**RESEARCH ARTICLE**

# Climate dictates microbial community composition and diversity in Australian biological soil crusts (biocrusts)

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**Abstract**

The soil surface of drylands can typically be colonized by cyanobacteria and other microbes, forming biological soil crusts or 'biocrusts'. Biocrusts provide critical benefits to ecosystems and are a common component of the largely arid and semi-arid Australian continent. Yet, their distribution and the parameters that shape their microbial composition have not been investigated. We present here the first detailed description of Australia's biocrust microbiome assessed from 15 sites across the continent using 16S rRNA sequencing. The most abundant bacterial phyla from all sites were Cyanobacteria, Proteobacteria, Actinobacteria, Chloroflexi and Bacteroidetes. Cyanobacterial communities from northern regions were more diverse and unclassified cyanobacteria were a noticeable feature of northern biocrusts. Segregation between northern and southern regions was largely due to the differential abundance of *Microcoleus* spp., with *M. paludosus* dominating in the north and *M. vaginatus* dominating in the south. The geographical shifts in bacterial composition and diversity were correlated to seasonal temperatures and summer rainfall. Our findings provide an initial reference for sampling strategies to maximize access to bacterial genetic diversity. As hubs for essential ecosystem services, further investigation into biocrusts in arid and semi-arid regions may yield discoveries of genetic mechanisms that combat increases in warming due to climate change.

## INTRODUCTION

Biological soil crusts ('biocrusts' hereafter) are complex communities of microorganisms and non-vascular plants, including cyanobacteria, algae, microfungi, lichens and bryophytes, which form a continuous organic profile within the top millimetres of surface soil (Belnap, 2001; Belnap et al., 2001). Biocrusts are often constrained to arid lands where they are centres for multiple ecosystem services such as carbon sequestration, atmospheric nitrogen fixation, nutrient enrichment, soil stabilization and soil hydrology regulation (Belnap, 2002; Bowker et al., 2013; Felde et al., 2014; Zhang, 2005; Zhao et al., 2016). Within arid lands,

biocrusts commonly form the dominant soil cover (Pointing & Belnap, 2012) while globally, biocrusts cover approximately 12% of Earth's terrestrial surface (Rodriguez-Caballero et al., 2018).

The forces underlying biocrust composition and distribution are scale-dependent. At the micro- to local-scale, biotic, topographic and edaphic (soil) factors have the greatest influence, while climatic and biogeographic factors operate at larger, intra-continental scales (Bowker et al., 2016). The primary basis for climatic and biogeographic forces has been derived from studies on the macrocomponents of biocrusts, such as lichens and mosses (Bowker et al., 2014; Concostrina-Zubiri et al., 2014; Martínez et al., 2006). Within Australia,
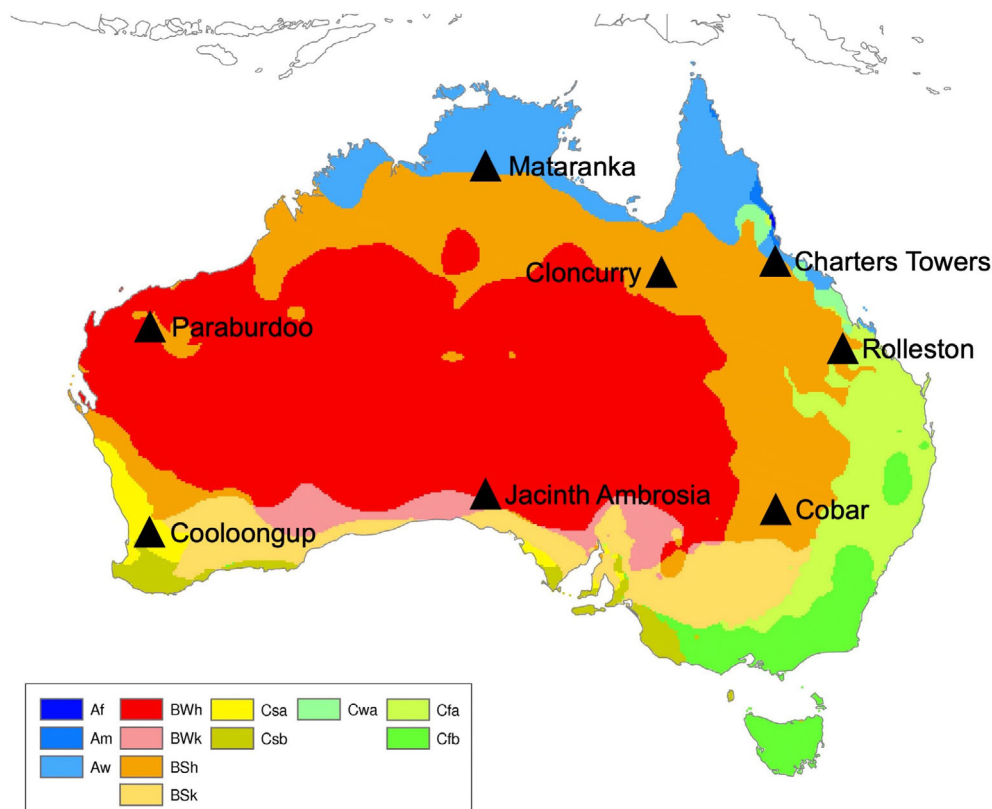
**FIGURE 1** Sample site locations across Australia with Köppen-Geiger climate classification zones shown. Where Af, tropical rainforest; Am, tropical monsoon; Aw, tropical savannah; BWh, arid desert, hot; BWk, arid desert, cold; BSh, arid steppe, hot; BSk, arid steppe, cold; Csa, temperate dry summer, hot summer; Csb, temperate dry summer, warm summer; Cwa, temperate dry winter, hot summer; Cfa, temperate without dry season, hot summer, and Cfb, temperate without dry season, hot summer. Climate zones based on criteria defined in Peel et al. (2007)

observable effects of climate on the distribution of dryland lichens manifest through seasonality of precipitation where lichens are mostly restricted to regions of winter rainfall (Eldridge, 1997; Rogers, 1972). For cyanobacteria, species richness within biocrusts of the northern Australian savannah has been observed to increase during the wet season, with links to nitrogen enrichment of the soil (Williams et al., 2018). Meanwhile, bacterial profiling of biocrusts from deserts of western America (Ferrenberg et al., 2015; Garcia-Pichel et al., 2013; Steven et al., 2015), the Mediterranean basin in Spain (Muñoz-Martín et al., 2019) and the tropical savannah in Brazil (Machado-De-Lima et al., 2019) has shown that the distribution of cyanobacteria in biocrusts is affected by seasonal temperatures and precipitation frequency. Similarly, morphological analyses along a transect in southern Africa showed that biocrusts exposed to high winter rainfall were richer in cyanobacteria than those exposed to predominantly summer rainfall (Büdel et al., 2008).

Previous studies investigating the bacterial communities comprising Australian biocrusts have relied on morphological classification using light microscopy (Strong et al., 2013; Williams et al., 2008; Williams et al., 2014; Williams et al., 2018) and genomic profiling, with sample sites spanning southern Australia (Abed et al., 2012), New South Wales (Chilton et al., 2018; Liu et al., 2017) and Western Australia (Moreira-Grez et al., 2019). However, comprehensive intra-continental scale studies have yet to be conducted for Australian biocrust bacteria.

