Islands in the sand: Are all hypolithic microbial communities the same?

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Abstract

Hypolithic microbial communities (hypolithons) are complex assemblages of phototrophic and heterotrophic organisms associated with the ventral surfaces of translucent minerals embedded in soil surfaces. Past studies on the assembly, structure and function of hypolithic communities have tended to use composite samples (i.e., bulked hypolithic biomass) with the underlying assumption that samples collected from within a 'homogeneous' locality are phylogenetically homogeneous. In this study, we question this assumption by analysing the prokaryote phylogenetic diversity of multiple individual hypolithons: i.e., asking the seemingly simple question of 'Are all hypolithons the same'? Using 16S rRNA gene-based phylogenetic analysis of hypolithons recovered for a localised moraine region in the Taylor Valley, McMurdo Dry Valleys, Antarctica, we demonstrate that these communities are heterogeneous at very small spatial scales (<5 m). Using null models of phylogenetic turnover, we showed that this heterogeneity between hypolithons is probably due to stochastic effects such as dispersal limitations, which is entirely consistent with the physically isolated nature of the hypolithic communities ('islands in the sand') and the almost complete absence of a liquid continuum as a mode of microbial transport between communities.

Keywords: Hypolithon; Small-scale heterogeneity; Phylogenetic turnover; Dispersal limitation; Functional variability; Core community

Introduction

Hypolithons are edaphic microbial-dominated communities found adhering to the undersides of translucent minerals, most typically quartz and marble, embedded in the soil surface. Such communities are a prominent feature of hot and cold deserts where quartz pebbles and rocks are common constituents of desert pavements (Cockell and Stokes, 2004; Chan et al. 2012). These cryptic niches provide micro-environmental conditions that favour microbial community development in 'extreme' edaphic habitats (Chan et al. 2012, Lebre et al. 2017). Such favourable parameters include protection from incident short-wavelength solar radiation and against desiccation, and the provision of thermal buffering and physical stability (Lebre et al. 2017).

The composition of hypolithic communities has been extensively studied, most intensively in the Antarctic McMurdo Dry Valleys (Wei et al. 2016; Cowan et al. 2010; Khan et al. 2011, Chan et al. 2013) and the Namib (Namibia) and Atacama (Chile) deserts (Warren-Rhodes et al. 2006, Van Goethem et al. 2017). Recent deep sequencing of prokaryote (16S rRNA gene) and lower eukaryote (18S rRNA and ITS genes) phylogenetic markers has shown that hypoliths form distinct communities depending on the dominant taxa (Cowan et al. 2011). 'Type I' hypolithons, the most common, are morphologically dominated by free-living photoautotrophic cyanobacteria (most commonly *Chroococcidiopsis* and *Phormidium*), but harbour complex heterotrophic microbial assemblages dominated by members of the phyla Actinobacteria, Proteobacteria, Bacteroidetes and Acidobacteria (Wei et al. 2016; Pointing 2016).

In desert soil ecosystems, where higher plants are largely or totally absent, hypolithons represent biological 'hotspots' which may make a substantial contribution to key ecosystem services, particularly carbon and nitrogen cycling. ^{14}C-radiolabelling experiments have suggested that hypolithic biomass may make a very significant contribution to C sequestration in Canadian High Arctic deserts (Cockell and Stokes, 2004), while studies have suggested that hypolithons in Antarctic and Namibian deserts may be the dominant source of nitrogen input in these ecosystems (Cowan et al 2011b, Ramond et al. 2018).

Little is known of either the rates or pathways of development of hypolithic communities. Studies have suggested that hypolithons recruit taxa from the surrounding soil (Makhalanyane et al, 2013), and that different types of hypolithons represent different steps in hypolith development, with cyanobacterial dominated hypolithons being the basal developmental stage (Makhalanyane et al, 2014). However, the kinetics of hypolithic community development is largely unquantified, with suggestions that the ages of hypolithons in cold slow-growth environments such as the Antarctic Dry Valleys to be decades or even centuries (Cowan 2014). In turn, a study of the processes driving

hypolith community development at a global scale using null probabilistic models (Caruso et al. 2011) postulated that hypolith structure at the global scale is dependent on a balance between deterministic and stochastic forces, which affect different functional taxa in distinct ways. Pointing and colleagues have suggested a hypolithic community development model (Pointing et al. 2007), where water availability is a critical driver of community establishment and growth. The implication for the development of hypolithic communities in desert soil ecosystems is that they will follow a 'static-step-static' growth profile, driven by the intermittent nature of rain events in such water-limited ecosystems.

Here, we have asked a basic, but largely unanswered question: are all hypolith prokaryote communities the same? Previous studies (Becker et al. 2006; Štursová et al. 2016, Franklin and Mills, 2003) showed that microbial communities can vary at very small scales in temperate soils, but no such study has been conducted with hypolithons. To this end, we sampled multiple individual hypolithic communities from localised moraine deposits in the Taylor Valley, Antarctic McMurdo Dry Valleys. The origins of the moraines in the Lower Taylor Valley are thought to be linked to the last glacial maximum (approx. 10ky; Vucetich and Robinson 1978). With subsequent slow soil turnover processes, driven by frost-heave (Bockheim, 2014), that may bring quartz pebbles to the surface for subsequent hypolithic colonization, it is reasonable to assume that the surface quartz minerals which are substrates for extant hypolithic communities have been exposed for very long periods (i.e., multiple decades or even centuries). Using 16S rRNA gene sequencing data from a collection of 30 independent and isolated hypolithons, we demonstrate that hypolithic communities form taxonomically distinct units at the local scale., In addition, we apply network analysis of taxonomical and predicted functional interactions, as well as ecological null models, to investigate the ecological processes driving the observed variation of individual hypolith communities at the small spatial scale.

Methods

Site description and sample acquisition: Fifteen quartz hypolithon samples were recovered from each of two sites, approximately 300m apart, during the January 2018 K080 field expedition to the lower Taylor Valley (New Harbour area), South Victoria Land, Antarctica (Table S1). The two sampling locations comprised areas of low-lying (3-10m asl) moraine deposits, where the morainal surface pavements, in which the hypolithons were embedded, consisted of pebbles and coarse sands of very mixed mineralogy typical of Taylor Valley moraines (principally granite, sandstone, gneiss and dolerite: Bockheim, 2002; Bockheim & McLeod, 2008; Bockheim et al., 2008) (Figure S1).

Within each site, all hypolithons were recovered within a radius of approximately 500 metres. The quartz pebbles with the attached hypolithic biomass were transferred into individual sterile Whirl-Pak bags, stored below 0°C in the field and during transport to South Africa, and at -80°C at the Centre for Microbial Ecology and Genomics (CMEG), Pretoria South Africa.

