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AN INVESTIGATION INTO THE DISTRIBUTION OF MYXOBACTERIA, THE EFFECT OF PREDATOR-PREY INTERACTIONS ON SECONDARY METABOLITE EXPRESSION, AND DRIVERS OF MICROBIAL COMMUNITY ASSEMBLY

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AN INVESTIGATION INTO THE DISTRIBUTION OF MYXOBACTERIA, THE EFFECT OF PREDATOR-PREY INTERATIONS ON SECONDARY METABOLITE EXPRESSION, AND DRIVERS OF MICROBIAL COMMUNITY ASSEMBLY

A DISSERTATION APPROVED FOR THE

DEPARMTMENT OF MICROBIOLOGY AND PLANT BIOLOGY

BY

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Abstract

The myxobacteria are Deltaproteobacteria capable of growing on a wide range of bacterial and fungal prey. To kill their prey, myxobacteria produce diverse secondary metabolites, many of which have medically desirable activities, including anti-viral, anti-fungal, anti-cancer, and antibacterial properties; however, the myxobacteria are difficult to cultivate because they do not form readily identifiable colonies on agar, do not disperse well in liquid, and grow slowly. As a consequence, the myxobacteria are poorly represented in culture, and as such their natural distribution and the effect predator-prey interactions on secondary metabolite expression are poorly understood. The purpose of the first part of this dissertation is to expand our knowledge of the natural distribution of the myxobacteria using cultivation-independent techniques, study the effect of predator-prey interactions on secondary metabolite expression, and improve their cultivation by supplementing growth media inhibitory dyes. The natural distribution of the myxobacteria was described using cultivation independent techniques. This analysis identified terrestrial, halophillic/ halotolerant, and generalist families of myxobacteria, highlighted the limitations of cultivation-based biogeography, and provided insights into the ecology of both cultivated and uncultivated clades of myxobacteria. To test the effect of predator-prey interactions on secondary metabolite production, cultures of *Myxococcus fulvus* were grown on media containing heat-killed prey biomass as the sole

carbon and energy source. *Myxococcus fulvus* produced more diverse LC-MS profiles when growing on prey that supported better growth, suggesting a link between predation rates and secondary metabolite expression. Finally, we report that a concentration of 1 mg/L crystal violet in standard growth media is useful for the isolation and purification of myxobacteria. Applied correctly, the insights from this dissertation will both improve cultivation of novel myxobacteria by directing sample site selection, and help unlock products of silent secondary metabolite gene clusters in existing cultures by leveraging the effects of predator-prey interactions.

The second half of this dissertation focuses on the effect of chemical and biological forces on bacterial diversity and community structure. In the last 50 years, world wide application of NPK fertilizers has increased ~500%, and the widespread use of fertilizers in agriculture has indirectly increased the input of inorganic nutrients into natural ecosystems. Because microbial communities are sensitive to changes in nitrogen, phosphorus, and potassium, we hypothesized that nutrient enrichment would suppress diversity and alter the structure of leaf litter bacterial communities by selecting for copiotrophic taxa. We demonstrated that bacterial communities in tropical rainforest leaf litter are more sensitive to changes in nitrogen compared to potassium, phosphorus, or micronutrients. In addition to chemical factors, macrobiotic plant-animal interactions may also shape the diversity and structure of bacterial communities. The Portal Project is a long-term rodent exclusion experiment and has shown that the exclusion of the

kangaroo rat (*Dipodomys spp.*) alters the density, diversity, and composition of the plant community through removal of granivory and soil disturbing behaviors; however, it was unknown if kangaroo rat removal has an effect on the soil microbial community. We hypothesized that granivorous rodent populations indirectly impact the diversity and structure of the bacterial community through regulation of the plant community. To test our hypothesis, we conducted a survey of soil bacterial communities, plant and rodent censuses, and soil chemistry analyses on rodent exclusion plots. Although the effect sizes were small, our results support the hypothesis that the direct and indirect effects of trophic interactions between above-ground populations can shape the diversity and structure of the below-ground bacterial community.

Chapter 1. Dissertation summary and contributions, author's declaration, and acknowledgements

Dissertation summary and contributions

Microorganisms provide essential services to health and society, including biogeochemical cycling, energy production, bioremediation, medicine, food, and many other industrial products. Yet, nearly 350 years after Antoine Van Leeuwenhoek first observed these "*wee little animalcules*," we have only just begun investigating the interactions between and among microbial populations, and interactions between microbial communities and the environment. Understanding how these interactions fit together is critical as we address emerging man-made problems, such as climate change, drought, famine, and antibiotic resistance.

This dissertation focuses on two broad themes: *the ecology and physiology of the myxobacteria* and *drivers of microbial assemblages*. In the first part, I describe my research on the biogeographical distribution of the myxobacteria, their interactions with prey, and strategies to improve their isolation. In the second, I focus on the effect of chemical and biological forces on bacterial diversity and community structure.

The ecology and physiology of the myxobacteria

Chapters 2-4 are focused on the ecology and physiology of bacteria belonging to the order *Myxococcales*. The myxobacteria are Deltaproteobacteria, known for their

complex life cycle and sophisticated cooperative behaviors. They grow in rippling swarms that degenerate into mounds as nutrients become scarce[1–7]. As the cell density within the mounds increases, the cells aggregate to form macro structures called "fruiting bodies," inside which a small proportion of cells develop into spores[2,3,8]. Upon germination, the life cycle begins again[2,3,7–9]. Many myxobacteria are predators and produce a diversity of secondary metabolites which serve the function of lysing prey cells[1,4,10–13]. They are nearly ubiquitous in nature and frequently occur in soils[14,15], decaying plant material[15], on living plants[16,17], the rhizosphere, animal dung, marine and freshwater[9,18], and are typically considered mesophiles and neutrophiles.

Chapter 2 describes the global distribution of families within the *Myxococcales*. Prior to this work, our knowledge of the biogeography of Myxobacteria was based on their presence and absence in cultivation-based studies[19]; however, cultivation of the myxobacteria is notoriously difficult[12,20–23], and only a fraction of their phylogenetic diversity based on 16S rRNA gene diversity has been cultivated[21–24]. To get around this limitation, we studied the distribution of the myxobacteria using cultivation-independent techniques. We described the distribution of families within the order *Myxococcales*, and identified terrestrial *(Myxococcaceae, Polyangiaceae, Cystobacteraceae, Bacteriap25, MSB-4B10, BIrii41, Mle1-27, Blfdi19,* and *KD3-10*), halophillic/halotolerant-aquatic (*PSB.29, VHS-B3-70, MidBa8, Eel-36e1D6,* and *UASB-TL25*), and generalist (*Haliangiaceae, Sandaracinaceae, P3OB-42, Phaselicystaceae,* and *Nannocystace*) myxobacteria. Our analysis highlighted the limitations of cultivation-based biogeography, and provided insights into the ecology of both cultivated and uncultivated

clades of myxobacteria. Applied correctly, these insights could improve cultivation of novel myxobacteria, thus providing targets for novel drug discovery. This study was conceived, designed, analyzed, and authored by me. I compiled data from ~81,000 publicly available 16S rRNA gene libraries from QIITA[25]. Bradley Stevenson, my PhD advisor, contributed computational resources and feedback.

Chapter 3 describes the effect of prey on secondary metabolite production by *Myxococcus fulvus.* Many myxobacteria are predators capable of growing on a wide range of bacterial and fungal prey[26–28]. To kill their prey, many myxobacteria produce diverse secondary metabolites[29,30]. Many of these secondary metabolites have medically desirable activities, such as anti-viral, anti-fungal, anti-cancer, and antibacterial properties, making the myxobacteria attractive targets for novel drug discovery[13,29]. We predicted that myxobacteria would produce different secondary metabolites when grown on different prey, and prey that promoted faster swarm expansion would produce more diverse profiles. To test this, metabolites were extracted from cultures of *M. fulvus* grown on media containing heat-killed prey biomass as the sole carbon and energy source. *Myxococcus fulvus* produced more diverse LC-MS profiles when growing on prey that better supported swarm expansion. Although this study was limited, it suggests a link between predation rates and secondary metabolite expression. This study was conceived and designed by me. The *Myxococcus fulvus* strain used was isolated by me from the University of Oklahoma duck pond. Prey bacteria were isolated by Maaz Khan and me from the OU duck pond. Swarm expansion assays were conducted by Maaz Khan under my mentorship. Organic

extracts of *Myxococcus fulvus* grown on prey and controls were collected by Maaz Khan and me. LC-MS traces of the crude extracts were generated by the University of Oklahoma Natural Products Discovery Group and analyzed by me. Bradley Stevenson contributed laboratory resources and feedback on the project.

Chapter 4 is a short communication that addresses the non-reproducibility of a published method to isolate and purify myxobacteria[31]. In 2003, Zhang *et al.* reported that crystal violet could be added to the standard myxobacteria growth media to improve the isolation and purification of myxobacteria[31]; however, we were unable to reproduce their results and were unable to find any other publication that reported using this method to isolate myxobacteria. The work in Chapter 4 includes an optimization experiment that demonstrates the concentration proposed by Zhang *et al.* was 1000x greater than myxobacteria could tolerate. We report that a concentration of 1 mg/L crystal violet in standard growth media is useful for the isolation and purification of myxobacteria, and that crystal violet can be used to enrich for previously uncultivated members of the family *Phaselicystis*. In addition, we report that the addition of acriflavine and brilliant green may be useful for the isolation and purification of myxobacteria. The study was designed collaboratively by Zainab Sandhu, Clayton Matthews, Bradley Stevenson, and myself. Zainab Sandhu and Clayton Matthews contributed to the data collection and analysis under my mentorship. Bradley Stevenson provided materials and feedback.

Drivers of bacterial assemblages

Chapters 5 and 6 are focused on chemical and biological forces that drive bacterial diversity and community structure. Microbial communities are sensitive to changes in nitrogen, phosphorus, and potassium (NPK)[32–41]. These three elements influence the rate of ecosystems processes, such as carbon, nitrogen, or phosphorus cycling[34,35,37–40,42,43], though most microbial communities are more sensitive to nitrogen than phosphorus or potassium[34–36,38,41]. Additionally, biological interactions can shape the diversity and structure of bacterial communities. Specifically, plant-microbe interactions play important roles in ecosystem processes[44–46]. The microbial community contributes to the diversity and productivity of the plant community through nutrient fixation, recycling, and mineralization[47–49]. In turn, the plant community supports the soil microbial community by providing carbon substrates (as soil exudates and litter) and microhabitats[44,46,50,51], as well as hosts for symbiotic and pathogenic relationships[45,46,48]. Thus, nutrient gradients and interactions with macroscopic organisms influence how bacterial communities assemble.

Chapter 5 describes the effect of long term NPK fertilization on bacterial communities in leaf litter from a lowland tropical rainforest. In the last 50 years, world wide consumption of NPK fertilizers has increased ~500%[52], and the widespread use of fertilizers in agriculture has indirectly increased the input of inorganic nutrients into natural ecosystems through the deposition of enriched dust, aerosols, surface water, and groundwater[53–56]. In this chapter, we report the effects of nine years of nitrogen, phosphorus, and potassium fertilization on the litter bacterial communities in a

Panamanian rainforest. We hypothesized that nutrient enrichment would suppress diversity and alter the structure of leaf litter bacterial communities by selecting for copiotrophic taxa. We demonstrated that that bacterial communities in tropical rainforest leaf litter are more sensitive to changes in nitrogen compared to potassium, phosphorus, or micronutrients. I contributed to data collection, analysis, and writing. Samples were collected by Dr. Bradley Stevenson, Dr. Jonathan Shik, and Dr. Michael Kaspari. Feedback on the writing was provided by Dr. Michael Kaspari, Dr. Bradley Stevenson, Dr. Jonathan Shik, and Dr. Joseph Wright.

Chapter 6 describes the effects of rodents on soil bacterial communities. This rodent exclusion experiment has been maintained since 1977, and has shown that the exclusion of the kangaroo rat (*Dipodomys spp.*) alters the density, diversity, and composition of the plant community through removal of granivory and soil disturbing behaviors[57–61]; however, it is unknown if kangaroo rat removal has an effect on the soil microbial community. We hypothesized that granivorous rodent populations indirectly impact the diversity and structure of the bacterial community through regulation of the plant community. To test our hypothesis, we conducted a survey of soil bacterial communities, plant and rodent censuses, and soil chemistry analyses on rodent exclusion plots. Although the effect sizes were small, our results support the hypothesis that the direct and indirect effects of trophic interactions between above-ground populations can shape the diversity and structure of the below-ground bacterial community. This study was designed collaboratively by Dr. Michael Kaspari, Dr. Bradley Stevenson, Dr. Morgan Ernest, Dr. Jane Lucas, Dr. Michael Weiser, and myself. I

contributed to sample collection, data generation, analysis, and writing. Dr. Jane Lucas and Dr. Michael Weiser assisted in sample collection.

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Author’s Declaration

I declare that this dissertation has been composed by myself, and that it has not been submitted in any previous application for any other degree of professional qualification. I confirm that the work is my own, except where stated as part of jointly-authored publications. My contribution and those of the other authors have been explicitly stated for each chapter below. I confirm that the appropriate credit has been given within this thesis where reference has been made to the work of others.

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Chapter 2. Geographical distribution of the Myxobacteria

Abstract

Current knowledge of Myxobacterial biogeography is based on the presence/absence of Myxobacteria from cultivation-based studies; however, these studies are limited by the high proportion of uncultivated diversity. We studied the global distribution and relative abundance of Myxobacteria using cultivation-independent techniques to gain insights into the ecology and physiology of uncultivated myxobacteria. Approximately 81,000 publicly available 16S rRNA gene sequence libraries were classified into a consistent ontology describing biome and sample type. Myxobacteria were present in ~20% of the libraries. They were most diverse in agrarian, wetland, and estuary biomes, and in samples taken from soil, the rhizosphere, and compost. The presence/absence and relative abundance of each family varied among biomes and sample types, suggesting geographical divergence among families in the order *Myxococcales*. Terrestrial (Myxococcaceae, Polyangiaceae, Cystobacteraceae, Bacteriap25, MSB-4B10, BIrii41, Mle1-27, Blfdi19, and KD3-10), hypersaline-aquatic (PSB.29, VHS-B3-70, MidBa8, Eel-36e1D6, and UASB-TL25), and generalist (Haliangiaceae, Sandaracinaceae, P3OB-42, Phaselicystaceae, and Nannocystace) myxobacteria were identified based on their incidence and relative abundance across libraries. Here, we make predictions about the ecology of clades within the order *Myxococcales*. The successful application of these predictions should aid in the cultivation of previously uncultivated myxobacteria.

Introduction

Members of the *Myxococcales* share a distinct lifestyle: vegetative cells grow in rippling swarms that degenerate into mounds as nutrients become scarce[1–7]. As the cell density within the mounds increases, the cells aggregate to form macro structures called “fruiting bodies,” inside which a small proportion of cells develop into spores[2,3,8]. During heterotrophic growth, many myxobacteria act as predators by producing a diversity of secondary metabolites to lyse prey cells[1,4,9–11,11,12]. Because of their ability to produce large bioactive secondary metabolites, cultivation of novel myxobacteria has become an area of interest for many drug discovery programs. In total, more than 600 distinct myxobacterial secondary metabolites have been described, several of which have developed into antibiotics or are at various stages of pre-clinical and clinical testing[11,12].

Despite much interest in their ability to synthesize medically important compounds, the natural distribution of the myxobacteria remains poorly understood. Current knowledge of their distribution is based on their presence or absence across environments using cultivation-based approaches. Cultivable myxobacteria are nearly ubiquitous in nature and frequently occur in soils[13–16], decaying and living plant material[14,16,17], the rhizosphere[14,16], or animal dung[16]. Most are considered mesophiles and neutrophiles, although some myxobacteria are capable of growing in psycrophilic[15,18] and thermophilic conditions, and in acidic or alkaline pHs[15,19]; however, cultivation studies are insufficient to describe biogeographical patterns of myxobacteria, especially considering the notorious difficulty of their cultivation[20–24] and the extent of

uncultivated diversity within the order[22–24].

Cultivation-independent techniques, such as high-throughput sequencing of 16S rRNA gene libraries, offer an alternative to the inherent biases of cultivation-based studies. These techniques have changed the way we view microbial community structures and revealed exciting spatial patterns, such as endemism of OTUs to regions and habitats[25–28], differential responses of taxa to environmental gradients[29–31], and genetic divergence among related species[32–35]. The information gleaned from community level molecular analysis has given insights into other difficult to cultivate groups of bacteria, such as the ecology and physiology of *Acidobacteria*[36–41]. Thus, leveraging the wealth of available sequence data to describe the distribution of uncultivated bacteria is a low-risk high-reward strategy for improving the cultivability of those groups.

The purpose of this project was to use cultivation-independent techniques to describe the global distribution of the myxobacteria. Recent advances in sequencing technologies have made monitoring microbial systems relatively easy and inexpensive[42–44]. The rapid accumulation of sequence data in publicly accessible repositories[45–47] presents an exciting opportunity for the evaluation of local, regional, and global microbial community assembly patterns[48–51]. Here, we analyzed publically available 16S rRNA sequence data to describe the global distribution of families belonging to the order *Myxococcales*. Using these patterns, we make inferences concerning the ecology and physiology of uncultivated myxobacteria, as well as present strategies to improve cultivation of novel myxobacteria.

Materials and Methods

Demultiplexed,quality-filtered, and preprocessed 16S rRNA gene sequence libraries were downloaded from QIITA[45] and compiled into a single database. Data was filtered to contain only 16S rRNA gene sequence libraries with sufficient metadata to classify each library into a consistent ontology describing biome and sample matter based on the author's descriptions and GPS coordinates. Inclusion criteria also required the clear documentation of sequencing platform, target subfragment, latitude, and longitude. Libraries consisting of mock communities were not included in the data set. Sequence data from selected studies was assigned into OTUs using closed reference OTU picking in QIIME[52] (version 1.9) and the non-redundant Silva reference database[53,54] (Release 123. Each library was rarefied to 1000 sequences to improve comparability across libraries by normalizing sequencing depth. In total, 80,903 16S rRNA gene libraries from 293 different projects met our quality standards.

To calculate the diversity and relative abundance of the myxobacteria, OTUs not belonging to the order *Myxococcales* were removed from the data. Alpha diversity of the myxobacteria was calculated using QIIME as the number of observed OTUs. The relative abundance of each family was calculated within each biome and sample matter as the median relative abundance and excluded libraries where the family was not detected. The incidence of each family was calculated as the percentage of libraries that contained at least 1 OTU belonging to that family within each biome and sample matter. For simplicity, the incidence of each family was categorized using the ACFOR rating within each biome and sample matter. A family was categorized as abundant (A)

if the incidence was greater than 40%, common (C) if it was greater than 20%, frequent (F) if it was greater than 10%, occasional (O) if it was greater than 5%, and rare (R) if it was less than 5%.

Results

After quality filtering and taxonomy assignment, 15,978 libraries of the 80,903 libraries contained at least one sequence belonging to the order *Myxococcales***.** The sources of libraries across biomes was highly variable, with the majority of samples collected from human-associated (49.7%), animal-associated (18.4%), agriculture-associated (7.7%), ocean and sea (6.5%), and pond/lake (6.2%) biomes (Appendix A. Supplemental Tables 1 and 2). The majority of samples were collected from feces (n = 33.1%), other animal material (e.g. sebum, mucous, hair, etc., 28.6%), soil (8.4%), and fresh water (8.2%). Myxobacteria belonging to the families *Haliangiaceae* (11.8%), *Polyangiaceae* (9.4%), P3OB.42 (8.9%), *Sandaracinaceae* (8.8%), *Blrii41* (8.0%), and *Cystobacteraceae* (5.4%) were detected most frequently across all samples (Appendix A. Supplemental Tables 1 and 2). The families *bacteriap25* (0.9%), *Vulgatibacteraceae* (0.6%), *MidBa8* (0.5%), *UASB.TL25* (0.5%), *Eel.36e1D6* (0.5%), *VHS.B3.70* (0.4%), *VHS.B4.70* (0.3%), and *PSB29* (0.2%) were detected in the fewest samples (Appendix A. Supplemental Tables 1 and 2).

The myxobacteria were widely distributed around the world (Fig. 1). Sequences belonging to the order were detected at latitudes between -88.2° and 78.9° and longitudes between -162.2° and 175.3°. Many families within the order were detected

across all latitudes; however, two clades, one representing the *PS.B29* and the other containing the *bacteriap25, VHS.B3.70, MidBa8, MSB,4B10,* and *UASB.TL25,* had reduced latitudinal ranges (Fig. 2). The *PS.B29* have the smallest range, and were only detected at latitudes between -3.73° and 51.65°.

The diversity of sequences belong to the myxobacteria followed a latitudinal gradient, with the most diverse samples occurring near the equator and the least diverse samples occurring closer to the poles (Fig. 3). Agriculture-associated biomes (23 OTUs/sample), wetlands (12 OTUs/sample), and estuaries (11 OTUs/sample) had the highest median diversity of myxobacteria, while mediterranean shrublands (1 OTU/sample), cold deserts (2 OTUs/sample), oceans and seas (2 OTUs/sample), ponds and lakes (2 OTUs/sample), saline lakes (2 OTUs/sample), and animal-associated biomes (2 OTUs/sample) had the lowest median diversity (Fig. 4). The myxobacteria were most diverse in samples taken from soil (21 OTUs/sample), the rhizosphere (20 OTUs/sample), compost (19 OTUs/sample), and other plant-associated materials (13 OTUs/sample). A median of 1 OTU/sample was detected in samples taken from algae, animal material, food products, fresh water, microbial mats, and seawater (Fig. 4).

The incidence and relative abundance of each family varied among biomes and sample matter, demonstrating geographical divergence among families in the order *Myxococcales* (Figs. 5 and 6). Several clades of myxobacteria were specific to terrestrial environments. In general, the *Myxococcaceae, Polyangiaceae, Cystobacteraceae, Bacteriap25, MSB-4B10, BIrii41, Mle1-27, Blfdi19,* and *KD3-10* were

mostly commonly found in sample matter from sediments, soils, and the rhizosphere. In contrast, OTUs belonging to the *PSB.29, VHS-B3-70, MidBa8, Eel-36e1D6,* and *UASB-TL25* groups were most frequently detected in saline environments, such as saline lakes, oceans, and seas, and from samples of saline water and sediments; however, their incidence and relative abundance were low across all biomes and matter, suggesting these groups occupy specialized niches in nature.

Multiple clades of myxobacteria were widely distributed to both terrestrial and aquatic habitats (Figs. 5 and 6). The *Haliangiaceae, Sandaracinaceae, P3OB-42, Phaselicystaceae,* and *Nannocystace* had generally higher incidence and relative abundance in terrestrial habitats, but were also regularly detected in saline aquatic biomes and sample matter. In particular, the *Haliangiaceae* had the widest distribution of habitats among myxobacteria. They were the most frequently detected family in our analysis (~12% global incidence), including >40% incidence in estuary, wetland, temperate forest, boreal forest, montane shrubland, semi-arid desert, cold desert, agricultural-associated biomes. Furthermore, they were rare or absent in only three of the 22 biomes surveyed.

Discussion

Our analysis of large, publicly available 16S rRNA gene sequence data allowed us to describe distributional patterns of myxobacteria. As expected, the myxobacteria followed a traditional latitudinal diversity gradient and had high incidence and abundance in soil and in association with plants, consistent with observations from

cultivation based research[15]; however, our analysis highlights the limitations of cultivation-based microbiogeography, and provides insights into the ecology of both cultivated and uncultivated clades of myxobacteria. For many uncultivated families of myxobacteria, this is the first analysis to shed light on their distribution. Carefully applied, our results should improve the probability of successful cultivation of novel myxobacteria. These findings allow us to summarize and make predictions about the ecology of families within the order *Myxococcales*:

PSB-29- OTUs belonging to this family were observed in fewer samples than any other family, so predictions about this family are made with limited confidence. Members of this clade have been detected in clone libraries from deep sea hydrothermal vents[55], submarine active mud volcano sediments[56], and hypersaline microbial mats[57]. This is consistent with our analysis that *PSB-29* are relatively abundant in saline lakes, oceans, and seas and from samples from saline water and sediments. *PSB-29* clones tend to appear in hypoxic-to-anaerobic environments with high concentrations of sulfides[55–57] and in methane enrichments[58,59], suggesting the possibility that this group may reduce sulfate and oxidize methane, as has been described previously in other deltaproteobacteria[60], but not in the myxobacteria.

Bacteriap25- This family was most frequently detected in agriculture-associated biomes and sample matter taken from sediments, soils, and the rhizosphere. Our observations were consistent with the literature, as clones from this family appear in surveys of agriculture associated soils[61–65], natural soils[63,66], under plants[67], deep sea sediments[68–71], and freshwater sediments[72–74]. Interestingly, clones from this family have

also been found in both aerobic[75] and anaerobic[76] sludge from wastewater treatment plants and in extreme environments, such as hot spring microbial mats[77]. Functionally, members of this family may be capable of formaldehyde metabolism[73], aliphatic hydrocarbon degradation[78,79], methane oxidation[80] and sulfate reduction[80]; however, with no cultivated representatives of this group, these predictions are at best speculation.

VHS-B3-70- The *VHS.B3.70* most frequently occured in saline lake biomes, in both sediments and saline water. Members of this group appear in clone library surveys in sediments from saline environments[71], particularly those with high sulfide and methane concentrations[56,57,80–82], or hydrocarbon contamination[79,83,84]. We predict that this group of myxobacteria are anaerobic halophiles, likely capable of polyaromatic hydrocarbon degradation, sulfate reduction, and/or methane oxidation.

MidBa8- Similarly to the *VHS.B3.70* clade, sequences belonging to *MidBa8* appeared most frequently in saline biomes in both sediments and water. Because of their presence in environments with high concentrations of sulfides[57,83,85–88] and hydrocarbon polluted environments[83,88], we suspect that members of the *MidBa8* clade participate in sulfur cycling and hydrocarbon degradation in saline environments.

MSB-4B10- Members of the *MSB.4D10* group appeared in more sample than any other members of the clade containing the uncultivated groups *bacteriap25, VHS.B3.70, MidBa8, MSB.4B10,* and *UASB.TL25* (Appendix A. Supplemental Tables 1 and 2). In contrast to those other groups, *MSB.4B10* most frequently occurred in wetlands, tropical forests, montane shrublands, temperate grasslands, and agrarian biomes, and from samples taken from sands, soils, plant material, and the rhizosphere.

Clones from this family have been detected in association with plants and the rhizosphere[67,89,90], and have been observed in high sulfate estuarine sediments[80].

UASB-TL25- The SSU rRNA of members of the *UASB.TL25* were most frequently observed in saline lake biomes and in samples taken from sediment and saline lake water. Data from clone libraries suggests that members of this family are found in polluted saline environments under anaerobic, methane oxidizing, and sulfate reducing conditions[83,91–94]. Interestingly, sequences belonging to this family was enriched in dechlorinate perchloroethene degradation RNA SIP experiments[93], indicating they may be involved in PCE dechlorination.

Sandaracinaceae- The *Sandaracinaceae* are another family of myxobacteria with few cultivated representatives. The only known species in culture, *Sandaracinus amylolyticus*, was isolated in India from soil containing plant residues[95]; however, our demonstrated that this family is much more widely distributed in nature, and is especially common in soil, sediments, the rhizosphere, and compost. The *Sandaracinaceae* had high incidence and relative abundance in saline biomes (estuaries, oceans, seas, and saline lakes), suggesting that some divisions in the family are halotolerant/halophilic. The high abundance of this family has been previously noted, including solid compost of dairy manure[96], the rhizosphere of yews[97], and marine sediments[98,99].

Mle1-27- *Mle1.27* was first detected in modified Ludzack-Ettinger waste water treatment reactors in 2000[100]. Since then, this family has been regularly detected in both anaerobic and aerobic wastewater reactors[100–102] and bulk soils in agriculture[61,62,67,90,103].

In each case, this family appears to be mesophillic and present in systems with high concentrations of nitrogen, phosphorus, and organic carbon[61,62,67,90,100–103]. Our data suggests that soil, compost, and rhizosphere samples taken from agricultural biomes may be the best targets to cultivate this group, and members of this family are not likely to be halophilic nor halotolerant.

Eel-36e1D6- The *Eel.36e1D6* family was first observed in clone libraries taken from anoxic methane-oxidizing marine sediments, suggesting the potential for methanotrophy[104,105]; however, this group has no cultivated representatives, and has only rarely been mentioned in the literature. Members of the *Eel.36e1D6* family were most commonly found in sediments and high saline biomes in our data, but were not detected regularly across other samples or biomes.

BIrii41- The *BIrii41* family was detected in ~8% of the total global libraries, including >40% of libraries from agriculture-associated biomes, wetlands, and montane shrubland biomes, and soil, compost, and rhizosphere sample matter. Sequences belonging to this group have been found in high relative abundance in manure compost[106,107] and are enriched when compost is treated with Flue Gas Desulphurization (FGD) Gypsum[107]. FGD Gypsum reduces N lost through ammonium volatilization, thus decreasing the C:N ratio in compost[108]. Furthermore, SIP experiments have demonstrated that the *BIrii41* are capable of incorporating nitrogen from monoammonium phosphate (MAP) and are enriched in MAP-amended soils[109]. Taken together, this suggests that the BIrii41 has evolved to specialize in environments with low C:N ratios; thus, cultivation programs targeting these uncultivated myxobacteria

should leverage nitrogen enrichments of manure compost to improve the likelihood of success.

Polyangiaceae- The *Polyangiaceae* are commonly cultivated from soil and decaying plant matter. Species from the most frequently isolated genus in the family, *Polyangium*, has nearly 40% global incidence in soil[15]. Cultivable diversity from this family is highest in the tropics and semi-arid biomes, and a variety of *Polyangiaceae* can be cultivated from a small amount of soil[15,110]. Although members of the *Polyangiaceae* have been cultivated from some extreme environments, no halotolerant nor halophilic species have been discovered. Our data confirms that the *Polyangiaceae* are not adapted for saline habitats, with sequences belonging to the *Polyangiaceae* only rarely being detected in saline biomes or sample matter.

Phaselicystidaceae- To date, representatives of the *Phaselicystidaceae* have only been cultivated from soil and decomposing plant material[111,112]. Our data confirmed that this family is common to those habitats. Although no halotolerant nor halophilic species have been discovered in this family, we detected sequences belonging to *Phaselicystaceae* in ~12% of samples taken from saline lake biomes. While their presence in hypersaline habitats is noteworthy, we hesitate to draw conclusions considering the small sample size from saline lake biomes.

Nannocystaceae- The Nannocystaceae are widely distributed in nature, and are regularly cultivated from soil containing decaying plant material[113]. Terrestrial *Nannocystaceae* isolates tend to have low salt tolerance, but the family includes halotolerant and halophilic genera commonly found in high saline muds, sands, and

sediments[113]. They are aerobic to microaerophilic, with some having broad temperature ranges[113]. Their wide distribution is reflected in our analysis, as the family *Nannocystaceae* was represented in nearly all of the biomes and sample types.

VHS-B4-70- Microbiome studies have previously detected the *VHS.B4.70* group in hypersaline environments[57,79,114,115]. In contrast, this group was rare or absent in our data with the exception of groundwater and sand samples. Interestingly, this group tends to be in high relative abundance when detected, suggesting the group is only able to persist within a narrow range of environmental conditions. Unfortunately, the ecology *VHS.B4.70* is likely to remain an enigma for some time considering the rarity of detection in the literature as well as in our analysis.

Haliangiaceae- Representatives of the *Haliangiaceae* have only rarely been cultivated. To date, only two members of this family have been isolated, both from marine environments[116]. The *Haliangiaceae* were the the most frequently detected family in our analysis, which is in stark contrast to their representation in culture. Their wide distribution, high relative abundance, and frequency of detection highlights the inability of current techniques to cultivate representatives from the *Haliangiaceae*. Because of their high abundance, wide distribution, and vast uncultivated diversity, the *Haliangiaceae* are excellent drug discovery targets, especially when considering the successful discovery of haliangicin and haliamide in *Haliangium*[117] and that 2.5% of *Haliangium ochraceum* SMP-2's recently sequenced genome codes for secondary metabolite biosynthesis[118].

Blfdi19- In our study, the *Blfdi19* group was observed mostly in terrestrial

environments. Although there are no cultivated representatives from this clade, this group has been frequently detected in soil[119,120] and rhizosphere community surveys[121–123]; however, in contrast to our results, the sequences belonging to the *Blfdi19* have also been regularly noted in lake and oceanic microbiome studies[56,73,74,124,125]. Functionally, some members of the *Blfdi19* may be capable of methanotrophy and sulfur reduction, given their presence in methane oxidizing[56,73,74,124] and sulfate reducing habitats[56,73,125].

KD3-10- Much like many of the uncultivated clades belonging to the *Myxococcales*, little published information exists for the *KD3-10* family. This group was detected in ~1% of the total libraries surveyed, including ~10% incidence in compost, soil, and rhizosphere samples; however, with the lack of corroborating evidence, inferences about the group's ecology or physiology should be made with caution.

P3OB-42- Of the clades with no cultivated representatives, the *P3OB-42* myxobacteria were the most frequently detected globally (~9%), including incidence greater than 40% in samples from sand, soil, compost, and rhizosphere samples, and from estuary, wetland, agriculture-associated, and semi-arid desert biomes. SIP experiments have suggested that the *P3OB-42* are capable of aerobic methanotrophy in rice fields[126], as well as using monoammonium phosphate as a nitrogen source[109]; however, nitrate and succinate amendments to rice field soil caused the group to disappear[65]. Much like the *BIrii41* myxobacteria, the *P3OB-42* are enriched in animal manure compost[106].

Vulgatibacteraceae- To date, only a single cultivated species represents the family *Vulgatibacteraceae*, and was discovered in forest soil from Yakushima Island,

Japan[127]. *Vulgatibacer incomptus* is a non-bacteriolytic, non-cellulolytic, obligate aerobe[127]. Outside of the successful cultivation of *V. incomptus*, little information is available on these myxobacteria; however, they have been previously noted in composting plants[128], and were detected in 54% of compost samples, but only rarely in other sample matter.

Cystobacteraceae- Members of the *Cystobacteraceae* have wide ranges of metabolic capabilities, including the ability to degrade proteins, nucleic acids, lipids, chitin, starch, xylan, and cellulose[129]. Cultivation based studies have suggested soil is the most common habitat for the *Cystobacteraceae*, but they are also found in dung and plant material[15,129]. Although the family is primarily aerobic, they do include the anaerobic genus *Anaeromyxobacter*. Our data demonstrates the wide distribution and broad habitat ranges for the *Cystobacteraceae*. In addition to occuring in soil and in association with plants, we also note that the group is detected regularly and in higher abundance in wetland, pond, and lake biomes.

