Abstract Word Count: 245 (Lines 26-46)

Importance: 139 (Lines 49-60)

Text Word Count: 4992 (Lines 65-394)
(Word limit, excluding the references, table footnotes, and figure legends)

Title: Long-term nutrient enrichment of an oligotroph-dominated wetland increases bacterial diversity in bulk soils and plant rhizospheres

Running title (54 chars and spaces): Root bacteria community response to nutrient additions

Authors: Regina B. Bledsoe[a#], Carol Goodwillie[a], and Ariane L. Peralta[a]

Affiliations: [a]Department of Biology, East Carolina University, Greenville, NC 27858, USA

Corresponding author: telephone: +1 252.328.2712, e-mail: bledsoer15@students.ecu.edu

Abbreviations: C: carbon, N: nitrogen, OTU: operational taxonomic unit, P: phosphorus, K: potassium

Author contributions: RBB, CG, and ALP conceived and designed the research; RBB collected and analyzed the data; RBB wrote the manuscript; all authors performed field work and edited the manuscript.

ABSTRACT

In nutrient-limited conditions, plants rely on rhizosphere microbial members to facilitate nutrient acquisition and in return plants provide carbon resources to these root-associated microorganisms. However, atmospheric nutrient deposition can affect plant-microbe relationships by changing soil bacterial composition and by reducing cooperation between microbial taxa and plants. To examine how long-term nutrient addition shapes rhizosphere community composition, we compared traits associated with bacterial (fast growing copiotrophs, slow-growing oligotrophs) and plant (C3 forb, C4 grass) communities residing in a nutrient poor wetland ecosystem. Results revealed that oligotrophic taxa dominated soil bacterial communities and that fertilization increased the presence of oligotrophs in bulk and rhizosphere communities. Additionally, bacterial species diversity was greatest in fertilized soils, particularly in bulk soils. Nutrient enrichment (fertilized vs. unfertilized) and plant association (bulk vs. rhizosphere) determined bacterial community composition; bacterial community structure associated with plant functional group (grass vs. forb) was similar within treatments but differed between fertilization treatments. The core forb microbiome consisted of 602 unique taxa and the core grass microbiome consisted of 372 unique taxa. Forb rhizospheres were enriched in potentially disease suppressive bacterial taxa and grass rhizospheres were enriched in bacterial taxa associated with complex carbon decomposition. Results from this study demonstrate that fertilization serves as a strong environmental filter on the soil microbiome, which leads to distinct rhizosphere communities and can shift plants' influence on the rhizosphere microbiome. These taxonomic shifts within plant rhizospheres could have implications for plant health and ecosystem functions involving carbon and nitrogen cycles.

Importance

Over the last century, humans have substantially altered nitrogen and phosphorus cycling. Use of synthetic fertilizer and burning of fossil fuels and biomass have increased nitrogen and phosphorous deposition, which results in unintended fertilization of historically low-nutrient ecosystems. With increased nutrient availability, plant biodiversity is expected to decline and bacterial communities are anticipated to increase in abundance of copiotrophic taxa. Here, we address how bacterial communities associated with different plant functional types (forb, grass) shift due to long-term nutrient enrichment. Unlike other studies, results revealed an increase in bacterial diversity, particularly, of oligotrophic bacteria in fertilized plots. We observed that nutrient addition strongly determines forb and grass rhizosphere composition, which could indicate different metabolic preferences in the bacterial communities. This study highlights how long-term fertilization of oligotroph-dominated wetlands could alter the metabolism of rhizosphere bacterial communities in unexpected ways.

Key words: Plant-microbe, rhizosphere, fertilization, copiotroph, oligotroph

Introduction

The soil microbiome is critical for plant health, fitness, and diversity, especially in nutrient-limited environments (1–4). In particular, within the rhizosphere plants provide carbon (C) resources to soil microorganisms in exchange for nutrients such as nitrogen (N) and phosphorus (P). However, nutrient enrichment has been documented to disrupt plant-microbe mutualisms (2). Over the last century, agricultural fertilization and the burning of fossil fuels and biomass have indirectly led to nutrient deposition onto historically low-nutrient ecosystems (5–8). Nutrient enrichment generally causes reduced plant species diversity (9, 10) sometimes as a shift in plant functional types with an increase in grass biomass and loss of forb diversity (11–13). Fertilization has also been shown to decrease soil microbial diversity across cropland, grassland, forest, and tundra ecosystems (14–16). Despite patterns that have emerged from these bulk soil studies, it is less clear how changes in soil microbial diversity due to nutrient additions influence rhizosphere microbial community assembly and diversity. We address this knowledge gap by comparing changes in rhizosphere bacterial community composition of a grass and forb within a long-term fertilization experiment.

Both bulk soil matrix (i.e., not in contact with plant roots) properties and plant identity influences rhizosphere microbial communities. Bulk soil is the reservoir of microbial diversity from which rhizosphere-associated microbial communities are selected; therefore, shifts in bulk soil microbial communities affect rhizosphere assemblages (17–19). In many cases N, N and P, and N-P-K fertilization decreases soil bacterial diversity (20–22). Nutrient enrichment selects for more copiotrophic (i.e., fast-growing, r-strategists) microbial heterotrophs that preferentially

metabolize labile carbon (C) sources versus oligotrophic (i.e., slow-growing, K-strategist) microbial species, which can metabolize complex C sources (14, 20, 23–25). Several studies indicate fertilization increases the abundance of copiotrophic bacterial groups within Actinobacteria, Alphaproteobacteria, and Gammaproteobacteria and decreases abundance in oligotrophic bacterial groups within Acidobacteria, Nitrospirae, Planctomycetes, and Deltaproteobacteria of bulk soils (14, 21, 26, 27). Additionally, copiotrophic taxa within Alpha-, Beta-, and Gamma- Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes are dominant members of some rhizosphere communities (17, 28, 29).

While the bulk soil environment is the primary source of rhizosphere diversity, plant species also influences rhizosphere bacterial community assembly (28–31). Plants release a large portion of photosynthetically fixed C through root exudation (32–34), and these root exudates (i.e., sugars, organic acids, phenolic compounds, and amino acids) can differentiate rhizosphere bacterial communities (1, 17, 35, 36). Therefore, differences in plant physiology influencing the quantity and quality of root exudates can affect rhizosphere bacterial community composition. For example, C4 grasses have higher photosynthetic rates (i.e., fix more C) and greater root biomass allocation compared to C3 plants, resulting in a greater quantity of root exudates (37, 38). Further, C3 plants contain more starches and sugars compared to C4 plants, which increases quality of root exudates (39). Thus, plant root exudation patterns of forbs and grasses are predicted to differentially affect rhizosphere bacterial community structure.

In this study, we address the following question: To what extent does long-term fertilization (N-P-K) of bulk soil shifts rhizosphere bacterial communities of two plant species

representing distinct functional types (i.e., a C3 forb and a C4 grass)? First, we hypothesize nutrient addition will decrease bacterial species diversity and increase the abundance of copiotrophic taxa in all soils, especially rhizosphere soils due to increased availability of labile C from root exudates. Second, we hypothesize fertilization will be the primary factor determining differences in rhizosphere communities due to changes in bulk soil community composition; and plant identity will secondarily influence the rhizosphere community. As a result, plant species are expected to associate with unique core microbiomes that differ between fertilization treatments.

To test these hypotheses, bulk and rhizosphere soils were sampled from two plant species (grass, forb) from fertilized and unfertilized plots at a long-term disturbance and fertilization experiment. Bacterial communities were identified using 16S rRNA amplicon sequencing which also allowed binning of bacterial taxa as copiotrophic or oligotrophic by estimating the average ribosomal RNA (*rrn*) gene copy number of bacterial taxon members. According to laboratory studies, bacterial *rrn* gene copy number is positively related to the organism's growth rate such that copiotrophs have higher *rrn* gene copy numbers than oligotrophs (24, 40, 41). By evaluating differences in taxonomic information and 16S rRNA gene copy numbers of bulk and rhizosphere soils of two plant species with associated soil properties (i.e., ammonium, nitrate, soil pH, carbon, and moisture), we can provide insight to biotic and abiotic processes that are contributing to rhizosphere bacterial community assembly.

RESULTS

Soil source, not fertilization treatment, distinguishes soil properties. The main effect of soil source (bulk vs. rhizosphere) was significantly different in physiochemical properties of moisture ($p = 0.02$), pH ($p < 0.001$), nitrate ($p<0.0001$), C percent ($p=0.03$), and N percent ($p=0.04$; Table S1). Rhizosphere soils were more similar to each other in soil properties than to bulk soils (Table 1, Tukey HSD, $p<0.05$). Specifically, soil C and N concentrations were lower in bulk compared to rhizosphere soils, while soil pH and moisture were lower in rhizosphere compared to bulk soils (Table 1, Tukey HSD, $p<0.05$).

Fertilization influenced soil bacterial diversity in bulk and rhizosphere soils. Chao1 bacterial richness and Shannon H' diversity were higher in fertilized soils compared to unfertilized soils (Table S2, Fig. 1A). In addition, the main effect of soil source influenced bacterial diversity; bulk soil diversity was significantly higher than rhizosphere soil diversity (Table S2, Fig. 1B). Finally, results revealed a positive relationship between Shannon H' diversity and pH, where pH explained 45% of the variation in bacterial diversity across all treatments and soil sources (Fig. 1C).

