Title: Locally-adapted *Mimulus* ecotypes differentially impact rhizosphere bacterial and archaeal communities in an environment-dependent manner

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Abstract

Plant root-microbe interactions influence plant productivity, health, and resistance to stress. Although there is evidence that plant species and even genotypes can alter soil microbial community structure, environmental conditions can potentially outweigh plant genetic effects. Here, we used a reciprocal transplant experiment to understand the contributions of the environment and the host plant to rhizosphere microbiome composition in locally-adapted ecotypes of *Mimulus guttatus* (syn. *Erythranthe guttata* (Fisch. ex DC.) G.L. Nesom). Two genotypes of a coastal ecotype and two genotypes of an inland ecotype were planted at coastal and inland sites. After three months, we collected rhizosphere and bulk soil and assessed microbial communities by 16S rRNA gene sequencing. We found that local environment (coastal versus inland site) strongly influenced rhizosphere communities, at least in part due to distinct local microbial species pools. Host identity played a smaller role: at each site, the ecotypes exhibited remarkably similar composition of microbial communities at the class level, indicating that divergent *M. guttatus* ecotypes recruit phylogenetically similar rhizosphere communities, even in environments to which they are maladapted. Nevertheless, the two ecotypes significantly differed in community composition at both sites due, in part, to an exclusive set of taxa associated with each ecotype. They also differed in alpha diversity at the inland site. Although this indicates that locally-adapted *M. guttatus* ecotypes are genetically diverged in factors shaping rhizosphere communities, our findings highlight the context-specific interactions between host identity and local environment that shape those communities.

Keywords: root microbiome; *Mimulus guttatus*; 16S rRNA gene; plant-microbe interactions

Introduction

The rhizosphere (the narrow zone of soil surrounding plant roots) is a highly diverse and active microenvironment. In addition to influencing soil structure, moisture, and nutrient availability (Marschner et al. 1987; Angers and Caron 1998; McKinney and Cleland 2014), plant roots continuously supply labile carbon to the soil through root exudation. These continual carbon inputs recruit a host of soil microbes to the rhizosphere (Bressan et al. 2009; Bulgarelli et al. 2012; Chaparro et al. 2014; Zhalnina et al. 2018), often resulting in distinct microbial communities compared to the surrounding bulk soil (Berendsen et al. 2012; Bever et al. 2012; Philippot et al. 2013). Rhizosphere microbial communities can strongly impact plant health and productivity, altering plant morphology (Friesen et al. 2011), phenology (Wagner et al. 2014), and plant resistance to both biotic (Santhanam et al. 2015; Busby et al. 2016; Ritpitakphong et al. 2016) and abiotic stresses (Lau and Lennon 2011, 2012). Nevertheless, despite the critical importance of rhizosphere communities for plant productivity, the factors shaping the rhizosphere microbiome are complex and not fully understood (Berg and Smalla 2009; Lareen et al. 2016; Sasse et al. 2018).

One factor that can strongly influence rhizosphere community composition is plant host identity. Plant species and even genotypes within species can differ in rhizosphere community structure when planted in a common environment (Aira et al. 2010; Bouffaud et al. 2012; Edwards et al. 2015; Mahoney et al. 2017; Berg et al. 2002; Bowen et al. 2017; Fitzpatrick et al. 2018). This finding is often suggested to result, at least in part, from species-specific root exudation patterns recruiting different community members (Marschner et al. 2001). Indeed, numerous studies suggest root exudation is the primary mechanism by which plants mediate rhizosphere community assembly and function (Broeckling et al. 2008; Haichar et al. 2008; Carvalhais et al. 2015; Hu et al. 2018). Other species- or genotype-specific factors could also contribute, such as differences in rooting depth (Aleklett et al. 2015) and root architecture (Pérez-Jaramillo et al. 2017), given that microbial community composition can shift with soil depth (Fierer et al. 2003; Ko et al. 2017).

In addition to the influence of plant host identity, environmental factors can also shape the rhizosphere microbiome. For example, the local environment directly affects rhizosphere communities by

79 determining the available source pool of microorganisms, since soil microbial communities are structured
80 by both spatial and environmental gradients (Fierer and Jackson 2006; Xue et al. 2018; Rath et al. 2019).
81 Local environmental conditions can also indirectly influence rhizosphere community composition by
82 affecting plant or microbial physiology (Aira et al. 2010). For example, many environmental factors can
83 influence rooting architecture (Lopez-Bucio et al. 2003; Niu et al. 2013; Kiba and Krapp 2016) and root
84 exudation (Zhang et al. 1991; Henry et al. 2007; Carvalhais et al. 2011; Gu et al. 2016; Gargallo-Garriga
85 et al. 2018), thereby influencing rhizosphere composition. As a result, environmental conditions can
86 outweigh the effects of plant host identity (i.e. differences among plant species or genotypes) in
87 structuring rhizosphere communities (Marschner et al. 2004; Peiffer et al. 2013). While considerable
88 recent microbiome research has been focused on economically important crops, less is known about the
89 interplay between plant host and the local environment for wild plants, which experience relatively higher
90 variability in their local environments than plants grown in managed systems.
91 In this study, we used a field reciprocal transplant experiment to better understand the
92 contributions of both the environment and host plant identity to rhizosphere microbiome composition. We
93 used two locally adapted ecotypes (coastal versus inland) of yellow monkeyflower, *Mimulus guttatu*s
94 (syn. *Erythranthe guttata* (Fisch. ex DC.) G.L. Nesom), a model species for ecological and evolutionary
95 genomics (Twyford et al. 2015; Wu et al. 2008). Coastal and inland ecotypes are highly locally adapted to
96 their respective habitats (Hall et al. 2010; Lowry et al. 2008; Lowry and Willis 2010; Hall and Willis
97 2006). Inland habitats of *M. guttatus* experience a hot summer drought, for which these populations have
98 evolved an early flowering, annual life-history strategy to escape from the long period of low soil water
99 availability (Lowry et al. 2008; Hall and Willis 2006). In contrast, coastal habitats typically are much
100 cooler as a result of proximity to the Pacific Ocean, which drives the production of summer sea fog.
101 However, coastal populations of *M. guttatus* contend with pervasive oceanic salt spray, for which they are
102 locally adapted (Lowry et al. 2008, 2009). Here, we planted coastal and inland ecotypes of *M. guttatus* in
103 both coastal and inland sites and investigated rhizosphere and bulk soil microbial community composition
104 after three months of growth.

Materials and Methods

Experimental Design

To establish the relative role of environment (coastal versus inland site) and ecotype (coastal perennial versus inland annual) on the *M. guttatus* microbial rhizosphere community, we leveraged a reciprocal transplant experiment conducted in Sonoma County, CA, USA in the spring of 2017 (Popovic and Lowry 2019). Briefly, accessions from two coastal perennial populations (SWB-11, 39.0359 N, -123.6905 W; MRR-13, 38.4564 N, -123.1409 W) and two inland annual populations (LMC-24, 38.8640 N, -123.0840 W; OCC-31, 38.4095 N, -122.9355 W) native in the same region of Northern California were used for the experiment. Source populations for the SWB and LMC seeds are in Mendocino County, CA, and have been used in many recent studies of genetics and local adaptation in this system (Lowry et al. 2008, 2009). The MRR and OCC source populations are located in Sonoma County, CA (Popovic and Lowry 2019). All accessions were grown for at least one generation in the Michigan State University greenhouses to control for maternal effects.

On February 1, 2017, seeds were planted on wet Sunshine Soil Mix #1 (SunGro Horticulture, Agawam, MA), which is a mix of sphagnum peat moss, perlite, and dolomitic lime, as well as macro- and micronutrients. Seeds were planted in two 54.28 x 27.94 cm potting trays per genotype. Seeds were then stratified at 4°C for 10-17 days (10 days for coastal accessions, 17 days for inland accessions), and subsequently germinated at University of California, Berkeley's Oxford Track greenhouse facilities under 16-hour days, supplied by supplemental lighting. Different lengths of stratification were used for the two ecotypes because the inland ecotype germinates earlier and grows faster than the coastal genotype early in development. This allowed seedlings to be transplanted to the field later at the same developmental stage. On February 28th, all seedlings were moved to the greenhouse at the Bodega Marine Reserve (bml.ucdavis.edu/bmr/) in Bodega Bay, CA. The Bodega greenhouse has no supplemental lights and minimal temperature controls.

We transplanted seedlings at the four-leaf stage into the coastal site on March 8th and into the inland site on March 9th. The coastal site was located at the Bodega Marine Reserve, Bodega Bay, CA, in a perennial seep at the south end of Horseshoe Cove (38.315716 N, -123.068625 W; ~60 m from the ocean). The inland site was planted in a seasonal grassland seep at the Pepperwood Preserve in Santa Rosa, CA (38.575545 N, -122.700851 W; 39.84 km from the ocean). Native populations of *M. guttatus* are located in both seeps. Prior to planting, three 1 x 1 m plots were cleared of native vegetation at each site using a hand-held hoe, such that both the aboveground and much of the belowground portion of native plants was removed. The experimental design was a complete randomized block design: each plot included a total of 100 plants *(N*=25 of each genotype), which were all equally spaced from one another and were haphazardly randomized throughout each plot (*N*=100 per plot, 300 per site, 600 total). Although most of the potting soil naturally fell free from the root system during transplant, a small amount of potting soil was unavoidably transplanted along with the roots. Plants were then grown for three months until being harvested for rhizosphere community analyses.