Myxococcaceae- The cultivated members of the Myxococcaceae are strictly aerobic, and grow in mesophilic temperatures and neutral to slightly alkaline pHs[130]. They are able to predate on other microorganisms and grow well on casitone containing media[130–132]. The most commonly documented habitats for the *Myxococcaceae* are soil, animal dung, shore sediments, or decaying plant material[15,130]. While some *Myxococcaceae* can tolerate salt, no obligate halophiles have been discovered in this family. Our data corroborates these observations, as their abundance and frequency of occurrence are highest in sand, sediment, soil, feces and the rhizosphere and from

temperate and tropical zones. Furthermore, the *Myxococcaceae* only rarely occurred in hypersaline biomes and sample matter, further supporting observations that the family contains no halophiles.

Summary and conclusions

Prior to this study, the natural distribution of the myxobacteria was poorly characterized despite much interest in their biosynthetic capabilities. Our analysis highlighted the limitations of cultivation-based microbiogeography, and provides insights into the ecology of both cultivated and uncultivated clades of myxobacteria. We described the distribution of families within the order *Myxococcales*, and identified terrestrial (*Myxococcaceae, Polyangiaceae, Cystobacteraceae, Bacteriap25, MSB-4B10, BIrii41, Mle1-27, Blfdi19,* and *KD3-10*), halophillic/halotolerant-aquatic (*PSB.29, VHS-B3-70, MidBa8, Eel-36e1D6,* and *UASB-TL25*), and generalist (*Haliangiaceae, Sandaracinaceae, P3OB-42, Phaselicystaceae,* and *Nannocystace*) myxobacteria. Applied correctly, these insights could improve cultivation of novel myxobacteria, thus providing targets for novel drug discovery.

Figures and Tables

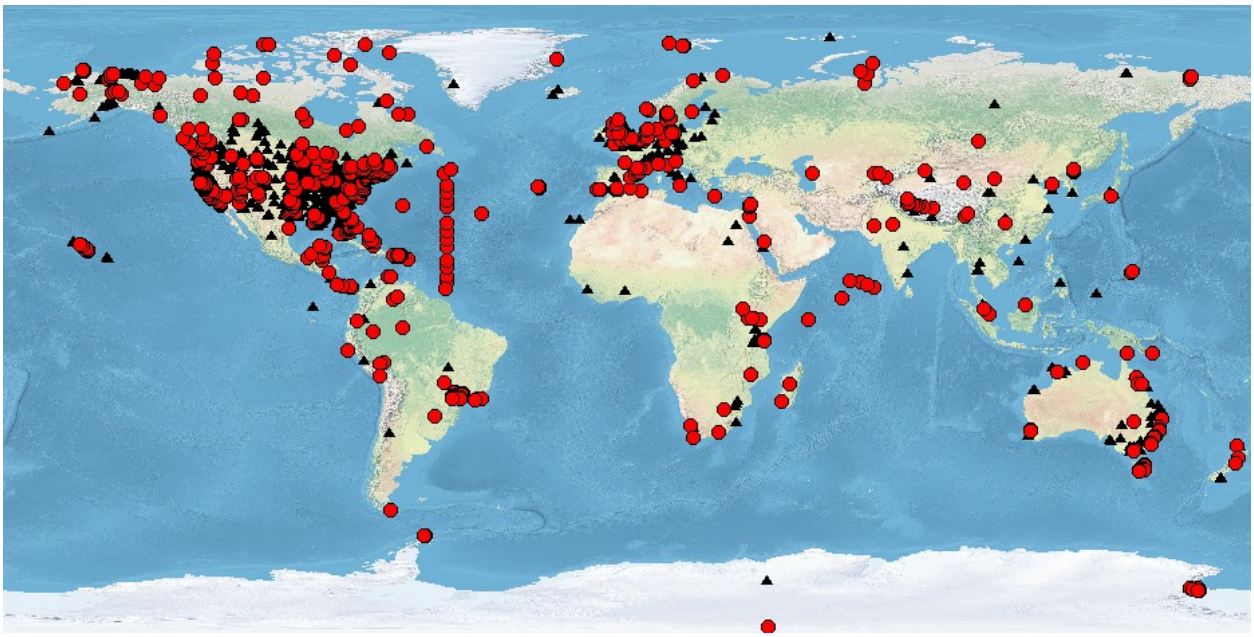

Fig. 1- Distribution of libraries. Red circles indicate sample sites that had at least one library containing a myxobacterium. Black triangles are locations where no libraries contained any myxobacteria.

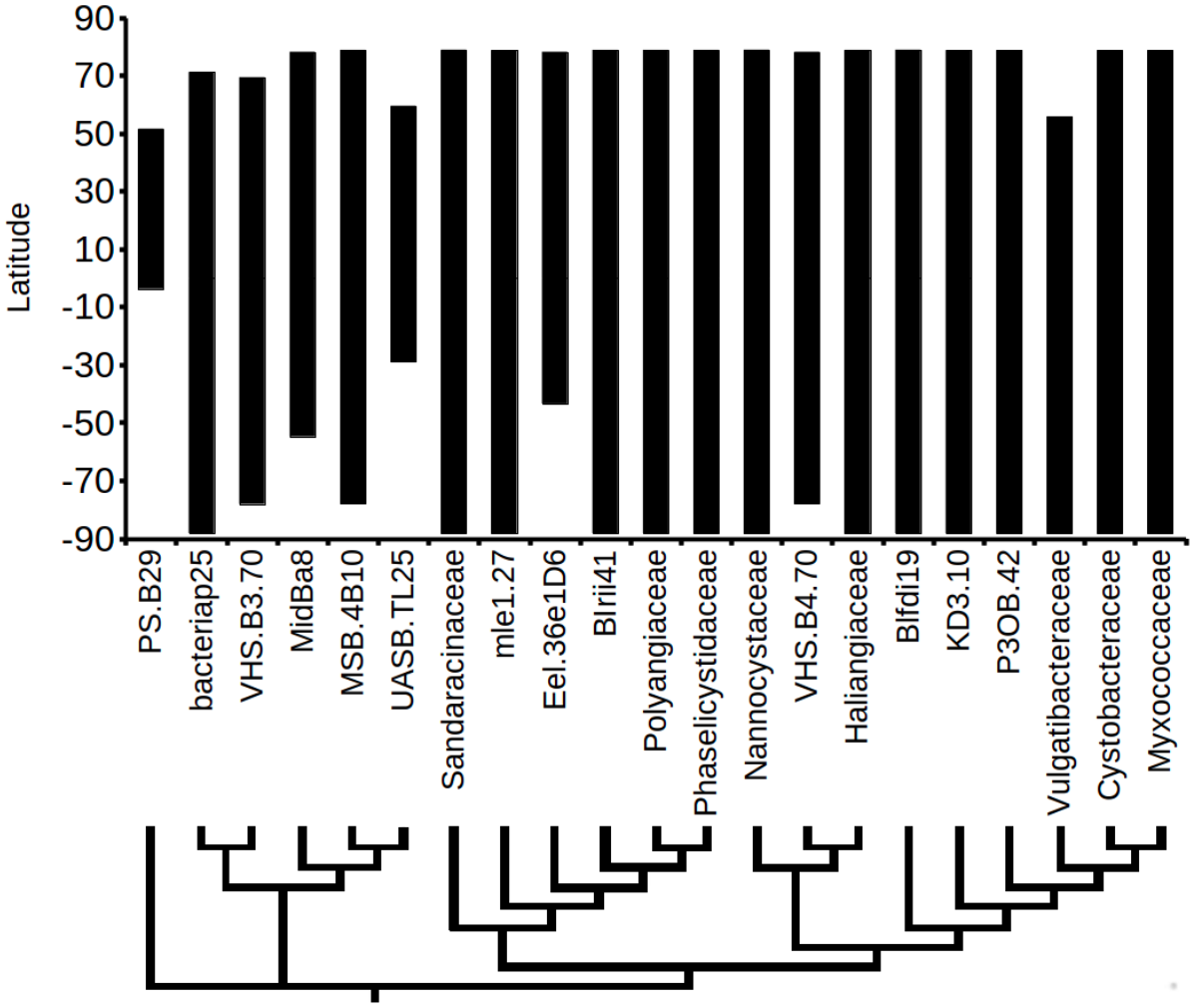

Fig. 2- Latitudinal ranges for families belonging to the order Myxococcaceae. Bars indicate inclusive ranges at which SSU rRNA gene sequences belonging to each family were detected.

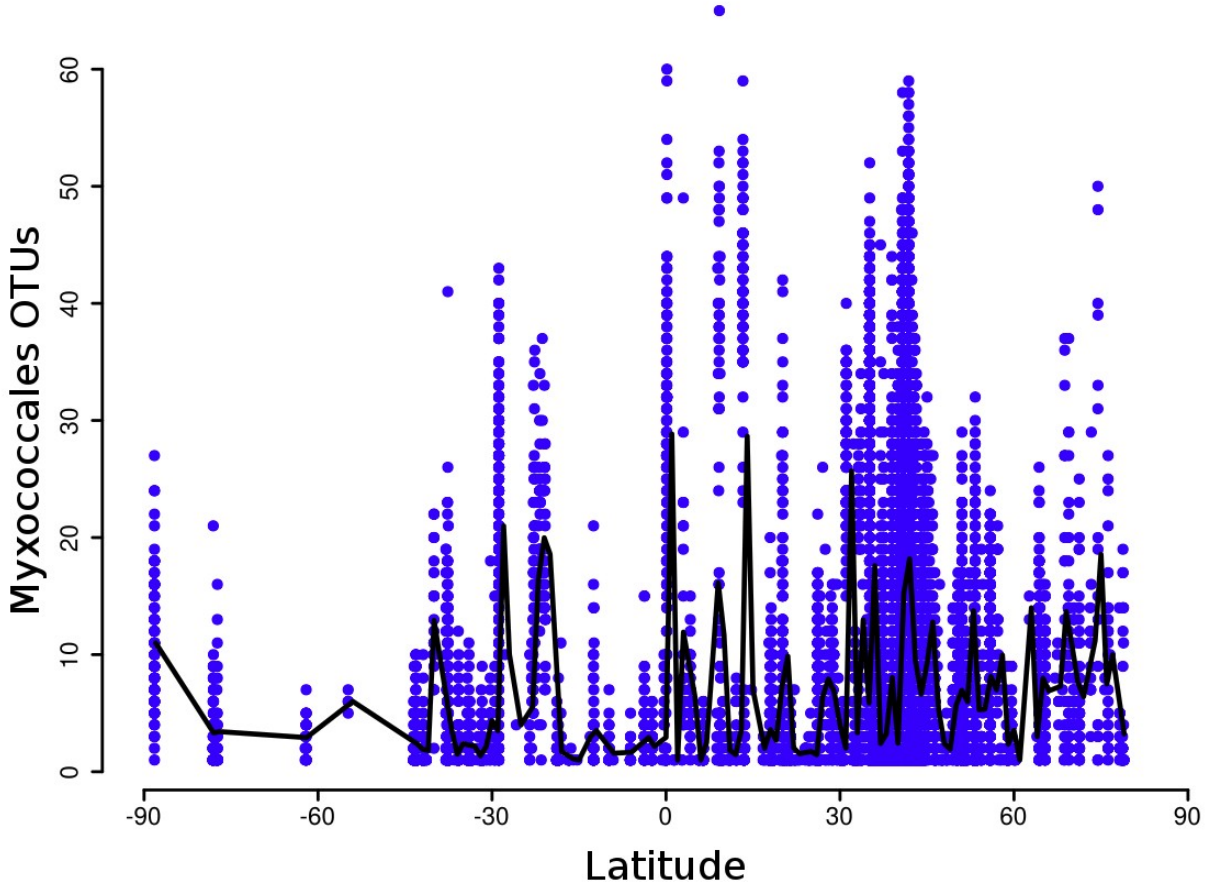

Fig. 3- Latitudinal diversity of the Myxobacteria. The black line is the mean diversity of myxobacteria binned at 1° intervals.

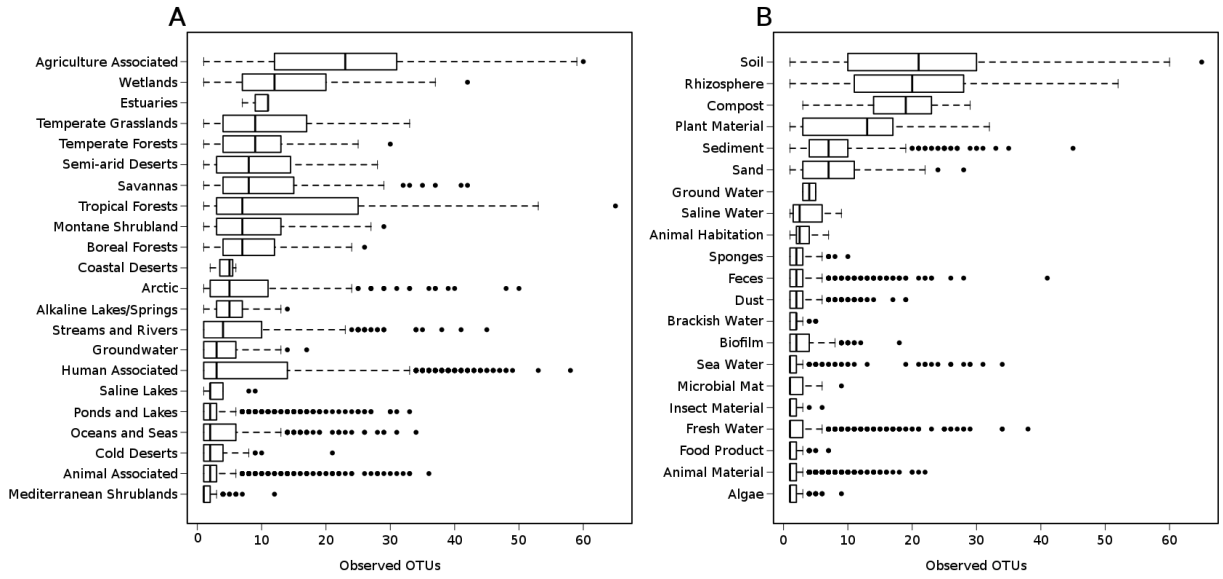

Fig. 4- Diversity of the Myxobacteria across biomes (A) and sample matter (B).

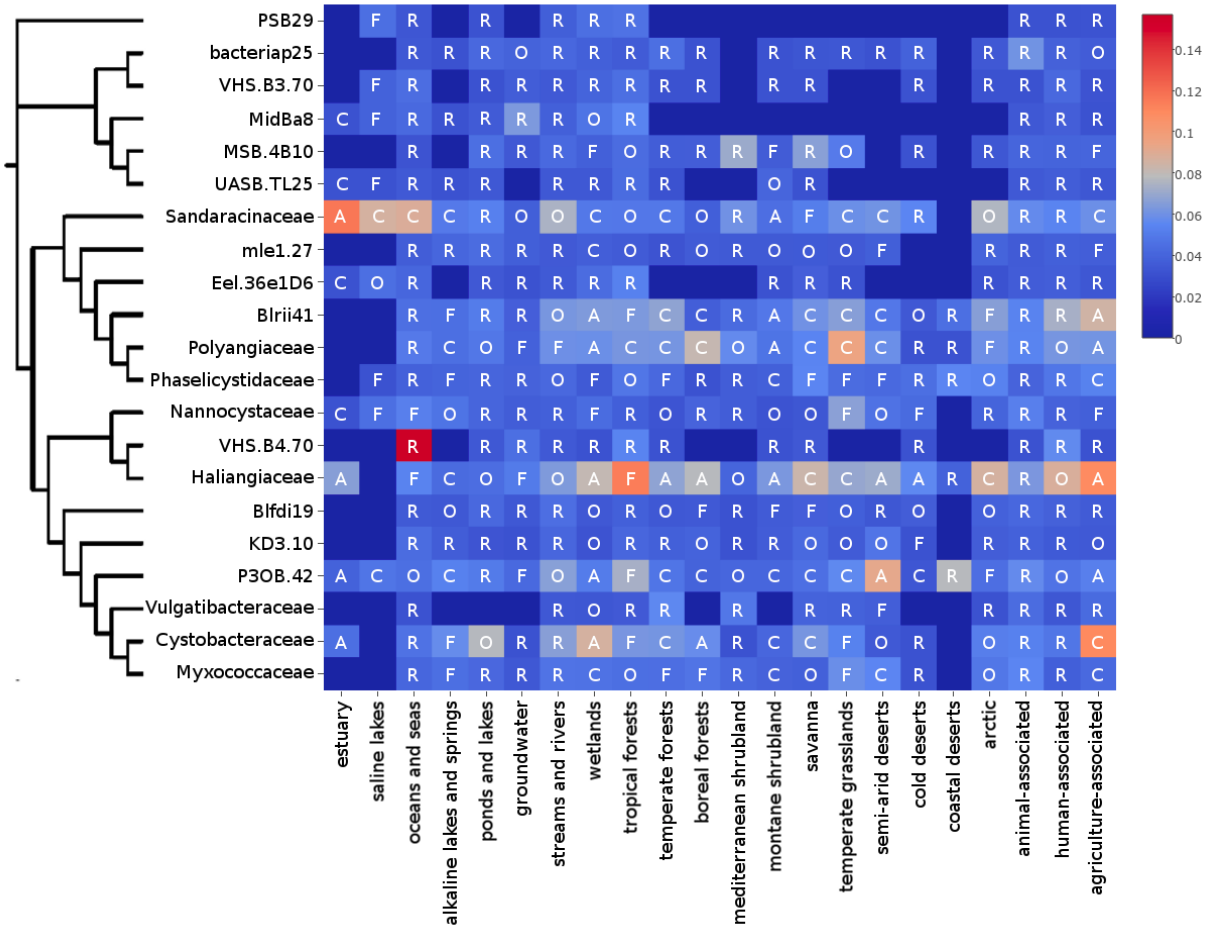

Fig. 5- Incidence and relative abundance of Myxococcaceae families by biome.

The median relative abundance of each family excluded libraries where the family was not detected. A family was categorized as abundant (A) if the incidence was greater than 40%, common (C) if it was greater than 20%, frequent (F) if it was greater than 10%, occasional (O) if it was greater than 5%, and rare (R) if it was less than 5%. Squares with no letter indicate the family was not detected in any samples from that biome.

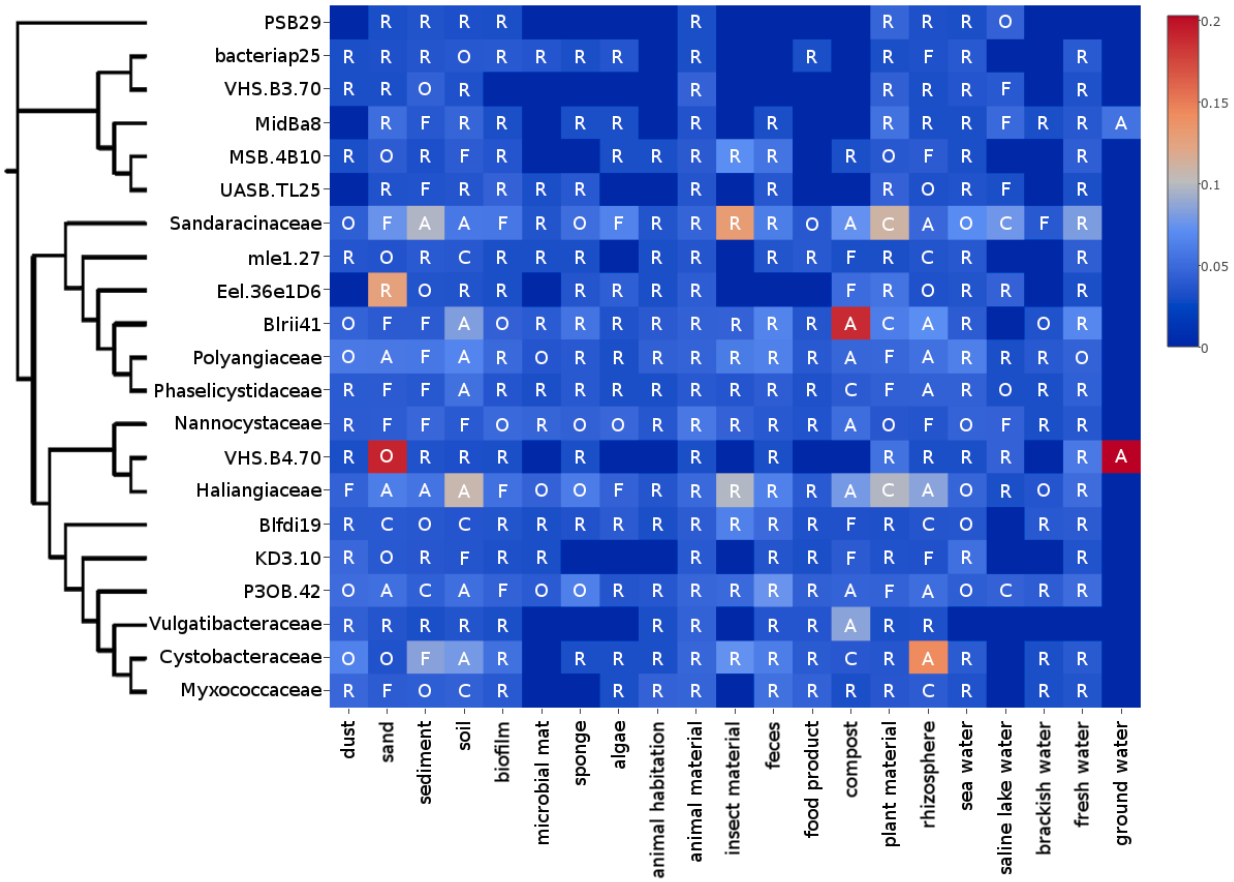

Fig. 6- Incidence and relative abundance of Myxococcaceae families by sample matter. The median relative abundance of each family excluded libraries where the family was not detected. A family was categorized as abundant (A) if the incidence was greater than 40%, common (C) if it was greater than 20%, frequent (F) if it was greater than 10%, occasional (O) if it was greater than 5%, and rare (R) if it was less than 5%. Squares with no letter indicate the family was not detected in any samples from that sample matter.

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Chapter 3. The effect of prey on secondary metabolite production by *Myxococcus fulvus*

Abstract

Many myxobacteria produce secondary metabolites to kill a wide range of bacterial and fungal prey. In total, more than 600 distinct secondary metabolites have been discovered in myxobacteria since 1967, including 42 new compounds between 2011 and 2016. Although some secondary metabolites are tied to predatory activities, it is unclear if the myxobacteria regulate secondary metabolite expression in response to signals from prey. The purpose of this project was to test the hypothesis that *Myxococcus fulvus* differentially regulates secondary metabolite production when encountering different prey. To test the effect of different prey on secondary metabolite production, metabolites were extracted from cultures of *Myxococcus fulvus* grown on media containing various heat-killed prey biomass as the sole carbon and energy source. *Myxococcus fulvus* produced more diverse secondary metabolite profiles when growing on prey that better supported their growth, suggesting that predator-prey interactions are linked to secondary metabolite expression.

Introduction

The *Myxococcales* are an order of *Deltaproteobacteria* known for their sophisticated lifecycle[1–4], large genomes[2,5], and complex cooperative behaviors[2,6–9].

Many myxobacteria are predators capable of growing on a wide range of bacterial and fungal prey[10–12]. Myxobacteria envelop prey in coordinated, gliding swarms[7,13,14], and deliver diverse secondary metabolites[15,16] packaged in outer membrane vesicles to kill their prey[17–19]. Many of these secondary metabolites have medically desirable activities, such as anti-viral, anti-fungal, anti-cancer, and antibacterial properties, making the myxobacteria attractive targets for novel drug discovery[15,20–22].

In total, more than 600 distinct secondary metabolites have been described in myxobacteria since 1967[15,20,23], including 42 new compounds between 2011 and 2016[15]. Additionally, high throughput genome sequencing coupled with *in silico* analyses have predicted that myxobacterial genomes contain high numbers of biosynthetic gene clusters encoding poly-keytide synthases (PKS) and non-ribosomal peptide synthases (NRPS)[17,24–26]. Iterative PKS–NRPS can generate highly diverse bioactive compounds that have great potential for new medicines; however, only a fraction of the predicted gene clusters in myxobacterial genomes encode for known products, and many of those products have no described function[21,24,27]. Because the maintenance and expression of these large gene clusters is resource and energetically expensive, they must be providing a selective advantage. Thus, leveraging natural ecological and physiological conditions may be the key to improving secondary metabolite discovery rates[28–30].

Although secondary metabolites are central to their predatory activities[16], it is unknown if the myxobacteria regulate secondary metabolite expression in response to signals from their prey. In other bacterial predators, such as the *Bdellovibrio*, predatory behaviors are tightly linked to prey recognition and quality cues[31,32]. In the

Streptomyces, interspecies interactions are required for the expression of some secondary metabolite gene clusters[33–36]. By comparison, little is known about the effect of interspecies interactions on the expression of secondary metabolite gene clusters in myxobacteria; however, the myxobacteria do respond to the presence and absence of different prey and quorum sensing molecules through a variety of behavioral changes, such as increasing motility[10,11,37], inducing spore germination[37], and changes in gene expression[38]. While the importance of secondary metabolites in predation and development have been well documented, it is unknown if the myxobacteria differentially regulate secondary metabolite production in the presence or absence of specific prey.

The purpose of this research was to test the hypothesis that *Myxococcus fulvus* differentially regulates secondary metabolite production when grown in the presence of different prey. We tested our hypothesis by growing *M. fulvus* in the presence of different prey and compared secondary metabolite profiles using LC-MS. We predicted that *M. fulvus* would produce more different secondary metabolites when grown on prey that allowed for rapid swarm expansion, a behavior previously correlated to prey-kill efficiency and predator growth rate in other myxobacteria[10–12]. We report the effect of prey on the production of secondary metabolites, and demonstrate that myxobacteria produce different secondary metabolite profiles when challenged by different prey.

Methods

Isolation and identification of prey organisms

Bacterial prey were isolated from soil samples collected near the University of Oklahoma duck pond (Norman, OK). Soil samples were suspended 1:10 (w/v) and serially diluted to 10^{-9} in a calcium chloride buffer (6.8 mM calcium chloride dihydrate and 20 mM HEPES), inoculated onto nutrient agar (3 g/L of beef extract, 5 g/L of peptone, and 15 g/L agar), and incubated for 48 hours at 30 °C. Colonies with different morphologies were successively transferred to fresh nutrient agar until pure isolates were obtained.

Each isolated prey species was identified by their 16S rRNA gene sequence. Genomic DNA was extracted from each pure culture using the MO BIO UltraClean© Microbial DNA Isolation Kit (Qiagen, MD, USA). These DNA extracts were used as templates for 30 cycles of PCR amplification of the 16S rRNA gene using 5PRIME HotMasterMix (Quanta bio, MA, USA) and the primers 8F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT).[13] The PCR program was conducted in a Techne TC-512 (Techne, Burlington, NJ, USA) thermocycler included an denaturation at 94°C for 30 seconds, followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 55°C for 45 seconds, and extension at 72°C for 45 seconds. A final extension was held at 72°C for 10 minutes and stored at 4°C. The resulting PCR products were sequenced at the University of Oklahoma Biology Core Molecular Lab (Norman, OK). The phylogeny of the genes was constructed using to the Ribosomal Database Project (RDP)[39,40].

Determination of swarming rate

The swarming rate of *Myxococcus fulvus* on different prey was measured in triplicate using a modified version of a previously described swarming assay[10,11]. Prey isolates were grown in 5 mL of nutrient broth (3 g/L beef extract and 5 g/L peptone) at 30°C for 48 hours. Cell biomass from each prey organism was collected by centrifugation at 10,000 ×*g* for two minutes. Cell pellets were washed twice and re-suspended in a calcium chloride buffer (6.8 mM calcium chloride dihydrate and 20 mM HEPES) and adjusted to an optical density of 0.500 ± 0.050 at 600 nm using a Thermo Scientific SPECTRONIC 20D+ spectrophotometer. Predation Assay (PA) plates were prepared by spreading 0.1 mL of each prey cell suspensions on a single quadrant of a WAT agar plate (15 g/L agar, 6.8 mM calcium chloride dihydrate, 20 mM HEPES) and sterile calcium chloride resuspension buffer on the opposite quadrant (Fig. 1). PA plates were inoculated in the center with 10 µL of a 1:100 diluted *Myxococcus fulvus* culture grown on *VY/2* agar (5 g/L Fleischmann's Active Dry Yeast, 6.8 mM calcium chloride dihydrate, 20 mM HEPES, 3.7 µM vitamin B12) at 30°C for one week. PA plates were incubated at 30°C for seven days. Predation rate effect sizes were calculated as the difference in average swarming area between the prey quadrant and the negative control quadrant on the PA plates after seven days, divided by the pooled standard deviation.

Secondary Metabolite Extraction

To test if prey species affected secondary metabolite production, the secondary metabolite profiles produced by *M. fulvus* grown on media containing different prey were compared. Three different prey organisms were selected based on the lowest, median, and highest effect sizes from the predation assay. Each prey organism was grown in an 2800 mL Ferncach flask containing 1000 mL nutrient broth (3 g/L beef extract and 5 g/L peptone) for 72 hours at 30°C and shaking at 200 RPM. Cell biomass was collected by centrifugation at 8,000 × *g* for 10 minutes. Cell pellets were washed twice and resuspended in 200 mL sterile 6.8 mM $CaCl_2 \bullet 2H_2O$ buffer. Direct cell counts with a hemocytometer were used to normalize each prey cell suspension to a final concentration of 10^6 cells/mL in a basal medium (6.8 mM $CaCl_2 \bullet 2H_2O$, 20 mM HEPES, and 3.7 µM vitamin B12, 15 g/L agar). Prey-agar was autoclaved, cooled, and poured into sterile petri dishes. The prey-agar plates and VY/2 agar plates (5 g/L Fleischmann's Active Dry Yeast, 6.8 mM calcium chloride dihydrate, 20 mM HEPES, 3.7 µM vitamin B12) were inoculated with 100 µL of a *Myxococcus fulvus* inoculum and incubated for seven days at 30 °C. The inoculum was prepared by recovering *Myxococcus fulvus* from a glycerol stock on *VY/2* agar at 30 °C. After seven days, *M. fulvus* was collected by scraping biomass into two mL of saline buffer. The resulting solution was diluted 1/100 in sterile saline and homogenized by vortexing.

Secondary metabolites were extracted with ethyl acetate from prey-agar, VY/2 agar, and negative controls in triplicate. The agar from each petri dish was blended in

ethyl acetate and incubated at room temperature overnight to extract secondary metabolites. Decanted ethyl acetate was evaporated in a rotary evaporator and the resulting extracts were resuspended in methanol to 10 mg crude extract/mL. Profiles from each extraction were analyzed using a Shimadzu LC-MS 2020 system (ESI quadrupole) coupled to a photodiode array detector with a Phenomenex Kintex column (2.6 µm C_{18} column, 100 Å, 75 × 3.0 mm). Peaks from the chromatograms were characterized using the LabSolutions LC-MS software and compared across chromatograms by their retention time, *m/z* base peak, and UV/Vis maxima, and putiative compound identification was assigned using the Dictionary of Natural Products[41]. The LC-MS profiles of prey treatments were compared to the negative controls to eliminate any peaks that may have originated from the prey or media components.

Results

Predation rates varied by prey species

The taxonomic identity of the nine prey bacteria was inferred by aligning partial 16S rRNA gene sequences to the Ribosomal Database Project database[39,40]. Seven of the prey isolates were Gram positive (M9, M14, M15, M19, M27, M29, and M34) and two were Gram negative (M31 and M20). The isolates were most closely related to *Lysinibacillus sphaericus* (M9), *Brevibacillus parabrevis* (M14), *Bacillus anthracis* (M15), *Paenibacillus lactis* (M19), *Spingobacterium sp.* (M20), *Staphylococcus cohnii* (M27), *Rhodococcus sp.* (M29), *Escherichia coli* (M31), and *Microbacterium kitamiense* (M34)

(Fig. 2A). All isolates shared 100% sequence similarity with their closest relative. All isolates were able to grow well aerobically and on nutrient agar or nutrient broth at 30 °C.

The predation rate by *M. fulvus* varied among prey (Fig. 2). In general, *M. fulvus* swarmed faster over prey than the sterile buffer; however, *M. fulvus* swarmed slower over M29 (Cohen's d = -1.40) than controls, although the difference was not significant (p = 0.160). Swarming rates were faster over Gram negative isolates (M20, M31) than Gram positive isolates (M9, M14, M15, M19, M27, M29, M34), with the highest swarming rate being observed on M20 (p = 0.014, Cohen's d = 3.41). Because swarm shape and size were highly variable, only swarming rates on M20 (*p* = 0.0140, Cohen's d = 3.41) and M34 (*p* = 0.036, Cohen's d = 2.55) were significantly different from controls, although the behaviors (i.e. faster over prey or faster over buffer) were always consistent.

Effect of prey on Myxococcus fulvus secondary metabolite profiles

To test the effect of prey species on secondary metabolite production, metabolites were extracted from cultures of *M. fulvus* grown on media containing heat-killed prey biomass as the sole carbon and energy source. *Myxococcus fulvus* produced different secondary metabolite profiles when grown on different prey (Fig. 3). In total, eleven unique peaks were identified across treatments on PA plates inoculated with *M. fulvus* compared to uninoculated controls by comparing retention time, UV/Vis maxima, and *m/z* base peaks among chromatograms (Table 1). The ability of *M. fulvus* to swarm

each prey may be a predictor of secondary metabolite production, as *Myxococcus fulvus* produced more diverse secondary metabolite profiles when growing on prey on which it swarmed faster in the predation assay (Fig. 4). The LC-MS profiles produced by *M. fulvus* on M29 more closely resembled negative controls than the other bacterial prey or on VY/2 medium. *Myxococcus fulvus* produced similar patterns when grown on M15 and M20, but were different compared to growth on VY/2 or on M29. Several compounds (B, E, G, H, I; Table 1) were typically only produced in the presence of M15 and M20, while others (C and F; Table 1) were produced in the presence of M29 (Fig. 4). Peak I (putatively identified as Myxothiazol A[42]) was produced in greater amounts for M15 and M20, but was only negligibly produced on agar containing M29. Peak G (putatively identified as Melithizole B) was produced on bacterial prey, but not on VY/2. No other peaks could be assigned putative IDs (Table 1).