Copiotroph to oligotroph ratios indicated oligotroph-dominated bacterial communities. Across all samples we detected 9 to 30 copiotrophic and 82 to 190 oligotrophic taxa at the class level. This resulted in copiotroph to oligotroph ratios of < 0.2 within all treatment combinations. Nutrient additions significantly decreased the ratio of copiotrophs to oligotrophs in bulk soils compared to rhizosphere soils (Tukey's HSD, $p < 0.05$; Table S4; Fig. 3). Finally, there was no relationship between bacterial Shannon H' diversity and copiotroph to oligotroph ratio (Fig. S1).

Fertilization treatment and soil source influenced bacterial community composition. Specifically, fertilization treatment (along PCoA axis 1) explained 31.6% of variation in bacterial community composition, while soil source (primarily bulk vs. rhizosphere) separated bacterial composition (along PCoA axis 2) and explained 22.5% of bacterial community variation (Fig. 2). Additionally, main effects of soil source (PERMANOVA R2=0.23, P=0.001) and fertilization treatment (PERMANOVA R2=0.281, P=0.001) influenced bacterial community composition (Table S3A). According to pairwise comparisons, rhizosphere bacterial community composition was similar between grass and forb rhizosphere samples within fertilization treatments (Table S3B). When examining relationships between community composition and soil characteristics, higher soil pH and moisture were correlated to fertilized bulk soils (Fig. 2). Further, higher concentrations of soil C and N were correlated with rhizosphere community composition (Fig. 2).

Different bacterial taxa (OTUs) characteristic of fertilization treatments and plant species. We compared bacterial community taxonomic shifts in unfertilized and fertilized bulk soils and then grass and forb rhizospheres, concluding with differences in microbiome structure between the two plant species. Within bulk soil samples important indicator species for bacterial communities within unfertilized plots are from the class Alphaproteobacteria with 1 OTU from the order Rhizobiales and 2 OTUs from Rhodospirillales and 3 OTUs from the class Spartobacteria (Table S5). In contrast, fertilized bulk soils are best represented by members of the class Actinobacteria with 1 OTU from the order Actinomycetales and 2 OTUs from the order Solirubrobacterales. While OTUs within Rhizobiales were identified as indicator species for

bacterial communities in unfertilized bulk soils, this order was in greatest relative abundance compared to other orders within both fertilization treatments (Fig. S2).

Comparisons of rhizosphere bacterial OTU presence/absence data revealed that forb (1,249 OTUs) and grass (1,019 OTUs) rhizospheres have distinct but overlapping microbiomes. Of the 1,621 total OTUs found in rhizosphere soils, 647 are broadly-distributed and are observed in all plant rhizospheres and bulk soils regardless of treatment. Therefore, less than half of the forb (48%) and grass (37%) rhizosphere members were unique to that plant functional type, and broadly-distributed species dominate plant microbiomes especially in grasses.

Of OTUs that were only represented in the grass microbiome (n=372), only 22 bacterial families are represented at > 0.075% relative abundance. Within those top OTUs, unfertilized grass rhizospheres were enriched in 9 families while fertilized plots were enriched in 19 families (Fig. 4). Indicator species for unfertilized grass rhizospheres included two OTUs, one in the genus *Singulisphaera* and family Planctomycetaceae (IndVal = 0.38, *P*=0.026) and an unclassified Spartobacteria (IndVal = 0.44, *P*=0.008; Table S5). Indicator species for fertilized grass rhizospheres included two OTUs, one in the genus *Planctomyces* and family Planctomycetaceae (IndVal = 0.42, *P*=0.011) and one in the genus *Actinoallomurus* and family Thermomonosporaceae (IndVal = 0.36, *P*=0.045; Table S5).

Of OTUs that were only represented in the forb microbiome (n=602), only 21 bacterial families are represented at > 0.1% relative abundance. Within those top OTUs, unfertilized forb rhizospheres were enriched in 10 families while fertilized plots were enriched in 16 families

(Fig. 5). Indicator species included two OTUs, Acidobacteria Gp1 (IndVal=0.42, *P*=0.02), and an unclassified Proteobacteria (IndVal=0.46, *P*=0.033; Table S5). Indicator species included an OTU in Acidobacteria Gp1 (IndVal= 0.34, *P*=0.041) class and an unclassified bacterial OTU (IndVal=0.60, *P*=0.017; Table S5).

Discussion

In this study, nutrient addition increased bacterial species diversity (H') and richness in bulk and rhizosphere soils (42). These results were similar to O'Brien et al. (42) but contrary to our prediction and the results of other studies (21). This may be due to the increase of pH in fertilized soil compared to unfertilized soil, which is known to be a strong driver of bacterial diversity (23, 43). Nitrogen additions can lead to base cation depletion resulting in decreases of soil pH (44), however in our study soil pH was significantly higher at fertilized compared to unfertilized plots. Acidic conditions in unfertilized plots indicate a depletion of base cations (Ca^{2+}, Mg^{2+}, and K^{+}) similar to peatland soils (44, 45). The addition of base cations, a component of the fertilizer used in this study, can increase the acid-buffering capacity of soils, which may account for the increase in pH in fertilized soils (44, 46). Furthermore, both Shannon H' diversity and Chao1 richness account for rare species; therefore, increases in bacterial diversity and richness in fertilized soils could indicate an increase in rare species (47). This increase in bacterial diversity is likely the result of niche differentiation due to fertilization increasing nutrient availability and rhizodeposition by plants, which introduces organic C resources for heterotrophs (17, 30).

Bacterial taxa identified in rhizosphere samples are putatively involved in nutrient cycling and disease suppressive functions. For example, fertilized forb rhizospheres were enriched in taxa from the family Streptomycetaceae, of which many produce antibiotics (48) and Sphingomonadaceae, which have disease suppression potential against fungal pathogens (49)(Fig. 5). This increase in disease suppressive bacterial taxa suggest a potential an increase in plant pathogenic taxa within fertilized rhizospheres; however, this study did not specifically address disease suppression in soils. In contrast, fertilized grass rhizospheres were enriched with taxa involved in N_2-fixation (Acetobacteraceae) (50) and also Chitiniphagaceae and Conexibacteraceae, which have been implicated in decomposition of recalcitrant C sources (51, 52)(Fig.4). Bacterial taxa in the Xanthomonadaceae family, which have previously been found in environments containing glyphosate (53), and Caulobacteraceae, which grows optimally on pesticides (54), are also more abundant in fertilized grass rhizospheres (Fig. 4). These results suggest nutrient addition enriches forb rhizospheres with putatively disease suppressive bacteria and grass rhizospheres with taxa capable of decomposing complex C sources.

Within bulk soil bacterial members, putative nitrogen cycling taxa in the order Rhizobiales were enriched across all fertilization treatments (55, 56). This is not surprising considering the limited amount of nitrogen in both unfertilized and fertilized soils at the study site. Despite the increase in taxa capable of N_2-fixation in fertilized rhizospheres, these taxa may be less cooperative with plant associates than the same taxa from unfertilized soils thereby reducing plant benefit (2, 57). This was not specifically tested in this study but could be an important future research topic.

Contrary to our prediction, bulk soils had a higher copiotroph to oligotroph ratio (based on *rrn* gene copy number) than rhizospheres. Characteristic of the copiotrophic life history strategy is the ability to rapidly decompose labile C sources, therefore we expected that C rich root exudates in the rhizosphere would support higher proportions of copiotrophic species (17). Additionally, fertilization did not increase the relative abundance of copiotrophic taxa. Rather, the observed copiotroph to oligotroph ratios were low in all samples with unfertilized bulk soils having the greatest proportion (22%) and unfertilized grass rhizospheres having the lowest (13%) copiotroph to oligotroph ratios. We suggest the dominance of oligotrophs reflects the low-nutrient history of this wetland (27, 58). This evidence also supports the idea that after nutrient enrichment bacterial community succession proceeds from copiotroph dominated to oligotroph dominated bacterial communities as nutrients are depleted from the system (58–60).

Comparisons of bulk and rhizosphere bacterial communities revealed that rhizospheres were more similar to each other than to bulk soil bacterial communities within fertilization treatments. This supports our first hypothesis that nutrient addition is the primary driver while plant identity is the secondary influence in shaping root microbiomes (17, 61). These results suggest that changes in the bulk soil bacterial community due to nutrient enrichment could determine which bacterial species are available for plant associations. Additionally, fertilization could be enriching for bacterial taxa that are metabolically different than bacteria in more nutrient-limited ecosystems resulting in less cooperative associations with plants. For example, Rhizobiales OTUs identified in fertilized plots may be less cooperative in plant associations than those from unfertilized plots (2, 57) but future experimental work is needed to confirm this.

In agreement with our second hypothesis, these results demonstrate that fertilization serves as a strong environmental filter on the bulk soil microbiome, which is the bacterial reservoir for rhizosphere microbiomes. Core plant microbiomes were predominantly composed of broadly-distributed taxa; therefore, changes in bulk soil bacterial composition due to nutrient enrichment can directly alter plant microbiome composition and indirectly diminish benefits to plants if nutrient enrichment selects for more competitive bacterial taxa. Specifically, fertilization increases the relative abundances of putative disease suppressive taxa within forb rhizospheres, and increases relative abundance of taxa involved in complex C decomposition in grass rhizospheres. While the taxonomic characterization sheds light on fertilization effects on plant-bacterial relationships in this study, the metabolic diversity of bacterial communities could be even more pronounced and warrants further investigation. Overall, this study suggests long-term fertilization of oligotroph-dominated soils in low-nutrient wetlands increases bacterial species diversity and differentially alters plant rhizosphere composition in a way that suggest metabolic changes within soil bacterial communities.