DNA extraction and sequencing: DNA from the hypolithon samples was extracted using the DNeasy PowerSoil Kit (QIAGEN, Germany) with 0.5 g of initial sample material. Extracted DNA was quantified using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and its quality was checked by PCR –amplification with 16S rRNA gene specific primers 341F (5′- CCTACGGGAGGCAGCAG-3′) and 908R (5′-CGTCAATTCMTTTGAGTT-3′). Thermocycling was conducted with a 25µl reaction volume following the protocol recommended by the polymerase provider (New England Biolabs, USA) (initial denaturation 95°C, 30 sec; 30 X (denaturation 95 °C, 15 sec; annealing 55 °C, 30 sec; elongation 68 °C, 60 sec); final extension 68 °C, 5 min; hold 4 °C). To check for contamination during DNA extraction, a blank negative control PCR reaction was performed using the kit elution buffer. Additionally, three samples that did not generate positive PCR signals were discarded. Extracted DNA from the remaining 27 samples was sent to the MRDNA Lab (Texas, USA) for 16S rRNA v3-v4 hypervariable region sequencing using 2x300 bps PE Illumina MiSeq technology with a read coverage of 20 000 reads per sample.

Phylogenetic Analysis: Sequenced reads were filtered and assembled using the QIIME2 pipeline (Bolyen et al. 2019), using DADA2 (Callahan et al. 2016) for read filtering and unique sequence inference, with a trunc-length (3′-terminus truncation length) of 280 bps for forward reads and 250 bps for reverse reads.   Taxonomy of the resulting assembled reads was carried out using the SILVA ver132 classifier (Quast et al. 2013) for prokaryotic species (with 99% similarity cut-off). The Amplicon Single Variants (ASVs)count table generated by the QIIME2 pipeline was manually curated to remove ASVs with less than 20 reads across all the samples. This step was performed to minimize the signal from potential false-positives originating from the sequencing platform. To assess if the sequencing depth for each sample was adequate, rarefaction curves of the sequence pool were generated using the Vegan (Oksanen, 2019) package in RStudio. After this analysis, sample H17 was discarded due to lack of sequencing depth.

Community composition Analysis: Alpha-diversity metrics, beta-diversity metrics and ordination for the remaining 26 samples were calculated using the Phyloseq (McMurdie and Holmes , 2013) and Vegan packages in R. The distribution of relative abundances and alpha-diversity indexes was tested using the Shapiro test (Royston, 1982), and the significance of difference in phylum relative abundances was calculated using ANOVA (for normally distributed data) (Chambers et al. 1992), and

the Kruskal-Wallis test (for non-normally distributed data) (McKight and Najab, 2010). To perform beta-diversity analyses, the ASVs count table was first rarefied using the sample with the lowest ASV count (53045 counts) as the reference sample, and counts were log(x+1) transformed. Samples were clustered into separate groups using the ward. D2 hierarchical method (Murtagh and Legendre, 2014). Beta-diversity between groups was then calculated using the weighted Unifrac distance metric (Lozupone et al. 2011), and visualized in a Principal Coordinates Analysis (PCoA) plot (Jolliffe and Cadima, 2016). PERMANOVA (Anderson and Walsh, 2013) was used to test for statistical differences between sample beta-diversity, while the variation within sample groups was tested using the analysis of multivariate homogeneity of group dispersions (β-disper) (Anderson, 2006). A significant β-disper value represents significantly different within-group variation in beta-diversity between groups, which might generate bias when analysing differences between groups. Therefore, for the PERMANOVA to be a reliable measure of significance of variation between groups, β-disper must be non-significant. The number of shared ASVs as a function of physical distance between samples was assessed by measuring zeta-diversity (the degree of shared ASVs between samples) to evaluate decay over distance (Hui and McGeoch, 2014). For analysis of 'generalists' and 'specialists', ASVs were clustered into their representative genera. 'Generalists' were manually curated as all genera present in at least 90% of the samples and in all groups defined by the hierarchical clustering analysis. The community composed of 'generalist' taxa was defined as the 'core' community, while the 'Specialists', considered as the the biomarkers for any specific group of hypoliths, were determined by calculating the $\log_2$ change of abundance between groups with the DESeq2 pipeline (Love et al. 2014), using the p-value threshold of 0.01.

Network clustering and function prediction: Interaction networks of taxa at the genus level were constructed using the CoNet (Faust and Raes, 2016) plugin in Cytoscape (Shannon et al. 2003). Both Pearson and Spearman correlation measures, as well as the Bray Curtis and Kullback-Leibler dissimilarity measures were used to infer synergistic and antagonistic relationships between taxa, with a p-value threshold of 0.05. The final network was generated from 1000 bootstraps, and the network was visualized in Gephi with a Fruchterman Reingold layout. Gephi was also used to calculate the network topology, including number of degrees, betweeness centrality and closeness centrality. Functional predictions of the genera used to generate the network were done by using the FAPROTAX (Louca et al. 2016) pipeline, the script of which is available at the developer's website (http://www.loucalab.com/archive/FAPROTAX/lib/php/index.php?section=Instructions).

Null modelling of phylogenetic turnover: A null modelling approach based on calculations of between-community mean-nearest taxon distance (β-NTI) and a Raup-Crick dissimilarity metric incorporating species relative abundance (RC_{bray}) was used to evaluate the relative influence of

deterministic and stochastic processes on community assembly, as previously described (Stegen et al. 2013). Observed β-NTI values that significantly deviate from β-NTI null distributions represent signals for variable selection (β-NTI > 2) and homogenous selection (β-NTI < -2), while those that fall within the null distribution represent compositional differences that arise from stochastic processes. The observed RC_{bray} values that significantly deviate from null distributions represent signals for dispersal limitation (2 > β-NTI > -2 and RC_{bray} > 0.95) and homogenizing dispersal (2 > β-NTI > -2 and RC_{bray} < -0.95). Comparisons that fall within the null expectations of both metrics (2 > β-NTI > -2 and 0.95 > RC_{bray} > -0.95) represent processes that are not dominated by selection or dispersal. A total of 999 randomizations were used for both β-NTI and RC_{bray} calculations. Null model assessments were performed on the complete dataset, and independently on the core and non-core datasets, with randomization based on full phylogeny for the complete dataset, and based on the separate phylogeny for each of the separate sets. The R script for null modelling analysis is available at (https://github.com/stegen/Stegen_etal_ISME_2013).

The R script for all other analyses is available at (https://github.com/PedroHLebre/Hypolith_script).

Results and Discussion

Hypolith communities are different across small spatial scales

A total of 26 Type I hypolithon communities (Type I; Cyanobacteria-dominated; following the classification set by Cowan et al. (2010)) were analyzed in order to explore the homogeneity of hypolithic microbial communities across small (<5m) spatial scales. Of the 20 prokaryotic phyla identified in the dataset, 9 phyla represented 99% of all assigned ASVs and 12 phyla were ubiquitous across all hypoliths (Figure 1A). Of these, Cyanobacteria were the most dominant (32.9% mean relative abundance, +/- 13% s.d.), followed by Proteobacteria (23.6 %, +/- 7% s.d.), Bacteroidetes (13.5%, +/- 7% s.d.) and Actinobacteria (10.2 %, +/- 9.3% s.d.). Analysis of the datasets at the genus level indicated that the cyanobacterial genus *Nostoc*, belonging to the *Nostocaceae* family, dominated microbial communities across all samples (20.6%, +/- 14% s.d.)(Figure S2). The dominance of cyanobacterial reads in the dataset was to be expected, as members of the order Nostocales have been previously reported as the dominant taxa in both Antarctic hypolithons and the surrounding soils (Pointing et al. 2010, Khan et al. 2011, Wood et al. 2008). It is important to note that members of other typically prevalent orders in these environments, such as Oscillatoriales and Chroococcales, were not detected in this particular dataset. This suggests that either these taxa are either not as widespread as previously observed, or are not represented in the sequenced pool of DNA due to differences in methodology compared to previous studies (eg. DNA extraction using

