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REGULAR ARTICLE

Bacterial communities differ between plant species and soil type, and differentially influence seedling establishment on serpentine soils

Alexandria N. Igwe · **Rachel L. Vannette**

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Abstract

Background and aim Root-associated microbial communities influence plant phenotype, growth and local abundance, yet the factors that structure these microbial communities are still poorly understood. California landscapes contain serpentine soils, which are nutrient-poor and high in heavy metals, and distinct from neighboring soils making them ideal for studying the factors that structure root microbiomes and their functions.

Method Here, we surveyed the rhizoplane of serpentine-indifferent plants, which grow on and off serpentine soil, to determine the relative influence of plant identity and soil chemistry on rhizoplane microbial community structure using 16S rRNA metabarcoding. Additionally, we experimentally examined if serpentine vs. non-serpentine microorganisms differentially affected plant growth in serpentine soil.

Results Rhizoplane bacterial communities differed among plant species, soil types, and the interaction between them in both the field and experimental soils. In the experiment, soil microbial community source influenced seedling survival, but plant growth phenotypes were largely invariant to microbial community with a few exceptions.

Conclusions Rhizosplane bacterial species composition differed between plant species and soil types, and Amplicon Sequence Variants (ASVs) from the phyla Acidobacteria and Proteobacteria (Genus: *Microvirga*) were characteristic of serpentine soils. While soil microbial community composition influenced seedling survival in the current study, further study is required to disentangle the role of microbial associations and plant tolerance to serpentine.

Keywords Plant-microbe interaction · Bodenvag · Biodiversity · Rhizoplane · Bacterial community · Plant-soil feedback

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A. N. Igwe (✉) · R. L. Vannette
Entomology and Nematology Department, University of California, Davis, One Shields Ave, Davis, CA 95616, USA
e-mail: aigwe@ucdavis.edu

Introduction

Root-associated microbial communities are important mediators of plant traits, often through effects on soil processes. For example, microorganisms can accelerate nutrient cycling (Baker et al. 2018) or fix nitrogen (Boyd and Peters 2013; Mus et al. 2016), which increases plant nutrition (Shakeel et al. 2015), and influences global nutrient cycles (Finzi et al. 2015). Further, root-associated microorganisms can alter plant traits such as disease tolerance (Santhanam et al. 2015), root architecture (Zhou et al. 2016) and drought tolerance (Lau and Lennon 2011). Despite the importance of belowground microbes, the factors that contribute to the structure of root-associated microbial communities are diverse and their relative influence across scales are poorly understood.

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Both plant identity and soil chemistry influence root microbiome composition (Burns et al. 2015; Erlandson et al. 2018; Leff et al. 2018), but their relative influence remains difficult to estimate. Plants can influence root-associated microbial communities by exuding carbon-rich compounds from their roots, which can select for beneficial plant-growth-promoting bacteria (Badri and Vivanco 2009; Chaparro et al. 2013) and in some cases, can feed back to increase plant biomass (Lally et al. 2017). Root-associated microbes can contribute to environmental tolerance of plant hosts via processes such as phosphorus solubilization (Govindasamy et al. 2010; Khan et al. 2014), production of extracellular polymeric substances (EPS) (Glick 2012), and metal complexation (Tak et al. 2013). Most work to date has examined soil or rhizosphere microbiome composition, but microbial communities on the immediate surface of the root (rhizoplane) can provide protection against soil-borne pathogens (Deora et al. 2005; Islam et al. 2005) and are an important barrier between microbial communities that reside in the area surrounding the root (rhizosphere) and area within the root (endosphere) communities (Edwards et al. 2015). Additionally, microbes in the rhizoplane may be more closely linked to plant benefits, including drought tolerance (Fitzpatrick et al. 2018) than microbes found in the rhizosphere. On the other hand, soil physical and chemical properties can also be strong drivers of rhizosphere and, subsequently, rhizoplane microbial community composition (Edwards et al. 2015), particularly on large scales or across soils that vary widely in composition. For example, pH (Bartram et al. 2014; Wu et al. 2017), salinity (Sardinha et al. 2003; Dillon et al. 2013), drought (Barnard et al. 2013; Chodak et al. 2015), and the presence of heavy metals (Wood et al. 2016) all influence soil and root-associated microbial community structure. Still, the relative importance of plant identity and soil chemistry across soil gradients remains difficult to predict in large part because plant species are often restricted to particular soil types.

Both soil and plant identity can shape not only structure but also function of root-associated microbial communities. Soil stressors, including drought, can promote the development of locally adapted microorganisms (Hawkes and Keitt 2015) which have been shown to be important for plant growth (Lau and Lennon 2012; Revillini et al. 2016). On the other hand, plant-induced changes in soil parameters, including microbial communities, can feed back to impact plant growth and microbial communities (Kulmatiski et al. 2008; Rúa et al. 2016). By associating with microbes from their home soil environment, plants are sometimes able to gain a greater fitness advantage than if they associated with microbes from a different soil environment (Rúa et al. 2016; Gehring et al. 2017), but plants do not always benefit from 'home' microbes (Doherty et al. 2008). Overall, there is still much to be learned about the role of locally adapted microorganisms for plant growth, particularly if, when and how they may influence plant hosts.

Serpentine soils are an ideal system in which to examine local adaptation and the relative contribution of plant identity and soil chemistry to microbial community structure. Serpentine soils are characterized by high concentrations of heavy metals including chromium, nickel, and cobalt, high concentrations of magnesium and iron, and low concentrations of essential plant nutrients (Safford et al. 2005). Combined, such characteristics contribute to poor plant productivity and high rates of plant endemism in serpentine soils (Brady et al. 2005). Most plants are unable to grow in serpentine soil, but there are a group of serpentine-indifferent plants that are able to grow in both serpentine and non-serpentine soils (Anacker 2014). By examining the microbial communities colonizing serpentine-indifferent plants on both serpentine and non-serpentine soils, we sought to determine the relative importance of plant identity and soil type on rhizoplane bacterial composition and role in growth and survival on serpentine soils.

