University of California, Merced

Plant Microbiome Structure Across the Species Range of *Mimulus laciniatus*

A Thesis submitted in partial satisfaction of the requirement for the degree of Master of Sciences

In

Quantitative Systems and Biology

by

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DEDICATION

I would like to dedicate my thesis to my grandparents– Sara and Doroteo Aguilar. Their unconditional love and support continue to be the foundation of all my accomplishments, even at a distance.

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ABSTRACT

Plant microbiomes are an essential component of the plant host and are widely known to ameliorate environmental stress response. In the California Sierra Nevada, the endemic cutleaf monkeyflower (*Mimulus laciniatus*) is experiencing and adapting to various levels of stress associated with climate change, however, it is unclear to what extent the *M. laciniatus* microbiome is engaged in responding to this stress. This thesis aims to determine the monkeyflower microbiome along with their growing substrate (moss or soil) across a steep elevation gradient in the species range during the 2019 growing season. Specifically, we collected monkeyflower plants and divided those collections into compartment subsamples (roots, shoots, soil, and moss substrate) to assess the bacterial communities of each compartment by 16S rRNA gene sequencing to answer the following: (i) Do the *M. laciniatus* endophyte communities differ in composition and diversity across the *M. laciniatus* species range, and does this composition vary by elevation? (ii) Does the *M. laciniatus* endophyte community differ between plant compartments (e.g. root and shoot)? (iii) Do soil and moss, from which *M. laciniatus* grows, serve as a source of *M. laciniatus* endophytes, and do soil and moss communities vary in structure across the *M. laciniatus* species range? We found that the bacterial community structure of both plant compartments (roots and shoots) and the substrate (soil, and moss) differed across the range of *M. laciniatus*. Compartment strongly influenced bacterial communities, supporting the idea that plant compartments provide micro-habitats for bacterial communities. Elevation played a role in community variation when all samples were analyzed together. When compartment subsets were analyzed separately, population and site (nested in population) were significant drivers of variation in all four compartments. The differences between sites and population may be explained by the effect on bacterial communities by local climate, which could in turn reflect that the plant host selects beneficial microbes to support plant responses to the local environment, leading to habitat-adapted symbiosis. Alternatively, differences in bacterial community structure across the *M. laciniatus* species range could be due to differences in the metacommunity of locally available bacterial taxa to colonize compartments. This thesis highlights the importance of plant compartment and local climate or metacommunity in shaping bacterial communities along the elevation gradient of plant species.

INTRODUCTION

Plants growing in mountain habitats adapt to changing climatic conditions to increase their chances of survival. As climate warming progresses rapidly, populations at lower elevations experience warmer and dryer environments (Walther et al., 2005). In order to cope with these environmental conditions, plant species exhibit range shifts as an adaptation response, shifting from low elevations with warmer temperatures to higher elevations with cooler temperatures (Chen et al., 2011; Walther et al., 2005). Plants species' ability to respond and adapt to environmental changes is linked to the plant's genetics (Hamann et al., 2018). Often, those plant species that adapt beyond their range display genetic plasticity, genetic variation, or both (Huenneke., 1991). However, plants don't rely solely on their genetics to withstand stress, they also rely on positive biotic interactions such as mutualistic relationships with microbial communities (Shay et al., 2021; Wu et al., 2008). Understanding how plant genetics along with their interactions with microbial species respond to climate change may be critical to predicting plant species ' responses to climate change (Rudgers et al., 2020).

All plants have a diverse microbiome of fungi and bacteria colonizing their tissues as endophytes, or the surface of leaves (i.e., the phyllosphere) or roots (i.e., the rhizosphere) (Berg et al., 2014). Microbiomes play roles in maintaining plant health and increasing fitness, with benefits ranging from nutrient uptake, hormone synthesis, and regulation to protect plant hosts against pathogens (Prakash et al., 2015). Plant microbiomes are also increasingly recognized for their role in mediating a range of plant responses to stressors, including those caused by extreme weather conditions, such as drought and flooding (Lemanceau et al., 2017; Martínez-Arias et al., 2022; Partida-Martinez & Heil., 2011). Another key property of microbiomes is that they are generalists and often colonize the plant from the environment, which leads to variation in microbiome composition and diversity across host species, space, and time (Compant et al., 2021; Fitzpatrick et al., 2020; Stopnisek & Shade., 2021). Therefore, the microbiome can provide a flexible buffer against plant stress, for both natural and agricultural plants experiencing climate stress (Kannenberg & Phillips. 2017).

The edge of a species range, in particular, presents a stressful habitat for wild plants due to rising temperatures and drought. Endophytes can help plants cope with this habitat stress (Afkhami et al., 2014) Plants can recruit specific microbes for the purpose of alleviating stress (Compant et al., 2019; Hardoim et al., 2015; Lemanceau et al., 2017), potentially leading to a higher abundance of beneficial interactions at range limits. In some cases, plant-endophyte interactions can cause some organisms to have a wider breadth of abiotic tolerance (Lata et al., 2018), including variations in the climatic niche (Afkhami et al. 2014). This can rresult in theoretically larger species ranges by expansion or stability in increasingly stressful environments, such as at the warming edge of a species range. Although several studies have explored the potential for endophytes to affect species ranges (Hille Ris Lambers et al., 2013; Van der Putten et al., 2010; Wisz et al., 2013), few have explored how endophyte communities vary naturally across the geographic range of a plant species.

Wildflower species belonging to the *Mimulus* genus serve as model systems for studying plant adaptation due to their high genetic diversity (Wu et al., 2008). However, some *Mimulus* species can also serve as model systems to help us understand microbial composition across host populations. In a transplant study, Bowsher et al. (2020) showed that Yellow Monkeyflower (*Mimulus guttatus*) displayed significant differences in rhizosphere community structure across habitats, with higher diversity at inland, warmer ecotypes compared to coastal, cooler ecotypes 12/8/22 5:58:00 PM. However, there are still many unknowns remaining on how the plant microbiome may contribute to plant adaptation to climate change across the host species range.

Mimulus laciniatus, or the cutleaf monkeyflower, is an ideal species for exploring these questions. This plant species, which grows on moist, mossy areas on granite soil, is endemic to the western slopes of the Sierra Nevada (Sexton et al., 2011). This western region is characterized by its Mediterranean climate, with warm, dry summers and cold, wet winters; however, over the last few decades, they have been experiencing increases in temperatures and decreases in precipitation (del Pozo et al., 2019). This leads to climate stress for plant species at the lower elevations of their natural range. Because *Mimulus laciniatus* occurs only in the western slopes of the Sierra Nevada, it is increasingly being studied to understand plant species range limits and climate change response (Dickman et al., 2019; Sexton et al., 2016). Its entire

species range occurs across an elevation-climate gradient covering different ecosystems, offering a model system to study climate change stress at climate extremes in natural field conditions.