Sample collection and processing

On June 13th-15th, five replicate *M. guttatus* rhizosphere soils were collected from each genotype at each field environment from plants that were spatially distributed across all three plots. Rhizosphere soil was isolated by uprooting the plant with a trowel, discarding excess soils from around the roots, and shaking what soil remained attached to the root into a sterile Whirl-Pak bag. It was impossible to avoid the original potting soil when collecting rhizosphere samples: we expect that the small amount of potting soil transplanted with the initial transplants blended quickly with the surrounding soil, as the plants were grown in very wet seeps with a constant low-level flow of water. Rhizosphere soils were homogenized with an ethanol-sterilized metal spatula, aliquoted into cryovials, flash frozen in liquid nitrogen, and stored on ice before being transferred to dry ice for transport to Michigan State University. Above- and belowground tissue for each plant was stored in a paper bag and transported at ambient temperature to the lab at Michigan State University, washed with distilled water, and dried for 1 week at 60°C before measuring dry biomass. In addition, bulk soil cores (10 cm x 2 cm) were collected at each site. Five

replicate soil samples (each comprised of three homogenized soil cores from a given plot) collected randomly from each site were collected, sieved, and stored in a sterile Whirl-Pak bag on ice, then transferred to dry ice for transport to Michigan State University. Bulk and rhizosphere soil samples were subsequently analyzed for phosphorus, potassium, calcium, magnesium, copper, percent organic matter, sodium, nitrate, ammonium, percent nitrogen, pH, and sulfur at the Michigan State University Soil and Plant Nutrient Laboratory following their standard protocols (http://www.spnl.msu.edu/). Gravimetric soil water content was determined from the loss of mass in soils dried for one week at 60°C.

DNA Extraction and Sequencing

DNA was extracted from the five replicate rhizosphere soil samples of each *M. guttatus* genotype from each environment (n=40 samples; five replicates of each of four genotypes at each of two sites), as well as from ten bulk soil samples (five replicates from each of two sites). We used the MoBio PowerSoil Total DNA Isolation Kit (Carlsbad, CA, USA) following the manufacturer's instructions, using 0.40 g soil per rhizosphere sample and 0.23 g per bulk soil sample. Extracted DNA was quantified fluorometrically with the Qubit (ThermoFisher, Waltham, MA, USA). DNA from each sample was diluted to < 10 ng μl^{-1} for paired-end amplicon sequencing using the dual-indexed primer pair 515F/806R (Kozich et al. 2013). Samples were prepared for sequencing by the Michigan State University Genomics Core (East Lansing, MI, USA) including PCR amplification and library preparation using the Illumina TruSeq Nano DNA Library Preparation Kit. Paired-end, 250bp reads were generated on an Illumina MiSeq at the Michigan State University Genomics Core, which also provided standard Illumina quality control and sample demultiplexing.

Sequence processing

The rhizosphere and bulk soil sequencing datasets were analyzed together. Paired-end reads were merged using USEARCH v10.0.240 (Edgar 2010) with *-fastq_maxdiffs* set to 10, *-fastq_minmergelen* set to 250, and *-fastq_maxmergelen* set to 300. Primer-binding regions were removed using cutadapt v1.18 (Martin 2011), then reads were quality-filtered (with *-fastq_maxee* set to 1), dereplicated, filtered of singletons, and clustered into zero-radius OTUs using the USEARCH v9.2.64/v10.0.240 and UNOISE

pipeline (Edgar 2016). Taxonomy annotations were assigned in QIIME v1.9.0 (Caporaso et al. 2010) using UCLUST (Edgar 2010) against the SILVA rRNA database v123 (Quast et al. 2013) at a similarity threshold of 0.9, and were added to the .biom file using the biom-format package (McDonald et al. 2012). Sequences that were unassigned at the phylum level, along with those matching chloroplasts or mitochondria, were excluded from analyses. Representative sequences were aligned using MUSCLE 3.8.1 (Edgar 2004) and FastTree v2.1.10 (Price et al. 2009, 2010) was used to build a phylogenetic tree. Samples were rarefied to the minimum number of sequences observed per sample (22,354) for all subsequent analyses. Although randomly sub-setting the data through rarefying can influence taxa abundances, the *single_rarefaction.py* command in QIIME was used for reproducibility of our analyses. We calculated species richness, Shannon diversity, and phylogenetic diversity in QIIME, as well as beta diversity using weighted UniFrac distance (Lozupone and Knight 2005) for principal coordinates analysis (PCoA). Jaccard distances (Jaccard 1901) were calculated using the *vegdist* function of the vegan v2.5-2 package (Oksanen et al. 2018) in R 3.5.0 (R Core Team 2018).

Statistical analysis

All statistical analyses were conducted in R. We assessed differences in soil chemistry between sites using t-tests. The homogeneity of variance assumption was assessed using both Bartlett's and Levene's tests (Levene 1960; Snedecor and Cochran 1989) in the car package (Fox and Weisberg 2011), while normality of residuals was assessed using the Shapiro-Wilk test. The Welch's t-test was used when the homogeneity of variance assumption was not met, while the Wilcoxon rank sum test was used when residuals were not normally distributed. Differences in alpha diversity metrics and plant traits were assessed using two-way ANOVA (with 'ecotype' and 'site' as main factors, and their interaction) followed by a Tukey post-hoc test. Shoot mass data was log-transformed in order to meet the assumptions of ANOVA. For Shannon diversity, a single outlier (greater than three standard deviations from the group mean) was removed to meet ANOVA assumptions; results are reported both including and excluding the outlier.

For the sequencing dataset, we assessed the effects of abiotic (phosphorus, potassium, calcium, magnesium, copper, percent organic matter, sodium, nitrate, ammonium, percent nitrogen, and sulfur) parameters on microbial community composition by fitting variables to the PCoA scores generated from weighted UniFrac (for phylogenetic community structure) as well as Jaccard distances (for presence/absence of taxa) using the *envfit* function of vegan (Oksanen et al. 2018). We included parameters that had significant explanatory value ($p < 0.05$) as vectors in the ordinations. Differences in community composition across categorical groups (rhizosphere versus bulk soil, inland versus coastal sites, as well as their interaction) were calculated with PERMANOVA using 999 iterations (Anderson 2001). We also tested for differences in group dispersions with PERMDISP (Anderson 2006). Next, we selected the twenty most abundant taxa at the Class level and tested for differences in abundance of these taxa using t-tests with an FDR-adjusted p-value for multiple comparisons. Within each site, we compared inland versus coastal ecotypes, as well as genotypes within ecotypes.

We next explored differences between ecotypes and sites at the individual OTU level to better understand the factors distinguishing their microbial communities. We conducted an indicator species analysis, which aims to determine which taxa are characteristic of a given treatment group, taking into account the abundances of a given taxon for each treatment group (specificity), as well as the proportion of samples in each treatment group in which that taxon occurs (fidelity) (De Cáceres and Legendre 2009; De Cáceres et al. 2010). We used the *multipatt* function (De Cáceres et al. 2010) in the R package indicspecies (De Cáceres and Legendre 2009). Next, we tested for ecotype differences in relative abundance of individual OTUs using t-tests with FDR-adjusted p-values for multiple comparisons. Finally, we generated Venn diagrams using the R packages gplots (Warnes et al. 2019) and VennDiagram (Chen 2018) to assess differences in the presence/absence of individual taxa between the two ecotypes. An OTU was designated as 'present' in a given ecotype if at least one sequencing read representing that OTU was found in at least one sample of that ecotype; otherwise it was 'absent'. Data were visualized using a combination of the R packages ggplot2 v2.2.1 (Wickham 2009), reshape2 v.1.4.3 (Wickham

2007), gridExtra 2.3 (Baptiste 2017), and cowplot v0.9.2 (Wilke 2017). Package plyr v.1.8.4 (Wickham 2011) was used for data summaries.

Data availability and computing workflows

Raw reads were submitted to the NCBI Sequence Read Archive under accession numbers PRJNA451377 (rhizosphere samples) and PRJNA526056 (bulk soil samples). All plant and environmental data, as well as computational workflows and custom scripts, are available on GitHub (https://github.com/ShadeLab/PAPER_MimulusRecipTransplant_Submitted).

Results

Soil characteristics and plant performance differ across sites

The coastal and inland sites had very different soil properties (Table 1). All measured abiotic parameters significantly differed between the coastal and inland sites, with the exception of pH, ammonium, nitrate, and percent nitrogen. Plants also performed differently in the coastal and inland sites. Plants in the coastal site were overall larger in both shoot mass (F=13.164, P<0.001) and root mass (F=23.359, P<0.001) than plants at the inland site (Figure S1). In addition, the coastal ecotype was overall significantly larger in both shoot mass (F=8.7571, P=0.006) and root mass (F=5.637, P=0.023) than the inland ecotype.

Sequencing summary

In total, 49 samples were sequenced, resulting in a 3,119,029 sequencing reads (average of 63,654 reads per sample). Of those, 2,503,095 sequences remained after merging (80.3%) and 2,433,943 of the merged sequences passed the ensuing quality filter (97.2%). After removing singletons and determining representative sequences, a total of 2,232,822 sequences mapped to OTU definitions, and 2,130,143 of those (95.4%) were assigned a taxonomy that was neither mitochondria nor chloroplast. Altogether, this resulted in a minimum of 22,354 reads per sample and a maximum of 94,669 reads per sample (average of 43,472 reads per sample). After rarefying (i.e., subsampling) the dataset to 22,354

reads per sample, there were an average of 3,902 OTUs per sample. See Table S1 for sequencing statistics for each sample.

Site and ecotype influence microbial community composition

A principal coordinates analysis based on weighted UniFrac distances found that two axes captured nearly 60% of the variation in the amplicon sequencing dataset (45.8% variation explained for PC1 and 13.9% for PC2) (Figure 1). Numerous abiotic parameters had significantly explanatory value for changes in microbiome community structure across samples (Table S2). Coastal site samples were associated with greater moisture content, sodium, phosphorus, and sulfur, while inland site samples were associated with greater potassium, calcium, magnesium, and copper (Figure 1).

