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SPECIAL FEATURE

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Soil type and precipitation level have a greater influence on fungal than bacterial diversity in serpentine and non-serpentine biological soil crusts

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Abstract

Serpentine soils are characterized by nutrient imbalances and high levels of potentially toxic metals (PTMs). These soils host depauperate plant communities of species with specialized adaptations. Initial studies showed that South African serpentine soils harbor distinct biocrust algal and cyanobacterial species compared to adjacent non-serpentine soils, with these communities further differing based on high and low precipitation levels. Here, we investigated the bacterial and fungal diversity of biological soil crusts from serpentine and non-serpentine soils at two precipitation levels. The bacterial and fungal communities were characterized using 16S rDNA and ITS metabarcoding, respectively. No significant differences could be found in bacterial richness and community structure. Nevertheless, bacterial taxa such as *Archangium*, *Candidatus Solibacter*, *Chthoniobacter*, and *Microvirga* were more abundant in serpentine biocrusts or biocrusts receiving lower precipitation. The fungal community structure was distinct between serpentine and non-serpentine soils ($p = 0.027$) and between high and low precipitation ($p = 0.018$). Furthermore, fungal diversity was lowest in the drier, serpentine biocrusts compared to non-serpentine ($p = 0.001$) and serpentine crusts receiving higher precipitation

($p = 0.002$). The fungal genera, *Ramimonilia* and *Vishniacozyma*, which are known to be resistant or tolerant to PTMs and other environmental extremes, were significantly more abundant ($p = 0.036$ and $p = 0.016$, respectively) in serpentine biocrusts, with the latter indicating serpentine habitats. This study concluded that soil type influenced the fungal alpha diversity, specifically in the serpentine soil, resulting in a decrease in fungal species richness. Furthermore, precipitation influenced fungal beta diversity by shaping distinct fungal communities found in the biocrusts of serpentine and non-serpentine soils.

KEYWORDS

biocrust, biological soil crust, metabarcoding, microbial diversity, serpentine geoecology

1 | INTRODUCTION

Serpentine soils, characterized by their nutrient imbalances (Ca:Mg molar ratio of <1, generally low levels of P, N, and K) and potentially toxic metals (PTMs) which include high levels of Cr, Cd, and Ni, are known to harbor specialized plant communities in many parts of the world. They often shape the diversity of soil biota by influencing both the colonization and persistence of species (Rajakaruna & Boyd, 2008). These harsh soils are known for the stressors they impose on plants, collectively called "serpentine syndrome" (Bini & Maleci, 2014; Jenny, 1980), resulting in low ecosystem productivity, high levels of endemism, and plants with lower competitive ability (Anacker, 2014). Serpentine soils are also of special interest for their potential to model solutions to degraded ecosystems, especially in mine restoration (Rajkumar et al., 2009; Robinson et al., 1999).

Despite the extensive work done on serpentine-soil-vascular-plant relations worldwide (Alexander et al., 2007; Galey et al., 2017; Rajakaruna et al., 2009; Teptina et al., 2018), research on serpentine substrate-microbe relations is limited to a few studies on mycorrhizal fungi (Schechter & Branco, 2014; Southworth et al., 2013), soil-dwelling bacteria (Ma et al., 2015; Oline, 2006), and saxicolous lichens (Favero-Longo et al., 2018; Mulroy et al., 2022; Rajakaruna et al., 2012). Some important distinctions between the microbial diversity of serpentine and non-serpentine soils have come to light in these and other studies. Microbial community structure may differ based on the presence of different plants in serpentine and non-serpentine soils (Pessoa-Filho et al., 2015), and local heterogeneity in soil characteristics and microclimatic gradients can impact the diversity of cyanobacteria and algae in serpentine soils (Venter et al., 2018). Actinobacteria is the most commonly isolated bacterial phylum from serpentine soils (Abou-Shanab et al., 2009; Khilyas et al., 2019; Mengoni et al., 2001; Turgay et al., 2012; Visioli et al., 2019) and exhibits K-strategist attributes that allow these species to thrive in resource-limited and high-competition environments, making them more abundant in soils low in organic matter and high in Ni concentrations (Brzeszcz et al., 2016; Visioli et al., 2019).

According to Muller and Hilger (2015), the richness of fungal communities in serpentine soils is not influenced by edaphic factors, but the community structure may differ significantly between serpentine and non-serpentine soils. This was supported by Branco and Ree (2010), concluding that serpentine soils host rich fungal communities with representatives from all fungal lineages and that these environments are not extreme for ectomycorrhizal fungi. Husna et al. (2017) also reported that arbuscular mycorrhizal fungi are influenced by soil chemical properties such as metal content, Ni, and Ca:Mg ratio. Therefore, serpentine soils are not necessarily depauperate and may host a rich assemblage of fungal species, with the structure of these communities strongly influenced by soil chemical and physical properties. Ortiz et al. (2020) furthermore found a significant influence of climate on rhizosphere microbial communities and Naidoo et al. (2022) found that precipitation is a key climatic factor that shapes the taxonomy and resulting ecosystem services of arid soil microbiomes.

However, serpentine soils are not only studied for their unique chemical and physical attributes and the consequential impacts on soil biota, but also for the microbial communities living in the upper millimeters of these and other harsh soils, namely biological soil crusts, or biocrusts. Biocrusts constitute communities of photoautotrophic cyanobacteria, algae, lichens, and bryophytes growing alongside heterotrophic fungi, bacteria, and archaea (Weber et al., 2016). They are an essential component of dryland and desert ecosystems and contribute to a range of ecosystem functions. Biocrusts play a fundamental role in soil aggregation and erosion resistance (Belnap et al., 2003; Chamizo et al., 2016), while also enhancing soil nutrient levels through N-fixation (Elbert et al., 2012), dust trapping (Reynolds et al., 2001), and nutrient cycling (Strauss et al., 2012). They further influence the hydrological

(Chamizo et al., 2016) and thermal properties of soil (Couradeau et al., 2016; Rutherford et al., 2017). Given that serpentine soils are often low in essential nutrients, especially N, P, and K, enriched with PTMs (e.g., Cd, Cr, and Ni), and generally water stressed due to poor soil texture and structure, as well as habitat openness/bareness (Rajakaruna & Boyd, 2014), biocrusts are likely to enrich serpentine soil with limited nutrients (such as N), minimize PTM stress by changing soil pH and increasing soil moisture content. Significant knowledge gaps in the composition of biocrusts for certain regions persist with data for African serpentine soils being very limited (Venter et al., 2015, 2018). Venter et al. (2015) characterized microbial communities using an isolation approach but no unique algal flora for serpentine soils was confirmed. Venter et al. (2018) then demonstrated, using a metabarcoding approach, that serpentine soils harbor distinct biocrust algal and cyanobacterial species compared to adjacent non-serpentine soils at different precipitation levels. Their study also documented, for the first time, nine genera of cyanobacteria from South African serpentine soils.

These findings prompted us to further survey the composition of biocrusts of serpentine soils in South Africa (Venter et al., 2015, 2018) across varying precipitation levels, via a DNA metabarcoding approach. Here, we investigate the differences and changes in the (i) bacterial and fungal biocrust community diversity and composition between serpentine and non-serpentine soils and (ii) bacterial and fungal diversity of biocrusts according to differences in precipitation. We hypothesize that the microbial diversity within biocrusts occurring on serpentine soils is significantly influenced by the combined effects of PTMs, low nutrients, and varying precipitation levels. Specifically, we predict that serpentine biocrusts will exhibit distinct microbial community composition relative to non-serpentine, with biocrusts of serpentine soils to be enriched with metal-tolerant or resistant microorganisms. Furthermore, we expect that the microbial diversity within serpentine biocrusts would be influenced by the interaction between the extreme soil conditions and the contrasting precipitation levels. Higher precipitation levels may potentially promote greater species richness and more even microbial community structures, while lower precipitation levels may lead to decreased diversity and increased abundance of stress-tolerant microbial taxa.

2 | MATERIALS AND METHODS

2.1 | Sampling sites

Biological soil crusts were sampled at eight different localities along the Barberton Greenstone belt in Mpumalanga, South Africa, paired with one on serpentine soil and one off serpentine to give 16 sampling sites in total (for more details, see Venter et al., 2018 and Table S1). Half of the localities (four serpentine and four non-serpentine) were sampled along the temperate Highveld escarpment with a cool mean annual temperature of 17°C with frequent fog and precipitation levels of more than 1000 mm per annum. The datasets resulting from the analysis of these biocrust samples are referred to as serpentine wet (SW: four sites), and non-serpentine wet (NSW: four sites). The other half of the paired samples (four serpentine and four non-serpentine) were obtained in subtropical Lowveld with higher mean annual temperature (19.7°C) and lower precipitation (<800 mm). The datasets resulting in the analysis of these biocrusts will be referred to as serpentine dry (SD: four sites) and non-serpentine dry (NSD: four sites). These defined substrate-climate groups (SW, NSW, NSD, and SD) were designated as "treatments" in subsequent statistical comparisons.

2.2 | Soil and biocrust sampling

Nine subsamples of soil and biocrusts were obtained randomly at each of the 16 sampling localities by placing three 20 × 20 m plots at each locality and taking three subsamples from each plot. Biocrust samples were collected using a sterile spatula to a depth of 3 mm, placed into sterile screw-cap falcon tubes, kept on ice during field collection and then stored at −80°C until DNA extraction. Soil samples for physical and chemical analyses were collected with a soil auger to a depth of 10 cm. The nine subsamples collected at each site were combined to obtain one composite sample per sampling site for soil and biocrusts, respectively. Because this is a continuation of the study of Venter et al. (2018), using the same study sites and samples, the chemical and physical soil parameters of the serpentine and non-serpentine soils analyzed in this study were already known (Tables S2 and S3).

Some important distinctions to note from Venter et al. (2018) include that biocrusts of serpentine soils had an overall higher chlorophyll-*a* content, and serpentine soils also had higher electrical conductivity (EC), higher Mg, and lower Ca concentrations than non-serpentine soils. Serpentine soils further had higher concentrations of PTMs such as Co, Cr, Fe, Mn, and Ni. There were no significant differences between nutrient levels of serpentine and non-serpentine soils which included N, S, and C percentages as well as concentrations (mg L^{-1} or ppm) of Cl, Na, N, P, K, and S.

2.3 | DNA extraction

DNA was extracted from 250 mg of each of the 16 composite biocrust samples (four independent replicates per treatment) using the Power® Soil DNA extraction kit

(MO-BIO Laboratories, Carlsbad, CA) according to the manufacturer's instructions. An additional step of a Proteinase K treatment was added. The DNA was eluted in 100 μL of the eluent buffer provided by the PowerMax Soil DNA kit. DNA samples were subsequently quantified using a Qubit 3.0 Fluorometer (Life Technologies, ThermoFisher Scientific Inc.).