Australia is the driest inhabited continent, with 70% of the land mass considered arid or semi-arid (Eldridge et al., 2018). Seasonal precipitation differences are observed across the latitudes where northern Australia experiences predominantly summer rainfalls while southern regions receive most precipitation during winter months. Determining landscape-scale distribution patterns can inform on the natural histories of biocrust communities, help delimit ecological regions and improve our understanding of the predicted impacts of climate change (Rodriguez-Caballero et al., 2018). In this study, we aimed to expand our understanding of the bacterial composition and biogeography of Australian biocrusts at the intra-continental scale through the application of 16S ribosomal RNA (16S rRNA) gene amplicon sequencing. We surveyed eight sites across the continent, with a geographical range spanning five states and territories and four distinct climate zones (Figure 1; Table 1). The influence of

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**TABLE 1** Sample site locations and collection details

| Sample site[a] | Climate classification[b] | Climate zone[c] | Sample location | Collection season | Date of collection | Date of DNA extraction |
|---|---|---|---|---|---|---|
| Bare, Jacinth Ambrosia Mine, SA | Arid desert, cold | Hot dry summer, cool winter | 30.5429 S, 132.1159 E | Winter | July, 2014 | December, 2015 |
| Early, Jacinth Ambrosia Mine, SA | Arid desert, cold | Hot dry summer, cool winter | 30.5429 S, 132.1159 E | Winter | July, 2014 | December, 2015 |
| Late, Jacinth Ambrosia Mine, SA | Arid desert, cold | Hot dry summer, cool winter | 30.5429 S, 132.1159 E | Winter | July, 2014 | December, 2015 |
| Bare, Cobar, NSW | Arid steppe, hot | Hot dry summer, cool winter | 31.4949 S, 145.8402 E | Autumn | April, 2013 | April, 2013 |
| Early, Cobar, NSW | Arid steppe, hot | Hot dry summer, cool winter | 31.4949 S, 145.8402 E | Autumn | April, 2013 | April, 2013 |
| Mid, Cobar, NSW | Arid steppe, hot | Hot dry summer, cool winter | 31.4949 S, 145.8402 E | Autumn | April, 2013 | April, 2013 |
| Late, Cobar, NSW | Arid steppe, hot | Hot dry summer, cool winter | 31.4949 S, 145.8402 E | Autumn | April, 2013 | April, 2013 |
| Rolleston, QLD | Arid steppe, hot | Hot dry summer, warm winter | 24.34325 S, 148.7129 E | Spring | September, 2011 | December, 2015 |
| Charters Towers, QLD | Arid steppe, hot | Hot dry summer, warm winter | 20.532817 S, 146.1167 E | Spring | September, 2011 | December, 2015 |
| Granada Station, Cloncurry, QLD | Arid steppe, hot | Hot dry summer, warm winter | 20.15185 S, 140.48405 E | Spring | October, 2011 | December, 2015 |
| Tara Station, Cloncurry, QLD | Arid steppe, hot | Hot dry summer, warm winter | 20.112267 S, 140.44155 E | Spring | October, 2011 | December, 2015 |
| Mataranka, NT | Arid steppe, hot | Hot humid summer, warm winter | 15.016367 S, 133.04985 E | Spring | October, 2011 | December, 2015 |
| Cell, Paraburdoo, WA | Arid desert, hot | Hot dry summer, warm winter | 23.004117 S, 116.963067 E | Spring | October, 2011 | December, 2015 |
| CTS, Paraburdoo, WA | Arid desert, hot | Hot dry summer, warm winter | 23.0239 S, 166.9816 E | Spring | September, 2011 | December, 2015 |
| Cooloongup, WA | Temperate dry summer, hot summer | Warm temperature | 32.3411 S, 115.7782 E | Spring | October, 2014 | December, 2015 |

[a]State locations for sample sites are as follows: SA, South Australia; NSW, New South Wales; QLD, Queensland; NT, Northern Territory; WA, Western Australia.
[b]Climate classifications are determined by the Köppen-Geiger climate classification scheme (Peel et al., 2007).
[c]Climate zones are identified based on the Australian Building Codes Board classifications based on humidity, temperature and rainfall characteristics (Australian Climate Zone Map, 2019).

selected environmental factors on bacterial composition and phylogeny was evaluated by multivariate analyses. Our investigation identified seasonality of temperature and precipitation as a primary force driving bacterial assemblages in biocrusts across Australia.

## EXPERIMENTAL PROCEDURES

### Biocrust sampling and processing

This study sampled 11 biocrust types in triplicate from eight geographically distinct semi-arid biomes across Australia between September 2011 and October 2014 (Table 1). The raw sequencing data from previously studied biocrusts from three sites within Cobar, NSW, were also included in our analysis (Chilton et al., 2018). For the Cobar datasets, replicates of each biocrust stage (bare, early, mid and late) from each site were merged resulting in three sequencing datasets per biocrust stage. In total, 15 biocrust communities were analysed in triplicate. Samples were collected to the depth of the biocrust, stored in paper bags, transported dry to the laboratory at the University of New South Wales and stored dry at room temperature until processing. Samples were confirmed visually as biocrusts based on the aggregation of soil into cohesive crust structures and the presence of microbial filaments. Environmental genomic DNA was extracted from 500 mg of homogenized biocrust sample using the FASTDNA Spin Kit for Soil (MP Bio Laboratories, USA) according to the manufacturer's instructions.

## Sequencing of 16S rRNA genes and bioinformatic analyses

Amplicon libraries of the hypervariable V1–V3 regions of the 16S rRNA gene were generated via PCR as previously described using unique combinations of indexed Illumina-compatible 27F/519R primers (Woodhouse et al., 2016). Libraries were normalized using SequalPrep Normalization Plates (Applied Biosystems) and equal volumes were subsequently pooled. The pooled library was submitted to the Ramaciotti Centre for Genomics (University of New South Wales, NSW, Australia) where sequencing was performed on an Illumina MiSeq using a MiSeq Reagent Kit v3 with a $2 \times 300$ bp run format. Sequencing data were received and de-multiplexed via the Illumina platform, BaseSpace, and are available on the National Center for Biotechnology Information's Sequence Read Archive under BioProject accession number PRJNA850665.

The forward read FASTQ files were pre-processed, quality-filtered and analysed using the Quantitative Insights into Microbial Ecology (QIIME) 2 pipeline v2020.2 (Bolyen et al., 2019). The DADA2 software package (Callahan et al., 2016) wrapped in QIIME2 was used for quality filtering (median Phred score ≥25), denoising and removing chimeric sequences, which resulted in a total of 3,044,055 high-quality sequences with an average length of 299 bp across 45 samples. The DADA2 denoising algorithm inferred those sequences into 31,169 exact amplicon sequence variants (ASVs), of which 30,205 ASVs with a minimum frequency of three were retained for further analysis.