PERMANOVA using weighted UniFrac distances revealed significant clustering of microbial communities by sample type (rhizosphere versus bulk soil; F=8.011, P=0.001) and site (coastal versus inland; F=43.227, P =0.001), as well as their interaction (F=4.307, P =0.006). We therefore investigated further by dividing the dataset by site and found that rhizosphere and bulk soils significantly differed in community composition at both the coastal and the inland sites (F=8.2951, P=0.001; and F=4.918, P =0.005, respectively), and differed in variability by PERMDISP at the coastal site (F=10.73, P=0.002). We next subdivided the rhizosphere samples by ecotype. We found that site influenced community composition for both the coastal (F=28.828, P=0.001) and inland ecotypes (F=16.319, P=0.001). In addition, the coastal ecotype differed in variability between the two sites (F=7.3244, P=0.013). Next, we found that inland ecotype rhizospheres differed from bulk soil in community composition at both the coastal and inland sites (F=6.2055, P=0.001; and F=5.2513, P=0.007, respectively), and differed in variability at the coastal site (F=13.198, P=0.004). Similarly, coastal ecotype rhizospheres differed from bulk soil at both the coastal and inland sites (F=10.474, P=0.001; and F=3.8461, P=0.004, respectively). We also tested for differences between ecotypes at each site and found that coastal and inland ecotypes differed in community composition at the inland site (F=3.279, P=0.006), but not at the coastal site (F=1.6859, P=0.095). Finally, we tested for differences between genotypes (within each ecotype at each site), and found that genotypes did not differ in any instance (all P>0.05).

A principal coordinates analysis based on Jaccard (binary presence/absence) distances found that two axes together captured 35.6% of the variation in the amplicon sequencing dataset (Figure 1). As with the PCoA of weighted UniFrac distances, all measured soil variables except ammonium were significantly associated with differences in community structure (Table S3), and primarily distinguished the coastal and inland sites along Axis 1 (Figure 1). As with weighted UniFrac distances (Figure 1), PERMANOVA using Jaccard distances revealed that sites differed in community composition for both the coastal (F=9.515, P=0.001) and inland ecotypes (F=8.612, P=0.001). In addition, the coastal ecotype differed in variability between the two sites (F=4.677, P=0.032). We also tested for differences between ecotypes at each site. Coastal and inland ecotypes differed in community composition at both the inland site (F=1.753, P=0.027) and the coastal site (F=1.431, P=0.047). Finally, we found that rhizosphere and bulk soils significantly differed in community composition at the coastal (F=2.060, P=0.001), but not the inland sites (F=.3189, P=0.099), and differed in variability by PERMDISP at the coastal site (F=32.73, P=0.001).

Ecotypes differ in rhizosphere community composition and diversity at inland site

Across environments and ecotypes, we detected 14,869 OTUs spanning a breadth of phylogenetic diversity. Species richness, phylogenetic diversity, and Shannon diversity were all significantly higher for the inland ecotype than the coastal ecotype at the inland site, while ecotypes did not differ at the coastal site (Figure 2). Similarly, all three alpha diversity metrics significantly differed between sites for the coastal ecotype, but not the inland ecotype. It should be noted, however, that differences in Shannon diversity were only significant when an extreme outlier (greater than three standard deviations from the ecotype/site mean) was excluded.

Next, we compared microbial abundances between ecotypes at each site, and found that the two ecotypes exhibited very similar relative abundances of microbial taxa at the class level (Figure 3). The two ecotypes did differ in the abundances of several highly abundant taxa, but only at the inland site. At the inland site, the inland ecotype had greater relative abundance of Cytophagia, Deltaproteobacteria,

Gammaproteobacteria, and Verrucomicrobiae, but lower relative abundance of Acidobacteria, than the coastal ecotype (Figure 3). Genotypes within each ecotype did not differ in relative abundances of taxa at either the coastal or the inland site (Figure S2).

Each ecotype exhibited some of the same compositional shifts in microbial communities (relative to bulk soil) in both sites. At both the coastal and inland sites, the inland ecotype exhibited lower relative abundance of Acidobacteria, Gemmatimonadetes, Nitrospira, and greater relative abundance of Planctomycetacia, compared to bulk soils (Figure S3). Similarly, at both sites, the coastal ecotype exhibited lower relative abundance of Nitrospira, and greater relative abundance of Planctomycetacia, compared to bulk soils. Within each site, both ecotypes influenced the relative abundance of numerous taxa in similar ways. At the coastal site, both ecotypes exhibited lower relative abundance of Acidobacteria, Anaerolineae, Gemmatimodetes, Nitrospira, Deltaproteobacteria, and OPB35-Soil, and greater relative abundance of Thermoleophilia, Cytophagia, Sphingobacteria, KD4-96, Planctomycetacia, and Alpha-proteobacteria, compared to bulk soil. Similarly, at inland site, both ecotypes exhibited lower relative abundance of Nitrospira and greater relative abundance of Planctomycetacia compared to bulk soil. There were exceptions to this rule, however. For example, at the inland site, the inland ecotype exhibited lower relative abundance of Acidobacteria, Gemmatimomdetes, Spartobacteria, and greater relative abundance of Actinobacteria compared to bulk soil, while the coastal ecotype did not.

Presence/absence of microbial taxa differs between coastal and inland ecotypes

We next explored differences between ecotypes and sites at the individual OTU level. Indicator species analysis revealed that no bacterial species were indicative of coastal versus inland ecotypes at either the coastal or the inland site (all adjusted $P>0.05$). In addition, the coastal and inland ecotypes did not differ in relative abundance of any individual OTUs at either site. However, the two ecotypes did differ in the presence/absence of numerous OTUs at each site. At the inland site, 1,157 OTUs were present in the coastal but not the inland ecotype, while 2,065 OTUs were present in the inland but not the coastal ecotype (Figure 4). Similarly, at the coastal site, 1,413 OTUs were present in the coastal but not the inland ecotype, while 1,395 OTUs were present in the inland but not the coastal ecotype (Figure 4). At

each site, these OTUs were in extremely low relative abundance (roughly ten-fold lower mean relative abundance) compared to the OTUs shared by the ecotypes and bulk soil. The OTUs distinguishing the ecotypes at each site also had very low occupancy (i.e. were present in a small proportion of samples per ecotype). For example, at the inland site, only 14 of the 1,157 OTUs unique to the coastal ecotype were present in at least half of the coastal ecotype samples, while only 99 of the 2,065 OTUs unique to the inland ecotype were present in at least half of the inland ecotype samples. Similarly, at the coastal site, only 39 of the 1,413 OTUs unique to the coastal ecotype were present in at least half of the coastal ecotype samples, while only 39 of the 1,395 OTUs unique to the inland ecotype were present in at least half of the inland ecotype samples. At both sites, a large number of OTUs were found in rhizosphere samples, but not the bulk soil, and vice versa (Figure 4).

Discussion

Interactions between plant roots and soil microorganisms strongly influence plant health and productivity, yet the relative role of host plant identity versus the local environment in shaping the rhizosphere microbiome is not well understood. To begin to unravel this we examined the rhizosphere communities of two ecotypes of *M. guttatus*, which are locally adapted to distinct environments, in a reciprocal transplant experiment.

The local environment (coastal versus inland site) strongly influenced rhizosphere microbial communities in *M. guttatus*. This effect is due, at least in part, to distinct microbial source pools in the bulk soil at each site. This finding was not surprising given that abiotic conditions strongly differed between the two sites, and microbial community structure is often influenced by environmental gradients (Lauber et al. 2009; Fierer et al. 2012; Xue et al. 2018; Sorensen et al. 2019). For example, both salinity (Rath et al. 2019) and moisture availability (Brockett et al. 2012), two of the major factors distinguishing the coastal and inland sites, can have substantial effects on microbial community structure. Indeed, numerous abiotic factors had significant explanatory value for the differences in microbial community structure across samples. In particular, the sharp differences between microbial communities at the coastal

and inland sites were related to differences in nutrient availability, salinity, and moisture between the sites.

Host plant identity also influenced rhizosphere community composition in *M. guttatus*, but to a smaller extent than the influence of environment. At each site, the two ecotypes exhibited remarkably similar abundances of microbial taxa at the Class level. Many of the shared lineages are commonly associated with rhizospheres, including Actinobacteria, Firmicutes, Alpha- and Beta-proteobacteria (Philippot et al. 2013), suggesting evolutionarily-conserved mechanisms for recruiting and/or sustaining these taxa. Our results indicate that divergent *M. guttatus* ecotypes recruit phylogenetically similar rhizosphere communities, even in environments to which they are maladapted. Nevertheless, the ecotypes differed in community composition at the inland site when considering weighted UniFrac distances (phylogenetic distance weighted by taxon relative abundance), and differed at both the coastal and inland sites when considering Jaccard distances (presence/absence of microbial OTUs). At both sites, numerous taxa of low abundance (rare) and low occupancy (found in a low proportion of samples) were found in one ecotype at the exclusion of the other, supporting the differences between ecotypes detected using Jaccard distances. Although the rarity of these OTUs suggests they may be present in the *M. guttatus* rhizosphere due to stochastic processes rather than deterministic recruitment by the plant host, rare microbial taxa have the potential to provide a reservoir of microbial functions that can support community stability despite environmental fluctuations (Shade et al. 2014; Shade and Gilbert 2015). The design of the present study does not allow us to determine whether differing rhizosphere communities are a cause or a consequence of the evolutionary divergence between the ecotypes, nor does it allow us to assess the biological relevance (if any) of the rare microbial taxa detected here. Future work could explore the potential role of the rhizosphere microbiome in local adaptation in this system by examining growth and fitness of the two ecotypes in sterilized and unsterilized 'home' and 'away' soil. For this type of experiment, a greater difference in fitness between the two ecotypes in the unsterilized soil would indicate that soil microbial communities contribute to local adaptation and ecotypic divergence in *M. guttatus*. Such experiments could be conducted in controlled greenhouse conditions to specifically isolate the

387 effects of soil communities on plant fitness, rather than environmental factors such as oceanic salt spray
388 and drought which strongly impact *M. guttatus* fitness in the field (Lowry et al. 2008, 2009; Popovic and
389 Lowry 2019).