We hypothesized that serpentine-indifferent plants host distinct microbiomes depending on both the plant species and soil type (non-serpentine or serpentine) and that locally adapted serpentine microorganisms influence plant growth on serpentine soil. We used a field survey and lathhouse experiment to answer the following questions: (1) Do serpentine-indifferent plants associate with similar microbial communities on serpentine or non-serpentine soil? (2) Is plant identity or soil chemistry the greater source of variation for soil rhizoplane bacterial communities? (3) Do microbes isolated from serpentine soils influence plant survival or growth on serpentine soil?

Materials and methods

Study system

Serpentine and non-serpentine soils for field samples were collected at two sites: McLaughlin Natural

Reserve (McLaughlin) and Hopland Research and Extension Center (Hopland). Both sites are characterized by a Mediterranean climate and hot and dry summers from April to October. Serpentine soils at McLaughlin are comprised by Henneke soil series with some Montara and Okiota series. The Henneke soils generally support chaparral, while grasslands are supported by Montara and Okiota. Hopland serpentine soil in this region is typically fine-loamy, mixed, active, mesic Typic Haploxeralfs. Representative serpentine and non-serpentine soils were sent to A&L Western Laboratories, Inc. (Modesto, CA) for testing using the S1B and S4 test packages to verify that chosen sites differed in soil chemistry, as these soils were also used in the lathhouse experiment (Supplementary Table 2).

Plant species were chosen based on their Serpentine Affinity Mean (SAM) (Safford et al. 2005), where plants with high SAM are typically serpentine endemic and rarely thrive outside of serpentine soil due to competition (Anacker 2014). In this study, we focused on California native, annual herbs that are serpentine-indifferent (SAM = 1) *Collinsia sparsiflora, Trifolium fucatum, Gilia tricolor, Plantago erecta, Trifolium willdenovii,* and *Gilia capitata* (Table 1; The Calflora Database (2016)), which occur on both serpentine and non-serpentine soils. Because plant species sampled from field sites were not available for purchase and field-collected seeds germinated poorly, we used congeners of plants sampled in the field including *P. erecta*, *T. willdenovii*, and *G. capitata* grown from seeds purchased from S&S Seeds (Carpinteria, CA) for our experiment (below).

Field survey

Plantago erecta, *T. fucatum*, *C. sparsiflora* and *G. tricolor* were collected on serpentine and non-serpentine plots at Hopland and McLaughlin in late March and early April 2017. Full sampling details are contained in Supplementary Methods 1. Briefly, plots within each site were chosen based on the presence and co-occurrence of serpentine-indifferent plant species, with each plant species sampled from at least 2 plots (Supplementary Fig. 1 and Supplementary Table 1). Plants were collected by excavating the whole plant, placing it into a 50-mL centrifuge tube and immediately putting the tube on ice. A garden trowel was used for sample collection and cleaned thoroughly with 70% ethanol between collections. Samples were stored at −20 °C until processing and DNA extraction, outlined below.

Lathhouse experiment

We altered soil microbial presence and source to examine if soil microbial communities influence plant establishment or growth on serpentine. Plants were grown in a partially shaded open-air propagation area (lathhouse). Soils for the experiment were collected from three serpentine and three non-serpentine plots at McLaughlin in late May 2016 where dense populations of *P. erecta*, *G. tricolor*, or *C. sparsiflora* were growing (Supplementary Fig. 1). Soils within each treatment were homogenized before planting (Supplementary Table 1).

To examine if serpentine microorganisms contribute to plant growth and survival on serpentine soils, plants were grown individually in one of four soil treatments: 1) live serpentine (serpentine), 2) autoclaved serpentine soil (autoclaved), 3) autoclaved serpentine soil amended with a serpentine microbial slurry (SS), 4) autoclaved serpentine soil amended with a non-serpentine microbial slurry (NSS). Slurries were prepared by extracting 3 kg of serpentine and non-serpentine soils separately with 4 L of autoclaved deionized water. Before planting, slurry solutions were added to autoclaved serpentine soil, thoroughly mixed in a large plastic tub and incubated at room temperature for 1 week. Live serpentine soil collected from the field was placed in a third tub and autoclaved serpentine soil from the field was placed in the fourth and final tub. Soils for the autoclaved treatment were sterilized at 121 °C for 60 min, sat overnight, then were autoclaved a second time (Dilly et al. 2004; Maignien et al. 2014; UNL Environmental Health and Safety 2018).

Soils were added to D16 deepots (262 ml) that were plugged with a paper towel, with each treatment replicated 24 times for a total of 288 pots (3 plant species × 4 soil treatments × 24 replicates). Three unplanted deepots containing each soil type was used to represent bulk soil. Seeds were vapor sterilized for 20 h (Clough and Bent 1998), and refrigerated in sterile petri dishes until germination. One seedling per pot was placed on damp soil and covered with dry potting soil. Seedlings that did not survive were replaced after 7 days, up to 6 weeks into the experiment. Every week we recorded seedling survival and the number of leaves on each plant. Shoots and roots were harvested after 11 weeks of growth.

Table 1 Plant characteristics of serpentine-indifferent plants

Scientific Name	Common Name	Family	Growing Season (months)	Bloom	SAM	Field	LH
C. sparsiflora	Spinster's blue-eyed Mary	Plantaginaceae	3 to 9	March through June	1.7	X	
G. capitata	Blue-thimble flower	Polemoniaceae	2 to 12	February through April	1.6		X
G. tricolor	Bird's eye gilia	Polemoniaceae	4 to 9	April through August	-	X	
P. erecta	California plantain	Plantaginaceae	4 to 12	March through April	1.0	X	X
T. fucatum	Bull clover	Fabaceae	4 to 12	April through June	1.3	X	
T. willdenovii	Tomcat clover	Fabaceae	3 to 12	April through March	1.3		X

SAM = Serpentine Affinity Mean; *LH* = Lathhouse

Roots were harvested over clean parchment paper, with roots shaken to remove loosely adhering soil. The total length of the plant was collected by measuring from the root tip to the apical meristem. Roots and shoots were separated, then aboveground plant height and maximum root length measured. Shoots were weighed immediately to determine wet weight then dried at 55 °C for at least 48 h and dry mass recorded. Roots were placed into a centrifuge tube and stored at −20 °C until processing.

