

Rocks support a distinctive and consistent mycobiome across contrasting dry regions of Earth

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One sentence summary: Rock-dwelling fungi play critical ecological roles in drylands, including soil formation and nutrient cycling; we found that the main drivers structuring their distribution were spatial distance and, to a larger extent, climatic factors regulating moisture and temperature, suggesting that these paramount and unique microorganisms might be particularly sensitive to increases in temperature and desertification.

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Abstract

Rock-dwelling fungi play critical ecological roles in drylands, including soil formation and nutrient cycling; however, we know very little about the identity, function and environmental preferences of these important organisms, and the mere existence of a consistent rock mycobiome across diverse arid regions of the planet remains undetermined. To address this knowledge gap, we conducted a meta-analysis of rock fungi and spatially associated soil communities, surveyed across 28 unique sites spanning four major biogeographic regions (North America, Arctic, Maritime and Continental Antarctica) including contrasting climates, from cold and hot deserts to semiarid drylands. We show that rocks support a consistent and unique mycobiome that was different from that found in surrounding soils. Lichenized fungi from class Lecanoromycetes were consistently indicative of rocks across contrasting regions, together with ascomycetous representatives of black fungi in Arthoniomycetes, Dothideomycetes and Eurotiomycetes. In addition, compared with soil, rocks had a lower proportion of saprobes and plant symbiotic fungi. The main drivers structuring rock fungi distribution were spatial distance and, to a larger extent, climatic factors regulating moisture and temperature (i.e. mean annual temperature and mean annual precipitation), suggesting that these paramount and unique communities might be particularly sensitive to increases in temperature and desertification.

Keywords: drylands, extremophiles, environmental factors, climate change

Introduction

Drylands are the largest terrestrial biome covering ~46.2% (±0.8%) of the land surface, which support ~40% of the global population (Cherlet *et al.* 2018) and include 35% of the global diversity hotspots (Gudka *et al.* 2014). Drylands, areas characterized by aridity index (AI; mean annual precipitation/mean annual potential evapotranspiration) values below 0.65, span hyperarid, arid, semiarid and dry subhumid regions of the world characterized by extreme temperatures, low and variable rainfall, and low soil fertility. These ecosystems are particularly sensitive to changes in climate and are expected to expand up to 56% by the end of the century (Cherlet *et al.* 2018). The progressive water depletion and fast degradation in these dry regions may further constrain the possibility to sustain active life, reducing carbon uptake by organisms and leading to a dramatic loss of biodiversity (Delgado-Baquerizo *et al.* 2020, García-Vega and Newbold 2020). Therefore, it is becoming urgent to improve our limited knowledge on the biodiversity and function of these globally relevant ecosystems,

in order to develop management policy to mitigate the effects of ongoing climate change in these vulnerable ecosystems.

In these regions, where terrestrial life is profoundly challenged by harsh environmental conditions, such as water limitation, high levels of radiation and temperature fluctuations, soil salinity and nutrient deficiency (Alsharif *et al.* 2020), many important terrestrial processes are supported by rock-inhabiting microbial communities. Indeed, rocks provide microorganisms with physical stability, protection against high ultraviolet (UV) and solar radiation, and water retention (e.g. Staley *et al.* 1982, Ruibal *et al.* 2008, Gueidan *et al.* 2011, Coleine *et al.* 2021), and often represent the ultimate refuge for microbial life in the harshest regions of the world. In turn, microbes able to develop within the rock substrate contribute to key environmental/biogeochemical processes, including metabolization of C, N and other macronutrients, and regulation of inputs and outputs of gases, water cycling and biological weathering (Mergelov *et al.* 2018). Despite the importance of these communities in regulating nutrient cycling and

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pedogenesis in drylands, most dryland microbial ecology studies have so far focused on describing the diversity of microbes in soil (e.g. Pointing and Belnap 2012, Delgado-Baquerizo *et al.* 2013, Steven *et al.* 2013, Ochoa-Hueso *et al.* 2018, Maestre *et al.* 2021), while much less attention has been paid to rock-dwelling microorganisms.