Discussion

Myxobacteria have large genomes that contain a number of secondary metabolite gene clusters with the potential to produce diverse, biologically active compounds[17,24–26]. Because many of these predicted gene clusters are silent[21,24,27], new cultivation strategies are needed to discover novel products[43]. We show that *M. fulvus* produces different secondary metabolite profiles when grown on different prey. This finding suggests that leveraging predator-prey interactions may contribute to drug discovery. Furthermore, swarm expansion rates on various prey may be a predictor of secondary metabolite profile diversity; however, we did not test this association, nor did

we measure bactericidal or bacteriostatic activity from any of our extracts. Overall, our data demonstrates a link between prey identity and secondary metabolite expression in *M. fulvus*.

The myxobacteria have a wide range of functional capabilities, including the ability to degrade macromolecules such as proteins, cellulose, peptidoglycan, lipids, and nucleic acids, and the production of antibiotics and bacteriocins[2,15,20,44]. These activities are central to their predatory lifestyle[7,20,44–47], and many myxobacteria have wide prey ranges in the laboratory. Moreover, the growth rate and prey-kill efficiency varies by strain and across prey species[10–12], suggesting that the myxobacteria adapt locally to prey. Swarm expansion is correlated to growth rate and prey kill efficiency[11], and our data suggests that swarm expansion rates might also predict secondary metabolite profile diversity, though we did not test this relationship. We believe that *M. fulvus* is producing more and different secondary metabolites in response to cues from the prey because interspecies interactions trigger the expression of different secondary metabolites in other systems[33–35]. The effect of interspecies interactions on secondary metabolite expression in the myxobacteria has received little attention, with most studies focusing on the production of defense compounds by prey in response to predation by *M. xanthus*, such as bacillaene in *Bacillus subtilis*[48] or actinorhodin by *Streptomyces coelicolor*[36]. The increase in secondary metabolite profile diversity may also be density dependent or the result of increased growth rates, especially considering that many bioactive metabolites produced by myxobacteria are expressed at different developmental stages. In either case, drug discovery programs should

benefit from leveraging the ecology of the myxobacteria.

Many myxobacterial genomes contains a large number of genes associated with predation[49]. Logically, the segregated expression of predatory and non-predatory genes in the presence and absence of suitable prey should confer fitness advantages. In other bacterial predators, such as the *Bdellovibrio*, attack and growth life phases are distinct and well regulated; for example, in *Bdellovibrio bacteriovorus* HD 100, 67% of genes were uniquely expressed during the growth phase and 15% of genes were exclusive to the attack phase[31]. Conversely, a recent transcriptomic study of *Myxococcus xanthus* DK1622 found that genes linked to predation were equally expressed in the presence and absence of live and dead *E. coli,* suggesting that *M. xanthus* expresses predatory genes constitutively rather than selectively[38]; however, because their study was limited to the interactions between a single strain of *Myxococcus* and *E. coli* Top10, Livingstone *et al*[38] would have been unable to detect expression patterns dependent on specific prey cues. Furthermore, considering that *M. xanthus* DK1622 has a doubling time between 4 to 8 hours[2,50,51] and that the experiment concluded after five hours[38], the expression of predatory genes during exponential growth would likely have been missed. In this study, *M. fulvus* differentially produced secondary metabolites in the presence of different prey. Specifically, *M. fulvus* produced compound I, putatively identified as Myxothiazol A, in large amounts when grown on the prey isolates M20 and M15 but not on M29. Although Myxothiazol A has reported activity against *B. subtilis*, it is generally considered anti-fungal rather than anti-bacterial, and functions by inhibiting reduction of cytochromes by succinate or NADH[42]. Still, the potential of leveraging

predator-prey interactions to express products of silent gene clusters is highlighted when considering that nine of the eleven compounds detected in this study had no known match to compounds previously described in myxobacteria.

Drug discovery programs employ a wide range of targeted and high-throughput strategies to increase the probability of discovery[35,52–54]. Targeted approaches use molecular tools to engineer metabolic systems to produce a product of interest. Gene knockouts, overexpression, and promoter exchange in both heterologous and homologous expression vectors have been somewhat successful in myxobacteria[55]; however, these techniques require a wealth of *a priori* sequence data and high quality putative function assignments, are relatively expensive and low throughput, and cannot take advantage of early activity screens. Conversely, high throughput strategies typically include growing potential drug producers in a wide range of growth conditions and screening for activity[56]. Many times high-throughput experiments test as many as 50 different growth conditions at a time[57,58], with activity detected in only a subset of conditions[57,58]. While this approach is high throughput and only requires a relatively small investment before screening for activity, rediscovery rates remain high, the techniques are labor intensive, and does not account for false negatives. Recently, ~2300 myxobacteria isolates were screened on 9 different media, leading to the discovery of rowithocin[27]. While the authors noted that medium composition had little to no effect on secondary metabolite production in the myxobacteria, their media did not contain whole cells of prey that the myxobacteria would likely encounter in nature[27]. We believe that predator-prey interactions can be leveraged to express some of these silent

pathways, and swarming assays against clinical pathogens may prove to be a valuable screen for drug discovery programs trying to find new drugs to combat emerging antibiotic resistant pathogens.

The myxobacteria have enormous potential to produce bioactive secondary metabolites. Myxobacterial genomes typically range from approximately 9 to 15 Mbp , with 8.6% the genomic content in *Myxococcus xanthus* is dedicated to secondary metabolites[59], compared to 4-6% in well studied *Streptomycetes*[60,61]; however, only a fraction of predicted gene clusters in myxobacteria produce known products. We propose leveraging the predatory nature of the myxobacteria to improve discovery of their secondary metabolite potential because the data presented here demonstrates that the myxobacteria produce different secondary metabolites when grown on different prey. Therefore, leveraging interspecies interactions within the ecological context of predator-prey interactions shows promise toward describing the product and function of large biosynthetic gene clusters in the myxobacteria, and may lead to significant advances in drug discovery.

Figures and Tables

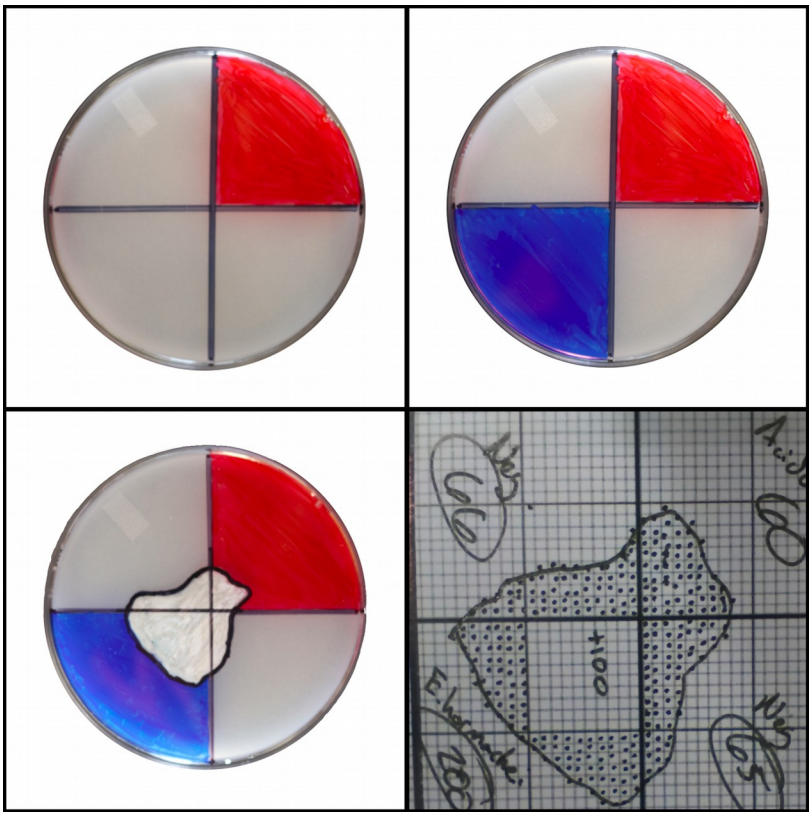

Fig. 1. Predation Assay Plates. Prey (blue) and sterile buffer (red) were spread on opposing quadrants. *M. fulvus* swarms from the center of the plate (white) over prey and negative control quadrants. Swarming patterns were measured by tracing the leading edge of the swarm onto graph paper after seven days.

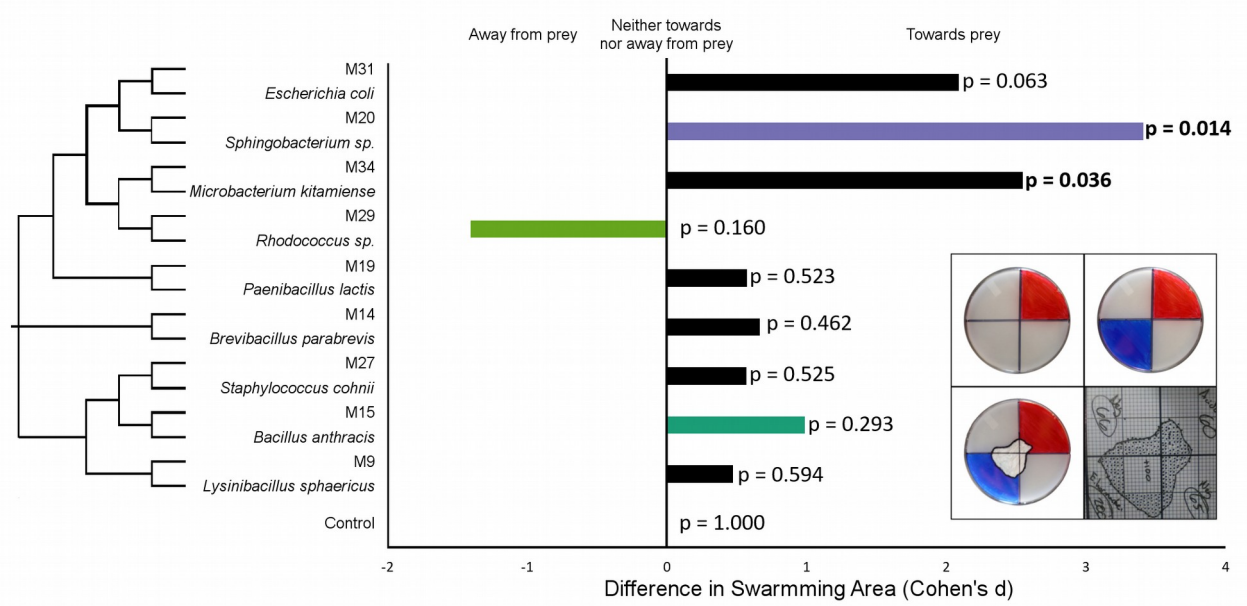

Fig. 2. Predation assay. Effect size (Cohen's d, length of bars) is calculated for each prey species as the standardized difference between mean swarming rate over prey vs controls. The cladogram (y-axis) was constructed using 16S rRNA gene sequence and indicates the closest relative of each isolate. The colored bars correspond to the isolates used to measure the effect of prey on secondary metabolite expression in Figs 3 and 4.

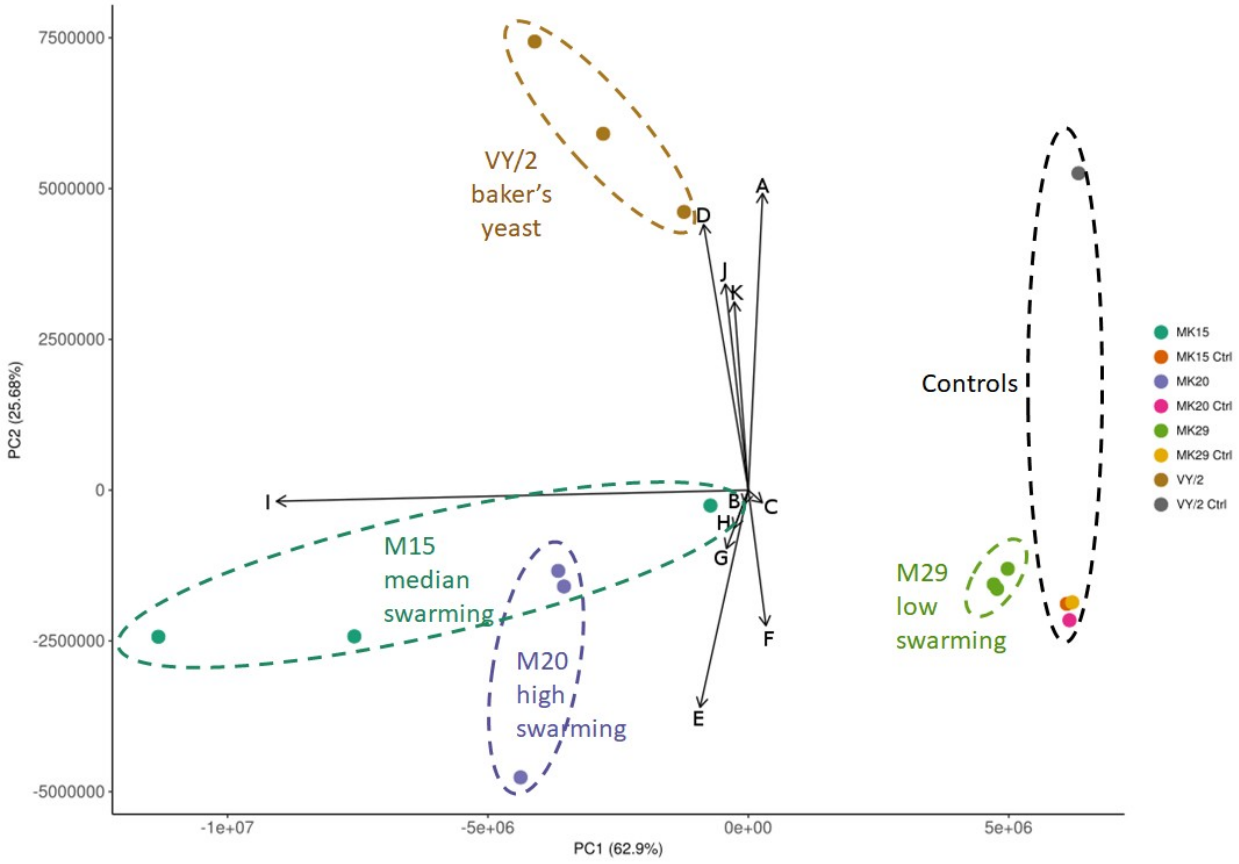

Fig. 3. The effect of prey on secondary metabolite production by *Myxococcus fulvus.* The letters on the vectors correspond to peaks identified in LC-MS chromatograms (Fig. 4 and Table 1).

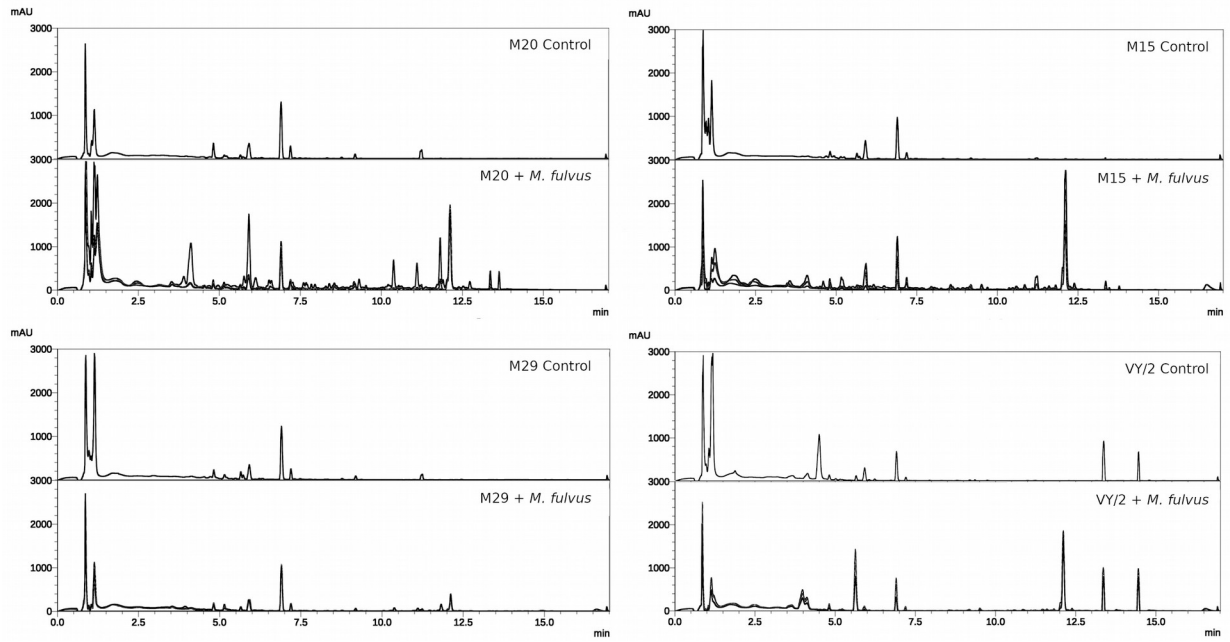

Fig. 4. LC chromatograms of PA medium with and without *Myxococcus fulvus.*

Table 1. LC-MS peaks produced by *Myxococcus fulvus*.

Peak	Retention Time (min)	UV/Vis Maxima (nm)	Base peak (*m/z*)	Putative ID*
A	4.0 ± 0.2	221	221	*No ID*
B	4.8 ± 0.1	213	157	*No ID*
C	5.1 ± 0.2	215	333	*No ID*
D	5.6 ± 0.1	260	205	*No ID*
E	5.9 ± 0.1	195	347	*No ID*
F	6.9 ± 0.2	216	391	*No ID*
G	7.2 ± 0.2	217	421	*Melithiazole B*
H	11.2 ± 0.3	234	332	*No ID*
I	12.1 ± 0.3	244, 313	488	*Myxothiazol A*
J	13.4 ± 0.1	256	1017	*No ID*
K	14.5 ± 0.2	273	1045	*No ID*

*Putative IDs were assigned using the Dictionary of Natural Products[41].

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Chapter 4. Improved enrichment and isolation of myxobacteria using inhibitory dyes

Abstract

The myxobacteria are difficult to cultivate because they do not form readily identifiable colonies on agar, do not disperse well in liquid, and grow slowly. We update a previously described cultivation technique and recommend supplementing media with 1 mg/L crystal violet to improve the cultivation of the myxobacteria.

The myxobacteria are a group of soil bacteria, many of which are predators of other bacteria. During vegetative growth, many myxobacteria produce bioactive secondary metabolites to kill prey, making the myxobacteria strong targets for novel antibiotic research[1–4]. Myxobacteria have long been known to produce antimicrobial compounds, with more than 600 distinct compounds described since the late 1960s[3–5]. Although the number of compounds described in myxobacteria is less than one-tenth than from the *Actinomycetes*[3,4], the myxobacteria are excellent targets for the discovery of novel drugs because their genomes contain high numbers of biosynthetic gene clusters capable of producing bioactive compounds[6–8].

Although the myxobacteria are prolific producers of bioactive secondary metabolites, the difficulty of their cultivation hampers discovery of novel compounds[3,9,10]. Most myxobacteria cannot be cultivated using traditional techniques because they do

not form colonies on agar, do not disperse well in liquid, and grow slowly. Furthermore, purifying myxobacteria is difficult because contaminating organisms stick to their exopolysaccharide matrix and can be propagated across multiple transfers.

Here, we present an update to a previously described method to improve the isolation of myxobacteria. In 2003, Zhang *et al.* reported that the addition of 0.1% (w/v) crystal violet (CV) aided in the cultivation of myxobacteria[11]; however, we were unable to reproduce their findings nor were we able to find other reports that successfully used this method. We suspected that the reported concentration of CV was too high to support growth of the myxobacteria, and tested our prediction by growing myxobacteria on standard media supplemented with a wide range of CV concentrations. Additionally, we tested the ability of myxobacteria to grow in the presence of two other dyes, acriflavine and brilliant green, which were selected for their ability to inhibit growth of common bacterial contaminants[12].

To determine the concentration range of each dye that supported growth, myxobacteria were grown on media supplemented with CV, brilliant green, or acriflavine. Nine different myxobacterial isolates from soil were recovered from glycerol stocks on Vy/2 agar[10] for seven days at 30 °C. Fruiting bodies were transferred onto WAT agar[10] and incubated for seven days at 30 °C, and the leading edge of each swarm was transferred to agar slants containing VY/2 or VY/2 supplemented with either 0.1 mg/L, 1.0 mg/L, or 10.0 mg/L CV, brilliant green, or acriflavine, and incubated at 30 °C. After seven days, eight of nine isolates grew on media supplemented with up to 1.0 mg/L CV, but only four of nine isolates grew at concentrations up to 10 mg/L (Table 1).

The optimal concentrations of brilliant green and acriflavine in VY/2 was 1.0 mg/L and 0.1 mg/L, respectively (Table 1). We found that supplementing WCX agar with 1.0 mg/L CV using the Coli spot method[13] worked well, and pure cultures of myxobacteria were typically obtained after two to three transfers.

To test if the addition of CV selected for different groups of myxobacteria, we use group-specific primers to produce clone libraries from biomass grown on media with and without CV. Activated sludge from the Norman, OK water reclamation facility was collected, diluted and plated onto WCX[10], WCX + CV, and CY-C10[10], and incubated at 30 °C. Microbial biomass was harvested from each plate after seven days by mechanical scraping into a saline buffer, and DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA). DNA extracts across each medium were screened for the presence of 16S rRNA gene sequences[14] belonging to the *Sorangineae* and *Nannocystineae* suborders using PCR and group specific primers (Table 2). Universal primers 8F and 1492R were used as positive controls and primers targeting the mglA gene were used to detect the presence or absence of all myxobacteria[15]. The presence of clones belonging to the *Nannocystineae* and *Sorangineae* varied by enrichment medium (Fig. 1). Members of the *Nannocystineae* were detected in enrichments on CY-C10, and members of the *Sorangineae* were detected in enrichments on WCX media, with and without the CV; however, 16S rRNA gene sequences from enrichments containing CV were closely related to the family *Phaselicystis*, while enrichments without CV tended to classify with the family *Polyangiaceae*. Furthermore, these sequences were between 92.7% and 97.3% similar

to their closest relative in the Silva non-redundant reference database[16,17], suggesting the presence of novel general and species of myxobacteria in these enrichments.

CV is commonly used as a selective pressure in microbiological growth media because of its wide range of inhibitory activity[12]. Bacteria with higher isoelectric points and fewer acidic membrane components are more resistant to inhibition by CV[18]. CV inhibits growth by disrupting a cell's membrane potential, thus reducing ATP production[19]. While the isoelectric point of myxobacteria has not been directly measured, we suspect that they can tolerate higher concentrations of CV because the myxobacteria accumulate high concentrations of proteins with high isoelectric points under starvation conditions[20,21]. In myxobacteria, starvation is detected by synthesis of guanosine pentaphosphate from GTP and ATP when uncharged tRNA binds to a ribosome[22]. Since binding an amino acid to tRNA requires ATP, this process also indirectly detects the cell's available ATP. Because CV diminishes a cell's ability to synthesize ATP[19], we would expect it to trigger a starvation response in myxobacteria by increasing the concentration of uncharged tRNA in the cell. In response to starvation, myxobacteria synthesize high concentrations of the protein hemagglutinin[23], a developmental protein with a high isoelectric point[20]. Hemagglutinin is localized in the periplasm and attached to the outer membrane[21], and could thus improve resistance to CV inhibition by increasing the cell's isoelectric point; however, this model has yet to be tested.

In summary, we report that supplementing media with 1 mg/L CV improves the cultivation of the myxobacteria by inhibiting the growth of contaminants. This differs

from the concentration previously reported, which was 100-1000x greater than the concentration our isolates could tolerate[11]. Furthermore, enrichment experiments containing CV selected for novel genera and families absent in controls, demonstrating that CV supplemented cultivation media has potential for discovering novel myxobacteria.

Table 1: Growth of myxobacteria on VY/2 supplemented with acriflavine, brilliant green, or crystal violet.

	Crystal Violet			Acriflavine			Brilliant Green		
	0.1 mg/mL	1.0 mg/mL	10.0 mg/mL	0.1 mg/mL	1.0 mg/mL	10.0 mg/mL	0.1 mg/mL	1.0 mg/mL	10.0 mg/mL
Myxococcus xanthus ATCC19368	+	+	+	+	+	+	+	+	+
Myxococcus sp. 1BB1	+	+	+	+	+	+	+	+	+
Myxococcus fulvus 1BB7	+	+	+	+	-	-	+	+	+
Myxococcus sp. 1BB10	-	-	-	+	+	-	+	+	-
Corallococcus sp. 1BB4	+	+	-	+	-	-	+	+	-
Corallococcus sp. 1BB8	+	+	-	-	-	-	-	-	-
Cystobacter sp. 1BB6	+	+	-	+	-	-	+	+	-
Archangium sp. 1BB9	+	+	-	+	-	-	+	+	-
Melitangium sp. 1BB3	+	+	+	+	+	-	+	+	+

Table 2: Group Specific Primers

Gene	Primer	Primer sequences (5’ → 3’)	Tm
mglA	mglA1F	CGCGAAATCAACTGCAAGAT	54.0 °C
mglA	mglA1R	GGCAGGTCGCGCTTGTTGTACTG	62.8 °C
16S rRNA	8F_Eub	AGAGTTTGATCCTGGCTCAG	54.3 °C
16S rRNA	1492R_Eub	GGTTACCTTGTTACGACT	48.7 °C
16S rRNA	Sora_1018R	CTCCGAAGAGCACCCCGS	59.5 °C
16S rRNA	Nann_429F	AAAGCTCTGTGGGGAGGG	57.1 °C

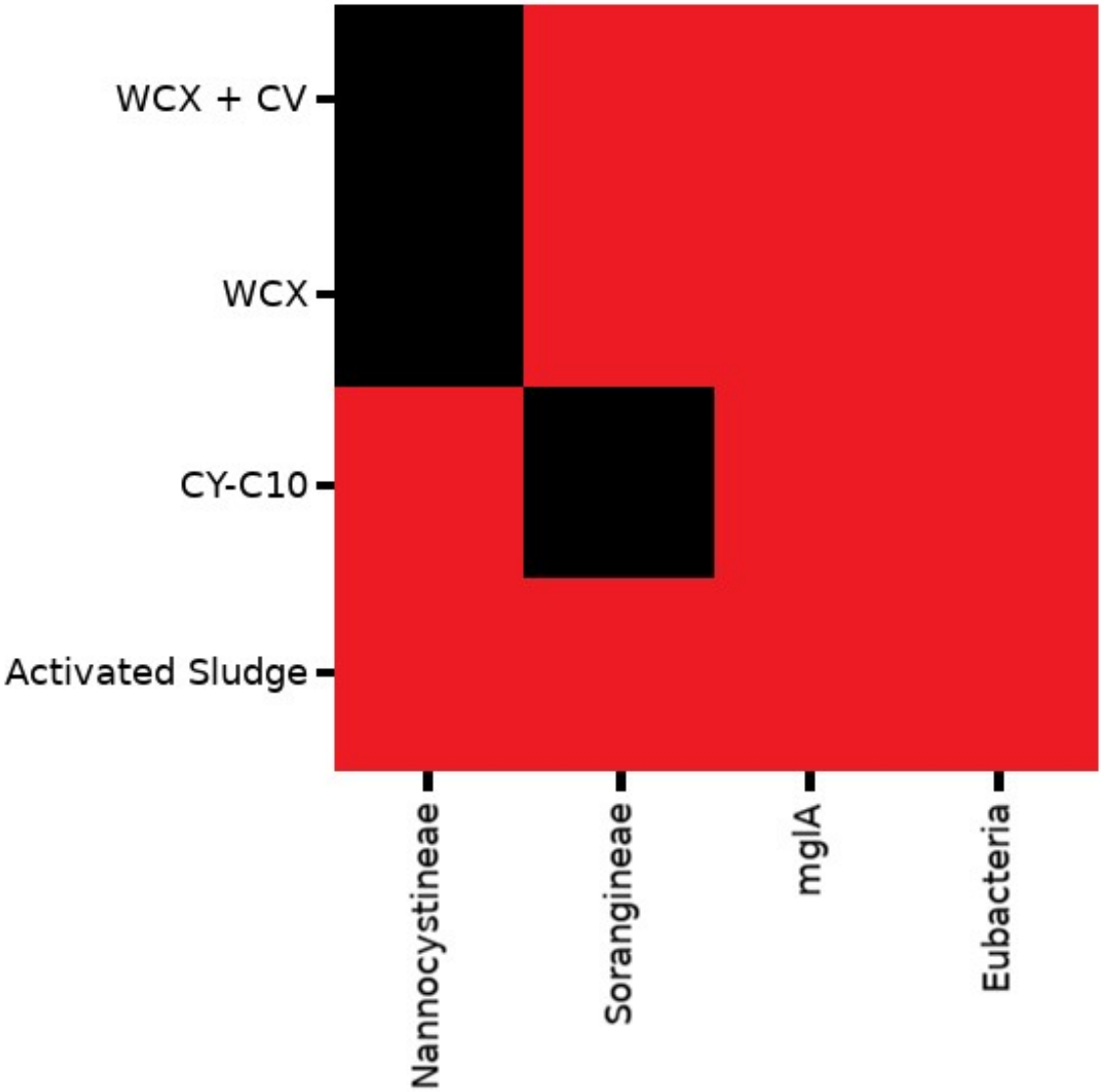

Figure 1: Heatmap of bacterial groups present (red) and absent (black) in enrichments from activated sludge.

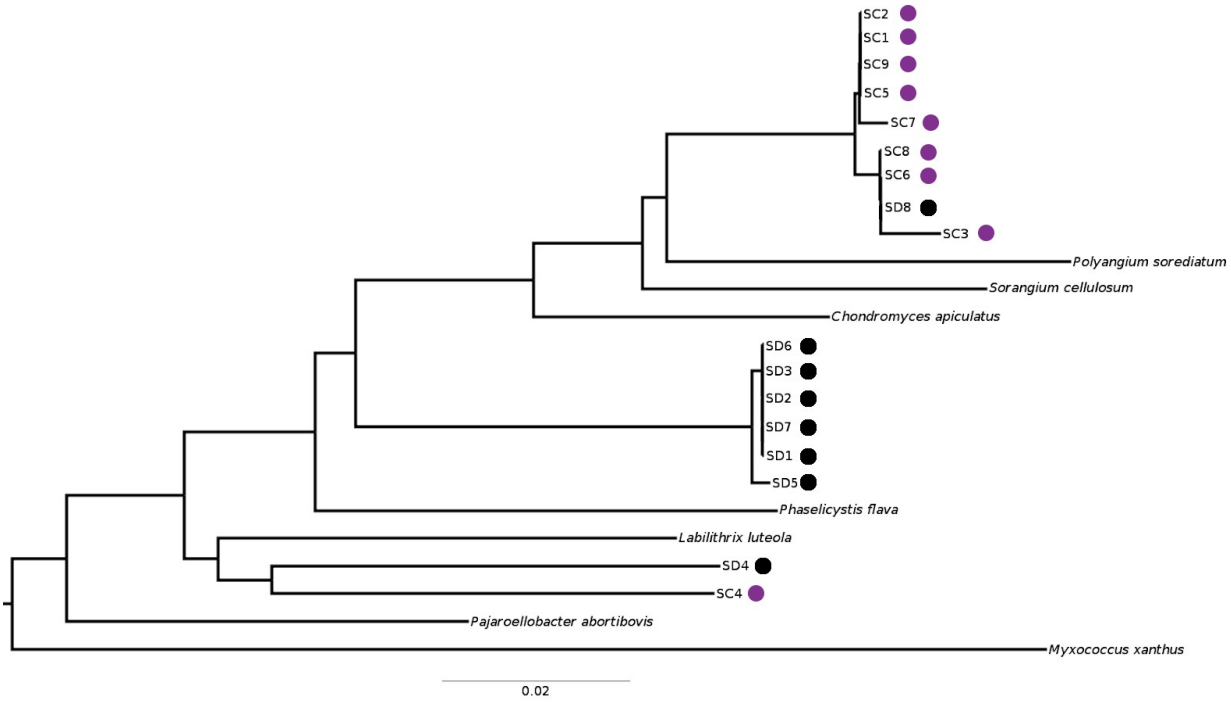

Figure 2: Phylogenetic tree of 16S rRNA gene clones from WCX (black circle) and WCX + CV (purple circle) enrichments.

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Chapter 5. The Effect of Long Term NPK Fertilization on Bacterial Communities in Leaf Litter from a Lowland Tropical Rainforest

Abstract

The widespread use of agricultural fertilizers has indirectly increased the input of inorganic nutrients in natural ecosystems through the deposition of enriched dust, aerosols, surface water, and groundwater. While much is known about the effects of nutrient enrichments on soil microbial communities, the effects of nutrient enrichment on microbial communities inhabiting leaf litter in tropical forests are poorly understood. Here, we report the effects of nine years of nitrogen, phosphorus, and potassium (NPK) fertilization on the litter bacterial communities in a Panamanian rainforest. We hypothesized that nutrient enrichment would suppress diversity and alter the structure of leaf litter bacterial communities by selecting for copiotrophic taxa. Leaf litter bacterial communities were characterized using high throughput sequencing of 16S rRNA gene libraries across a factorial network of NPK fertilization plots. Nitrogen and nitrogen:phosphorus additions reduced the diversity of the leaf litter bacterial community compared to controls. No other treatments had a significant effect on bacterial diversity. Nitrogen additions had the greatest effect on community structure relative to controls, but nitrogen:potassium, phosphorus:potassium, and nitrogen:phosphorus:potassium amendments also altered community structure when compared to unfertilized plots. These results demonstrate that bacterial communities in leaf litter are more sensitive to

changes in nitrogen compared to potassium, phosphorus, or micronutrients.

Introduction

Nutrient cycling in rainforests is governed by a complex feedback loop between above- and below-ground nutrient pools (Proctor 1987). Nutrients naturally enter the rainforest ecosystem through the deposition of dust and aerosols (Yu *et al* 2015), weathering of parent material (Walker and Syers 1976, Yavitt and Wieder 1988, Yavitt 2000), and fixation by microorganisms (Yavitt and Wieder 1988, Yavitt 2000). These inorganic components are rapidly consumed by vegetation, incorporated into biomass, and returned to the soil through the decomposition of fallen leaves, wood, fruit, and dead bodies; however, the nutrients contained in leaf litter are unavailable for direct use by plants, and, as the forest ages, the soil becomes depleted in a variety of geologically and biologically derived chemical elements (Walker and Syers 1976, Wardle et al 2004, Yavitt 2000, Yavitt and Wieder 1988). The organic nutrients sequestered in leaf litter are slowly returned to the soil through mechanical and biochemical processes involving herbivores (Coley and Barone 1996), litter invertebrates (Kaspari and Yanoviak 2009, Moore *et al.* 1988), and microbial decomposers (Kim *et al* 2014, Allison et al. 2009, Bell et al. 2005, Matulich and Martiny 2015).

