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Research Article

RESEARCH ARTICLE

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

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One sentence summary: Phylogenetic analysis of hypolithic communities reveals high degree of small scale heterogeneity, driven by stochastic processes.

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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 localized 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 favor microbial community development in 'extreme' edaphic habitats (Chan *et al.* 2012, Lebre, De Maayer and Cowan 2017). Such favorable parameters include protection

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from incident short-wavelength solar radiation and against desiccation, and the provision of thermal buffering and physical stability (Lebre, De Maayer and Cowan 2017).

The composition of hypolithic communities has been extensively studied, most intensively in the Antarctic McMurdo Dry Valleys (Cowan *et al.* 2010; Khan *et al.* 2011; Chan *et al.* 2013; Wei *et al.* 2016) 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 harbor 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; Šťursová *et al.* ; 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 localized 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 1976). 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

A total of 15 quartz hypolithon samples were recovered from each of two sites, approximately 300 m apart, during the January 2018 K080 field expedition to the lower Taylor Valley (New Harbour area), South Victoria Land, Antarctica (Table S1, Supporting Information). 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 and McLeod 2008; Bockheim, Prentice and McLeod 2008; Figure S1, Supporting Information).

Within each site, all hypolithons were recovered within a radius of approximately 500 m. 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, Waltham, Massachusetts, 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 25 μL reaction volume following the protocol recommended by the polymerase provider (New England Biolabs,Ipswich, Massachusetts, USA); initial denaturation 95°C, 30 s; 30 X denaturation 95°C, 15 s; annealing 55°C, 30 s; elongation 68°C, 60 s; 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 (Shallowater, Texas, USA) for 16S rRNA v3–v4 hypervariable region sequencing using 2 × 300 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 trunclength (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 *et al.* 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, Freeny and Heiberger 1992), and the Kruskal–Wallis test (for non-normally distributed data; cKight and Najab 2010). To perform beta-diversity analyses, the ASVs count table was first rarefied using the sample with the lowest ASV count (53 045 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, Huber and Anders 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, Parfrey and Doebeli 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 ($2 > \beta$-NTI > -2 and $RC_{bray} > 0.95$) and homogenizing dispersal ($2 > \beta$-NTI > -2 and $RC_{bray} < -0.95$). Comparisons that fall within the null expectations of both metrics ($2 > \beta$-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 (<5 m) 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 (Fig. 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, Supporting Information). 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