390 Our finding that plant host identity impacts rhizosphere communities in both common garden
391 sites strongly suggests that the *M. guttatus* ecotypes are genetically diverged in the factors shaping those
392 communities. Although numerous studies have documented genetic differentiation for rhizosphere
393 microbiome communities in crops and model species in controlled environments (Costa et al. 2006;
394 Micallef et al. 2009; Aira et al. 2010; Peiffer et al. 2013; Mahoney et al. 2017), our work is one of only a
395 few studies reporting genotype-specific effects of wild plants in natural environments (Kuske et al. 2002;
396 Osanai et al. 2013; Aleklett et al. 2015). Our study differs in that it utilized seedlings germinated in a
397 common controlled environment, then transplanted to natural environments for a portion of the growing
398 season, rather than sampling from established plant communities as in previous work of wild plants
399 (Kuske et al. 2002; Osanai et al. 2013; Aleklett et al. 2015). We hypothesize that variable root exudate
400 composition and/or root morphology between *M. guttatus* ecotypes acts to differentially shape
401 rhizosphere community structure in these ecotypes.

402 Interestingly, the effect of host plant identity reported here was environment-dependent: the two
403 ecotypes only differed in alpha diversity at the inland site. The ability of the inland ecotype to harbor a
404 more OTU-rich and phylogenetically-diverse rhizosphere could potentially contribute to its greater fitness
405 at the inland site compared to the coastal ecotype. Previous work in the *M. guttatus* system has found that
406 the coastal ecotype exhibits extremely low fitness in inland sites due to near-zero survival-to-flowering
407 rates (Lowry et al. 2008; Lowry and Willis 2010). Although the sample collections made here were
408 completed before the inland site dried out for the summer (and the ecotypes did not differ in biomass at
409 the inland site), it is possible that the early stages of physiological stress at the inland environment
410 contributed to the differences in rhizosphere composition between the two ecotypes seen here. In any
411 case, the complex interplay between host identity and environment is consistent with the contrasting
412 results seen in studies of cultivated crops. For example, some studies report that differences in

413 rhizosphere community composition across species or genotypes are environment-dependent (Marschner
414 et al. 2004; Costa et al. 2006; Peiffer et al. 2013), while others find that differences across species or
415 genotypes are maintained regardless of environment (Mahoney et al. 2017; Marschner et al. 2001).
416 Although the two ecotypes are indeed genetically diverged in factors shaping the rhizosphere
417 microbiome, environmental factors can outweigh genetic factors in shaping the *M. guttatus* microbiome at
418 least for the field sites examined in our study.

419 It is worth noting that numerous taxa were detected in the *M. guttatus* rhizosphere that were not
420 detected in bulk soil. For example, rhizosphere and bulk soil communities differed at both coastal and
421 inland sites, suggesting the presence of *M. guttatus* strongly influences soil microbiome structure. An
422 important caveat of this finding is that the horticultural potting soil in which the seedlings were
423 germinated likely contributed to the difference between rhizosphere and bulk communities detected here,
424 thought we suggest that this influence was minimal given that care was taken to not transplant notable
425 amounts of potting soil. Nevertheless, we suggest that the differences between rhizosphere and bulk
426 communities at both sites was not solely an artefact of the germination media, given the general
427 observation that plants play a major role in regulating soil microbial community composition and function
428 (reviewed in (Bulgarelli et al. 2013; Lareen et al. 2016; Coskun et al. 2017). In any case, our study was
429 primarily designed to assess the relative roles of host plant identity and the local environment in shaping
430 the rhizosphere microbiome, which were detectable in spite of the common germination environment.

431 In summary, we found that the local environment (coastal versus inland site) strongly influenced
432 rhizosphere communities, at least in part due to distinct composition of the microbial source pool at each
433 site. Although host plant identity also influenced rhizosphere community composition, it was to a much
434 smaller extent than the influence of the environment. At each site, the two ecotypes exhibited remarkably
435 similar abundances of microbial taxa at the Class level, indicating that divergent *M. guttatus* ecotypes
436 recruit phylogenetically similar rhizosphere communities, even in foreign habitats. Nevertheless, the two
437 ecotypes did differ in rhizosphere community composition at both sites, due, at least in part, to differences
438 in the presence or absence of microbial taxa, particularly rare (low abundance and low occupancy) taxa.

In addition, the ecotypes differed in alpha diversity at the inland site, but not the coastal site. This indicates an element of environment-dependence in the plant genetic factors that regulate the *M. guttatus* microbiome, and highlights the context-specific interactions between host identity and local environment in shaping those communities.

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Soil Variable	Coastal site	Inland site	p-value
pH	6.08 (0.1)	6.16 (0.15)	0.398
Phosphorus (ppm)	17.4 (2.3)	3.4 (0.6)	0.001
Potassium (ppm)	49.2 (9.3)	171.4 (16.4)	<0.001
Calcium (ppm)	788.2 (121.7)	2518.4 (91.8)	<0.001
Magnesium (ppm)	227.4 (19.7)	1603.8 (162.4)	0.008
Copper (ppm)	2.42 (0.4)	21.68 (2.6)	<0.001
Percent Organic Matter	3.46 (0.57)	4.9 (1.07)	0.029
Sodium (ppm)	135.8 (17.0)	50.4 (2.7)	<0.001
Nitrate (ppm)	0 (0)	0.6 (0.51)	0.060
Ammonium (ppm)	5.26 (1.37)	5.64 (1.54)	0.828
Percent Moisture	34.24 (4.64)	17.82 (4.08)	<0.001
Total-N (%)	0.139 (0.04)	0.1888 (0.05)	0.107
Sulfur (ppm)	23.6 (4.6)	17.4 (2.9)	0.034

Table 1. Soil characteristics (mean with standard error in parentheses) for bulk soils collected from *Mimulus guttatus* planted in two environments.

Supplementary Table 1. Sequencing results and read counts through the sequencing analysis pipeline.

Supplementary Table 2. Environmental variables fitted to differences in microbiome community structure computed using weighted UniFrac distances. Significant variables were plotted as vectors in Figure 1.

Supplementary Table 3. Environmental variables fitted to differences in microbiome community structure computed using Jaccard distances. Significant variables were plotted as vectors in Figure 6.

716 **Figure Legends**

717

718 **Figure 1.** Principal coordinates analysis based on (A) weighted UniFrac distances and (B) Jaccard
719 distances of bacterial and archaeal community structure. The strength of statistically significant ($p < 0.05$)
720 explanatory variables are shown with solid arrows.

721

722 **Figure 2**. Metrics of alpha diversity in bulk soil and rhizosphere of coastal (genotypes MRR and SWB
723 pooled) and inland (genotypes LMC and OCC pooled) ecotypes of *Mimulus guttatus* planted in two
724 environments. For each alpha diversity measure, ecotype-site combinations that significantly differed by
725 the Tukey post-hoc test are indicated by a different letter above the black lines. Note that differences in
726 Shannon diversity were only significant when an extreme outlier (greater than three standard deviations
727 from the mean) was excluded. Bulk soil values are shown for comparison.

728

729 **Figure 3**. Relative abundance (mean ± SD) of the top 20 most abundant bacterial and archaeal classes in
730 the rhizospheres of coastal (genotypes MRR and SWB pooled) and inland (genotypes LMC and OCC
731 pooled) ecotypes of *Mimulus guttatus* planted in two environments. Less abundant taxa were pooled into
732 a single group ("Less Abundant Classes"). Taxa which significantly differed between ecotypes at a given
733 site are indicated by an asterisk.