To investigate how endophyte communities vary across an elevation-climate gradient, we surveyed *M. laciniatus* endophyte communities across the host species range, assessing two potential drivers of microbiome community structure–plant compartment and population range. We attempt to answer the following questions: 1. Do the *M. laciniatus* endophyte communities differ in composition and diversity across the *M. laciniatus* species range and its elevation gradient? 2. Does the *M. laciniatus* endophyte community differ between plant compartments (e.g., root and shoot)? 3. Do soil and moss, from which *M. laciniatus* grows, serve as a source of *M. laciniatus* endophytes, and do soil and moss communities vary in structure across the *M. laciniatus* species range? To answer these questions, we collected samples at nine populations within the *M. laciniatus* species range, spanning the low and high elevation edges of the species range, which represent climatically stressful habitats, and including several interior populations (Sexton et al., 2016). Samples were collected as a whole plant, including, soil, moss, root, and shoot during the flowering stage. By sampling the wild plant, *M. laciniatus*, we aim to gain insights into how endophyte communities are structured across a geographic gradient across the entire species range during a single growing season and whether population range, compartment, or both play a role in shaping these microbial communities.

METHODS

Experimental and Sampling Design

Populations of *M. laciniatus* were sampled across the species range in the California Sierra Nevada during the growing season (April through September) in 2019. We sampled across a wide elevation gradient within three regional categories that represent the warmest and coldest peripheries and interior areas of the species range: at three sites of the warm-edge of the *M. laciniatus* distribution (elevation 938-1352m), averaging 13.1 °C, at three sites within the interior of the distribution (elevation 1376-2170m) averaging 9.8 °C; and at three sites of the cold-edge of the distribution (elevation 2515-2922m) averaging 2.13 °C (refer to Table 1 for elevations and geo-coordinates). At each established site (Table 1.), 10 plants were collected at least 5 meters apart at each visit (3 visits total), totaling 270 plants at varying life stages, including flowering,

fruiting, and senescing. Samples were collected aseptically as a whole plant (shoot and root tissue). Each individual plant was stored in sterile bags and transported to UC Merced on ice. Growing substrate, which can be either soil, low-lying true mosses or vascular spike mosses, which we collectively refer to as "moss," *or a combination of both,* was collected for each individual plant using the same techniques. Every collection had at least one substrate medium although most had two.

At each site, we recorded the following: geo-coordinates, elevation, type of growing substrate, plant height, plant life stage, and flower count. Plant fitness data were taken upon collection and included the total number of flowers (as a proxy for annual lifetime fitness) (Sexton et al., 2016; Dickman et al., 2019), height, and life stage (flowering, fruiting, or senescing). Soil and moss substrate were measured to acquire the gravimetric water soil and plant substrate profile: Gravimetric soil water content (%) = [mass of moist soil (g) − mass of oven-dried soil (g)/mass of oven-dried soil (g)] × 100 (Schmugge et al. 1980). The substrate was then oven-dried at 105 °C and measured again after 24 hours.

Plant sterilization and DNA extraction

Within 48 hours of collection, plants were surface sterilized, and both plant tissues and soil/moss substrate were homogenized to prepare for DNA extraction. We used chemical surface sterilization following the protocol by Schultz et al. (1993) recommended for herbaceous plants: Plants were first cleaned in a 70% ethanol bath for 30 seconds, followed by 0.5% bleach (NaOCL) for 1 minute, and then a second 70% ethanol bath for 30 seconds, concluding with four rinses with sterile, autoclaved water (Agbessenou et al., 2020; Schulz et al., 1993). The effectiveness of surface sterilization was confirmed by plating sterilized leaves and final rinses on agar (Schulz et al. 1998). After sterilization, every individual plant was separated into root and shoot samples with a sterile razor at the point where the stem differentiated from the root. Once separated, samples were flash-frozen in liquid nitrogen, mechanically grounded, and stored in a -80 °C freezer.

DNA extraction and Sequencing

We next selected a subset of plants that were all sampled in the fruiting stage to continue with microbial analysis, along with their soil and substrate moss (n= 258 samples; 71 plants with shoot and root, and 1-2 substrate samples for 68 of those plants). We performed DNA extractions using the DNeasy PowerSoil Pro Kits (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. To assess the quality of extractions and quantify DNA concentration from each sample, we utilized the Qubit dsDNA high sensitivity kit (Thermo Fisher Scientific, Wilmington, Delaware USA). Extractions ready for library preparation were then stored at -20 °C.

We performed PCR amplification on samples following the Earth Microbiome Project (EMP) 16S Illumina Amplicon Protocol. We used the primer pair 515F/926R (Parada et al., 2016; Quince et al., 2011) with Peptide Nucleic Acid (PNA) added to suppress the amplification of chloroplast and mitochondrial DNA (Lundberg et al., 2013).To start library preparation, we checked and visualized the quality of the amplified DNA for each sample through gel electrophoresis. Amplified DNA from each sample was diluted to 20 ng ml^{-1} and pooled for paired-end amplicon sequencing. The pooled library was purified to remove excess of unwanted material, following the Agencourt AMPure XP protocol (Beckman Coulter, Inc. California, CA), and sequenced on an Illumina MiSeq at the University of California Davis, Genome Center (Davis, CA) using 2 × 250 bp paired-end chemistry.

Sequence Processing

Initial processing of demultiplexed data was performed using the QIIME2 (v.2020.2.0) package (Bolyen et al., 2019). Reverse reads were discarded due to low sequence quality. We used the DADA2 pipeline (Callahan et al., 2016)to quality filter forward reads and detect amplicon sequence variants (ASVs). Chimeras were removed, and ASVs were assigned taxonomy using the Silva database (Robeson et al., 2020). ASVs assigned to mitochondria, chloroplast, and Eukarya were filtered out using QIIME2. Representative sequences were aligned using the program MAFFT (Katoh & Standley, 2013), and a maximum-likelihood phylogenetic tree was built using FastTree (Price et al., 2010). The remaining data analysis was done using R (v.4.2.0; RStudio Team, 2020), and the phyloseq package (v.1.18.0; McMurdie & Holmes, 2013).

Putative contaminants introduced during DNA extractions and PCR were removed using the package decotam (v.1.14.0; Davis et al. 2018) with the 'isContaminant' function (method=prevalence) using our blank (no DNA for PCR and no sample for extraction). We disaggregated samples for each plant compartment (shoot, root, soil, and moss substrate) using the function 'subset_samples' from the phyloseq package. Abundance plots in Class taxonomic rank for shoot, root, soil, and moss substrate were built using the microbiome package (v.1.18.0; Lahti et al, 2012-219) with the 'aggregate_rare' function. To begin the statistical analysis, we normalized the data through cumulative sum scaling using the metagenomeSeq package (v.1.38.0; Paulson et al, 2013).

Statistical Analysis

All statistical tests were performed in RStudio (v.4.2.0; RStudio Team, 2020). Rarefaction analysis was performed on all samples and subset samples with the 'rarefy_even_depth' function from the phyloseq package. We used the vegan package (v.2.6-2) (Oksanen, J. et al., 2007) to analyze alpha- and beta diversity. For alpha diversity, we calculated observed species and Shannon diversity, and used the Kruskal-Wallis rank sum test with the coin package (v.1.4-2; Hothorn et al, 2006) to test for statistical significance among samples. To investigate the relationship between bacterial community composition and compartment (shoot, root, soil substrate, moss substrate), elevation, population (warm, interior, cool), and site (nested in population) we used the adonis2 function in the vegan package to performed a non-parametric multivariate statistical permutation test (PERMANOVA) (Anderson, 2001) and Bray Curtis dissimilarity matrix. Beta diversity was visualized via principal coordinate analysis (PCoA) on Bray-Curtis distances.