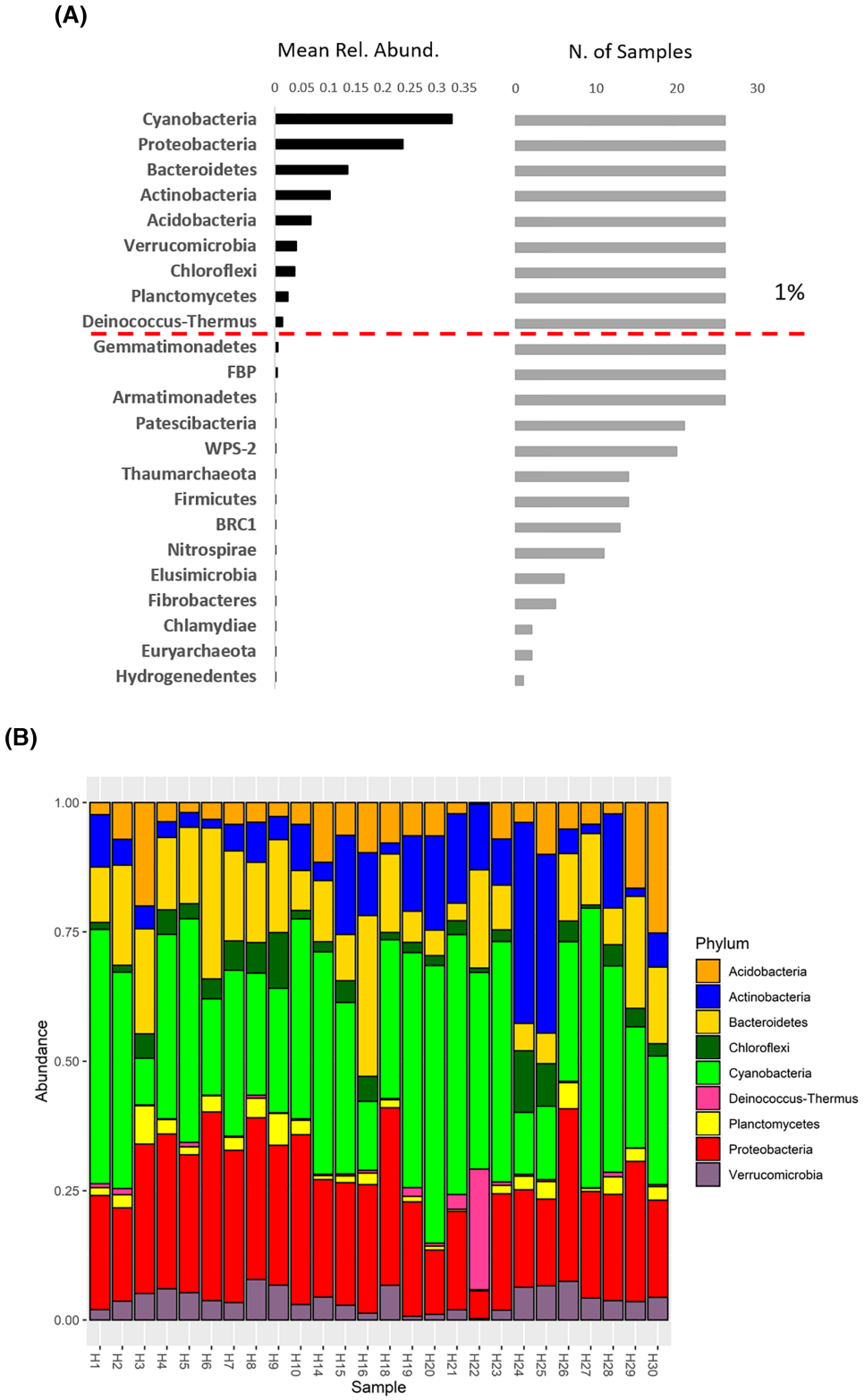

Figure 1. Prevalence of phyla in hypolithic communities **(A)** and distribution of dominant phyla across samples **(B)**. Total abundance of each phylum for the dataset was expressed as mean relative abundance (the mean of relative abundances across samples), while the relative abundances of dominant 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.