For taxonomic analysis, a Naïve Bayes classifier was trained using the QIIME2's q2-feature-classifier plugin (Bokulich et al., 2018) and taxonomy was assigned using reference sequences from SILVA (v138; Pruesse et al., 2007) and Greengenes 13_8 (DeSantis et al., 2006) databases. It has been shown that when a Naïve Bayes classifier is trained exclusively with sequences representing the target region, the accuracy of taxonomic classification of 16S rRNA gene sequences improves (Werner et al., 2012); therefore, we used the 27F/519R primer pair to trim the SILVA and Greengenes reference sequences to the V1–V3 region and used these fragments for classifier training. The SILVA classifier was used for all steps of taxonomic analysis, whereas the Greengenes classifier was used for filtering chloroplast sequences as our manual curation of taxonomic assignment suggests that Greengenes was more sensitive than SILVA in detecting chloroplast-matching ASVs. All ASVs that were identified as mitochondria or chloroplast sequences were removed from the feature table. *De novo* phylogeny was constructed from the remaining 29,782 ASVs (hereafter, 'observed ASVs') by MAFFT alignment v7.475 (Katoh et al., 2002) and FastTree v2.1.10 (Price et al., 2010) as implemented in QIIME2 with default masking options.

To improve the poor taxonomic resolution for cyanobacteria obtained with the SILVA database, cyanobacterial ASVs were filtered from the QIIME2 feature table and then aligned to reference sequences from the Cydrasil cyanobacteria database (v3, Roush et al., 2021) using the short-read alignment algorithm, PaPaRa (Berger & Stamatakis, 2011), placed into the reference tree using the RaxMLB algorithm (Stamatakis, 2014) and visualized with iTOL3 server (Letunic & Bork, 2016). The NCBI database was also used to examine the similarity of those ASVs compared to publicly available cyanobacterial sequences. ASVs that were placed in a branch of the Cydrasil reference tree with >70% certainty (Roush & Garcia-Pichel, 2020) and >94.5% similarity to an identified sequence (Yarza et al., 2014) were considered to represent the same genus.

## Statistical analyses

Rarefaction analysis was performed and visualized in QIIME2 using the alpha-rarefaction function. Rarefaction analysis based on either observed ASVs or the Shannon diversity index demonstrated that adequate 16S rRNA gene sequence diversity was captured using the selected primer set (Supplementary Figure S1). For alpha and beta diversity analysis, data were rarefied to a minimum sampling depth of 6,062 to account for unequal sequencing depth. Alpha diversity metrics were estimated based on the number of ASVs observed, Shannon's diversity indices (Shannon, 1948) and Faith's Phylogenetic Diversity (Faith, 1992) using the q2-diversity plugin and visualized using the *ggplot2* package in R statistical software (Oksanen et al., 2015). To determine the broad taxonomic sources of diversity for each site, cyanobacterial and non-cyanobacterial ASVs were also examined separately. Differences (between sites and between regions) in alpha diversity metrics were tested using one-way ANOVA followed by examination with Tukey's HSD multiple comparisons for significant differences using the *agricolae* package in R (de Mendiburu, 2019).

A nMDS plot based on the Bray–Curtis dissimilarity matrix derived from rarefied, square-root transformed ASVs was created to visualize differences in community composition for each sample. Differences in community structure across sites were assessed by conducting a PERMANOVA using the command 'adonis2' in the package *vegan* in R using the following grouping factors: site, temperature, precipitation season, collection year, collection season, climate zone, and extraction method. For temperature, sample sites were categorized on a *post hoc* basis as either cool,

cool-warm, warm, or very warm depending on their average maximum winter temperature (Supplementary Table S1). For precipitation season, sample sites were categorized on a *post hoc* basis as either winter, summer, or even depending on when they received most precipitation (Supplementary Table S1). For site, different biocrust types from the same sampling site were assigned the same location (Table 1). However, as PERMANOVA can be sensitive to dispersion, a test of homogeneity of multivariate dispersion (Anderson et al., 2008) was performed on PERMANOVA outcomes using the command 'permdisp' in the package *vegan*. A significant PERMDISP result [p(perm) > 0.05] suggests differences may be due to within-group heterogeneity of dispersion rather than true community structure variation. Site, temperature and precipitation season were found to explain groupings. To further examine the effects of seasonal temperature and precipitation PCoA based on the Bray–Curtis dissimilarity matrix was overlaid with vectors for site precipitation and temperature using data sourced from the Australian Government Bureau of Meteorology (Australian Government Bureau of Meteorology, 2014, accessed 2017) (Supplementary Table S1), using the functions 'ordinate' and 'envfit' from the package *vegan* in R. Weighted UniFrac dissimilarity matrix was computed from rarefied, square-root transformed ASV feature table using the function 'phyloseq::distance' from the package *phyloseq* in R (McMurdie & Holmes, 2013).

To statistically quantify the difference in relative abundance of bacterial composition between southern and northern biocrusts and determine which taxa contribute to the segregation between the two groups, we used pairwise Wald tests as implemented in the DESeq2 package (Love et al., 2014). Over-abundant ASVs with Benjamini–Hochberg corrected *p*-values <0.05 and $\log_2$ fold change >1.5 or <−1.5 were retained for further analysis. The top 102 ASVs with baseMean values >35 were visualized in a heatmap using the function 'ComplexHeatmap::pheatmap' in the package *ComplexHeatmap* in R (Gu et al., 2016). Sites (*x*-axis) and bacterial taxa (*y*-axis) of the heatmap were clustered using the function 'vegdist' and 'hclust' in the package *vegan* (Oksanen et al., 2015).

To identify the main taxa driving the grouping in biocrust bacterial compositions across sites, we used DEICODE (Martino et al., 2019) via the q2-DEICODE plugin implemented in QIIME2 as a complementary approach to DESeq2. DEICODE is a compositionally aware method that utilizes a form of robust Aitchison distances to create a species abundance distance matrix of ASVs that can be projected onto a PCA biplot. The ordination file output from DEICODE was exported and the top 50 features with the highest magnitudes, i.e., those expected to be important in causing separation in the data set, were put into R and visualized using the *ggplot2* package (Wickham, 2016).

# RESULTS

## Microbial diversity within Australian biocrusts

The alpha diversity of the bacterial community in the 45 biocrust samples from 15 locations was examined by the number of observed aASVs (richness), the Shannon diversity index (a measure accounting for both taxon richness and evenness; Shannon, 1948) and the Faith's PD index (a measure incorporating phylogenetic distances between taxa; Faith, 1992). When the diversity was compared between the two regions, on average, southern biocrusts were slightly richer and more diverse than northern samples [Figure 2(A–C)]; however, the difference was not statistically supported. Nonetheless, when only cyanobacterial communities were considered, biocrusts from northern Australia were significantly richer and more diverse than those from southern Australia (ANOVA; observed ASVs: $p = 0.02$; Shannon: $p = 0.01$; Faith: $p = 0.004$). Compared with all the other sites, the cyanobacterial communities sampled from Cooloongup, and to a lesser extent, Paraburdoo (both located in Western Australia), had the lowest richness, evenness and diversity [Figure 2(D–F)]. Sites from Cloncurry and Charters Towers, both located in Queensland and in the 'arid steppe, hot' climate zone, had biocrust communities that were significantly richer and more diverse in their compositions of cyanobacteria [Figure 2(D–F)].