In soil, the microbial community is sensitive to changes in nitrogen, phosphorus, and potassium (Kaspari et al 2017, Ramirez et al 2012, Ramirez et al 2010, Nemergut et al 2010, Lammel et al 2015a, Lammel et al 2015b, Pan et al 2014, Leff et al 2015, Allison and Martiny 2008, Turner and Wright 2013). These three elements influence the

rate of ecosystems processes, such as carbon, nitrogen, or phosphorus cycling (Lammel et al 2015a, Turner and Wright 2013, Lammel et al 2015b, Ramirez et al 2012, Pan et al 2014, Allison et al 2009, Allison and Martiny 2008, Fierer et al 2007), though the microbial community structure is more sensitive to nitrogen than phosphorus or potassium (Ramirez et al 2012, Ramirez et al 2010, Nemergut et al 2010, Lammel et al 2015b, Pan et al 2014, Leff et al 2015). Nutrient addition tends to favor faster-growing copiotrophic microorganisms over slower-growing oligotrophic microorganisms (Fierer et al 2007, Leff et al 2015); for example, the population proportion of oligotrophs, such as the *Acidobacteria, Aquificae, Chlorobi, Cyanobacteria, Nitrospirae,* and *Verrucomicrobia*, often decreases with nitrogen addition, while the population proportion of copiotrophic phyla, such as the *Actinobacteria, Alphaproteobacteria,* and *Gammaproteobacteria*, increases with nitrogen addition (Ramirez et al 2012, Nemergut et al 2010, Leff et al 2015, Pan et al 2014).

In contrast to the established effects of nutrient enrichment on soil microbe communities, little is known about the effects of nutrient enrichment on microbial communities in leaf litter. The structure of microbial communities in leaf litter does change with nitrogen enrichment (Kaspari *et al.* 2010), but it is not clear which taxa are responding. Many of these taxa are in involved in litter decomposition, which is limited by nitrogen (Berg and Matzner 1997, Matulich and Martiny 2015, Fanin et al. 2012, Allison et al. 2009), phosphorus (Berg and Matzner 1997, Kaspari *et a*l 2008, Kaspari and Yanoviak 2009, Kaspari and Yanoviak 2008, Fanin et al. 2012), and potassium (Kaspari *et al* 2008a). Measuring the effect of nutrient enrichment on the microbial

community in leaf litter is particularly important because mineralization rates of leaf litter, and thus emissions of CO_2, are predicted to increase with global temperatures (Prescott 2010, Zhang et al 2008, Bothwell et al. 2014, Hobbie et al. 2002, Rustad et al. 2001) and inorganic nutrient availability (Berg and Matzner 1997, Matulich and Martiny 2015, Kaspari *et a*l 2008a, Kaspari et al 2008, Kaspari and Yanoviak 2008). Because the diversity and structure of the microbial community influences decomposition of leaf litter (Bell et al. 2005, Matulich and Martiny 2015, Schimel and Gulledge 1998, Strickland et al. 2009), incorporation of microbial community data may improve predictions of changes to overall ecosystem functions (Ingwersen et al. 2008, Moorhead and Sinsabaugh 2006).

Here we report the effects of nine years of fertilization on leaf litter bacterial communities in a Panamanian rainforest. We hypothesized that nutrient enrichment would suppress diversity and alter the structure of leaf litter bacterial communities by selecting for copiotrophic over oligotrophic taxa. To test our hypothesis, leaf litter bacterial communities were characterized using high throughput sequencing of 16S rRNA gene libraries across a factorial treatment of nitrogen, phosphorus, and potassium fertilizations as part of the Smithsonian Tropical Research Institutes' Gigante Fertilization Experiment at the Barro Colorado Nature Monument, Republic of Panama. This work allowed us to identify which taxa responded to nutrient enrichment changes in addition to changes in the diversity and structure of the leaf litter bacterial community.

Materials and Methods

Leaf litter sampling and characterization

Leaf litter was sampled from the Smithsonian Tropical Research Institute's Gigante Fertilization Experiment (9°06'31" N, 79°50'37" W) in the Barro Colorado Nature Monument, Republic of Panama, in September, 2007. The experiment consisted of 36 plots (40 x 40 m) located 30 – 40 m apart over a total area of 38.4 ha. Each plot has received one of 9 treatments (+N, +P, +K, +NP, +NK, +PK, +NPK, micronutrients (+M), and no addition), each replicated four times. The fertilization treatments were arranged as a stratified random design with four replicate strata treated as statistical blocks. Fertilization was applied four times a year during the rainy season (May - October) since 1998, with 6 – 8 weeks between applications. Nitrogen amendments were 150 kg urea (NH_2CONH_2) ha^{-1} $year^{-1}$, phosphorus amendments were 50 kg of triple super phosphate ($Ca(H_2PO_4)_2 \bullet H_2O$) ha^{-1} $year^{-1}$, potassium amendments were 50 kg KCl ha^{-1} $year^{-1}$, and micronutrients amendments were 25 kg ha^{-1} $year^{-1}$ Scotts soluble trace element mix (The Scotts Miracle-Gro Company, Marysville, OH). These fertilization treatments have significantly increased the amount of extractable inorganic nutrients from soil (Turner *et al.* 2013) and leaf litter (Turner et al. 2015).

Surface leaf litter was sampled at five sites within each plot within a 0.09 m^2 quadrat. Three sites were located 1 m, 10 m, and 20 m from the Southwest corner of the plot, and the remaining two samples were taken 1 m east of each of the 1 m and 20 m sites. Litter adjacent to each sample site was used to field rinse the sifter prior to

collection to reduce noise from previous samples. The litter depth was measured with a thin, scored plastic rod at the four corners and the center of each quadrat. All litter within the quadrat was collected down to mineral soil and sifted for 30 s through a 1 cm screen to produce a homogeneous residuum. The residuum was stored in plastic Ziploc bags and processed within 12 hrs or stored at 3 °C for 24 h prior to processing.

The leaf litter was homogenized by mixing and mechanical shearing to ≤3 mm^2. Moisture content was calculated by measuring wet and dry mass of a 50 mL subsample before and after drying to a constant mass at 60 °C. The pH of the dried subsample was measured by a pH meter after mixing with 30 – 50 mL of deionized water (in ~10 mL excess of litter volume) and incubating for 10 min in the dark at 22 °C.

Microbial community analysis

The diversity, composition, and structure of the bacterial community across each fertilization treatment plot was examined. Total DNA was extracted from duplicate subsamples of each homogenized litter residuum sample (0.5 – 1.0 g wet mass) using the PowerSoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol. Duplicate DNA extractions were pooled and stored at -20 °C.

A portion of the V4 region of the bacterial/archaeal 16S rRNA gene was amplified from each DNA extract using PCR with composite primers S-D-Arch-0519-a-S-15/S-D-Bact-0785-b-A-18 (Klindworth *et al* 2012). The primer S-D-Arch-0519-a-S-15 was modified to include a 16 bp adapter sequence (GTAAAACGACGGCCAG) at the 5' end

to allow for the attachment of a unique 12 bp barcode in a subsequent PCR reaction. Each PCR amplification contained 2 µl of 1:10 diluted template DNA, 1x Taq buffer with KCl (Thermo Scientific, Waltman, MA, USA), 1.5 mM $MgCl_2$ (Thermo Scientific), 0.2 µM of the forward and reverse primer, 0.25 mM of each dNTP (Thermo Scientific), and 1.25 U of Taq DNA Polymerase (Thermo Scientific). The PCR thermal cycling was carried out in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, NJ, USA). Initial denaturation was held at 96 °C for 3 min, followed by 30 cycles, each consisting of 96 °C for 30 s, 52 °C for 45 s, and 72 °C for 45 s. The final extension was held for 10 min at 75 °C. The fidelity of PCR product sizes was verified visually using agarose gel electrophoresis. PCR products were purified using SPRIselect beads (Beckman Coulter, Brea, CA, USA) following the manufacturer's protocol.

A unique “barcode” was attached to each library of purified PCR products with a 6 cycle PCR reaction. The forward primer consisted of a unique 12 bp “barcode” (Appendix A. Supplemental Table 3), two spacer nucleotides, and the 16 bp adapter sequence (5’ to 3’). The reverse primer was S-D-Bact-0785-b-A-18. Each “barcoding” reaction contained 4 µl of the purified PCR product, 1x Taq buffer with KCl (Thermo Scientific), 1.5 mM $MgCl_2$ (Thermo Scientific), 0.2 µM each primer, 0.25 mM of each dNTP (Thermo Scientific), and 1.25 U of Taq DNA Polymerase (Thermo Scientific). Six cycles of PCR thermal cycling were carried out in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, NJ, USA) using the amplification schedule described above. The resulting PCR products were purified using SPRIselect beads (Beckman Coulter) and quantified using the Qubit fluorometer and dsDNA HS assay kit (Life

Technologies, Grand Island, NY, USA). Equimolar amounts of each uniquely barcoded PCR product were pooled. The final multiplexed PCR sample was submitted for Illumina MiSeq using TruSeq 250 bp PE v2 chemistry.

Sequence data were demultiplexed and analyzed using QIIME (Caporaso *et al* 2010). All sequence reads were screened to remove those that contained any errors in the reverse primer or barcode regions, ambiguities, homopolymers (greater than 6 nucleotides in length), or an average quality score < 25. Paired-end reads were merged with a minimum overlap of 100 bp, and each sequence was binned according to its unique barcode. Primer sequences were trimmed, chimeric sequences were removed using USEARCH version 6.1 (Edgar 2010) and the RDP "gold" reference database, and unique sequences were clustered into *de novo* OTUs at 97% similarity using USEARCH version 6.1 (Edgar 2010). Phylogenetically consistent taxonomy was assigned by comparing a representative sequence from each OTU to the SILVA SSU database (Release 111; Quast *et al* 2013) using the RDP naïve Bayesian rRNA classifier (Wang *et al* 2007). Representative sequences were aligned against the SILVA reference database (Quast *et al* 2013) using the pyNAST aligner.

Diversity Measures

Microbial diversity responses to fertilization were measured using two metrics and two spatial scales. Simple counts of the number of OTUs and phylogenetic diversity (Faith 1992) were calculated for each sample and averaged within each plot. Diversity across the whole plot was estimated by pooling sequence data within each plot *in silico*. Diversity metrics at both spatial grains were calculated from a randomly sampled subset

of sequences from each library, rarified to 3000 reads. The diversity estimates were compared using an ANCOVA across the factorial design of +N, +P, and +K treatments, plus micronutrients, pH, and moisture content. Preliminary analyses of the effects of pH and moisture content did not account for variance, and pH and moisture content were removed from subsequent analyses ($p > 0.05$). Data normality and equal variance were tested using Shapiro-Wilk and Bartlett's tests, respectively, and both assumptions were satisfied ($p > 0.05$). The ANCOVA, ANOVA, and tests of normality and variance were calculated in R (R Core Team 2014).

Changes in community structure were measured using Weighted UniFrac (Lozupone and Knight 2005) and Bray Curtis (Bray and Curtis 1957) distance matrices. Both the Weighted UniFrac and Bray Curtis indices were calculated from a randomly sampled subset of sequences from each library and rarified to 3000 reads. The community dissimilarity among treatments was compared using pairwise ANOSIM tests (1000 permutations). The significance cutoff for these comparisons was lowered to $p < 0.006$ using the Bonferroni correction (Dunn 1961) to adjust for the inflation of false discovery.

The effect of nutrient enrichment on the relative abundance of each taxonomic group was modeled using a generalized linear model with a quasi-binomial error structure. To test if a treatment significantly affected the relative abundance of a taxon, each treatment was removed from the full model, and reduced models were compared to the full model using a Chi-squared test in R. Multicollinearity of each model was evaluated using the vif function in the Companion to Applied Regression package in R

(Fox and Weisberg 2010). Effect sizes were calculated as Cohen's d between treatments and controls to standardize changes in relative abundance (Kaspari et al 2017).

Results

Bacterial Diversity in Leaf Litter

De novo clustering of 1,724,535 sequence reads formed a total of 63,058 OTUs across all plots. At the 0.09 m^2 grain, the number of OTUs ranged from 1740 ± 85 OTUs on +NK treatment plots to 1885 ± 83 OTUs on +K treatment plots, and phylogenetic diversity (Faith 1992) ranged from 71.9 ± 5.3 on +N treatment plots to 76.9 ± 3.8 on +K treatment plots. Plots that received no nutrient amendments had 1811 ± 123 OTUs and a phylogenetic diversity of 76.8 ± 5.9 (Fig. 1). Nitrogen addition significantly reduced the phylogenetic diversity of the microbial leaf litter community relative to controls ($p = 0.009$, Cohen's $d = -2.67$) but did not suppress the total number of OTUs ($p = 0.088$, Fig. 1). Neither the number of OTUs ($p = 0.859$) nor the phylogenetic diversity ($p = 0.962$) were affected by phosphorus additions. Interestingly, the combination of phosphorus and nitrogen increased the observed microbial diversity slightly ($p = 0.038$, Cohen's $d = 0.029$) but not the phylogenetic diversity ($p = 0.052$), even through nitrogen addition reduced phylogenetic diversity and phosphorus had no effect on diversity.

To simulate species accumulation over a larger spatial grain (40 m^2 plots), sample data was summed by plot *in silico*. On average, diversity summed across 40 m^2 plots was ~5% higher than the diversity detected at the 0.9 m^2 sampling grain. In the 40

m^2 samples, the number of OTUs ranged from 1798 ± 29 OTUs on +N treatment plots to 1969 ± 84 OTUs on potassium treatment plots, and the phylogenetic diversity (Faith 1992) ranged from 73.5 ± 3.4 on + N treatment plots to 81.4 ± 4.4 on potassium treatment plots. Control plots had 1945 ± 78 OTUs and a phylogenetic diversity of 81.4 ± 4.5 (Fig. 1). The addition of nitrogen to treatment plots significantly suppressed the microbial species richness ($p = 0.010$, Cohen's $d = -8.224$) and Faith's phylogenetic diversity index ($p = 0.003$, Cohen's $d = -5.488$). The combination of phosphorus and nitrogen significantly decreased the total number of OTUs ($p = 0.032$, Cohen's $d = -1.266$), but not phylogenetic diversity ($p = 0.078$), compared to controls. No other treatments had a significant effect on the diversity of the bacterial community. Thus, nitrogen fertilization had the greatest effect of the treatments on microbial diversity at both 0.09 m^2 and 40 m^2, and the suppression of diversity accumulated with area.

Community Structure

The nine years of nutrient addition to the forest floor altered the leaf litter bacterial community structure at the 0.09 m^2 (weighted UniFrac, $p < 0.001$; Bray-Curtis, $p < 0.001$) and 40 m^2 spatial grains (weighted UniFrac, $p = 0.006$; Bray-Curtis, $p = 0.007$). For the 0.09 m^2 samples, nitrogen additions significantly altered the phylogenetic ($r = 0.544$) and compositional ($r = 0.590$) community structure, the combination of +NK altered the phylogenetic ($r = 0.267$) and compositional ($r = 0.328$) community structure, but combinations of +PK ($r = 0.245$) and +NPK ($r = 0.238$) only

altered the compositional community structure, not the phylogenetic community structure. At the 40 m^2 scale, the phylogenetic community structures only changed marginally from controls (Table 1) for +N ($p = 0.028$, $r = 0.625$), +K ($p = 0.056$, $r = 0.323$), +NP ($p = 0.067$, $r = 0.271$), and +NPK ($p = 0.053$, $r = 0.396$); however, the magnitude of the differences were greater between the 40 m^2 communities relative to the 0.09 m^2 communities. Likewise, the compositional community structure changed only marginally at the 40 m^2 scale for +N ($p = 0.028$), +K ($p = 0.036$), +NK ($p = 0.053$), +PK ($p = 0.065$), and +NPK ($p = 0.025$). The magnitude of the differences between compositional community structures were similar between 40 m^2 and 0.09 m^2 samples. These changes in composition rather than phylogeny likely reflect the stochasticity in hyper-diverse microbial communities.

The community structure at higher taxonomic ranks was similar across all treatments (Fig. 2) and were composed of the phyla Proteobacteria (44.8% - 54.8%), Acidobacteria (9.6% - 13.6%), Actinobacteria (5.3% - 8.4%), Planctomycetes (4.6% - 8.8%), Bacteroidetes (5.3% - 8.8%), Verrucomicrobia (3.7% - 7.1%), Chloroflexi (2.3% - 4.0%), Nitrospirae (1.1% - 2.7%), and Gemmatimonadetes (1.0% - 1.6%). Sequences belonging to unassigned phyla made up 3.0% - 3.8% of the total community. No other phyla represented more than 1% of the total community. In general, few shifts in relative abundance occurred between treatments and controls; the Acidobacteria (Cohen's $d = 1.258$) and Nitropirae (Cohen's $d = 0.512$) increased significantly on +N addition plots, Bacteroidetes (Cohen's $d = 1.426$) and Proteobacteria (Cohen's $d = 1.506$) increased on +K, Proteobacteria increased on +NK (Cohen's $d = 1.506$), and Bacteroidetes

increased on +NPK (Cohen's d = 0.722). The Proteobacteria decreased with +NK (Cohen's d = -0.093) and +N (Cohen's d = -0.144).

Because non-significant responses of phyla may be a net zero sum of significant responses of constituent taxa, a hierarchical dissection of taxonomic responses was conducted (Appendix A. Supplemental Table 4). We observed significant shifts in the relative abundance of taxa belonging to the same higher order taxonomic group, particularly within orders and families. For example, we noticed a relative abundance trade-off pattern among four specific orders within the class Acidobacteria: the Acidobacteriales, DA052, DS100, and DA023 (Fig. 3). These varied responses of the orders within the Acidobacteria resulted in a net-zero and non-significant change in relative abundance across all treatments except +N (Cohen's d = 1.369); however, the Acidobacteriales and DA052 both increased on +PN, but decreased on +N , +P, +K, +PK, +NK, and +NPK. The response of DA023 and DS100 were the opposite, as both orders decreased on +PN , and increased on +N, +P, +K, +PK, +NK, and +NPK. These trade-offs in relative abundance are likely to shed new light on taxa whose natural history is largely unknown.

Differences between the responses in higher and lower order taxa were not always a result of clear trade-offs in relative abundance. For example, although the class Alphaproteobacteria significantly increased on +K (Cohen's d = 1.542) and +N (Cohen's d = 0.432) and decreased on +NK (Cohen's d= -0.332), the responses of orders and families within the class varied across nutrient additions. Within the Alphaproteobacteria, the relative abundance of the order Sphingomonadales only

increased on +K treatments (Cohen's d = 2.271), but the constituent families of the order Sphingomonadales had variable responses; of the seven families detected within the order Sphingomonadales, only the Sphingomonadaceae (Cohen's d = 2.219) and GOBB3.C201 (Cohen's d = 2.108) increased on +K treatments, while the GOBB3.C201 (Cohen's d = 1.026), SD04E11 (Cohen's d = 0.751), and DSSF69 (Cohen's d = 0.751) increased on +N treatments. Surprisingly, none of the families in the order significantly decreased on +NK, and GOBB3.C201 increased (Cohen's d = 0.423). Additionally, GOBB3.C201 significantly increased on +P (Cohen's d = 0.302), +PK (Cohen's d = 1.642), and +NPK (Cohen's d = 0.627), and decreased on +PN (Cohen's d = -3.236). In contrast to the order Sphingomonadales, the order Caulobacterales (also within the class Alphaproteobacteria) increased on +K (Cohen's d = 2.272), +N (Cohen's d = 1.190), and +NK (Cohen's d = 0.122). Within the Caulobacterales, the family Caulobacteraceae increased on +K (Cohen's d = 0.390), +N (Cohen's d = -1.073), +NK (Cohen's d = -0.950), +NPK (Cohen's d = -2.873), +P (Cohen's d = -3.178), +PK (Cohen's d = 0.774), and +PN (Cohen's d = 0.487). The only other family detected in the order, the Hyphomonadaceae, only increased significantly on +K (Cohen's d = 2.432), +N (Cohen's d = 1.915), and +NK (Cohen's d = 0.580). Because shifts in relative abundance occur within lower-rank taxonomic groups and the response of constituent taxa varies with respect to geochemistry, hierarchical dissections of taxa are critical when describing the effects of a treatment on the community.

Because nutrient enrichment affects the abundance of functional groups in other systems (Leff et al 2015, Lammel et al 2015a, Lammel et al 2015b), we compared the

change in relative abundance of members of a polyphyletic functional group of "nitrifiers" (Belser 1979, Koops *et al* 2006) between controls and nitrogen amended plots. Seven families were detected in our samples: the Nitrospiraceae, O319.6A21, and 4.29 of order Nitrospirales within the class Nitrospira of the phylum Nitrospirae, the Nitrosomonadaceae of the order Nitrosomonadales within the class Betaproteobacteria of the phylum Proteobacteria, the Nitrospinaceae of the order Desulfobacterales within the class Deltaproteobacteria of the phylum Proteobacteria, the Ectothiorhodospiraceae of order Chormatiales within the class Gammaproteobacteria, and the Bradyrhizobiaceae of order Rhizobiales within the class Alphaproteobacteria. The relative abundance of families Nitrosomonadaceae, Nitrospiraceae, and 4.29 (order Nitrospirales) were not significantly different from controls; families Nitrospinaceae (Cohen's d = 0.211), 0319.6A21 (order Nitrospirales, Cohen's d = 0.515), and Ectothiorhodospiraceae (Cohen's d = 0.141) increased on +N treatment plots relative to controls. The relative abundance of 0319.6A21 did not increase significantly on any other treatment. The Nitrospinaceae increased on +NK treatments (Cohen's d = 0.788), decreased on +K treatments (Cohen's d = -1.600), but did not change on any other treatment. The Ectothiorhodospiraceae increased on +P (Cohen's d = 0.627) and +PK treatments (Cohen's d = 0.200), but decreased with +K treatments (Cohen's d = -0.496). As predicted, the addition of nitrogen affected the relative abundance of OTUs belonging to a polyphyletic group of nitrifiers.

Discussion

Bacterial leaf-litter communities are structured around nitrogen

Fertilizers containing nitrogen, phosphorous, and potassium are motile in nature and can impact natural ecosystem processes in a variety of ways (Smith et al 1999, Matson et al. 1997, Howarth et al. 1996, Galloway et al. 1995). From 1963 to 2013, world wide consumption of NPK fertilizers has increased 486% (IFADATA 2014), and the widespread use of fertilizers in agriculture has indirectly increased the input of inorganic nutrients into natural ecosystems through the deposition of enriched dust, aerosols, surface water, and groundwater (Smith et al 1999, Matson et al. 1997, Howarth et al. 1996, Galloway et al. 1995). In this study, we show that nitrogen enrichment reduced diversity and significantly altered the structure of tropical leaf litter bacterial communities. These changes to the leaf litter bacterial community are consistent with other nitrogen fertilization experiments in leaf litter (Kaspari *et al* 2010) and soils (Allison and Martiny 2008, Nemergut *et al* 2010, Leff *et al* 2015, Ramirez *et al* 2012, Ramirez *et al* 2010). Our observations suggest that microbial leaf litter communities are more sensitive to nitrogen enrichment than potassium or phosphorus, similar to soil microbial communities (Kaspari et al 2017, Ramirez et al 2012, Ramirez et al 2010, Nemergut et al 2010, Lammel et al 2015b, Pan et al 2014, Leff et al 2015).

The high diversity of microbial communities in tropical leaf-litter highlights the enormous niche space that is a composite of chemical, spatial, and temporal gradients found across the floor of a tropical rainforest. Our measures of diversity were ~50% higher than reported in another study (Kim *et al* 2014); however, this was likely an effect

of differences in DNA extraction protocol, the amount of material used, sequencing, and rarefaction depth. Regardless, the major phyla detected (Proteobacteria, Actinobacteria, Bacteroidetes, and Acidobacteria) were found in similar abundances to those found in other tropical leaf litter communities (Kim *et al* 2014).

Responses to fertilization were detected more frequently in lower-rank taxa

Bacteria and archaea are ideal candidates for a hierarchical dissection of the relationship between phylogeny and population responses. “Ecological coherence” (Philippot et al 2009, Philippot et al 2010) is emerging as a predictive property of ecological function in microorganisms. This hypothesis predicts that phylogenetically related taxa share traits that distinguish them from other taxa (Fierer et al 2007, Kaspari 2001, O’Brien et al 1998, Philippot et al 2009, Philippot et al 2010). In microbial systems, evidence continues to emerge demonstrating correlations between phylogeny and spatial distribution (Cho and Tiedje 2000, Kim et al 2014, Lozupone and Knight 2007, Nemergut et al 2010, Philippot et al 2009, Takacs-Vesbach et al 2008), responses to gradients (Fierer et al 2007, Horner-Devine et al 2003, Morrissey and Franklin 2015, Oton et al 2016, Pan et al 2014, Allison *et al.* 2009, Hewson and Fuhrman 2014), responses to stresses (Amend et al 2016, Evans and Wallenstein 2014), and conservation of genes (Martiny et al 2013) and functional characteristics (Morrissey et al 2016). We show that compositional shifts occur within lower-rank taxonomic groups, revealing new associations between biogeochemistry and the bacterial community (Appendix A. Supplemental Table 4). Such analyses are likely to

shed new light on taxa whose natural history is largely unknown. For example, high throughput sequencing of microbial communities can be used to infer the natural history of poorly characterized lineages. For example, the functional ecology of the Acidobacteria has been mostly inferred from incidence and relative abundance in molecular surveys (Barns *et al* 2007, Eichorst *et al* 2011, He *et al* 2006, Jones *et al* 2009, Sait *et al* 2006, Ward *et al* 2009). Acidobacteria tend to increase in low pH soils (Lauber *et al* 2009, Rousk *et al* 2010, Sait *et al* 2006). Differentiation of lower rank taxonomic groups within the Acidobacteria may provide additional insight to the functional ecology of this diverse and common group of organisms. We observed a co-occurrence and trade-off pattern among four families of Acidobacteria, three of which have no cultivated representatives. In each treatment plot in which the relative abundance of the Acidobacteriales increased, DA052 also increased. Conversely, when the relative abundance of the Acidobacteriales and DA052 increased, the relative abundance of DA023 and DS100 decreased, and vice-versa. Little is known about the physiology of these uncultivated acidobacteria; however, DA052 was present in high abundance in a litter addition/removal experiment and decreased in relative abundance as nitrate concentrations increased (Yarwood et al. 2013). DS100 was present in a wastewater system with high nitrate, ammonium, and phosphate concentrations (Hoshino et al. 2006). This suggests that DS100 and DA023 might be adapted to high nutrient systems, while low nutrient conditions may favor DA052.

The varied responses of the families belonging to the same order demonstrate the ability of phylogenetically related microorganisms to specialize along nutrient

gradients. The class Alphaproteobacteria is diverse in ecology, distribution, genome size, and genetic content (Boussau *et al* 2004, Ettema and Andersson 2009). The Alphaproteobacteria were common in our samples, in lowland tropical forest soil (Kim *et al* 2014), and other soils rich in C (Smit *et al* 2001, Thompson *et al* 2010). We examined the responses of two orders within the Alphaproteobacteria to nutrient amendments: the Sphingomonadales and the Caulobacterales. In particular, members of the Sphingomonadales can degrade a diversity of recalcitrant natural and xenobiotic compounds (White *et al* 1996) and are up to 10-times more abundant in leaf litter than soil (Murakami *et al* 2010). The Caulobacterales are dimorphic prosthecate bacteria common in nutrient poor, structured environments and have a high affinity for organic carbon from the decomposition of plant matter (Poindexter 2006). Members within these orders differentiated in response to nutrient additions in leaf litter, demonstrating that specialization of phylogenetically related microbes does occur at finer taxonomic resolutions. Specifically, the relative abundance of the order Sphingomonadales only increased on +K treatments, and the order Caulobacterales increased on +K, +N, and +NK, while the constituent taxa belonging to either order had variable responses to nutrient addition.

Natural nitrogen cycles are dependent upon microbial chemolithoautotrophic nitrification, the key process in oxidizing ammonium to nitrate (Prosser 1989). Archaea are typically associated with oxidation of ammonia, especially when produced by mineralization of organic matter (Offre *et al* 2009, Prosser and Nicol 2008, Zhang *et al* 2010, Stahl and de la Torre 2012), and ammonium concentration has been correlated to

the abundance of ammonium oxidizing bacteria and archaea (Lammel et al 2015b). However, we did not detect any known ammonium oxidizing Archaea in our data, likely due to the low coverage of the Phylum Thaumarchaeota with these primers (Klindworth *et al* 2012, Stahl and de la Torre 2012). Conversely, we detected shifts in taxa associated with the oxidation of nitrite to nitrate, the rate limiting step in nitrification (Yavitt and Wieder 1988). The Nitrospinaceae, Ectothiorhodospiraceae, and 0319.6A21 (order Nitrospirales) all increased with +N amendments. We suspect that bacteria belonging to 0319.6A21 (order Nitrospirales) are capable of nitrite oxidation because they are closely related to other nitrite oxidizing families (Juretschko et al 1998, Watson et al 1986), and increased in relative abundance on nitrogen addition plots. This suggests that members of 0319.6A21 are able to compete for nitrite when other nutrients are limiting; however, this competitive advantage may be lost when nitrite and potassium are abundant, as the relative abundance of 0319.6A21 decreased relative to other members of the Nitrospinaceae on NP fertilization treatments.

In summary, we measured the effects of nutrient enrichment on the diversity and structure of bacterial communities in the leaf litter of a tropical rainforest. The combination of replicated, factorial additions of nutrients with a high throughput, sequence-based survey of bacterial community composition allowed for the detection and characterization of significant shifts in diversity and structure, as well as a hierarchical dissection of taxonomic response to fertilization. We found that nitrogen fertilization had the greatest impact on microbial compositional and phylogenetic richness and structure. These changes in diversity were caused by specialization at

finer taxonomic resolution (e.g. orders and families). Data describing the effects of nutrient enrichment on microbial communities may improve predictions of changes to the overall ecosystem function (Ingwersen et al. 2008, Moorhead and Sinsabaugh 2006) because the diversity and structure of the microbial community influences the decomposition of leaf litter (Bell et al. 2005, Matulich and Martiny 2015, Schimel and Gulledge 1998, Strickland et al. 2009). Because leaf litter and deadwood can account for ~12% of the carbon in a tropical forest (Pan et al 2011), improving the accuracy of mineralization rate models is critical for predictions and policy, especially considering that mineralization rates are predicted to increase with global temperatures (Prescott 2010, Zhang et al 2008, Bothwell et al. 2014, Hobbie et al. 2002, Rustad et al. 2001) and inorganic nutrient availability (Berg and Matzner 1997, Matulich and Martiny 2015, Kaspari *et a*l 2008a, Kaspari et al 2008, Kaspari and Yanoviak 2008).

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Tables and Figures

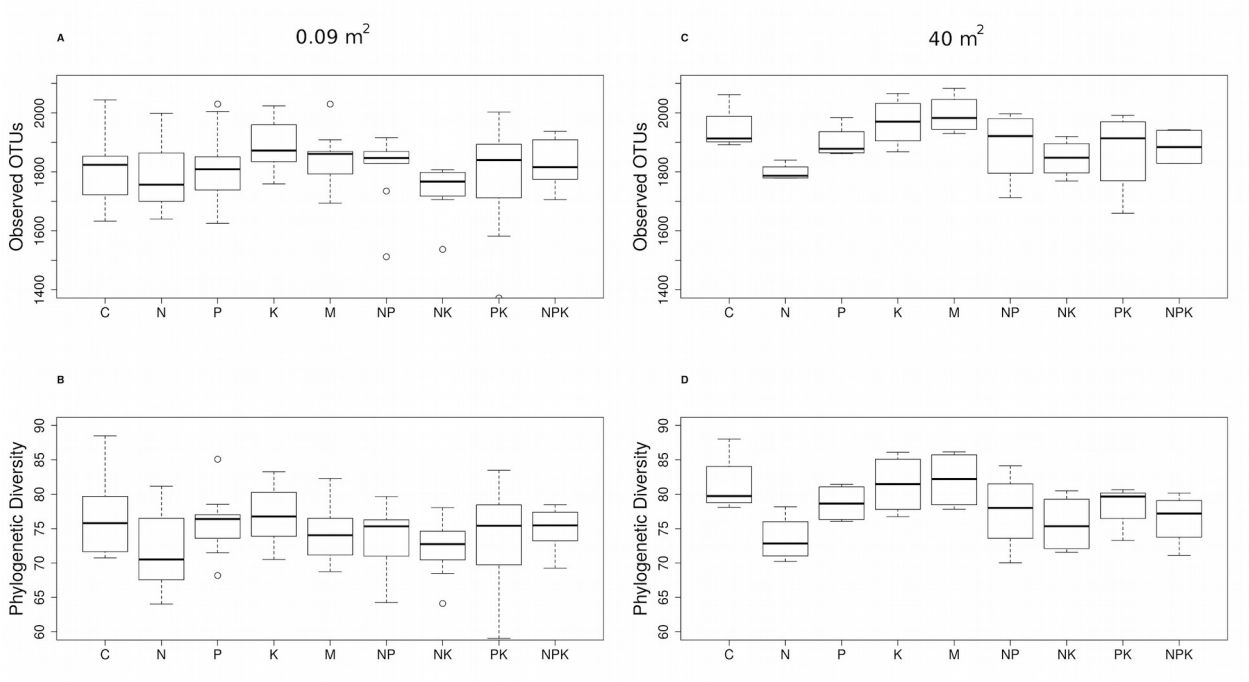

Figure 1: Boxplots of observed (top, A and C) and phylogenetic (bottom, B and D) diversity at 0.09 m^2 (A and B) and 40 m^2 (C and D) spatial grains.

Table 1: The effect of nutrient additions on the leaf litter microbial community structure. Comparisons are pairwise ANOSIM tests between the treatment and control. Bolded values indicate significance.