Material and Methods

Study site and experimental design. A long-term experimental sit established in 2003 to test the effects of fertilization, mowing, and the interaction on wetland plant communities. The site is located at East Carolina University's West Research Campus in Greenville, North Carolina, USA (35.6298N, -77.4836W). A description of the study site and experimental design can be found in Goodwillie and Franch (62) and is summarized here. This site is classified as a jurisdictional wetland but historically described as a mosaic of wet pine flatwood habitat, pine

savanna, and hardwood communities. Soils were characterized as fine, kaolinitic, thermic Typic Paleaquults (Coxville series) with a fine sandy loam texture which are ultisols that are acidic and moderate to poorly drained soil types (https://soilseries.sc.egov.usda.gov/osdname.aspx). The annual mean temperature is 17.2°C and annual precipitation is 176 cm (https://www.climate.gov/maps-data/dataset/). Treatments are replicated on eight 20×30 m blocks, and the N-P-K 10-10-10 pellet fertilizer is applied 3× per year (February, June, and October) for a total annual supplementation of 45.4 kg ha^{-1} for each nutrient. Plots are mowed by bush-hog and raked annually to simulate a fire disturbance (62).

We compared rhizosphere and bulk soil microbiomes in mowed unfertilized and fertilized plots, where herbaceous species dominated. Soil samples were collected at mowed/unfertilized and mowed/fertilized plots in four out of eight replicate blocks to reduce variability due to hydrology. Half the site is located adjacent to a ditch (drier soils) compared to away from the ditch, where soil conditions are wetter. Since this hydrologic gradient has resulted in distinct plant communities (C. Goodwillie M.W. McCoy and A. L. Peralta, submitted for publication), we collected samples from the wetter plots (away from the drainage ditch).

Bulk and rhizosphere soil sampling. For a single composite bulk soil sample, we collected two soil cores (12 cm depth, 3.1 cm diameter) near each of the three permanently installed 1 m^2 quadrats used for annual plant surveys. Each composite bulk soil sample was homogenized with a 4 mm sieve before further analysis. At each plot, rhizosphere soils were collected from the C3 forb *Euthamia caroliniana* (L.) Greene ex Porter & Britton and C4 grass *Andropogon virginicus* L. Rhizosphere soils were a composite of three root systems of the same

species. Roots were gently dislodged from soil and neighboring roots and placed in a paper bag. After vigorous shaking, soil in the bag was processed for abiotic analysis. The roots were placed into 50 mL centrifuge tubes with 30 mL sterilized Nanopure® water and shaken at 100 RPM for 1 hour. Washed roots were removed, and the soil and water mixture was freeze-dried to remove water. Freeze-dried rhizosphere samples were stored at -80 °C until DNA extraction.

Soil chemical and physical characteristics. We measured gravimetric soil moisture by drying 20-30 g of field-moist soil at 105 °C for 24 hours. We calculated percent moisture as the difference in weight of moist and dried soils divided by the oven-dried soil weight. Oven-dried samples were ground and measured for pH by mixing a 1:1 (soil:water) solution. A subsample of oven-dried soil was sieved with a 500 µm mesh and analyzed for total carbon and total nitrogen (TC, TN) using an elemental analyzer (2400 CHNS Analyzer; Perkin Elmer; Waltham, Massachusetts, USA) at the Environmental and Agricultural Testing Service laboratory (Department of Crop and Soil Sciences at NC State). Approximately 5 g of field moist soil was extracted with 45 ml of 2 M KCl, and available ammonium (NH_4^+) and nitrate (NO_3^-) ions were colorimetrically measured using a SmartChem 200 auto analyzer (Unity Scientific Milford, Massachusetts, USA) at the East Carolina University Environmental Resources Laboratory.

Bacterial community analyses. We extracted DNA from soils using the Qiagen DNeasy PowerSoil Kit. We used this DNA as template in PCR reactions using barcoded primers (bacterial/archaeal 515FB/806R) originally developed by the Earth Microbiome Project to target the V4 region of the bacterial 16S subunit of the ribosomal RNA gene (63). For each sample, three 50 µL PCR libraries were combined and cleaned using the AMPure XP magnetic bead

protocol (Axygen, Union City, California, USA). Cleaned PCR product were quantified using QuantIT dsDNA BR assay (Thermo Scientific, Waltham, Massachusetts, USA) and diluted to a concentration of 10 ng μL^{-1} before pooling libraries in equimolar concentration of 5 ng μL^{-1}. We sequenced pooled libraries using the Illumina MiSeq platform using paired end reads (Illumina Reagent Kit v2, 500 reaction kit) at the Indiana University Center for Genomics and Bioinformatics Sequencing Facility. Sequences were processed using MOTHUR (v1.40.1) (64) MiSeq pipeline (65). We assembled contigs from the paired end reads, quality trimmed using a moving average quality score (minimum quality score 35), aligned sequences to the SILVA rRNA database (v128) (66), and removed chimeric sequences using the VSEARCH algorithm (67). We created operational taxonomic units (OTUs) by first splitting sequences based on taxonomic class and then binning into OTUs based on 97% sequence similarity.

Samples were rarefied to 43,811 OTUs and resampled. We determined bacterial species diversity by calculating Shannon diversity index (H´) because it accounts for species abundance and evenness and accounts for rare species (68, 69). Next, we used the abundance-based Chao1 estimator to determine species richness because it is non-parametric and also considers rare species (69, 70). Shannon diversity was calculated using the *vegan::diversity* function and Chao1 OTU richness using *vegan::estimate* (71). We assigned gene copy number to each OTU using RDP classifier (v2.12) (72) integrated with the *rrn* operon database developed by the Schimdt Laboratory at the Michigan Microbiome Project, University of Michigan (24, 40). Higher gene copy numbers (>=5) represent the copiotrophic lifestyle and lower gene copy numbers (<3) represent the oligotrophic lifestyle (23, 73). The number of copiotrophs and oligotrophs were

summed for each soil sample to calculate the copiotroph to oligotroph ratio within a soil bacterial community.

Statistical Analyses. All statistical analyses were performed in the R statistical environment (RStudio v1.1.383, Rv3.4.0) (74). To test for differences in OTU diversity and richness, copiotroph to oligotroph ratios, and soil parameters (soil pH, total carbon, extractable ammonium and nitrate total nitrogen, soil moisture) in response to long-term fertilization, we used the two-way model of analysis of variance (ANOVA) to compare the main effects of soil source and fertilization treatment and the interaction. Significant interactions were compared with Tukey's post-hoc analysis using the *agricolae::HSD.test* R function (75). We examined beta diversity by visualizing bacterial community responses to nutrient additions and rhizosphere association using principal coordinates of analysis (PCoA) of bacterial community composition based on Bray-Curtis dissimilarity. We used permutational multivariate analysis of variance (PERMANOVA) to test for differences in bacterial community composition among treatments and within treatment using pairwise comparisons. Hypothesis testing using PERMANOVA was performed using the *vegan::adonis* function (71). We examined the relationship between soil parameters and bacterial Bray-Curtis dissimilarity patterns using the *vegan::envfit* function (71). Soil parameters with $p<0.05$ were represented on the PCoA plot as vectors scaled by the strength of their correlation. To identify specific community members that represented each soil source and fertilization treatment, we performed a Dufrene-Legendre indicator species analysis using the *labdsv::indval* function (76). Because we were interested in differences between fertilization treatments within a soil source, indicator species analysis was conducted on bulk soils and rhizosphere soils independently.

Acknowledgments

We thank M. Beamon, C. Eakins, J. LeCrone, J. Stiller, and S. Wilkinson for laboratory and field assistance. We thank J. Gill and the East Carolina University grounds crew for their efforts in maintaining the long-term ecological experiment. This work was supported by the National Science Foundation (GRFP to RBB) and East Carolina University. All code and data used in this study can be found in a public GitHub repository (https://github.com/PeraltaLab/WRC15_Rhizo) and the NCBI SRA (BioProject PRJNA599142).

References

1. Van Der Heijden MGA, Bardgett RD, Van Straalen NM. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett.

2. Weese DJ, Heath KD, Dentinger BTM, Lau JA. 2014. Long-term nitrogen addition causes the evolution of less-cooperative mutualists. Evolution (N Y) 69:631–642.

3. Regus JU, Wendlandt CE, Bantay RM, Gano-Cohen KA, Gleason NJ, Hollowell AC, O'Neill MR, Shahin KK, Sachs JL. 2017. Nitrogen deposition decreases the benefits of symbiosis in a native legume. Plant Soil 414:159–170.

4. Jach-Smith LC, Jackson RD. 2018. N addition undermines N supplied by arbuscular mycorrhizal fungi to native perennial grasses. Soil Biol Biochem 116:148–157.

5. Fowler D, Coyle M, Skiba U, Sutton MA, Cape JN, Reis S, Sheppard LJ, Jenkins A, Grizzetti B, Galloway JN, Vitousek P, Leach A, Bouwman AF, Butterbach-Bahl K, Dentener F, Stevenson D, Amann M, Voss M. 2013. The global nitrogen cycle in the Twentyfirst century. Philos Trans R Soc B Biol Sci 368.