2.4 | 16S rRNA and ITS gene amplification and MiSeq sequencing

This study employed 16S rRNA gene sequencing for bacterial community exploration and the nuclear ribosomal internal transcribed spacer (ITS) for fungi. The MiSeq341F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′) and MiSeq785R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′) primer pair (Klindworth et al., 2012) was used to amplify the variable V3–V4 region of the 16S ribosomal RNA gene which resulted in amplicon lengths of 464 bp. Fungal library preparation was performed using the ITS1 (5′-5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCTCCGCTTATTGATATGC-3′) primer set (White et al., 1990) that spans the whole ITS region (ITS1, 5.8S rDNA, and ITS2) creating amplicons of 350–880 bp in length. Both 16S and ITS primer pairs incorporated the Illumina overhang adaptor. Library preparation and pooling were done per the methods described by Venter et al. (2018).

2.5 | Sequencing data processing

CutAdapt (v.4: Martin, 2011) was used to remove Illumina overhang adapters and sequencing primers. The Divisive Amplicon Denoising Algorithm 2 (DADA2, v.1.24: Callahan et al., 2016) was used to analyze the 16S and ITS Illumina amplicon sequence data. This analysis encompasses denoising (removal of sequencing errors and PCR artifacts), quality control (removal of low-quality reads and sequences with ambiguous bases), error correction via a learned error model, inference of amplicon sequence variants (ASVs) and the creation of an ASV table that summarizes the abundance of each identified variant across samples. During the DADA2 analysis, forward and reverse reads are merged to create a consensus sequence, but this could only be done for the 16S dataset due to low-quality reverse reads for the ITS sequences. Additionally, the sequences of the ITS region targeted for fungal community exploration were too long for MiSeq sequencing since only 301 bp from each end could be sequenced, therefore, sequences could not be generated with a sufficient overlap to create a consensus sequence. Consequently, only forward ITS reads were represented in the analysis. The ASVs and the ASV tables created for 16S and ITS were subsequently used to explore the microbial diversity and community structure of the biocrust samples.

To determine the sum of branch lengths (SBL) of the bacterial and fungal datasets respectively, the ASVs were aligned in the Multiple alignment program for amino acid or nucleotide sequences (MAFFT, v.7: Katoh & Standley, 2013) and then used to reconstruct neighbor-joining (NJ) phylogenetic trees for the 16S and ITS datasets, as implemented by the R package *ape* (v.5.6.2: Paradis & Schliep, 2018) with bootstrap testing of 1000 replicates using the Jukes–Cantor model. The taxonomy of the ASVs was assigned according to the SILVA (Quast et al., 2012) and NCBI genomic databases for bacteria (http://www.ncbi.nlm.nih.gov) and the UNITE genomic database (Nilsson et al., 2019) for fungi. Taxonomic assignments were followed by visualization in R (v.4.3.1: R Core Team, 2023) via bar plots to enable a broad perspective of the fungal and bacterial datasets. These bar plots showed the relative abundance of ASVs detected for each marker dataset per treatment by the R package, *phyloseq* (v.1.4: McMurdie & Holmes, 2013).

2.6 | Statistical analyses

Alpha-diversity metrics calculated species richness based on the number of distinct taxa (ASVs) observed in a sample, which were then grouped according to treatment, soil type (serpentine and non-serpentine) and precipitation level (high and low). This calculation was facilitated by the *estimate_richness* function of the *phyloseq* package. This metric provided insights into the diversity of microbial communities: higher observed species richness indicated a greater diversity of taxa in a sample. Statistical analyses were performed to assess the significant differences in the observed ASV richness between groups. The normality of the data distribution was assessed by the Shapiro–Wilk test. In the case of normally distributed data, parametric tests such as ANOVA (Analysis of Variance) were used. Conversely, if the data was not normally distributed, non-parametric tests such as the Kruskal–Wallis test were used. In the case of significance, post-hoc tests such as Tukey's HSD (Honestly Significant Difference) or the Mann–Whitney *U* test were used to facilitate pairwise comparisons, respectively. All statistical analyses and subsequent visualizations were performed in R and the significance level was set at $p = 0.05$.

To assess the dissimilarities in microbial community structure between treatments, soil types and precipitation levels, beta-diversity analyses, including non-metric multidimensional scaling (NMDS), Permutational Multivariate Analysis of Variance (PerMANOVA), and canonical correspondence analysis (CCA) were conducted using presence-absence matrices based on fungal and bacterial ASV tables by various functions of the R package, *vegan* (v.2.3: Oksanen et al., 2022). NMDS ordinations were performed on entire, unfiltered datasets. This approach allowed for the representation of the entire microbial community present in the biocrust samples, including ASVs that could not be taxonomically assigned in genomic reference databases. NMDS analysis was executed via the *metaMDS* function based on Bray–Curtis dissimilarities calculated with the *vegdist* function. To determine the statistical significance of environmental variables in structuring microbial communities, the non-parametric PerMANOVA was employed via the *adonis2* function. PerMANOVA tests the association between groups and co-variates using permutation procedures, providing robust statistical support for the observed differences. This analysis assessed the association of environmental variables with microbial community structure within the different treatments. The analysis included the separate consideration of soil type and precipitation level.

CCA was performed using filtered datasets that only represented taxa occurring at least five times in 25% of the samples. This filtering step was included to focus the analysis of beta diversity on the most robust and abundant taxa, thereby reducing the introduction of noise and potential biases by rare or spurious ASVs (e.g., Gabay et al., 2023; Pombubpa et al., 2020). CCA was then conducted at the order level to identify key environmental variables that drive the community structure of bacteria and fungi biocrusts across serpentine (sites 1, 3, 5, 7, 9, 11, 13, and 15) and non-serpentine (sites 2, 4, 6, 8, 10, 12, 14, and 16) localities using CANOCO software for windows, v.4.5 (Ter Braak & Smilauer, 2002). To determine which environmental variables to include in the CCA, Kruskal–Wallis tests and inspections of variance inflation factors (VIFs) were used to identify those variables that significantly differed between serpentine and non-serpentine sites and to remove environmental variables that may be inflating the correlations. Monte Carlo permutation tests were conducted using 499 random permutations to determine the statistical validity of the CCAs.

Following the CCA, further investigations were conducted to determine the ecological relevance of the identified environmental variables. Specifically, genera whose abundances significantly differed between treatments, soil type and precipitation levels were examined to identify taxa that may be especially sensitive to specific environmental conditions. Kruskal–Wallis tests were used to determine which genera differed significantly in abundance across different treatments and environmental conditions. Only genera with *p*-values lower than 0.05 were represented in boxplots showing their abundance. An analysis of indicator species was also conducted via the *multipatt* function in the *indicspecies* package (De Cáceres & Legendre, 2009) to find taxa that function as markers of environmental conditions and preferred habitats.

The analysis of significantly abundant genera and indicator species relied on the same filtered dataset as the CCA, however, instead of presence-absence data, abundance data was used. The centered log-ratio (CLR) transformation was applied to the abundance data to address PCR bias, which refers to the selective or disproportionate amplification of specific taxa. The CLR transformation was applied via the *transform_sample_counts* function of the *phyloseq* package (Barlow et al., 2020; Silverman et al., 2021). This transformation addresses the compositional nature of the sequencing data (Gloor et al., 2017), that is, that the observed relative abundance of certain taxa is dependent on the relative abundances of all other taxa within a sample. CLR transformation describes an approach which uses the geometric mean of the read counts of all taxa within a sample as a denominator for that sample. The total taxon read counts are then divided by this denominator and the log fold changes in this ratio between samples are compared (Aitchison & Greenacre, 2002). A reliable framework for the evaluation of abundance data was therefore created that allowed for meaningful comparisons among samples. To further mitigate PCR bias, genera that had significant differences in abundance among treatments, soil types and precipitation levels, were compared across these environmental groupings and not within them, assuming that PCR biases are consistent within a taxon. Unless stated otherwise all statistical analyses were performed in R with R-base packages and visualized with *ggplot2* (Wickham, 2016).

3 | RESULTS

3.1 | Microbial abundance

The 16S rRNA metabarcoding yielded 5989 ASVs of which 5973 could be assigned to phylum level. Overall, the bacterial dataset consisted of 30 phyla, 65 classes, 134 orders, 179 families, 295 genera and 72 species with the most abundant phyla being Actinobacteria (31.21%), Proteobacteria (21%), and Acidobacteriota (13.51%). These phyla and others that had a relative abundance of more than 2% across the dataset were included in a stacked bar plot

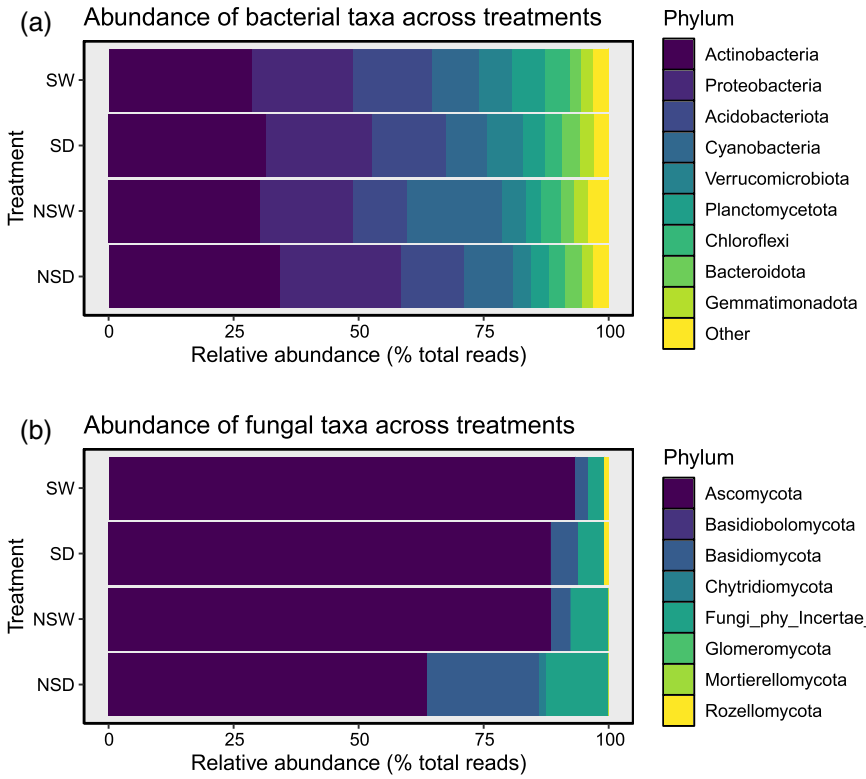

FIGURE 1 Relative abundance of (a) phyla constituting more than 2% of the bacterial dataset, and (b) all fungal phyla present in the different treatments of serpentine biocrusts receiving high and low levels of precipitation (SW and SD, respectively) and non-serpentine biocrusts receiving high and low levels of precipitation (NSW and NSD, respectively). Relative taxa abundances were calculated per treatment. NSD, dry, non-serpentine soils with low rainfall; NSW, cool, non-serpentine soils with high rainfall; SD, dry, serpentine soils with low rainfall; SW, cool serpentine soils with high rainfall.