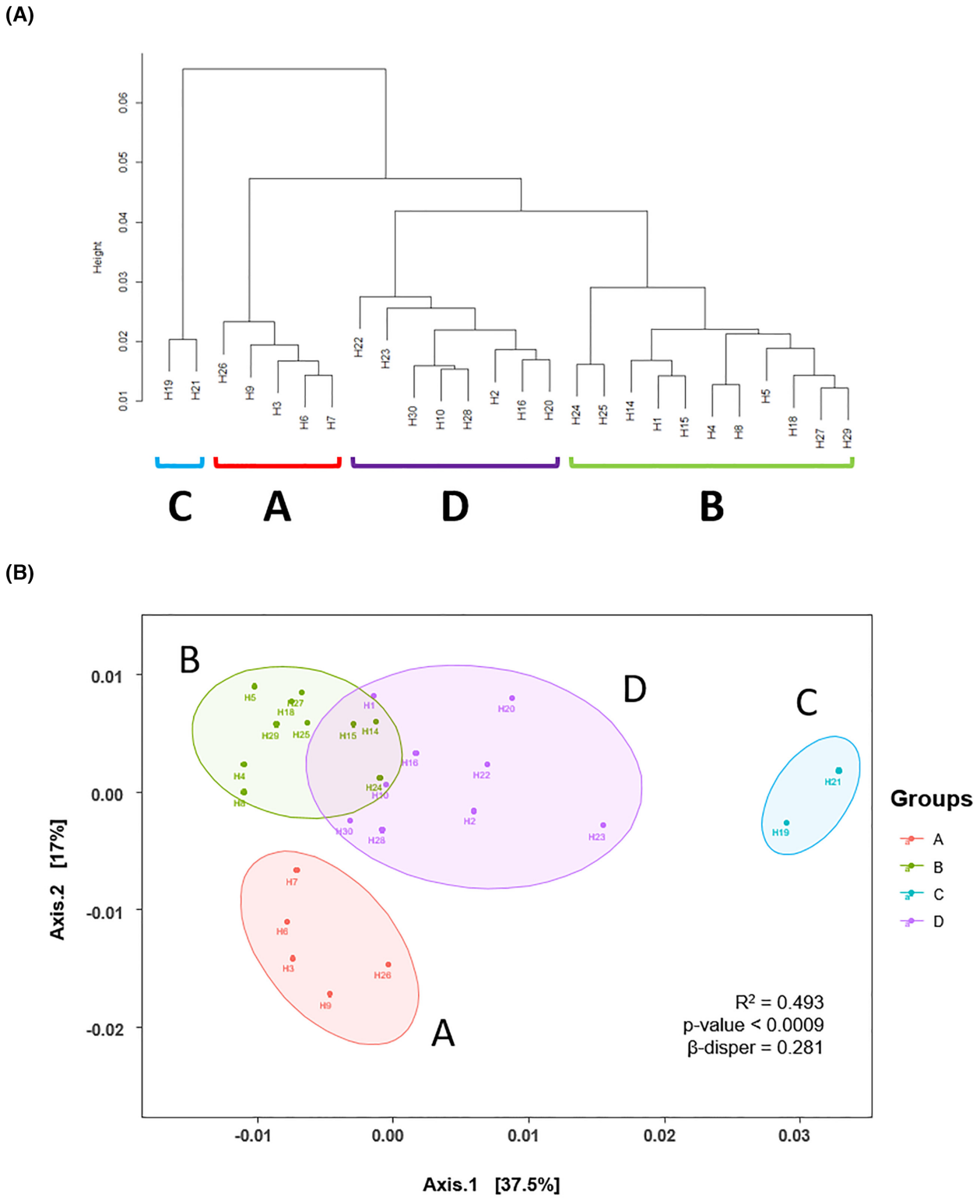

Figure 2. Sample clustering according to weighted Unifrac distances **(A)** and ordination of distances between samples in a PCoA plot **(B)**. The four groups resulting from the clustering are labelled as A, B, C and D, and colored as red, green, blue and purple, respectively. The same color code was 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.

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 (e.g. 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 (Fig. 1B). This result indicates that hypolith communities can vary over even short (meter) distances.

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 (Fig. 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 $= 0.281$).

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, Supporting Information). Functional prediction of this core community based on analysis using the FAPROTAX database tool (Louca, Parfrey and Doebeli 2016) revealed that, aside from the ubiquitous photoautotrophic cyanobacteria, several genera predicted to be involved in nitrate reduction, nitrogen respiration and ureolyis, were also found to be present (Table S2, Supporting Information). The relationships between these taxa were further inferred by calculating potential interactions between them, visualized as a network of significant relationships between genera (Fig. 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; Lebre, De Maayer and Cowan 2017; Van Goethem *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 (Fig. 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.