6. Guignard MS, Leitch AR, Acquisti C, Eizaguirre C, Elser JJ, Hessen DO, Jeyasingh PD, Neiman M, Richardson AE, Soltis PS, Soltis DE, Stevens CJ, Trimmer M, Weider LJ, Woodward G, Leitch IJ. 2017. Impacts of Nitrogen and Phosphorus: From Genomes to Natural Ecosystems and Agriculture. Front Ecol Evol 5.

7. Wang R, Balkanski Y, Boucher O, Ciais P, Peñuelas J, Tao S. 2015. Significant contribution of combustion-related emissions to the atmospheric phosphorus budget. Nat Geosci 8:48–54.

8. Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ. 2004. Nitrogen cycles: Past, present, and futureBiogeochemistry.

9. Harpole WS, Sullivan LL, Lind EM, Firn J, Adler PB, Borer ET, Chase J, Fay PA, Hautier Y, Hillebrand H, MacDougall AS, Seabloom EW, Williams R, Bakker JD, Cadotte MW, Chaneton EJ, Chu C, Cleland EE, D'Antonio C, Davies KF, Gruner DS, Hagenah N, Kirkman K, Knops JMH, La Pierre KJ, McCulley RL, Moore JL, Morgan JW, Prober SM, Risch AC, Schuetz M, Stevens CJ, Wragg PD. 2016. Addition of multiple limiting resources reduces grassland diversity. Nature 537:93–96.

10. Wallis DeVries MF, Bobbink R. 2017. Nitrogen deposition impacts on biodiversity in terrestrial ecosystems: Mechanisms and perspectives for restoration. Biol Conserv 212:387–389.

11. Dickson TL, Foster BL. 2011. Fertilization decreases plant biodiversity even when light is not limiting. Ecol Lett 14:380–388.

12. Song L, Bao X, Liu X, Zhang Y, Christie P, Fangmeier A, Zhang F. 2011. Nitrogen enrichment enhances the dominance of grasses over forbs in a temperate steppe ecosystem. Biogeosciences 8:2341–2350.

13. Stevens CJ, Dise NB, Gowing DJG, Mountford JO. 2006. Loss of forb diversity in relation to nitrogen deposition in the UK: Regional trends and potential controls. Glob Chang Biol 12:1823–1833.

14. Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE,

Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proc Natl Acad Sci 112:10967–10972.

15. Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Glob Chang Biol 18:1918–1927.

16. Liu J, Liu M, Wu M, Jiang C, Chen X, Cai Z, Wang B, Zhang J, Zhang T, Li Z. 2018. Soil pH rather than nutrients drive changes in microbial community following long-term fertilization in acidic Ultisols of southern China. J Soils Sediments 18:1853–1864.

17. Bulgarelli D, Schlaeppi K, Spaepen S, Ver Loren van Themaat E, Schulze-Lefert P. 2013. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–38.

18. Mendes LW, Kuramae EE, Navarrete AA, Van Veen JA, Tsai SM. 2014. Taxonomical and functional microbial community selection in soybean rhizosphere. ISME J 8:1577–1587.

19. de Ridder-Duine AS, Kowalchuk GA, Klein Gunnewiek PJA, Smant W, Van Veen JA, De Boer W. 2005. Rhizosphere bacterial community composition in natural stands of Carex arenaria (sand sedge) is determined by bulk soil community composition. Soil Biol Biochem 37:349–357.

20. Su JQ, Ding LJ, Xue K, Yao HY, Quensen J, Bai SJ, Wei WX, Wu JS, Zhou J, Tiedje JM, Zhu YG. 2015. Long-term balanced fertilization increases the soil microbial functional

diversity in a phosphorus-limited paddy soil. Mol Ecol 24:136–150.

21. Wang C, Liu D, Bai E. 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol Biochem 120:126–133.

22. Zeng J, Liu X, Song L, Lin X, Zhang H, Shen C, Chu H. 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biol Biochem 92:41–49.

23. Noah Fierer MA. B and RB. J. 2007. Toward an Ecological Classification of Soil Bacteria. Ecology 88:1354–1364.

24. Stoddard SF, Smith BJ, Hein R, Roller BRK, Schmidt TM. 2015. rrnDB: Improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development. Nucleic Acids Res 43:D593–D598.

25. Zhou J, Jiang X, Wei D, Zhao B, Ma M, Chen S, Cao F, Shen D, Guan D, Li J. 2017. Consistent effects of nitrogen fertilization on soil bacterial communities in black soils for two crop seasons in China. Sci Rep 7:1–10.

26. Francioli D, Schulz E, Lentendu G, Wubet T, Buscot F, Reitz T. 2016. Mineral vs. organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. Front Microbiol 7:1–16.

27. Ho A, Di Lonardo DP, Bodelier PLE. 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol Ecol 93.

28. Zarraonaindia I, Owens S, Weisenhorn P, West K, Hampton-Marcell J, Lax S, Bokulich N, Mills D, Martin G, Taghavi S, Lelie, van der D, Gilbert J. 2015. The Soil Microbiome

Influences Grapevine-Associated Microbiota. MBio.

29. Matthews A, Pierce S, Hipperson H, Raymond B. 2019. Rhizobacterial Community Assembly Patterns Vary Between Crop Species. Front Microbiol 10:1–13.

30. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL. 2012. Defining the core Arabidopsis thaliana root microbiome. Nature 488:86–90.

31. Uroz S, Buée M, Murat C, Frey-Klett P, Martin F. 2010. Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288.

32. Hütsch BW, Augustin J, Merbach W. 2002. Plant rhizodeposition ± an important source for carbon turnover in soils Summary ± Zusammenfassung Pflanzliche Rhizodeposition ± eine wichtige Quelle für den Kohlenstoffumsatz in Böden. J Plant Nutr Soil Sci 165:397–407.

33. Jones DL, Hodge A, Kuzyakov Y. 2004. Plant and mycorrhizal regulation of rhizodeposition\rTansley Review 163:3 (September 2004). New Phytol 163:459–480.

34. Haichar F el Z, Santaella C, Heulin T, Achouak W. 2014. Root exudates mediated interactions belowground. Soil Biol Biochem 77:69–80.

35. Bertin C, Yang X, Weston LA. 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83.

36. Dakora FD, Phillips DA. 2002. Root exudates as mediators of mineral acquisition in low-

nutrient environments. Plant Soil 245:35–47.

37. Kellogg EA. 2013. C4 photosynthesis. Curr Biol 23:R594–R599.

38. Schmitt MR, Edwards GE. 1981. Photosynthetic Capacity and Nitrogen Use Efficiency of Maize , Wheat , and Rice : A Comparison Between C 3 and C 4 Photosynthesis content in a trusted digital archive . We use information technology and tools to increase productivity and facilitate new fo. J Exp Bot 32:459–466.

39. Barbehenn R V., Chen Z, Karowe DN, Spickard A. 2004. C3 grasses have higher nutritional quality than C4 grasses under ambient and elevated atmospheric CO2. Glob Chang Biol 10:1565–1575.

40. Roller BRK, Stoddard SF, Schmidt TM. 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nat Microbiol 1:1–19.

41. Klappenbach J, Saxman P, Cole J, TM S. 2001. rrndb: the Ribosomal RNA Operon Copy Number Database. Nucleic Acids Res 29:181–184.

42. O'Brien SL, Gibbons SM, Owens SM, Hampton-marcell J, Johnston ER, Jastrow JD, Gilbert JA, Meyer F, Antonopoulos DA. 2016. Spatial scale drives patterns in soil bacterial diversity 18:2039–2051.

43. Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631.

44. Tian D, Niu S. 2015. A global analysis of soil acidification caused by nitrogen addition. Environ Res Lett 10:24019.

45. Bridgham, Scott D. and Richardson CJ. 1993. Hydrology and nutrient gradients in North Carolina Peatlands. Wetlands 13:207–218.

46. Moore TR, Knorr KH, Thompson L, Roy C, Bubier JL. 2019. The effect of long-term fertilization on peat in an ombrotrophic bog. Geoderma 343:176–186.

47. Wang Q, Ma M, Jiang X, Guan D, Wei D, Zhao B, Chen S, Cao F, Li L, Yang X, Li J. 2019. Impact of 36 years of nitrogen fertilization on microbial community composition and soil carbon cycling-related enzyme activities in rhizospheres and bulk soils in northeast China. Appl Soil Ecol 136:148–157.

48. Kinkel LL, Schlatter DC, Bakker MG, Arenz BE. 2012. Streptomyces competition and co-evolution in relation to plant disease suppression 163:490–499.

49. Chapelle E, Mendes R, Bakker PAHM, Raaijmakers JM. 2015. Fungal invasion of the rhizosphere microbiome 10:265–268.

50. Saravanan VS, Madhaiyan M, Osborne J, Thangaraju M, Sa TM. 2008. Ecological occurrence of Gluconacetobacter diazotrophicus and nitrogen-fixing Acetobacteraceae members: Their possible role in plant growth promotion. Microb Ecol 55:130–140.

51. Deng J, Gu Y, Zhang J, Xue K, Qin Y, Yuan M, Yin H, He Z, Wu L, Schuur EAG, Tiedje JM, Zhou J. 2015. Shifts of tundra bacterial and archaeal communities along a permafrost thaw gradient in Alaska. Mol Ecol 24:222–234.