Rhizoplane soil collection

Rhizoplane soil was collected from field-sampled and lathhouse-sampled roots. Briefly, roots were shaken in a 0.9% (*w*/*v*) NaCl and autoclaved water solution in either 15-ml or 50-mL tube depending on root size. Tubes were shaken on a lateral shaker at 10 strokes per minute (spm) for 90 min, then roots were removed, dried and massed. The roots were removed from the tubes after shaking and placed into a clean centrifuge tube of the same size as the original. To obtain the rhizoplane soil, 10 ml of the 0.1% (*v*/*v*) Tween80 in 0.9%NaCl solution was added to a 15-mL tube or 20 mL of the Tween80 solution was added to a 50-mL tube depending on the size of the root (Barillot et al. 2012). The tube and solution were shaken on a lateral shaker at 10 spm for 90 min. After shaking, the roots were removed from the tube and placed into a labeled coin envelope, dried at 55 °C for at least 48 h, then weighed. The tubes containing rhizoplane soil were centrifuged at 200 g for 10 min. The supernatant was discarded, and the pellet used to extract DNA using ZR Soil Microbe DNA MicroPrep following manufacturer's instructions (Zymo Research, Irvine, CA).

Library prep and sequencing

From DNA extracts, the V4 region of the 16S SSU rRNA was amplified using primers 515F-806R (515F: 5′ - GTGCCAGCMGCCGCGGTAA - 3′; 806R: 5′ - GGACTACHVGGGTWTCTAAT - 3′) (Caporaso et al. 2011). PCR was carried out in 25 μl reactions including 1 μl genomic DNA, 0.5 μl of each 10 μM primer, 12.5 μl of MyTaq Hot Start Red Mix (Bioline), and 10.5 μl of dH_2O. PCR reactions were set up on ice to minimize non-specific amplification and primer dimerization. PCR conditions were: denaturation at 94 °C for 2 min; 34 amplification cycles of 30 s at 94 °C, 30 s at 51 °C and 30 s at 72 °C; followed by a 10 s final extension at 72 °C. PCR products were visualized using gel electrophoresis and successful samples cleaned using Carboxyl-modified Sera-Mag Magnetic Speedbeads in a PEG/NaCl buffer (Rohland and Reich 2012).

Cleaned PCR products were quantified using the Qubit hs-DS-DNA kit (Invitrogen, Carlsbad CA), pooled in equimolar concentration and sequenced by the Genome Center at UC Davis on the Illumina MiSeq platform (500 cycles v2 PE250). Raw sequences are available at https://www.ncbi.nlm.nih.gov/sra/SRP152892.

Bioinformatics

Amplicon sequence variants (ASVs) from 16S rRNA amplicons were identified using DADA2 (v1.7.2) (Callahan et al. 2016a). Briefly, paired-end fastq files were processed by filtering and truncating forward and reverse reads at position 200. Sequences were dereplicated, merged and error-corrected. Chimeras were removed, and the taxonomy assigned using the SILVA database (v128) (Quast et al. 2012; Yilmaz et al. 2014; Glöckner et al. 2017). A phylogenetic tree

based on 16S sequences was created using the DECIPHER package (v2.8.1) in R to perform multi-step alignment and phangorn (v2.4.0) to construct the tree (Schliep 2011; Wright 2016). The sequence table and taxonomy, phylogenetic tree and metadata, were combined into a phyloseq object and used for further analysis (phyloseq v1.22.3) (McMurdie and Holmes 2013; Callahan et al. 2016b).

Using phyloseq, the mitochondria and chloroplast were removed from samples. Low-abundance samples (<1000 reads) were removed and the count data normalized. Count data were normalized via a relative abundance method where the proportion of counts per sample were taken against the sum of all counts. Alpha-diversity metrics were calculated on the ASV table using Shannon diversity index.

Statistical analysis

Field survey

To examine if bacterial communities differed in alpha diversity, we used ANOVA with soil type, plant species and their interaction as predictors. To visualize the similarity between groups, non-metric multidimensional scaling (NMDS) plots were created based on Bray-Curtis dissimilarity metrics (Bray and Curtis 1957; Kruskal 1964). To test for differences in multivariate dispersion among rhizoplane communities using the 'betadisper' function from the vegan (v2.5.3) package in R (Oksanen et al. 2018). To determine if soil or plant species differed in rhizoplane bacterial composition, we used the 'adonis' function from the vegan package in R (v3.4.4) with Bray-Curtis dissimilarities as the response variable and plant identity, soil type, collection site, and collection plot as predictors. We used the randomForest package (v4.6–14) to identify characteristic ASVs for each soil type (Liaw and Wiener 2002; Delgado-Baquerizo et al. 2017).

Lathhouse experiment

To determine the effects of soil treatments on seedling establishment, survival analysis was conducted on plant presence/absence data using a Cox proportional hazards regression model ('coxph') on a survival object ('Surv') in the survival package (v2.42.6) (Therneau 2015). The time, in weeks, from plant absence (seeding failure to establish after weekly seedling addition) to plant presence (successful establishment) was used as the event. Differences in the hazard ratio were visualized using 'ggforest' in the survminer package (v0.4.3) (Kassambara and Kosinski 2018).

To determine the effects of soil treatments on plant growth, each plant trait was analyzed using a general linearized model with plant species and soil treatment as predictors and differences between group means were identified using likelihood ratio tests. Tukey HSD was used as a post-hoc test to identify differences among groups.

Bacterial community alpha diversity and community composition were compared among lathhouse soil treatments using methods outlined above. Alpha diversity (Shannon index) was compared among soil treatments using ANOVA with soil type as a predictor. Community composition was visualized using NMDS based on Bray-Curtis dissimilarity metrics and analyzed using 'betadisper' function and 'adonis' function, with Bray-Curtis dissimilarity as the response variable and plant identity, soil, and week of planting as predictors.