## Microbial community composition

From all sites a total of 35 phyla were detected, including Cyanobacteria (relative abundance, 23.7%–65.5%), Proteobacteria (13.8%–28.2%), Actinobacteria (4.6%–21.8%), Chloroflexi (5.4%–14.6%), Acidobacteria (0.5%–5.4%), and Bacteroidetes (0.4%–5.2%). There were 21 rare phyla (average abundance <0.01%), including candidate divisions WSP-2, SAR324 clade, RCP2-54, WS2, and WS4 (Supplementary Figure S2 and Table S2). At the family and genus levels, the relative abundance of each taxon, particularly those belonging to the phyla Cyanobacteria and Chloroflexi, varied greatly across the sample sites and between regions (Supplementary Figures S2 and S3), reflecting the significant differences observed for community richness and evenness as revealed by alpha-diversity analysis (Figure 2). The most abundant families were Coleofasciculaceae, Scytonemataceae, Nostocaceae, Microcoleaceae, Geodermatophilaceae (belonging to the phylum Cyanobacteria), Beijerinckiaceae, Acetobacteraceae, Sphingomonadaceae (Proteobacteria), Geodermatophilaceae, Rubrobacteriaceae (Actinobacteria), and AKIW781 (Chloroflexi), which together accounted for over 50% of all taxa (Supplementary Figure S3;
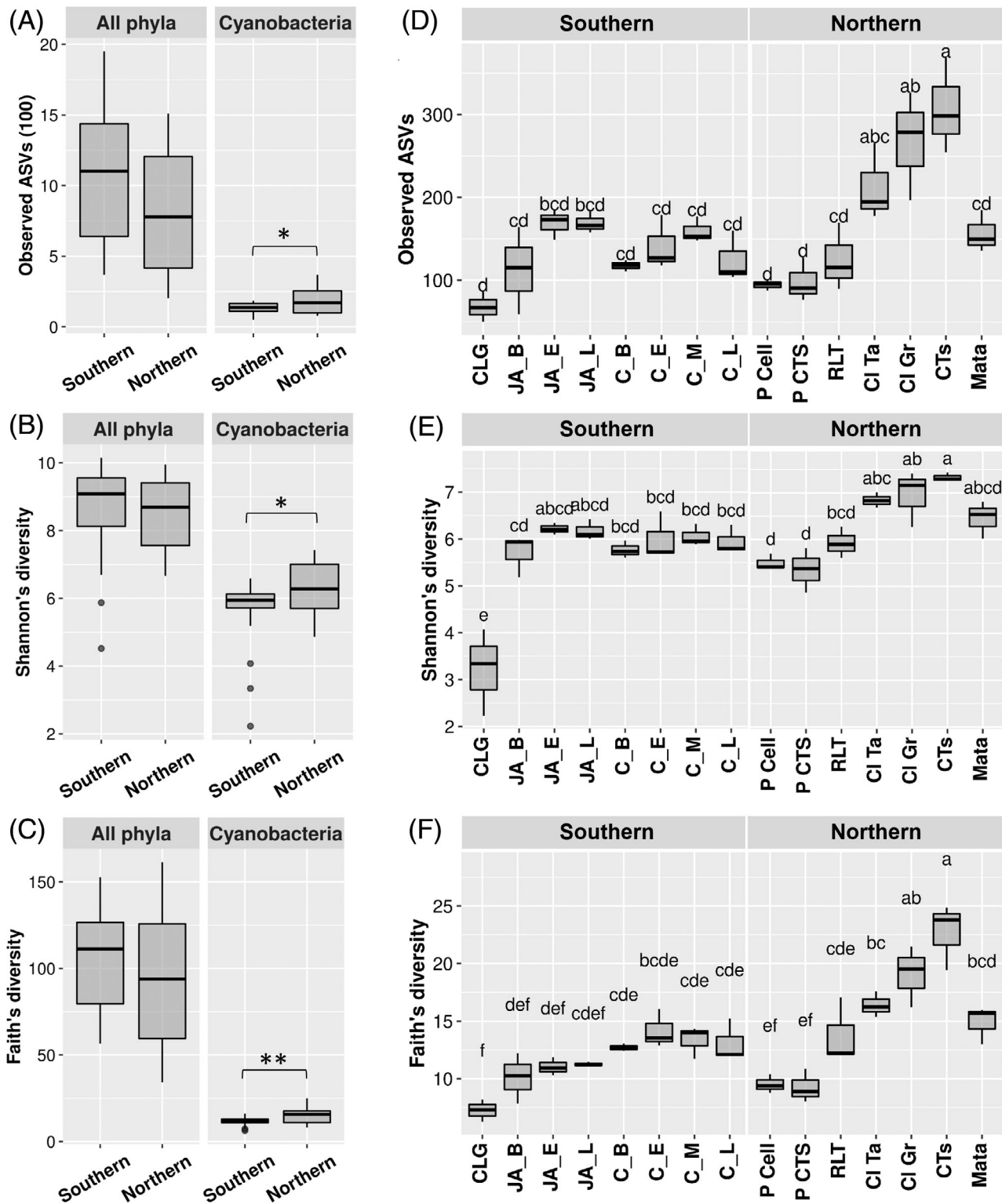
**FIGURE 2** Alpha-diversity measures comparing southern versus northern samples. Figures are separated into those comparing cyanobacteria only and all phyla at northern and southern regions (A–C); and those comparing cyanobacteria only at each sample site (D–F). For panels A–C, asterisks indicate the level of statistical significance: ANOVA, $p < 0.05$ (*), $p < 0.01$ (**). For panel D–F, samples are grouped as southern (CLG = Cooloongup, JA = Jacinth Ambrosia, C = Cobar; 'B', 'E', 'M' and 'L' stand for bare, early, middle and late stage of biocrust development, respectively) and northern (P cell = Paraburdoo cell, P CTS = Paraburdoo CTS, RLT = Rolleston, Cl Ta = Cloncurry Tara, Cl Gr = Cloncurry Granada, CTs = Charters Towers and Mata = Mataranka). Small letters represent the level of significance between sites (Tukey HSD test).

Supplementary Table S3). Biocrusts from Cooloongup were the most compositionally distinct, having the highest relative abundance of cyanobacteria

(65.5%), particularly Scytonemataceae species (43.3%). Cyanobacteria were much less abundant in 'young' (bare) biofilms from Jacinth Ambrosia

(JA) and Cobar compared to mature biocrusts from these regions, with the exception being the JA-bare samples, which were enriched with Gomontiellaceae species (5.6%; Supplementary Figure S3). Biocrusts from northern regions of Australia had higher abundances of Calotrichaceae, Hyellaceae, Microcoleaceae, Nostocaceae (Cyanobacteria), and Roseiflexaceae (Chloroflexi) species compared to those from southern sites. A large component of the cyanobacterial communities (60.1%) were unclassified at the genus level when the SILVA database was used as a taxonomic reference (52.7% and 67.6% for southern and northern biocrusts, respectively; data not shown). Further curation of cyanobacterial ASVs using both the phylogenetic placement tool incorporated into the Cydrasil Cyanobacteria database (Roush et al., 2021) and NCBI BLAST (Madden, 2002) significantly reduced unclassified ASVs in southern biocrusts to 20.3%. However, unclassified cyanobacteria in northern samples remained high at 52.2% of all detected cyanobacterial ASVs, of which 82.5% were unclassified at the phylum level (Supplementary Table S4).