(Figure 1a), including Cyanobacteria, Verrumicrobiota, Planctomycetota, Chloroflexi, Bacteroidota, and Gemmatimonadota. The remaining 21 phyla were grouped into "Other." The same phyla that were most abundant in the overall dataset were also most abundant in NSD, SD, and SW, respectively. NSW did not follow this trend, with Cyanobacteria being the second most abundant (18.98%) after Actinobacteria (30.27%), followed by Proteobacteria (18.58%) and then Acidobacteriota (10.87%) (Figure 1a). The other treatments had lower relative abundances of Cyanobacteria with NSD having 9.82%, SD having 8.39%, and SW having 9.49%.

Of the 1433 ASVs created for the ITS metabarcoding dataset, 1373 could be assigned to phylum level. Overall, the fungal dataset consisted of 8 phyla, 28 classes, 73 orders, 157 families, 286 genera, and 191 species with the most abundant phyla being Ascomycota (83.5%), Basidiomycota (8.53%), and Fungi_phy_Incertae_sedis (7.11%) (Figure 1b). The treatments receiving lower precipitation (NSD and SD) had the same most abundant phyla as the overall dataset, but the treatments with higher precipitation (NSW and SW) had Ascomycota (88.5% and 93.37%, respectively), Fungi_phy_Incertae_sedis (7.59% and 3.24%, respectively), and then Basidiomycota (3.68% and 2.51%, respectively). From Figure 1b, it is also clear that the serpentine treatments (SW and SD) had a higher abundance of Rozellomycota and NSD had a lower abundance of Ascomycota and a higher abundance of Basidiomycota than the other treatments.

TABLE 1 The sum of branch lengths (SBL) of the neighbor-joining trees constructed for bacterial (16S) and fungal (ITS) amplicon sequence variants for serpentine biocrusts receiving high and low frequencies of precipitation (SW and SD, respectively) and non-serpentine biocrusts receiving high and low precipitation frequencies (NSW and NSD, respectively).

Treatment	SBL
16S	
SW	35.51
SD	45.72
NSW	37.94
NSD	42.54
ITS	
SW	27.46
SD	22.44
NSW	69.00
NSD	48.45

3.2 | Community diversity

To estimate the phylogenetic distances of communities sampled within each treatment, the SBL of the constructed NJ trees from each treatment for the 16S and the ITS ASVs were compared. The phylogenetic distances (SBL) decreased for ITS ASVs as follows: NSW (69) > NSD (48) > SW (27) > SD (22) and 16S ASVs as

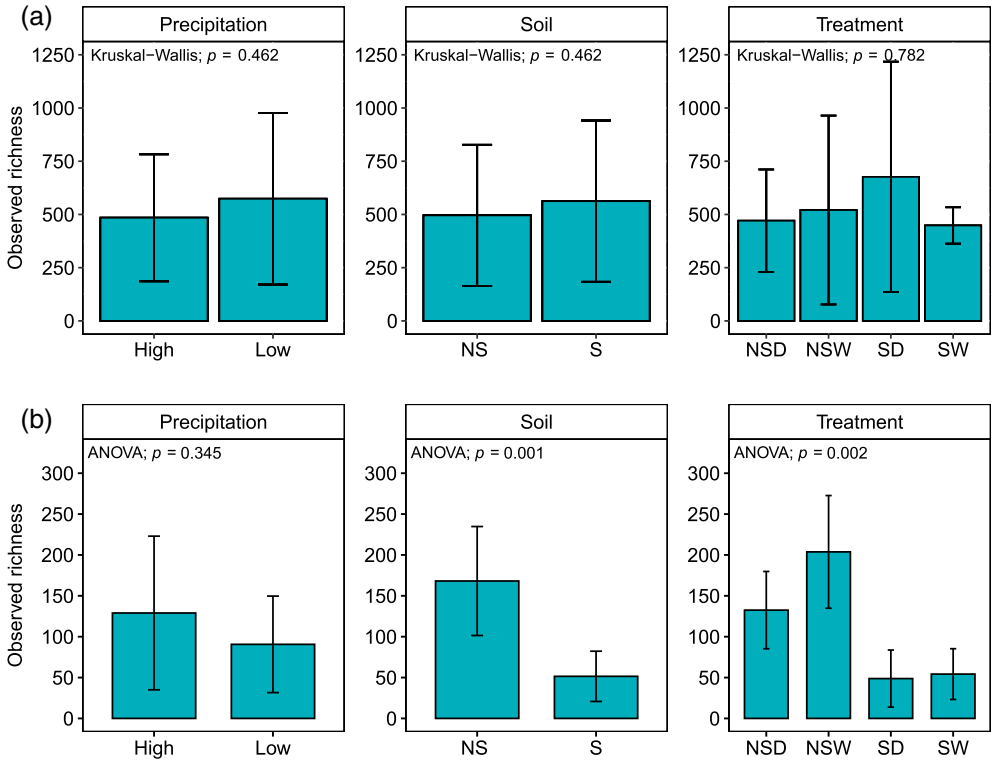

FIGURE 2 Bar plots representing the number of unique ASVs (observed species richness) for high and low precipitation frequencies ($n = 8 \pm$ std), non-serpentine and serpentine soils ($n = 8 \pm$ std), and treatments, ($n = 4 \pm$ std), for the (a) bacterial, and (b) fungal datasets. The whiskers indicate the standard deviation. Observed ASV richness in the bacterial dataset (a) was assessed for differences across precipitation levels, soil type and treatment using the Kruskal–Wallis test. For comparisons of fungal ASV richness (b), different letters indicate statistical differences between treatments according to a one-way ANOVA combined with Tukey post-hoc tests ($p < 0.05$). ASV, amplicon sequence variant; NS, non-serpentine soil; NSD, dry, non-serpentine soils with low rainfall; NSW, cool, non-serpentine soils with high rainfall; S, serpentine soil; SD, dry, serpentine soils with low rainfall; SW, cool serpentine soils with high rainfall.

follows: SD (46) > NSD (43) > NSW (38) > SW (36) (Table 1).

3.3 | Alpha diversity

Alpha diversity metrics were calculated to assess the observed species richness in microbial communities (Figure 2). The observed species richness was highest in non-serpentine biocrusts, biocrusts receiving higher precipitation, and particularly high in NSW for fungal communities (Figure 2b). An inverse trend was observed for bacterial communities since diversity was higher in serpentine biocrusts, lower precipitation and especially low in SD (Figure 2a). The Shapiro–Wilk test confirmed that the 16S data was not normally distributed and the subsequent Kruskal–Wallis analysis showed that there was no significant difference in the observed species richness between treatments, soil types or precipitation levels. The Shapiro–Wilk tests showed that the ITS data is normally distributed and the subsequent ANOVA results revealed that there was a significant difference in fungal species richness for treatments (precipitation and soil type) ($p = 0.002$) and soil type ($p = 0.001$). Pairwise comparisons by the Tukey HSD test showed that NSW is significantly different from SD ($p = 0.003$) and SW ($p = 0.004$) in terms of observed species richness. This test also showed that the observed fungal species richness of serpentine and non-serpentine soils was significantly different ($p = 0.001$).

3.4 | Beta diversity

Microbial communities in the different treatments could be differentiated using metabarcoding data. NMDS ordinations constructed based on Bray–Curtis dissimilarities confirmed that the community structure differed between the different serpentine and non-serpentine treatments. However, the PerMANOVA indicated that the difference observed was only a result of the difference in the fungal communities present in different treatments (Figure 3a) and precipitation levels (Figure 3b). This difference in the community structure of the fungi for the different

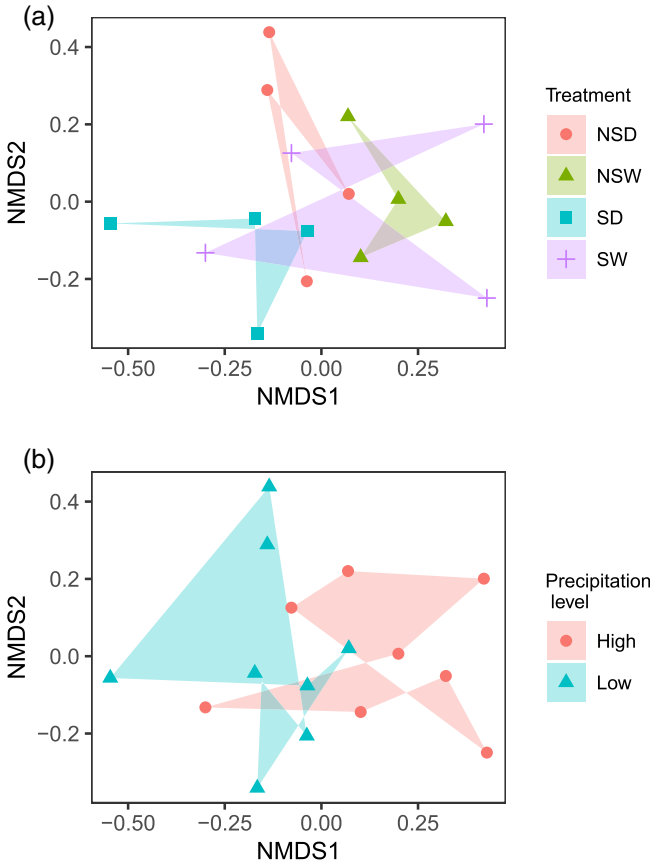

FIGURE 3 Non-metric multidimensional scaling (NMDS) ordination plot of Bray–Curtis dissimilarities for fungal communities across (a) treatments and (b) precipitation frequencies. Analyses were based on presence-absence amplicon sequence variant data matrices. The stress value for the ordination is 0.172. NSD, dry, non-serpentine soils with low rainfall; NSW, cool, non-serpentine soils with high rainfall; SD, dry, serpentine soils with low rainfall; SW, cool serpentine soils with high rainfall.