Results

Field survey

After quality filtering and removal of non-target sequences, we recovered 4,114,382 reads (average 15,825 per sample) that were grouped into 8903 amplicon sequence variants (Supplementary Data 1). Sampling curves within samples were saturating, indicating a robust sampling of the microbial diversity associated with individual plants (Supplementary Fig. 2).

Across both soil types, microbial communities of all plants were dominated by Proteobacteria and Actinobacteria (Supplementary Fig. 3a). Serpentine rhizoplane communities were less diverse than rhizoplane communities formed on non-serpentine (Supplementary Fig. 3b; $P = 0.007$). Plant species differed from each other in rhizoplane diversity (Supplementary Fig. 3b, $P = 0.02$). Specifically, communities on *T. fucatum* were significantly less diverse than either *G. tricolor* and *P. erecta*.

Rhizoplane bacterial species composition differed among plant species (Fig. 1; $F_{4,98} = 2.97$; $P < 0.001$; $R^2 = 0.09$), soil types ($F_{1,98} = 5.33$; $P < 0.001$; $R^2 = 0.04$) and their interaction (species x soil, $F_{4,98} = 1.92$; $P = 0.004$; $R^2 = 0.07$). Plant species and soil types also

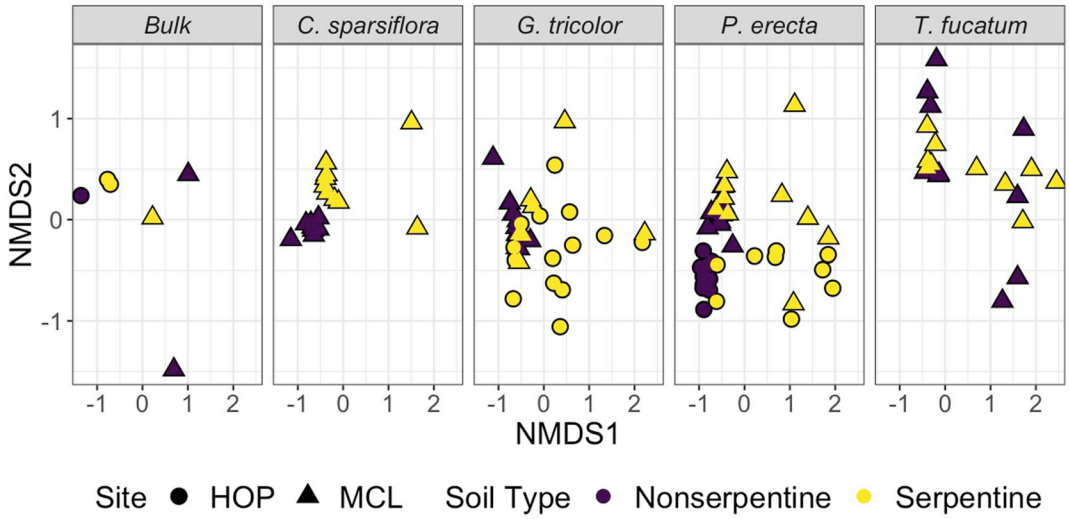

Fig. 1 Non-metric dimensional scaling (NMDS) plot of field-sampled bacterial rhizoplane communities associated with the plant species *Collinsia sparsiflora*, *Gilia tricolor*, *Plantago erecta*, and *Trifolium fucatum* using Bray-Curtis dissimilarity. Symbols indicate the sampling site (HOP = Hopland Reserve, MCL = McLaughlin Natural Reserve). Community composition was influenced by plant species identity ($F_{4,98} = 2.97$; $P < 0.001$; $R^2 =$

differed in dispersion (betadisper soil: $P = 0.01$; plant: $P = 0.01$). *Trifolium fucatum* was the only plant in the study that did not associate with distinct microbial communities when grown on disparate soil types ($P = 0.13$), while *C. sparsiflora* ($P < 0.001$), *P. erecta* ($P < 0.001$), and *G. tricolor* ($P = 0.03$) all associate with distinct microbial communities when grown on serpentine or non-serpentine soil.

Random forest correctly classified samples 78% of the time based on soil type and identified taxa that distinguished soil types. In particular, *Acidibacter, Blastococcus, Methylobacterium, Pseudonocardia, Skermanella, Sphingomonas*, an unidentified Proteobacteria and Firmicute classified non-serpentine rhizoplane communities. In contrast, *Microvirga* and RB41 were major features that characterized serpentine rhizoplane communities (Fig. 2; Supplementary Fig. 4).

Lathhouse study

Seedling survival varied among plant species ($P < 0.001$) and soil treatments ($P = 0.003$; Fig. 3) and was typically highest in autoclaved serpentine soil and serpentine soils (live or slurry-amended). Additionally, *P. erecta* took longer to establish than *G. capitata* while *T. willdenovii* established the more quickly than either *P. erecta* or *G. capitata*. Across all species, seedling survival was lowest in soils augmented with non-serpentine slurries.

Plant growth also varied among species and among soil treatments. Plant species differed in the number of leaves (Fig. 4a, $X^2(2, N = 227) = 16.56$, $P < 0.001$;), and although the main effect of soil treatment was not significant ($X^2(3, N = 227) = 2.76$, $P = 0.43$), plant species responded differentially to soil treatments (Plant x Soil: $X^2(6, N = 227) = 12.96$, $P = 0.04$). Soil type significantly influenced the root length of the plants (Fig. 4b, $X^2(3, N = 215) = 9.95$, $P = 0.02$). When compared to plants grown in autoclaved soil, plant roots amended with non-serpentine slurries were, on average, 10.8% shorter, those with live serpentine soil were 2.2% longer and those amended with serpentine slurries were 15.6% shorter. Plant species varied in root length ($X^2(2, N = 215) = 6.25$, $P = 0.04$), but plant species did not respond differently to soils (Plant x Soil: $X^2(6, N = 215) = 1.97$, $P = 0.90$).