Rock surfaces are typically barren and free of vegetation or continuous soil mantle, and are constantly subjected to changes in atmospheric conditions, including temperature, humidity and UV radiation (Coleine *et al.* 2021). Given their extremely changing nature, rocks are considered too constrained for the development of most of the cosmopolitan and copiotrophic members of the soil microbiome (Gorbushina 2007), and their colonization is often restricted to highly specialized microbes capable to resist, develop and adapt to the rock microhabitat (Coleine and Selbmann 2021).

Many of these rock specialists belong to the kingdom Fungi. Rock-dwelling fungi are ubiquitous in all drylands, including the hyperarid drylands ($AI < 0.05$), where they represent the ultimate life form surviving before extinction (Wierzos *et al.* 2015, 2018). Thanks to their exceptional adaptations and resistance (Coleine *et al.* 2022), these organisms can still persist up to the limits of water availability, playing pivotal roles in nutrient cycles and driving positive feedback loops for further colonization and soil formation (Mergelov *et al.* 2018). However, a comprehensive understanding of the diversity and functional composition of fungal communities in rocks from global drylands is still missing. Indeed, previous studies have mainly focused on describing the biodiversity of rock fungi from individual drylands or biomes (e.g. Santiago *et al.* 2018, Garrido-Benavent *et al.* 2020), or in particular fungal groups (Coleine *et al.* 2018), which gave only a limited perspective of the patterns and drivers of the rock mycobiome on a larger scale. For example, lichenized fungi, rock-inhabiting microcolonial fungi (RIF) and yeasts are considered rock specialists and are commonly retrieved from different rock substrates (e.g. Fanelli *et al.* 2021, Choe *et al.* 2021, Ertekin *et al.* 2021). Yet, whether a consistent membership of these rock-associated fungi exists across different biomes and continents is still undetermined (Coleine *et al.* 2021). Additionally, while previous studies suggest differential colonization and succession of fungal communities in rock and soil substrates (Garrido-Benavent *et al.* 2020, Choe *et al.* 2021), these differences might be less pronounced in less constrained habitats, where higher heterogeneity might be facilitated due to more permissive environmental conditions. Further, a plethora of environmental attributes, including both climatic features, spatial distance and solar radiation (Antony *et al.* 2012), have been associated with patterns of change in rock fungal communities (e.g. Choe *et al.* 2021), but their relative importance in determining rock fungal distributions is nowadays undetermined.

To fill this gap, we conducted a meta-analysis of rock fungal communities from different dryland regions of the globe. Since both rocks and soils are critical to sustain fungal life in drylands and are often located in proximity to each other, to highlight the unique microbiological nature of the lithic niche, surrounding soils were also included.

We reanalyzed multiple published datasets from four major different biogeographical regions and biomes from dry regions of the world (i.e. hot and cold, hyperarid, arid and semiarid), where both soil and rock fungal community data were available (both Continental and Maritime Antarctica, Mojave Desert and Arctic) to assess the peculiarity of the rock mycobiome in their biodiversity, functional traits and composition. Further, we identified and characterized the distribution of rock fungi and environmental variables that drive their abundances and habitat preferences in

relation to spatial, environmental and climate parameters. Given the distinct microenvironmental nature of rocks and soils, and the specific climatic and geographical differences of regions analyzed within the present study, we hypothesize that (i) compared with soil, rocks support a distinct community of specialized fungi; (ii) there are regional differences in the distribution of taxonomically and functionally different groups of rock-inhabiting fungi; and (iii) UV radiation and climate have a great influence on diversity and structure of the rock fungal communities occurring across different biomes.

Our work presents a unique perspective of the patterns and drivers of fungal distributions in rocks at large scale that provides critical insights toward prediction of ecosystem-level changes in these fundamental terrestrial biomes.

Materials and methods

Literature selection

To investigate and compare rock-inhabiting and soil fungal communities collected in the same area, we created a dataset by collecting literature on soil and rock-inhabiting fungal communities in drylands across the globe. We searched on Web of Knowledge (www.webofknowledge.com), Google Scholar (<https://scholar.google.it/>) and PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), including studies between 2017 and 2021 (last update in February 2021).