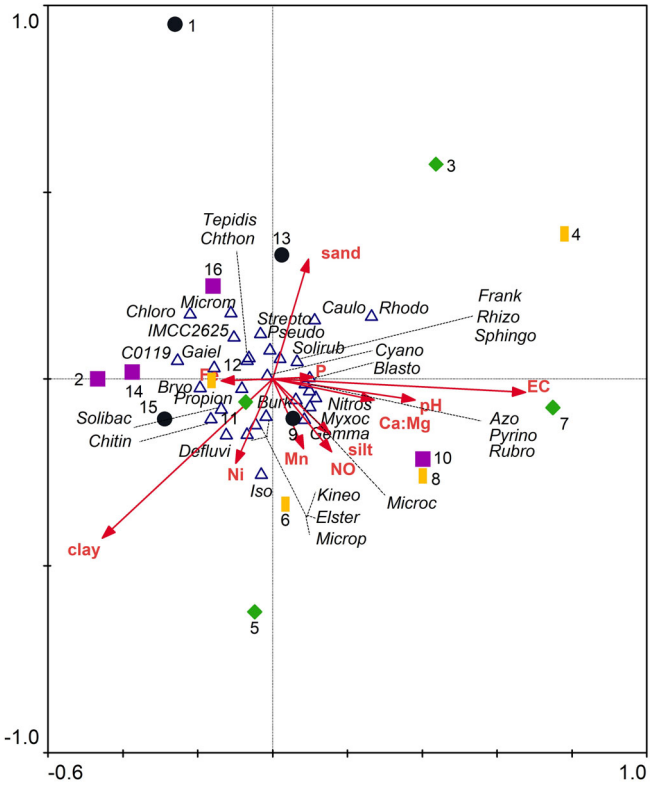

FIGURE 4 Environmental factors driving the bacterial composition variation. Canonical correspondence analysis (CCA) triplot based on amplicon sequence variants assigned to bacterial orders (blue triangles) concerning the environmental variables (red arrows) and serpentine and non-serpentine soils. The sites are represented as follows: SW (cool serpentine soils with high rainfall) includes sites 1, 11, 13, and 15 (black circles); NSW (cool, non-serpentine soils with high rainfall) includes sites 2, 12, 14, and 16 (purple squares); SD (dry, serpentine soils with low rainfall) includes sites 3, 5, 7, and 9 (green diamonds); NSD (dry, non-serpentine soils with low rainfall) includes sites 4, 6, 8, and 10 (yellow rectangles). Azo, Azospirillales; Blasto, Blastocatellales; Bryo, Bryobacterales; Burk, Burkholderiales; Caulo, Caulobacterales; Chitin, Chitinophagales; Chloro, Chloroplast; Chthon, Chthoniobacterales; Cyano, Cyanobacteriales; Defluvi, Defluviicoccales; Elster, Elsterales; Frank, Frankiales; Gaiel, Gaiellales; Gemma, Gemmatimonadales; Iso, Isosphaerales; Kineo, Kineosporiales; Microc, Micrococcales; Microm, Micromonosporales; Microp, Micropepsales; Myxoc, Myxococcales; Nitros, Nitrospirales; Propion, Propionibacteriales; Pseudo, Pseudonocardiales; Pyrino, Pyrinomonadales; Rhizo, Rhizobiales; Rhodo, Rhodobacterales; Rubro, Rubrobacterales; Solibac, Solibacterales; Solirub, Solirubrobacterales; Sphingo, Sphingomonadales; Strepto, Streptomycetales; Tepidis, Tepidisphaerales.

treatments was statistically supported by a PerMANOVA with $p = 0.027$, and $p = 0.018$ for the differences in fungal community structures observed with higher and lower precipitation.

CCA was employed to visualize the species distributions concerning different sites and to represent how different edaphic factors correlated with bacterial (Figure 4) and fungal (Figure 5) taxa. The environmental variables chosen for the ordinations were based on Kruskal–Wallis tests and analysis of VIFs. The Kruskal–Wallis analysis showed that serpentine and non-serpentine biocrusts can be differentiated based on soil metal content, with serpentine soil being enriched with Cr ($p = 0.023$), Mn ($p = 0.015$), Fe ($p = 0.017$), Co ($p = 0.012$), and Ni ($p = 0.017$). The Ca:Mg ratio of serpentine soil was also significantly lower than that of their non-serpentine counterparts ($p = 0.031$). Other environmental variables were included/excluded based on the VIFs and the final environmental variables were chosen to explain the variation in microbial composition across the different sampling sites were soil texture (percentage of sand, silt, and clay), PTM concentrations (Mn, Ni, and Fe), pH, EC, soil

nutrients (P and the nitrogen nutrient factor represented by "NO" which is the sum of NO^-_2 and NO^-_3 concentrations) and the Ca:Mg ratio.

The CCA for the bacterial dataset was performed for the 11 environmental variables and 34 bacterial orders left after filtering (Figure 4). A summary of Eigenvalues for the first four axes of the CCA is displayed in Table 2. The environmental variables accounted for 43.94% of the total variability in the occurrence of bacterial taxa across biocrust samples. The most important environmental variables shaping the bacterial community structure across different sites were clay, EC and sand. Furthermore, the ordination plot showed EC to be most strongly, and positively associated with the first ordination axis ($r = 0.668$), whereas clay was most strongly, and negatively related to the second axis ($r = -0.397$). The analysis revealed that PTMs (Mn and Ni) were positively associated with each other and the presence of silt and high Ca:Mg ratios was strongly associated with high pH values and high EC. The biocrust samples were also scattered across the ordination, making it challenging to identify clear groups. Efforts to link specific species to serpentine and non-serpentine sites were thus hampered by the lack of cohesion in the ordination. Some species associated closely with serpentine sites and some serpentine conditions, including Burkholderiales, Gemmatimonadales, Myxococcales to SD site 9, high silt percentages, high nitrogen nutrient concentrations, and high Mn concentrations, as well as Propionibacteriales, Solibacterales, Chitinophagales, and Defluviicoccales to SW site 11 and Kineosporiales, Elsterales, Micropepsales which also associated closely with serpentine sites (9 and 12) as well as high concentrations of Ni. Bryobacterales and Gaiellales were closely associated with NSD site 12 and Micromonosporales and C0119 were closely associated with NSW sites 16 and 14, respectively. Taxa that associated closely with high Ca:Mg ratios, EC, pH, and P concentrations were Blastocatellales, Azospirillales, Pyrinomonadales, and Rubrobacterales. Cyanobacteriales was found very close to the origin of axes in the CCA ordination demonstrating ubiquitous distribution across environmental variables and sites. However, caution should be exercised when establishing direct associations between these microbial taxa and specific environmental conditions and sites solely based on the ordination results.

CCA analysis was also performed for the fungal dataset for all samples and 11 statistically significant different environmental variables for six taxa (Figure 5). A summary of Eigenvalues for the first four axes of the CCA for the fungal dataset is displayed in Table 3. The environmental variables, soil texture, PTM concentrations, pH, electrical conductivity, soil nutrients, and the Ca:Mg ratio, could explain 70.36% of the total variability in the

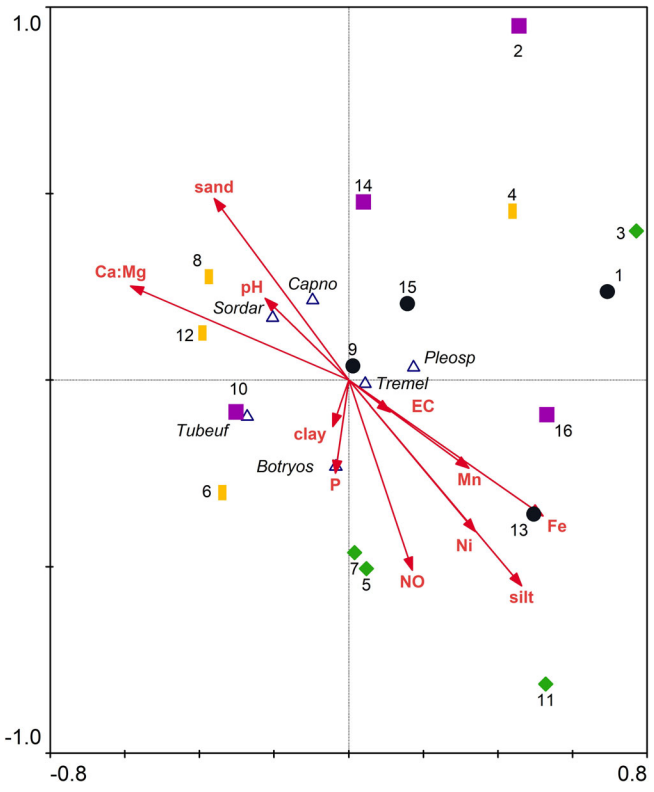

FIGURE 5 Environmental factors driving the fungal community composition variation. Canonical correspondence analysis (CCA) triplot based on amplicon sequence variants assigned to fungal orders (blue triangles) concerning the environmental variables (red arrows) and serpentine and non-serpentine soils. The sites are represented as follows: SW (cool serpentine soils with high rainfall) includes sites 1, 11, 13, and 15 (black circles); NSW (cool, non-serpentine soils with high rainfall) includes sites 2, 12, 14, and 16 (purple squares); SD (dry, serpentine soils with low rainfall) includes sites 3, 5, 7, and 9 (green diamonds); NSD (dry, non-serpentine soils with low rainfall) includes sites 4, 6, 8, and 10 (yellow rectangles). Botryos, Botryosphaeriales; Capno, Capnodiales; Pleosp, Pleosporales; Sordar, Sordariales; Tremel, Tremellales; Tubeuf, Tubeufiales.

occurrence of fungal taxa in the dataset. Silt, Fe, and the Ca:Mg ratio were identified as the most important variables in shaping fungal community structure. The Ca:Mg ratio is furthermore shown to be most strongly, and negatively associated with the first ordination axis ($r = -0.51$), whereas the percentage of silt was most strongly, and negatively associated with the second ordination axis ($r = -0.47$).

SD sites (5, 7, and 11) were characterized by the presence of high percentages of silt and clay, low pH values and Ca:Mg ratios, as well as a close correlation with high concentrations of PTMs and soil nutrients. The SD site 3, while also positively correlated with high percentages of silt, was also associated with sandy soil texture and low clay percentages. This site was also negatively

TABLE 2 Summary of results from canonical correspondence analysis (CCA) of the bacterial taxa-environment relation.

Axes	1	2	3	4
Eigenvalues	0.094	0.069	0.052	0.041
Species-environment correlations	0.998	0.934	0.981	0.894
Cumulative percentage of variance of species data	18.4	31.8	41.9	49.8
Cumulative percentage variance of species-environment relation	25.4	44.1	58.0	69.0
Sum of all eigenvalues = 0.513				
Sum of all canonical eigenvalues = 0.371				
Total inertia = 0.513				
Test of significance of all canonical axes: Trace = 0.371 *F*-ratio = 1.301 *p*-Value = 0.0600				

TABLE 3 Summary of results from canonical correspondence analysis (CCA) of the fungal taxa-environment relation.