	0.09 m^2				40 m^2			
	Weighted UniFrac		Bray Curtis		Weighted UniFrac		Bray Curtis	
Treatment	p.value	r	p.value	r	p.value	r	p.value	r
N	**< 0.001**	**0.544**	**0.001**	**0.590**	0.028	0.625	0.028	0.589
P	0.108	0.078	0.083	0.089	0.343	0.094	0.322	0.099
K	0.046	0.115	0.116	0.062	0.056	0.323	0.036	0.255
M	0.180	0.055	0.099	0.083	0.715	-0.052	0.477	0.016
NP	0.066	0.092	0.009	0.146	0.067	0.271	0.368	0.063
NK	**0.003**	**0.267**	**< 0.001**	**0.328**	0.254	0.125	0.053	0.200
PK	0.009	0.199	**0.002**	**0.245**	0.193	0.167	0.065	0.292
NPK	0.056	0.137	**0.004**	**0.238**	0.053	0.396	0.025	0.359

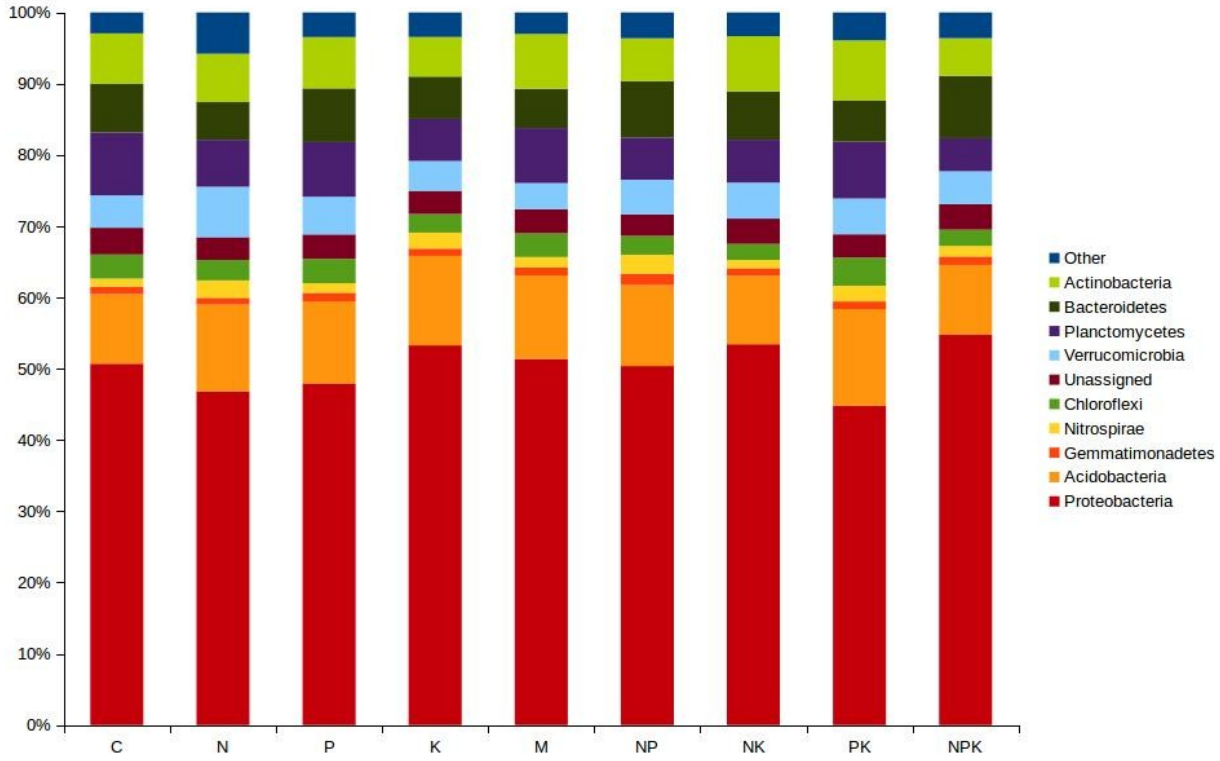

Figure 2: The phylum level taxonomic composition of leaf litter microbial communities on enrichment plots fertilized with nitrogen (N), phosphorus (P), potassium (K), micronutrient (M), nitrogen and phosphorus (NP), nitrogen and potassium (NK), phosphorus and potassium (PK), and nitrogen, phosphorus, and potassium (NPK). Control plots (C) received no nutrient fertilization.

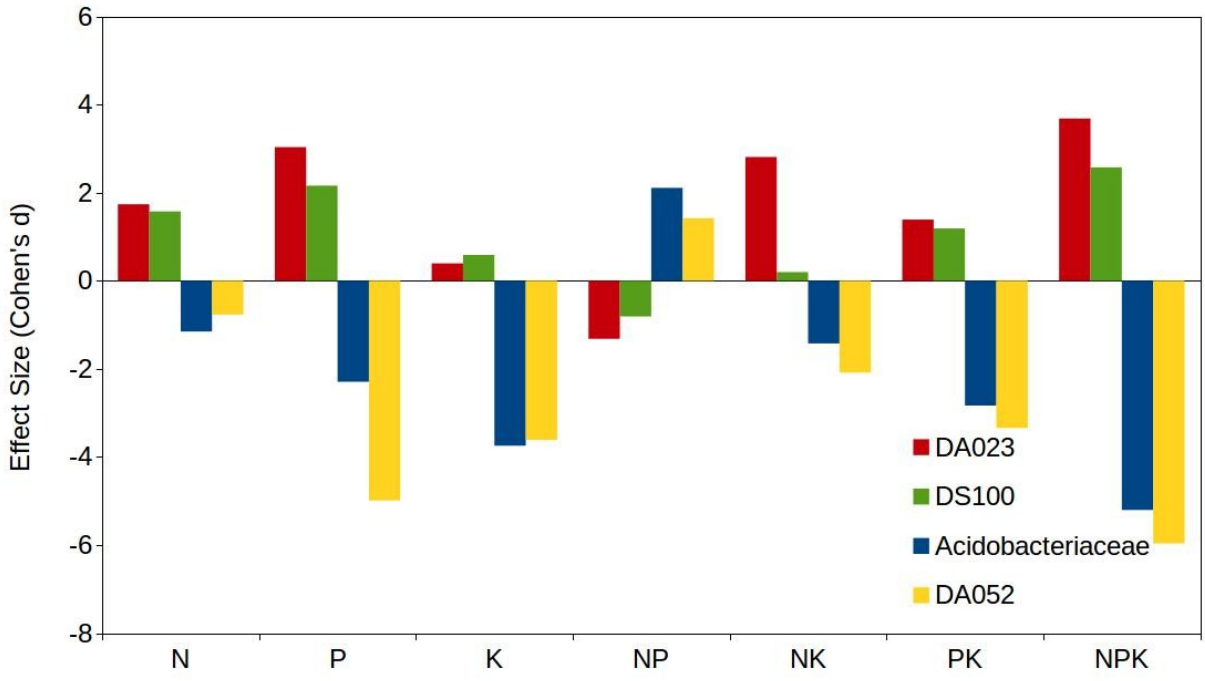

Figure 3: The change in relative abundance (expressed above as Cohen's d) for families within the Acidobacteria in response nutrient addition. All responses shown above are significant ($p < 0.05$).

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Chapter 6. Effect of rodents on soil bacterial communities in the Chihuahuan desert

Abstract

The diversity and structure of biological communities are shaped by interactions between populations and their environment. Interactions that affect plant and bacterial communities are of particular interest because of their strong impact on ecosystem processes; however, the importance of interactions among plant and animal populations on soil microbial communities remains largely unexplored. We hypothesized that granivorous rodent populations indirectly impact the diversity and structure of the bacterial community through regulation of the plant community. To test our hypothesis, we conducted a survey of soil bacterial communities, plant and rodent censuses, and soil chemistry analyses on rodent exclusion plots as part of a long-term rodent exclusion experiment in the Chihuahuan Desert. Rodent exclusion, plant species richness, and plant community structure had no effect on bacterial species richness, and neither plant species richness nor plant community structure predicted bacterial species richness. The soil bacterial community structure shifted when rodents were excluded from treatment plots, and changes in the plant community structure and soil chemistry predicted changes in the bacterial community structure; however, the effect sizes were small. Our results support the hypothesis that the direct and indirect effects of trophic interactions between above-ground populations can shape the diversity and structure of the below-ground bacterial community, although the mechanisms behind the

relationships remain to be tested.

Introduction

Interactions between populations shape the diversity and structure of biological communities. In some cases, the presence or absence of a particular species significantly alters the ecosystem by changing the dynamics of trophic, competitive, and mutualistic interactions. “Species-level cascades” occur when changes in a species’ abundance affect a small number of other species in the community (Polis 1999). Contrastly, “community-level cascades” occur when changes in a species’ abundance substantially the distribution of species in the entire system (Polis 1999). Such species whose presence or absence have large effects on an ecosystem’s community structure are known as “keystone species.” Keystone species are important drivers in the identity, productivity, and resilience of an ecosystem, and their removal can have effects that cascade throughout the food web (Polis 1999, Polis et al 2000).

Community-level cascades are of particular importance because of their strong impact on ecosystem processes (Polis *et al* 2000). In many cases, community-level cascades are driven by the disruption of predator-prey interactions. Predator-prey interactions can have direct (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Saleem et al 2016) and indirect (Schmitz *et al* 2000, Letourneau and Dyer 1998) effects on ecosystem productivity, diversity, and species composition. Direct effects occur when a predator differentially consumes primary producers, whereas indirect effects typically involve the regulation of herbivory by higher predators. Although

community level cascades are well documented in the literature, it is unknown if keystone species removal has an effect on the soil microbial community.

Plant-microbe interactions play important roles in ecosystem processes (Bardgett and Shine 1999, Lou *et al* 2014, Schlatter et al 2015). The microbial community contributes to the diversity and productivity of the plant community through nutrient fixation, recycling, and mineralization (Heijden *et al* 2008, Hooper *et al* 2000, Prashar *et al* 2014). In turn, the plant community supports the soil microbial community by providing carbon substrates (as soil exudates and litter) and microhabitats (Schlatter et al 2015, Bardgett and Shine 1999, Ball et al 2009, Wardle et al 2006), as well as hosts for symbiotic and pathogenic relationships (Schlatter et al 2015, Luo et al 2014, Hooper et al 2000). Thus, if keystone species removal affects the plant community, it should have a cascading effect on the diversity and structure of the microbial community.

We hypothesized that granivorous rodent populations indirectly impact the diversity and structure of the bacterial community through regulation of the plant community. To test these indirect effects, we conducted a 16S rRNA gene survey of soil bacterial communities, plant and rodent censuses, and soil chemistry analyses on rodent exclusion and control plots as part of a long-term rodent exclusion experiment in the Chihuahuan Desert. This rodent exclusion experiment has been maintained since 1977 (Brown 1998), and has shown that the exclusion of the kangaroo rat (*Dipodomys* spp.) alters the density, diversity, and composition of the plant community through removal of granivory and soil disturbing behaviors (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Samson et al. 1992, Guo and Brown 1996). Our study is the

first to examine the effects of the rodent exclusions on the diversity and structure of the soil bacterial community. These data allowed us to explore the importance of macro-biological interactions on the soil bacterial community.

Methods

Study Site

The Portal Project field site is located in the Chihuahuan Desert near Portal, Arizona, USA. The site consists of twenty-four, 50 m x 50 m plots. Each plot is surrounded by 60 cm tall 6.25 mm wire mesh buried 20 cm in the ground and topped with a 15-cm strip of aluminum flashing. Rodent access is controlled by sixteen gates, four gates cut into each side of the fence. Ten plots have large gates that allow all rodents to enter and leave freely, eight plots have smaller gates to exclude kangaroo rats, and six plots have no gates to exclude all rodents.

Plant Community Census

The plant communities on the rodent exclusion plots were censused between 30 August and 03 September 2014 by counting stems per species on sixteen, 0.25-m^2 quadrats within each plot. The percent coverage of forb/grass and shrub plants at each soil sample location was measured using a spherical crown densiometer at ground level.

Soil Sample Collection

Soil samples were collected from 29-31 in August 2014. Two surface soil samples (200 g each) were collected from the top 6 cm of soil, 1 m apart, and approximately 21 m diagonally towards the center from the SW, SE, and NE corners of each plot. Each sample was homogenized by mechanical mixing and ~0.25 g of soil was immediately sub-sampled and preserved in the field using the Xpedition Lysis/Stablization Solution and ZR BashingBead Lysis Tubes following the manufacturer's protocol (Zymo Research, Irvine, CA). All tools were washed with 95% ethanol, dried, and field rinsed prior to collecting each sample to reduce contamination. Total DNA was extracted from the subsampled soil using the Xpedition Soil/Fecal DNA MiniPrep kit following manufacturer's protocol (Zymo Research).

The remaining soil was submitted for chemical analysis to measure the effects of rodent manipulation on soil chemistry. The concentrations of sodium, calcium, magnesium, potassium, boron, phosphorus, iron, zinc, boron, copper, manganese, total nitrogen, total carbon, and the electrical conductivity of the soil were measured for each soil sample. The effects of rodent manipulation on soil chemical properties were compared using a MANOVA.

Bacterial Community Analysis

Bacterial diversity of the soil samples was measured using high-throughput DNA sequencing of a ~250 bp fragment spanning the V4 hypervariable region of the 16S

rRNA gene. The composite primers S-D-Arch-0519-a-S-15/S-D-Bact-0785-b-A-18 (Klindworth *et al* 2013) were used with PCR to amplify a region of 16S rRNA genes from most bacteria and archaea present in each DNA extract. The primer S-D-Arch-0519-a-S-15 was modified to include a 16 bp adapter sequence (GTAAAACGACGGCCAG) at the 5' end to allow for the attachment of a unique 12 bp barcode in a subsequent 6-cycle PCR reaction (Hamady et al 2008, Stamps et al 2016). PCR amplification was performed in 50 µl reactions containing 2 µl of 1:10 diluted template DNA, 1x Taq buffer with KCl (Thermo Scientific, Waltman, MA, USA), 1.5 mM MgCl2 (Thermo Scientific), 0.2 µM of the forward and reverse primer, 0.25 mM of each dNTP (Thermo Scientific), and 1.25 U of Taq DNA Polymerase (Thermo Scientific). The PCR was performed in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, NJ, USA). Initial denaturation was held at 96 °C for 3 min, followed by 30 cycles, each consisting of 96 °C for 30 s, 52 °C for 45 s, and 72 °C for 45 s. The final extension was held for 10 min at 75 °C. The fidelity of PCR product sizes was verified visually using agarose gel electrophoresis, and each was purified using SPRIselect beads (Beckman Coulter, Brea, CA, USA) following the manufacturer's protocol.

A unique "barcode" was attached to each library of purified PCR products with a 6 cycle PCR reaction (Hamady et al 2008, Stamps et al 2016). The forward primer consisted of a unique 12 bp "barcode" (Appendix A. Supplemental Table 5), two spacer nucleotides, and the 16 bp adapter sequence (5' to 3'). The reverse primer was S-D-Bact-0785-b-A-18. Each "barcoding" reaction was a total of 50 µL and contained 4 µl of the purified PCR product, 1x Taq buffer with KCl (Thermo Scientific), 1.5 mM MgCl2

(Thermo Scientific), 0.2 µM each primer, 0.25 mM of each dNTP (Thermo Scientific), and 1.25 U of Taq DNA Polymerase (Thermo Scientific). Six cycles of PCR thermal cycling were carried out in a Techne TC-512 Gradient Thermal Cycler (Techne Inc., Burlington, NJ, USA) using the PCR program described above. The resulting PCR products were purified using SPRIselect beads (Beckman Coulter) and quantified using the Qubit fluorometer and dsDNA HS assay kit (Life Technologies, Grand Island, NY, USA). Equimolar amounts of each uniquely barcoded PCR product were pooled. The final multiplexed PCR sample was submitted for sequencing on an Illumina MiSeq using TruSeq 250 bp PE V2 chemistry at the Oklahoma Medical Research Foundation (Oklahoma City, Oklahoma, USA).

Sequence data were demultiplexed and analyzed using QIIME (Caporaso *et al* 2010). All sequence reads were screened to remove those containing any errors in the reverse primer or barcode regions, ambiguities, homopolymers (greater than 6 nucleotides in length), or an average quality score less than 25. Paired-end reads were merged with a minimum overlap of 100 bp, and each sequence was binned according to its unique barcode. Primer sequences were trimmed, chimeric sequences were removed using USEARCH version 6.1(Edgar 2010) and the RDP “gold” reference database, and unique sequences were clustered into *de novo* Operational Taxonomic Units (OTUs) at 97% similarity using USEARCH version 6.1 (Edgar 2010).

A representative sequence from each OTU was assigned a phylogenetically consistent taxonomy using the SILVA SSU database (Release 111; Quast *et al* 2013) and RDP naïve Bayesian rRNA classifier (Wang *et al* 2007). Representative sequences

were aligned against the SILVA reference database (Quast *et al* 2013) using the pyNAST aligner. The effects of rodent manipulation on the abundance of each OTU were tested using DESeq2 in R (Love *et al.* 2014).

Bacterial and plant diversity were calculated using simple and abundance-weighted counts (Shannon's Index, Shannon 1948). Phylogenetic diversity was calculated using Faith's Index for the bacterial communities (Faith 1992). The effect of rodent exclusion treatments on the species richness of the soil bacterial community was compared using ANOVA for the total observed OTUs and phylogenetic diversity. The assumptions of homogeneity of variance and normality were tested using Bartlett's test ($p > 0.05$ for all conditions) and Shapiro-Wilk's test ($p > 0.05$ for all conditions), respectively. Correlations between bacterial and plant community simple and abundance-weighted diversity were measured using Spearman's rank correlation coefficient. All diversity values were calculated from randomly sampled subsets of sequences from each library, rarified to 1500 reads using the QIIME software package for the bacterial community, and from raw count data for the plant community.

The effects of rodent manipulation on the plant and bacterial community structures were measured using Bray Curtis (Bray and Curtis 1957) distance matrices. Additionally, weighted UniFrac (Lozupone and Knight 2005) was used to calculate differences in phylogenetic diversity for the bacterial community across treatments. All distance matrices were calculated from randomly sampled subsets of sequences from each library, rarified to 1500 reads using the QIIME software package for the bacterial communities and raw count data for the plant community. ANOSIM (1000 permutations)

was used to measure changes in the bacterial community structures among treatments using both the Bray-Curtis and weighted UniFrac distance matrices. Relationships between soil chemistry, plant diversity, and plant community structure to the bacterial community structure were measured using ADONIS in QIIME.

Results

De novo clustering of 1,276,162 sequence reads formed a total of 70,853 bacterial OTUs. Rodent exclusion had no effect ($p > 0.05$) on the number of OTUs, abundance weighted diversity, or phylogenetic diversity (Fig. 1); however, rodent manipulation significantly altered the phylogenetic soil bacterial community structure (Weighted UniFrac; $p = 0.008$, $r = 0.076$), but the differences among the communities were small (Fig. 2). Phylogenetic structure of the bacterial communities on kangaroo rat exclusion treatment plots varied significantly from the all rodent exclusion ($p = 0.010$, $r = 0.073$) and no rodent exclusion ($p = 0.002$, $r = 0.136$) treatments. There was no significant difference in the soil bacterial communities between all rodent and no rodent exclusion treatments (Table 1). The composition of the bacterial communities did not vary with rodent exclusion (Bray-Curtis; $p = 0.600$), but varied between pairwise comparisons of kangaroo rat exclusion and no rodent exclusion treatments ($p = 0.007$, $r = 0.051$).

The phyla represented in the soil communities were similar among rodent exclusion treatments, and were most numerically represented by the Proteobacteria

(28%-35%), Acidobacteria (15%-17%), Actinobacteria (13%-18%), Firmicutes (5%-7%), Bacteroidetes (1.5% - 7.7%), Planctomycetes (1.9% - 5.7%), Gemmatimonadetes (2.1% - 5.3%), Verrucomicrobia (1.5% - 4.6%), and Armatimonadetes (1.1% - 4.5%). Sequences belonging to unassigned phyla ranged from 4%-5% relative abundance.

Rodent manipulation significantly affected the relative abundance of 56 Acidobacteria OTUs, 9 Actinobacteria OTUs, 13 Armatimonadetes OTUs, 30 Bacteroidetes OTUs, 16 Gemmatimonadetes OTUs, 22 Planctomycetes OTUs, 159 Proteobacteria OTUs, and 14 Verrucomicrobia OTUs (Fig. 3). The exclusion of all rodents decreased the relative abundance of 93 OTUs and increased the relative abundance of 17 OTUs, while the exclusion of only kangaroo rats increased the relative abundance of 51 OTUs and decreased the relative abundance of 6 OTUs. More OTUs belonging to the Proteobacteria were affected by rodent exclusion than any other phylum, with the abundance of 42 OTUs decreasing from no rodent to all rodent exclusion plot, 118 OTUs decreasing from kangaroo rat to all rodent exclusion plots, 14 OTUs increasing from no rodent to all rodent exclusion plots, and 32 OTUs increasing from no rodent to kangaroo rat exclusion plots (Fig. 3).

Bacterial and plant species richness were not significantly correlated (Fig. 4). Plant community structure did not predict the number of bacterial OTUs ($p = 0.396$) or phylogenetic diversity (Faith's index, $p = 0.697$), nor did the bacterial community structure predict the plant species richness ($p = 0.457$), weighted richness (Shannon's index, $p = 0.175$), or abundance ($p = 0.125$, Table 2); however, changes in plant community structure weakly predicted (rho = 0.217) changes in the bacterial community

structure ($p < 0.001$, Fig. 5). The percentage of open ground cover correlated weakly to the bacterial community structure ($R^2 = 0.012$, $p = 0.036$), but the percentage of shrub, grass, and forb did not (Table 2).

Soil nutrient concentrations (Appendix A. Supplemental Table 5)) varied with rodent manipulation, percent forb and grass cover, and percent shrub cover (Table 3). Rodent exclusion treatments significantly increased the concentration of manganese by 161% and 170% when all rodents or kangaroo rats were excluded, respectively. Forb and grass cover significantly affected the concentrations of calcium, magnesium, total nitrogen, potassium, copper, and total carbon ($p < 0.002$). Copper varied with the percentage of shrub cover ($p < 0.001$). Boron, sodium, iron, zinc, and phosphorus did not significantly change with rodent manipulation, forb and grass cover, or shrub cover. The electrical conductivity of the soil varied with the percentage of forb and grass cover ($p < 0.002$) and the percentage of shrub cover ($p < 0.05$).

The composition and phylogenetic structure of the bacterial communities were weakly predicted by variation in soil chemistry (Table 4). The electrical conductivity and concentrations of calcium, copper, iron, manganese, sodium, and zinc all correlated significantly to the phylogenetic and compositional structure of the bacterial community ($p < 0.050$); however, the variance explained by each was low ($R^2 < 0.100$). The concentrations of boron, phosphorus, and magnesium also correlated with the compositional community structure, but the associations were weak for each element individually ($R^2 < 0.100$). The concentration of potassium did not predict neither the phylogenetic nor compositional community structure.

Discussion

The impact of trophic interactions on ecosystem processes has long been a focus in ecology. Our study is the first to investigate the possible indirect effects of granivory on the bacterial community diversity and structure. In this system, the kangaroo rat regulates the plant community through seed consumption and soil disturbance (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Brown 1998). The bacterial community responded to changes in the plant community and soil chemistry; however, the effects of rodent removal on the soil bacterial community were modest. Our data was collected in the summer, which may account for the small effect sizes observed because rodent removal has a greater effect on the plant community in the winter (Supp et al. 2012, Heske et al. 1993, Brown and Heske 1990, Samson et al. 1992, Guo and Brown 1996). Thus, we predict that measuring temporal responses of the bacterial community to rodent manipulations will reveal a divergence-convergence pattern in bacterial community structure, and these changes may correlate to changes in the plant community.

Plant and soil microbial communities are linked through nutrient cycles (Van Der Heijden *et al* 2008, Hooper *et al* 2000, Prashar *et al* 2014, Schlatter *et al* 2015, Bardgett and Shine 1999, Ball et al 2009, Wardle *et al* 2006), species specific affinity relationships (Schlatter *et al* 2015, Luo *et al* 2014, Hooper *et al* 2000), and environmental factors (Fierer and Jackson 2006, Fierer *et al.* 2011, Soininen 2012). The nature of these interactions suggest plant and microbial species richness should be

positively correlated; however, empirical evidence has suggested the relationship between plants and soil microbes is either uncoupled (Prober et al 2015, Wardle 2006, Millard and Singh 2010, Gao et al. 2013) or plant community species richness has less impact on bacterial community diversity than geochemical, climatic, disturbance, or time-dependent factors (Prober et al. 2015, Tedersoo et al. 2014, Ge *et al* 2008). Our results are consistent with these other studies, suggesting bacterial richness is not correlated to the plant community species richness (Fig. 3) or structure (Table 2).

Although it appears species richness of the plant and bacterial communities are not correlated, the structure of the soil bacterial community may vary with the structure of the plant community at local (Mitchell *et al.* 2010), regional (Griffiths *et al.* 2011), and global (Prober et al 2015, Opik *et al.* 2006) scales. In the Chihuahuan desert, selective granivory by kangaroo rat populations regulates the diversity, structure, and density of the local plant community through granivory and soil disturbing behaviors. The presence of the kangaroo rat suppresses summer annual dicot diversity and increases winter annual density (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Brown 1998). We found that local variation in vegetation predicted variation in the bacterial community (Fig. 4), i.e. compositional differences between two plant communities correlated to differences in the underlying bacterial community, and these differences are not necessarily related to community richness. Changes in the bacterial community between rodent exclusion treatments were caused by shifts in the abundance of several OTUs belonging to multiple phyla associated with the rhizosphere and plant disease, including those belonging to the phyla Actinobacteria, Acidobacteria,

Gemmatimonadetes, Proteobacteria, Bacteroidetes, and Verrucomicrobia (DeAngelis et al. 2005, Teixeira et al. 2010, Uroz et al. 2010, Mendes et al. 2011, Weinert et al. 2011, Torres-Cortez et al. 2012, Bulgarelli et al. 2012, Lundberg et al. 2012, Roesch et al. 2007, Mirete et al. 2007), but not OTUs associated with other wild mice (Weldon et al 2015). This might suggest that the bacterial community is changing in response to the plant species present (Fig. 5) and total plant cover (Table 2), rather than a relationship between richness or as a direct effect of rodent exclusion.

The low variation in soil chemistry among rodent exclusion treatments may also explain the low variation among bacterial communities within each treatment. Soil chemistry influences the microbial community structure, diversity, and function (Fierer *et al* 2007, Leff *et al* 2015, Pan *et al* 2014, Lammel *et al*, 2015a, Lammel *et al*, 2015b, Nemergut 2010) because microorganisms specialize along gradients in soil (Allison *et al.* 2009, Pan *et al.* 2014, Hewson and Fuhrman 2014, Claire Horner-Devine et al. 2003). Rodent populations have tremendous impact on soil resource heterogeneity through the deposition of fecal material, foraging, and burrowing (Davidson and Lightfoot 2006, Gurney et al. 2015, Garkaklis et al. 1998). Furthermore, the distribution of soil nutrients is associated with plant cover and varies by species and distance (Charley et al 1975, Jackson and Caldwell 1993a, Jackson and Caldwell 1993b, Johnson et al 2016, Schlesinger and Pilmanis 1998, Vinton and Burke 1995). In this study, we only detected an effect of rodent exclusion on the concentration of manganese; however, more variation in soil chemistry may occur during the winter months at this site, especially considering the greater variation in plant communities

among these treatments during the winter months (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Samson et al. 1992, Guo and Brown 1996).

In summary, the removal of kangaroo rats from plots in the Chihuahuan desert altered the structure of the below-ground bacterial community; however, the effect size was small and the mechanisms for the shifts were not tested. Although the structure of the bacterial community changes with the structure of the plant community, it is unclear if the shifts in structure are a result of plant-microbe interactions, rodent-microbe interactions, or bacterial responses to changes in soil chemistry. We predict that changes in the bacterial community will be greater in the winter than in the summer, as has been observed in the plant community (Heske *et al* 1993, Supp *et al* 2012, Brown and Heske 1990, Samson et al. 1992, Guo and Brown 1996).

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Figures and Tables

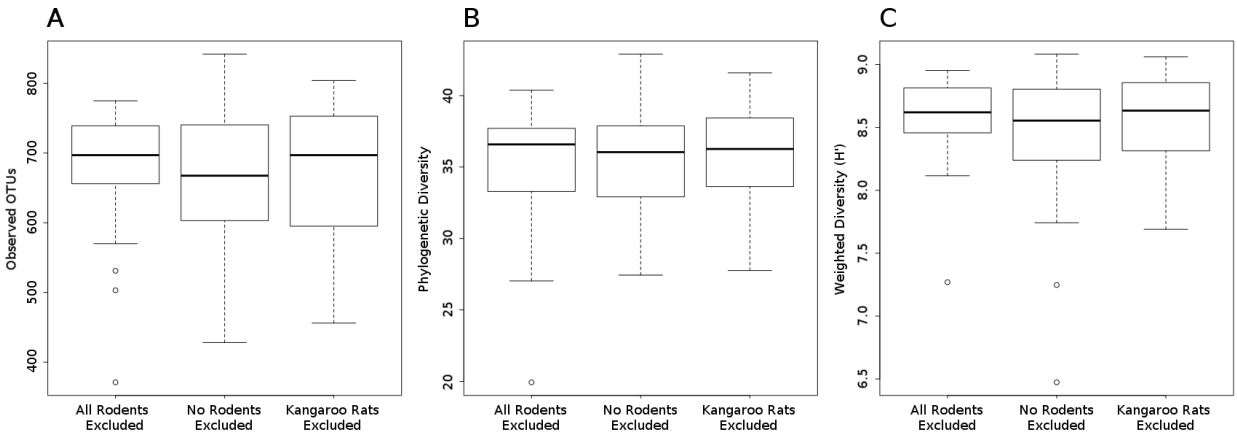

Figure 1- Boxplot of changes in microbial diversity on rodent exclusion plots in the Chihuahuan desert. Comparison of the number of observed OTUs (A), phylogenetic diversity (B, Faith's Index), and abundance-weighted diversity (Shannon's Index, C) found in libraries of 16S rRNA sequences from soil collected from rodent exclusion plots in the Chihuahuan desert, AZ.

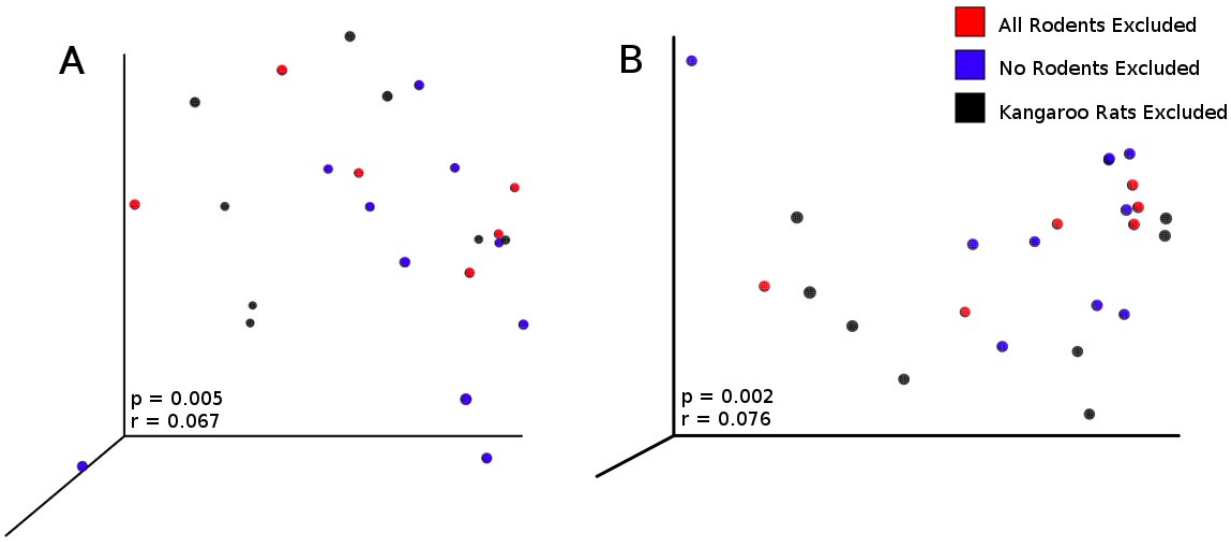

Figure 2- Principal coordinates analysis ordinations comparing the soil microbial communities on rodent exclusion treatment plots. Comparison of the structure of the microbial community when kangaroo rats (black), all rodents (red), or no rodents (blue) were excluded using Bray-Curtis (A) and weighted UniFrac (B) indices. P and r values were calculated using an ANOSIM with 1000 permutations.

Table 1- Pairwise comparisons among soil microbial communities located on rodent exclusion plots in the Chihuahuan desert.