A total of 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, Supporting Information): 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 (Fig. 4). The over-representation in group A of the Proteobacterial genus *Rhodomicrobium*, which is known to contain species capable of phototrophic metabolism (Wright and Madigan 1991; Miot *et al.* 2009; Ramana *et al.* 2013) may be particularly significant. A total of 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

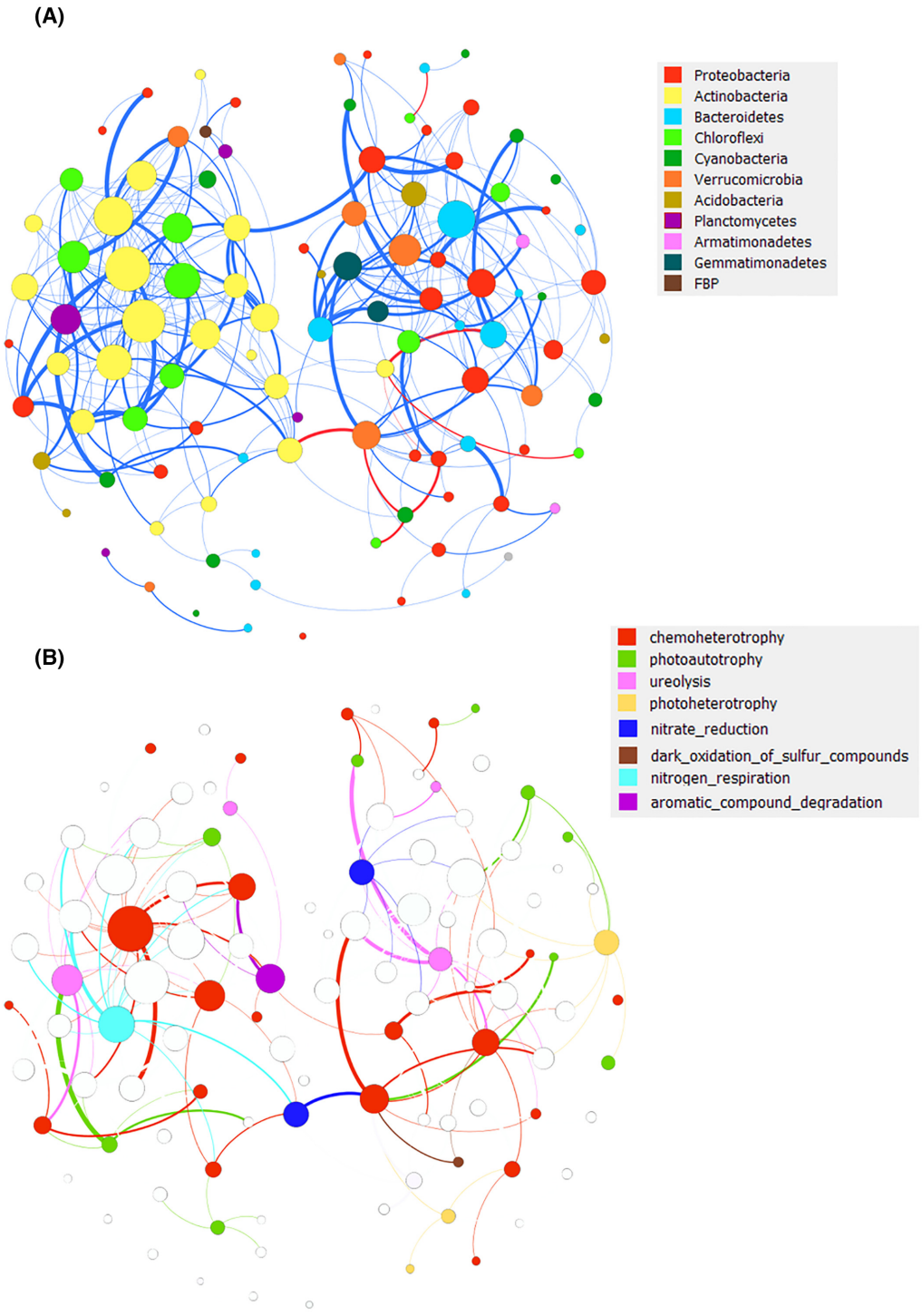

Figure 3. Co-occurrence network of the core hypolithic community, according to the taxonomy **(A)** and functional predictions **(B)** of the genera in the core community. Co-occurrence analysis was performed using Pearson. Spearman and Bray–Curtis correlations, with a p-value threshold of 0.05. The 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 colored as blue or red, respectively.