Axes	1	2	3	4
Eigenvalues	0.169	0.142	0.083	0.033
Species-environment correlations	0.873	0.853	0.791	0.707
Cumulative percentage of variance of species data	26.4	48.6	61.4	66.6
Cumulative percentage variance of species-environment relation	38.2	70.3	89.0	96.5
Sum of all eigenvalues = 0.641				
Sum of all canonical eigenvalues = 0.442				
Total inertia = 0.641				
Test of significance of all canonical axes: Trace = 0.442 *F*-ratio = 1.116 *p*-Value = 0.3400				

correlated with P. SW sites (11, 13, and 15) were, like SD sites, characterized by the absence of clay, with site 1 associating with high silt percentages and 9 and 25 with sand. All SW sites were characterized by high concentrations of Fe and Mn and sites 1 and 13 further correlated with high Ni concentrations and high nitrogen. SW sites 9 and 15 also showed a weak positive association with high pH values and Ca:Mg ratios. Overall, serpentine sites were associated with low Ca:Mg ratios, low pH values, the silt/sand soil texture, high concentrations of soil nutrients, especially nitrogen, as well as high concentrations of PTMs. Furthermore, serpentine sites with the above-mentioned characteristics were positively associated with the presence of fungal orders Tremellales and Pleosporales (SW sites 9 and 15: high EC, high concentrations of Mn and Fe) and Botryosphaeriales (SD sites 7 and 5: high concentrations of soil nutrients and high percentages of clay). All non-serpentine soils were positively associated with high percentages of sand (except NSW site 16, which was associated with high silt percentages), high Ca:Mg ratios (except NSD site 4 and NSW site 16) and high pH values (except NSW site 16). The fungal orders Capnodiales and Sordariales were also positively associated with these characteristics by being near NSD sites 8 and 12 in the CCA ordination. NSW sites 10 and 16 and NSD site 6 were further positively associated with high concentrations of soil nutrients. The fungal order Tubeufiales was closely associated with NSW site 10 (sand/clay soil texture, high Ca:Mg ratios, high pH values and high concentrations of soil nutrients). NSW site 16 and NSD site 4 were also associated with high EC and high concentrations of Mn and Fe.

The analysis of significant abundant genera, across treatments, soil type and precipitation level revealed some bacterial and fungal species that may be sensitive to certain environmental conditions (Figure 6). Eight bacterial genera showed significant abundance across treatments, soil types (serpentine and non-serpentine) and precipitation levels (high and low) (Figure 6a). *Archangium* (Order: Myxococcales) was significantly more abundant in "harsh" treatments: NSD, SD, and SW ($p = 0.48$), and biocrusts receiving lower precipitation ($p = 0.021$). This genus was also identified as an indicator species of biocrusts receiving lower precipitation ($p = 0.019$).

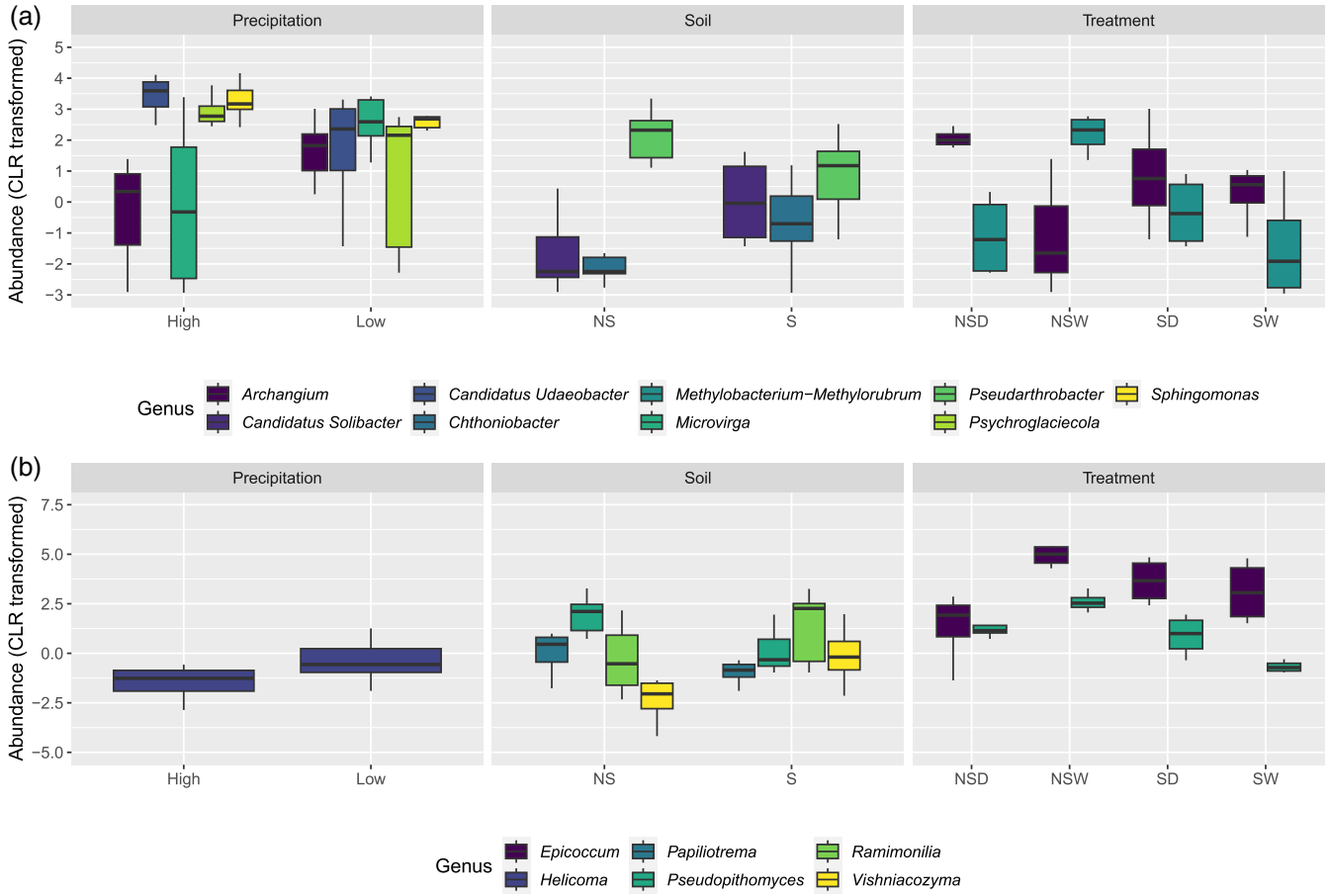

FIGURE 6 Boxplots illustrating the centered log-ratio (CLR) transformed abundance of significant (a) bacterial and (b) fungal genera ($p < 0.05$), stratified by precipitation levels (high and low) ($n = 8 \pm$ std), soil type (serpentine and non-serpentine) ($n = 8 \pm$ std), and treatment ($n = 4 \pm$ std). NS, non-serpentine soil; NSD, dry, non-serpentine soils with low rainfall; NSW, cool, non-serpentine soils with high rainfall; S, serpentine soil; SD, dry, serpentine soils with low rainfall; SW, cool serpentine soils with high rainfall. For the bacterial dataset (a), the genera appear in the following order from left to right: for precipitation: *Archangium*, *Candidatus Udeobacter*, *Microvirga*, *Psychroglaciecola*, and *Sphingomonas*; for soil: *Candidatus Solibacter*, *Chthoniobacter*, and *Pseudarthrobacter*; for treatment: *Archangium* and *Methylobacterium-Methylorubrum*. For the fungal dataset (b), the genera appear in the following order from left to right: for precipitation: *Helicoma*; for soil: *Papiliotrema*, *Pseudopithomyces*, *Ramimonilia*, and *Vishniacozyma*; for treatment: *Epicoccum* and *Pseudopithomyces*.

Methylobacterium-Methylorubrum (Order: Rhizobiales) was significantly more abundant in NSW ($p = 0.028$) and was also identified as an indicator species of biocrusts belonging to this treatment ($p = 0.013$). Other genera belonging to this order that were identified as significantly abundant, were *Psychroglaciecola* and *Microvirga*, with the former being more abundant in biocrusts receiving higher precipitation ($p = 0.021$) whereas *Microvirga* was more abundant in biocrusts receiving lower precipitation ($p = 0.036$). *Microvirga* was also an indicator species of biocrusts receiving lower precipitation ($p = 0.026$). Genera that were significantly more abundant in biocrusts of serpentine soils were *Candidatus Solibacter* (Order: Solibacterales) ($p = 0.036$) and *Chthoniobacter* (Order: Chthoniobacterales) ($p = 0.027$). *Pseudarthrobacter* (Order: Micrococcales) was more abundant in biocrusts of non-serpentine soils ($p = 0.046$). *Candidatus Udeobacter* (Order: Chthoniobacter) and *Sphingomonas* (Order: Sphingomonadales) were more abundant in biocrusts receiving higher precipitation.

Six fungal genera showed significant abundance across treatments, soil type, and precipitation levels (Figure 6b). The genera *Epicoccum* and *Pseudopithomyces* (order: Pleosporales) were significantly more abundant in NSW ($p = 0.042$ and $p = 0.008$, respectively), and *Pseudopithomyces* was also an indicator of biocrusts of non-serpentines ($p = 0.046$). *Papiliotrema* (Order: Tremellales) was significantly more abundant in non-serpentine biocrusts ($p = 0.046$) and was also an indicator of NSD ($p = 0.038$). *Ramimonilia* (order: Botryosphaeriales) was more abundant in biocrusts of serpentine soils ($p = 0.036$) and another genus from Tremellales, *Vishniacozyma*, was also significantly more abundant in serpentine biocrusts ($p = 0.016$) and was also an indicator of this condition ($p = 0.031$). The only

genus more abundant across precipitation levels was *Helicoma* (Order: Tubeufiales), whose abundance was significantly higher for biocrusts receiving lower precipitation ($p = 0.036$). The genus *Pyronchaetopsis* (Order: Pleosporales), although not significantly more abundant in biocrusts receiving higher precipitation, was an indicator of this condition ($p = 0.033$).

4 | DISCUSSION

The serpentine and non-serpentine soil harboring biocrusts differed significantly in terms of PTM content, with serpentine soils being enriched with Cr, Mn, Fe, Co, and Ni as well as having characteristically lower Ca:Mg ratios than their non-serpentine counterparts. The bacterial phyla Actinobacteria, Proteobacteria and Acidobacteriota were most abundant for NSD, SW and SD, which is consistent with the results of dominant phyla from serpentine and non-serpentine soils from a previous study (Khilyas et al., 2019). Cyanobacteria was less abundant in the serpentine soils since this phylum is sensitive to increased concentrations of metals (Lu et al., 2000), but not entirely intolerant to such conditions in the presence of moisture (Venter et al., 2018), as also reflected in the CCA ordination where it plotted close to the origin of axes, demonstrating ubiquitous distribution.