RESULTS

Overview of Taxonomy. Shoot tissue and root tissue showed the lowest bacterial diversity, consisting of four main orders including *Pseudomonadales, Burkholderiales, Rhizobiales,* and *Sphingomonadales.* However, *Sphingomonadales* (5 %) *dominate* in shoot tissue along with *Rhizobiales* (15% %) while *Burkholderiales* (10%) dominate in root tissue also with *Rhizobiales* (20%) (Figure 1). Moss substrate and soil showed higher diversity compared to plant tissue (shoot and root), with soil having the highest. Soil and Moss substrate were dominated by two

orders with high relative abundance, including *Burkholderiales* (7% for moss and 8% for soil) and *Rhizobiales* (9% for moss and 12 % for soil) (Figure 1). These were followed by the class orders *Chitinophagales* (6%) in moss substrate and soil, and by *Frankiales* (6%) in soil. We observed more differences in the relative abundance of bacterial orders across populations in plant samples (root and shoot) than in substrate samples (soil and moss). The relative abundance at the Order level, also differed between sites at each plant compartment, particularly in root and shoot tissue compartments. For example, *Rhizobiales* dominated roots in warm-edge sites but not at the interior or cool-edge sites. The relative abundance of orders in soil and moss was more similar across warm-edge, interior, and cool-edge sites.

Alpha Diversity. Using the observed and Shannon diversity indices, we tested the effects of plant compartment and population (low warm-edge, interior, high cool-edge) on alpha diversity. Alpha diversity was significantly different among compartments (Kruskal-Wallis test, $p=0.00978$), with the highest diversity observed in soil and moss substrate bacterial communities, followed by root and shoot (Figure 2; Table 2). Diversity was not significantly different among populations, including when compartments were analyzed separately (Table 2), although Alpha diversity was slightly higher in all compartments in the low warm-edge populations (Figure 2).

Beta Diversity. Next, we tested the effect of plant compartment and population on bacterial community variation. Plant compartments significantly structured endophytic bacterial communities. To capture the variation explained by compartment, population site (nested in population range), and elevation across all samples, we used permutational multivariate analysis of variance (PERMANOVA) on a weighted UniFrac distance matrix. Our results revealed that when all samples were analyzed together, all four factors structured the bacterial communities, but elevation was the strongest driver of variation effect ($R^2 = 0.29929$), explaining a third of the variation across samples. We then divided samples into two distinct groups–plant tissue (shoot and root) and growing substrate (soil and moss). For both plant tissue and growing substrate, all four factors still structured bacterial communities with elevation remaining a strong driver of variation effect for both groups ($R^2 = 0.53462$; $R^2 = 0.56211$ respectively). However, elevation was not a strong driver of variation effect at each compartment subset (Table 4). At the subset

level for shoot, root, and moss substrate, population range and site were significant drivers of variation. Shoot in particular, displayed high Beta diversity, whereas soil had no significant drivers of variation.

To observe bacterial community structure, we generated a principal coordinate analysis (PCoA) based on Bray Curtis dissimilarities for all plant compartments, capturing a total variation of 12% between all samples (8.4% explained by Axis 1 and 3.6% explained by Axis 2). We observed an overlap between all plant compartments with low inter-variability between them. However, soil and moss substrate showed the lowest intra-variability within samples (Figure 3), and shoot samples showed the highest intra-variability between samples.

We then divided the dataset, to further investigate variation across the population range at each compartment. Shoot tissue showed the highest total variation of 20.7% (Figure 4) with high intra-variability within samples in all population ranges. Root tissue showed a similar trend with low inter-variability between population ranges but captured a lower total variation of 11.4%. Both soil and moss substrate showed lower inter-variability between samples at the cool edge and high inter-variability at the warm edge. Additionally, samples at the interior edge had low inter-variability with those at the warm edge.

DISCUSSION

In our study, we sampled root and shoot endophyte communities of *Mimulus laciniatus* in the fruiting stage, along with growing substrate (moss, soil, or a mix of both) across three populations (warm, interior, cool) in its species range distribution in the Sierra Nevada Mountain range. We found that compartments (shoot, root, soil, moss substrate) shaped bacterial community composition. Overall community variation was influenced strongly by elevation. When compartments were analyzed separately, communities were structured by population and site, but not elevation.

Soil and moss communities showed higher diversity than endophytic communities. This is an expected result for soil, given that plant rhizosphere (soil surrounding plant tissue), is known to host highly diverse bacterial communities compared to plant tissue (Rosenblueth & Martínez-

Romero, 2006). Endophyte communities were composed of known plant-associated orders, including *Rhizobiales, Spingomonodales, Pseudomondales, and Spingomondales*. Soil communities also consisted of taxa orders commonly represented in soil belonging to the *Rhizobiales, Burkholderiales, Frankiales, and Kineosporiales*. Although moss substrate communities shared similar taxa with those in soil, we observed a higher relative abundance of the order *Cyanobacteriales* in moss compared to soil. The presence of this bacterial order in moss substrate was not surprising due to the known symbiosis between cyanobacteria and moss species (Rousk et al., 2013). However, it was unexpected to observe the presence of this nitrogen-fixing bacteria in shoot tissue since they are not common members of the plant microbiome. We suggest that moss act as a vector of transmission of bacterial endophytes to plant tissue, although this has yet to be studied.

Alpha and beta diversity varied across compartments. Although, soil bacterial community had the highest alpha diversity, whereas shoot displayed the lowest diversity, we found that beta diversity (turnover of taxa among samples) was the highest in shoot, followed by root and moss, and soil which had the lowest beta diversity. We speculate that the possible presence of soil traces in moss samples contributed to lower beta diversity in moss compared to shoot and root. Additionally, the colonization of leaves from surrounding air tends to be stochastic (Maignien et al., 2014; Stegen et al., 2012; Zhou & Ning, 2017) and may depend less on soil and substrate communities, which could explain higher variation among shoot than root communities. Endophyte communities in root might also be selected by the plant host in response to the local environment. Roots in particular recruit specific taxa— usually those beneficial to the plant host– – in response to stress (Bucci, 2015; Hartmann et al., 2009; Singer et al., 2019). For example, roots of *Arabidopsis thaliana* have been shown to recruit beneficial bacteria as a defense mechanism (Lebeis et al., 2015). However, to our knowledge recruitment of specific taxa has not been shown in the above-ground plant endosphere.

We found that elevation was the strongest driver of variation when all samples were analyzed together, explaining a third of the variation. When plant (shoot and root) and substrate (soil and moss) were analyzed separately, elevation explained half of the variation in both (R^2 = 50%; R^2 = 57% respectively). However, when shoot, root, soil, and moss were analyzed separately,

elevation was a strong driver of variation for shoot and root, but not in soil or moss. It is possible that shoot and root endosphere are colonized by locally climate-adapted bacterial communities selected by the plant. However, it is still not definitive if elevation per se or climate and/or other factors may co-vary. We suggest that incorporating the Mantel test would allow us to further investigate the effect of geography and climate on bacterial endophytes. Moreover, other studies show no clear trend in soil bacterial communities with elevation. Soil bacterial composition in mountainous regions has been shown to be more strongly correlated with surrounding vegetation rather than elevation (Looby & Martin, 2020).