Plant biomass varied among species (Fig. 4c, $X^2(2, N = 193) = 17.48$ $P < 0.001$), but neither soil ($X^2(3, N = 193) = 2.85$, $P = 0.42$) nor species-specific responses to soils influenced plant biomass at harvest (Plant x Soil: $X^2(6, N = 193) = 6.73$, $P = 0.35$). Similarly, plant species differed in shoot-to-root ratio (Fig. 4d, $X^2(2, N = 215) = 9.21$, $P = 0.01$), but neither soil ($X^2(3, N = 215) = 4.14$, $P = 0.25$) nor species-specific responses to soil influenced shoot height (Plant x Soil: $X^2(6, N = 215) = 9.75$, $P = 0.13$).

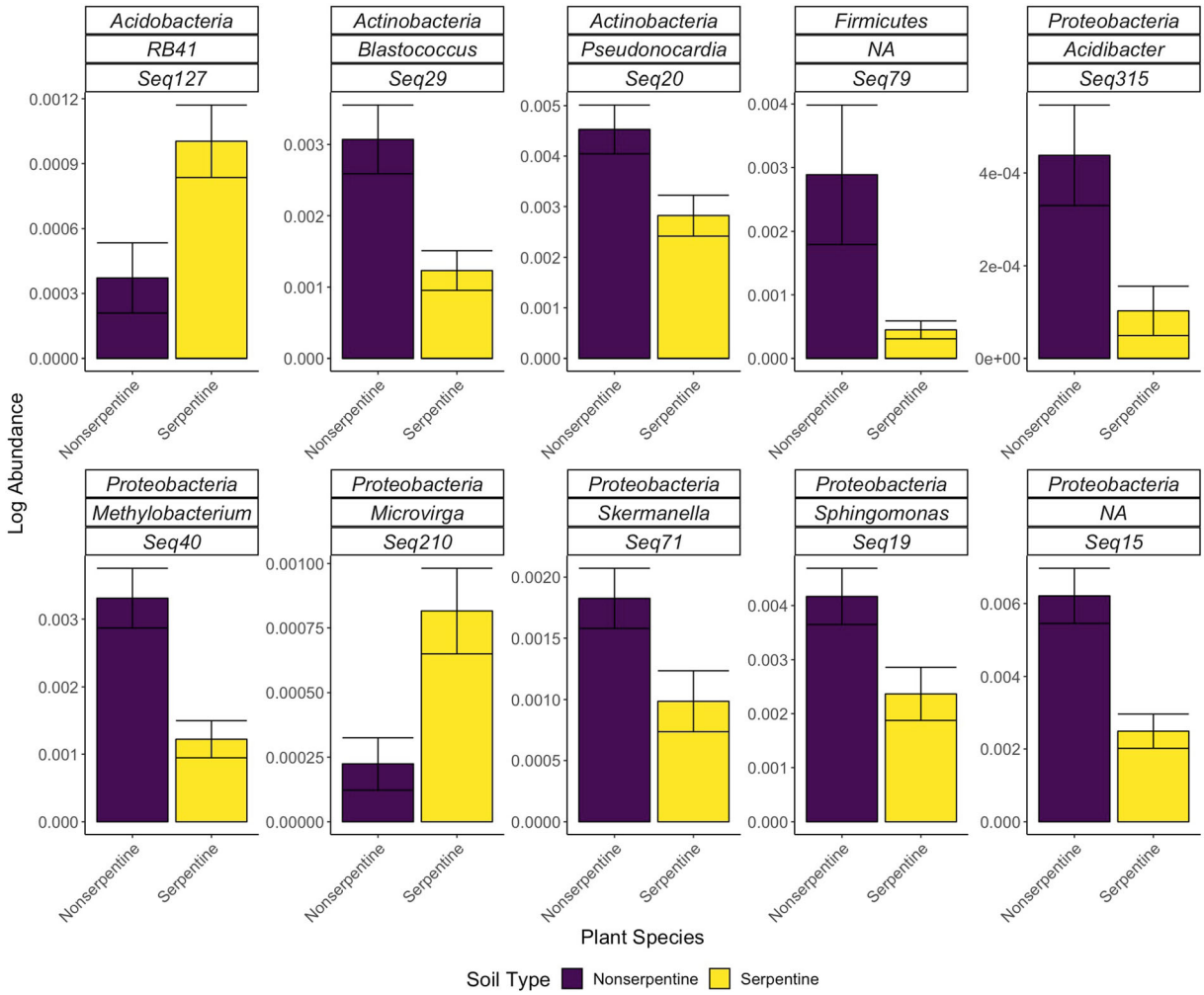

Fig. 2 Bacterial Actual Sequence Variants (ASVs) that were shown to be defining features of serpentine and non-serpentine field soils using random forest analysis. The mean abundance of the top ten features in serpentine and non-serpentine samples (bulk soil and rhizoplane samples) are shown here. Bars indicate the mean value +/-SE

Across soil types and plant species, microbial communities in the lathhouse experiment were dominated by Proteobacteria (Supplementary Fig. 5a). In contrast to the field study, rhizoplane samples amended with the non-serpentine slurry were less diverse than those that received live serpentine soil ($P = 0.03$) and *T. willdenovii* were more diverse than *P. erecta* ($P = 0.00$). Consistent with the field study, bacterial species composition in the rhizoplane differed with soil type (Supplementary Fig. 5b, $P = 0.02$) and plant species ($P = 0.00$). Plant species ($P = 0.01$), soil type ($P = 0.01$), and week of planting ($P = 0.01$) also differed in dispersion.

In the lathhouse experiment, bacterial community composition differed between plant species (Supplementary Fig. 4, $F_{3,87} = 4.23$, $P = 0.001$; $R^2 = 0.09$), soil treatments ($F_{3,87} = 3.11$, $P = 0.001$; $R^2 = 0.07$) and their interaction ($F_{9,87} = 1.33$, $P = 0.001$; $R^2 = 0.09$). Week of planting also influenced bacterial community composition ($F_{4,87} = 1.59$, $P = 0.001$; $R^2 = 0.04$), but interactions with week of planting were not significant (Plant:Week: $F_{4,87} = 1.04$, $P = 0.373$; $R^2 = 0.03$; Soil:Week: $F_{8,87} = 0.89$, $P = 0.890$; $R^2 = 0.05$; Plant:Soil:Week: $F_{2,87} = 1.31$, $P = 0.08$; $R^2 = 0.02$). When plant species were examined individually, both *G. capitata* ($P < 0.001$) and *T. willdenovii* ($P < 0.001$) associated with distinct bacterial communities depending on soil type while the community of *P. erecta* was invariant to the microbial source ($P = 0.26$).