PowerSoil Kit vs Phenol-Chloroform used in other studies). Despite the presence of half of the phyla in all hypolithons, the relative abundance of the dominant phyla (defined as phyla accounting for more than 1% of total ASV abundance) varied between samples (Figure 1B). This result indicates that hypolith communities can vary over even short (meter) distances.
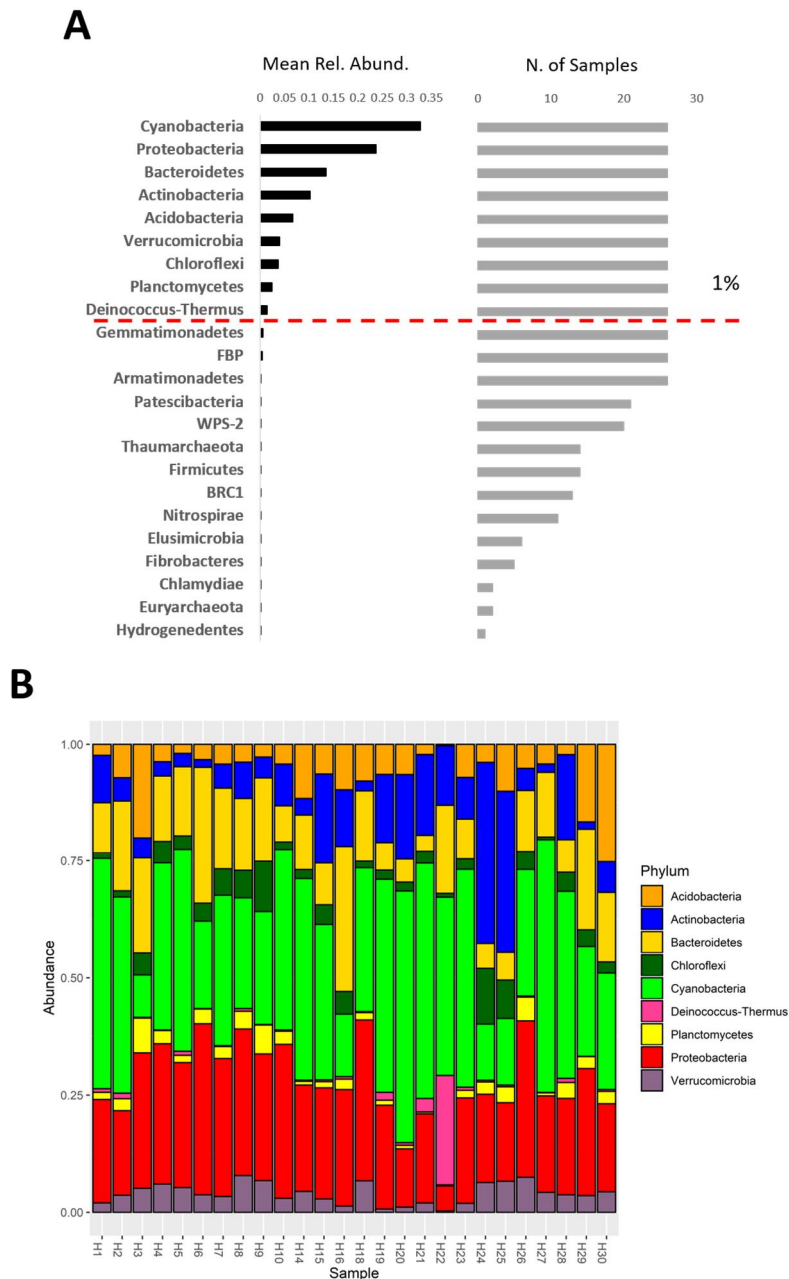

Figure 1. Prevalence of phyla in hypolithic communities (A) and distribution of dominant phyla across samples (B). Total abundance of each phylum for the dataset was expressed as mean relative abundance (the mean of relative abundances across samples), while the relative abundances of dominant phyla per sample were calculated as a fraction of total ASV counts for each sample. The dashed red line in (A) represents the threshold for phyla accounting for more than 1% of the mean relative abundance of the dataset, which were classified as dominant phyla.

The degree of community heterogeneity was further reflected in the grouping of the phylogenetic datasets into four distinct clusters (A, B, C, and D) according to microbial composition differences between samples, as expressed by weighted unifrac beta-dissimilarity scores (Figure 2). PERMANOVA analysis showed the clustering to be highly significant (R^2 = 0.493, p-value < 0.009), and not an artefact of intra-group variation, as indicated by the non-significant beta-dispersivity value (β-disper = 0.281).
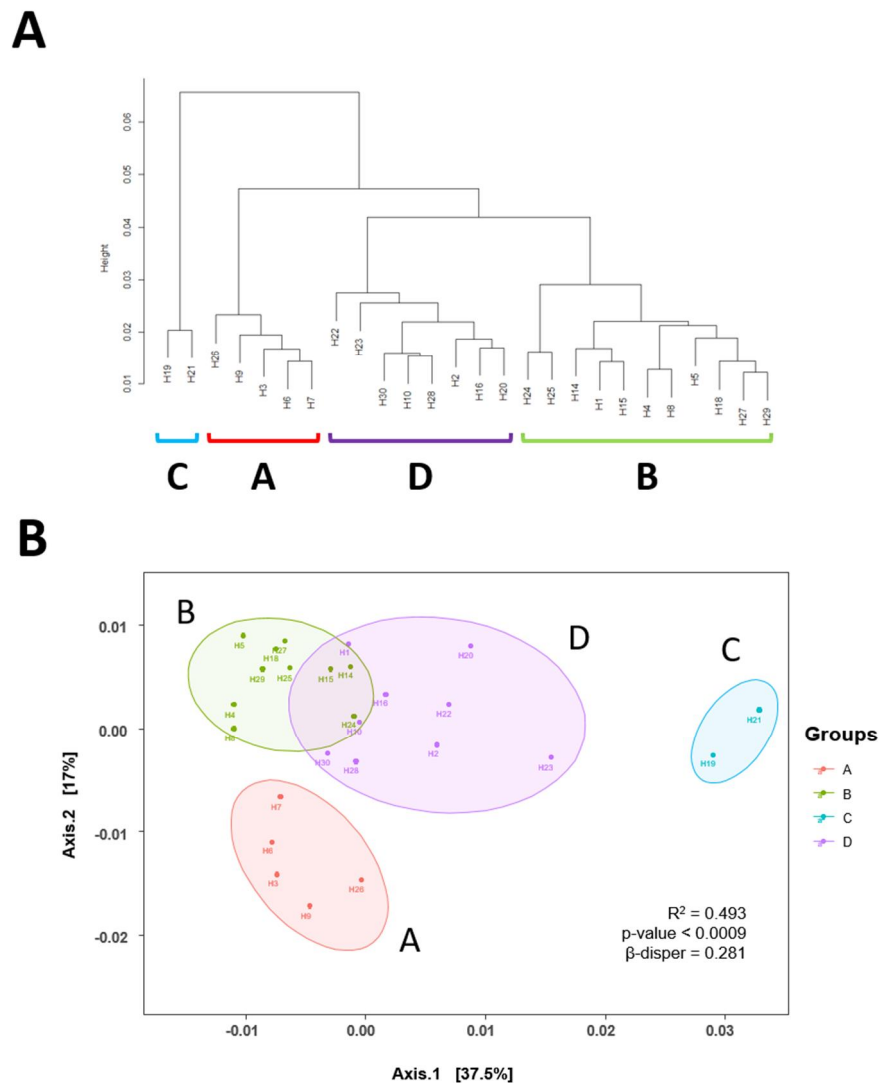