In addition to elevation, population (low-warm edge, interior, high-cool edge) and site (nested in population) were also significant drivers of variation for both endophyte and substrate bacterial communities. Population and site were significant drivers of variation when plant (shoot and root) and substrate parts (soil and moss) were analyzed separately. Additionally, when compartments (shoot, root, soil, and moss) were analyzed separately, population and site were significant drivers for all compartments. However, site nested in population had a stronger effect than compartment when samples were analyzed together. Interestingly, site nested in population also had a stronger effect than compartment on plant parts analyzed separately, suggesting that substrate type could be a more important factor in shoot and root community variation, possibly as a result of colonization of plant parts from soil or moss. On the other hand, compartment had a stronger effect on substrate compared to site.

We observed differences in community structure in both plant parts and substrate types across the range of *M. laciniatus*. Differences between sites and populations could be explained by the effect on bacterial communities by local climate. It could reflect habitat-adapted symbiosis, where plants partner up with the most beneficial microbes to support them in the local environment (Baltrus, 2020). Furthermore, it could also reflect varying dispersal patterns across populations and sites, leading to different source pools of microbial communities available to colonize (Carper et al., 2018). For example, the rhizosphere microbiome composition of *Mimulus guttatus* displayed differences between ecotypes (warm inland vs. cool coastal), suggesting that local environment influenced rhizosphere communities (Bowsher et al., 2020). In this transplant study, the local environment at each site shaped bacterial communities, whereas plant host had a

weaker influence on microbiome composition. Additionally, similarly to our results, alpha diversity was higher at the warmer sites (inland ecotype) compared to the cool coastal sites. These results were in part due to different abiotic factors at each site, however, in our results we did not take into account abiotic factors in each population. We suggest that in future studies, the effect of abiotic factors on bacterial endophytes across a plant species range should be considered to further understand how plant microbiome composition is structured across a plant species, in addition to an indicator species analysis to explore significantly associated bacterial families with the plant, at particular sites and populations.

CONCLUSION

It is widely recognized that plants depend on microbial communities to respond to environmental stressors. Yet, it is poorly understood how microbes help plants alleviate these stressors, particularly across climate gradients in mountainous regions. To our knowledge, this is one of the first studies to look at the effects of a climate gradient on plant-associated bacteria across an herbaceous plant host species' range, that spans a very wide environmental gradient. Here, we explored the bacterial community structure and composition of *Mimulus laciniatus* across its elevation gradient along the California Sierra Nevada. By collecting *M. laciniatus* plant parts (shoot and root) along with their corresponding substrate type (soil and moss), we revealed that compartment was the main predictor of bacterial community composition across all samples and at the subset level. Whereas, elevation was the main predictor of overall bacterial community structure, and at the shoot and root subset. Population and site were the main drivers of variation for soil and moss substrate. Here, we observed differences in community structure in both plant parts and substrate types across the range of M. laciniatus suggesting that local factors could be important predictors of bacterial communities across population. These differences could reflect the effects on bacterial communities by local climate, local adaptation between plants and microbes and local source of microbial communities available to colonize across sites. We suggest that including temperature and vegetation type data in future studies could further our understanding of how local climate shapes interactions between bacterial communities and plant host in the wild.

REFERENCES

Afkhami, M. E., McIntyre, P. J., & Strauss, S. Y. (2014). Mutualist-mediated effects on species' range limits across large geographic scales. *Ecology Letters*, *17*(10), 1265–1273. https://doi.org/10.1111/ele.12332

Agbessenou, A., Akutse, K. S., Yusuf, A. A., Ekesi, S., Subramanian, S., & Khamis, F. M. (2020). Endophytic fungi protect tomato and nightshade plants against Tuta absoluta (Lepidoptera: Gelechiidae) through a hidden friendship and cryptic battle. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-78898-8

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Baltrus, D. A. (2020). Bacterial dispersal and biogeography as underappreciated influences on phytobiomes. *Current Opinion in Plant Biology*, *56*, 37–46. https://doi.org/10.1016/j.pbi.2020.02.010

Berg, G., Grube, M., Schloter, M., & Smalla, K. (2014). Unraveling the plant microbiome: Looking back and future perspectives. *Frontiers in Microbiology*, *5*, 148. https://doi.org/10.3389/fmicb.2014.00148

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), Article 8. https://doi.org/10.1038/s41587-019-0209-9

Bowsher, A. W., Kearns, P. J., Popovic, D., Lowry, D. B., & Shade, A. (2020). Locally Adapted Mimulus Ecotypes Differentially Impact Rhizosphere Bacterial and Archaeal Communities in an Environment-Dependent Manner. *Phytobiomes Journal*, *4*(1), 53–63. https://doi.org/10.1094/PBIOMES-05-19-0026-R

Bucci, M. (2015). Root recruiting. *Nature Chemical Biology*, *11*(9), Article 9. https://doi.org/10.1038/nchembio.1900

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), Article 7. https://doi.org/10.1038/nmeth.3869

Carper, D. L., Carrell, A. A., Kueppers, L. M., & Frank, A. C. (2018). Bacterial endophyte communities in Pinus flexilis are structured by host age, tissue type, and environmental factors. *Plant and Soil*, *428*(1), 335–352. https://doi.org/10.1007/s11104-018-3682-x

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid Range Shifts of Species Associated with High Levels of Climate Warming. *Science*, *333*(6045), 1024–1026. https://doi.org/10.1126/science.1206432

Compant, S., Cambon, M. C., Vacher, C., Mitter, B., Samad, A., & Sessitsch, A. (2021). The plant endosphere world – bacterial life within plants. *Environmental Microbiology*, *23*(4), 1812–1829. https://doi.org/10.1111/1462-2920.15240

del Pozo, A., Brunel-Saldias, N., Engler, A., Ortega-Farias, S., Acevedo-Opazo, C., Lobos, G. A., Jara-Rojas, R., & Molina-Montenegro, M. A. (2019). Climate Change Impacts and Adaptation Strategies of Agriculture in Mediterranean-Climate Regions (MCRs). *Sustainability*, *11*(10), Article 10. https://doi.org/10.3390/su11102769

Dickman, E. E., Pennington, L. K., Franks, S. J., & Sexton, J. P. (2019). Evidence for adaptive responses to historic drought across a native plant species range. *Evolutionary Applications*, *12*(8), 1569–1582. https://doi.org/10.1111/eva.12803

Davis, N.M., Proctor, D.M., Holmes, S.P. *et al.* Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome* **6**, 226 (2018). https://doi.org/10.1186/s40168-018-0605-2

Fitzpatrick, C. R., Salas-González, I., Conway, J. M., Finkel, O. M., Gilbert, S., Russ, D., Teixeira, P. J. P. L., & Dangl, J. L. (2020). The Plant Microbiome: From Ecology to Reductionism and Beyond. *Annual Review of Microbiology*, *74*(1), 81–100. https://doi.org/10.1146/annurev-micro-022620-014327

Hamann, E., Kesselring, H., & Stöcklin, J. (2018). Plant responses to simulated warming and drought: A comparative study of functional plasticity between congeneric mid and high elevation species. *Journal of Plant Ecology*, *11*(3), 364–374. https://doi.org/10.1093/jpe/rtx023

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews : MMBR*, *79*(3), 293–320. https://doi.org/10.1128/MMBR.00050-14

Hartmann, A., Schmid, M., Tuinen, D. van, & Berg, G. (2009). Plant-driven selection of microbes. *Plant and Soil*, *321*(1), 235–257. https://doi.org/10.1007/s11104-008-9814-y

HilleRisLambers, J., Harsch, M. A., Ettinger, A. K., Ford, K. R., & Theobald, E. J. (2013). How will biotic interactions influence climate change-induced range shifts? *Annals of the New York Academy of Sciences*, *1297*, 112–125. https://doi.org/10.1111/nyas.12182

Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2006). "A Lego system for conditional inference." *The American Statistician*, **60**(3), 257–263. doi:10.1198/000313006X118430.