Random forest correctly classified samples 65% of the time based on soil type, and identified taxa that

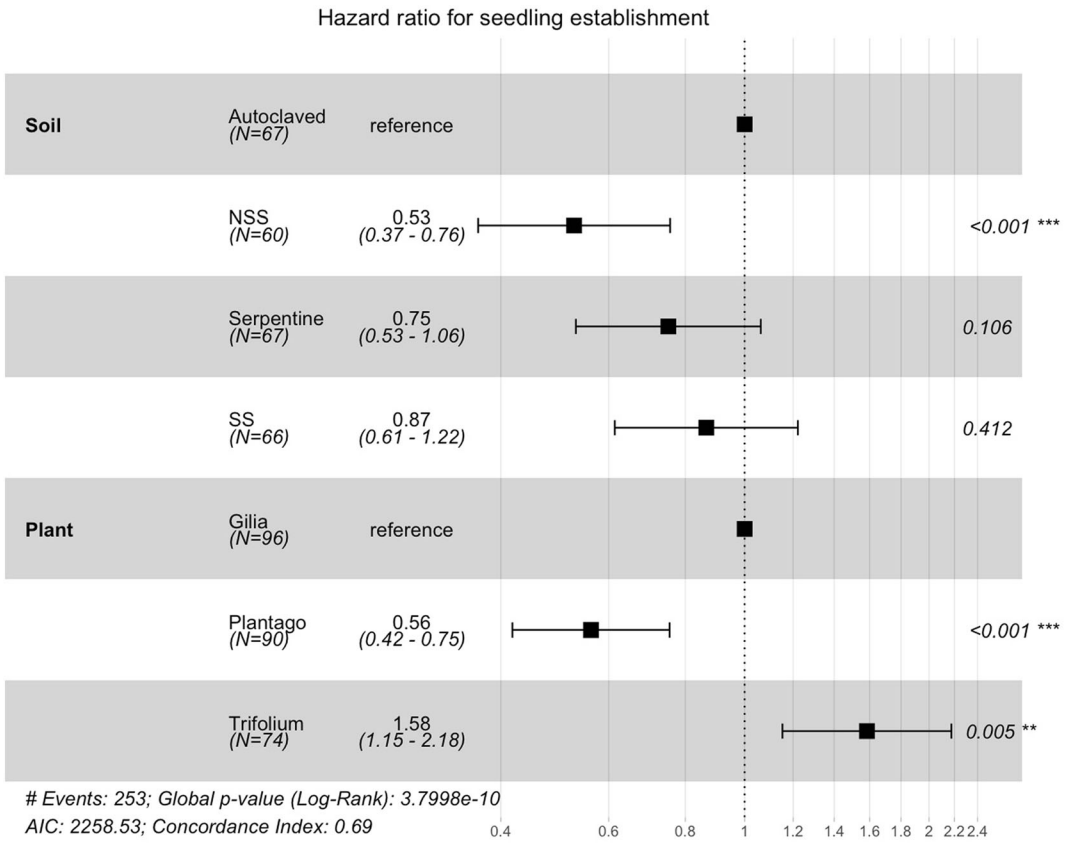

Fig. 3 Hazard ratio of seedling establishment in lathhouse study by soil type and plant species conducted using Cox proportional-hazards model. A HR > 1 indicates an increased likelihood of establishment while an HR < 1, on the other hand, indicates a decreased likelihood of establishment. For example, *Trifolium willdenovii* has a HR = 1.58 with a confidence interval of 1.15–2.18 showing that seedlings are more likely to establish relative to the reference

distinguished soil types. *Blastococcus, Microvirga*, and an unidentified Actinobacteria characterized the autoclaved and live serpentine soil bacterial communities. While *Micromonospora*, *Paenarthrobacter, Pseudomonas,* and two unidentified Firmicutes were major features of the non-serpentine slurry (NSS) soil treatment. Finally, *Streptomyces* characterized the serpentine slurry (SS) soil bacterial communities (Supplementary Fig. 6 and Supplementary Fig. 7).

Discussion

Taken together, our results demonstrate that both plant species identity and soil type shape bacterial species composition in the rhizoplane. Further, we show that microbes isolated from serpentine soil influence seedling survival on serpentine soils and microbial communities differentially influence some but not most plant phenotypic characteristics.

Relative influence of plant identity or soil chemistry on rhizoplane bacterial communities

Similar to previous findings across other soil types, both plant species (current study variance explained in Field: 9%, Lathhouse: 9%) and soil type (Field: 4%, Lathhouse: 7%) were important in determining bacterial species composition associated with plant roots. In our experiment, much of the variation in bacterial species composition was explained by plot identity (Field: 13%), week of planting (Lathhouse: 4%), or remained unexplained (Field and Lathhouse: 62%). Previous research has shown strong influence of plant species