function of distance. No decay was observed in zeta-diversity across the sampled distance (Figure S5, Supporting Information), 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 by Stegen *et al.* (2013; Figure S6, Supporting Information). The calculated β-Nearest Taxon

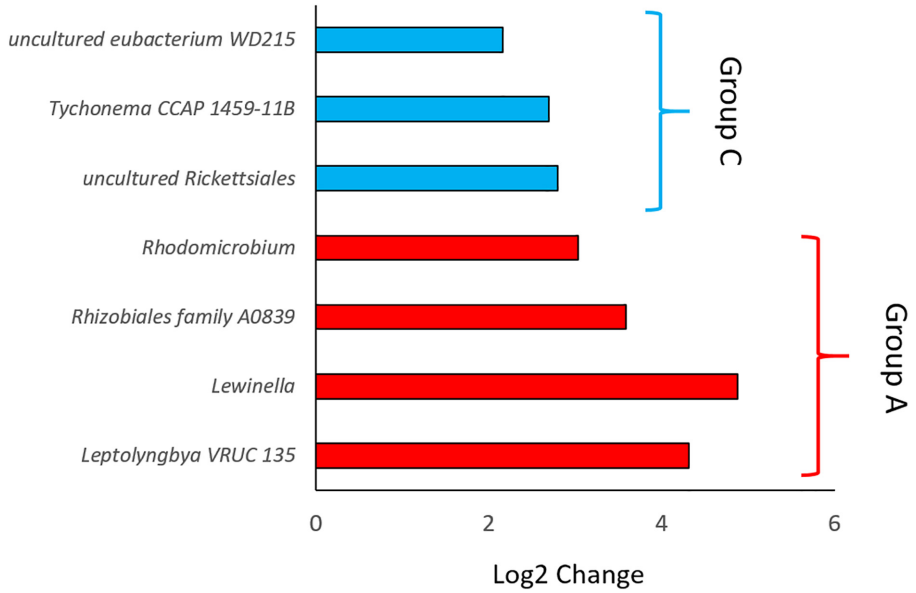

Figure 4. 'Specialist' genera in the different hypolithic groups. Log change values were calculated as pair-wise comparisons between groups, with all values represented in the plot being significant (adjusted P-value < 0.001).

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 ($-2 < \beta\text{-NTI}_{null} < 2$) 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 ($-2 < \beta$-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 (Fig. 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 $\beta\text{-NTI}_{core}$ average of -2.5. Conversely, 70% of the turnover in the non-core community is driven by stochastic processes, with a $\beta\text{-NTI}_{Noncore}$ 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.

To 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 (Table S3, Supporting Information) 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.

The results from this study have led us to propose a model for the evolutionary development of Antarctic hypolithic communities at the local scale (Fig. 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 (Zhou *et al.* 2002; Becker *et al.* 2006; Štursová *et al.* 2016), 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 harbor rich and distinct microbial