Studies by Branco and Ree (2010), Urban et al. (2008), and Venter et al. (2015, 2018) indicate that microbial communities in serpentine soils do not follow the general pattern of low diversity and high specialization seen among higher plants (Harrison & Rajakaruna, 2011), but are rich in species and phylogenetic distance compared to non-serpentine communities. The serpentine dry (SD) treatment showed the highest diversity of bacterial species richness and phylogenetic diversity. However, the non-significant statistics for the alpha and beta diversity of the bacterial community composition suggest several implications. First, the absence of significant differences in the observed alpha diversity across treatments, precipitation level, and soil type suggest that bacterial diversity may not be strongly influenced by the specific conditions considered in this study. This could be explained by the ubiquitous nature of bacteria, which are generally less specialized (Chen et al., 2022) and more adaptable compared to fungi. Fungi often exhibit distinct ecological niches and show specialized adaptations, while bacteria may be less affected by dispersal limitations and less prone to differentiation based on environmental conditions alone. Second, the lack of differentiation in bacterial community structure between serpentine and non-serpentine treatments and precipitation levels suggests that other unmeasured variables or ecological processes may play a more important role in shaping these communities. Additionally, the lack of distinguishable patterns and unexpected correlations observed in the CCA could indicate that the environmental variables considered in this study (concentrations of soil nutrients, PTM concentrations, pH, electrical conductivity, and the Ca: Mg ratio), do not fully capture the complexity of factors that drive bacterial community structure across serpentine and non-serpentine biocrusts and implies a complex and potentially multifaceted relationship between bacterial communities and the environmental conditions.

While the non-significant statistical results suggest that the considered environmental conditions and variables do not fully explain the bacterial community composition, specific taxa correlations were identified in the CCA that may provide insights into the ecological dynamics of these communities. For instance, Myxococcales and Solibacterales, which were closely associated with serpentine treatments had genera, *Archangium* and *Candidatus Solibacter*, respectively, known to be significantly abundant in, or indicators of, harsh environmental conditions. *Candidatus Solibacter* was significantly abundant in serpentine biocrusts and *Archangium* in NSD, SD, and SW and was also an indicator of biocrusts receiving lower precipitation. *Candidatus Solibacter* produces enzymes that can break down organic carbon (Ward et al., 2009; Dedysh & Yilmaz, 2018), is adapted to low-nutrient environments (Eichorst et al., 2011) and provides conditions to benefit other bacteria that degrade organic compounds (Rime et al., 2015). Myxococcales, or Myxobacteria, can survive under extremes such as broad pH and temperature ranges. Members of this order also have highly tolerant fruiting bodies when stressed by starvation (Findlay, 2016). This may explain why *Archangium* was significantly more abundant and an indicator of sites receiving lower precipitation. Other genera that were significantly abundant in harsh environments (serpentine and/or lower precipitation) were *Chthoniobacter* and *Microvirga*. *Chthoniobacter flavus* is the sole member of the genus *Chthoniobacter* and can grow at pH values of 4.0 up to 7.5, plays an important role in the decomposition of plant material and transformation of organic carbon compounds in the soil (aids in preventing nutrient leaching and soil erosion) and is unable to grow on amino acids or organic acids other than pyruvate (Sangwan et al., 2004). *Chthoniobacter* belongs to the class Spartobacteria which has members that are highly active and abundant in soil habitats (Sangwan et al., 2004). Species contributing to the *Microvirga* genus are versatile and widely distributed. These members have been distinguished into two clades, categorized by isolation from roots or soil (Li et al., 2020). Members

belonging to the soil clade are characterized by a high abundance of heat- or radiation-resistant genes. Bacterial taxa that correlate closely with serpentine environments or were found to be more abundant in serpentine than non-serpentine treatments were also commonly associated with having adaptations to extreme environments.

Genera that were abundant in non-serpentine soils or sites receiving higher precipitation were two genera from the order Rhizobiales, *Methylobacterium-Methylorubrum*, and *Psychroglaciecola*, as well as a genus from the order Sphingomonadales, *Sphingomonas*. Members belonging to both *Methylobacterium* and *Sphingomonas* are anoxygenic phototrophs and several of their isolates have been reported in biocrusts (Csotonyi et al., 2010). *Methylobacterium-Methylorubrum* is a methanotrophic Alphaproteobacteria that lives freely in water, soil or air and they are especially associated with the phyllosphere (Delmotte et al., 2009; Knief et al., 2008) and are known plant colonizers (Knief et al., 2010). Members of the family Beijerenckiaceae, *Microvirga* and *Psychroglaciecola*, are described as symbionts of bryophytes and they could contribute to biocrust formation in environments of lower and higher precipitation levels, respectively. Environmental conditions such as elevated moisture levels could lead to the enrichment of *Sphingomonas* in biocrust since this genus is known to increase with biocrust development (Zhang et al., 2016). *Candidatus_Udaeobacter* (order: Chthoniobacterales) was significantly abundant in sites receiving higher precipitation and is an organic matter-degrading bacterium that has been observed in non-serpentine soil (Böhmer et al., 2020).

In contrast to Branco and Ree's (2010) finding that fungal diversity is not limited by the chemical characteristics of serpentine soils, we found that serpentine soils have a distinct composition and are less diverse when compared to non-serpentine soils. Serpentine environments can have reduced leaf litter and soil organic matter that may lead to greater extremes of temperature, less water holding capacity and soil aeration, and more limited nutrient cycling and therefore less favorable conditions for fungal growth (Southworth et al., 2013) The fungal community structure also differed according to higher and lower precipitation levels with greater species richness observed for biocrusts receiving higher precipitation. According to Chen et al. (2019), drought stress increases the relative abundance of Ascomycota, which is reflected in the present study by this phylum being most abundant in SD and NSD. Wang et al. (2014) also note that changes in soil total nitrogen and pH due to precipitation played important roles in shaping the fungal community structure in a temperate forest. The most prevalent fungal orders across the serpentine and non-serpentine treatments, as represented in the CCA ordination (Figure 5), were Botryospirales, Capnodiales, Pleosporales, Sordariales, Tremellales, and Tubeufiales. The Botryospirales were associated with high concentrations of soil nutrients (phosphorous) and clay. Botryospirales species are important pathogens of woody plants but have been found on lichens (Denman et al., 2000) and in lichen biocrusts growing on limestone and dolomite outcrops on high elevations (2026 m) of the Al-Jabal Al-Akhdar mountain range (Abed et al., 2013). A genus belonging to this order, *Ramimonilia*, was found to be significantly more abundant in serpentine soils. *R. apicalis* is the sole member of this genus which is a rock-inhabiting fungus first isolated from Patones, Central Mountain System, Spain, that tolerates harsh conditions on rock surfaces (Ruibal et al., 2009). Capnodiales and Sordariales were closely associated with sites 8 and 10 (NSD) and soil nutrients. The order Capnodiales includes fungi known as the sooty molds and, like Sordariales, encompasses a wide array of metabolically diverse species (Crous et al., 2007; Kruys et al., 2015). The fungal orders Pleosporales and Tremellales were associated with serpentine soils with high concentrations of Fe and Mn and high electrical conductivity. However, significantly abundant genera belonging to Pleosporales, *Epicoccum* and *Pseudopithomyces*, favored NSW and *Pseudopithomyces* was also an indicator of non-serpentine biocrusts. Other studies have shown that members of Pleosporales can be present in serpentine and non-serpentine soils (Daghino et al., 2012). Genera belonging to Tremellales was significantly abundant across serpentine and non-serpentine treatments with *Papiliotrema* being significantly abundant in non-serpentine biocrusts and an indicator of NSD and *Vishniacozyma* being significantly abundant in serpentine biocrusts. *Papiliotrema* is a yeast whose species are associated with nutrient-rich habitats (Ladino et al., 2019) as well as being an indicator of bare soils sampled next to moss biocrusts and is therefore exposed to environmental stress (García-Carmona et al., 2022). *Vishniacozyma* is highly enriched in root rhizospheric samples from acid mine drainage sites, which are extreme environments rich in PTMs (Kalu et al., 2021).

5 | CONCLUSION

The bacterial and fungal community composition of serpentine and non-serpentine soils differ significantly and serpentine soils were found to be enriched with metal-tolerant or resistant microorganisms. Bacterial community structures were not statistically distinguishable between serpentine and non-serpentine soil. Nonetheless, taxa resistant to extreme environments were more abundant in serpentine biocrusts or those receiving lower precipitation and included the genera *Archangium*, *Candidatus Solibacter*, Chthoniobacter, and *Microvirga*.

Fungal species richness, however, was higher at the non-serpentine localities than at serpentine localities. A significant difference was found between the fungal community structure of serpentine and non-serpentine treatments (beta diversity) as well as biocrusts receiving higher and lower precipitation. Fungal biocrusts of serpentine soils exhibited a notable abundance of genera adapted to harsh environmental conditions and high concentrations of PTMs. *Ramimonilia* and *Vishniacozyma* were significantly more abundant in serpentine biocrusts than their non-serpentine counterparts and *Vishniacozyma* was also identified as an indicator species specifically associated with serpentine habitats. In summary, our findings highlight two key influences on fungal communities in the studied environments. First, soil type determines the richness of fungal species within biocrusts. We observed a decrease in fungal species richness (alpha diversity) on serpentine soils, suggesting a selective filter effect for specific fungal species adapted to harsh soil environments. Second, precipitation levels played a crucial role in species turnover (beta diversity), influencing the differentiation of fungal biocrust communities between sites. Distinct fungal community structure was observed for serpentine and non-serpentine biocrusts. We conclude that a complex interplay exists between serpentine soil characteristics and precipitation to shape fungal diversity within and across habitats.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

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REFERENCES

Abed, R. M. M., Al-Sadi, A. M., Al-Shehi, M., Al-Hinai, S., & Robinson, M. D. (2013). Diversity of free-living and lichenized fungal communities in biological soil crusts of the Sultanate of Oman and their role in improving soil properties. *Soil Biology and Biochemistry*, *57*, 695–705. https://doi.org/10.1016/j.soilbio.2012.07.023

Abou-Shanab, R. A. I., Van Berkum, P., Angle, J. S., Delorme, T. A., Chaney, R. L., Ghozlan, H. A., Ghanem, K., & Moawad, H. (2009). Characterization of Ni-resistant bacteria in the rhizosphere of the hyperaccumulator *Alyssum murale* by 16S rRNA gene sequence analysis. *World Journal of Microbiology and Biotechnology*, *26*, 101–108. https://doi.org/10.1007/s11274-009-0148-6

Aitchison, J., & Greenacre, M. (2002). Biplots of compositional data. *Journal of the Royal Statistical Society: Series c (Applied Statistics)*, *51*, 375–392. https://doi.org/10.1111/1467-9876.00275

Alexander, E. B., Coleman, R. G., Keeler-Wolfe, T., & Harrison, S. P. (2007). *Serpentine Geoecology of Western North America: Geology, soils, and vegetation*. Oxford University Press.