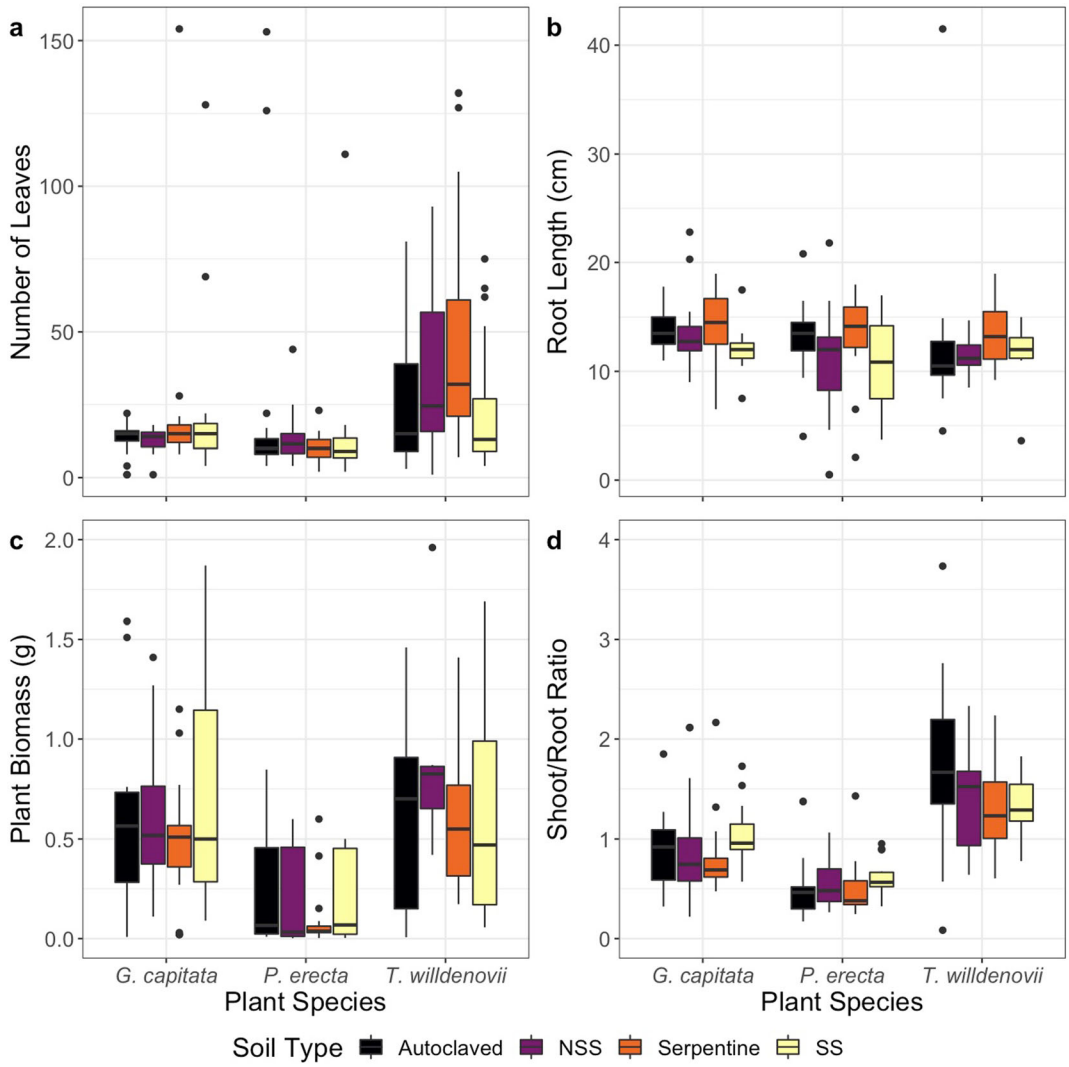

Fig. 4 Plant growth traits vary among *Gilia capitata*, *Plantago erecta*, and *Trifolium wildenovii* and soil treatment including: autoclaved serpentine soil, non-serpentine slurry added to autoclaved serpentine soil, live serpentine, and serpentine slurry added to autoclaved soil in lathhouse experiment. Box and whiskers plots indicate the interquartile range and points indicate alpha diversity measurements for individual samples. Soil type did not significantly impact (a) number of leaves ($X^2(2, N = 227) = 16.56$, $P < 0.001$) (c) plant biomass ($X^2(2, N = 193) = 17.48$ $P < 0.001$) or (d) shoot-to-root ratio ($X^2(2, N = 215) = 9.21$, $P = 0.01$), but (b) root length was slightly longer in the live serpentine treatment ($X^2(2, N = 193) = 17.48$ $P < 0.001$)

identity on root-associated bacterial community structure (Burns et al. 2015; Aleklett et al. 2015; Jorquera et al. 2016; Leff et al. 2018), however, the result documented here is particularly surprising given the large difference in physicochemical properties between serpentine and non-serpentine soils. In the field, this result was primarily driven by *T. fucatum* which associated with similar microbial communities in both serpentine and non-serpentine soils. Indeed, when *T. fucatum* is removed from the dataset, soil chemistry explains as much of the variation as plant species (soil chemistry: $F_{1,92} = 6.24$; $P = 0.001$; $R^2 = 0.06$; plant species: $F_{3,92} = 2.27$; $P = 0.001$; $R^2 = 0.06$). Nevertheless, it is notable that differentiation among plant species was still detected despite large differences in soil chemistry.

For the other serpentine-indifferent plant species including *C. sparsiflora*, *P. erecta*, and *G. tricolor*, bacterial species composition was strongly influenced by soil type, with communities largely distinct between serpentine and non-serpentine soil. Previous research showed

C. sparsiflora associates with distinct fungal communities on serpentine and non-serpentine soils (Schechter and Bruns 2008) and the current study showed that bacterial communities are distinct between soil types as well. Differentiation in *C. sparisiflora* or *P. erecta* could be due to variation in soil properties (Supplemental Table 1) or distinct plant ecotypes found on each soil type (Espeland and Rice 2007; Wright and Stanton 2007). Although ecotypic differentiation may contribute to the variation described here, the lathhouse experiment suggests that plant species identity, regardless of ecotypic variation, influences rhizoplane composition. In both the field and lathhouse studies, plant species explained slightly more variation in rhizoplane composition than soil type, suggesting specific recruitment or growth promotion of certain bacterial taxa (e.g. Figure 2, Supplementary Data 2).

Trifolium fucatum did not associate with distinct communities when grown on different soil types. Associations between *Rhizobium* and *T. fucatum* may be partially responsible for the similarity between root-association microbial communities across soil types. Still, when *Rhizobium* ASVs were removed from the data set, communities were still not distinct between soil types (data not shown; $P = 0.18$), indicating the presence of other microorganisms also contribute to the similarity seen across soil types, and the strong influence of *T. fucatum* on rhizosphere communities across soil types. The variation among plant species in their effects on microbial structure is notable and demonstrates that plant species differ in the extent to which soil conditions vs. plant effects influence their root-associated microbial communities.