Examination of ASVs contributing to inter-regional variation via DESeq2 (Love et al., 2014) showed that Cyanobacteria explained most of the segregation observed between southern and northern sample sites (Figure 3). Other taxa belonging to the phyla Proteobacteria, Actinobacteria, Chloroflexi and Bacteroidetes also contributed considerably to the south–north segregation. Interestingly, within southern sites, the segregation was also observed among Cooloongup, Cobar and JA sites, whereas differentially abundant ASVs were distributed more uniformly across northern biocrusts (Figure 3). Consistent with DESeq2 analysis, PCoA with Aitchison distance also identified cyanobacterial ASVs as the main taxa driving the differences along the first two axes in the compositional PCoA biplot (Supplementary Figure S4). Among the top 50 contributing ASVs that explained 91.1% variation in the Australian biocrust community composition (Supplementary Figure S4), 35 ASVs were cyanobacterial, half of which belonged to the genera *Microcoleus* and *Scytomema* (Supplementary Table S5).

## Seasonal temperature and precipitation are the main factors driving the segregation of Australian biocrust assemblies.

Unconstrained ordination of each sample via non-metric multidimensional scaling (nMDS) showed that biocrust communities remained tightly grouped according to sample site within two dimensions (Supplementary Figure S5). A low 2D stress value of 0.11 suggests that this ordination is robust and that the display of the model in two dimensions is reliable. The positioning of samples according to Bray–Curtis dissimilarity corresponded with a geographical gradient. Specifically, a general trend from left to right confirmed with sampling from north to south [Supplementary Figure S5(A)] and from regions with high winter temperatures and predominantly summer rainfall to regions with cooler winter temperatures and predominantly winter precipitation [Supplementary Figure S5(B,C)]. Consistent with nMDS ordination plots, permutational multivariate analyses of variance (PERMANOVA, Table 2) indicated sample site and seasonality of both temperature and precipitation (factors 'site', 'temperature' and 'precipitation', respectively) had significant effects on biocrust community structure. We observed a significant PERMDISP result for site ($p = 0.0003$, Table 2), suggesting that within-group variability was not equal across all sites, and this may account for the significant PERMANOVA finding. To examine other factors and identify if differences in sample collection and processing had significant effects on community contribution patterns, 'collection year', 'collection season', 'climate zone', and 'extraction method' were also tested as factors (data not shown). These factors poorly explained the sample ordination and significant PERMANOVA results. These were likely due to significant sample dispersions [PERMDISP $p$ (perm) $= 0.000001$ for all three factors, Table 2] and thus were not pursued.

To examine the effects of temperature and precipitation on biocrust community composition, we performed principal coordinate analysis (PCoA) based on the Bray–Curtis dissimilarity matrix and overlaid correlated variables as vectors. Correlation with environmental variables showed that the community composition of northern samples were most affected by winter temperature ($r = 0.76$; $p = 0.01$), summer temperature ($r = 0.35$; $p = 0.001$) and high summer rainfall ($r = 0.32$; $p = 0.001$), whereas biocrusts sampled from southern regions were not correlated with any of the examined variables (Figure 4).

## Biocrust phylogeny and biogeography

Phylogenetic relationships between samples were determined by calculating weighted UniFrac distances and visualized with a PCoA plot. Greater homogeneity among sites compared to Bray–Curtis measures suggested that communities, while compositionally distinct, were composed of related species (Figure 5). Significant PERMANOVA results indicate the geographical factors of site ($p = 0.001$) and precipitation ($p = 0.001$) influence community patterns (Table 2). Linear regressions used to examine distance–decay relationships showed community composition (based on Bray–Curtis dissimilarity) correlated more strongly (Mantel statistic $r = 04$, $p = 0.0001$) than weighted UniFrac measure

**FIGURE 3** Heatmap of the top 102 differentially abundant amplicon sequence variants (ASVs) between southern and northern regions. Heatmap data were identified from DESeq2 analysis which identifies those ASVs which are differentially expressed at sample sites. Details of taxonomic assignment for each ASV can be viewed by zooming into the heatmap.

(Mantel statistic $r = 0.17$, $p = 0.0044$) with geographical distance (Figure 6).

## DISCUSSION

Biocrusts occur globally in a wide range of primarily arid biomes; accordingly, they exhibit spatial variability in community structure. In this study, we explored the continent-wide 16S rRNA gene dataset in Australian biocrusts to study their bacterial components. Multivariate analyses revealed patterns in community

composition and phylogeny consistent with emerging biogeographical models detailing the natural history of biocrusts. We observed that climatic forces govern biocrust assembly, and communities, while compositionally distinct, are composed of related species.

## Intra-continental patterns of biocrust microbiome diversity

Overall trends in cyanobacterial diversity showed that biocrusts sampled from northern Australian sites,

**TABLE 2** PERMANOVA and PERMDISP results for factors.

| Factor | PERMANOVA | | PERMDISP | |
|---|---|---|---|---|
| | Pseudo-*F* | *p*-value | *F*-value | *p*-value |
| Site | 4.712 | 0.001 | 2.7982 | 0.003 |
| Temperature | 5.1808 | 0.001 | 6.8318 | 0.087 |
| Precipitation | 4.3468 | 0.001 | 0.6447 | 0.645 |
| Collection year | 6.5759 | 0.001 | 38.7361 | 0.006 |
| Collection season | 7.0550 | 0.001 | 38.6577 | 0.001 |
| Extraction method | 6.7600 | 0.001 | 343.5401 | 0.001 |
| Site climate | 4.3589 | 0.001 | 50.2077 | 0.013 |
| Site (UniFrac) | 7.3315 | 0.001 | 0.6843 | 0.061 |
| Precipitation (UniFrac) | 5.8116 | 0.001 | 1.3726 | 0.286 |