Kannenberg, S. A., & Phillips, R. P. (2017). Soil microbial communities buffer physiological responses to drought stress in three hardwood species. *Oecologia*, *183*(3), 631–641. https://doi.org/10.1007/s00442-016-3783-2

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Lata, R., Chowdhury, S., Gond, S. k., & White Jr, J. f. (2018). Induction of abiotic stress tolerance in plants by endophytic microbes. *Letters in Applied Microbiology*, *66*(4), 268–276. https://doi.org/10.1111/lam.12855

Lebeis, S. L., Paredes, S. H., Lundberg, D. S., Breakfield, N., Gehring, J., McDonald, M., Malfatti, S., Glavina del Rio, T., Jones, C. D., Tringe, S. G., & Dangl, J. L. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, *349*(6250), 860–864. https://doi.org/10.1126/science.aaa8764

Lemanceau, P., Blouin, M., Muller, D., & Moënne-Loccoz, Y. (2017). Let the Core Microbiota Be Functional. *Trends in Plant Science*, *22*(7), 583–595. https://doi.org/10.1016/j.tplants.2017.04.008

Looby, C. I., & Martin, P. H. (2020). Diversity and function of soil microbes on montane gradients: The state of knowledge in a changing world. *FEMS Microbiology Ecology*, *96*(9), fiaa122. https://doi.org/10.1093/femsec/fiaa122

Lundberg, D. S., Yourstone, S., Mieczkowski, P., Jones, C. D., & Dangl, J. L. (2013). Practical innovations for high-throughput amplicon sequencing. *Nature Methods*, *10*(10), Article 10. https://doi.org/10.1038/nmeth.2634

Maignien, L., DeForce, E. A., Chafee, M. E., Eren, A. M., & Simmons, S. L. (2014). Ecological Succession and Stochastic Variation in the Assembly of Arabidopsis thaliana Phyllosphere Communities. *MBio*, *5*(1), e00682-13. https://doi.org/10.1128/mBio.00682-13

Martínez-Arias, C., Witzell, J., Solla, A., Martin, J. A., & Rodríguez-Calcerrada, J. (2022). Beneficial and pathogenic plant-microbe interactions during flooding stress. *Plant, Cell & Environment*, *45*(10), 2875–2897. https://doi.org/10.1111/pce.14403

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. (M. Watson, Ed.)*PLoS ONE*, *8*(4), e61217.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., & Oksanen, M.J. (2007). *vegan: Community ecology package*. https://CRAN.R-project.org/package=vegan

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental Microbiology*, *18*(5), 1403–1414. https://doi.org/10.1111/1462-2920.13023

Partida-Martinez, L. P., & Heil, M. (2011). The Microbe-Free Plant: Fact or Artifact? *Frontiers in Plant Science*, *2*. https://www.frontiersin.org/articles/10.3389/fpls.2011.00100

Prakash, O., Sharma, R., Rahi, P., & Karthikeyan, N. (2015). Role of Microorganisms in Plant Nutrition and Health. In A. Rakshit, H. B. Singh, & A. Sen (Eds.), *Nutrient Use Efficiency: From Basics to Advances* (pp. 125–161). Springer India. https://doi.org/10.1007/978-81-322-2169-2_9

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 – Approximately Maximum-Likelihood Trees for Large Alignments. *PLOS ONE*, *5*(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Quince, C., Lanzen, A., Davenport, R. J., & Turnbaugh, P. J. (2011). Removing Noise From Pyrosequenced Amplicons. *BMC Bioinformatics*, *12*(1), 38. https://doi.org/10.1186/1471-2105-12-38

Robeson, M. S., O'Rourke, D. R., Kaehler, B. D., Ziemski, M., Dillon, M. R., Foster, J. T., & Bokulich, N. A. (2020). RESCRIPt: Reproducible sequence taxonomy reference database management for the masses. *BioRxiv*, 2020.10.05.326504. https://doi.org/10.1101/2020.10.05.326504

Rosenblueth, M., & Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions: MPMI*, *19*(8), 827–837. https://doi.org/10.1094/MPMI-19-0827

Rousk, K., Jones, D., & DeLuca, T. (2013). Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. *Frontiers in Microbiology*, *4*. https://www.frontiersin.org/articles/10.3389/fmicb.2013.00150

Rudgers, J. A., Afkhami, M. E., Bell-Dereske, L., Chung, Y. A., Crawford, K. M., Kivlin, S. N., Mann, M. A., & Nuñez, M. A. (2020). Climate Disruption of Plant-Microbe Interactions. *Annual Review of Ecology, Evolution, and Systematics*, *51*(1), 561–586. https://doi.org/10.1146/annurev-ecolsys-011720-090819

R Development Core Team. (2013). A language and Environment for Statistical Computing. *Vienna, Austria*. Retrieved from http://www.R-project.org/

Schmugge, T. J., Jackson, T. J., & McKim, H. L. (1980). Survey of methods for soil moisture determination. *Water Resources Research*, *16*(6), 961–979. https://doi.org/10.1029/WR016i006p00961

Schulz, B., Guske, S., Dammann, U., & Boyle, C. (n.d.). *Endophyte-Host Interactions. II. Defining Symbiosis of the Endophyte-Host Interaction*. 15.

Schulz, B., Wanke, U., Draeger, S., & Aust, H.-J. (1993). Endophytes from herbaceous plants and shrubs: Effectiveness of surface sterilization methods. *Mycological Research*, *97*(12), 1447–1450. https://doi.org/10.1016/S0953-7562(09)80215-3

Sexton, J. P., Hufford, M. B., C.Bateman, A., Lowry, D. B., Meimberg, H., Strauss, S. Y., & Rice, K. J. (2016). Climate structures genetic variation across a species' elevation range: A test of range limits hypotheses. *Molecular Ecology*, *25*(4), 911–928. https://doi.org/10.1111/mec.13528

Sexton, J. P., Strauss, S. Y., & Rice, K. J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proceedings of the National Academy of Sciences*, *108*(28), 11704–11709. https://doi.org/10.1073/pnas.1100404108

Shay, J. E., Pennington, L. K., Mandussi Montiel-Molina, J. A., Toews, D. J., Hendrickson, B. T., & Sexton, J. P. (2021). Rules of Plant Species Ranges: Applications for Conservation

Strategies. *Frontiers in Ecology and Evolution*, *9*. https://www.frontiersin.org/articles/10.3389/fevo.2021.700962

Singer, E., Bonnette, J., Kenaley, S. C., Woyke, T., & Juenger, T. E. (2019). Plant compartment and genetic variation drive microbiome composition in switchgrass roots. *Environmental Microbiology Reports*, *11*(2), 185–195. https://doi.org/10.1111/1758-2229.12727

Stegen, J. C., Lin, X., Konopka, A. E., & Fredrickson, J. K. (2012). Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal*, *6*(9), Article 9. https://doi.org/10.1038/ismej.2012.22

Stopnisek, N., & Shade, A. (2021). Persistent microbiome members in the common bean rhizosphere: An integrated analysis of space, time, and plant genotype. *The ISME Journal*, *15*(9), Article 9. https://doi.org/10.1038/s41396-021-00955-5

Van der Putten, W. H., Macel, M., & Visser, M. E. (2010). Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2025–2034. https://doi.org/10.1098/rstb.2010.0037

Walther, G.-R., Beißner, S., & Burga, C. A. (2005). Trends in the upward shift of alpine plants. *Journal of Vegetation Science*, *16*(5), 541–548. https://doi.org/10.1111/j.1654-1103.2005.tb02394.x

Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., Dormann, C. F., Forchhammer, M. C., Grytnes, J.-A., Guisan, A., Heikkinen, R. K., Høye, T. T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.-C., Normand, S., Öckinger, E., Schmidt, N. M., … Svenning, J.-C. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: Implications for species distribution modelling.