Figure 2. Sample clustering according to weighted Unifrac distances (A) and ordination of distances between samples in a PCoA plot (B). The four groups resulting from the clustering are labelled as A, B, C and D, and colored as red, green, blue and purple, respectively. The same color code was used in the PCoA ordination. Significance between weighted Unifrac distances was calculated using PERMANOVA, and expressed by the coefficient of determination (R^2) and adjusted p-value (p-adj). Intra-group variation in beta-dispersion (betadisper) was non-significant, which is a requirement for the validity of the PERMANOVA analysis. Ellipses on the PCoA plot highlight the clustering of the hypolithon samples into different groups.

Hypolithon community structure is driven by synergy between different taxa

To explore in more detail the relationships between the hypolithic community sample clusters, community similarities and differences were assessed by determining "generalists" (i.e., taxa that present in ≥90% of samples) and "specialists" (i.e., taxa that were over-represented in a specific group). This analysis identified 132 genera (of a total of 431) that were present in 22 or more samples (Figure S3). Functional prediction of this core community based on analysis using the FAPROTAX database tool (Louca et al. 2016) revealed that, aside from the ubiquitous photoautotrophic cyanobacteria, several genera predicted to be involved in nitrate reduction and nitrogen respiration, and ureolyis, were also found to be present (Table S2). The relationships between these taxa were further inferred by calculating potential interactions between them, visualized as a network of significant relationships between genera (Figure 3A). Of the 132 core genera, 105 were found to be significantly correlated (adjusted p-value <0.05), with most correlations (98%) being classified as synergistic. The network topology was organized around two principal self-contained clusters dominated by Actinobacteria-Chloroflexi and Verrucomicrobia-Bacteroidetes-Proteobacteria interactions, respectively. The two principal clusters were only sparsely connected by both synergistic (n=6) and antagonistic interactions (n=1) between these main heterotrophic groups.

Surprisingly, cyanobacterial members of the core community were positioned at the periphery of the network with only a limited number of connections to other taxa, and *Nostoc*, which accounted for a fifth of all ASV counts, did not exhibit significant correlations with any other taxa in the core community. This result suggests that while photoautotrophic cyanobacteria may play an important role as primary producers and keystone taxa in hypolithic communities, as suggested by several studies (Lacap-Bugler et al. 2017, Van Goethem et al. 2017, Lebre et al. 2017), they may not be important determinants of compositional differences in community structure. This is further emphasized by mapping of the predicted functions of each genus into the network (Figure 3B), showing that most taxa capable of photoautotrophy were located on the edge of the network, with low connectivity (average number of interactions = 3.3). One possible explanation for this result would be the lack of competition between the dominant photosynthetic taxa and the less abundant portion of the community. By comparison, the functional network suggests that taxa with the predicted potential for nitrogen acquisition and ureolysis play a prominent role in the trophic relationships within the core community, as indicated by the high number of connections linking taxa with these functions within the network. The implication is therefore that N acquisition, rather than C supply, is the dominant trophic driver of community structuring. Nitrogen metabolism has been shown to be a key function in hypolithons (Ramond et al. 2018, Cowan et al. 2011b), and it is

therefore consistent that genera with this metabolic capability would show high connectivity in the co-occurrence network. However, it is important to note that while they could not be functionally mapped, four genera in the group of hub taxa (taxa with highest number of connections) belong to the class *Chloroflexia*, which is exclusively composed of anoxygenic phototrophic bacteria (Hanada, 2014). This observation suggests that photoautotrophs other than Cyanobacteria may play an important role in the structuring of the core hypolithic community. Together, the network analysis of taxonomic and functional interactions within the core community suggests that less abundant taxa have a disproportionately dominant role in shaping the core hypolithic community structure.
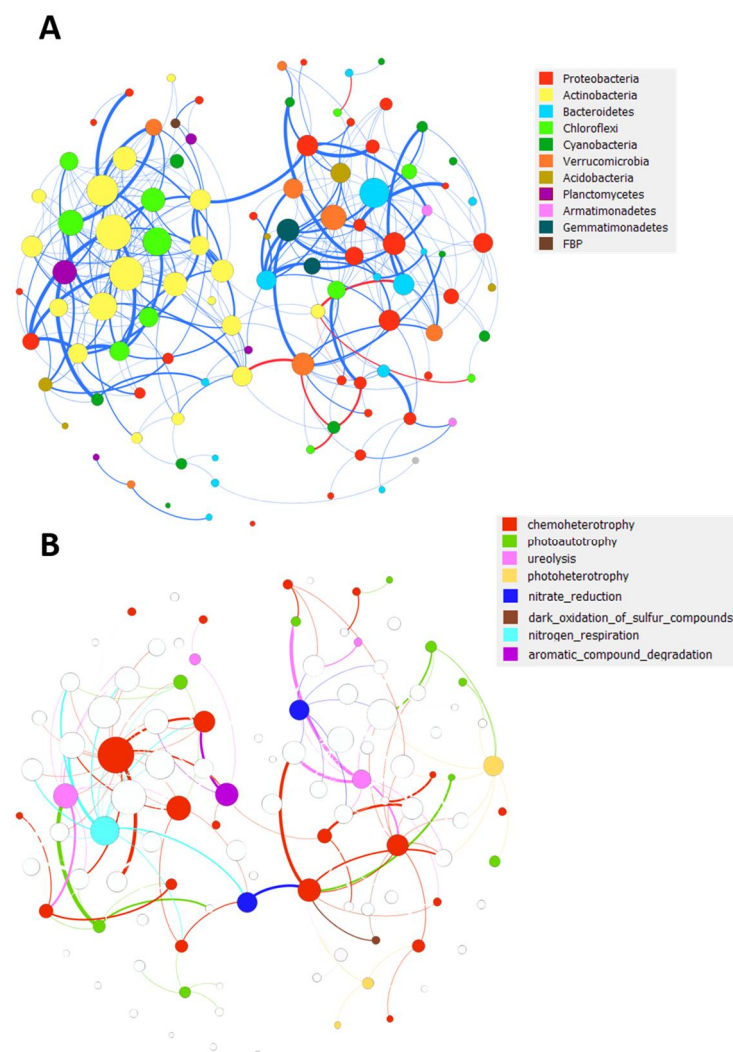