Anacker, B. L. (2014). The nature of serpentine endemism. *American Journal of Botany*, *101*, 219–224. https://doi.org/10.3732/ajb.1300349

Barlow, J. T., Bogatyrev, S. R., & Ismagilov, R. F. (2020). A quantitative sequencing framework for absolute abundance measurements of mucosal and lumenal microbial communities. *Nature Communications*, *11*, 2590. https://doi.org/10.1038/s41467-020-16224-6

Belnap, J., Büdel, B., & Lange, O. L. (2003). Biological soil crusts: Characteristics and distribution. In O. L. Lange (Ed.), *Biological soil crusts: Structure, function and management* (pp. 3–30). Springer.

Bini, C., & Maleci, L. (2014). The "Serpentine Syndrome" (H. Jenny, 1980): A proxy for soil remediation. *DOAJ (DOAJ: Directory of Open Access Journals)*, *15*, 1–13. https://doi.org/10.6092/issn.2281-4485/4547

Böhmer, M., Ozdín, D., Račko, M., Lichvár, M., Budiš, J., & Szemes, T. (2020). Identification of bacterial and fungal communities in the roots of orchids and surrounding soil in heavy metal contaminated area of mining heaps. *Applied Sciences*, *10*, 7367. https://doi.org/10.3390/app10207367

Branco, S., & Ree, R. H. (2010). Serpentine soils do not limit mycorrhizal fungal diversity. *PLoS One*, *5*, e11757. https://doi.org/10.1371/journal.pone.0011757

Brzeszcz, J., Steliga, T., Kapusta, P., Turkiewicz, A., & Kaszycki, P. (2016). R-strategist versus K-strategist for the application in bioremediation of hydrocarbon-contaminated soils. *International Biodeterioration & Biodegradation*, *106*, 41–52. https://doi.org/10.1016/j.ibiod.2015.10.001

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581–583. https://doi.org/10.1038/nmeth.3869

Chamizo, S., Cantón, Y., Rodríguez-Caballero, E., & Domingo, F. (2016). Biocrusts positively affect the soil water balance in semiarid ecosystems. *Ecohydrology*, *9*, 1208–1221. https://doi.org/10.1002/eco.1719

Chen, H., Zhao, X., Lin, Q., Li, G., & Kong, W. (2019). Using a combination of PLFA and DNA-based sequencing analyses to detect shifts in the soil microbial community composition after a simulated spring precipitation in a semi-arid grassland in China. *Science of the Total Environment*, *657*, 1237–1245. https://doi.org/10.1016/j.scitotenv.2018.12.126

Chen, Y., Xi, J., Xiao, M., Wang, S., Chen, W., Liu, F., Shao, Y., & Yuan, Z. (2022). Soil fungal communities show more specificity

than bacteria for plant species composition in a temperate forest in China. *BMC Microbiology*, *22*, 208. https://doi.org/10.1186/s12866-022-02591-1

Couradeau, E., Karaoz, U., Lim, H. C., Nunes, U., Northen, T. R., Brodie, E. L., & Garcia-Pichel, F. (2016). Bacteria increase arid-land soil surface temperature through the production of sunscreens. *Nature Communications*, *7*, 1–7. https://doi.org/10.1038/ncomms10373

Crous, P. W., Braun, U., & Groenewald, J. Z. (2007). Mycosphaerella is polyphyletic. *Studies in Mycology*, *58*, 1–32. https://doi.org/10.3114/sim.2007.58.01

Csotonyi, J. T., Swiderski, J., Stackebrandt, E., & Yurkov, V. (2010). A new environment for aerobic anoxygenic phototrophic bacteria: Biological soil crusts. *Environmental Microbiology Reports*, *2*, 651–656. https://doi.org/10.1111/j.1758-2229.2010.00151.x

Daghino, S., Murat, C., Sizzano, E., Girlanda, M., & Perotto, S. (2012). Fungal diversity is not determined by mineral and chemical differences in serpentine substrates. *PLoS One*, *7*, e44233. https://doi.org/10.1371/journal.pone.0044233

De Cáceres, M., & Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. *Ecology*, *90*, 3566–3574. https://doi.org/10.1890/08-1823.1

Dedysh, S. N., & Yilmaz, P. (2018). Refining the taxonomic structure of the phylum *Acidobacteria*. *International Journal of Systematic and Evolutionary Microbiology*, *68*, 3796–3806. https://doi.org/10.1099/ijsem.0.003062

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., Von Mering, C., & Vorholt, J. A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences*, *106*, 16428–16433. https://doi.org/10.1073/pnas.0905240106

Denman, S., Crous, P. W., Taylor, J. E., Kang, J. C., Pascoe, L., & Wingfield, M. J. (2000). An overview of the taxonomic history of *Botryosphaeria*, and a re-evaluation of its anamorphs based on morphology and ITS rDNA phylogeny. *Studies in Mycology*, *45*, 129–140.

Eichorst, S. A., Kuske, C. R., & Schmidt, T. M. (2011). Influence of plant polymers on the distribution and cultivation of bacteria in the phylum Acidobacteria. *Applied and Environmental Microbiology*, *77*, 586–596. https://doi.org/10.1128/aem.01080-10

Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Büdel, B., Andreae, M. O., & Pöschl, U. (2012). Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nature Geoscience*, *5*, 459–462. https://doi.org/10.1038/ngeo1486

Favero-Longo, S. E., Matteucci, E., Giordani, P., Paukov, A., & Rajakaruna, N. (2018). Diversity and functional traits of lichens in ultramafic areas: A literature-based worldwide analysis integrated by field data at the regional scale. *Ecological Research*, *33*, 593–608. https://doi.org/10.1007/s11284-018-1573-5

Findlay, B. (2016). The chemical ecology of predatory soil bacteria. *ACS Chemical Biology*, *11*, 1502–1510. https://doi.org/10.1021/acschembio.6b00176

Gabay, T., Petrova, E., Gillor, O., Ziv, Y., & Angel, R. (2023). Only a minority of bacteria grow after wetting in both natural and post-mining biocrusts in a hyperarid phosphate mine. *The Soil*, *9*, 231–242. https://doi.org/10.5194/soil-9-231-2023

Galey, M. L., Van der Ent, A., Iqbal, M. C. M., & Rajakaruna, N. (2017). Ultramafic geoecology of South and Southeast Asia. *Botanical Studies*, *58*, 18. https://doi.org/10.1186/s40529-017-0167-9

García-Carmona, M., Lepinay, C., García-Orenes, F., Baldrian, P., Arcenegui, V., Cajthaml, T., & Mataix-Solera, J. (2022). Moss biocrust accelerates the recovery and resilience of soil microbial communities in fire-affected semi-arid Mediterranean soils. *Science of the Total Environment*, *846*, 157467. https://doi.org/10.1016/j.scitotenv.2022.157467

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*, 1–6. https://doi.org/10.3389/fmicb.2017.02224

Harrison, S., & Rajakaruna, N. (2011). *Serpentine: The evolution and ecology of a model system*. University Of California Press.

Husna, Tuheteru, F. D., & Arif, A. (2017). *Arbuscular mycorrhizal fungi and plant growth on serpentine soils* (pp. 293–303). Springer EBooks. https://doi.org/10.1007/978-981-10-4115-0_12

Jenny, H. (1980). *The soil resource: Origin and behavior*. Springer Science & Business Media.

Kalu, C. M., Oduor Ogola, H. J., Selvarajan, R., Tekere, M., & Ntushelo, K. (2021). Fungal and metabolome diversity of the rhizosphere and endosphere of *Phragmites australis* in an AMD-polluted environment. *Heliyon*, *7*, e06399. https://doi.org/10.1016/j.heliyon.2021.e06399

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780. https://doi.org/10.1093/molbev/mst010

Khilyas, I. V., Sorokina, A. V., Elistratova, A. A., Markelova, M., Siniagina, M., Шарипова, M. P., Shcherbakova, T. A., D'Errico, M. E., & Cohen, M. F. (2019). Microbial diversity and mineral composition of weathered serpentine rock of the Khalilovsky massif. *PLoS One*, *14*, e0225929. https://doi.org/10.1371/journal.pone.0225929

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2012). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*, e1. https://doi.org/10.1093/nar/gks808

Knief, C., Frances, L., Cantet, F., & Vorholt, J. A. (2008). Cultivation-independent characterization of *Methylobacterium* populations in the plant phyllosphere by automated ribosomal intergenic spacer analysis. *Applied and Environmental Microbiology*, *74*, 2218–2228. https://doi.org/10.1128/aem.02532-07

Knief, C., Ramette, A., Frances, L., Alonso-Blanco, C., & Vorholt, J. A. (2010). Site and plant species are important determinants of the *Methylobacterium* community composition in the plant phyllosphere. *The ISME Journal*, *4*, 719–728. https://doi.org/10.1038/ismej.2010.9

Kruys, Å., Huhndorf, S. M., & Miller, A. N. (2015). Coprophilous contributions to the phylogeny of *Lasiosphaeriaceae* and allied taxa within *Sordariales* (Ascomycota, Fungi). *Fungal Diversity*, *70*, 101–113. https://doi.org/10.1007/s13225-014-0296-3

Ladino, G., Ospina-Bautista, F., Estévez Varón, J., Jerabkova, L., & Kratina, P. (2019). Ecosystem services provided by bromeliad plants: A systematic review. *Ecology and Evolution*, *9*, 7360–7372. https://doi.org/10.1002/ece3.5296

Li, J., Gao, R., Chen, Y., Xue, D., Han, J., Wang, J., Dai, Q., Lin, M., Ke, X., & Zhang, W. (2020). Isolation and identification of *Microvirga thermotolerans* HR1, a novel thermo-tolerant bacterium, and comparative genomics among *Microvirga* species. *Microorganisms*, *8*, 101. https://doi.org/10.3390/microorganisms8010101

Lu, C. M., Chau, C. W., & Zhang, J. H. (2000). Acute toxicity of excess mercury on the photosynthetic performance of cyanobacterium, *S. platensis* – Assessment by chlorophyll fluorescence analysis. *Chemosphere*, *41*, 191–196. https://doi.org/10.1016/s0045-6535(99)00411-7

Ma, Y., Rajkumar, M., Rocha, I., Oliveira, R. S., & Freitas, H. (2015). Serpentine bacteria influence metal translocation and bioconcentration of *Brassica juncea* and *Ricinus communis* grown in multi-metal polluted soils. *Frontiers in Plant Science*, *5*, 1–13. https://doi.org/10.3389/fpls.2014.00757

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet Journal*, *17*, 10. https://doi.org/10.14806/ej.17.1.200

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An r package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*, e61217. https://doi.org/10.1371/journal.pone.0061217