The following keywords were selected using a variety of expressions for describing fungal biodiversity investigated by high-throughput sequencing in soils and rock communities of worldwide drylands: (global* OR worldwide* OR Arctic* OR Antarctica* OR Polar regions* OR hot*) AND (drylands* OR desert* OR arid/semi-arid/hyper-arid/dry-humid region/area*) (soil* OR rock* OR endolith* OR lithic communities* OR rock-inhabiting*) AND (fung* OR ITS* OR fungal communities*) AND (high-throughput sequencing* OR amplicon sequencing* or metabarcoding*). In total, we examined 116 papers. The following selection criteria were used for the inclusion of samples into the dataset: (i) samples came from soils and rocks; (ii) the precise geographic location of each sample was recorded (GPS coordinates); (iii) the whole fungal community was subject to amplicon sequencing (studies using group-specific primers were excluded); (iv) the internal transcribed spacer regions (ITS1, ITS2 or both) were amplified and sequenced on Illumina platforms; and (v) sequencing data (either in fasta or fastq format) were publicly available or provided by the authors of the study upon request, and the sequences were unambiguously assigned to samples.

In total, 12 publications and 1 unpublished study contained samples that matched our criteria (see Table S1, Sheet 1, Supporting Information).

Sample description and environmental metadata

In total, 13 studies, encompassing four global biogeographic regions (DV, Dry Valleys in Continental Antarctica; MA, Maritime Antarctica; MD, Mojave Desert; and SV, Svalbard in Arctic), were identified and included in the analysis (Table S1, Sheet 1, Supporting Information and Fig. S1, Supporting Information) for a total of 361 samples (197 rock and 164 soil samples, respectively). We gathered all samples to individual sites with unique geographic coordinates, resulting in a final dataset of 28 sites with unique geographic coordinates. This allowed us to encompass three continents, and most of dryland subtypes (semiarid, $AI = 0.20-0.50$; arid, $AI = 0.05-0.20$; and hyperarid, $AI < 0.05$), spanning a wide

range of environmental conditions, including arid hot drylands (MD, California; AI = 0.038–0.089) hyperarid cold drylands (DV, Antarctica; AI = 0.01), arid cold drylands (MA, Antarctica; AI = 0.15) and semiarid cold drylands (SV, Arctic; AI = 0.17).

Our meta-analysis included eight environmental variables, which include UV radiation, mean annual temperature (MAT), mean annual precipitation (MAP) and precipitation seasonality (PSEA), collected from the WorldClim database (<https://www.worldclim.org>; ~1 km resolution) (Hijmans et al. 2005). The AI was obtained from the global maps of Zomer et al. (2008), which provides the averaged AI of the period 1970–2000, and has a spatial resolution of 30 arcsec.

Sample metadata were collected from the published papers and/or public repositories; the complete list is presented in Table S1, Sheet 1 (Supporting Information).

Bioinformatic processing of sequencing data

A graphical summary of bioinformatic processing steps is reported in Fig. S2 (Supporting Information). Most of studies were available on National Center for Biotechnology Information (NCBI); the Sequence Read Archive (SRA) files sequences were converted to FASTQ file using `fasterq-dump` v2.9.1 (<https://github.com/ncbi/sra-tools/wiki/HowTo:-fasterq-dump>), a tool of NCBI SRA Toolkit (<https://github.com/ncbi/sra-tools>). FASTQ files were directly downloaded from the NCBI SRA repository.

Raw reads were trimmed and filtered based on their quality using `BBDuk` v.38.79 (<http://sourceforge.net/projects/bbmap/>, parameters `maq = 10 qtrim = r trimq = 6 mlf = 0.5 minlen = 50`). Overlapping paired-end reads were merged with the `VSEARCH` v.2.14.2 `mergepairs` command (Rognes et al. 2016) (parameters `-fastq_minovlen 16 -fastq_maxdiffct 10 -fastq_allowmergestagger`). ITS1 and ITS2 sequences were detected and extracted using `ITSx` (Bengtsson-Palme et al. 2013) v.1.1.2 (parameters `-t 'fungi' -graphical F`), resulting in a total of 12 485 769 sequences. Dereplication and taxonomic classification was performed by `MICCA` (Albanese et al. 2015) v.1.7.2. Briefly, for each sample, ITS sequences were dereplicated using the subcommand `otu` (parameters `-m denovo_greedy -d 1.0 -s 1`). Finally, the resulting sequences were taxonomically classified using the Ribosomal Database Project (RDP) Classifier v.2.11 (Wang et al. 2007, Albanese et al. 2015) (`'micca classify'` command, parameters `-m rdp -rdp-gene fungalits_unite -rdp-minconf 0.8`). Samples were randomly subsampled to 1000 sequences using the command `'micca table rare'`, to minimize effects of differential sampling intensity across studies. Samples with <1000 sequences were not considered. We also used the number of reads as a proxy to estimate the relative abundance of each taxa sequence in a given sample (Giner et al. 2016, Weiss et al. 2017).