*Note*: Tests were conducted on Bray-Curtis Dissimilarity matrix created from rarefied, square-root transformed data. Tests for groupings with UniFrac in brackets were conducted on weighted UniFrac distances created from rarefied, square-root transformed data.
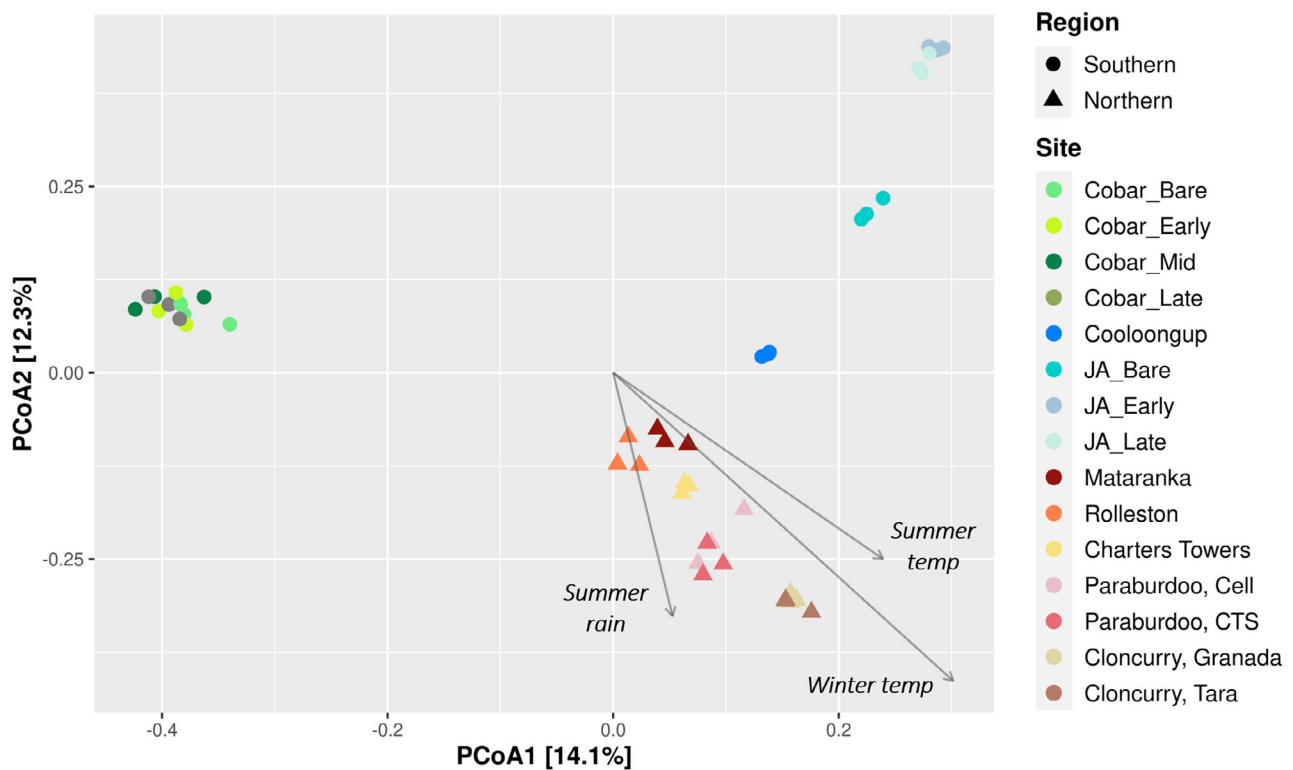


**FIGURE 4** Principal coordinate analysis (PCoA) of microbial community composition at sites in relation to climate variables. Microbial community data were rarefied and transformed before conversion to a Bray–Curtis dissimilarity matrix. Correlated climate variables were overlaid with diversity data. Vectors indicate those variables with the strongest correlation to the microbial community in the direction of correlation.

characterized by warm winters and predominantly summer rainfalls, were more diverse than biocrusts from southern Australian sites, characterized by cool winters and high winter rainfalls [Figure 2(A–C)]. This contrasts with findings based on traditional morphological classification of cyanobacteria from southern Africa where greater diversity was found in sites with higher winter rainfall (Büdel et al., 2008). The discord may be due to the different approaches used, whereby community sequencing is more sensitive to genotypic diversity masked by homogenous morphology.

The abundant higher-level taxa identified here are common constituents of arid soils worldwide (Makhalanyane et al., 2015) and specifically of biocrusts (Kuske et al., 2011; Moreira-Grez et al., 2019; Thomas & Dougill, 2007). The defining community signature distinguishing biocrust microbiomes from non-biocrust arid microbiomes is the abundance of Cyanobacteria and Bacteroidetes (Kuske et al., 2011; Steven
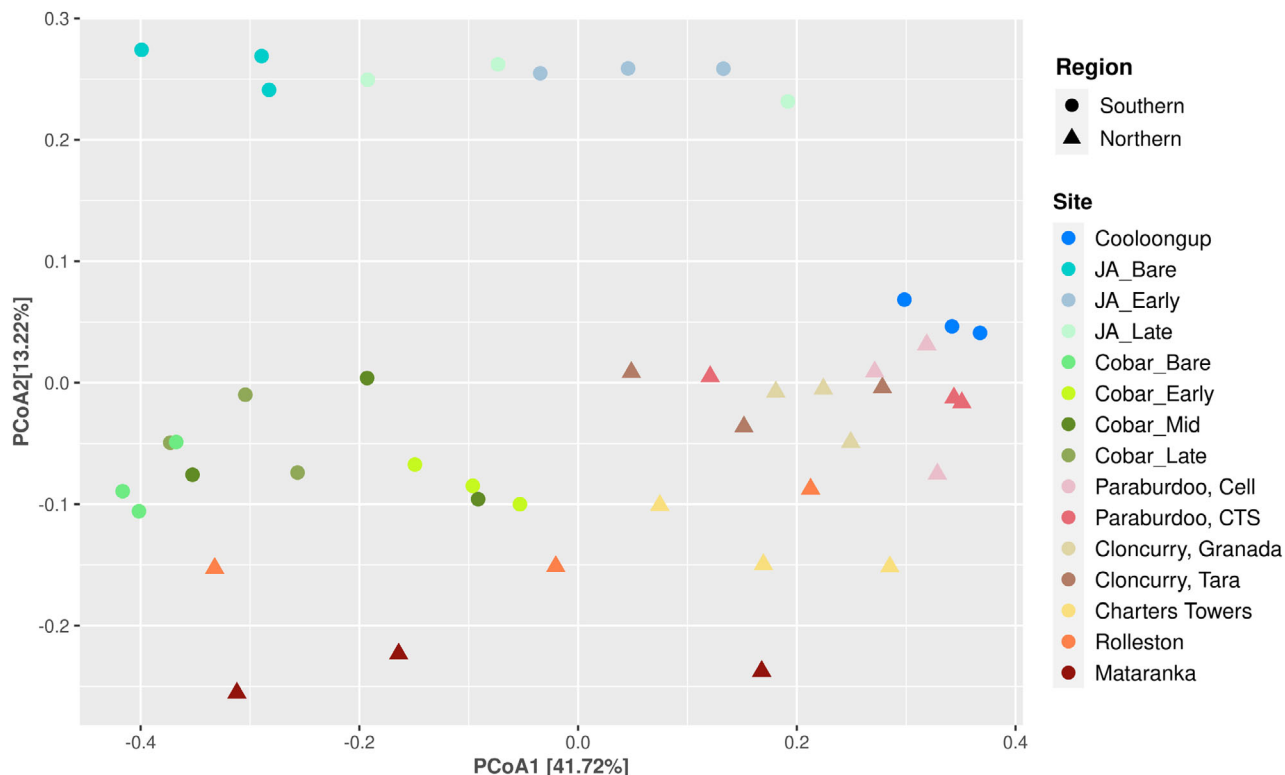
**FIGURE 5** Principal coordinate analysis (PCoA) of microbial community composition at sites and regions. Microbial community data were rarefied and transformed before conversion to a weighted UniFrac dissimilarity matrix between samples which considers the abundance and phylogenetic distance of each amplicon sequence variant (ASV) in a sample.

et al., 2013; Thomas & Dougill, 2007). While Proteobacteria, Chloroflexi and Actinobacteria are also abundant within biocrusts, these phyla are typically more enriched within sub-crust communities (Steven et al., 2013; Thomas & Dougill, 2007). This stratification likely represents metabolic niches driven by light and photosynthesis (Garcia-Pichel et al., 2003). As expected, all sites sampled here were dominated by filamentous cyanobacteria.