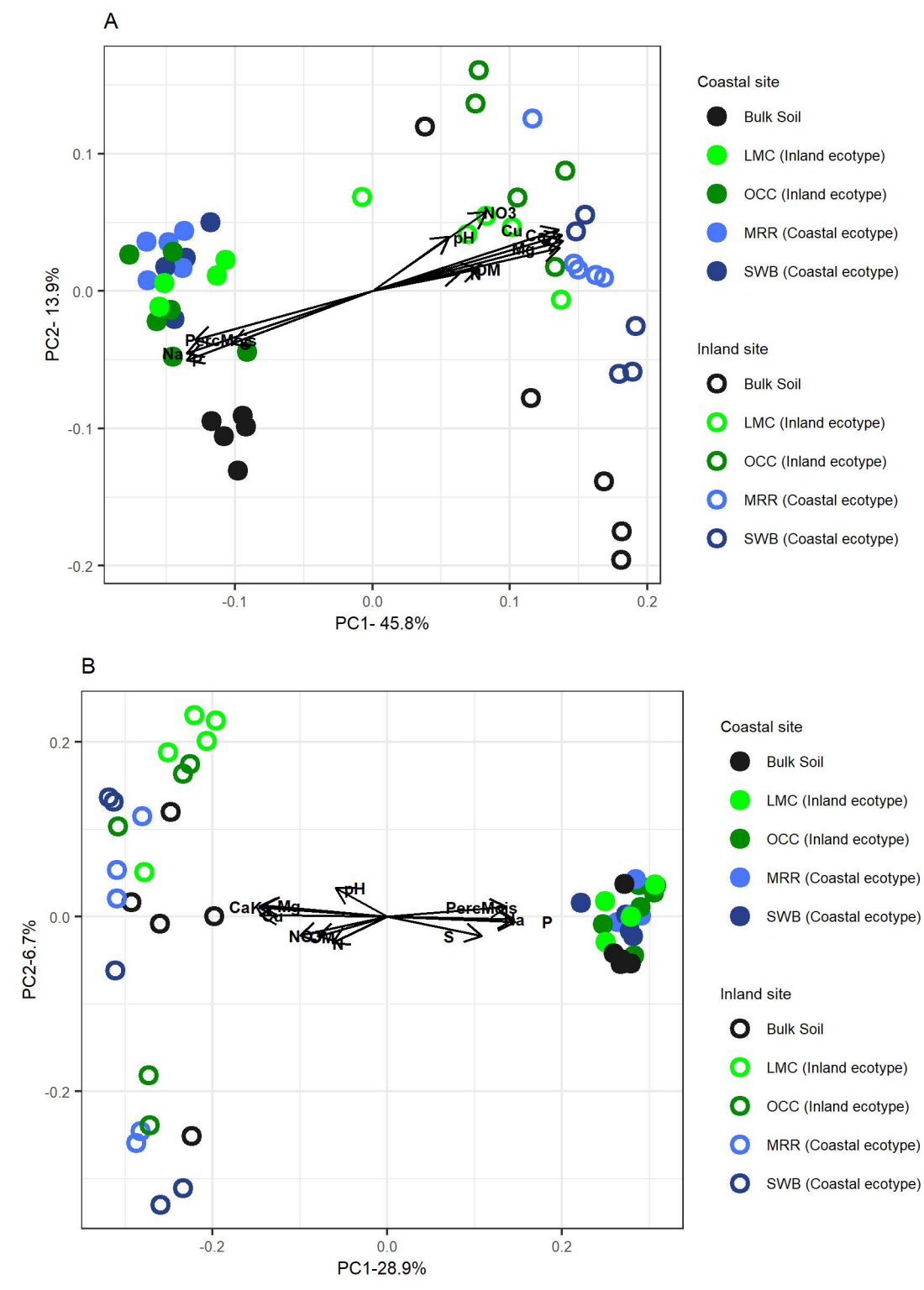
734

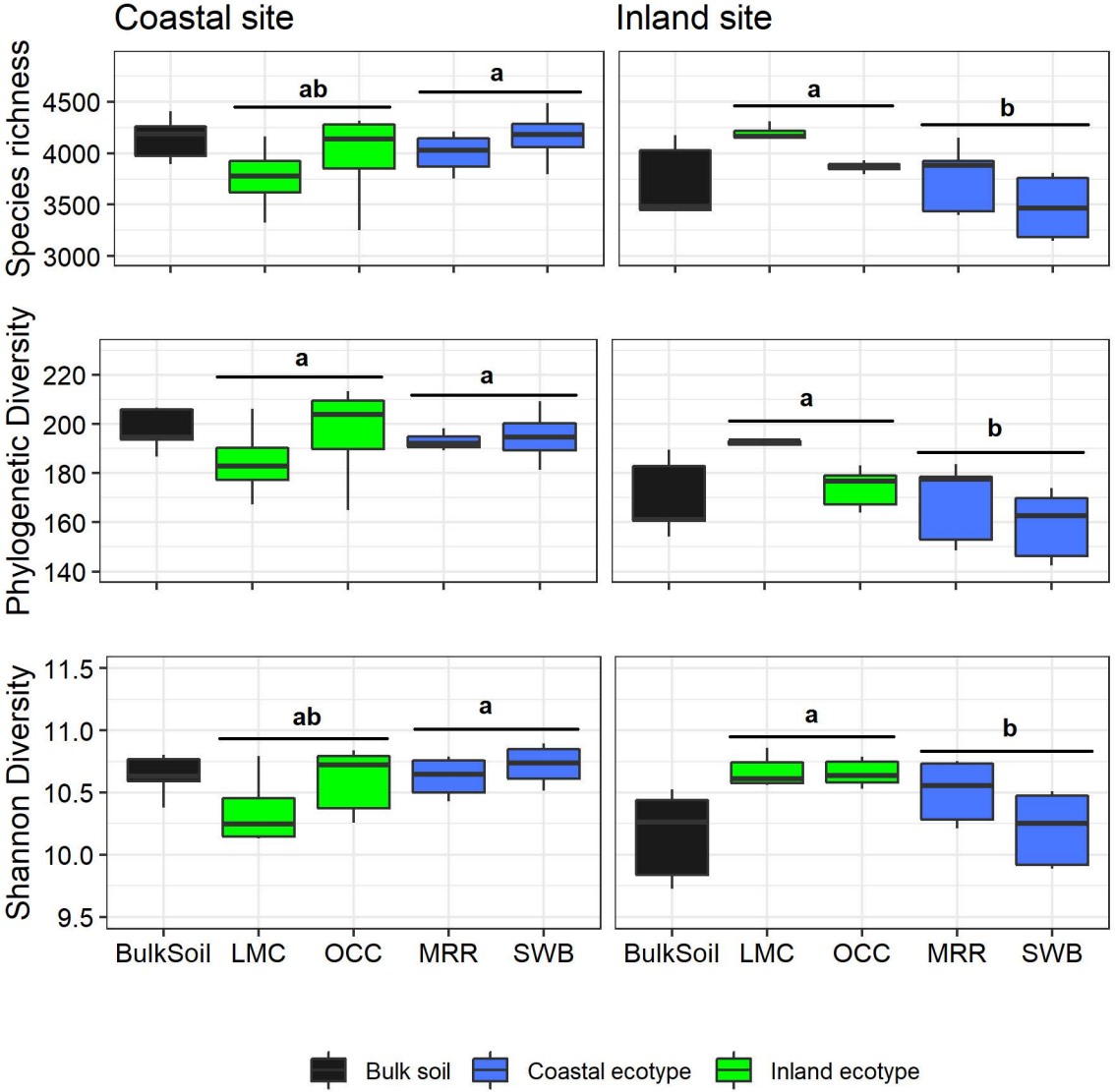
735 **Figure 4.** Presence and relative abundance of microbial OTUs in each ecotype rhizosphere and bulk soil
736 at the inland and coastal sites. Labels indicate the number of OTUs unique to a given set, as well as the
737 mean relative abundance of those OTUs across the full dataset.