52. Seki T, Matsumoto A, Shimada R, Inahashi Y. 2019. Conexibacter arvalis sp . nov ., isolated from a cultivated field soil sample 2400–2404.

53. Newman MM, Hoilett N, Lorenz N, Dick RP, Liles MR, Ramsier C, Kloepper JW. 2016.

Glyphosate effects on soil rhizosphere-associated bacterial communities. Sci Total Environ 543:155–160.

54. Lingens F, Blecher R, Blecher H, Blobel F, Frohner C, Gorisch H, Gorisch H, Layh ANDG. 1985. Phenylobacterium immobile gen. nov. sp. nov. a Gram-Negative Bacterium That Degrades the Herbicide Chloridazon. Int J Syst BACTERIOLO 26–39.

55. Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR. 2011. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia (Jena) 54:309–320.

56. Rilling JI, Acuña JJ, Sadowsky MJ, Jorquera MA. 2018. Putative nitrogen-fixing bacteria associated with the rhizosphere and root endosphere of wheat plants grown in an andisol from southern Chile. Front Microbiol 9:1–13.

57. Ai C, Liang G, Sun J, Wang X, He P, Zhou W, He X. 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol Biochem 80:70–78.

58. Song W, Kim M, Tripathi BM, Kim H, Adams JM. 2016. Predictable communities of soil bacteria in relation to nutrient concentration and successional stage in a laboratory culture experiment. Environ Microbiol 18:1740–1753.

59. Nemergut DR, Knelman JE, Ferrenberg S, Bilinski T, Melbourne B, Jiang L, Violle C, Darcy JL, Prest T, Schmidt SK, Townsend AR. 2016. Decreases in average bacterial community rRNA operon copy number during succession. ISME J 10:1147–1156.

60. Fierer N, Nemergut D, Knight R, Craine JM. 2010. Changes through time: Integrating

microorganisms into the study of succession. Res Microbiol 161:635–642.

61. Delgado-Baquerizo M, Reich PB, Khachane AN, Campbell CD, Thomas N, Freitag TE, Abu Al-Soud W, Sørensen S, Bardgett RD, Singh BK. 2017. It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environ Microbiol 19:1176–1188.

62. Goodwillie C, Franch WR. 2006. An experimental study of the effects of nutrient addition and mowing on a ditched wetland plant community: Results of the first year. J North Carolina Acad Sci 122:106–117.

63. Caporaso JG, Lauber CL, Walters W a, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert J a, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624.

64. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski R a., Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541.

65. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. Appl Environ Microbiol 79:5112–5120.

66. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and

web-based tools. Nucleic Acids Res 41:590–596.

67. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584.

68. Shannon CE. 1948. A Mathematical Theory of Communication. Bell Syst Tech J 27:379–423.

69. Kim BR, Shin J, Guevarra RB, Lee JH, Kim DW, Seol KH, Lee JH, Kim HB, Isaacson RE. 2017. Deciphering diversity indices for a better understanding of microbial communities. J Microbiol Biotechnol 27:2089–2093.

70. Chao A. 1984. Board of the Foundation of the Scandinavian Journal of Statistics Nonparametric Estimation of the Number of Classes in a Population Author (s): Anne Chao Source : Scandinavian Journal of Statistics , Vol . 11 , No . 4 (1984), pp . 265-270 Published by. Scand J Stat 11:265–270.

71. Oksanen J. 2015. Vegan : ecological diversity 1:1–12.

72. Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267.

73. Roller BRK, Schmidt TM. 2015. The physiology and ecological implications of efficient growth. ISME J 9:1481–1487.

74. R Core Team. 2019. R: A language and environment for statistical computing. R Found Stat Comput Vienna, Austria.

75. de Mendiburu F. 2019. R Package ‘ agricolae .’

76. Roberts ADW, Roberts MDW. 2016. Package ‘ labdsv .’

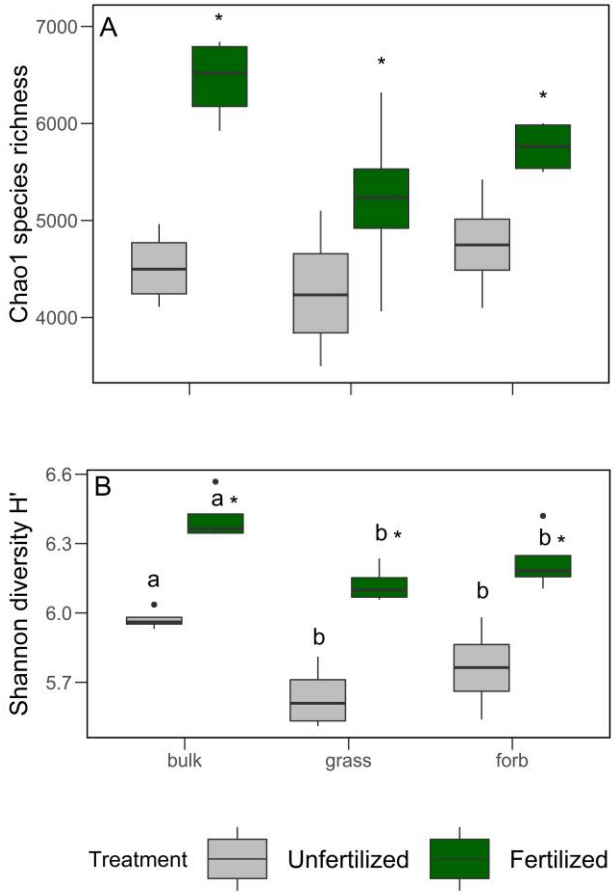
FIGURES AND TABLES

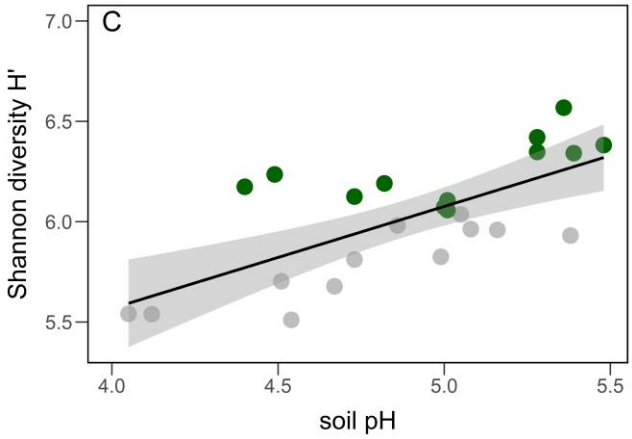
Table 1. (A) Soil physiochemical properties after 12 years of fertilization and mowing disturbance. Average (mean ± SD) soil properties (temperature, gravimetric moisture, pH, extractable nitrate and ammonium concentrations, total C% and N%, and C:N ratio) across unfertilized and fertilized plots and among soil sources (bulk, forb rhizosphere, and grass rhizosphere). Soil source main effects that are significantly differently (ANOVA $p<0.05$) are bolded. Letters represent significant differences between soil sources (Tukey's HSD $p < 0.05$).

	Unfertilized			Fertilized		
	Bulk	Forb	Grass	Bulk	Forb	Grass
Temperature °C	23.3 ± 0.4	-	-	22.8 ± 0.6	-	-
Moisture (%)	19.53 ± 0.39 ***a***	19.18 ± 0.13 ***b***	19.18 ± 0.10 ***b***	19.45 ± 0.26 ***a***	19.18 ± 0.15 ***b***	19.15 ± 0.10 ***b***
pH	5.17 ± 0.15 ***a***	4.62 ± 0.39 ***b***	4.50 ± 0.31 ***b***	5.38 ± 0.08 ***a***	4.88 ± 0.37 ***b***	4.81 ± 0.25 ***b***
NO_3^--N (µg/g dry soil)	0.31 ± 0.26 ***b***	0.97 ± 0.28 ***a***	1.83 ± 0.55 ***a***	0.41 ± 0.28 ***b***	0.92 ± 0.42 ***a***	0.97 ± 0.25 ***b***
NH_4^+-N (µg/g dry soil)	2.51 ± 0.71	2.37 ± 0.14	2.45 ± 0.90	2.64 ± 0.95	2.89 ± 0.65	2.53 ± 0.82
Total C (%)	3.52 ± 0.86 ***a***	5.00 ± 1.02 ***ab***	5.24 ± 1.03 ***b***	3.81 ± 0.59 ***a***	4.20 ± 0.46 ***ab***	5.82 ± 2.71 ***b***

Total N (%)	0.20 ± 0.05 ***a***	0.27 ± 0.06 ***ab***	0.29 ± 0.06 ***b***	0.22 ± 0.03 ***a***	0.24 ± 0.02 ***ab***	0.33 ± 0.15 ***b***
Soil C:N (wt:wt)	17.84 ± 1.21	18.91 ± 0.35	18.13 ± 1.02	17.31 ± 1.47	17.86 ± 0.38	17.62 ± 0.49

Figure 1. Boxplots of bacterial diversity based on mean (A) Chao1 richness and (B) Shannon H′ Diversity Index associated with soil source (bulk, grass rhizosphere, forb rhizosphere). Colors indicate fertilization treatment (gray = unfertilized, green = fertilized) at mowed plots. Asterisks (*) indicate significant differences between fertilization treatments and letters represent significant differences between soil sources (Tukey's HSD, $p < 0.05$). (C) Linear regression of soil pH and bacterial community diversity by fertilization treatment with 95% confidence intervals.