Downstream analysis, unless otherwise specified, were performed in R environment v.4 and using the Genus taxonomy table (Table S1, Sheet 3, Supporting Information).

Analysis of community composition, biodiversity and dominant taxa

To explore the main compositional gradients and relate them to sample type and sites, we ordinated the gathered samples through nonmetric multidimensional scaling (NMDS). In this analysis, we were interested in general patterns of compositional variation among sites, therefore, we analyzed the whole dataset of 700 genera occurring on the 358 aggregated samples. Bray–Curtis dissimilarity index was calculated on Hellinger-transformed SH abundances. Significance testing between soil and rock communities for beta diversity was assessed using permutational multivari-

ate analysis of variance (PERMANOVA) using the R 'vegan' (Dixon 2003) v.2.5-6. To test for differences in multivariate dispersion (i.e. within-region variability) of community composition between rock and soil communities, we used `PermDISP` (betadisper function in 'vegan') function, followed by `permutest` to determine statistical significance (Anderson et al. 2006). `PermDISP` was performed separately for each bioregion.

For each sample, we calculated three alpha biodiversity indices, i.e. Shannon index, Simpson index and Chao1 index. Pairwise comparison of community biodiversity between sample types within each site was assessed by Wilcoxon test with Benjamini–Hochberg FDR multiple test correction. Alpha diversities were calculated using 'phyloseq' v.1.32.0 (McMurdie and Holmes 2013).

To pinpoint those fungal genera that were enriched within each sample type, an indicator species analysis combining both the abundance and occurrence of a given genus across all sites was used. Indicator values (`IndVal`) were calculated by comparing rock and soil communities within each site, using the `multipatt` function in the 'indicspecies' v.1.7.9. The significance of each genus–niche association was tested using a permutation test ($n = 9999$) and nonsignificant associations ($P > 0.05$) were discarded.

Assignment and analysis of fungal ecological lifestyles

Lifestyles for fungal communities were determined by classifying ITS sequences into ecological guilds, using `FungalTraits` (Pöhlme et al. 2020) (<https://github.com/traitecoevo/fungaltraits>; accessed February 2021). Furthermore, where necessary, the functional assignments were corrected on assessments given in primary research literature and manually checked afterward based on ecological metadata of the corresponding sequences in UNITE v.8.3 for genera that are known to comprise species with diverse functions. Where appropriate, alternative guild assignments were also chosen based on our own experience. We created the functional group Rock-Inhabiting black Fungi ('RIF'), (also known as 'black yeasts', 'black meristematic fungi', and 'microcolonial fungi') that encompass an ecologically defined group of stress-tolerant specialists that share morphological similarity despite diverse phylogenetic placement in the Classes Eurotiomycetes (order Chaetothiales), Arthoniomycetes, and Dothideomycetes (order Capnodiales) (Coleine and Selbmann 2021). Yet, lichens and lichenicolous fungi were grouped in the 'lichenized' group.

In total, we assigned ecological functions into 15 guilds, as follows: 'lichenized', 'soil saprotroph', 'litter saprotroph', 'wood saprotroph', 'unspecified saprotroph', 'nectar/tap saprotroph', 'dung saprotroph', 'plant pathogen', 'RIF', 'ectomycorrhizal', 'root endophyte', 'foliar endophyte', 'mycoparasite', 'animal parasite' and 'sooty mold' (Table S1, Sheet 7, Supporting Information).