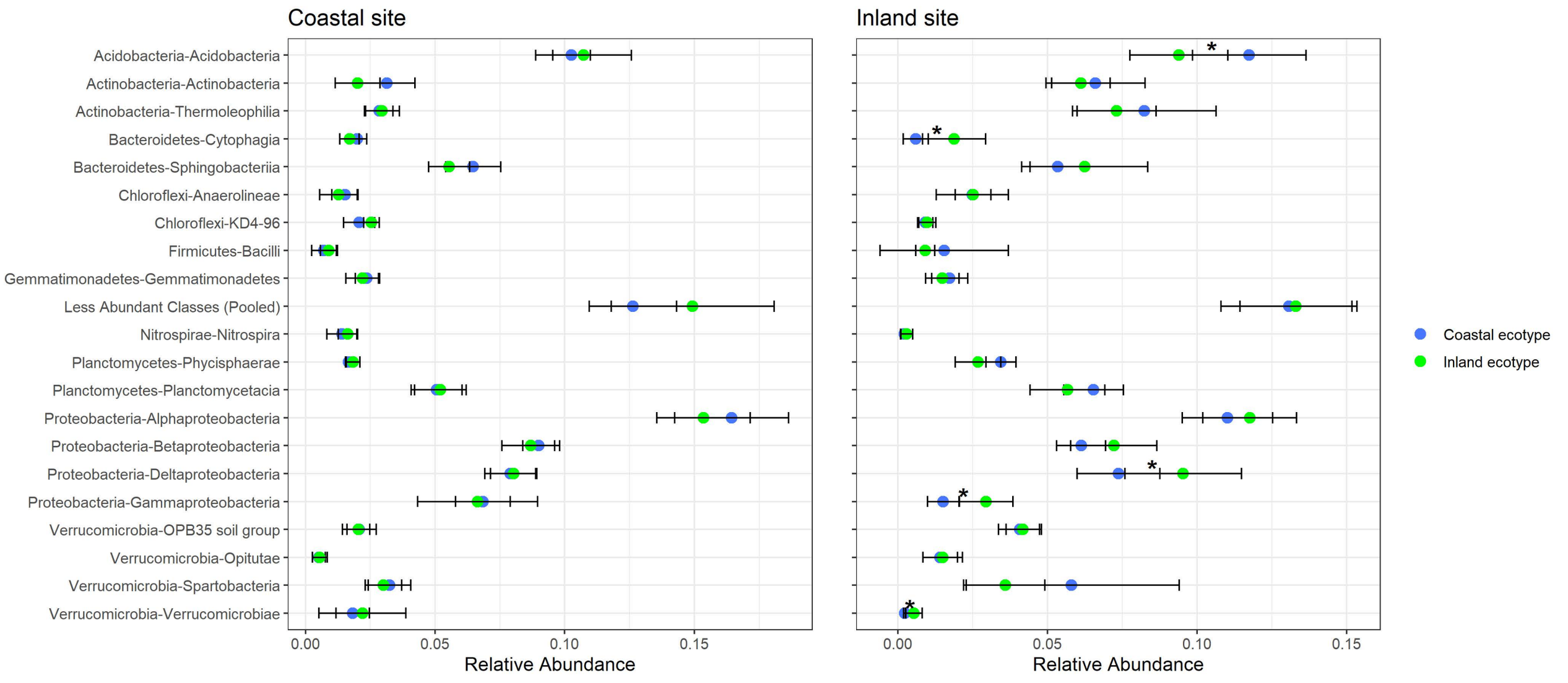
738

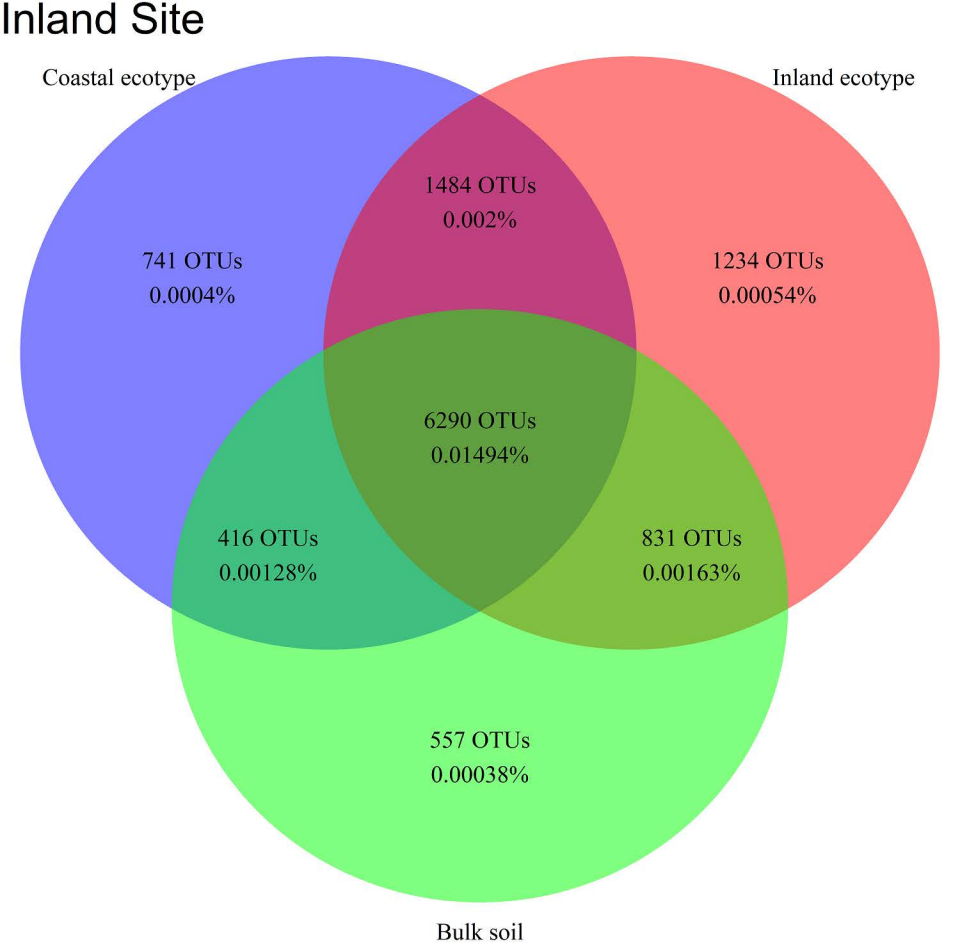
739 **Supplementary Figure 1**. Shoot and root biomass of coastal (genotypes MRR, SWB) and inland
740 (genotypes LMC, OCC) ecotypes of *Mimulus guttatus* planted in two environments. For each biomass

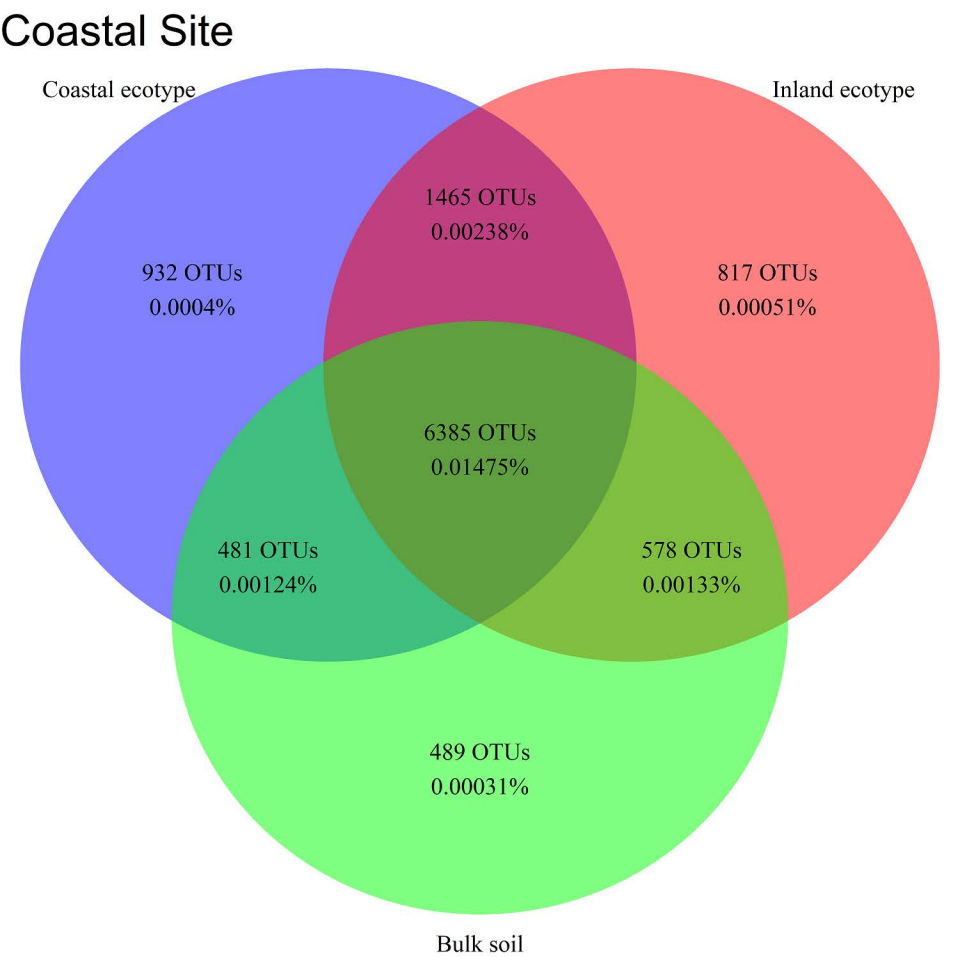
741 variable, ecotype-site combinations that significantly differed by the Tukey post-hoc test are indicated by
742 a different letter above the black lines.
743
744 **Supplementary Figure 2.** Relative abundance (mean ± SD) of the top 20 most abundant bacterial and
745 archaeal classes in the rhizospheres of coastal (MRR, SWB) and inland (LMC, OCC) genotypes of
746 *Mimulus guttatus* planted in two environments. Less abundant taxa were pooled into a single group
747 ("Less Abundant Classes"). None of the taxa depicted here significantly differed between genotypes at
748 either site.
749
750 **Supplementary Figure 3**. Relative abundance (mean ± SD) of the top 20 most abundant bacterial and
751 archaeal classes in bulk soil and rhizosphere communities of *Mimulus guttatus* planted in two
752 environments. Less abundant taxa were pooled into a single group ("Less Abundant Classes"). Taxa
753 which significantly differed between a specific ecotype and bulk soil are indicated by an asterisk.
754
755

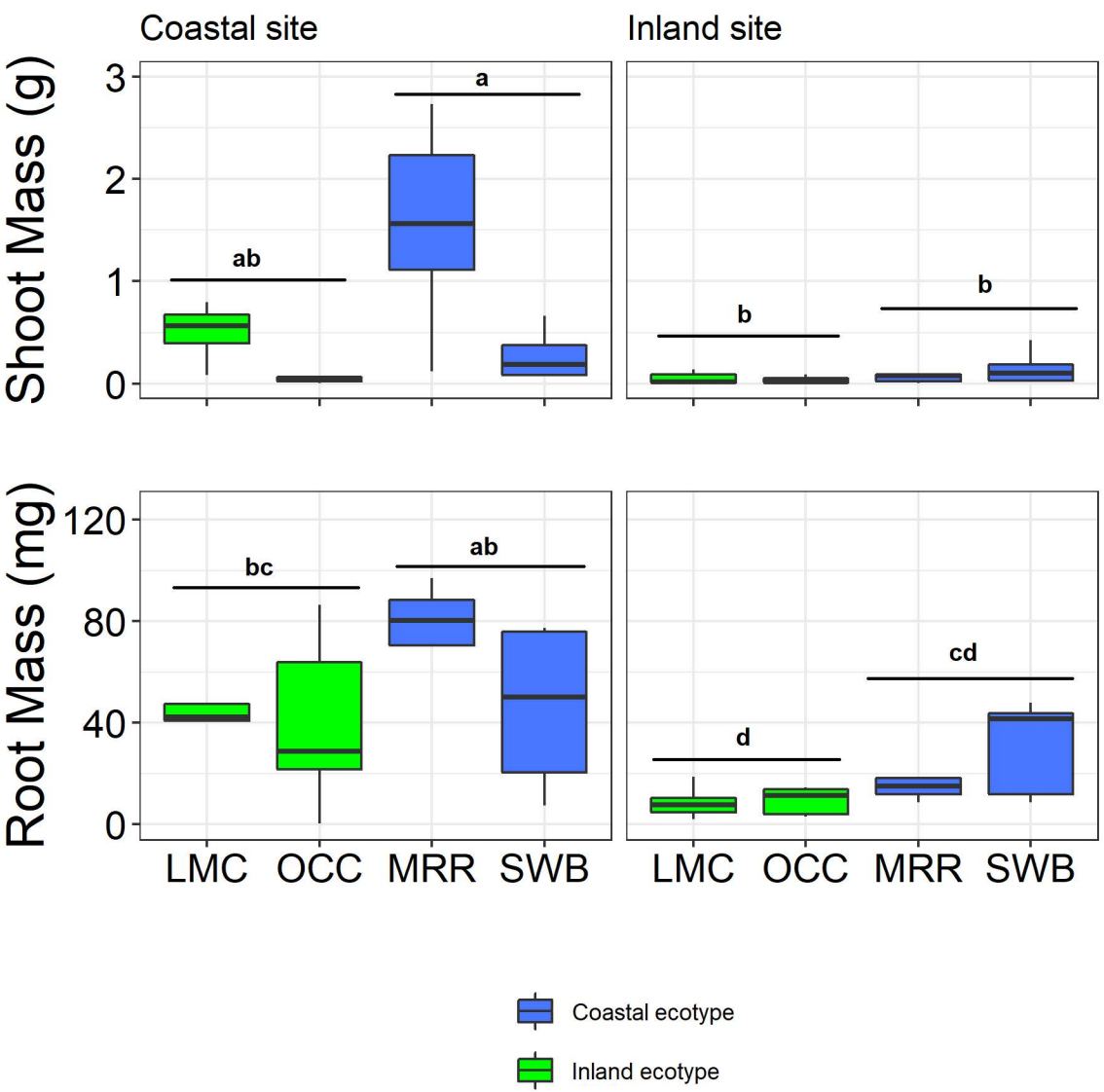

A
PC2- 13.9%
PC1- 45.8%
0.1
0.0
-0.1
-0.2
-0.1
0.0
0.1
0.2
NO3
pH
Cu
Mg
OM
Na
PercMois
Coastal site
Bulk Soil
LMC (Inland ecotype)
OCC (Inland ecotype)
MRR (Coastal ecotype)
SWB (Coastal ecotype)
Inland site
Bulk Soil
LMC (Inland ecotype)
OCC (Inland ecotype)
MRR (Coastal ecotype)
SWB (Coastal ecotype)
B
PC2-6.7%
PC1-28.9%
0.2
0.0
-0.2
-0.2
0.0
0.2
pH
Ca
Mg
Cu
NO3
OM
K
PercMois
Na
P
S
Coastal site
Bulk Soil
LMC (Inland ecotype)
OCC (Inland ecotype)
MRR (Coastal ecotype)
SWB (Coastal ecotype)
Inland site
Bulk Soil
LMC (Inland ecotype)
OCC (Inland ecotype)
MRR (Coastal ecotype)
SWB (Coastal ecotype)