Serpentine-associated bacteria

Serpentine and non-serpentine soils could be classified by different ASVs (Fig. 2). On serpentine, *Microvirga* was in high abundance in the rhizoplane of most plant species, except *Trifolium* (data not shown) and was also abundant in both serpentine and autoclaved serpentine soils in the lathhouse experiment (Supplementary Fig. 7). *Microvirga* has been found free-living in soils with heavy metals and endosymbiotically as a nitrogen-fixing bacteria (Kelly et al. 2014; Msaddak et al. 2017; Tapase and Kodam 2018). Although its function remains to be examined, the presence of this genus across treatments exhibiting enhanced seed survival (Fig. 3) combined with previous work linking it to nurse plants in harsh environments (Rodríguez-Echeverría et al. 2016) suggests it may be associated with plant benefit. The bacterial genus *RB41,* also more abundant in serpentine, has been associated with petroleum-contaminated soil (Shen et al. 2018) and its can be a biomarker for low-nutrient soil (Ai et al. 2018). Microbes in higher abundance on non-serpentine included *Blastococcus* and *Pseudonocardia,* which have previously been associated with nickel hyperaccumulating plants (Touceda-González et al. 2018). Although these taxa were found in higher abundance in non-serpentine soils in the current study, it is possible for the same species to be differentiated by the presence of accessory genes which could be detected with whole genome sequencing (Porter et al. 2017). Other ASVs more abundant in non-serpentine included *Skermanella,* which has been associated with polluted soil (Luo et al. 2012) and *Pseudonocardia*, associated with hydrocarbon degradation (Zhu et al. 2018). Other taxa more abundant in non-serpentine have been associated with plant nutrient acquisition (e.g. *Sphingomonas* (Videira et al. 2009) or enhance plant growth (e.g. *Methylobacterium* (Giamminola et al. 2017).

Previous studies suggest that differentiation in microbial communities between serpentine and non-serpentine soils depends on the resolution of the methods employed. For example, phospholipid fatty acid analysis suggested that *Avenula sulcata*, a serpentine tolerant grass does not associate with distinct bacterial or fungal communities (Fitzsimons and Miller 2010). Similarly, 16S rRNA gene sequencing was used to show that *Acmispon wrangelianus* (Chilean bird's foot trefoil) associated with *Mesorhizobium* when grown on serpentine or non-serpentine soil (Porter and Rice 2012). However, full genome sequencing revealed that *Mesorhizobium* isolated from serpentine harbored accessory genes, which provided a fitness advantage when grown in a high-nickel environment (Porter et al. 2016). Because the 16S barcoding used here may mask important functional variation in microbial communities or even populations found on each soil type, it will be important for future experiments to consider the functions or whole genomes of microorganisms (e.g. shotgun sequencing) in both soil environments.

Effects of serpentine microbes on plant survival and growth

Local adaptation of plants to soils has been studied in serpentine systems, yet the importance of locally

adapted serpentine microorganisms to plant performance has not received the same attention (Wright et al. 2006; Sambatti and Rice 2006). Here, our results demonstrate that microbial composition influences seedling establishment in serpentine soil, suggesting distinct effects of microbial communities on plant establishment on serpentine. This is in line with previous research which showed that seedlings grown in local soils were more than twice as likely to survive as seedlings grown in non-local soil (Smith et al. 2012). Other studies have found that soil fungi and/or bacteria can mediate seedling establishment (Thrall et al. 2007; Wagg et al. 2011; Pickles et al. 2015). A large body of theory suggests that microbial effects can drive plant population dynamics at small and large scales (Comita et al. 2014; Christian et al. 2017),with consequences for plant coexistence, distribution and large-scale patterns of biodiversity.

Some previous work has shown that microbes can influence plant growth in the presence of heavy metals (Branco 2009; Jing et al. 2014; Mesa et al. 2015), but most plant growth traits in the current experiment were largely invariant with soil microbial treatment. However, plants grown with serpentine microbes had consistently longer roots, which could enhance plant survival under drought conditions (Comas et al. 2013). Comparing to previous work in serpentine, serpentine microbial communities do not always enhance the growth or survival of plant hosts. For example, serpentine arbuscular mycorrhizal (AM) and serpentine whole microbial communities decreased plant biomass relative to uninoculated plants and did not improve nickel tolerance (Doherty et al. 2008). In our study, it is possible that different soil inoculation methods could introduce different, possibly more beneficial bacteria. In addition, it is likely that effects of microbial communities are context-dependent and may not be revealed under our experimental conditions.

Although few effects of microbial source were observed on plant phenotype, soil microbial communities influenced seedling establishment, a critical barrier to survival in serpentine and non-serpentine soils. We identified ASVs that vary among soil types, but cannot conclusively identify specific microorganisms associated with variable establishment. Previous work has shown that pathogens can strongly mediate seeding survival (Packer and Clay 2003; Mendes et al. 2013). Nonetheless, it is clear that the strength of plant-soil feedbacks, which can be mediated by microbial effects on seedling survival, are variable among soil types (Ehrenfeld et al. 2005), it remains difficult to predict when feedbacks are important in predicting community dynamics, and if pathogens, mutualists or other microbes contribute to these effects. One promising approach may be to examine the microbial effects on seedling survivorship across soil types and mechanistic basis of local adaptation using shotgun metagenomics and whole genome sequencing of isolated bacteria in serpentine.

Conclusions

Overall, our results show that plant species vary in the degree to which soil type influences rhizoplane bacterial composition, but species and soil type both influenced bacterial structure. Microbial communities also influenced plant traits relevant to establishment that may scale to influence community or population dynamics including seedling establishment and root length. These results suggest that both plant identity and the source microbial pool can explain variation in the structure of microbial communities, with consequences for their function. The presence of key taxa, including *Microvirga*, that consistently distinguish serpentine rhizosphere communities, suggests that microbial contribution to plant serpentine tolerance should be investigated further. Finally, these results improve our understanding of the relative influence of soil chemistry and plant identity in structuring the rhizoplane microbial community with implications for population dynamics.

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