	Phylogenetic Structure[a]		Compositional Structure[b]	
Comparison	r[c]	p value[c]	r[c]	p value[c]
All Rodents Excluded: No Rodents Excluded	0.025	0.183	0.035	0.826
Kangaroo Rats Excluded: All Rodents Excluded	**0.073**	**0.010**	0.021	0.810
Kangaroo Rats Excluded: No Rodents Excluded	**0.136**	**0.002**	**0.051**	**0.007**

[a]Calculated using Weighted UniFrac index
[b]Calculated using Bray-Curtis index
[c]ANOSIM, 1000 permutations

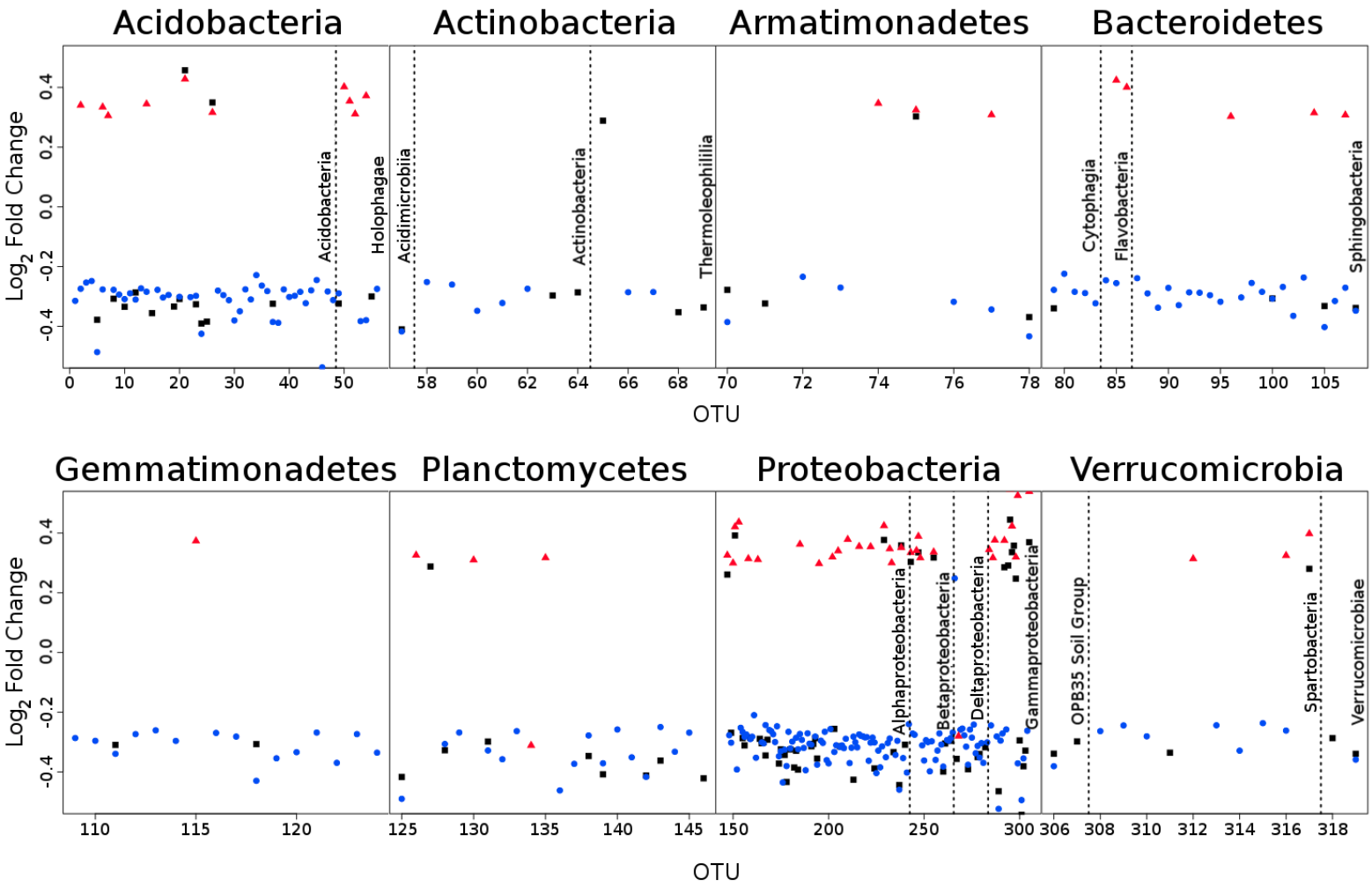
Acidobacteria
Actinobacteria
Armatimonadetes
Bacteroidetes
Log2 Fold Change
0.4
0.2
0.0
-0.2
-0.4
Acidobacteria
Holophagae
Acidimicrobiia
Actinobacteria
Thermoleophilia
Cytophagia
Flavobacteria
Sphingobacteria
0 10 20 30 40 50
58 60 62 64 66 68
70 72 74 76 78
80 85 90 95 100 105
OTU
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia
Log2 Fold Change
0.4
0.2
0.0
-0.2
-0.4
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
Gammaproteobacteria
OPB35 Soil Group
Spartobacteria
Verrucomicrobiae
110 115 120
125 130 135 140 145
150 200 250 300
306 308 310 312 314 316 318
OTU

Figure 3- The log_2 fold changes of soil microbial OTUs belonging to the phyla Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Proteobacteria, and Verrucomicrobia in response to rodent exclusion treatments. Black squares are the shift in abundance from no rodent exclusions to all rodent exclusions, blue circles are the shift in abundance from kangaroo rat exclusions to all rodent exclusions, and red triangles are the shift in abundance from no rodent exclusions to kangaroo rat exclusions. Only OTUs that significantly changed with rodent treatments are shown.

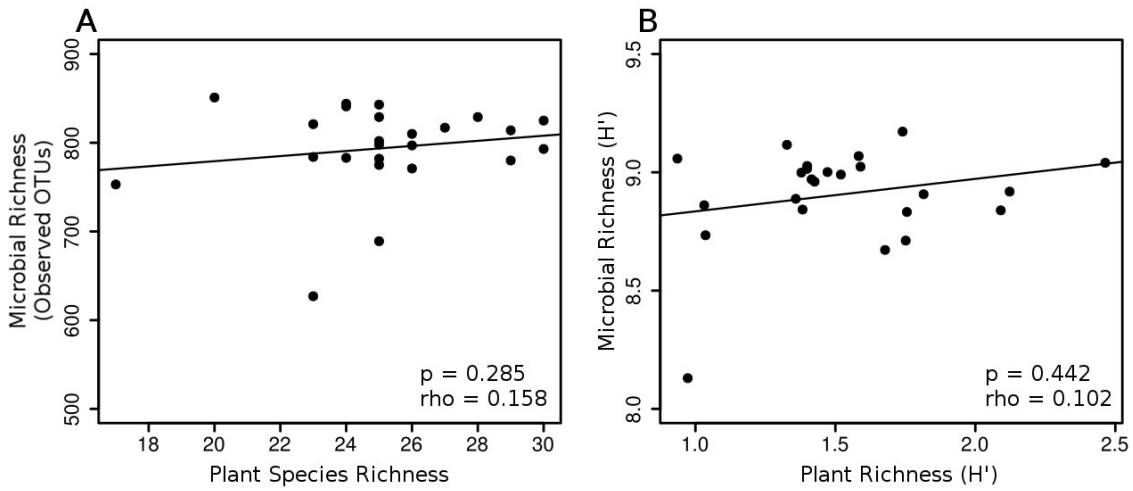

Figure 4- Spearman's correlation between the species richness of the microbial and plant communities. Comparison of the observed (A) and abundance weighted (B, Shannon's index) richness of the microbial and plant communities located within the same plots at the Portal long term rodent exclusion experiment.

Table 2- Correlation between plant community structure and microbial diversity and microbial community structure and plant diversity, abundance, and cover.

	$R^{2,a}$	p value[a]
Plant Community Structure[b]		
Observed OTUs	0.045	0.396
Phylogenetic Diversity	0.027	0.697
Microbial Community Structure[b]		
Plant Abundance	0.057	0.125
Plant Species Richness	0.044	0.457
Plant Species Richness (H')	0.055	0.175
Forb and Grass Cover (%)	0.010	0.128
Shrub Cover (%)	0.010	0.182
Open Cover (%)	**0.012**	**0.036**

[a] ADONIS, 1000 permutations
[b] Calculated using Bray-Curtis index

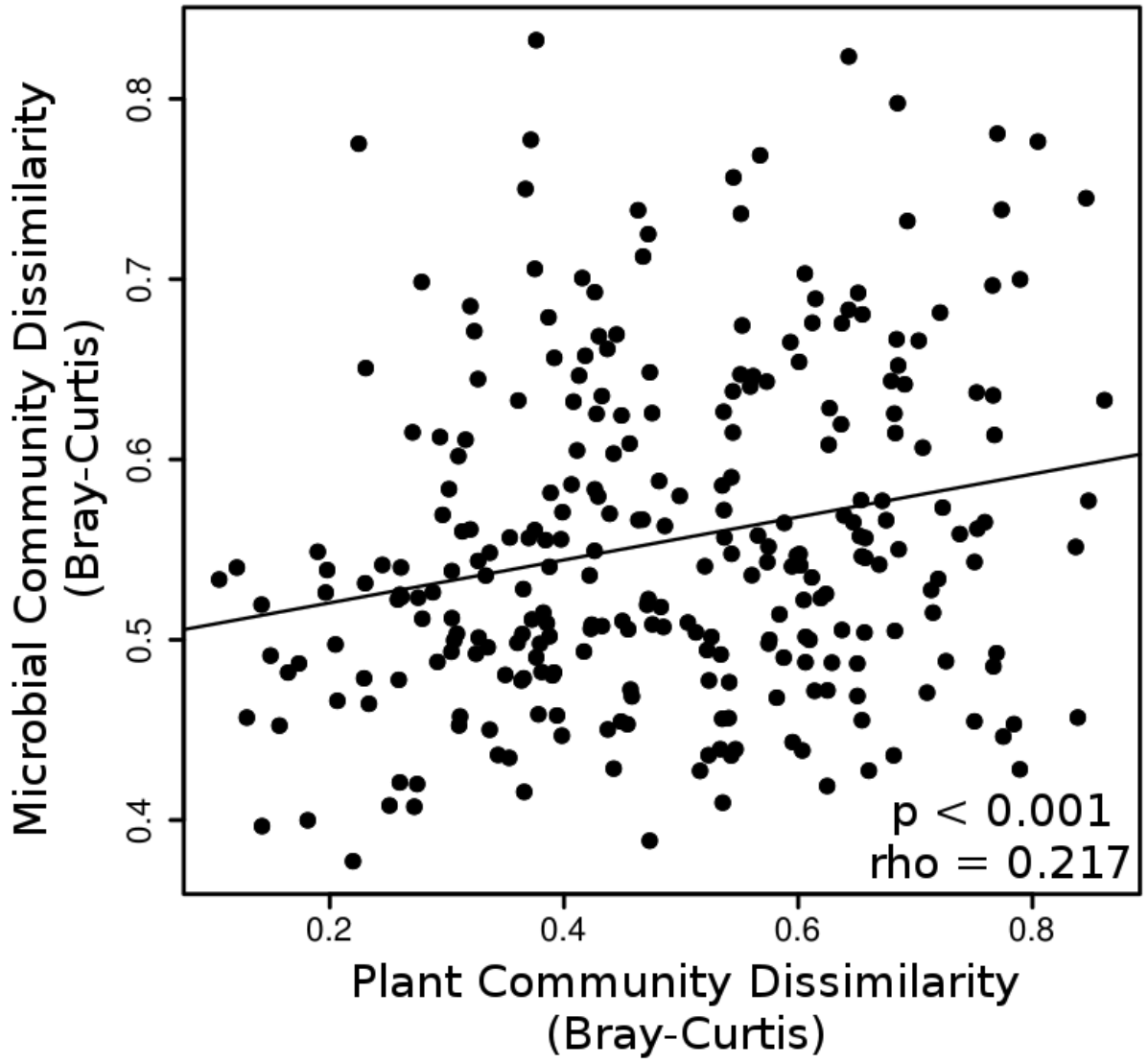

Figure 5- Spearman's correlation between plant and microbial community Bray-Curtis dissimilarity matrices.

Table 3- The effect of rodent manipulation and plant cover on the concentrations of various soil chemical properties. The values in the table are p values from MANOVA testing.

	B (ppm)	Ca (ppm)	Cu (ppm)	Fe (ppm)	P (mg/kg)	K (ppm)	Mg (ppm)	Mn (ppm)	Na (ppm)	Zn (ppm)	Total Carbon (%)	Total Nitrogen (%)	Conductivity (µS/cm)
							Pr (>F)						
Treatment	0.163	0.647	0.074	0.236	0.502	0.061	0.553	**0.004**	0.344	0.368	0.733	0.515	0.196
Forb and Grass Cover (%)	0.632	**< 0.001**	**0.003**	0.104	0.349	**0.002**	**0.001**	0.166	0.964	0.686	**< 0.001**	**< 0.001**	**< 0.001**
Shrub Cover (%)	0.164	0.063	0.596	0.227	0.897	0.077	0.051	0.642	0.191	0.868	0.192	0.052	**0.030**

Table 4- Correlation between soil chemistry and soil microbial community structure.

	Phylogenetic Structure[a]		Compositional Structure[b]	
	$R^{2,c}$	p value[c]	$R^{2,c}$	p value[c]
B (ppm)	0.013	0.109	**0.015**	**0.002**
Ca (ppm)	**0.028**	**0.001**	**0.014**	**0.006**
Cu (ppm)	**0.063**	**0.001**	**0.021**	**0.002**
Fe (ppm)	**0.088**	**0.001**	**0.031**	**0.001**
P (mg/kg)	0.016	0.051	**0.015**	**0.005**
K (ppm)	0.011	0.173	0.011	0.085
Mg (ppm)	0.015	0.066	**0.014**	**0.005**
Mn (ppm)	**0.024**	**0.006**	**0.019**	**0.001**
Na (ppm)	**0.030**	**0.005**	**0.014**	**0.004**
Zn (ppm)	**0.029**	**0.003**	**0.030**	**0.001**
Percent Total Carbon	**0.024**	**0.008**	**0.014**	**0.006**
Percent Total Nitrogen	**0.029**	**0.003**	**0.018**	**0.002**
Conductivity (µS/cm)	**0.016**	**0.034**	**0.012**	**0.018**

[a] Calculated using Weighted UniFrac index
[b] Calculated using Bray-Curtis index
[c] ADONIS, 1000 permutations

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Appendix A. Supplementary Information

Supplementary Information for Chapter 2. Geographical distribution of the Myxobacteria

Table 1: Number of observations of uncultivated families of Myxobacteria within each biome and sample matter.

		Observations within Family												
	Total Observations	PSB29	bacteriap25	VHS.B3.70	MidBa8	MSB.4B10	UASB.TL25	Mle1.27	Eel.36e1D6	Blrii41	VHS.B4.70	Blfdi19	KD3.10	P3OB.42
Sample Site Biome														
Agriculture-associated	6216	64	406	63	1	843	132	948	138	2958	11	919	535	2511
Air	68	0	0	0	0	0	0	0	0	0	0	0	0	0
Alkaline Lakes and Springs	80	0	1	0	1	0	1	3	0	9	0	6	3	18
Alpine Tundra	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Animal-associated	14873	3	3	1	18	28	19	44	32	213	3	93	44	404
Arctic	988	0	5	2	0	21	0	34	1	145	0	82	25	113
Boreal Forest	276	0	2	2	0	11	0	18	0	62	0	37	22	68
Coastal Desert	49	0	0	0	0	0	0	0	0	1	0	0	0	1
Cold Desert	137	0	2	3	0	2	0	0	0	10	1	11	8	35
Estuary	4	0	0	0	1	0	1	0	1	0	0	0	0	2
Groundwater	571	0	34	3	1	5	0	17	7	14	1	12	2	57
Human-associated	40205	8	154	48	13	351	9	587	40	1655	20	773	215	2010
Mediterranean Shrubland	571	0	0	0	0	1	0	4	0	17	0	5	3	47
Montane Shrubland	321	0	3	3	0	42	28	29	1	179	3	48	16	112
Oceans and Seas	5245	53	50	85	201	35	128	18	102	213	165	111	25	514
Ponds and Lakes	5014	3	4	4	84	28	70	86	41	109	6	103	22	244
Saline Lakes	16	2	0	2	2	0	3	0	1	0	0	0	0	4
Savanna	264	0	10	1	0	10	1	23	7	60	5	28	14	62
Semi-arid	143	0	1	0	0	0	0	20	0	48	0	7	31	75

Deserts														
Streams and Rivers	3954	12	21	40	23	23	23	82	16	242	9	177	85	372
Temperate Forests	433	0	3	5	0	12	3	17	0	133	1	43	16	96
Temperate Grasslands	342	0	7	0	0	19	0	26	3	85	0	33	29	87
Tropical Forests	719	14	14	16	22	40	7	40	27	72	20	33	30	126
Wetlands	410	2	9	7	39	46	6	84	16	232	8	39	22	239
Sample Matter														
Algae	1389	0	3	0	1	1	0	0	27	14	0	5	0	7
Animal Habitation	830	0	0	0	0	1	0	1	1	13	0	1	0	10
Animal Material	23122	11	5	36	23	26	21	59	23	460	3	156	49	713
biofilm	1234	2	2	0	4	3	1	2	1	97	1	24	14	130
Brackish water	236	0	0	0	3	0	0	0	0	19	0	8	0	7
compost	50	0	0	0	0	1	0	6	6	48	0	8	5	31
dust	2007	0	3	1	0	9	0	21	0	138	1	37	28	181
feces	26784	0	0	0	7	11	5	24	0	109	1	97	23	209
freshwater	6657	0	50	15	18	40	4	73	8	126	18	98	30	249
groundwater	6	0	0	0	4	0	0	0	0	0	4	0	0	0
insect material	913	0	0	0	0	1	0	0	0	2	0	1	0	6
lichen	16	0	0	0	0	0	0	0	0	0	0	0	0	0
microbial mat	462	0	3	0	0	0	1	4	0	8	0	4	1	26
plant material	782	14	5	19	22	68	6	11	23	159	20	22	7	102
rhizosphere	941	25	97	23	1	119	59	201	57	823	6	223	119	664
saline water	23	2	0	4	3	0	3	0	1	0	1	0	0	5
sand	1326	3	30	9	47	67	14	97	2	156	105	274	77	549
sea water	1396	1	2	3	4	5	5	9	30	36	10	82	9	128
sediment	1366	58	45	88	214	33	177	57	85	161	57	107	22	362
soil	6834	45	482	87	54	1132	133	1492	149	3960	25	1398	760	3617
Food Product	2428	0	1	0	0	0	0	21	0	112	0	9	3	52
sponge	1920	0	1	0	1	0	2	2	20	15	1	6	0	150

Table 2: Number of observations of cultivated families of Myxobacteria within each biome and sample matter.

		Observations within Family							
	Total Observations	Sandaracinaceae	Polyangiaceae	Phaselicystidaceae	Nannocystaceae	Haliangiaceae	Vulgatibacteraceae	Cystobacteraceae	Myxococcaceae
Sample Site Biome									
Agriculture-associated	6216	2298	2703	2087	922	3146	277	1788	1277
Air	68	0	0	0	0	0	0	0	0
Alkaline Lakes and Springs	80	19	21	12	5	29	0	12	9
Alpine Tundra	3	0	0	0	0	0	0	0	0
Animal-associated	14873	558	258	97	297	439	16	161	76
Arctic	988	62	168	57	26	273	1	84	65
Boreal Forest	276	19	93	4	9	187	0	130	28
Coastal Desert	49	0	1	1	0	2	0	0	0
Cold Desert	137	4	4	1	16	56	0	3	3
Estuary	4	2	0	0	1	3	0	2	0
Groundwater	571	29	69	25	21	75	0	3	6
Human-associated	40205	1910	2303	1166	706	2498	106	999	560
Mediterranean Shrubland	571	28	35	6	12	36	3	5	12
Montane Shrubland	321	129	166	103	30	241	0	84	72
Oceans and Seas	5245	1061	63	48	550	921	7	46	28
Ponds and Lakes	5014	143	419	192	51	336	0	266	81

Saline Lakes	16	5	0	2	3	0	0	0	0
Savanna	264	35	71	44	15	102	11	87	22
Semi-arid Deserts	143	55	49	26	13	61	16	9	37
Streams and Rivers	3954	381	409	222	93	344	11	140	99
Temperate Forests	433	87	175	60	25	215	10	127	47
Temperate Grasslands	342	90	117	51	43	129	4	56	56
Tropical Forests	719	56	159	56	26	143	17	99	58
Wetlands	410	147	297	62	53	289	39	233	137
Sample Matter									
Algae	1389	224	2	0	101	172	0	2	1
Animal Habitation	830	4	11	2	5	11	3	1	8
Animal Material	23122	881	557	177	335	701	18	261	179
biofilm	1234	173	58	9	82	221	2	28	30
Brackish water	236	29	2	4	5	15	0	1	1
compost	50	36	28	17	22	36	27	12	2
dust	2007	183	141	85	49	238	6	121	60
feces	26784	212	303	123	36	158	6	80	61
freshwater	6657	229	432	153	94	324	0	116	20
groundwater	6	0	0	0	0	0	0	0	0
insect material	913	10	5	3	1	10	0	3	0
lichen	16	0	0	0	0	0	0	0	0
microbial mat	462	8	24	1	7	23	0	0	0
plant material	782	176	148	109	68	180	2	23	38
rhizosphere	941	511	703	503	139	867	35	474	309
saline water	23	7	1	2	3	1	0	0	0
sand	1326	253	631	184	197	742	23	105	158
sea water	1396	120	25	11	71	82	0	14	7
sediment	1366	556	200	174	263	668	1	260	100
soil	6834	3166	4256	2744	1224	4885	325	2784	1681
Food Product	2428	160	46	18	96	51	70	42	19
sponge	1920	181	6	2	119	139	0	7	0

Supplementary Information for Chapter 5. The Effect of Long Term NPK Fertilization on Bacterial Communities in Leaf Litter from a Lowland Tropical Rainforest

Table 3. Sample locations and barcodes used for 16S rRNA gene community analysis.

SampleID	LinkerPrimerSequence	Plot	Distance from SW Corner	Block	Treatment
1-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	1	1	1	P
2-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	2	1	1	K
3-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	3	1	1	NP
4-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	4	1	1	N
5-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	5	1	1	NPK
6-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	6	1	1	C
7-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	7	1	1	PK
8-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	8	1	1	M
9-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	9	1	1	NK
10-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	10	1	2	NK
11-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	11	1	2	N
12-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	12	1	2	C
13-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	13	1	2	PK
14-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	14	1	2	P
15-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	15	1	2	NP
16-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	16	1	2	M
17-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	17	1	2	NPK
18-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	18	1	2	K
19-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	19	1	3	K
20-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	20	1	3	NPK
21-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	21	1	3	PK
22-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	22	1	3	NP
23-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	23	1	3	N
24-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	24	1	3	P
25-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	25	1	3	M
26-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	26	1	3	C
27-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	27	1	3	NK
28-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	28	1	4	N
29-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	29	1	4	NPK
30-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	30	1	4	P
31-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	31	1	4	M
32-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	32	1	4	K
33-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	33	1	4	PK
34-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	34	1	4	NK
35-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	35	1	4	NP
36-1a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	36	1	4	C
1-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	1	1	1	P
2-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	2	1	1	K
3-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	3	1	1	NP
4-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	4	1	1	N
5-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	5	1	1	NPK

6-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	6	1	1	C
7-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	7	1	1	PK
8-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	8	1	1	M
9-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	9	1	1	NK
10-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	10	1	2	NK
11-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	11	1	2	N
12-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	12	1	2	C
13-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	13	1	2	PK
14-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	14	1	2	P
15-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	15	1	2	NP
16-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	16	1	2	M
17-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	17	1	2	NPK
18-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	18	1	2	K
19-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	19	1	3	K
20-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	20	1	3	NPK
21-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	21	1	3	PK
22-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	22	1	3	NP
23-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	23	1	3	N
24-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	24	1	3	P
25-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	25	1	3	M
26-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	26	1	3	C
27-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	27	1	3	NK
28-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	28	1	4	N
29-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	29	1	4	NPK
30-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	30	1	4	P
31-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	31	1	4	M
32-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	32	1	4	K
33-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	33	1	4	PK
34-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	34	1	4	NK
35-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	35	1	4	NP
36-1b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	36	1	4	C
1-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	1	10	1	P
2-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	2	10	1	K
3-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	3	10	1	NP
4-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	4	10	1	N
5-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	5	10	1	NPK
6-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	6	10	1	C
7-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	7	10	1	PK
8-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	8	10	1	M
9-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	9	10	1	NK
10-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	10	10	2	NK
11-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	11	10	2	N
12-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	12	10	2	C
13-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	13	10	2	PK
14-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	14	10	2	P
15-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	15	10	2	NP
16-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	16	10	2	M

17-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	17	10	2	NPK
18-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	18	10	2	K
19-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	19	10	3	K
20-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	20	10	3	NPK
21-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	21	10	3	PK
22-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	22	10	3	NP
23-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	23	10	3	N
24-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	24	10	3	P
25-2	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	25	10	3	M
26-2	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	26	10	3	C
27-2	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	27	10	3	NK
28-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	28	10	4	N
29-2	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	29	10	4	NPK
30-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	30	10	4	P
31-2	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	31	10	4	M
32-2	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	32	10	4	K
33-2	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	33	10	4	PK
34-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	34	10	4	NK
35-2	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	35	10	4	NP
36-2	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	36	10	4	C
1-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	1	20	1	P
2-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	2	20	1	K
3-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	3	20	1	NP
4-3a	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	4	20	1	N
5-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	5	20	1	NPK
6-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	6	20	1	C
7-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	7	20	1	PK
8-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	8	20	1	M
9-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	9	20	1	NK
10-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	10	20	2	NK
11-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	11	20	2	N
12-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	12	20	2	C
13-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	13	20	2	PK
14-3a	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	14	20	2	P
15-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	15	20	2	NP
16-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	16	20	2	M
17-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	17	20	2	NPK
18-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	18	20	2	K
19-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	19	20	3	K
20-3a	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	20	20	3	NPK
21-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	21	20	3	PK
22-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	22	20	3	NP
23-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	23	20	3	N
24-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	24	20	3	P
25-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	25	20	3	M
26-3a	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	26	20	3	C
27-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	27	20	3	NK

28-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	28	20	4	N
29-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	29	20	4	NPK
30-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	30	20	4	P
31-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	31	20	4	M
32-3a	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	32	20	4	K
33-3a	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	33	20	4	PK
34-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	34	20	4	NK
35-3a	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	35	20	4	NP
36-3a	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	36	20	4	C
1-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	1	20	1	P
2-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	2	20	1	K
3-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	3	20	1	NP
4-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	4	20	1	N
5-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	5	20	1	NPK
6-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	6	20	1	C
7-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	7	20	1	PK
8-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	8	20	1	M
9-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	9	20	1	NK
10-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	10	20	2	NK
11-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	11	20	2	N
12-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	12	20	2	C
13-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	13	20	2	PK
14-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	14	20	2	P
15-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	15	20	2	NP
16-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	16	20	2	M
17-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	17	20	2	NPK
18-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	18	20	2	K
19-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	19	20	3	K
20-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	20	20	3	NPK
21-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	21	20	3	PK
22-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	22	20	3	NP
23-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	23	20	3	N
24-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	24	20	3	P
25-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	25	20	3	M
26-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	26	20	3	C
27-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	27	20	3	NK
28-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	28	20	4	N
29-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	29	20	4	NPK
30-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	30	20	4	P
31-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	31	20	4	M
32-3b	CCCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	32	20	4	K
33-3b	CCCGTAAAACGACGGCCAGCACMGCCGCGGTAA	33	20	4	PK
34-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	34	20	4	NK
35-3b	CGTAAAACGACGGCCAGCACMGCCGCGGTAA	35	20	4	NP
36-3b	CCGTAAAACGACGGCCAGCACMGCCGCGGTAA	36	20	4	C

Table 4. Hierarchical taxonomic responses to NPK fertilzation treatments.

Taxon	Treatment	p value	Cohen's d	Dispersion
Archaea; Euryarchaeota	N:P:K	0.046	2.568	0.000
Archaea; Euryarchaeota; Halobacteria	P:N	0.050	-2.481	0.000
Archaea; Euryarchaeota; Halobacteria	P:K	0.039	-0.124	0.000
Archaea; Euryarchaeota; Halobacteria	K	0.050	0.619	0.000
Archaea; Euryarchaeota; Halobacteria	N:P:K	0.021	2.405	0.000
Archaea; Euryarchaeota; Halobacteria; Halobacteriales	P:N	0.050	-2.481	0.000
Archaea; Euryarchaeota; Halobacteria; Halobacteriales	P:K	0.039	-0.124	0.000
Archaea; Euryarchaeota; Halobacteria; Halobacteriales	K	0.050	0.619	0.000
Archaea; Euryarchaeota; Halobacteria; Halobacteriales	N:P:K	0.021	2.405	0.000
Archaea; Euryarchaeota; Halobacteria; Halobacteriales; Deep_Sea_Hydrothermal_Vent_Gp_6.DHVEG.6.	N:P:K	0.025	2.107	0.000
Archaea; Euryarchaeota; Methanobacteria	N	0.010	0.751	0.000
Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales	N	0.010	0.751	0.000
Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae	N	0.010	0.751	0.000
Archaea; Euryarchaeota; Thermoplasmata	N	0.014	-4.157	0.000
Archaea; Euryarchaeota; Thermoplasmata	P	0.016	-1.712	0.000
Archaea; Euryarchaeota; Thermoplasmata	P:N	0.012	0.982	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales	N	0.014	-4.157	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales	P	0.016	-1.712	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales	P:N	0.012	0.982	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales; Marine_Group_II	N	0.045	-2.828	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales; Terrestrial_Miscellaneous_Gp.TMEG.	P	0.010	-2.828	0.000
Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales;	N	0.025	-2.828	0.000

Terrestrial_Miscellaneous_Gp.TMEG. Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales;				
Terrestrial_Miscellaneous_Gp.TMEG. Archaea; Euryarchaeota; Thermoplasmata; Thermoplasmatales;	K	0.038	-2.828	0.000
Terrestrial_Miscellaneous_Gp.TMEG.	P:N	0.016	0.980	0.000
Archaea; Thaumarchaeota	K	0.009	-4.290	0.002
Archaea; Thaumarchaeota; Marine_Group_I	N	0.006	0.817	0.000
Archaea; Thaumarchaeota; Marine_Group_I	P:N	0.033	1.254	0.000
Archaea; Thaumarchaeota; Marine_Group_I	N:P:K	0.019	1.404	0.000
Archaea; Thaumarchaeota; Marine_Group_I	N:K	0.029	1.458	0.000
Archaea; Thaumarchaeota; Marine_Group_I; o	N	0.006	0.817	0.000
Archaea; Thaumarchaeota; Marine_Group_I; o	P:N	0.033	1.254	0.000
Archaea; Thaumarchaeota; Marine_Group_I; o	N:P:K	0.019	1.404	0.000
Archaea; Thaumarchaeota; Marine_Group_I; o	N:K	0.029	1.458	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.	K	0.009	-5.631	0.002
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera	K	0.034	-6.560	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera	N	0.006	-2.421	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera	N:K	0.025	-1.342	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera; f	K	0.034	-6.560	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera; f	N	0.006	-2.421	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; Candidatus_Nitrososphaera; f	N:K	0.025	-1.342	0.000
Archaea; Thaumarchaeota; Soil_Crenarchaeotic_Group.SCG.; o	K	0.011	-5.190	0.001
Archaea; Thaumarchaeota; terrestrial_group	N	0.012	0.410	0.000
Archaea; Thaumarchaeota; terrestrial_group	P	0.049	0.765	0.000
Archaea; Thaumarchaeota; terrestrial_group; o	N	0.012	0.410	0.000
Archaea; Thaumarchaeota; terrestrial_group; o	P	0.049	0.765	0.000
Bacteria; Acidobacteria	N	0.037	1.258	0.007
Bacteria; Acidobacteria; Acidobacteria	N	0.048	1.137	0.006
Bacteria; Acidobacteria; Acidobacteria; 32.21	N	0.048	0.746	0.001

Bacteria; Acidobacteria; Acidobacteria; 32.21; f	N	0.048	0.746	0.001
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	N:P:K	0.002	-5.204	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	K	0.000	-3.745	0.010
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	P:K	0.005	-2.832	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	P	0.004	-2.294	0.010
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	N:K	0.009	-1.423	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	N	0.006	-1.151	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales	P:N	0.001	2.111	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	N:P:K	0.002	-5.204	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	K	0.000	-3.745	0.010
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	P:K	0.005	-2.832	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	P	0.004	-2.294	0.010
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	N:K	0.009	-1.423	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	N	0.006	-1.151	0.009
Bacteria; Acidobacteria; Acidobacteria; Acidobacteriales; Acidobacteriaceae	P:N	0.001	2.111	0.009
Bacteria; Acidobacteria; Acidobacteria; AT.s3.28	N:P:K	0.038	1.254	0.000
Bacteria; Acidobacteria; Acidobacteria; AT.s3.28; f	N:P:K	0.038	1.254	0.000
Bacteria; Acidobacteria; Acidobacteria; BPC015	K	0.019	-4.156	0.000
Bacteria; Acidobacteria; Acidobacteria; BPC015; f	K	0.019	-4.156	0.000
Bacteria; Acidobacteria; Acidobacteria; Candidatus_Solibacter	K	0.008	-1.077	0.000
Bacteria; Acidobacteria; Acidobacteria; Candidatus_Solibacter; f	K	0.008	-1.077	0.000
Bacteria; Acidobacteria; Acidobacteria; DA023	P:N	0.002	-1.318	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023	K	0.009	0.396	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023	P:K	0.050	1.389	0.006
Bacteria; Acidobacteria; Acidobacteria; DA023	N	0.001	1.738	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023	N:K	0.005	2.811	0.006
Bacteria; Acidobacteria; Acidobacteria; DA023	P	0.009	3.037	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023	N:P:K	0.017	3.684	0.006
Bacteria; Acidobacteria; Acidobacteria; DA023; f	P:N	0.002	-1.318	0.007

Bacteria; Acidobacteria; Acidobacteria; DA023; f	K	0.009	0.396	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023; f	P:K	0.050	1.389	0.006
Bacteria; Acidobacteria; Acidobacteria; DA023; f	N	0.001	1.738	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023; f	N:K	0.005	2.811	0.006
Bacteria; Acidobacteria; Acidobacteria; DA023; f	P	0.009	3.037	0.007
Bacteria; Acidobacteria; Acidobacteria; DA023; f	N:P:K	0.017	3.684	0.006
Bacteria; Acidobacteria; Acidobacteria; DA052	N:P:K	0.005	-5.963	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	P	0.013	-4.988	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	K	0.000	-3.613	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	P:K	0.017	-3.343	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	N:K	0.012	-2.085	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	N	0.009	-0.768	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052	P:N	0.002	1.421	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	N:P:K	0.005	-5.963	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	P	0.013	-4.988	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	K	0.000	-3.613	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	P:K	0.017	-3.343	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	N:K	0.012	-2.085	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	N	0.009	-0.768	0.004
Bacteria; Acidobacteria; Acidobacteria; DA052; f	P:N	0.002	1.421	0.004
Bacteria; Acidobacteria; Acidobacteria; DS.100	P:N	0.001	-0.809	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	N:K	0.002	0.198	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	K	0.012	0.586	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	P:K	0.003	1.189	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	N	0.005	1.575	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	P	0.000	2.161	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100	N:P:K	0.001	2.576	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	P:N	0.001	-0.809	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	N:K	0.002	0.198	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	K	0.012	0.586	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	P:K	0.003	1.189	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	N	0.005	1.575	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	P	0.000	2.161	0.000
Bacteria; Acidobacteria; Acidobacteria; DS.100; f	N:P:K	0.001	2.576	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573	P:N	0.014	-1.840	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573	K	0.006	-1.838	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573	P	0.007	-1.003	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573	N	0.041	0.714	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573	P:K	0.013	1.949	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573; f	P:N	0.014	-1.840	0.000

Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573; f	K	0.006	-1.838	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573; f	P	0.007	-1.003	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573; f	N	0.041	0.714	0.000
Bacteria; Acidobacteria; Acidobacteria; Elev.16S.573; f	P:K	0.013	1.949	0.000
Bacteria; Acidobacteria; Acidobacteria; FFCH5909	N:K	0.030	0.820	0.000
Bacteria; Acidobacteria; Acidobacteria; FFCH5909	K	0.031	1.325	0.000
Bacteria; Acidobacteria; Acidobacteria; FFCH5909; f	N:K	0.030	0.820	0.000
Bacteria; Acidobacteria; Acidobacteria; FFCH5909; f	K	0.031	1.325	0.000
Bacteria; Acidobacteria; Acidobacteria; GOUTB8	N	0.004	0.751	0.000
Bacteria; Acidobacteria; Acidobacteria; GOUTB8; f	N	0.004	0.751	0.000
Bacteria; Acidobacteria; Acidobacteria; JG37.AG.116	K	0.013	-5.980	0.000
Bacteria; Acidobacteria; Acidobacteria; JG37.AG.116	N	0.014	-0.002	0.000
Bacteria; Acidobacteria; Acidobacteria; JG37.AG.116; f	K	0.013	-5.980	0.000
Bacteria; Acidobacteria; Acidobacteria; JG37.AG.116; f	N	0.014	-0.002	0.000
Bacteria; Acidobacteria; Acidobacteria; KF.JG30.18	P:K	0.042	-2.930	0.000
Bacteria; Acidobacteria; Acidobacteria; KF.JG30.18	K	0.000	-2.523	0.001
Bacteria; Acidobacteria; Acidobacteria; KF.JG30.18; f	P:K	0.042	-2.930	0.000
Bacteria; Acidobacteria; Acidobacteria; KF.JG30.18; f	K	0.000	-2.523	0.001
Bacteria; Acidobacteria; Acidobacteria; Order_Incertae_Sedis	N:P:K	0.050	-4.803	0.000
Bacteria; Acidobacteria; Acidobacteria; Order_Incertae_Sedis	K	0.002	-1.754	0.000
Bacteria; Acidobacteria; Acidobacteria; Order_Incertae_Sedis; Family_Incertae_Sedis	N:P:K	0.050	-4.803	0.000
Bacteria; Acidobacteria; Acidobacteria; Order_Incertae_Sedis; Family_Incertae_Sedis	K	0.002	-1.754	0.000
Bacteria; Acidobacteria; Acidobacteria; RB41	K	0.013	0.967	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41	N	0.007	1.825	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41	N:P:K	0.050	2.196	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41	N:K	0.012	2.812	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41; f	K	0.013	0.967	0.002

Bacteria; Acidobacteria; Acidobacteria; RB41; f	N	0.007	1.825	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41; f	N:P:K	0.050	2.196	0.002
Bacteria; Acidobacteria; Acidobacteria; RB41; f	N:K	0.012	2.812	0.002
Bacteria; Acidobacteria; Acidobacteria; S035	P:N	0.017	-1.180	0.000
Bacteria; Acidobacteria; Acidobacteria; S035	P:K	0.009	-0.351	0.000
Bacteria; Acidobacteria; Acidobacteria; S035	K	0.000	-0.031	0.001
Bacteria; Acidobacteria; Acidobacteria; S035	N	0.000	0.766	0.001
Bacteria; Acidobacteria; Acidobacteria; S035	N:K	0.000	1.925	0.001
Bacteria; Acidobacteria; Acidobacteria; S035	P	0.010	2.566	0.001
Bacteria; Acidobacteria; Acidobacteria; S035	N:P:K	0.005	3.123	0.000
Bacteria; Acidobacteria; Acidobacteria; S035; f	P:N	0.017	-1.180	0.000
Bacteria; Acidobacteria; Acidobacteria; S035; f	P:K	0.009	-0.351	0.000
Bacteria; Acidobacteria; Acidobacteria; S035; f	K	0.000	-0.031	0.001
Bacteria; Acidobacteria; Acidobacteria; S035; f	N	0.000	0.766	0.001
Bacteria; Acidobacteria; Acidobacteria; S035; f	N:K	0.000	1.925	0.001
Bacteria; Acidobacteria; Acidobacteria; S035; f	P	0.010	2.566	0.001
Bacteria; Acidobacteria; Acidobacteria; S035; f	N:P:K	0.005	3.123	0.000
Bacteria; Acidobacteria; Holophagae; 43F.1404R	P:N	0.045	2.125	0.001
Bacteria; Acidobacteria; Holophagae; 43F.1404R	N	0.033	2.380	0.001
Bacteria; Acidobacteria; Holophagae; 43F.1404R; f	P:N	0.045	2.125	0.001
Bacteria; Acidobacteria; Holophagae; 43F.1404R; f	N	0.033	2.380	0.001
Bacteria; Acidobacteria; Holophagae; CA002	P:N	0.000	-3.736	0.000
Bacteria; Acidobacteria; Holophagae; CA002	P:K	0.001	-0.408	0.000
Bacteria; Acidobacteria; Holophagae; CA002	N:K	0.001	-0.174	0.000
Bacteria; Acidobacteria; Holophagae; CA002	N:P:K	0.000	0.916	0.000
Bacteria; Acidobacteria; Holophagae; CA002	P	0.003	1.669	0.000
Bacteria; Acidobacteria; Holophagae; CA002	K	0.003	1.673	0.000
Bacteria; Acidobacteria; Holophagae; CA002	N	0.002	1.922	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	P:N	0.000	-3.736	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	P:K	0.001	-0.408	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	N:K	0.001	-0.174	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	N:P:K	0.000	0.916	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	P	0.003	1.669	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	K	0.003	1.673	0.000
Bacteria; Acidobacteria; Holophagae; CA002; f	N	0.002	1.922	0.000
Bacteria; Acidobacteria; Holophagae; Sva0725	P	0.025	1.933	0.000
Bacteria; Acidobacteria; Holophagae; Sva0725; f	P	0.025	1.933	0.000
Bacteria; Acidobacteria; Holophagae; TPD.58	N	0.007	1.104	0.000
Bacteria; Acidobacteria; Holophagae; TPD.58; f	N	0.007	1.104	0.000
Bacteria; Acidobacteria; Holophagae.Other	P:N	0.031	-5.180	0.000
Bacteria; Acidobacteria; Holophagae.Other	N	0.025	-0.469	0.000
Bacteria; Acidobacteria; RB25	N	0.008	0.650	0.001
Bacteria; Acidobacteria; RB25	K	0.007	0.719	0.001
Bacteria; Acidobacteria; RB25; o	N	0.008	0.650	0.001
Bacteria; Acidobacteria; RB25; o	K	0.007	0.719	0.001
Bacteria; Actinobacteria; Acidimicrobiia; Acidimicrobiales; Acidimicrobiaceae	K	0.041	-0.449	0.000

Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Catenulisporaceae	N	0.005	-4.213	0.000
Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Catenulisporaceae	P	0.006	-4.213	0.000
Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Catenulisporaceae	K	0.010	-4.213	0.000
Bacteria; Actinobacteria; Actinobacteria; Catenulisporales; Catenulisporaceae	P:N	0.001	-0.309	0.000
Bacteria; Actinobacteria; Actinobacteria; Corynebacteriales; Tsukamurellaceae	P	0.000	1.106	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales	K	0.003	-4.025	0.003
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Acidothermaceae	K	0.000	-4.507	0.004
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Cryptosporangiaceae	P:K	0.047	0.433	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Geodermatophilaceae	N:K	0.011	-0.904	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Geodermatophilaceae	N	0.041	1.062	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Nakamurellaceae	N	0.025	0.446	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Nakamurellaceae	N:P:K	0.046	1.228	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; Nakamurellaceae	K	0.005	1.852	0.000
Bacteria; Actinobacteria; Actinobacteria; Frankiales; uncultured	K	0.011	-3.102	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales	N	0.008	-1.277	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales	N:K	0.014	0.057	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales	K	0.029	0.301	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Beutenbergiaceae	N	0.001	0.916	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Beutenbergiaceae	K	0.000	1.269	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Bogoriellaceae	P	0.004	1.106	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Bogoriellaceae	N	0.002	1.178	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Intrasporangiaceae	P:N	0.036	1.112	0.000

Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Intrasporangiaceae	N	0.010	1.789	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Intrasporangiaceae	P	0.026	2.474	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Microbacteriaceae	N	0.021	-2.075	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Micrococcaceae	N	0.034	-1.402	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Micrococcaceae	P:K	0.006	0.118	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Micrococcaceae	K	0.033	1.799	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales; Micrococcaceae	P	0.018	2.224	0.000
Bacteria; Actinobacteria; Actinobacteria; Micrococcales.Other	N:K	0.046	0.751	0.000
Bacteria; Actinobacteria; Actinobacteria; PeM15	N	0.000	-4.275	0.001
Bacteria; Actinobacteria; Actinobacteria; PeM15	K	0.000	1.295	0.001
Bacteria; Actinobacteria; Actinobacteria; PeM15; f	N	0.000	-4.275	0.001
Bacteria; Actinobacteria; Actinobacteria; PeM15; f	K	0.000	1.295	0.001
Bacteria; Actinobacteria; Actinobacteria; Propionibacteriales	P:N	0.043	-2.162	0.001
Bacteria; Actinobacteria; Actinobacteria; Propionibacteriales	N	0.019	-0.233	0.001
Bacteria; Actinobacteria; Actinobacteria; Propionibacteriales; Nocardioidaceae	P:N	0.044	-2.162	0.001
Bacteria; Actinobacteria; Actinobacteria; Propionibacteriales; Nocardioidaceae	N	0.019	-0.233	0.001
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales	P:N	0.045	1.236	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae	P:N	0.030	-0.585	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae	N:K	0.032	-0.042	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae	P:K	0.035	0.737	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae	N	0.048	1.029	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Streptosporangiaceae	N:P:K	0.010	2.540	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae	P:K	0.049	-2.959	0.000
Bacteria; Actinobacteria; Actinobacteria; Streptosporangiales; Thermomonosporaceae	K	0.019	-2.500	0.000
Bacteria; Actinobacteria; Actinobacteria;	N	0.008	-2.158	0.000

Streptosporangiales; Thermomonosporaceae Bacteria; Actinobacteria; Actinobacteria;				
Streptosporangiales; Thermomonosporaceae Bacteria; Actinobacteria; Actinobacteria;	P	0.024	-1.087	0.000
Streptosporangiales; Thermomonosporaceae Bacteria; Actinobacteria; Actinobacteria;	N:K	0.026	-0.147	0.000
Streptosporangiales; Thermomonosporaceae Bacteria; Actinobacteria; Actinobacteria;	P:N	0.006	1.452	0.000
Streptosporangiales.Other	N	0.004	0.751	0.000
Bacteria; Actinobacteria; MB.A2.108	K	0.029	-0.075	0.000
Bacteria; Actinobacteria; MB.A2.108	N	0.027	-0.020	0.000
Bacteria; Actinobacteria; MB.A2.108	N:K	0.021	1.639	0.000
Bacteria; Actinobacteria; MB.A2.108; o	K	0.029	-0.075	0.000
Bacteria; Actinobacteria; MB.A2.108; o	N	0.027	-0.020	0.000
Bacteria; Actinobacteria; MB.A2.108; o	N:K	0.021	1.639	0.000
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae	N	0.026	0.559	0.001
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae	K	0.022	0.912	0.001
Bacteria; Actinobacteria; Thermoleophilia; Gaiellales; Gaiellaceae	N:K	0.007	1.382	0.001
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; 288.2	N	0.036	0.864	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae	P:K	0.009	-9.720	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae	K	0.000	-4.985	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae	P	0.007	-1.189	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Conexibacteraceae	P:N	0.022	0.756	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Elev.16S.1332	P:K	0.001	1.073	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Elev.16S.1332	K	0.000	2.338	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Elev.16S.1332	P	0.004	2.365	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Q3.6C1	P	0.002	-4.404	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Q3.6C1	K	0.004	-4.404	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; Q3.6C1	P:K	0.001	-2.003	0.000
Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales; YNPFFP1	K	0.011	-5.251	0.001

Bacteria; Actinobacteria; Thermoleophilia; Solirubrobacterales.Other	K	0.001	-3.346	0.000
Bacteria; Aquificae	P	0.000	1.106	0.000
Bacteria; Aquificae; Aquificae	P	0.000	1.106	0.000
Bacteria; Aquificae; Aquificae; Aquificales	P	0.000	1.106	0.000
Bacteria; Aquificae; Aquificae; Aquificales; Aquificaceae	P	0.000	1.106	0.000
Bacteria; Armatimonadetes; Chthonomonadetes; Chthonomonadales; Chthonomonadaceae	K	0.019	0.008	0.000
Bacteria; Bacteroidetes	N:P:K	0.050	0.722	0.006
Bacteria; Bacteroidetes	K	0.020	1.426	0.006
Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Marinilabiaceae	N	0.014	-2.828	0.000
Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Marinilabiaceae	K	0.022	-2.828	0.000
Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae	K	0.000	0.894	0.000
Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae	P	0.000	1.106	0.000
Bacteria; Bacteroidetes; Cytophagia	P:N	0.009	-1.840	0.002
Bacteria; Bacteroidetes; Cytophagia	N	0.029	-1.831	0.002
Bacteria; Bacteroidetes; Cytophagia	P	0.048	-0.457	0.002
Bacteria; Bacteroidetes; Cytophagia	K	0.041	1.310	0.002
Bacteria; Bacteroidetes; Cytophagia	N:P:K	0.022	1.446	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales	P:N	0.008	-1.840	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales	N	0.025	-1.831	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales	P	0.044	-0.469	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales	K	0.041	1.310	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales	N:P:K	0.021	1.446	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae	N	0.024	-1.826	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae	P:N	0.007	-1.826	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae	P	0.042	-0.449	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae	K	0.040	1.330	0.002
Bacteria; Bacteroidetes; Cytophagia; Cytophagales; Cytophagaceae	N:P:K	0.020	1.489	0.002
Bacteria; Bacteroidetes; Cytophagia;	N:K	0.048	0.874	0.000

Order_II_Incertae_Sedis				
Bacteria; Bacteroidetes; Cytophagia; Order_II_Incertae_Sedis; Rhodothermaceae	N:K	0.048	0.874	0.000
Bacteria; Bacteroidetes; Flavobacteria	N	0.029	-2.444	0.002
Bacteria; Bacteroidetes; Flavobacteria	P:K	0.045	0.502	0.002
Bacteria; Bacteroidetes; Flavobacteria	K	0.021	0.698	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales	N	0.029	-2.444	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales	P:K	0.045	0.502	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales	K	0.021	0.698	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	P:N	0.027	-4.807	0.000
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	N:K	0.036	-4.076	0.000
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	N:P:K	0.012	-1.949	0.000
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	P:K	0.024	-1.004	0.000
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae	N	0.015	-0.635	0.000
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae	P	0.015	-3.588	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae	N	0.026	-3.371	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae	P:K	0.042	0.555	0.002
Bacteria; Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae	K	0.014	0.624	0.002
Bacteria; Bacteroidetes; SB.5	N	0.005	1.251	0.000
Bacteria; Bacteroidetes; SB.5	P	0.007	1.586	0.000
Bacteria; Bacteroidetes; SB.5; o	N	0.005	1.251	0.000
Bacteria; Bacteroidetes; SB.5; o	P	0.007	1.586	0.000
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; AKYH767	P:N	0.016	-2.561	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; AKYH767	N	0.023	0.280	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; AKYH767	K	0.011	0.935	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; AKYH767	P	0.032	2.167	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; CWT_CU03.E12	P	0.048	-1.762	0.000
Bacteria; Bacteroidetes; Sphingobacteriia;	N	0.040	-0.008	0.000

Sphingobacteriales; CWT_CU03.E12				
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; env.OPS_17	N:K	0.031	-1.819	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; env.OPS_17	N	0.033	0.045	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; env.OPS_17	K	0.039	1.788	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; KD3.93	K	0.026	1.901	0.000
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; NS11.12_marine_group	K	0.003	2.756	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales; PHOS.HE51	K	0.019	1.298	0.001
Bacteria; Bacteroidetes; Sphingobacteriia; Sphingobacteriales.Other	P	0.031	-0.201	0.000
Bacteria; Bacteroidetes; VC2.1_Bac22	K	0.018	0.165	0.000
Bacteria; Bacteroidetes; VC2.1_Bac22; o	K	0.018	0.165	0.000
Bacteria; Bacteroidetes.Other	K	0.000	0.894	0.000
Bacteria; Candidate_division_OP11	K	0.001	-4.205	0.000
Bacteria; Candidate_division_OP11	P	0.009	-4.205	0.000
Bacteria; Candidate_division_OP11	N:K	0.002	-3.847	0.000
Bacteria; Candidate_division_OP11	N	0.012	0.260	0.000
Bacteria; Candidate_division_OP11	P:K	0.008	0.780	0.000
Bacteria; Candidate_division_OP11; c	K	0.001	-4.205	0.000
Bacteria; Candidate_division_OP11; c	P	0.009	-4.205	0.000
Bacteria; Candidate_division_OP11; c	N:K	0.002	-3.847	0.000
Bacteria; Candidate_division_OP11; c	N	0.012	0.260	0.000
Bacteria; Candidate_division_OP11; c	P:K	0.008	0.780	0.000
Bacteria; Candidate_division_SR1	K	0.012	2.053	0.000
Bacteria; Candidate_division_SR1; c	K	0.012	2.053	0.000
Bacteria; Candidate_division_WS3	K	0.006	-0.940	0.004
Bacteria; Candidate_division_WS3	N	0.005	0.027	0.004
Bacteria; Candidate_division_WS3	N:K	0.002	1.683	0.004
Bacteria; Candidate_division_WS3; c	K	0.006	-0.940	0.004
Bacteria; Candidate_division_WS3; c	N	0.005	0.027	0.004
Bacteria; Candidate_division_WS3; c	N:K	0.002	1.683	0.004
Bacteria; Chlamydiae; Chlamydiae; Chlamydiales.Other	P	0.000	1.106	0.000
Bacteria; Chloroflexi; Anaerolineae	P:K	0.043	1.343	0.001
Bacteria; Chloroflexi; Anaerolineae; Anaerolineales	P:K	0.043	1.343	0.001
Bacteria; Chloroflexi; Anaerolineae; Anaerolineales; Anaerolineaceae	P:K	0.043	1.343	0.001
Bacteria; Chloroflexi; Caldilineae	P:N	0.013	-0.318	0.001
Bacteria; Chloroflexi; Caldilineae	N:K	0.030	1.316	0.001
Bacteria; Chloroflexi; Caldilineae	N:P:K	0.013	1.774	0.001
Bacteria; Chloroflexi; Caldilineae	N	0.023	1.775	0.001

Bacteria; Chloroflexi; Caldilineae	P:K	0.037	1.960	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales	P:N	0.013	-0.318	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales	N:K	0.030	1.316	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales	N:P:K	0.013	1.774	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales	N	0.023	1.775	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales	P:K	0.037	1.960	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae	P:N	0.013	-0.318	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae	N:K	0.030	1.316	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae	N:P:K	0.013	1.774	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae	N	0.023	1.775	0.001
Bacteria; Chloroflexi; Caldilineae; Caldilineales; Caldilineaceae	P:K	0.037	1.960	0.001
Bacteria; Chloroflexi; Chloroflexi; Herpetosiphonales	N:K	0.041	-0.008	0.000
Bacteria; Chloroflexi; Chloroflexi; Herpetosiphonales; Herpetosiphonaceae	N:K	0.041	-0.008	0.000
Bacteria; Chloroflexi; KD4.96	P:N	0.019	-2.450	0.002
Bacteria; Chloroflexi; KD4.96	K	0.039	0.224	0.002
Bacteria; Chloroflexi; KD4.96	N	0.027	0.252	0.002
Bacteria; Chloroflexi; KD4.96	N:K	0.035	1.573	0.002
Bacteria; Chloroflexi; KD4.96; o	P:N	0.019	-2.450	0.002
Bacteria; Chloroflexi; KD4.96; o	K	0.039	0.224	0.002
Bacteria; Chloroflexi; KD4.96; o	N	0.027	0.252	0.002
Bacteria; Chloroflexi; KD4.96; o	N:K	0.035	1.573	0.002
Bacteria; Chloroflexi; Ktedonobacteria	K	0.026	-1.343	0.001
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9	K	0.000	-2.121	0.000
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9	P:K	0.041	-1.481	0.000
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9	P	0.045	2.030	0.000
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9; f	K	0.000	-2.121	0.000
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9; f	P:K	0.041	-1.481	0.000
Bacteria; Chloroflexi; Ktedonobacteria; JG30.KF.AS9; f	P	0.045	2.030	0.000
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales	K	0.019	-1.198	0.001
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; FCPS473	K	0.003	-2.828	0.000

Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; HSB_OF53.F07	K	0.000	-6.271	0.000
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae	N:P:K	0.031	-1.834	0.000
Bacteria; Chloroflexi; Ktedonobacteria; Ktedonobacterales; Ktedonobacteraceae	P:N	0.034	1.973	0.000
Bacteria; Chloroflexi; P2.11E	K	0.037	0.381	0.000
Bacteria; Chloroflexi; P2.11E	N:K	0.030	0.504	0.000
Bacteria; Chloroflexi; P2.11E	N:P:K	0.044	1.164	0.000
Bacteria; Chloroflexi; P2.11E; o	K	0.037	0.381	0.000
Bacteria; Chloroflexi; P2.11E; o	N:K	0.030	0.504	0.000
Bacteria; Chloroflexi; P2.11E; o	N:P:K	0.044	1.164	0.000
Bacteria; Chloroflexi; SAR202_clade	N:K	0.023	0.751	0.000
Bacteria; Chloroflexi; SAR202_clade	K	0.011	0.894	0.000
Bacteria; Chloroflexi; SAR202_clade; o	N:K	0.023	0.751	0.000
Bacteria; Chloroflexi; SAR202_clade; o	K	0.011	0.894	0.000
Bacteria; Chloroflexi; SHA.26	N	0.004	0.751	0.000
Bacteria; Chloroflexi; SHA.26; o	N	0.004	0.751	0.000
Bacteria; Chloroflexi; Thermomicrobia; AKYG1722	N	0.024	0.751	0.000
Bacteria; Chloroflexi; TK10	K	0.007	-3.324	0.002
Bacteria; Chloroflexi; TK10	N	0.028	-0.397	0.002
Bacteria; Chloroflexi; TK10	N:P:K	0.047	-0.333	0.002
Bacteria; Chloroflexi; TK10	P:N	0.025	1.499	0.002
Bacteria; Chloroflexi; TK10; o	K	0.007	-3.324	0.002
Bacteria; Chloroflexi; TK10; o	N	0.028	-0.397	0.002
Bacteria; Chloroflexi; TK10; o	N:P:K	0.047	-0.333	0.002
Bacteria; Chloroflexi; TK10; o	P:N	0.025	1.499	0.002
Bacteria; Cyanobacteria	N:P:K	0.045	-2.739	0.002
Bacteria; Cyanobacteria	P:N	0.036	1.280	0.002
Bacteria; Cyanobacteria; 4C0d.2	K	0.030	-2.828	0.000
Bacteria; Cyanobacteria; 4C0d.2; o	K	0.030	-2.828	0.000
Bacteria; Cyanobacteria; Cyanobacteria	P	0.012	-1.931	0.000
Bacteria; Cyanobacteria; Cyanobacteria	N	0.016	-0.576	0.000
Bacteria; Cyanobacteria; Cyanobacteria	P:N	0.003	0.277	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV	P	0.006	-4.216	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV	N	0.006	-2.676	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV	P:N	0.003	0.488	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV; FamilyI	N	0.002	-4.216	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV; FamilyI	P	0.002	-4.216	0.000
Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV; FamilyI	P:N	0.001	0.488	0.000

Bacteria; Cyanobacteria; Cyanobacteria; SubsectionIV; FamilyII	N	0.004	0.751	0.000
Bacteria; Cyanobacteria; MLE1.12	P	0.000	-7.230	0.001
Bacteria; Cyanobacteria; MLE1.12	N:P:K	0.000	-5.229	0.001
Bacteria; Cyanobacteria; MLE1.12	K	0.000	-3.606	0.001
Bacteria; Cyanobacteria; MLE1.12	P:K	0.000	-2.324	0.001
Bacteria; Cyanobacteria; MLE1.12	N	0.000	-1.384	0.001
Bacteria; Cyanobacteria; MLE1.12	N:K	0.000	-0.775	0.001
Bacteria; Cyanobacteria; MLE1.12	P:N	0.000	0.787	0.001
Bacteria; Cyanobacteria; MLE1.12; o	P	0.000	-7.230	0.001
Bacteria; Cyanobacteria; MLE1.12; o	N:P:K	0.000	-5.229	0.001
Bacteria; Cyanobacteria; MLE1.12; o	K	0.000	-3.606	0.001
Bacteria; Cyanobacteria; MLE1.12; o	P:K	0.000	-2.324	0.001
Bacteria; Cyanobacteria; MLE1.12; o	N	0.000	-1.384	0.001
Bacteria; Cyanobacteria; MLE1.12; o	N:K	0.000	-0.775	0.001
Bacteria; Cyanobacteria; MLE1.12; o	P:N	0.000	0.787	0.001
Bacteria; Cyanobacteria; WD272	P	0.016	-5.915	0.002
Bacteria; Cyanobacteria; WD272	K	0.001	-5.008	0.002
Bacteria; Cyanobacteria; WD272	N:P:K	0.006	-3.649	0.002
Bacteria; Cyanobacteria; WD272	P:K	0.021	-1.957	0.002
Bacteria; Cyanobacteria; WD272	N:K	0.018	-0.819	0.002
Bacteria; Cyanobacteria; WD272	N	0.002	-0.003	0.002
Bacteria; Cyanobacteria; WD272	P:N	0.002	2.408	0.002
Bacteria; Cyanobacteria; WD272; o	P	0.016	-5.915	0.002
Bacteria; Cyanobacteria; WD272; o	K	0.001	-5.008	0.002
Bacteria; Cyanobacteria; WD272; o	N:P:K	0.006	-3.649	0.002
Bacteria; Cyanobacteria; WD272; o	P:K	0.021	-1.957	0.002
Bacteria; Cyanobacteria; WD272; o	N:K	0.018	-0.819	0.002
Bacteria; Cyanobacteria; WD272; o	N	0.002	-0.003	0.002
Bacteria; Cyanobacteria; WD272; o	P:N	0.002	2.408	0.002
Bacteria; Deferribacteres; Deferribacteres; Deferribacterales; PAUC34f	P	0.000	1.106	0.000
Bacteria; Deinococcus.Thermus; Deinococci; KD3.62	P	0.003	1.106	0.000
Bacteria; Deinococcus.Thermus; Deinococci; KD3.62; f	P	0.003	1.106	0.000
Bacteria; Elusimicrobia; Elusimicrobia; Elusimicrobiales	N:K	0.023	1.260	0.000
Bacteria; Elusimicrobia; Elusimicrobia; Elusimicrobiales	K	0.002	1.319	0.000
Bacteria; Elusimicrobia; Elusimicrobia; Elusimicrobiales; Elusimicrobiaceae	N:K	0.023	1.260	0.000
Bacteria; Elusimicrobia; Elusimicrobia; Elusimicrobiales; Elusimicrobiaceae	K	0.002	1.319	0.000
Bacteria; Fibrobacteres; Fibrobacteria; KD2.123	P:K	0.029	1.106	0.000
Bacteria; Fibrobacteres; Fibrobacteria; KD2.123; f	P:K	0.029	1.106	0.000

Bacteria; Firmicutes; Bacilli; Bacillales; Family_XI_Incertae_Sedis	P	0.034	1.402	0.000
Bacteria; Firmicutes; Bacilli; Bacillales; Family_XII_Incertae_Sedis	N	0.000	1.099	0.000
Bacteria; Firmicutes; Bacilli; Bacillales; Pasteuriaceae	K	0.000	1.328	0.000
Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae	N:K	0.004	-2.192	0.000
Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae	N	0.003	0.847	0.000
Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae	K	0.007	1.296	0.000
Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae	N	0.006	0.751	0.000
Bacteria; Firmicutes; Bacilli; Lactobacillales; Carnobacteriaceae	P	0.001	1.106	0.000
Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae	N:K	0.043	1.016	0.000
Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae	N	0.048	-5.308	0.000
Bacteria; Firmicutes; Bacilli; Lactobacillales; Leuconostocaceae	K	0.024	1.184	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Christensenellaceae	K	0.036	-3.066	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	N	0.003	-3.662	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	K	0.006	-3.662	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	P	0.007	-3.662	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	N:P:K	0.011	-3.662	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	N:K	0.026	-3.045	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	P:N	0.012	-0.867	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Family_XIII_Incertae_Sedis	P:K	0.003	-0.037	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Gracilibacteraceae	N:K	0.003	0.751	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	N:P:K	0.008	-5.725	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	N	0.039	-1.521	0.000

Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	K	0.012	-0.868	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	P:K	0.010	-0.187	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	N:K	0.023	0.305	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	P:N	0.029	0.781	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae	P	0.050	1.057	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; OPB54	N	0.005	0.751	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; OPB54	N:K	0.001	1.027	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; OPB54	P:K	0.001	1.432	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; OPB54	P:N	0.001	1.622	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	N:P:K	0.003	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	K	0.014	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	N	0.017	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	P	0.031	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	P:N	0.009	-1.257	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	P:K	0.012	-0.594	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; Peptostreptococcaceae	N:K	0.003	0.391	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; uncultured	P	0.010	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; uncultured	K	0.013	-2.828	0.000
Bacteria; Firmicutes; Clostridia; Clostridiales; uncultured	P:K	0.005	-1.477	0.000
Bacteria; Firmicutes; Clostridia; Thermoanaerobacterales	K	0.024	-0.403	0.000
Bacteria; Firmicutes; Clostridia; Thermoanaerobacterales; Thermodesulfobiaceae	K	0.024	-0.403	0.000
Bacteria; Firmicutes; Erysipelotrichi	P:K	0.049	-0.051	0.000
Bacteria; Firmicutes; Erysipelotrichi;	P:K	0.049	-0.051	0.000

Erysipelotrichales Bacteria; Firmicutes; Erysipelotrichi; Erysipelotrichales; Erysipelotrichaceae	P:K	0.049	-0.051	0.000
Bacteria; Fusobacteria; Fusobacteria; BS1.0.74	K	0.031	-2.828	0.000
Bacteria; Fusobacteria; Fusobacteria; BS1.0.74; f	K	0.031	-2.828	0.000
Bacteria; GAL08	K	0.001	0.894	0.000
Bacteria; GAL08; c	K	0.001	0.894	0.000
Bacteria; Gemmatimonadetes; Gemmatimonadetes; AT425.EubC11_terrestrial_group	P	0.047	1.636	0.000
Bacteria; Gemmatimonadetes; Gemmatimonadetes; AT425.EubC11_terrestrial_group; f	P	0.047	1.636	0.000
Bacteria; HDB.SIOH1705	N	0.009	0.751	0.000
Bacteria; HDB.SIOH1705; c	N	0.009	0.751	0.000
Bacteria; Lentisphaerae; Lentisphaeria; c5LKS8	K	0.000	0.894	0.000
Bacteria; Lentisphaerae; Lentisphaeria; c5LKS8; f	K	0.000	0.894	0.000
Bacteria; MVP.21	N	0.023	1.095	0.000
Bacteria; MVP.21	P	0.015	1.106	0.000
Bacteria; MVP.21; c	N	0.023	1.095	0.000
Bacteria; MVP.21; c	P	0.015	1.106	0.000
Bacteria; Nitrospirae	N	0.030	0.512	0.009
Bacteria; Nitrospirae; Nitrospira	N	0.030	0.512	0.009
Bacteria; Nitrospirae; Nitrospira; Nitrospirales	N	0.030	0.512	0.009
Bacteria; Nitrospirae; Nitrospira; Nitrospirales; 0319.6A21	N	0.036	0.515	0.009
Bacteria; NPL.UPA2	P:N	0.009	-1.188	0.000
Bacteria; NPL.UPA2	P:K	0.024	0.591	0.000
Bacteria; NPL.UPA2	N	0.005	1.231	0.000
Bacteria; NPL.UPA2	K	0.023	1.299	0.000
Bacteria; NPL.UPA2	N:K	0.019	1.728	0.000
Bacteria; NPL.UPA2	P	0.048	2.121	0.000
Bacteria; NPL.UPA2	N:P:K	0.006	2.420	0.000
Bacteria; NPL.UPA2; c	P:N	0.009	-1.188	0.000
Bacteria; NPL.UPA2; c	P:K	0.024	0.591	0.000
Bacteria; NPL.UPA2; c	N	0.005	1.231	0.000
Bacteria; NPL.UPA2; c	K	0.023	1.299	0.000
Bacteria; NPL.UPA2; c	N:K	0.019	1.728	0.000
Bacteria; NPL.UPA2; c	P	0.048	2.121	0.000
Bacteria; NPL.UPA2; c	N:P:K	0.006	2.420	0.000
Bacteria; Planctomycetes; OM190	P:N	0.002	-3.486	0.002
Bacteria; Planctomycetes; OM190	P:K	0.002	-2.467	0.002
Bacteria; Planctomycetes; OM190	K	0.000	-1.037	0.002
Bacteria; Planctomycetes; OM190	N	0.000	0.527	0.002
Bacteria; Planctomycetes; OM190	N:K	0.000	1.017	0.002
Bacteria; Planctomycetes; OM190	P	0.010	1.657	0.002

Bacteria; Planctomycetes; OM190	N:P:K	0.001	2.179	0.002
Bacteria; Planctomycetes; OM190; o	P:N	0.002	-3.486	0.002
Bacteria; Planctomycetes; OM190; o	P:K	0.002	-2.467	0.002
Bacteria; Planctomycetes; OM190; o	K	0.000	-1.037	0.002
Bacteria; Planctomycetes; OM190; o	N	0.000	0.527	0.002
Bacteria; Planctomycetes; OM190; o	N:K	0.000	1.017	0.002
Bacteria; Planctomycetes; OM190; o	P	0.010	1.657	0.002
Bacteria; Planctomycetes; OM190; o	N:P:K	0.001	2.179	0.002
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	N:K	0.011	-3.088	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	P:N	0.003	-2.321	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	K	0.020	-0.550	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	N:P:K	0.003	-0.249	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	P:K	0.010	0.048	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	N	0.011	0.922	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage	P	0.007	1.218	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	N:K	0.011	-3.088	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	P:N	0.003	-2.321	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	K	0.020	-0.550	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	N:P:K	0.003	-0.249	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	P:K	0.010	0.048	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	N	0.011	0.922	0.000
Bacteria; Planctomycetes; Phycisphaerae; Pla1_lineage; f	P	0.007	1.218	0.000
Bacteria; Planctomycetes; Phycisphaerae; S.70	P	0.021	-1.613	0.000
Bacteria; Planctomycetes; Phycisphaerae; S.70; f	P	0.021	-1.613	0.000
Bacteria; Planctomycetes; Pla3_lineage	K	0.016	0.402	0.000
Bacteria; Planctomycetes; Pla3_lineage	N	0.006	1.388	0.000
Bacteria; Planctomycetes; Pla3_lineage	N:K	0.015	1.721	0.000
Bacteria; Planctomycetes; Pla3_lineage; o	K	0.016	0.402	0.000
Bacteria; Planctomycetes; Pla3_lineage; o	N	0.006	1.388	0.000
Bacteria; Planctomycetes; Pla3_lineage; o	N:K	0.015	1.721	0.000
Bacteria; Planctomycetes; vadinHA49	P:K	0.040	-3.634	0.000