Mengoni, A., Barzanti, R., Gonnelli, C., Gabbrielli, R., & Bazzicalupo, M. (2001). Characterization of nickel-resistant bacteria isolated from serpentine soil. *Environmental Microbiology*, *3*, 691–698. https://doi.org/10.1046/j.1462-2920.2001.00243.x

Muller, L. A. H., & Hilger, H. H. (2015). Insights into the effects of serpentine soil conditions on the community composition of fungal symbionts in the roots of *Onosma echioides*. *Soil Biology and Biochemistry*, *81*, 1–8. https://doi.org/10.1016/j.soilbio.2014.10.027

Mulroy, M., Fryday, A. M., Gersoff, A., Dart, J., Reese Næsborg, R., & Rajakaruna, N. (2022). Lichens of ultramafic substrates in North America: A review. *Botany*, *100*, 593–617. https://doi.org/10.1139/cjb-2021-0187

Naidoo, Y., Valverde, A., Pierneef, R. E., & Cowan, D. A. (2022). Differences in precipitation regime shape microbial community composition and functional potential in Namib Desert soils. *Microbial Ecology*, *83*, 689–701. https://doi.org/10.1007/s00248-021-01785-w

Nilsson, R. H., Larsson, K.-H., Taylor, A. F., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*, D259–D264. https://doi.org/10.1093/nar/gky1022

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Wagner, H., & Oksanen, M. J. (2022). Vegan: Community ecology package. R Package Version 2.6-4. https://cran.r-project.org/package=vegan

Oline, D. K. (2006). Phylogenetic comparisons of bacterial communities from serpentine and nonserpentine soils. *Applied and Environmental Microbiology*, *72*, 6965–6971. https://doi.org/10.1128/aem.00690-06

Ortiz, Y., Restrepo, C., Vilanova-Cuevas, B., Santiago-Valentin, E., Tringe, S. G., & Godoy-Vitorino, F. (2020). Geology and climate influence rhizobiome composition of the phenotypically diverse tropical tree *Tabebuia heterophylla*. *PLoS One*, *15*, e0231083. https://doi.org/10.1371/journal.pone.0231083

Paradis, E., & Schliep, K. (2018). Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics*, *35*, 526–528. https://doi.org/10.1093/bioinformatics/bty633

Pessoa-Filho, M., Barreto, C. C., dos Reis Junior, F. B., Fragoso, R. R., Costa, F. S., de Carvalho Mendes, I., & de Andrade, L. R. M. (2015). Microbiological functioning, diversity, and structure of bacterial communities in ultramafic soils from a tropical savanna. *Antonie Van Leeuwenhoek*, *107*, 935–949. https://doi.org/10.1007/s10482-015-0386-6

Pombubpa, N., Pietrasiak, N., De Ley, P., & Stajich, J. E. (2020). Insights into dryland biocrust microbiome: Geography, soil depth and crust type affect biocrust microbial communities and networks in Mojave Desert, USA. *FEMS Microbiology Ecology*, *96*, 1–16. https://doi.org/10.1093/femsec/fiaa125

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*, D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2023). *R: A language and environment for statistical computing*. R-Project.org; R Foundation for Statistical Computing. http://www.r-project.org/

Rajakaruna, N., & Boyd, R. S. (2008). Edaphic factor. In S. E. Jørgensen & D. D. Fath (Eds.), *Encyclopedia of ecology* (pp. 1201–1207). Elsevier Science.

Rajakaruna, N., & Boyd, R. S. (2014). Serpentine soils. In D. Gibson (Ed.), *Oxford Bibliographies in Ecology*. Oxford University Press. https://doi.org/10.1093/obo/9780199830060-0055

Rajakaruna, N., Harris, T. B., & Alexander, E. B. (2009). Serpentine geoecology of eastern north america: A review. *Rhodora*, *111*, 21–108. https://doi.org/10.3119/07-23.1

Rajakaruna, N., Knudsen, K., Fryday, A. M., O'Dell, R. E., Pope, N. S., Olday, F. C., & Woolhouse, S. (2012). Investigation of the importance of rock chemistry for saxicolous lichen communities of the New Idria serpentinite mass, San Benito County, California, USA. *The Lichenologist*, *44*, 695–714. https://doi.org/10.1017/s0024282912000205

Rajkumar, M., Vara Prasad, M. N., Freitas, H., & Ae, N. (2009). Biotechnological applications of serpentine soil bacteria for phytoremediation of trace metals. *Critical Reviews in Biotechnology*, *29*, 120–130. https://doi.org/10.1080/07388550902913772

Reynolds, R., Belnap, J., Reheis, M., Lamothe, P., & Luiszer, F. (2001). Aeolian dust in Colorado Plateau soils: Nutrient inputs and recent change in source. *Proceedings of the National Academy of Sciences*, *98*, 7123–7127. https://doi.org/10.1073/pnas.121094298

Rime, T., Hartmann, M., Brunner, I., Widmer, F., Zeyer, J., & Frey, B. (2015). Vertical distribution of the soil microbiota along a successional gradient in a glacier forefield. *Molecular Ecology*, *24*, 1091–1108. https://doi.org/10.1111/mec.13051

Robinson, B., Brooks, R. R., & Clothier, B. E. (1999). Soil amendments affecting nickel and cobalt uptake by *Berkheya coddii*: Potential use for phytomining and phytoremediation. *Annals of Botany*, *84*, 689–694. https://doi.org/10.1006/anbo.1999.0970

Ruibal, C., Gueidan, C., Selbmann, L., Gorbushina, A. A., Crous, P. W., Groenewald, J. Z., Muggia, L., Grube, M.,

Isola, D., Schoch, C. L., Staley, J. T., Lutzoni, F., & de Hoog, G. S. (2009). Phylogeny of rock-inhabiting fungi related to Dothideomycetes. *Studies in Mycology*, *64*, 123–133. https://doi.org/10.3114/sim.2009.64.06

Rutherford, W. A., Painter, T. H., Ferrenberg, S., Belnap, J., Okin, G. S., Flagg, C., & Reed, S. C. (2017). Albedo feedbacks to future climate via climate change impacts on dryland biocrusts. *Scientific Reports*, *7*, 1–9. https://doi.org/10.1038/srep44188

Sangwan, P., Chen, X., Hugenholtz, P., & Janssen, P. H. (2004). *Chthoniobacter flavus* gen. nov., sp. nov., the first pure-culture representative of subdivision two, Spartobacteria classis nov., of the phylum Verrucomicrobia. *Applied and Environmental Microbiology*, *70*, 5875–5881. https://doi.org/10.1128/aem.70.10.5875-5881.2004

Schechter, S. P., & Branco, S. (2014). The ecology and evolution of mycorrhizal fungi in extreme soils. In N. Rajakaruna, R. S. Boyd, & T. B. Harris (Eds.), *Plant ecology and evolution in harsh environments* (pp. 33–53). Nova Publishers.

Silverman, J. D., Bloom, R. J., Jiang, S., Durand, H. K., Dallow, E., Mukherjee, S., & David, L. A. (2021). Measuring and mitigating PCR bias in microbiota datasets. *PLoS Computational Biology*, *17*, e1009113. https://doi.org/10.1371/journal.pcbi.1009113

Southworth, D., Tackaberry, L. E., & Massicotte, H. B. (2013). Mycorrhizal ecology on serpentine soils. *Plant Ecology & Diversity*, *7*, 445–455. https://doi.org/10.1080/17550874.2013.848950

Strauss, S. L., Day, T. A., & Garcia-Pichel, F. (2012). Nitrogen cycling in desert biological soil crusts across biogeographic regions in the Southwestern United States. *Biogeochemistry*, *108*, 171–182. https://doi.org/10.1007/s10533-011-9587-x

Teptina, A., Paukov, A., & Rajakaruna, N. (2018). Ultramafic vegetation and soils in the circumboreal region of the Northern Hemisphere. *Ecological Research*, *33*, 609–628. https://doi.org/10.1007/s11284-018-1577-1

Ter Braak, C. J. F., & Smilauer, P. (2002). *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5)*. https://edepot.wur.nl/405659

Turgay, O. C., Görmez, A., & Bilen, S. (2012). Isolation and characterization of metal resistant-tolerant rhizosphere bacteria from the serpentine soils in Turkey. *Environmental Monitoring and Assessment*, *184*, 515–526. https://doi.org/10.1007/s10661-011-1984-z

Urban, A., Puschenreiter, M., Strauss, J., & Gorfer, M. (2008). Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil. *Mycorrhiza*, *18*, 339–354. https://doi.org/10.1007/s00572-008-0189-y

Venter, A., Levanets, A., Siebert, S. J., & Rajakaruna, N. (2015). A preliminary survey of the diversity of soil algae and cyanoprokaryotes on mafic and ultramafic substrates in South Africa. *Australian Journal of Botany*, *63*, 341. https://doi.org/10.1071/bt14207

Venter, A., Siebert, S., Rajakaruna, N., Barnard, S., Levanets, A., Ismail, A., Allam, M., Peterson, B., & Sanko, T. (2018). Biological crusts of serpentine and non-serpentine soils from the Barberton Greenstone Belt of South Africa. *Ecological Research*, *33*, 629–640. https://doi.org/10.1007/s11284-017-1546-0

Visioli, G., Sanangelantoni, A. M., Conti, F. D., Bonati, B., Gardi, C., & Menta, C. (2019). Above and belowground biodiversity in adjacent and distinct serpentine soils. *Applied Soil Ecology*, *133*, 98–103. https://doi.org/10.1016/j.apsoil.2018.09.013

Wang, M., Shi, S., Lin, F., & Jiang, P. (2014). Response of the soil fungal community to multi-factor environmental changes in a temperate forest. *Applied Soil Ecology*, *81*, 45–56. https://doi.org/10.1016/j.apsoil.2014.04.008

Ward, N. L., Challacombe, J. F., Janssen, P. H., Henrissat, B., Coutinho, P. M., Wu, M., Xie, G., Haft, D. H., Sait, M., Badger, J., Barabote, R. D., Bradley, B., Brettin, T. S., Brinkac, L. M., Bruce, D., Creasy, T., Daugherty, S. C., Davidsen, T. M., DeBoy, R. T., … Kuske, C. R. (2009). Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology*, *75*, 2046–2056. https://doi.org/10.1128/aem.02294-08

Weber, B., Büdel, B., & Belnap, J. (2016). *Biological soil crusts: An organizing principle in drylands* (pp. 3–13). Springer International Publishing.

White, T. J., Bruns, T. D., Lee, S. B., & Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). Elsevier Science.

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. https://ggplot2.tidyverse.org

Zhang, B., Kong, W., Wu, N., & Zhang, Y. (2016). Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. *Journal of Basic Microbiology*, *56*, 670–679. https://doi.org/10.1002/jobm.201500751

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