Figure 3. Co-occurrence network of the core hypolithic community, according to the taxonomy (A) and functional predictions (B) of the genera in the core community. Co-occurrence analysis was performed using Pearson. Spearman, and Bray-Curtis correlations, with a p-value threshold of 0.05. The final network was drawn from a 100 bootstraps using the CoNet plugin in Cytoscape. Genera are represented as nodes, which are sized proportionally to the number of connections Synergistic and antagonistic connections are coloured as blue or red, respectively.
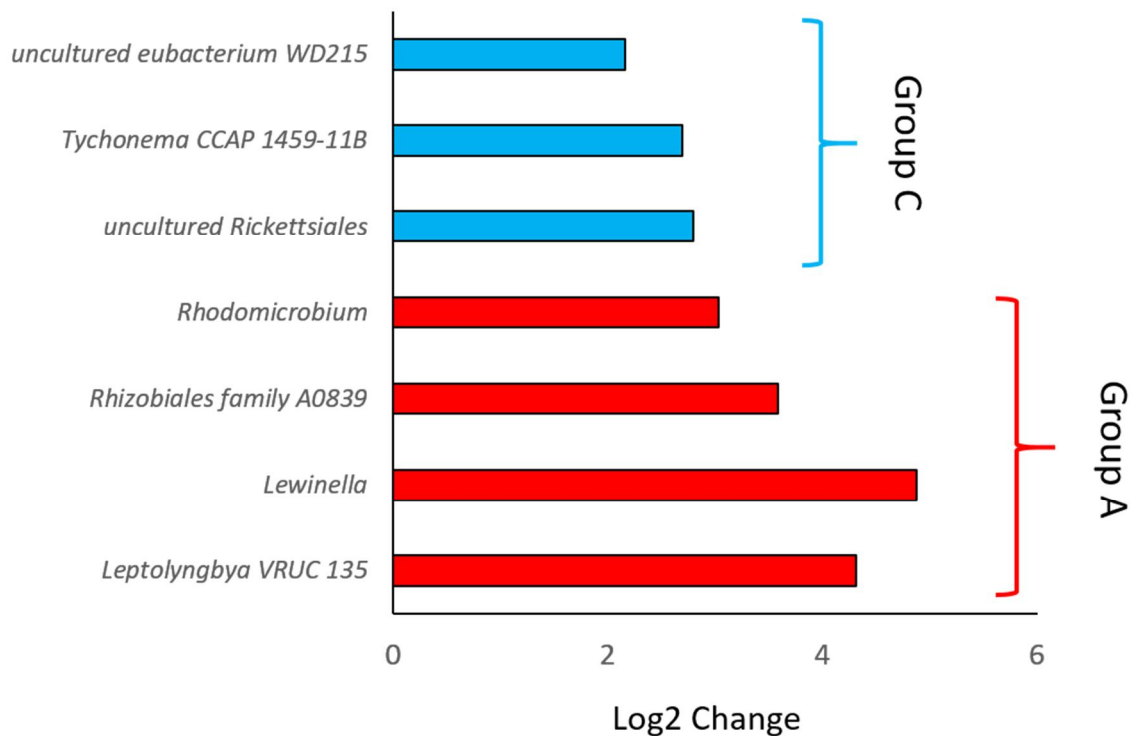
Figure 4. "Specialist" genera in the different hypolithic groups. Log change values were calculated as pair-wise comparisons between groups, with all values represented in the plot being significant (adjusted p-value < 0.001).

Four phyla were found to be differentially abundant, at a statistically significant level, between the four different clusters (A, B, C, and D) previously determined from weighted unifrac beta-dissimilarity scores (Figure S4): Abditibacteriota, Verrucomicrobia, Deinococcus-Thermus and Planctomycetes. As the average relative abundance for these phyla was below 10%, this result suggests that the greatest degree of variation between communities occurs in the lower abundance taxa. A search for "specialist" taxa, defined as taxa that are over-represented in any specific group of hypoliths, led to the identification of a small number of genera specific to groups A and C. In particular, group A was found to be the most distinct group, containing 4 of the 7 identified "specialist" genera (Figure 4). The over-representation in group A of the Proteobacterial genus *Rhodomicrobium*, which is known to contain species capable of phototrophic metabolism (Miot et al, 2009; Wright and Madigan 1991; Ramana et al. 2013) may be particularly significant. Two other Cyanobacterial genera, *Leptolyngbya* and *Tychonema*, were found to be over-represented in groups A and C, respectively. The fact that all these "specialist" genera are potentially involved in phototrophic metabolism, but represent less than 1% of the total genera across all samples

(therefore are considered to be 'rare' taxa), re-enforces the concept that rare taxa might play important roles in the structure and evolution of microbial communities (Shade et al. 2014).

Stochastic processes explain differences between hypolith communities

The role that physical distance might play in the variation between hypolithic community compositions was evaluated by assessing the zeta-diversity, which measures the average number of species shared between sites across the entire sample set. If distance were to play a significant role in the clustering of hypolith communities, zeta-diversity should decay linearly as a function of distance. No decay was observed in zeta-diversity across the sampled distance (Figure S5), indicating that differences between hypolithon communities are not linearly correlated to the distance between the spatially separated communities.

To infer the relative roles of stochastic and deterministic processes in shaping community composition, deviations in phylogenetic turnover across the sample set were calculated using the null model methodology developed be Stegen et al. (2013) (Figure S6). The calculated β-Nearest Taxon Index (β-NTI) quantifies the degree to which observed the β-Mean Nearest Taxon Distance between pairs of communities deviates from values expected when *selection* does not influence turnover in community composition. Values of β-NTI above or below the expected null model (-2 < β-NTI$_{null}$ <2) can be interpreted as evidence for deterministic processes shaping community composition. In the sample set used in this study, 56% of community turnover was primarily attributed to *homogenizing selection*, with β-NTI values being below those expected if turnover was dominated by stochastic processes (-2 < β-NTI); i.e., communities were found to be more similar to each other than would be expected by chance. This result is attributed to the existence of shared selective pressures, such as similar micro-environmental conditions provided by the quartz substrate, and by similar abiotic stresses (such as low a$_w$). By comparison, 39.1% of community turnover fell within the expected values under the null model, and therefore was considered to be governed by stochastic processes such as dispersal limitation, homogenizing dispersal, drift or a combination of these processes. This result is consistent with a study by Makhalanyane et al. (2014), whereby deterministic processes were postulated to drive community composition of Type I hypolithons. In turn, comparison of phylogenetic turnover between the core community and the non-core community (Figure 5) revealed that most turnover in the core community, which is dominated by cyanobacteria (80% of the turnover), is driven by homogenizing selection, with a β-NTI$_{core}$ average of -2.5. Conversely, 70% of the turnover in the non-core community is driven by stochastic processes, with a β-NTI$_{Noncore}$ average of -1.2. These data suggest that the deterministic