Bacteria; Planctomycetes; vadinHA49; o	P:K	0.040	-3.634	0.000
Bacteria; Planctomycetes.Other	N	0.009	0.751	0.000
Bacteria; Proteobacteria	N	0.013	-0.144	0.005
Bacteria; Proteobacteria	N:K	0.005	-0.093	0.005
Bacteria; Proteobacteria	K	0.016	1.506	0.005
Bacteria; Proteobacteria; Alphaproteobacteria	N:K	0.007	-0.332	0.007
Bacteria; Proteobacteria; Alphaproteobacteria	N	0.007	0.432	0.007
Bacteria; Proteobacteria; Alphaproteobacteria	K	0.033	1.542	0.007
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales	N:K	0.001	0.122	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales	N	0.001	1.190	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales	K	0.005	2.272	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	P	0.018	-3.178	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	N:P:K	0.004	-2.873	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	N	0.002	-1.073	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	N:K	0.002	-0.950	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	K	0.011	0.390	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	P:N	0.007	0.487	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae	P:K	0.011	0.774	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae	N:K	0.006	0.580	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae	N	0.003	1.915	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; Caulobacterales; Hyphomonadaceae	K	0.022	2.432	0.004
Bacteria; Proteobacteria; Alphaproteobacteria; MNG3	N:K	0.033	-2.481	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; MNG3; f	N:K	0.033	-2.481	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Parvularculales	N:P:K	0.041	-6.502	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Parvularculales; Parvularculaceae	N:P:K	0.041	-6.502	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; A0839	N:K	0.041	0.091	0.001
Bacteria; Proteobacteria; Alphaproteobacteria;	K	0.030	1.718	0.002

Rhizobiales; A0839				
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; alphaI_cluster	K	0.001	-5.637	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; alphaI_cluster	N:P:K	0.026	-4.857	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; alphaI_cluster	P:K	0.050	-3.268	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; alphaI_cluster	P:N	0.029	1.689	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Bartonellaceae	P	0.000	1.106	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; DUNssu044	N	0.030	1.543	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; DUNssu044	P:K	0.020	2.651	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; DUNssu371	N	0.000	1.014	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; F0723	N:K	0.003	-0.437	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; F0723	K	0.012	0.747	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; F0723	N	0.010	1.288	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Family_Incertae_Sedis	N:P:K	0.033	-1.579	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Family_Incertae_Sedis	K	0.011	-1.104	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Family_Incertae_Sedis	P:K	0.019	-0.547	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Rhizobiaceae	N	0.005	0.120	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	P	0.001	-4.781	0.003
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	N:P:K	0.006	-3.279	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	P:K	0.018	-2.993	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	N:K	0.015	-1.716	0.002
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	N	0.003	-1.583	0.003
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales	K	0.004	-1.107	0.003
Bacteria; Proteobacteria; Alphaproteobacteria;	P:N	0.000	3.054	0.003

Rhodospirillales				
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Acetobacteraceae	K	0.007	-1.791	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; DA111	N:P:K	0.043	-4.588	0.005
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; DA111	K	0.000	-4.016	0.006
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; DA111	P	0.041	-3.174	0.005
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; DA111	N	0.045	-1.266	0.005
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; DA111	P:N	0.008	1.031	0.005
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; f	N	0.004	0.751	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Family_Incertae_Sedis	P	0.007	-5.323	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; Family_Incertae_Sedis	K	0.018	-1.052	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; JG37.AG.20	K	0.001	-1.533	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rhodospirillales; JG37.AG.20	P:N	0.024	1.517	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	N:P:K	0.002	-2.825	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	P	0.001	-2.400	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	N	0.000	-1.436	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	N:K	0.000	-0.912	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	P:K	0.003	-0.172	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	K	0.001	1.165	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales	P:N	0.001	3.290	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Candidatus_Odyssella	N:P:K	0.036	-4.351	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Candidatus_Odyssella	N:K	0.014	-0.456	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Candidatus_Odyssella	N	0.029	0.201	0.000
Bacteria; Proteobacteria; Alphaproteobacteria;	P:N	0.036	1.161	0.000

Rickettsiales; EF100.94H03				
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; EF100.94H03	N	0.006	1.431	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; EF100.94H03	N:K	0.011	1.444	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; f	N	0.007	0.751	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; f	N:K	0.032	1.588	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; f	K	0.001	1.665	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Family_Incertae_Sedis	P:K	0.018	-2.968	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Family_Incertae_Sedis	K	0.012	0.478	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Holosporaceae	N	0.049	1.024	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; RB446	P:K	0.012	0.894	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; RB446	K	0.014	1.237	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; RB446	N:K	0.028	1.249	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; RB446	P:N	0.027	1.254	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; RB446	N:P:K	0.008	2.265	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	P	0.001	-3.802	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	N:P:K	0.002	-2.771	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	N	0.000	-2.130	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	N:K	0.001	-1.041	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	P:K	0.004	-0.363	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	K	0.004	1.144	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; SM2D12	P:N	0.000	3.060	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales	K	0.005	2.271	0.006
Bacteria; Proteobacteria; Alphaproteobacteria;	N	0.005	0.751	0.000

Sphingomonadales; DSSF69				
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	P:N	0.017	-3.236	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	P	0.049	0.302	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	N:K	0.004	0.423	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	N:P:K	0.011	0.627	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	N	0.006	1.026	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	P:K	0.040	1.642	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; GOBB3.C201	K	0.001	2.108	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; SD04E11	N	0.001	0.751	0.000
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae	K	0.003	2.188	0.005
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales.Other	P:N	0.011	-2.041	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales.Other	P	0.002	0.141	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales.Other	N	0.011	1.935	0.001
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales.Other	K	0.017	3.231	0.001
Bacteria; Proteobacteria; Alphaproteobacteria.Other	N:P:K	0.013	-3.051	0.000
Bacteria; Proteobacteria; Alphaproteobacteria.Other	P:K	0.003	-2.935	0.000
Bacteria; Proteobacteria; Alphaproteobacteria.Other	K	0.008	-2.473	0.000
Bacteria; Proteobacteria; Alphaproteobacteria.Other	P	0.015	-1.337	0.000
Bacteria; Proteobacteria; Alphaproteobacteria.Other	N:K	0.045	-0.215	0.000
Bacteria; Proteobacteria; Alphaproteobacteria.Other	P:N	0.040	1.428	0.000
Bacteria; Proteobacteria; Betaproteobacteria; B1.7BS	K	0.035	-2.629	0.001
Bacteria; Proteobacteria; Betaproteobacteria; B1.7BS	N	0.033	0.054	0.001
Bacteria; Proteobacteria; Betaproteobacteria;	N:K	0.020	0.506	0.001

B1.7BS Bacteria; Proteobacteria; Betaproteobacteria; B1.7BS; f	K	0.035	-2.629	0.001
Bacteria; Proteobacteria; Betaproteobacteria; B1.7BS; f	N	0.033	0.054	0.001
Bacteria; Proteobacteria; Betaproteobacteria; B1.7BS; f	N:K	0.020	0.506	0.001
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae	P:K	0.045	0.539	0.000
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae	N	0.007	1.269	0.000
Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Oxalobacteraceae	N:P:K	0.019	-2.938	0.001
Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales	P	0.029	-1.508	0.001
Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales	K	0.001	1.083	0.001
Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae	P	0.029	-1.508	0.001
Bacteria; Proteobacteria; Betaproteobacteria; Rhodocyclales; Rhodocyclaceae	K	0.001	1.083	0.001
Bacteria; Proteobacteria; Betaproteobacteria; SC.I.84	N	0.037	0.448	0.001
Bacteria; Proteobacteria; Betaproteobacteria; SC.I.84; f	N	0.037	0.448	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfarculales	K	0.000	1.197	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfarculales; Desulfarculaceae	K	0.000	1.197	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales	K	0.013	-1.596	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales	N	0.011	0.211	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales	N:K	0.009	0.805	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae	K	0.013	-1.596	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae	N	0.011	0.211	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfobacterales; Nitrospinaceae	N:K	0.010	0.788	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales	P:N	0.003	-3.492	0.000
Bacteria; Proteobacteria; Deltaproteobacteria;	P	0.014	-0.513	0.000

Desulfuromonadales				
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales	N	0.011	0.176	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae	P	0.040	-4.608	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae	K	0.015	-3.831	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae	P:N	0.018	-3.019	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; Geobacteraceae	P:K	0.028	0.549	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR.WP33.58	P:N	0.005	-1.928	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR.WP33.58	P	0.025	0.902	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR.WP33.58	N	0.000	1.095	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales; GR.WP33.58	N:K	0.037	2.618	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	N:P:K	0.002	-4.202	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	P	0.009	-4.202	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	K	0.010	-4.202	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	N	0.010	-2.479	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	P:N	0.001	-0.851	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	N:K	0.007	0.427	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Desulfuromonadales.Other	P:K	0.006	0.643	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; GR.WP33.30	N	0.044	0.173	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; GR.WP33.30; f	N	0.044	0.173	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; *Myxococcales*	N:P:K	0.023	-0.966	0.004
Bacteria; Proteobacteria; Deltaproteobacteria; *Myxococcales*; Cystobacteraceae	P	0.008	-5.214	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae	N:P:K	0.000	-4.353	0.001
Bacteria; Proteobacteria; Deltaproteobacteria;	K	0.006	-2.946	0.001

Myxococcales; Cystobacteraceae				
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae	N	0.004	-0.617	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae	P:K	0.001	-0.141	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae	N:K	0.001	0.152	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacteraceae	P:N	0.001	2.068	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	P	0.001	-4.618	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	K	0.008	-2.045	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	N:P:K	0.000	-2.042	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	N	0.002	-1.783	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	P:K	0.002	-1.405	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	N:K	0.002	-0.193	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Cystobacterineae	P:N	0.000	0.737	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Elev.16S.1158	N	0.038	-3.221	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Elev.16S.1158	N:K	0.022	-0.699	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	P	0.037	-2.017	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	N:P:K	0.002	-0.654	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	K	0.043	-0.575	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	N	0.018	0.049	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	P:K	0.007	0.379	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	N:K	0.010	1.261	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Haliangiaceae	P:N	0.009	2.596	0.002
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Nannocystaceae	P:N	0.037	-2.728	0.001
Bacteria; Proteobacteria; Deltaproteobacteria;	N:P:K	0.028	0.465	0.001

Myxococcales; Nannocystaceae				
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Nannocystineae	P:N	0.023	-3.975	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Nannocystineae	N	0.001	-1.485	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Nannocystineae	K	0.007	1.532	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	N:P:K	0.000	-3.218	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	P	0.000	-3.002	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	K	0.000	-1.960	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	N	0.000	-1.023	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	P:K	0.000	-0.820	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	N:K	0.000	0.783	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Myxococcales; Polyangiaceae	P:N	0.000	5.006	0.001
Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophobacteraceae	N:P:K	0.031	-2.828	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophobacteraceae	N:K	0.044	-2.157	0.000
Bacteria; Proteobacteria; Deltaproteobacteria; Syntrophobacterales; Syntrophobacteraceae	P:K	0.041	0.505	0.000
Bacteria; Proteobacteria; Epsilonproteobacteria	N	0.003	-0.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria	P	0.007	0.290	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria	K	0.003	1.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales	N	0.003	-0.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales	P	0.007	0.290	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales	K	0.003	1.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae	P	0.006	-2.828	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae	N	0.003	-0.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae	K	0.002	1.344	0.001
Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae	P	0.000	1.106	0.000
Bacteria; Proteobacteria; Gammaproteobacteria;	P	0.011	-4.216	0.000

BD72BR169 Bacteria; Proteobacteria; Gammaproteobacteria; BD72BR169; f	P	0.011	-4.216	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae	K	0.024	-0.496	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae	N	0.006	0.141	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae	P:K	0.013	0.200	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Chromatiales; Ectothiorhodospiraceae	P	0.002	0.627	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; EC3	P	0.010	-2.142	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; EC3; f	P	0.010	-2.142	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales	P	0.028	0.523	0.003
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae	P	0.028	0.523	0.003
Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales	N	0.041	-1.245	0.005
Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales	K	0.027	0.895	0.006
Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae	N	0.047	-1.108	0.006
Bacteria; Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae	K	0.025	0.892	0.006
Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales	P:K	0.045	-0.992	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales	N:P:K	0.030	1.142	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales; f	N	0.004	0.751	0.000
Bacteria; Proteobacteria; Gammaproteobacteria; Oceanospirillales; oc58	K	0.018	1.233	0.000
Bacteria; Proteobacteria; Gammaproteobacteria.Other	P:K	0.048	-0.257	0.000
Bacteria; Proteobacteria; JTB23	P:N	0.044	-2.828	0.000
Bacteria; Proteobacteria; JTB23; o	P:N	0.044	-2.828	0.000
Bacteria; Proteobacteria; SPOTSOCT00m83	P	0.036	-0.082	0.000
Bacteria; Proteobacteria; SPOTSOCT00m83; o	P	0.036	-0.082	0.000
Bacteria; Proteobacteria.Other	N:K	0.003	0.836	0.000
Bacteria; Proteobacteria.Other	N	0.005	2.069	0.000
Bacteria; Proteobacteria.Other	K	0.001	2.888	0.000
Bacteria; SM2F11	N:K	0.007	-2.233	0.000

Bacteria; SM2F11	N	0.009	0.335	0.000
Bacteria; SM2F11	K	0.012	0.572	0.000
Bacteria; SM2F11; c	N:K	0.007	-2.233	0.000
Bacteria; SM2F11; c	N	0.009	0.335	0.000
Bacteria; SM2F11; c	K	0.012	0.572	0.000
Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Leptospiraceae	N	0.043	-2.746	0.000
Bacteria; TA06	N:P:K	0.032	-2.828	0.000
Bacteria; TA06; c	N:P:K	0.032	-2.828	0.000
Bacteria; Tenericutes; Mollicutes; RF9	K	0.000	0.894	0.000
Bacteria; Tenericutes; Mollicutes; RF9; f	K	0.000	0.894	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group	N	0.015	-2.248	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group	P	0.041	-1.789	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group	N:P:K	0.024	-0.151	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group	P:N	0.011	3.540	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group; o	N	0.019	-2.227	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group; o	N:P:K	0.029	-0.117	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group; o	P:N	0.014	3.465	0.003
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	P	0.001	-1.692	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	N:P:K	0.001	-1.604	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	K	0.003	-1.487	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	N	0.002	-1.267	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	P:K	0.002	-0.556	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	N:K	0.004	0.609	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera	P:N	0.000	2.789	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	P	0.001	-1.692	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	N:P:K	0.001	-1.604	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	K	0.003	-1.487	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	N	0.002	-1.267	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	P:K	0.002	-0.556	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group; Pedosphaera; f	N:K	0.004	0.609	0.000
Bacteria; Verrucomicrobia; OPB35_soil_group;	P:N	0.000	2.789	0.000

Pedosphaera; f				
Bacteria; Verrucomicrobia; Opitutae; vadinHA64	P	0.000	1.660	0.000
Bacteria; Verrucomicrobia; Opitutae; vadinHA64; f	P	0.000	1.660	0.000
Bacteria; Verrucomicrobia; S.BQ2.57_soil_group	N	0.032	2.799	0.000
Bacteria; Verrucomicrobia; S.BQ2.57_soil_group; o	N	0.032	2.799	0.000
Bacteria; Verrucomicrobia; Spartobacteria	K	0.044	-3.955	0.007
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales	K	0.044	-3.955	0.007
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; DA101_soil_group	N	0.037	-4.076	0.009
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; DA101_soil_group	K	0.019	-3.857	0.009
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; DA101_soil_group	N:K	0.041	-2.375	0.008
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; FukuN18_freshwater_group	K	0.012	1.106	0.000
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales; Xiphinematobacteraceae	K	0.001	-2.892	0.002
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales.Other	P:N	0.003	1.254	0.000
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales.Other	N	0.002	1.607	0.000
Bacteria; Verrucomicrobia; Spartobacteria; Chthoniobacterales.Other	P	0.006	2.461	0.000
Bacteria; Verrucomicrobia; Verrucomicrobiae	P:N	0.003	-3.768	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	N:K	0.003	-0.679	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	N	0.014	0.233	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	P:K	0.002	0.446	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	P	0.017	0.578	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	N:P:K	0.001	1.289	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae	K	0.002	2.268	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	P:N	0.003	-3.768	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	N:K	0.003	-0.679	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	N	0.014	0.233	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	P:K	0.002	0.446	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	P	0.017	0.578	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales	N:P:K	0.001	1.289	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae;	K	0.002	2.268	0.001

Verrucomicrobiales				
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	P:N	0.003	-3.904	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	N:K	0.003	-0.682	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	N	0.013	0.233	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	P:K	0.002	0.446	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	P	0.015	0.578	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	N:P:K	0.001	1.289	0.001
Bacteria; Verrucomicrobia; Verrucomicrobiae; Verrucomicrobiales; Verrucomicrobiaceae	K	0.002	2.268	0.001
Bacteria; WCHB1.60	P	0.030	-0.403	0.000
Bacteria; WCHB1.60	N	0.029	0.403	0.000
Bacteria; WCHB1.60	N:K	0.009	0.650	0.000
Bacteria; WCHB1.60	K	0.042	0.717	0.000
Bacteria; WCHB1.60	N:P:K	0.031	1.043	0.000
Bacteria; WCHB1.60; c	P	0.030	-0.403	0.000
Bacteria; WCHB1.60; c	N	0.029	0.403	0.000
Bacteria; WCHB1.60; c	N:K	0.009	0.650	0.000
Bacteria; WCHB1.60; c	K	0.042	0.717	0.000
Bacteria; WCHB1.60; c	N:P:K	0.031	1.043	0.000
Bacteria.Other	P:N	0.042	-3.733	0.000
Bacteria.Other	P:K	0.008	-1.838	0.000
Bacteria.Other	N:K	0.015	-1.341	0.000
Bacteria.Other	P	0.019	-1.266	0.000
Bacteria.Other	K	0.005	-1.159	0.000
Bacteria.Other	N	0.021	-1.109	0.000
Bacteria.Other	N:P:K	0.016	2.670	0.000
Eukaryota; Stramenopiles; Bicosoecida; Bicosoecidae	N	0.006	0.751	0.000
Eukaryota; Stramenopiles; Bicosoecida; Bicosoecidae; Bicosoeca	N	0.006	0.751	0.000
Eukaryota; Stramenopiles; MAST.12	P:N	0.033	-0.931	0.000

Supplementary Information for Chapter 6. Effect of rodents on soil bacterial communities in the Chihuahuan desert

Table 5. Sample locations and barcodes used for 16S rRNA gene community analysis.

SampleID	LinkerPrimerSequence	Plot	Treatment	Corner
1.A	TCCCTTGTCTCCCCGTAAAACGACGGCCAG	1	Control	SW
1.B	ACGAGACTGATTCCGTAAAACGACGGCCAG	1	Control	SE
1.C	GCTGTACGGATTCCGTAAAACGACGGCCAG	1	Control	NE
1.D	ATCACCAGGTGTCCGTAAAACGACGGCCAG	1	Control	SW
1.E	TGGTCAACGATACCGTAAAACGACGGCCAG	1	Control	SE
1.F	ATCGCACAGTAACCGTAAAACGACGGCCAG	1	Control	NE
2.A	GTCGTGTAGCCTCCGTAAAACGACGGCCAG	2	Control	SW
2.B	AGCGGAGGTTAGCCGTAAAACGACGGCCAG	2	Control	SE
2.C	ATCCTTTGGTTCCCGTAAAACGACGGCCAG	2	Control	NE
2.D	TACAGCGCATACCCGTAAAACGACGGCCAG	2	Control	SW
2.E	ACCGGTATGTACCCGTAAAACGACGGCCAG	2	Control	SE
2.F	AATTGTGTCGGACCGTAAAACGACGGCCAG	2	Control	NE
3.A	TGCATACACTGGCCGTAAAACGACGGCCAG	3	KRatExclosure	SW
3.B	AGTCGAACGAGGCCGTAAAACGACGGCCAG	3	KRatExclosure	SE
3.C	ACCAGTGACTCACCGTAAAACGACGGCCAG	3	KRatExclosure	NE
3.D	GAATACCAAGTCCCGTAAAACGACGGCCAG	3	KRatExclosure	SW
3.E	GTAGATCGTGTACCGTAAAACGACGGCCAG	3	KRatExclosure	SE
3.F	TAACGTGTGTGCCCGTAAAACGACGGCCAG	3	KRatExclosure	NE
4.A	CATTATGGCGTGCCGTAAAACGACGGCCAG	4	Control	SW
4.B	CCAATACGCCTGCCGTAAAACGACGGCCAG	4	Control	SE
4.C	GATCTGCGATCCCCGTAAAACGACGGCCAG	4	Control	NE
4.D	CAGCTCATCAGCCCGTAAAACGACGGCCAG	4	Control	SW
4.E	CAAACAACAGCTCCGTAAAACGACGGCCAG	4	Control	SE
4.F	GCAACACCATCCCCGTAAAACGACGGCCAG	4	Control	NE
5.A	GCGATATATCGCCCGTAAAACGACGGCCAG	5	AllRodentExclosure	SW
5.B	CGAGCAATCCTACCGTAAAACGACGGCCAG	5	AllRodentExclosure	SE
5.C	AGTCGTGCACATCCGTAAAACGACGGCCAG	5	AllRodentExclosure	NE
5.D	GTATCTGCGCGTCCGTAAAACGACGGCCAG	5	AllRodentExclosure	SW
5.E	CGAGGGAAAGTCCCGTAAAACGACGGCCAG	5	AllRodentExclosure	SE
5.F	CAAATTCGGGATCCGTAAAACGACGGCCAG	5	AllRodentExclosure	NE
6.A	AGATTGACCAACCCGTAAAACGACGGCCAG	6	KRatExclosure	SW
6.B	AGTTACGAGCTACCGTAAAACGACGGCCAG	6	KRatExclosure	SE
6.C	GCATATGCACTGCCGTAAAACGACGGCCAG	6	KRatExclosure	NE
6.D	CAACTCCCGTGACCGTAAAACGACGGCCAG	6	KRatExclosure	SW
6.E	TTGCGTTAGCAGCCGTAAAACGACGGCCAG	6	KRatExclosure	SE
6.F	TACGAGCCCTAACCGTAAAACGACGGCCAG	6	KRatExclosure	NE
7.A	CACTACGCTAGACCGTAAAACGACGGCCAG	7	AllRodentExclosure	SW
7.B	TGCAGTCCTCGACCGTAAAACGACGGCCAG	7	AllRodentExclosure	SE
7.C	ACCATAGCTCCGCCGTAAAACGACGGCCAG	7	AllRodentExclosure	NE
7.D	TCGACATCTCTTCCGTAAAACGACGGCCAG	7	AllRodentExclosure	SW
7.E	GAACACTTTGGACCGTAAAACGACGGCCAG	7	AllRodentExclosure	SE
7.F	GAGCCATCTGTACCGTAAAACGACGGCCAG	7	AllRodentExclosure	NE

8.A	TTGGGTACACGTCCGTAAAACGACGGCCAG	8	Control	SW
8.B	AAGGCGCTCCTTCCGTAAAACGACGGCCAG	8	Control	SE
8.C	TAATACGGATCGCCGTAAAACGACGGCCAG	8	Control	NE
8.D	TCGGAATTAGACCCGTAAAACGACGGCCAG	8	Control	SW
8.E	TGTGAATTCGGACCGTAAAACGACGGCCAG	8	Control	SE
8.F	CATTCGTGGCGTCCGTAAAACGACGGCCAG	8	Control	NE
9.A	TACTACGTGGCCCCGTAAAACGACGGCCAG	9	Control	SW
9.B	GGCCAGTTCCTACCGTAAAACGACGGCCAG	9	Control	SE
9.C	GATGTTCGCTAGCCGTAAAACGACGGCCAG	9	Control	NE
9.D	CTATCTCCTGTCCCGTAAAACGACGGCCAG	9	Control	SW
9.E	ACTCACAGGAATCCGTAAAACGACGGCCAG	9	Control	SE
9.F	ATGATGAGCCTCCCGTAAAACGACGGCCAG	9	Control	NE
10.A	GTCGACAGAGGACCGTAAAACGACGGCCAG	10	AllRodentExclosure	SW
10.B	TGTCGCAAATAGCCGTAAAACGACGGCCAG	10	AllRodentExclosure	SE
10.C	CATCCCTCTACTCCGTAAAACGACGGCCAG	10	AllRodentExclosure	NE
10.D	TATACCGCTGCGCCGTAAAACGACGGCCAG	10	AllRodentExclosure	SW
10.E	AGTTGAGGCATTCCGTAAAACGACGGCCAG	10	AllRodentExclosure	SE
10.F	ACAATAGACACCCCGTAAAACGACGGCCAG	10	AllRodentExclosure	NE
11.A	CGGTCAATTGACCCGTAAAACGACGGCCAG	11	Control	SW
11.B	GTGGAGTCTCATCCGTAAAACGACGGCCAG	11	Control	SE
11.C	GCTCGAAGATTCCCGTAAAACGACGGCCAG	11	Control	NE
11.D	AGGCTTACGTGTCCGTAAAACGACGGCCAG	11	Control	SW
11.E	TCTCTACCACTCCCGTAAAACGACGGCCAG	11	Control	SE
11.F	ACTTCCAACTTCCCGTAAAACGACGGCCAG	11	Control	NE
12.A	CTCACCTAGGAACCGTAAAACGACGGCCAG	12	Control	SW
12.B	GTGTTGTCGTGCCCGTAAAACGACGGCCAG	12	Control	SE
12.C	CCACAGATCGATCCGTAAAACGACGGCCAG	12	Control	NE
12.D	TATCGACACAAGCCGTAAAACGACGGCCAG	12	Control	SW
12.E	GATTCCGGCTCACCGTAAAACGACGGCCAG	12	Control	SE
12.F	CGTAATTGCCGCCCGTAAAACGACGGCCAG	12	Control	NE
13.A	GGTGACTAGTTCCCGTAAAACGACGGCCAG	13	KRatExclosure	SW
13.B	ATGGGTTCCGTCCCGTAAAACGACGGCCAG	13	KRatExclosure	SE
13.C	TAGGCATGCTTGCCGTAAAACGACGGCCAG	13	KRatExclosure	NE
13.D	AACTAGTTCAGGCCGTAAAACGACGGCCAG	13	KRatExclosure	SW
13.E	ATTCTGCCGAAGCCGTAAAACGACGGCCAG	13	KRatExclosure	SE
13.F	AGCATGTCCCGTCCGTAAAACGACGGCCAG	13	KRatExclosure	NE
14.A	GTACGATATGACCCGTAAAACGACGGCCAG	14	Control	SW
14.B	GTGGTGGTTTCCCCGTAAAACGACGGCCAG	14	Control	SE
14.C	TAGTATGCGCAACCGTAAAACGACGGCCAG	14	Control	NE
14.D	TGCGCTGAATGTCCGTAAAACGACGGCCAG	14	Control	SW
14.E	ATGGCTGTCAGTCCGTAAAACGACGGCCAG	14	Control	SE
14.F	GTTCTCTTCTCGCCGTAAAACGACGGCCAG	14	Control	NE
15.A	CGTAAGATGCCTCCGTAAAACGACGGCCAG	15	KRatExclosure	SW
15.B	GCGTTCTAGCTGCCGTAAAACGACGGCCAG	15	KRatExclosure	SE
15.C	GTTGTTCTGGGACCGTAAAACGACGGCCAG	15	KRatExclosure	NE
15.D	GGACTTCCAGCTCCGTAAAACGACGGCCAG	15	KRatExclosure	SW
15.E	CTCACAACCGTGCCGTAAAACGACGGCCAG	15	KRatExclosure	SE

15.F	CTGCTATTCCTCCCGTAAAACGACGGCCAG	15	KRatExclosure	NE
16.A	TACCGCTTCTTCCCGTAAAACGACGGCCAG	16	AllRodentExclosure	SW
16.B	TGTGCGATAACACCGTAAAACGACGGCCAG	16	AllRodentExclosure	SE
16.C	GATTATCGACGACCGTAAAACGACGGCCAG	16	AllRodentExclosure	NE
16.D	GCCTAGCCCAATCCGTAAAACGACGGCCAG	16	AllRodentExclosure	SW
16.E	GATGTATGTGGTCCGTAAAACGACGGCCAG	16	AllRodentExclosure	SE
16.F	ACTCCTTGTGTTCCGTAAAACGACGGCCAG	16	AllRodentExclosure	NE
17.A	GTCACGGACATTCCGTAAAACGACGGCCAG	17	Control	SW
17.B	GCGAGCGAAGTACCGTAAAACGACGGCCAG	17	Control	SE
17.C	ATCTACCGAAGCCCGTAAAACGACGGCCAG	17	Control	NE
17.D	ACTTGGTGTAAGCCGTAAAACGACGGCCAG	17	Control	SW
17.E	TCTTGGAGGTCACCGTAAAACGACGGCCAG	17	Control	SE
17.F	TCACCTCCTTGTCCGTAAAACGACGGCCAG	17	Control	NE
18.A	GCACACCTGATACCGTAAAACGACGGCCAG	18	KRatExclosure	SW
18.B	GCGACAATTACACCGTAAAACGACGGCCAG	18	KRatExclosure	SE
18.C	TCATGCTCCATTCCGTAAAACGACGGCCAG	18	KRatExclosure	NE
18.D	AGCTGTCAAGCTCCGTAAAACGACGGCCAG	18	KRatExclosure	SW
18.E	GAGAGCAACAGACCGTAAAACGACGGCCAG	18	KRatExclosure	SE
18.F	TACTCGGGAACTCCGTAAAACGACGGCCAG	18	KRatExclosure	NE
19.A	CGTGCTTAGGCTCCGTAAAACGACGGCCAG	19	KRatExclosure	SW
19.B	TACCGAAGGTATCCGTAAAACGACGGCCAG	19	KRatExclosure	SE
19.C	CACTCATCATTCCCGTAAAACGACGGCCAG	19	KRatExclosure	NE
19.D	GTATTTCGGACGCCGTAAAACGACGGCCAG	19	KRatExclosure	SW
19.E	TATCTATCCTGCCCGTAAAACGACGGCCAG	19	KRatExclosure	SE
19.F	TTGCCAAGAGTCCCGTAAAACGACGGCCAG	19	KRatExclosure	NE
20.A	AGTAGCGGAAGACCGTAAAACGACGGCCAG	20	KRatExclosure	SW
20.B	GCAATTAGGTACCCGTAAAACGACGGCCAG	20	KRatExclosure	SE
20.C	CATACCGTGAGTCCGTAAAACGACGGCCAG	20	KRatExclosure	NE
20.D	ATGTGTGTAGACCCGTAAAACGACGGCCAG	20	KRatExclosure	SW
20.E	CCTGCGAAGTATCCGTAAAACGACGGCCAG	20	KRatExclosure	SE
20.F	TTCTCTCGACATCCGTAAAACGACGGCCAG	20	KRatExclosure	NE
21.A	GCTCTCCGTAGACCGTAAAACGACGGCCAG	21	KRatExclosure	SW
21.B	GTTAAGCTGACCCCGTAAAACGACGGCCAG	21	KRatExclosure	SE
21.C	ATGCCATGCCGTCCGTAAAACGACGGCCAG	21	KRatExclosure	NE
21.D	GACATTGTCACGCCGTAAAACGACGGCCAG	21	KRatExclosure	SW
21.E	GCCAACAACCATCCGTAAAACGACGGCCAG	21	KRatExclosure	SE
21.F	ATCAGTACTAGGCCGTAAAACGACGGCCAG	21	KRatExclosure	NE
22.A	TCCTCGAGCGATCCGTAAAACGACGGCCAG	22	Control	SW
22.B	ACCCAAGCGTTACCGTAAAACGACGGCCAG	22	Control	SE
22.C	TGCAGCAAGATTCCGTAAAACGACGGCCAG	22	Control	NE
22.D	AGCAACATTGCACCGTAAAACGACGGCCAG	22	Control	SW
22.E	GATGTGGTGTTACCGTAAAACGACGGCCAG	22	Control	SE
22.F	CAGAAATGTGTCCCGTAAAACGACGGCCAG	22	Control	NE
23.A	GTAGAGGTAGAGCCGTAAAACGACGGCCAG	23	AllRodentExclosure	SW
23.B	CGTGATCCGCTACCGTAAAACGACGGCCAG	23	AllRodentExclosure	SE
23.C	GGTTATTTGGCGCCGTAAAACGACGGCCAG	23	AllRodentExclosure	NE
23.D	GGATCGTAATACCCGTAAAACGACGGCCAG	23	AllRodentExclosure	SW

23.E	GCATAGCATCAACCGTAAAACGACGGCCAG	23	AllRodentExclosure	SE
23.F	GTGTTAGATGTGCCGTAAAACGACGGCCAG	23	AllRodentExclosure	NE
24.A	TTAGAGCCATGCCCGTAAAACGACGGCCAG	24	AllRodentExclosure	SW
24.B	TGAACCCTATGGCCGTAAAACGACGGCCAG	24	AllRodentExclosure	SE
24.C	AGAGTCTTGCCACCGTAAAACGACGGCCAG	24	AllRodentExclosure	NE
24.D	ACAACACTCCGACCGTAAAACGACGGCCAG	24	AllRodentExclosure	SW
24.E	CGATGCTGTTGACCGTAAAACGACGGCCAG	24	AllRodentExclosure	SE
24.F	ACGACTGCATAACCGTAAAACGACGGCCAG	24	AllRodentExclosure	NE