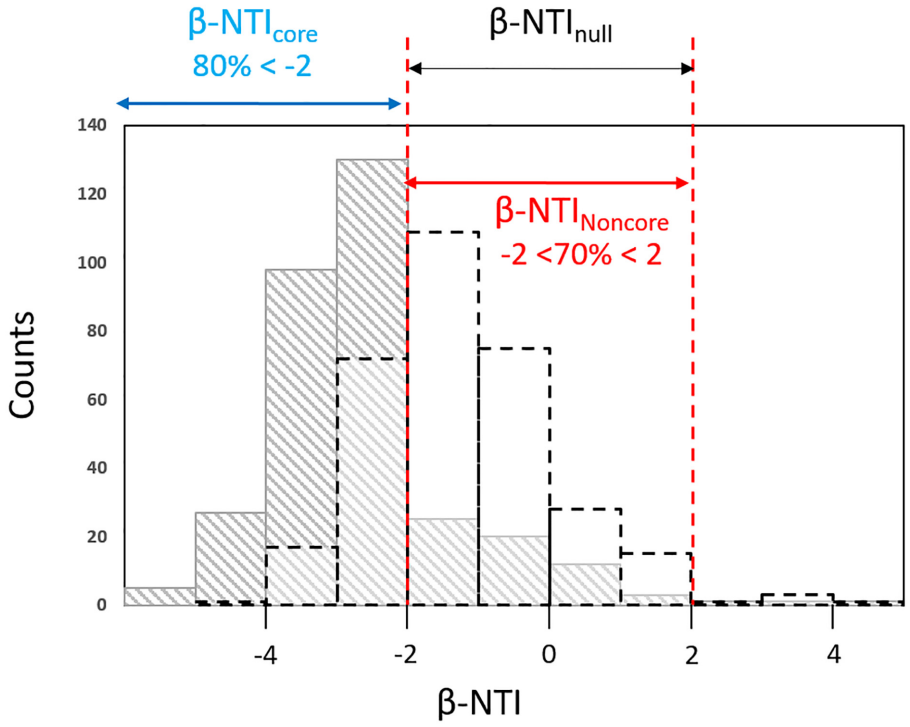

Figure 5. β-NTI distribution of the core (striped-grey bars) and non-core (white, black-traced bars) communities. The β-NTI range for stochastic processes ($-2 < \beta$-NTI < 2) is highlighted by the trace red lines, while the ranges in which most of the β-NTI values for core and non-core communities are distributed are highlighted by the blue and red arrows, respectively.

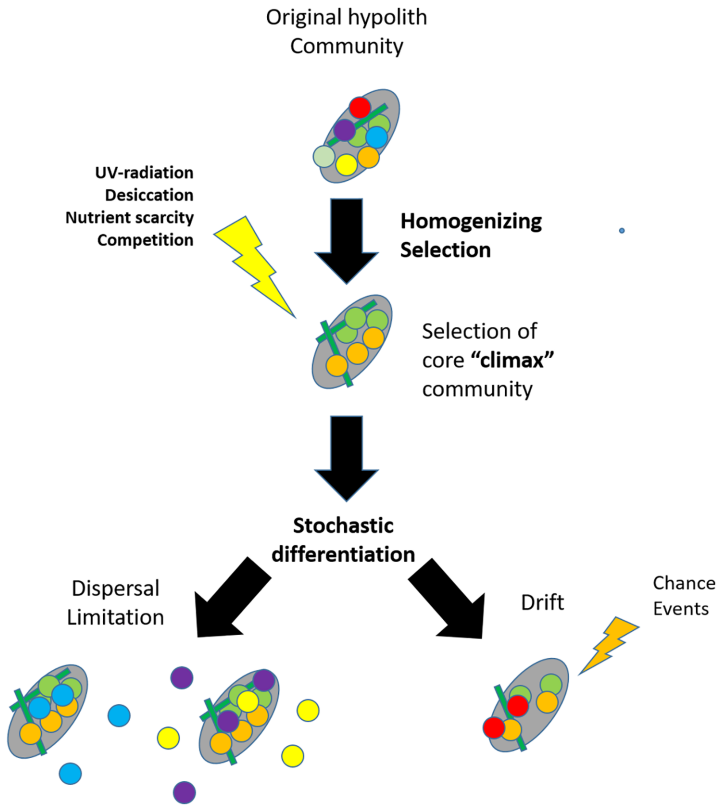

Figure 6. Model for the evolutionary development of Antarctic hypolith communities. Variation of communities at the local scale is driven by stochastic processes, while a core 'climax' community is maintained through the effects of selective abiotic and biotic pressures.

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 (Cowan *et al*. 2011; Warren-Rhodes *et al*. 2013), 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.

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SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

Conflicts of interest. None declared.

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.

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