processes driving hypolith community turnover mostly affect the core community that is ubiquotously distributed accross hypoliths, while differences between hypolith communities (represented by the non-core portion of the communities) is driven by stochastic processes. Together with the non-significant zeta-diversity, these results suggest that the limitations of dispersal that lead to the stochastic variability of microbial communities in hypolithons apply even at very small spatial (meter) scales.
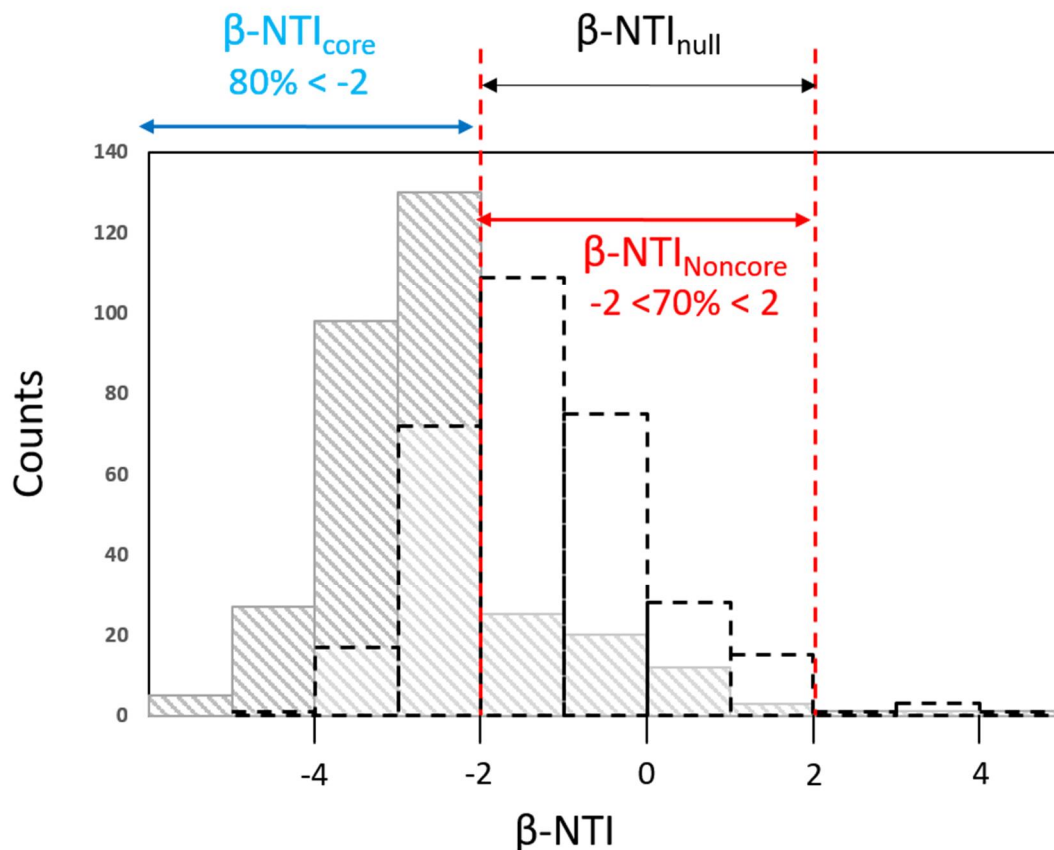

Figure 5. β-NTI distribution of the core (striped-grey bars) and non-core (white, black-traced bars) communities. The β-NTI range for stochastic processes (-2 < β-NTI < 2) is highlighted by the trace red lines, while the ranges in which most of the β-NTI values for core and non-core communities are distributed are highlighted by the blue and red arrows, respectivelyTo further clarify the stochastic processes that are likely to play a dominant role in the phylogenetic turnover of communities, the Raup-Crick dissimilarity (RC_{bray}) of sample pairs with absolute β-NTI values below 2 was calculated. According to Stegen et al. (2013), values of $RC_{bray} > 0.95$ and $RC_{bray} < -0.95$ represent turnovers driven by dispersal limitation together with drift, and homogenizing dispersal, respectively. This analysis (TableS3) indicated that the majority of stochastic turnover between hypoliths was driven by dispersal limitation together with drift (92.2%), while only 8.8% of stochastic turnover was driven by drift alone.

Original hypolith
Community

UV-radiation
Desiccation
Nutrient scarcity
Competition

**Homogenizing
Selection**

Selection of
core **"climax"**
community

**Stochastic
differentiation**

Dispersal
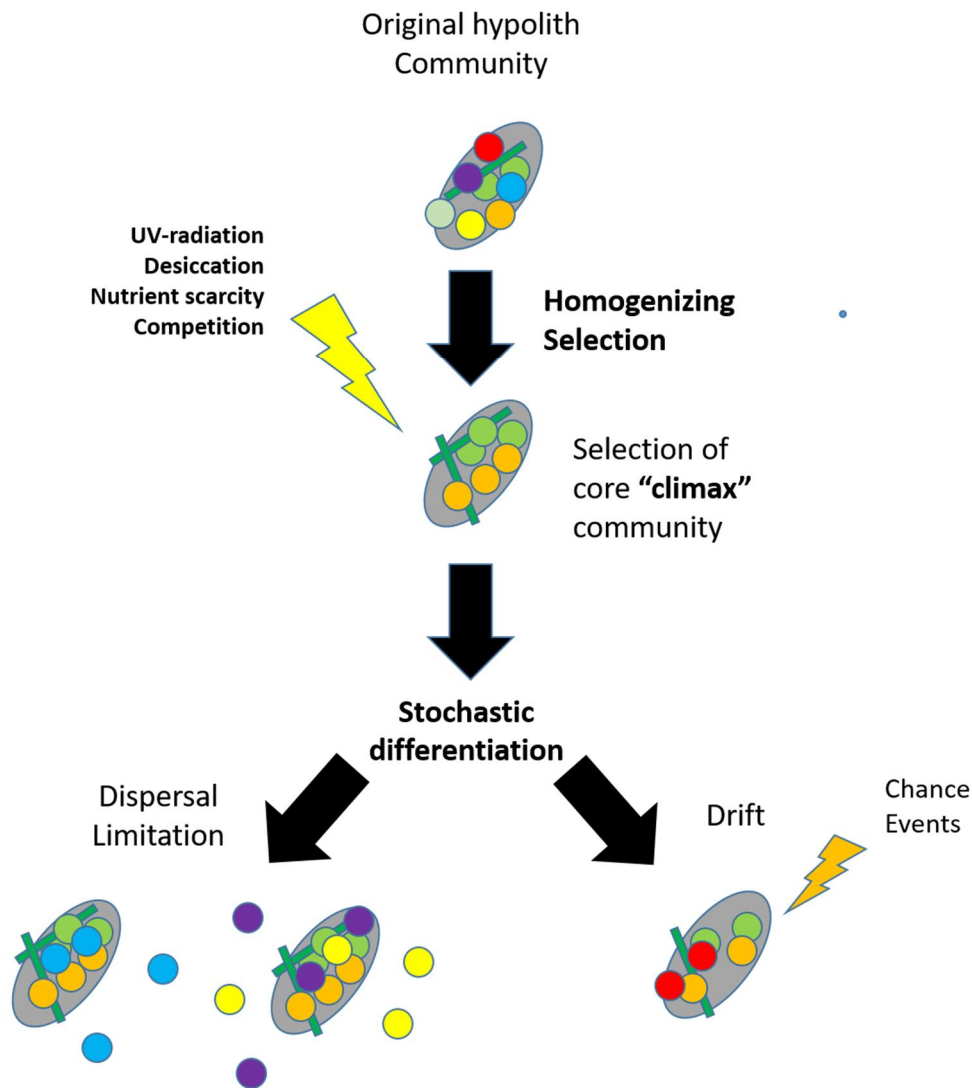Limitation

Drift

Chance
Events

Figure 6. Model for the evolutionary development of Antarctic hypolith communities. Variation of communities at the local scale is driven by stochastic processes, while a core "climax" community is maintained through the effects of selective abiotic and biotic pressures.