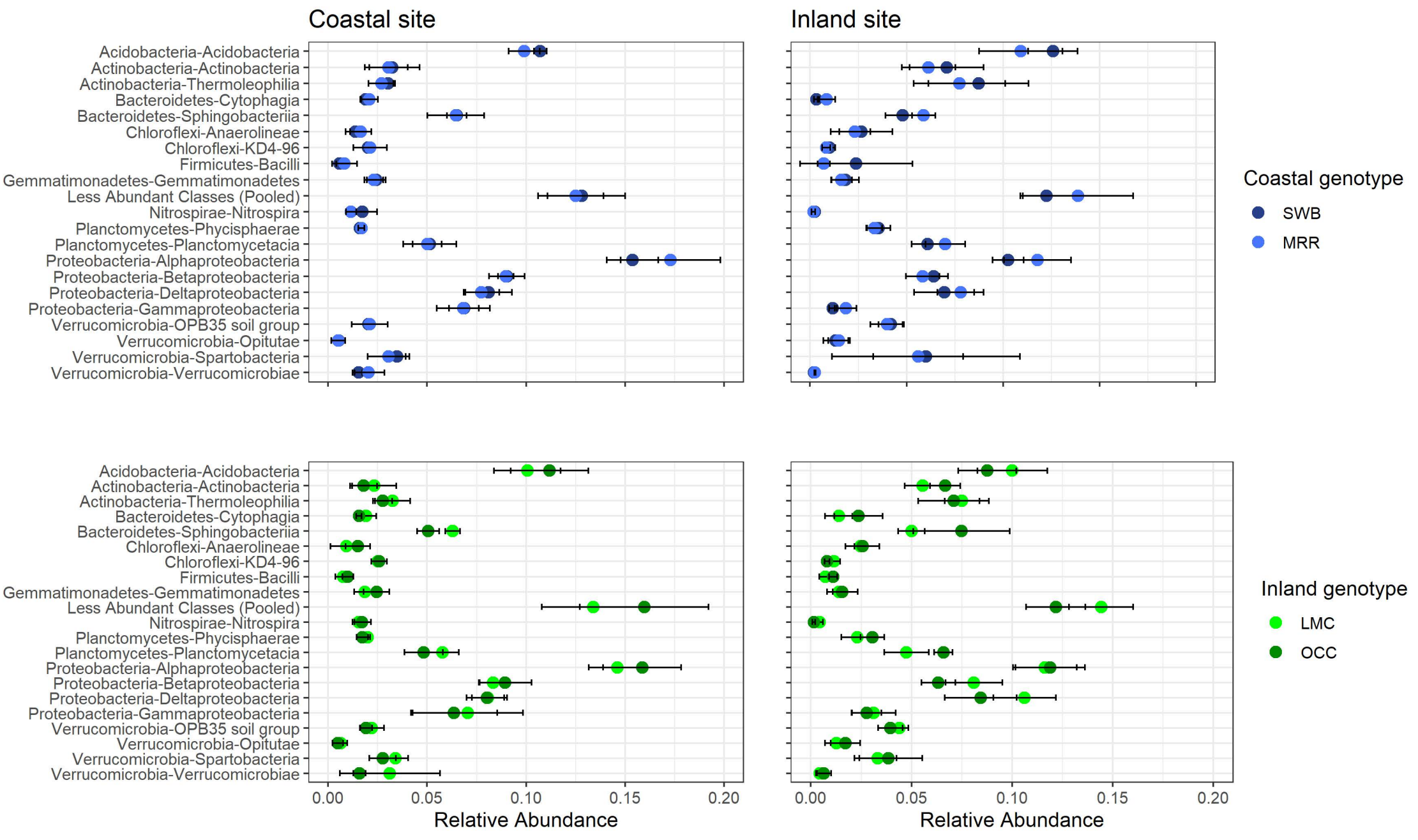

Coastal site
Inland site
Species richness
4500
4000
3500
3000
ab
a
a
b
Phylogenetic Diversity
220
200
180
160
140
a
a
a
b
Shannon Diversity
11.5
11.0
10.5
10.0
9.5
ab
a
a
b
BulkSoil
LMC
OCC
MRR
SWB
BulkSoil
LMC
OCC
MRR
SWB
Bulk soil
Coastal ecotype
Inland ecotype

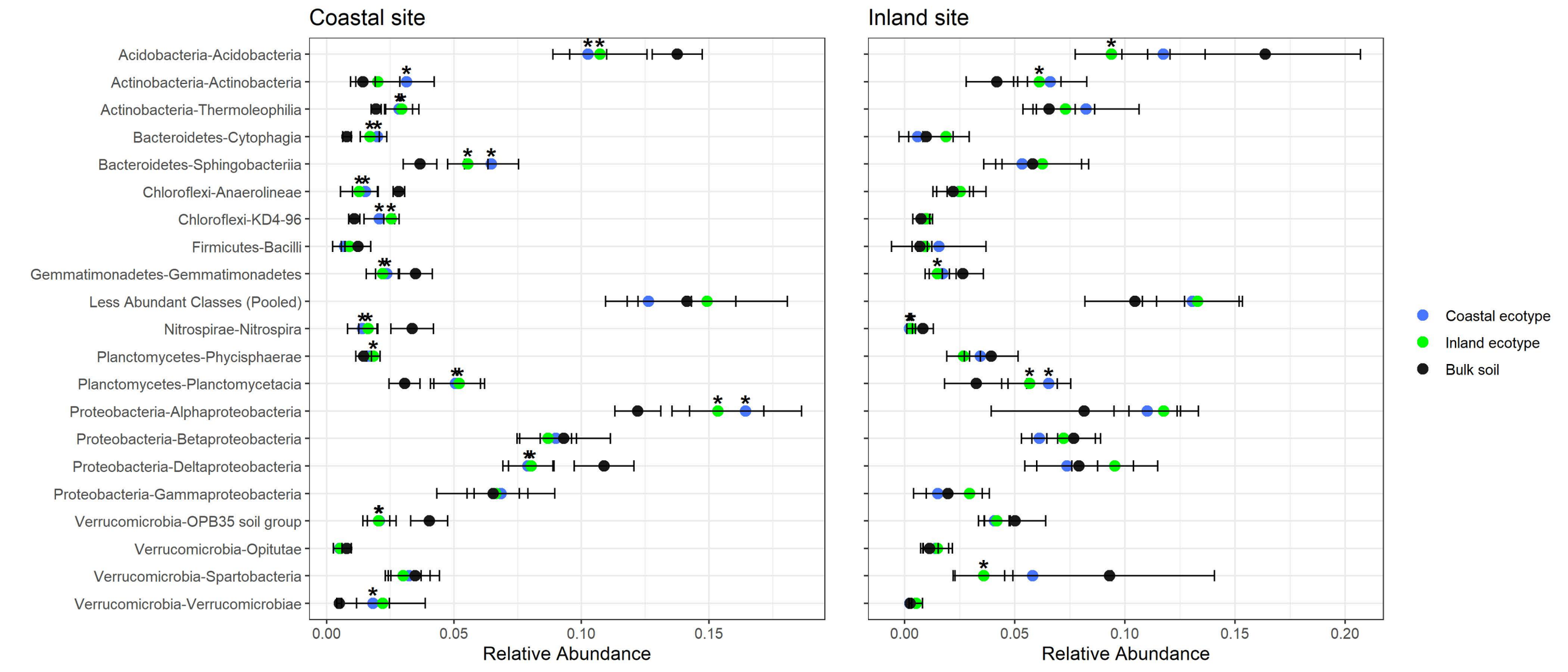

Coastal site
Inland site
Acidobacteria-Acidobacteria
Actinobacteria-Actinobacteria
Actinobacteria-Thermoleophilia
Bacteroidetes-Cytophagia
Bacteroidetes-Sphingobacteriia
Chloroflexi-Anaerolineae
Chloroflexi-KD4-96
Firmicutes-Bacilli
Gemmatimonadetes-Gemmatimonadetes
Less Abundant Classes (Pooled)
Nitrospirae-Nitrospira
Planctomycetes-Phycisphaerae
Planctomycetes-Planctomycetacia
Proteobacteria-Alphaproteobacteria
Proteobacteria-Betaproteobacteria
Proteobacteria-Deltaproteobacteria
Proteobacteria-Gammaproteobacteria
Verrucomicrobia-OPB35 soil group
Verrucomicrobia-Opitutae
Verrucomicrobia-Spartobacteria
Verrucomicrobia-Verrucomicrobiae
0.00
0.05
0.10
0.15
Relative Abundance
Coastal ecotype
Inland ecotype