Biological Reviews of the Cambridge Philosophical Society, *88*(1), 15–30. https://doi.org/10.1111/j.1469-185X.2012.00235.x

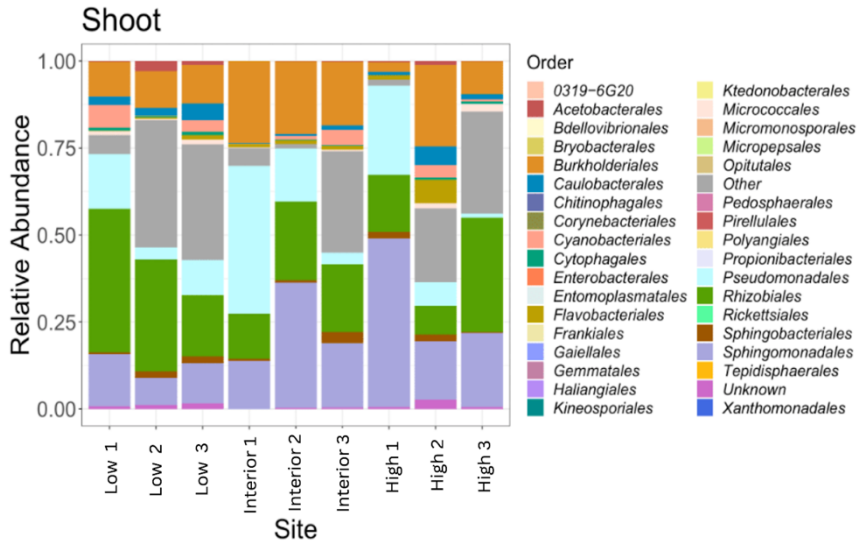
Wu, C. A., Lowry, D. B., Cooley, A. M., Wright, K. M., Lee, Y. W., & Willis, J. H. (2008). Mimulus is an emerging model system for the integration of ecological and genomic studies. *Heredity*, *100*(2), Article 2. https://doi.org/10.1038/sj.hdy.6801018

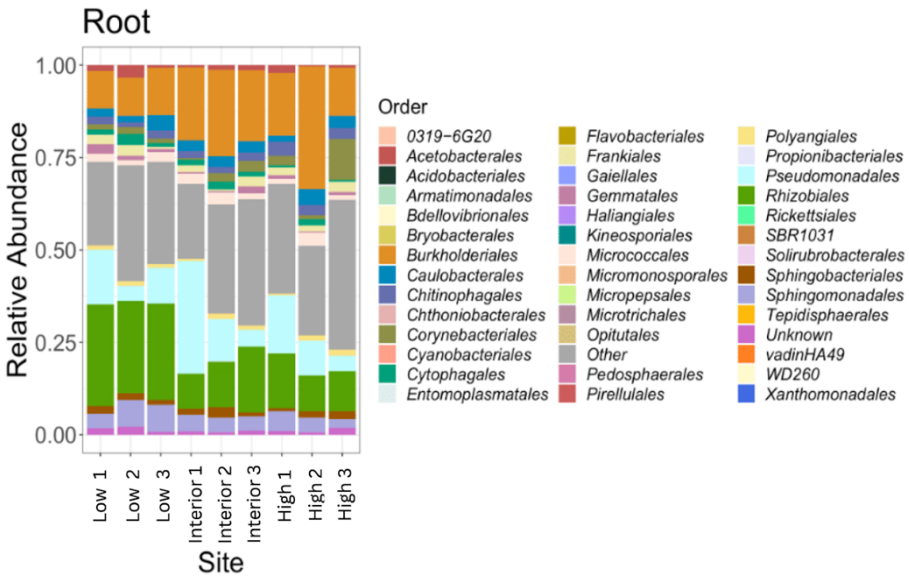
Zhou, J., & Ning, D. (2017). Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiology and Molecular Biology Reviews : MMBR*, *81*(4), e00002-17. https://doi.org/10.1128/MMBR.00002-17

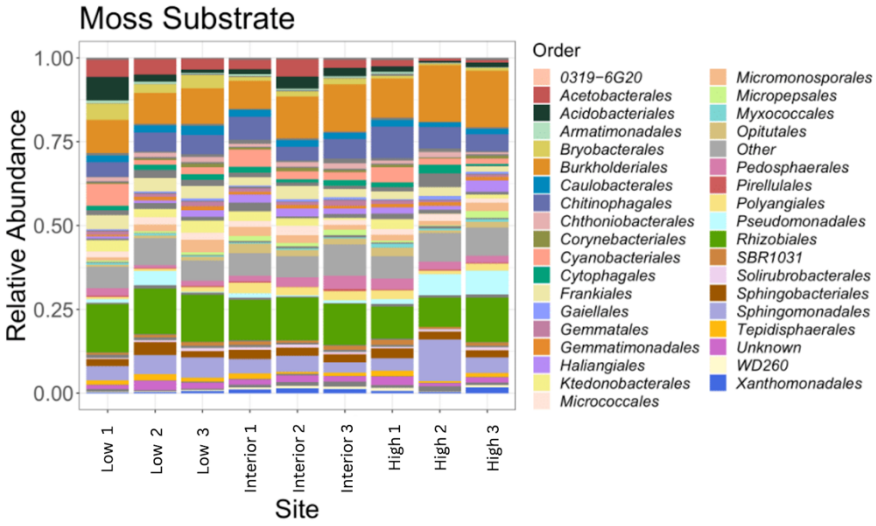
TABLES AND FIGURES

Position	Population (Code)	Elevation (m)	Latitude	Longitude	County
Low	Yosemite Forks (ZP)	938	37.38136	-119.66533	Madera
Low	Highway 168 (HW)	1024	37.24300	-119.24300	Fresno
Low	Hetch Hetchy (HH)	1352	37.89389	-119.84903	Tuolumne
Interior	Wawona Tunnel (WA)	1376	37.71429	-119.67638	Mariposa
Interior	Grand Bluff (GB)	1670	37.07225	-119.23030	Fresno
Interior	Jackass Meadow (JM)	2170	37.50694	-119.33867	Madera
High	Tenaya Lake (TL)	2515	37.83631	-119.45561	Mariposa
High	Mammoth Edge (ME)	2815	37.69477	-119.09480	Madera
High	Pear Lake (PL)	2922	36.61323	-118.66703	Tulare

Table 1. Population, climate, and geo-coordinates for each sampling site.