The results from this study have led us to propose a model for the evolutionary development of Antarctic hypolithic communities at the local scale (Figure 6), whereby common abiotic and biotic stresses such as desiccation, nutrient limitation and inter-taxon competition are strong primary drivers for the homogenous selection of dominant taxa, such as Cyanobacteria, which might constitute the core community. By comparison, dispersal limitation is the main stochastic process driving the differentiation of less abundant, heterotrophic taxa. In turn, differentiation within the rare taxon fraction of the community might lead to functional differentiation of hypolithons, as suggested by the enrichment of different taxa capable of phototrophy. We postulate that local physicochemical heterogeneity (Becker et al. 2006; Štursová et al. 2016; Zhou et al. 2002), together

with the limitations in transport due to the absence of a liquid continuum, leads to the differential small-scale recruitment of taxa by the hypolith community (Makhalanyane et al. 2013).

Conclusions:

In this study, we addressed the question of whether hypolithons, at local scales, share the same microbial community compositions. This question is relevant to most of the ecological studies of hypolithic microbiomics, where the assumption of community homogeneity across multiple samples is inherent. The results in this study show that rather than being homogenous communities, hypolithons harbour rich and distinct microbial communities, dominated by a core assemblage of bacteria. Analysis of the possible interactions between taxa suggests that synergy between less abundant taxa, particularly those with the capacity to photosynthesize and metabolize nitrogen, is an important driver in shaping the community. The application of null models of phylogenetic turnover also led us to infer that the majority of community variation between hypolithons is driven by stochastic effects, more specifically, dispersal limitation.

We acknowledge that the reliance on sequencing data based on short 16S rRNA gene hyper-variable regions, which has been shown to have several short-comings including the difficulty of assigning ASVs to species or genera with high confidence (Poretsky et al. 2014) and the use of incomplete databases for taxonomic assignment (Edgar, 2018), represent limitations in this study, but do not affect the central conclusions.

In the absence of data on the micro-environment of each hypolithon, we cannot draw definitive conclusions on the drivers of hypolithon heterogeneity. In addition to the spatial segregation of the microbial communities, other drivers could also be involved in the evolution of community heterogeneity. For example, variations in the physical or chemical properties of the over-lying hypolithic rock substrate, which has been documented in polar and non-polar deserts (Warren-Rhodes et al. 2013; Cowan et al. 2011), could lead to differences in light penetration and moisture retention (Schlesinger et al. 2003), which in turn would result different selective pressures being exerted on the underlying hypolithic community. Alternatively, divergent successional development (Makhalanyane et al. 2013) of individual communities, possibly due to variations in the micro-environment, could lead to the spatial heterogeneity described in this study. Future studies should complement these findings with more quantitative methodologies such as sequencing of the total hypolithon DNA and mRNA, combined with the detailed characterization of the hypolithic micro-environment.

Acknowledgements

Data availability

The raw sequencing reads can be found in the NCBI Bioproject database (BioProject ID:PRJNA659625, https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA659625&o=acc_s%3Aa). In addition, the metadata of the sequencing output can be found in the github webpage https://github.com/PedroHLebre/Hypolith_script

References

Anderson MJ. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics*, 2006;62:245--253.

Anderson MJ, Walsh DCI. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monograph*s. 2013;83(4):557-574.

Becker JM, Parkin T, Nakatsu CH *et al*. Bacterial Activity, Community Structure, and Centimeter-Scale Spatial Heterogeneity in Contaminated Soil. *Microb Ecol*, 2006;51:220–231.

Bockheim JG. Antarctic Soil Properties and Soilscapes. In: Cowan DA (ed). *Microbiology of Antarctic Soils*, Springer publ, 2014, 293-316

Bockheim, JG. Landform and soil development in the McMurdo Dry Valleys, Antarctica: a regional synthesis*. Arctic, Antarctic & Alpine Res.* 2002;34:308-317.

Bockheim, JG, McLeod, M. Soil distribution in the McMurdo Dry Valleys, Antarctica. *Geoderma.* 2008; 144:43-49.

Bockheim, JG, Prentice, ML, McLeod, M. Distribution of glacial deposits, soils, and permafrost in Taylor Valley, Antarctica. Arctic, Antarctic & Alpine Res. 2008;40:279-286.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*, 2016;13(7):581–583. https://doi.org/10.1038/nmeth.3869

Caruso T, Chan Y, Lacap D *et al.* Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. *ISME J*, 2011;5:1406–1413.

Chambers JM, Freeny A and Heiberger, RM. (1992) Analysis of variance; designed experiments. Chapter 5 of Statistical Models in S. In: Chambers JM and Hastie TJ (ed). Wadsworth & Brooks/Cole.

Chan Y, Lacap DC, Lau MCY *et al.* Hypolithic microbial communities: between a rock and a hard place. *Environ. Microbiol*, 2012;14:2272–2282.

Chan Y, Van Nostrand JD, Zhou J *et al.* Functional ecology of an Antarctic Dry Valley. *Procs Natl Acad Sci USA*, 2013;110(22);8990-8995

Cockell C, Stokes M. Widespread colonization by polar hypoliths. *Nature*, 2004;431(7007):414 .

Cowan D, Khan N, Pointing SB *et al.* Diverse hypolithic refuge communities in the McMurdo Dry Valleys. *Antarctic Sci,* 2010;*22*(6):714-720.

Cowan, DA, Pointing, SB., Stevens, MI *et al.* Distribution and abiotic influences on hypolithic microbial communities in an Antarctic Dry Valley. *Polar Biol*, 2011a;34:307–311.

Cowan DA., Sohm JA, Makhalanyane TP *et al.* Hypolithic communities: important nitrogen sources in Antarctic desert soils. *Environ Microbio Rep*, 2011b;3:581-586.

Cowan DA. Introduction. In: Cowan, DA (ed). *Antarctic terrestrial microbiology.* Springer-Verlag publ, Berlin, 2014, 1-8.

Edgar R. Taxonomy annotation and guide tree errors in 16S rRNA databases. PeerJ. 2018:6:e5030.

Faust K and Raes J. CoNet app: inference of biological association networks using Cytoscape. *F1000Research*, 2016;5:1519.

Franklin RB, Mills AL. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. *FEMS Microbiol Ecol.* 2003;44(3):335-346.

Hanada S. The Phylum Chloroflexi, the Family Chloroflexaceae, and the Related Phototrophic Families Oscillochloridaceae and Roseiflexaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E and Thompson F (eds). *The Prokaryotes.* Springer publ, Berlin, 2014, 515-532.