11

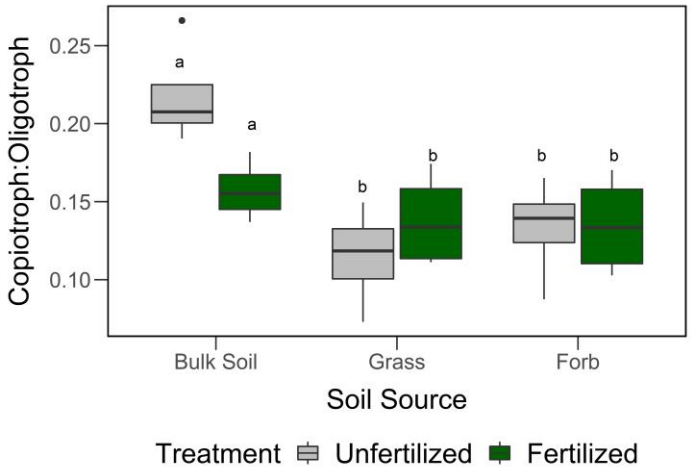
12

13

14

15

16 **Figure 2.** Comparison of bacterial life history traits according to fertilization treatment. Average
17 copiotroph to oligotroph ratios (based on 16S rRNA sequences) of soil sources (bulk, grass
18 rhizosphere, forb rhizosphere). Boxplots are colored according to fertilization treatment (gray =
19 unfertilized, green = fertilized). Letters indicate significant differences among soil sources
20 (Tukey's HSD, $p < 0.05$).



21

22

23

Figure 3. Ordination based on Principal Coordinates Analysis depicting bacterial community composition. Colors represent fertilization treatment (gray = unfertilized, green = fertilized) and symbols represent soil source (bulk soil = circle, grass rhizosphere = open square, forb rhizosphere = filled square). Vectors represent soil factors that are correlated to patterns in bacterial community composition (See Table S2; pH = soil pH, moisture = soil gravimetric moisture percent, C% = percent soil carbon, N%= percent soil nitrogen).

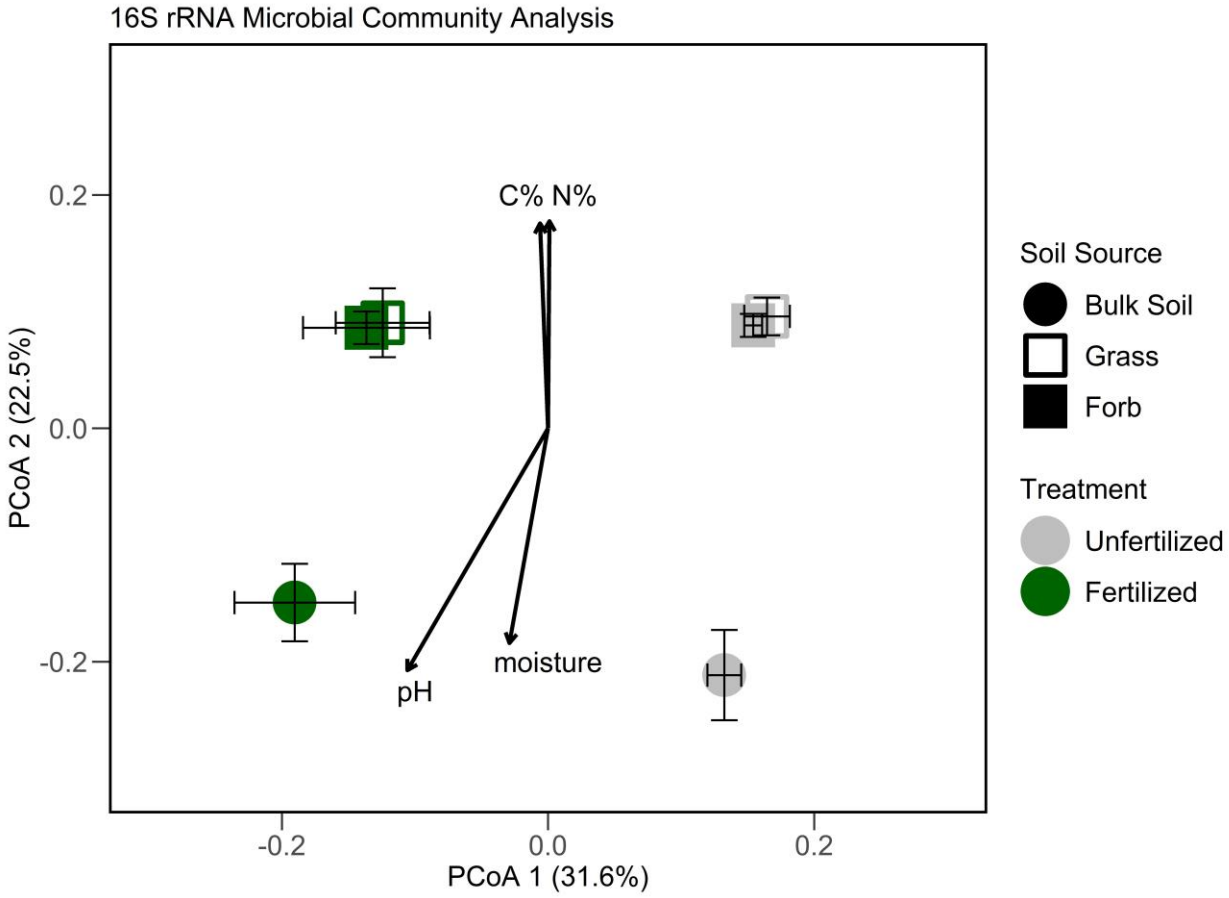
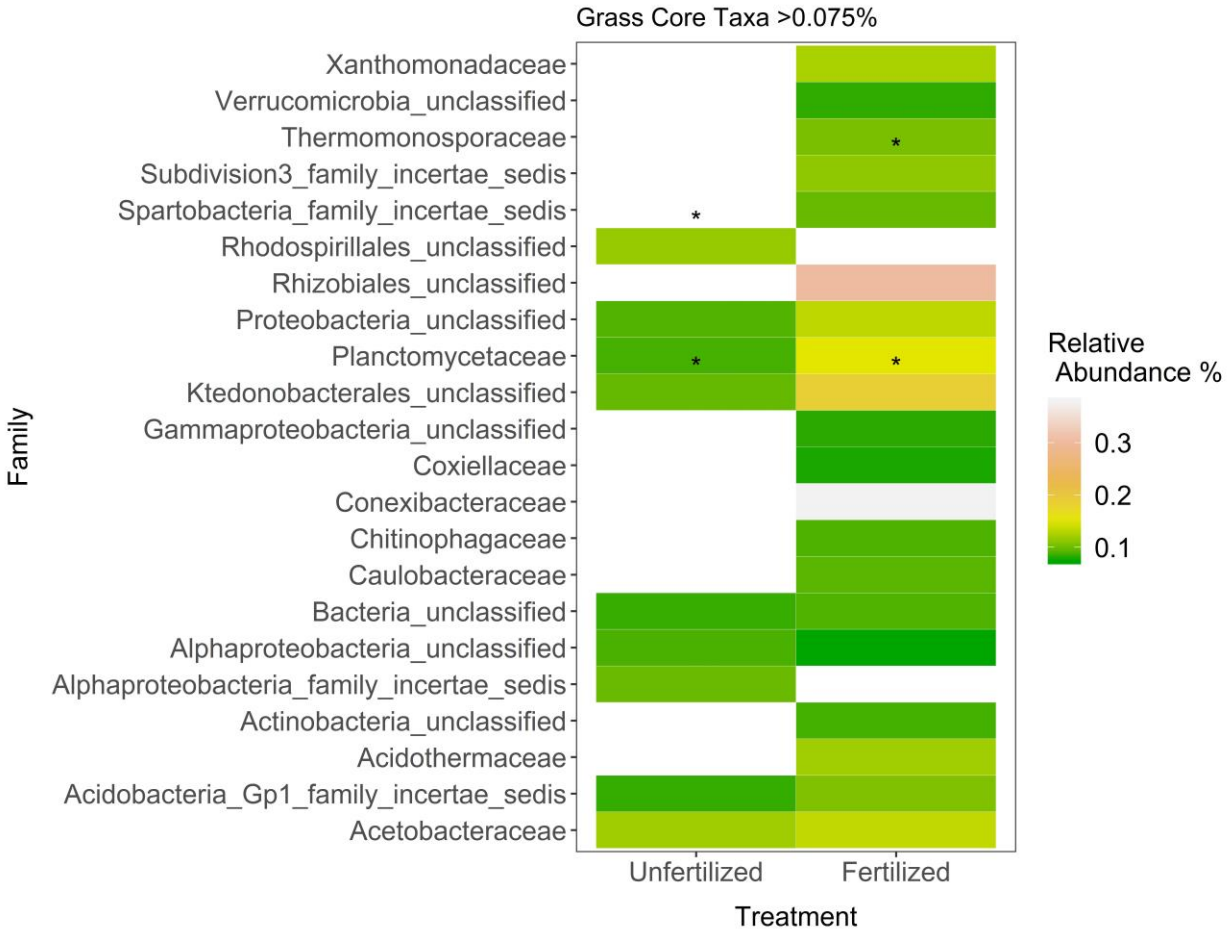
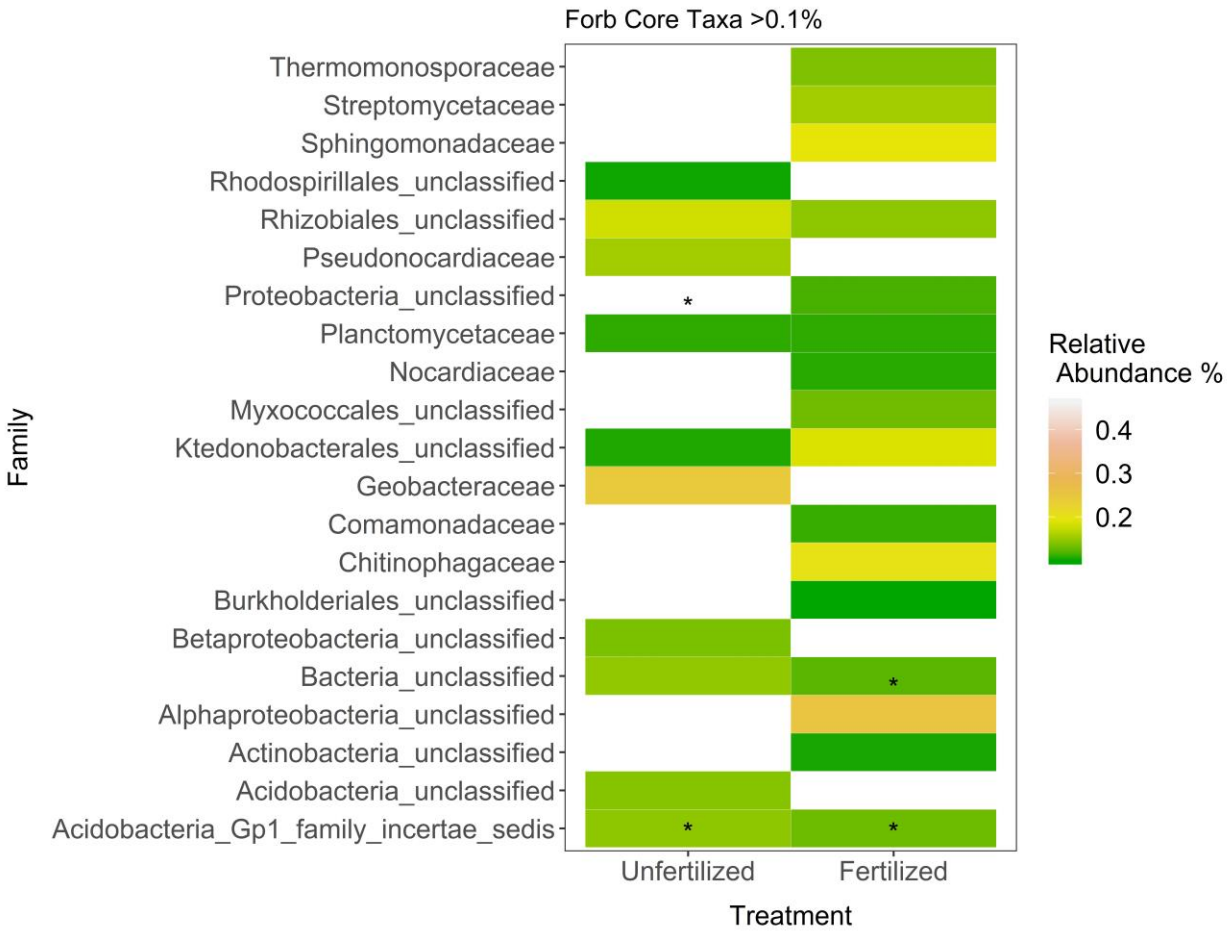