Root
Relative Abundance
1.00
0.75
0.50
0.25
0.00
Low 1
Low 2
Low 3
Interior 1
Interior 2
Interior 3
High 1
High 2
High 3
Site
Order
0319−6G20
Acetobacterales
Acidobacteriales
Armatimonadales
Bdellovibrionales
Bryobacterales
Burkholderiales
Caulobacterales
Chitinophagales
Chthoniobacterales
Corynebacterales
Cyanobacterales
Cytophagales
Entomoplasmatales
Flavobacteriales
Frankiales
Gaiellales
Gemmatales
Haliangiales
Kineosporiales
Micrococcales
Micromonosporales
Micropepsales
Microtrichales
Opitutales
Other
Pedosphaerales
Pirellulales
Polyangiales
Propionibacteriales
Pseudomonadales
Rhizobiales
Rickettsiales
SBR1031
Solirubrobacterales
Sphingobacteriales
Sphingomonadales
Tepidisphaerales
Unknown
vadinHA49
WD260
Xanthomonadales


Moss Substrate
Relative Abundance
1.00
0.75
0.50
0.25
0.00
Low 1
Low 2
Low 3
Interior 1
Interior 2
Interior 3
High 1
High 2
High 3
Site
Order
0319−6G20
Acetobacterales
Acidobacteriales
Armatimonadales
Bryobacterales
Burkholderiales
Caulobacterales
Chitinophagales
Chthoniobacterales
Corynebacterales
Cyanobacterales
Cytophagales
Frankiales
Gaiellales
Gemmatales
Gemmatimonadales
Haliangiales
Ktedonobacterales
Micrococcales
Micromonosporales
Micropepsales
Myxococcales
Opitutales
Other
Pedosphaerales
Pirellulales
Polyangiales
Pseudomonadales
Rhizobiales
SBR1031
Solirubrobacterales
Sphingobacteriales
Sphingomonadales
Tepidisphaerales
Unknown
WD260
Xanthomonadales

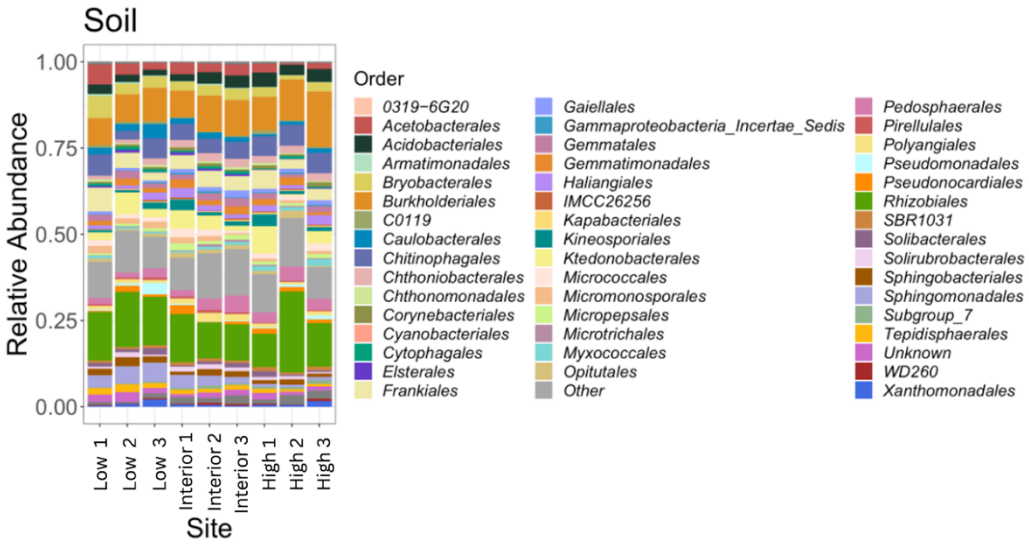


Figure 1. Relative abundances of bacterial orders in root, shoot, soil, and moss substrate across sites.

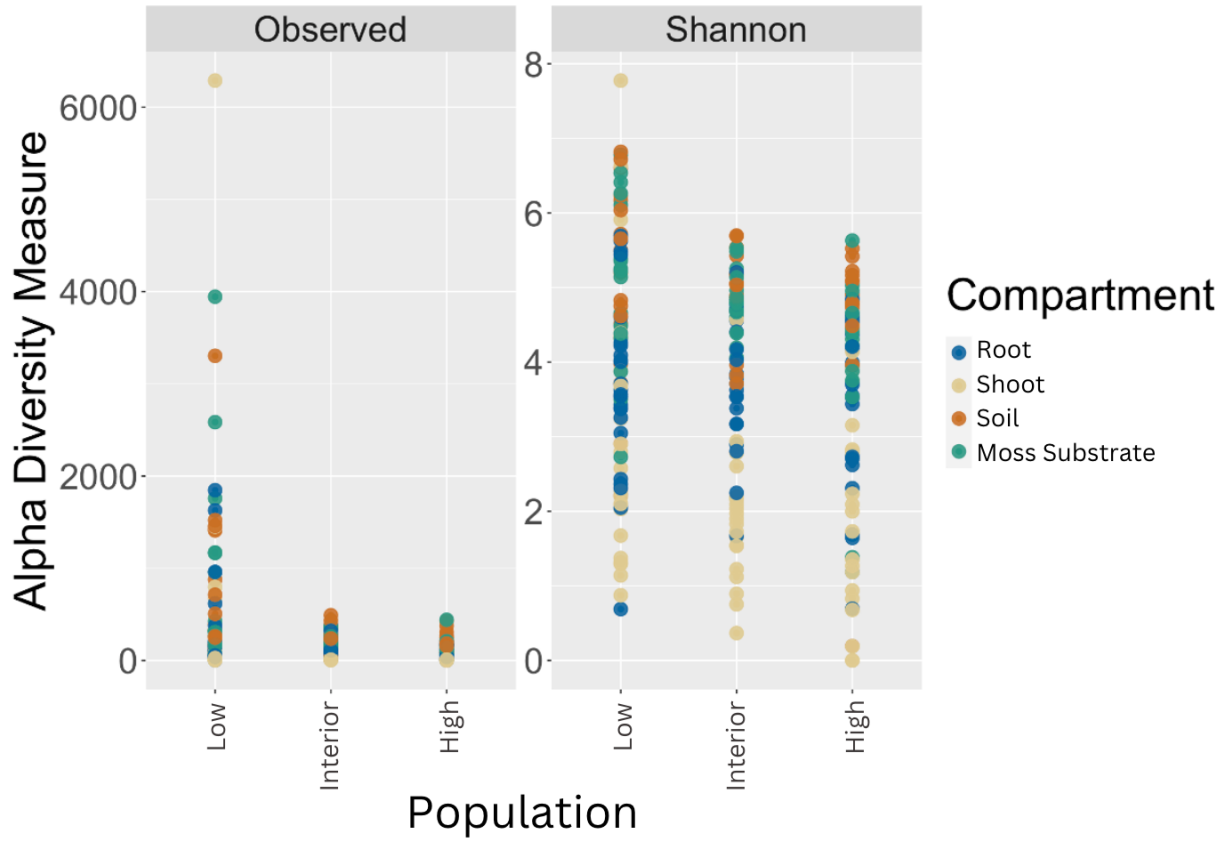


Figure 2. Observed and Shannon alpha diversity indices colored by compartment (root, shoot, soil, and moss substrate) across Populations (low, interior, high).