In contrast with our previous study that investigated biocrust bacterial communities in the Cobar site and showed no operational taxonomic units assigned to *Microcoleus* (Chilton et al., 2018), in the present study, several *Microcoleus* species were found in all examined samples and were particularly abundant in the Cobar biocrusts. This discord was due to previous taxonomic analysis approaches based on the Greengenes database (v13_8) that resulted in poor taxonomic resolution (Chilton et al., 2018), in which sequences corresponding to *Microcoleus* spp. were incorrectly assigned to *Phormidium* or *Leptolyngbya* (data not shown). The presence of this bundle-forming genus in Australian biocrusts is in accordance with previous consideration that *Microcoleus* is among the most representative cyanobacterial genus in biocrusts across the globe (Büdel, 2001), typically in North America (Garcia-Pichel et al., 2001; Giraldo-Silva et al., 2020), South

America (Becerra-Absalón et al., 2019; Machado-De-Lima et al., 2019), southern Africa (Gundlapally & Garcia-Pichel, 2006), Europe (Schulz et al., 2016), Israel (Hagemann et al., 2014), and north Iran (Dulic et al., 2016). However, unlike other parts of the world in which *M. steenstrupii* was prevalent in arid soil biocrusts [e.g. accounting for 55%–84% of microbial phototrophs in the cooler sites of the American southwestern region (Garcia-Pichel et al., 2003)], this species was absent in most of the examined sites in this study, and was only found in two northern sites, Cloncurry and Charter Towers (0.5% and 2%, of the total population, respectively, Supplementary Table S6). Nonetheless, *M. paludosus*, which is not commonly found in biocrusts around the globe, was abundant in many samples including those from Cobar, Cloncurry, Paraburdoo and Rolleston (Supplementary Table S6). The observed substitution of *M. steenstrupii* for *M. paludosus* in samples from several sites further highlights the importance of this study in establishing an Australian framework for biocrust research.

The high abundance of unclassified cyanobacteria in biocrusts from northern Australia supports the theory that this group may harbour novel extremotolerant strategies pertinent for sustaining biocrust coverage in a warming climate (Garcia-Pichel et al., 2013). Lack of classification of the ASVs below the phylum levels
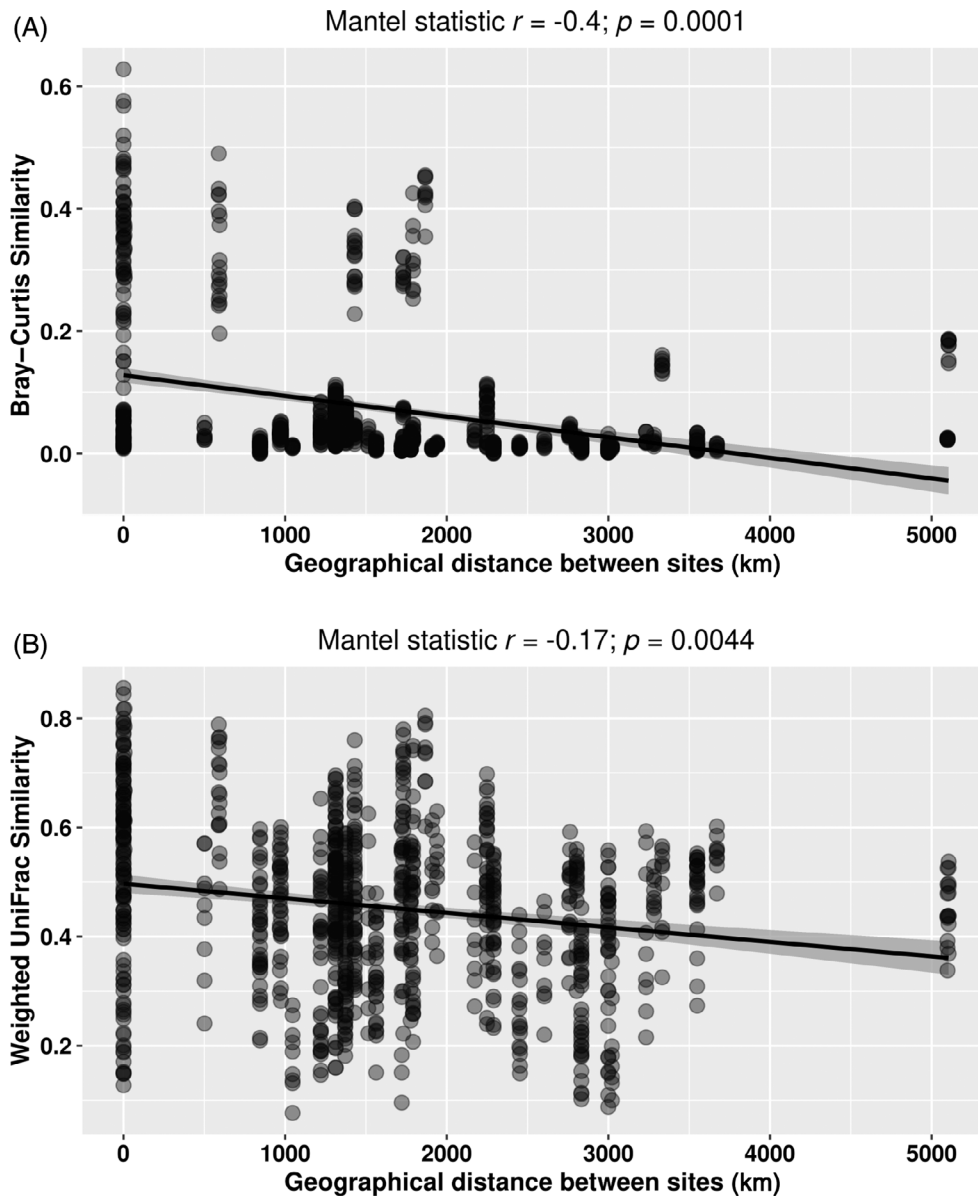
**FIGURE 6** Linear regression of community differences in relation to site geographical distance. Panel A indicates community composition based on Bray–Curtis dissimilarity matrix and Panel B indicates community difference based on weighted UniFrac distances which includes phylogenetic distance along with relative abundance.

reflects a paucity of arid cyanobacterial sequences within all the databases used in this study including SILVA, NCBI and Cydrasil. Comparison of the most abundant unclassified cyanobacterial ASVs to the NCBI nucleotide database showed that these sequences were most similar to previously undescribed environmental sequences that are not included in curated databases for taxonomic classification (data not shown). These groups may contain novel species that are potentially endemic to northern Australia, given that the proportion of unclassified cyanobacteria was significantly reduced for southern biocrusts (from 52.7% to 20.3%) when refined taxonomic analysis employing the NCBI and Cydrasil databases was

applied, whereas this figure remained high for northern samples. Alternatively, the unclassified cyanobacteria in northern Australia may not represent true endemism but rather reflect the gaps in our current knowledge of cyanobacterial diversity that is partially due to the lack of genetic verification for many taxa. Indeed, the concept of cyanobacterial endemism in Antarctica has been challenged when strains of *Wilmottia murrayi*, previously believed to be endemic to the Antarctic Peninsula, have recently been identified through 16S rRNA gene amplicon sequencing using cyanobacteria-specific primers (99%–100% identity) in America, China, New Zealand, Spain, Ireland, and Bolivia (Pessi et al., 2018a, 2018b). Interestingly, in the present study,

15 ASVs were classified as *Wilmottia murrayi* Ant-Ph58 based on similarity to sequences in the SILVA database. Placement of some of these ASVs on the Cydrasil reference tree showed that they can be assigned to various *Wilmottia murrayi* strains with total certainty of 88% (data not shown). Notably, these ASVs were detected in most of the examined sites, including Paraburdoo and Mataranka, which are exposed to the hottest summers (39.8°C) and winters (30.8°C), respectively, compared to all the other sites. This observation supports the hypothesis that most microorganisms are ubiquitous and warrants further analysis of extended sampling sites using a polyphasic approach combining traditional and molecular techniques to better define the status of endemism for northern Australian cyanobacteria.