Figure 4: Comparisons of top OTU relative abundances (>0.1%) at the family level between fertilization treatments for grass rhizospheres bacterial communities. Asterisk (*) represents indicator species present within family (Table S5). Colors indicate relative abundance increases from cool to warm (green yellow, orange, and red). White boxes indicate taxa present at <0.075% relative abundance.



43 **Figure 5:** Comparisons of top OTU relative abundances (>0.1%) at the family level between
44 fertilization treatments for forb rhizospheres bacterial communities. Asterisk (*) represents
45 indicator species present within family (Table S5). Colors indicate relative abundance increases
46 from cool to warm (green yellow, orange, and red). White boxes indicate taxa present at < 0.1%
47 relative abundance.

48



49

50 **Supplementary Material**

51 **Supplemental Table S1.** Summary of two-way ANOVA comparing soil properties among soil

52 source (bulk, grass rhizosphere, forb rhizosphere) and fertilization treatments.

	Treatment		Source		T*S	
	F-Value	P	F-Value	P	F-Value	P
Moisture (%)	0.28	0.84	**5.14**	**0.02**	0.05	0.95
pH	2.11	0.13	**12.14**	**0.0003**	0.07	0.93
NO3--N (µg/g dry soil)	0.81	0.51	**12.51**	**0.0003**	3.23	0.06
NH4+-N (µg/g dry soil)	0.25	0.86	0.07	0.93	0.21	0.81
Total C (%)	0.33	0.81	**4.37**	**0.03**	0.54	0.59
Total N (%)	0.27	0.85	**3.96**	**0.04**	0.42	0.66
Soil C:N (wt:wt)	1.37	0.28	1.54	0.24	0.22	0.81

53
54
55

Supplemental Table S2. Summary of two-way ANOVA comparing bacterial community Chao1 richness (A) and Shannon H' diversity (B) metrics among soil source and fertilization treatments. Source represents bulk, grass rhizosphere, and forb rhizosphere and treatment represents fertilized and unfertilized mowed treatments. Treatment effects that were significantly different (ANOVA $p<0.05$) are bolded.

(A) Chao1 richness

Fixed Effect	SumSq	MeanSq	NumDF	F value	Pr(>F)
Source	2323441	1161721	2	3.40	0.056
Treatment	10062645	10062645	1	29.476	**<0.0001**
Source:treatment	202762	101381	2	1.79	0.195

(B) Shannon diversity

Fixed Effect	SumSq	MeanSq	NumDF	F value	Pr(>F)
Source	0.399	0.199	2	12.901	**0.0003**
Treatment	1.278	1.278	1	82.705	**<0.0001**
Source:treatment	0.003	0.015	2	0.082	0.922

Supplemental Table S3. Summary of (A) PERMANOVA main effects (soil source and fertilization treatment) and interaction, (B) pairwise PERMANOVA comparisons of soil sources (bulk, grass rhizosphere, forb rhizosphere) within fertilization treatments and (C) pairwise PERMANOVA comparisons of soil sources between fertilization treatments.

(A) Main effects

	SumSq	F-value	R^2	p-value
Source	0.466	4.924	0.234	**0.001**
Treatment	0.558	11.80	0.281	**0.001**
Source * Treatment	0.114	1.202	0.057	0.257

(B) Pairwise PERMANOVA within fertilization treatments

	Unfertilized				Fertilized			
Soil Sources	SumSq	F-value	R^2	p-value	SumSq	F-value	R^2	p-value
Bulk x Forb	0.214	4.839	0.446	**0.033**	0.189	2.987	0.332	**0.024**
Bulk x Grass	0.215	5.123	0.461	**0.034**	0.169	3.011	0.334	**0.036**
Forb x Grass	0.035	0.819	0.120	0.557	0.072	1.223	0.169	0.186

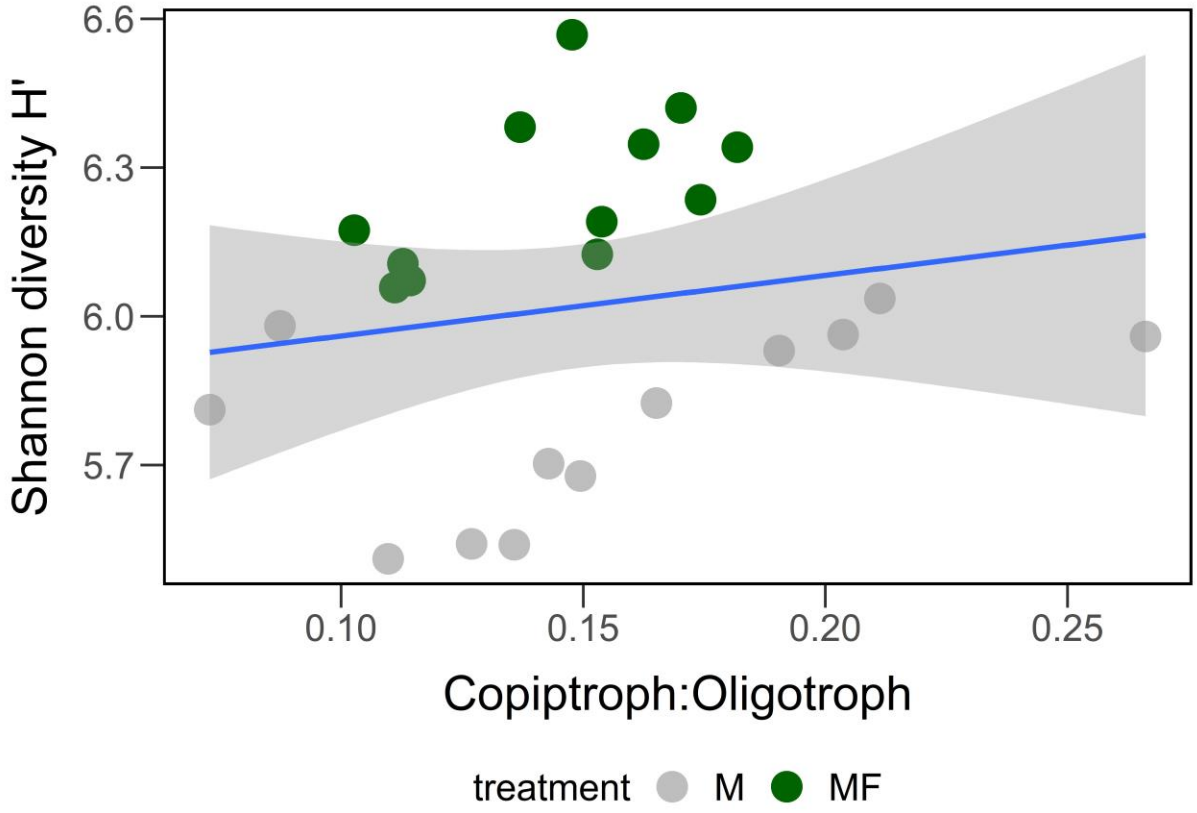
83 **Supplemental Table S4**. Summary of two-way ANOVA comparing bacterial community

84 copiotroph to oligotroph ratio among soil source and fertilization treatments.