Inland Site
Coastal ecotype
Inland ecotype
741 OTUs
0.0004%
1484 OTUs
0.002%
1234 OTUs
0.00054%
6290 OTUs
0.01494%
416 OTUs
0.00128%
831 OTUs
0.00163%
557 OTUs
0.00038%
Bulk soil


Coastal Site
Coastal ecotype
Inland ecotype
932 OTUs
0.0004%
1465 OTUs
0.00238%
817 OTUs
0.00051%
6385 OTUs
0.01475%
481 OTUs
0.00124%
578 OTUs
0.00133%
489 OTUs
0.00031%
Bulk soil


Coastal site
Inland site
Shoot Mass (g)
3
2
1
0
a
ab
b
b
Root Mass (mg)
120
80
40
0
bc
ab
cd
d
LMC
OCC
MRR
SWB
LMC
OCC
MRR
SWB
Coastal ecotype
Inland ecotype


Coastal site
Inland site
Acidobacteria-Acidobacteria
Actinobacteria-Actinobacteria
Actinobacteria-Thermoleophilia
Bacteroidetes-Cytophagia
Bacteroidetes-Sphingobacteriia
Chloroflexi-Anaerolineae
Chloroflexi-KD4-96
Firmicutes-Bacilli
Gemmatimonadetes-Gemmatimonadetes
Less Abundant Classes (Pooled)
Nitrospirae-Nitrospira
Planctomycetes-Phycisphaerae
Planctomycetes-Planctomycetacia
Proteobacteria-Alphaproteobacteria
Proteobacteria-Betaproteobacteria
Proteobacteria-Deltaproteobacteria
Proteobacteria-Gammaproteobacteria
Verrucomicrobia-OPB35 soil group
Verrucomicrobia-Opitutae
Verrucomicrobia-Spartobacteria
Verrucomicrobia-Verrucomicrobiae
Coastal genotype
SWB
MRR
Inland genotype
LMC
OCC
0.00
0.05
0.10
0.15
0.20
Relative Abundance


Coastal site
Inland site
Acidobacteria-Acidobacteria
Actinobacteria-Actinobacteria
Actinobacteria-Thermoleophilia
Bacteroidetes-Cytophagia
Bacteroidetes-Sphingobacteriia
Chloroflexi-Anaerolineae
Chloroflexi-KD4-96
Firmicutes-Bacilli
Gemmatimonadetes-Gemmatimonadetes
Less Abundant Classes (Pooled)
Nitrospirae-Nitrospira
Planctomycetes-Phycisphaerae
Planctomycetes-Planctomycetacia
Proteobacteria-Alphaproteobacteria
Proteobacteria-Betaproteobacteria
Proteobacteria-Deltaproteobacteria
Proteobacteria-Gammaproteobacteria
Verrucomicrobia-OPB35 soil group
Verrucomicrobia-Opitutae
Verrucomicrobia-Spartobacteria
Verrucomicrobia-Verrucomicrobiae
0.00
0.05
0.10
0.15
0.20
Relative Abundance
Coastal ecotype
Inland ecotype
Bulk soil

Sample	Genotype	Ecotype	Site	Raw reads	Merged	Quality-
Mim109_S9(	LMC	Inland	Inland	63237	51320	49780
Mim116_S6{	BulkSoil	Inland	Inland	92117	73507	71962
Mim11_S62_	MRR	Coastal	Inland	46370	38304	37339
Mim124_S9(	BulkSoil	Inland	Inland	116335	84850	83322
Mim129_S12	BulkSoil	Inland	Inland	105007	82453	80943
Mim12_S86_	SWB	Coastal	Inland	52997	43197	42024
Mim132_S24	BulkSoil	Inland	Inland	38412	31631	30985
Mim139_S7(	BulkSoil	Inland	Inland	100894	80074	78468
Mim141_S11	MRR	Coastal	Coastal	58560	48934	47545
Mim143_S13	MRR	Coastal	Coastal	60010	49464	48038
Mim151_S16	SWB	Coastal	Coastal	62187	51106	49403
Mim152_S18	SWB	Coastal	Coastal	68668	54072	52321
Mim161_S2(	MRR	Coastal	Coastal	60611	50945	49584
Mim162_S44	MRR	Coastal	Coastal	51918	44253	42805
Mim172_S6{	SWB	Coastal	Coastal	53640	45224	43764
Mim176_S92	SWB	Coastal	Coastal	58541	48967	47434
Mim181_S11	OCC	Inland	Coastal	41296	35130	34216
Mim182_S14	OCC	Inland	Coastal	59776	50001	48556
Mim190_S16	OCC	Inland	Coastal	69430	57929	56135
Mim191_S18	LMC	Inland	Coastal	72317	57145	55507
Mim1_S14_L	OCC	Inland	Inland	49512	40082	39064
Mim212_S46	MRR	Coastal	Coastal	51561	42694	41225
Mim21_S11(	MRR	Coastal	Inland	55008	45426	44259
Mim224_S7(	OCC	Inland	Coastal	66714	54150	52402
Mim228_S94	OCC	Inland	Coastal	59663	47589	45990
Mim22_S134	LMC	Inland	Inland	50349	41776	40751
Mim237_S11	LMC	Inland	Coastal	62680	50855	49394
Mim238_S14	LMC	Inland	Coastal	63685	50765	49207
Mim250_S16	OCC	Inland	Coastal	70053	55438	53699
Mim255_S19	LMC	Inland	Coastal	79122	58754	57139
Mim261_S11	BulkSoil	Coastal	Coastal	82406	64675	63320
Mim266_S35	BulkSoil	Coastal	Coastal	50466	40915	40032
Mim271_S23	BulkSoil	Coastal	Coastal	146584	117936	115290
Mim277_S57	BulkSoil	Coastal	Coastal	32041	26314	25771
Mim286_S46	BulkSoil	Coastal	Coastal	31647	24798	24312
Mim2_S38_L	MRR	Coastal	Inland	50566	42241	41072
Mim31_S158	SWB	Coastal	Inland	61825	50348	49068
Mim32_S182	OCC	Inland	Inland	66083	51228	49959
Mim46_S16_	MRR	Coastal	Inland	56211	44264	42852
Mim47_S40_	SWB	Coastal	Inland	39488	31807	30673
Mim57_S64_	SWB	Coastal	Inland	51506	41369	39971
Mim58_S88_	MRR	Coastal	Inland	67059	52610	50884
Mim66_S112	OCC	Inland	Inland	56586	45340	44001
Mim67_S136	OCC	Inland	Inland	62529	48854	47287

Mim83_S160	LMC	Inland	Inland	71604	55996	54117
Mim87_S184	LMC	Inland	Inland	71278	52525	50915
Mim93_S18_	LMC	Inland	Inland	63201	49735	48174
Mim97_S42_	SWB	Coastal	Inland	56107	45840	44257
Mim98_S66_	OCC	Inland	Inland	61172	50265	48727

Reads	Reads with	Number of
47011	46016	4313
64617	63061	3483
34042	33247	4152
69373	68712	4176
75361	74387	4028
38531	38240	3464
27227	27072	3450
69238	68668	3450
43742	41276	4144
44579	41478	4211
46107	44204	4220
47939	46362	3794
46352	37118	3867
39232	37593	4027
39257	37506	4486
44113	42728	4144
30509	26606	4060
44174	40140	4296
51743	46358	4219
51383	44841	4164
37083	35412	3932
37481	28804	3756
41240	40443	3881
49053	45290	3786
43827	40795	3255
37018	35709	3903
45946	42477	3328
46141	43312	3717
49845	47165	4317
53730	45962	3845
56527	55997	4191
36119	35584	3896
96371	94669	4410
23038	22620	4261
23093	22354	3975
38669	37001	3923
45898	44978	3757
47155	45658	3894
39596	39181	3437
27629	27143	3151
37125	36927	3182
49133	48596	3396
40827	37989	3796
44623	43964	3870

50772	49803	4150
47839	47005	4216
45016	44044	4161
41672	40995	3807
45826	44653	3846

Soil Variable	Axis 1 Scores	Axis 2 Scores	r2	p-value
pH	0.81496	0.57952	0.2066	0.006
Phosphorous	-0.93741	-0.34824	0.924	0.001
Potassium (p	0.96679	0.25559	0.915	0.001
Calcium (ppn	0.95878	0.28416	0.9194	0.001
Magnesium (	0.9747	0.22351	0.8675	0.001
Copper (ppm	0.9497	0.31317	0.9048	0.001
Percent Orga	0.97922	0.2028	0.2775	0.004
Sodium (ppm	-0.94855	-0.31663	0.9056	0.001
Nitrate (ppm	0.82135	0.57043	0.4514	0.001
Ammonium (	0.03331	-0.99945	0.0172	0.678
Percent Mois	-0.96437	-0.26455	0.7961	0.001
Total-N (%)	0.98011	0.19844	0.1853	0.012
Sulfur (ppm)	-0.93685	-0.34973	0.5296	0.001

Soil Variable	Axis 1 Scores	Axis 2 Scores	r2	p-value
pH	-0.87326	0.48725	0.2035	0.003
Phosphorous	0.99918	-0.04037	0.9512	0.001
Potassium (p	-0.99726	0.07399	0.9563	0.001
Calcium (ppn	-0.99674	0.08069	0.9611	0.001
Magnesium (	-0.99586	0.09085	0.916	0.001
Copper (ppm	-0.99991	0.01373	0.9446	0.001
Percent Orga	-0.9608	-0.27724	0.3009	0.001
Sodium (ppm	0.99983	-0.01833	0.9376	0.001
Nitrate (ppm	-0.97781	-0.20952	0.4635	0.001
Ammonium (	0.04147	-0.99914	0.0124	0.738
Percent Mois	0.99666	0.08166	0.8334	0.001
Total-N (%)	-0.90286	-0.42994	0.2162	0.005
Sulfur (ppm)	0.97985	-0.19975	0.5404	0.001