Hibbing ME, Fuqua, C, Parsek, MR *et al.* Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev. Microbiol*, 2010;8(1):15–25.

Hui C and McGeoch MA. Zeta diversity as a concept and metric that unifies incidence-based biodiversity patterns. *Am Nat*, 2014;184(5):684-694.

Khan N, Tuffin M, Stafford W *et al.* Hypolithic microbial communities of quartz rocks from Miers Valley, McMurdo Valleys, Antarctica. *Pol Biol*, 2011;34:1657-68.

Lazzarini A, Cavaletti L, Toppo G *et al.* Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie van Leeuwenhoek*, 2000;78(3-4):399-405.

Lebre P, De Maayer P and Cowan DA. Xerotolerant bacteria: surviving through a dry spell. *Nat Rev Microbiol*, 2017;15:285–296.

Lozupone C, Lladser ME, Knights D *et al.* UniFrac: an effective distance metric for microbial community comparison. *ISME J*, 2011;*5*(2):169–172.

Louca S, Parfrey LW and Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. *Science*, 2016;353:1272-1277

Love MI, Huber W and Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*, 2014;15(12):550.

Makhalanyane TP, Valverde A, Lacap DC *et al.* Evidence of species recruitment and development of hot desert hypolithic communities. *Environ Microbiol Rep*, 2013;5(2):219-24.

Makhalanyane TP, Valverde A, Birkeland NK *et al.* Evidence for successional development in Antarctic hypolithic bacterial communities. *ISME J*, 2014;8(4):952.

Štursová M, Bárta J, Šantrůčková H *et al.* Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. *FEMS Microbiol Ecol*, 2016;92(12): fiw185.

McKnight PE and Najab J. (2010). Kruskal-Wallis Test. In: Weiner IB and W.E. Craighead WE (eds). *The Corsini Encyclopedia of Psychology*, Wiley Online Library, 2010.

McMurdie PJ and Holmes S. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE*, 2013;8(4): e61217.

Miot J, Benzerara K, Obst M *et al.* Extracellular Iron Biomineralization by Photoautotrophic Iron-Oxidizing Bacteria. *Appl Environ Microbiol*, 2009;75(17):5586-5591.

Murtagh F and Legendre P. Ward's Hierarchical Agglomerative Clustering Method: Which Algorithms Implement Ward's Criterion?. *J Classif*, 2014;31:274-295.

Oksanen J, Blanchet FG, Friendly M *et al.* Vegan: Community Ecology Package. R package version 2.5-5, 2019. https://CRAN.R-project.org/package=vegan

Pointing SB, Warren-Rhodes KA, Lacap DC *et al.* Hypolithic community shifts occur as a result of liquid water availability along environmental gradients in China's hot and cold hyperarid deserts. *Environ Microbiol*, 2007;9(2):414–424.

Pointing SB, Chan Y, Lacap DC *et al.* Highly specialized microbial diversity in hyper-arid polar desert. *PNAS*, 2010;106(47):19964-69.

Poretsky R, Rodriguez-R LM, Luo C et *al.* Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One, 2014;9(4):e93827.

Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*, 2013;41(Database issue):D590–D596.

Ramana VV, Raj PS, Tushar L *et al.* Rhodomicrobium udaipurense sp. nov., a psychrotolerant, phototrophic alphaproteobacterium isolated from a freshwater stream. *Int J Syst Evol Microbiol*, 2013;63(Pt 7):2684-9.

Royston JP. Algorithm AS 181: The W test for Normality. *Appl Stat*, 1982;31:176--180.

Schlesinger WH, Pippen JS, Wallenstein MD *et al.* Community composition and photosynthesis by photoautotrophs under quartz pebbles, Southern Mojave Desert. *Ecology.* 2003:84: 3222-3231.

Shade A, Hannes P, Allison SD *et al.* Fundamentals of Microbial Community Resistance and Resilience. *Front Microbiol*, 2012;3:417.

Shannon P, Markiel A, Ozier O *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*, 2003;13(11):2498–2504.

Shade A, Jones SE, Caporaso G *et al.* Conditionally Rare Taxa Disproportionately Contribute to Temporal Changes in Microbial Diversity. *mBio*, 2014;5(4): e01371-14.

Stegen JC, Lin X, Konopka AE *et al.* Stochastic and deterministic assembly processes in subsurface microbial communities. *ISME J*, 2012;6(9):1653–1664.

Stegen JC, Lin X, Fredrickson JK *et al.* Quantifying community assembly processes and identifying features that impose them. *ISME J*, 2013;7(11):2069–2079.

Štursová M, Bárta J, Šantrůčková H, Baldrian P. Small-scale spatial heterogeneity of ecosystem properties, microbial community composition and microbial activities in a temperate mountain forest soil. *FEMS Microbiol Ecol.* 2016;92(12): fiw185.

Jolliffe IT and Cadima J. Principal component analysis: a review and recent developments. *Philos Trans A Math Phys Eng Sc*, 2016;374(2065):20150202.

Van Goethem, MW, Makhalanyane TP, Cowan DA *et al*. Cyanobacteria and Alphaproteobacteria May Facilitate Cooperative Interactions in Niche Communities. *Front Microbiol*, 2017;8:2099.

Vucetich, CG and Robinson PH. Quaternary stratigraphy and glacial history of the Lower Taylor Valley, Antarctica. *New Zealand J Geol Geoph*, 1976;21(4):467-482.

Warren-Rhodes, KA, McKay CP, Boyle LN *et al*. Physical ecology of hypolithic communities in the central Namib Desert: The role of fog, rain, rock habitat, and light, *J. Geophys. Res. Biogeosci.* 2013:118:1451– 1460.

Warren-Rhodes KA, Rhodes KL, Pointing SB *et al*. Hypolithic cyanobacteria, dry limit of photosynthesis and microbial ecology in the hyperarid Atacama Desert, Chile. *Microb Ecol*, 2006;52: 389–398.

Wei STS, Lacap-Bugler DC, Lau MCY *et al*. Taxonomic and Functional Diversity of Soil and Hypolithic Microbial Communities in Miers Valley, McMurdo Dry Valleys, Antarctica. *Front Microbiol*, 2016;7:1642.

Welz PJ, Ramond JB, Cowan DA *et al*. Minor differences in sand physicochemistry lead to major differences in bacterial community structure and function after exposure to synthetic acid mine drainage. *Biotech Bioproc Eng*, 2014;19(2):211-20.

Wright GE and Madigan MT. Photocatabolism of Aromatic Compounds by the Phototrophic Purple Bacterium Rhodomicrobium vannielii. *Appl Environ Microbiol*, 1991;57(7):2069–2073.

Zhou J, Xia B, Treves DS *et al*. (2002). Spatial and Resource Factors Influencing High Microbial Diversity in Soil. *Appl Environ Microbiol*, 2002:68(1);326-334.

Zothanpuia, Passari AK, Leo VV *et al*. Bioprospection of Actinobacteria Derived from Freshwater Sediments for Their Potential to Produce Antimicrobial Compounds. *Microb Cell Fact*, 2018;17(1):68.