Fixed Effect	SumSq	MeanSq	NumDF	F value	Pr(>F)
Source	0.757	0.379	2	7.257	**0.005**
Treatment	0.007	0.007	1	0.136	0.717
Source:treatment	0.939	0.142	2	2.716	0.093

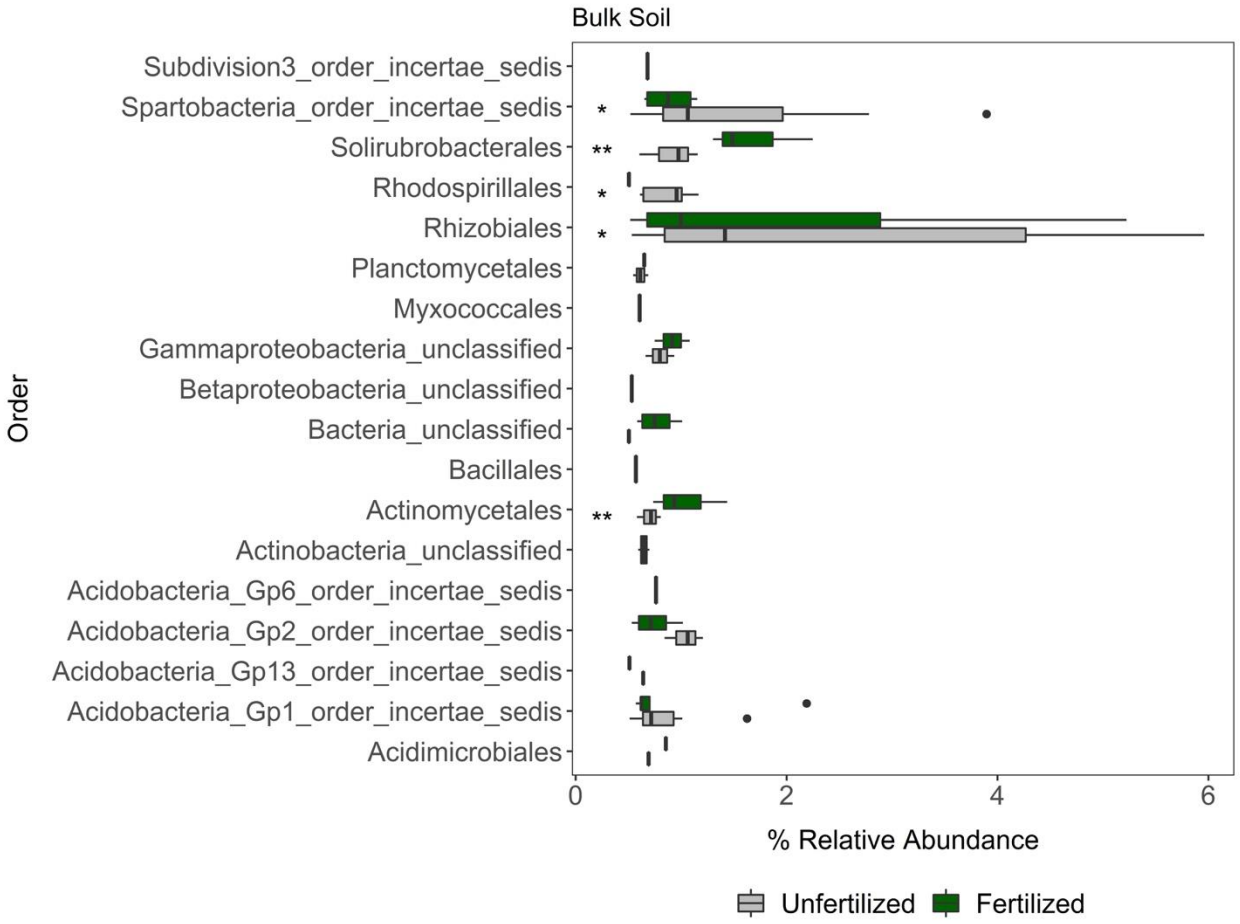
85
86

87 **Supplemental Figure SF1:** Linear regression of copiotroph to oligotroph ratio and Shannon
88 diversity H' by fertilization treatment. Gray confidence bands represent 95% confidence
89 intervals.



90
91

92 **Supplemental Figure SF2:** Comparisons of top OTU relative abundances (>1%) of bulk soils.
93 Single asterisk (*) = indicator taxa for unfertilized treatment and double asterisk (**) = indicator
94 taxa for fertilized plots (Table S5). Boxplots are colored according to fertilization treatment (gray
95 = unfertilized, green = fertilized).



96
97

98 **Supplemental Table S5**: Summary of bacterial taxa (OTUs) characteristic to each soil source
99 and fertilization treatment based on indicator species analysis. These are the top OTUs that are
100 significantly associated with each soil source and treatment group.

OTU	Cluster	IndVal	Prob	Classification Phylum; Class; Order; Family; Genus
Otu00016	fertilized bulk	0.716	0.028	Actinobacteria; Actinobacteria; Solirubrobacterales; Solirubrobacterales_unclassified; Solirubrobacterales_unclassified
Otu00005	fertilized bulk	0.697	0.038	Actinobacteria; Actinobacteria; Solirubrobacterales; Solirubrobacterales_unclassified; Solirubrobacterales_unclassified
Otu00013	fertilized bulk	0.640	0.030	Actinobacteria; Actinobacteria; Actinomycetales; Thermomonosporaceae; Actinoallomurus
Otu00001	unfertilized bulk	0.592	0.022	Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiales_unclassified; Rhizobiales_unclassified
Otu00039	unfertilized bulk	0.763	0.030	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillales_unclassified; Rhodospirillales_unclassified
Otu00029	unfertilized bulk	0.713	0.034	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillales_unclassified; Rhodospirillales_unclassified
Otu00064	unfertilized bulk	0.768	0.034	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillales_unclassified; Rhodospirillales_unclassified
Otu00018	unfertilized bulk	0.723	0.030	Verrucomicrobia; Spartobacteria; Spartobacteria_order_incertae_sedis; Spartobacteria_family_incertae_sedis; Spartobacteria_genera_incertae_sedis
Otu00010	unfertilized bulk	0.642	0.033	Verrucomicrobia; Spartobacteria; Spartobacteria_order_incertae_sedis; Spartobacteria_family_incertae_sedis; Spartobacteria_genera_incertae_sedis
Otu00003	unfertilized bulk	0.706	0.035	Verrucomicrobia; Spartobacteria; Spartobacteria_order_incertae_sedis; Spartobacteria_family_incertae_sedis; Spartobacteria_genera_incertae_sedis
Otu00026	fertilized forb	0.341	0.041	Acidobacteria; Acidobacteria_Gp1; Acidobacteria_Gp1_order_incertae_sedis; Acidobacteria_Gp1_family_incertae_sedis; Gp1
Otu00023	fertilized	0.595	0.017	Bacteria_unclassified; Bacteria_unclassified;

	forb			Bacteria_unclassified; Bacteria_unclassified; Bacteria_unclassified
Otu00044	unfertilized forb	0.418	0.020	Acidobacteria; Acidobacteria_Gp1; Acidobacteria_Gp1_order_incertae_sedis; Acidobacteria_Gp1_family_incertae_sedis; Gp1
Otu00034	unfertilized forb	0.455	0.033	Proteobacteria; Proteobacteria_unclassified; Proteobacteria_unclassified; Proteobacteria_unclassified; Proteobacteria_unclassified
Otu00027	fertilized grass	0.415	0.011	Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Planctomyces
Otu00013	fertilized grass	0.358	0.045	Actinobacteria; Actinobacteria; Actinomycetales; Thermomonosporaceae; Actinoallomurus
Otu00024	unfertilized grass	0.384	0.026	Planctomycetes; Planctomycetacia; Planctomycetales; Planctomycetaceae; Singulisphaera
Otu00003	unfertilized grass	0.441	0.008	Verrucomicrobia; Spartobacteria; Spartobacteria_order_incertae_sedis; Spartobacteria_family_incertae_sedis; Spartobacteria_genera_incertae_sedis