## Biogeography of bacteria within Australian biocrusts

In terms of community assembly, the exploration of diversity patterns presented here indicates that Australian biocrusts are structurally distinct from each other yet phylogenetically similar at the ASV level. Sample ordinations derived from Bray–Curtis dissimilarity and weighted UniFrac distances showed that biocrust microbiomes exhibit spatial variability on an intracontinental scale and group strongly according to site. Similar findings have been reported for cyanobacteria from North American deserts, for example, biocrusts from Tehuaćan, Mexico were composed of assemblages distinct from other deserts despite sharing cosmopolitan species (Garcia-Pichel et al., 2013; Rivera-Aguilar et al., 2006).

These findings begin to inform the factors which govern the assembly of microbiomes (Nemergut et al., 2013). The different patterns between composition and phylogeny suggest that as communities, biocrusts may first adapt to a new niche via compositional changes, that is, the selected enrichment of dominant taxa before diversification via evolutionary changes. The capacity for dispersal is an important consideration regarding such adaptations. Current studies suggest that arid cyanobacteria do not disperse well (Abed et al., 2012; Bahl et al., 2011). Temporal analysis of the desert cyanobacterium *Chroococcidiopsis* by Bahl et al. (2011) indicated that global populations of arid cyanobacteria diverged before the formation of the modern continents. Specifically, within Australia, taxonomic profiling of a large dust storm, a mechanism of bacterial dispersal, by Abed et al. (2012) showed that biocrust-cyanobacteria represented only 2% of the dust microbiome despite representing over 50% of the source community. Alternatively, filamentous, crust-forming cyanobacteria have been shown to migrate through top soils at a rate of up to 0.02 m per month (Sorochkina et al., 2018). Given the

ancient histories of both cyanobacteria as colonizers of land and Australia as a geographically isolated continent (Beraldi-Campesi & Garcia-Pichel, 2011; Bowker et al., 2016), Australian biocrusts may be composed of an endemic communal cohort of bacteria structurally differentiated over ecological distances and timescales (Eldridge et al., 2018).

## Seasonal temperature and precipitation drive biocrust diversity

Seasonal temperature and rainfall were the major factors explaining the biogeographical distribution of bacteria within the examined biocrusts (Table 2). While Australian biocrusts are composed of bacteria of related lineages, certain environmental conditions may enrich specific members resulting in compositional distinctions between locations. Specifically, northern sites characterized by warm winters, very hot summers and high summer rainfalls were enriched with *Microcoleus paludosus* but largely lacked *M. vaginatus*. Whereas southern sites characterized by cool winters, moderately hot summers and high winter rainfalls were enriched with *M. vaginatus* (Figure 3; Supplementary Table S6). Such patterns have also been observed within American hot and cold deserts where prevailing temperatures dictated the dominance of certain *Microcoleus* spp. in biocrusts (Garcia-Pichel et al., 2013). Niche partitioning was also observed for other genera including *Chroococcidiopsis*, *Nostoc*, *Phormidesmis*, and *Tolypothrix*, which were highly abundant in southern biocrusts. This finding is consistent with the nitrogen fixation heat sensitivity trait observed for *Nostoc* and *Tolypothrix* under laboratory conditions and in nature (Giraldo-Silva et al., 2020). The genera *Crassifilum*, *Parifilum* and particularly *Scytonema* were more heat tolerant and abundant in both southern and northern regions of Australia, supporting previous predictions that *Scytonema* spp. could become increasingly dominant because of global warming (Giraldo-Silva et al., 2020).

These observations also support a growing body of research showing that climatic patterns of precipitation and temperature significantly affect biocrust structure and coverage (Bowker et al., 2016; Deane-Coe et al., 2012; Escolar & Maestre, 2015; Fernandes et al., 2018; Garcia-Pichel et al., 2013; Machado-De-Lima et al., 2019; Rogers, 1972; Samolov et al., 2020; Steven et al., 2015). Specifically, the coordination of precipitation events and high temperatures can significantly influence the diversity and relative abundance of photosynthetic organisms (Bowker et al., 2016). Arid-adapted photosynthetic organisms often enter a state of dormancy during periods of drought or extreme evaporation. Upon rehydration, carbon storage molecules are mobilized to power the reactivation of photosystems, resulting in a pulse of

$CO_2$ (Lange, 2001). However, if the conditions after rehydration are not conducive to productive photosynthesis (e.g., if the wetting period is too short or the temperatures are too high), a cellular carbon deficit can result and the organism will fail to resurrect during the next wet–dry cycle (Deane-Coe et al., 2012). While we did not directly investigate desiccation survival strategies in this study, it is likely that they play an important role in shaping the Australian biocrust cyanobacteria cohort observed in each region (Ferrenberg & Reed, 2017).

Previous drought-tolerance trials of biocrust-cyanobacteria from across the northern Australian savannah suggest that they are resistant to resurrection during the summer season despite rehydration, a strategy that avoids premature reactivation of photosystems and heat-stressed photosynthesis (Williams et al., 2014; Williams & Büdel, 2012). The authors postulated that this is made possible through water-regulation by extracellular polymeric substances; however, more recent studies suggest that seasonal light exposure may also be important (Oren et al., 2017). These strategies likely impart an advantage allowing strains to colonize hostile soils with less competition from macro-components. Indeed, the restriction of lichens and mosses to winter rainfall areas (Rogers, 1972) may account for the higher diversity of cyanobacteria observed in the northern Australian samples.

The northern biocrusts contained a high proportion of genetically unclassified cyanobacteria (twice as much as southern samples). These poorly documented groups may prove important as climate change takes effect and precipitation patterns shift (Muñoz-Martín et al., 2019; Rodriguez-Caballero et al., 2018). Rainfall manipulation studies conducted over several years have shown that the increasing incidence of summer rainfall events has significantly modified the structure and function of biocrusts from the Colorado Plateau, North America (Steven et al., 2015). Macro-components were greatly impacted which reduced the visible coverage of biocrusts while, after an initial loss, cyanobacteria recovered both in abundance and biomass after six years. In Australia, northern biocrusts may serve as reservoirs of biological and functional diversity that prove critical in maintaining biocrusts on climate-impacted arid lands (Garcia-Pichel et al., 2013; Rodriguez-Caballero et al., 2018). Future work will benefit from both culture-based methods and next-generation sequencing approaches to enumerate and describe these bacteria (Büdel et al., 2016).

## CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.