Sample Group	Effect of	Chi-Square	df	P-value
All	Population	4.1249	2	0.1271
	Compartment	11.393	3	0.00978
Shoot	Population	0.062156	2	0.9694
Root	Population	0.048912	2	0.9758
Moss Substrate	Population	3.9683	2	0.1375
Soil	Population	1.0996	2	0.5771

Table 2. Results of the Kruskal-Wallis rank sum test from the effect of population and compartment on all samples and the effect of population on each compartment subset.

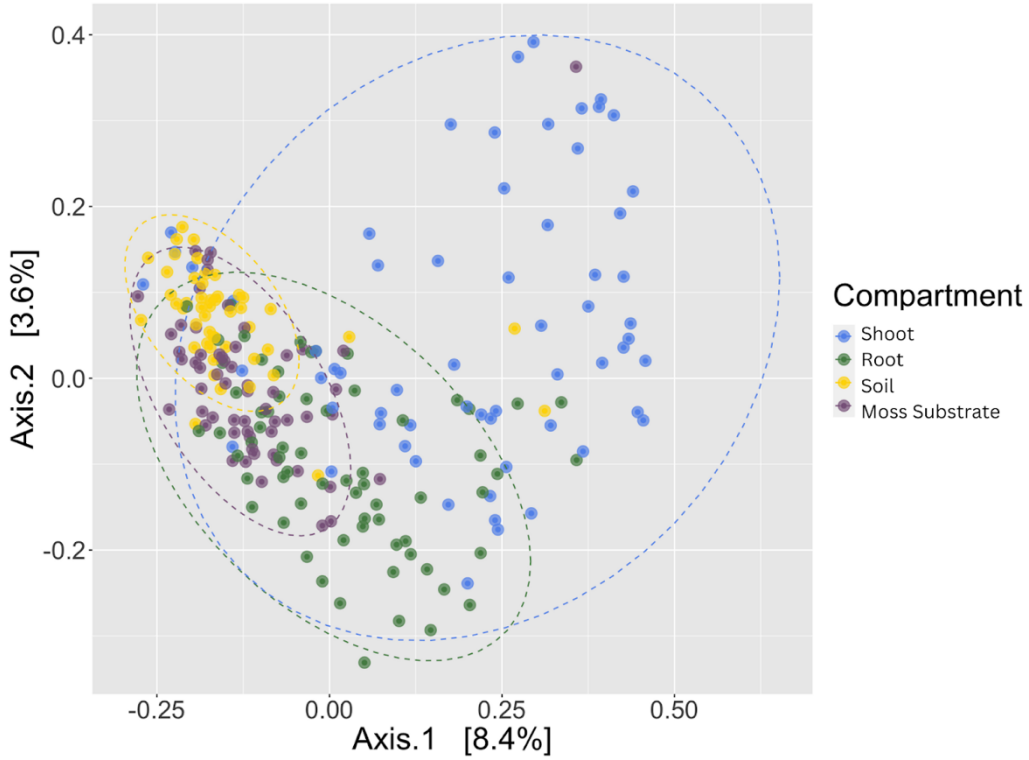


Figure 3. Principal coordinate analysis (Bray-Curtis distance index) plots for visualization of community structure between plant compartments.

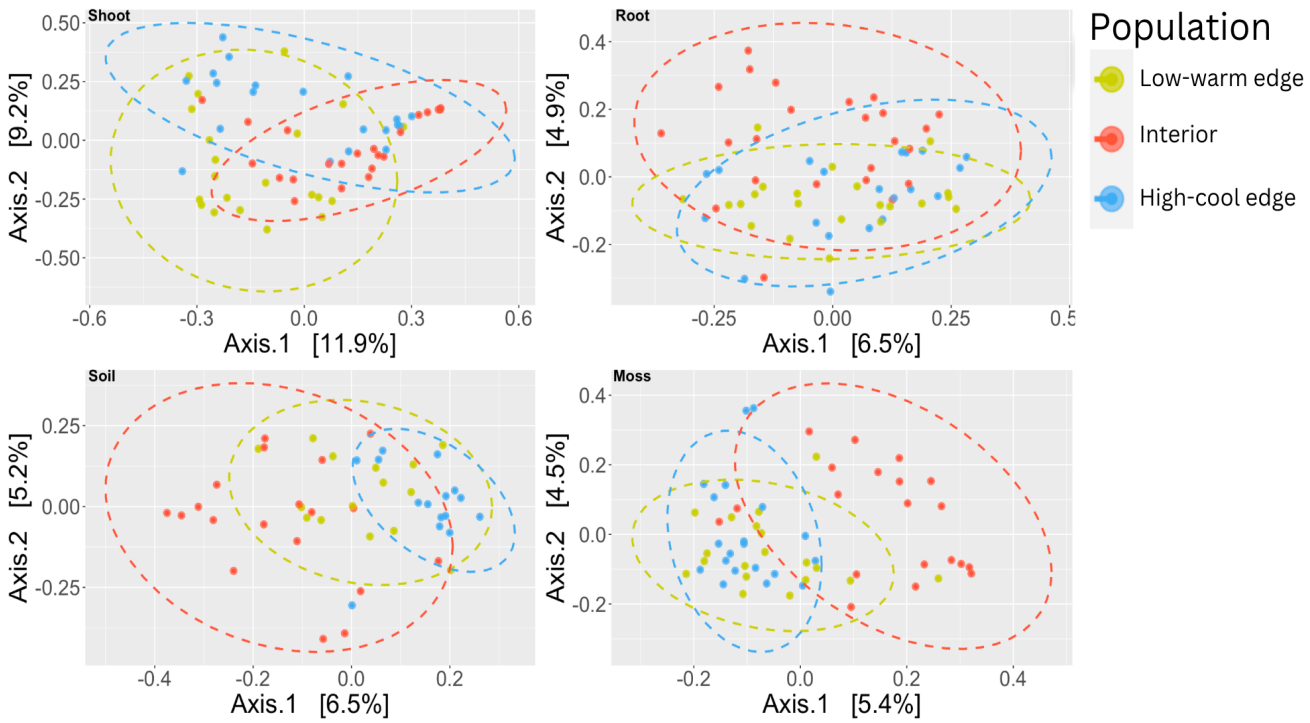


Figure 4. Principal coordinate analysis (Bray-Curtis distance index) plot for visualization of community structure across Population for each compartment subset (shoot, root, soil, moss substrate).

Sample group	**Variable**	**Bray Curtis Dissimilarity R^2**
All	Compartment	0.06505***
	Population	0.02697
	Site nested in population	0.04617
	Elevation	0.29765***
Plant (root, shoot)	Compartment	0.04006***
	Population	0.03916***
	Site nested in population	0.07834***
	Elevation	0.50755***
Substrate (soil, moss)	Compartment	0.01847***
	Population	0.04539***

	Site nested in population	0.08135***
	Elevation	0.57308***
Root	Population	0.0486***
	Site nested in population	0.11069***
	Elevation	0.85846*
Shoot	Population	0.08069***
	Site nested in population	0.1452***
	Elevation	0.88277**
Soil	Population	0.06836*
	Site nested in population	0.15321*
	Elevation	0.89447
Moss Substrate	Population	0.06146*
	Site nested in population	0.10981*
	Elevation	0.89845

Table 3. Results of PERMANOVA analysis for all samples, Plant and Substrate, and individual compartments (shoot, root, soil, and moss). *** $p<0.001$, ** $p<0.01$, * $p<0.5$, NS $p>0.1$.