedar Creek, long-term N amendments have resulted in decreased plant diversity and increased productivity [34, 59]. Low diversity plant communities in N-amended plots may supply a less diverse array of plant-derived resources to microbes in soil than high diversity plant communities. As a result, *Streptomyces* in N-amended soils may have adapted to the more limited suite of available resources, as reflected in narrower niche widths among *Streptomyces* from N-amended

versus non-amended plots. Greater primary productivity among plant communities in N-amended versus non-amended soils and the subsequent increases in total soil C may also have contributed to the negative relationships between niche width and growth efficiency with soil C described above.

Selection for *Streptomyces* with different resource use strategies in soil under N amendment may reflect a reduced need for *Streptomyces* to decompose compounds to access N in soil organic matter [60]. Alternatively, N amendment may alter the recalcitrance or quality of organic compounds in soil via changes in plant-derived carbon inputs [61]. It has been hypothesized that N amendments shift the composition of soil microbial communities to favor more specialized and less efficient (copiotrophic) microbes [10, 30, 62]. Different community composition in combination with greater similarity in resource use patterns among *Streptomyces* in N-amended versus non-amended soils is consistent with the hypotheses that reductions in microbial activity frequently observed under N amendment are due in part to shifts in communities towards copiotrophic microbes. However, among *Streptomyces* from the same OTU, those from N-amended soils had smaller niches and less efficient growth than *Streptomyces* from non-amended soils. This suggests that, in addition to shifts in community composition towards OTUs with smaller niche widths and less efficient growth, *Streptomyces* in long-term N-amended soils have been selected to use fewer resources in comparison to closely related isolates from non-amended soils. In total, these data suggest that reductions in microbial activities under long-term N amendment result from both shifts in phylogenetic composition and selection for more specialized resource use phenotypes both within and among phylogenetic groups (OTUs).

Nitrogen fertilizers are routine in most agricultural systems, yet little is known about how N amendments influence interactions among soil bacteria [33]. Smaller *Streptomyces* niche widths and altered resource use preferences under long-term N amendment are likely to significantly impact species interactions among *Streptomyces*, especially competition for resources. In particular, if smaller niche widths lead to more intense resource competition, then N amendment may indirectly select for *Streptomyces* that produce antibiotics to defend resources. Alternatively, if smaller resource use niches increase the likelihood of *Streptomyces* strains having distinct resource use patterns, *Streptomyces* may be less likely to engage in resource competition (i.e., niche differentiation). However, N-amended plots did not always harbor *Streptomyces* with smaller niche widths and less efficient growth patterns than every non-amended plot. Rather, reductions in niche widths and growth efficiency were observed between plots paired in space. This highlights the importance of understanding drivers of microbial phenotypes at small spatial scales and suggests that variation in bacterial phenotypes prior

to N amendment may be important for predicting the outcomes of N amendment on soil communities.

Resource use is a critical aspect of the ecological niche of microbes in soil and plays a key role in determining species interactions, adaptation, and community assembly. Here we document that *Streptomyces* have diverse resource use patterns that vary with space, phylogeny, and N amendment. Moreover, soil C and *Streptomyces* population densities were correlated with niche width and growth efficiency, suggesting that resource use among *Streptomyces* is linked to resource competition. Further study of resource use, adaptation, and competition among *Streptomyces* and other microbial groups will provide critical insight into microbial population dynamics and species interactions in natural systems.

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