To investigate how different niches shaped fungal lifestyles, we implemented a Random Forest (RF) approach using the package 'rfPermute' v.2.1.81. A significant P -value ($P < 0.05$) and high increase in the mean square error ($MSE > 15$) were used to determine the important fungal guilds ($ntree = 500, 999$ permutations). We next assessed whether the relative abundance of these fungal lifestyles significantly changed in the lithic versus soil niche using Wilcoxon test with Benjamini–Hochberg FDR multiple test correction.

To further identify the fungal lifestyles associated with each sample type, the first two ordination axes of a canonical (constrained) correspondence analysis (CCA) were fit to fungal lifestyles identified in RF modeling using the `envfit` function in 'vegan'.

The rock mycobiome

We then aimed to identify those fungal taxa consistently associated with the mycobiome of rocks *cfr.* soils. We conducted PERMANOVA analyses aiming to test for significant differences in the proportion of fungal genus in rocks vs soils, while statistically ($P < 0.01$) accounting for the region of study. We used the function `adonis` in the R package 'vegan' and the term `strata` (region of study) to conduct these analyses. We then kept those fungal genera that were relatively more abundant in rocks *cfr.* soils. The potential effect of technical differences between studies on the abundance and occurrence of the rock subcommunity was further evaluated using a random forest approach (see Note S2 and Fig. S11, Supporting Information).

Environmental modeling

We used correlation (Pearson) analyses and PERMANOVA to identify the most important environmental factors associated with the distribution of the rock mycobiome. The proportion of the fungal microbiome of rocks was calculated as the standardized average (0–1) of the proportion of all fungal genera classified as such. This information was used in our environmental modeling based on a variation partitioning analysis. Predictors included niche type (soil vs rock), spatial parameters (latitude and longitude), elevation, UV index and climatic data (MAT, MAP, PSEA, diurnal temperature range, temperature seasonality).

Results

A unique mycobiome distinguishes rock-inhabiting fungal communities

Among the five fungal phyla identified, Ascomycota was the most abundant in rock communities of Dry Valleys, while a decrease in Basidiomycota was reported (Fig. 1A; see also Table S1, Sheet 3, Supporting Information). At a finer taxonomic level, among the 26 classes retrieved (Table S1, Sheet 3, Supporting Information; mainly in Ascomycota and Basidiomycota), lichenized fungi belonging to Lecanoromycetes represented the most abundant in rock communities across all contrasting regions ($P < 0.05$). In particular, the Dry Valleys presented a higher number of differentially abundant lichenized families compared with the other three regions; there, members of Lecideaceae, Lecanoraceae, Parmeliaceae and Acarosporaceae (in the order Lecidiales, Lecanorales and Acarosporales, respectively) predominated, while Verrucariaceae (order Verrucariales) were exclusive for the Arctic. Conversely, members of Trichomaceae, Filobasidiaceae, Mortierellaceae and Helotiaceae were linked to soil communities and almost absent in the Antarctic rocks (Fig. 1A; $P < 0.05$).

A number of genera were dominant in either rock or soil-associated communities; for instance, yeasts as the genera *Cryptococcus*, *Rhodotorula*, Lecanoromycetes, and black fungi in both Chaetothyriales and Capnodiales were particularly abundant in rock samples (Figs S3–S5, Supporting Information; Table S1, Sheet 4, Supporting Information). Conversely, only few genera were shared between rock and soil communities (Fig. S6, Supporting Information). The relative abundance of the top ten most abundant genera demonstrated that almost all are unique to one region (Fig. 1A); for instance, rocks communities of McMurdo Dry Valleys were enriched in three lichenized genera (*Carbonea*, *Lecidea* and *Acarospora*), while *Lecidella* genus was associated with Svalbard rock-inhabiting communities ($P < 0.05$).

Bray–Curtis dissimilarities also confirmed a significant differentiation in the overall mycobiome structure between rocks and

soil across locations as shown in Fig. 2B and Fig. S7 (Supporting Information) (PERMANOVA, $P < 0.05$; a full list of PERMANOVA results by sites are included in Table S1, Sheets 5 and 6, Supporting Information and Note S1, Supporting Information). Further analysis of dispersion among all pairwise sample type comparisons (pairwise `betadisper`) showed that there was no significant difference in dispersion within each bioregion between soil and fungal communities, except for Svalbard and Maritime Antarctica (Fig. S8, Supporting Information). These findings suggested an higher degree of heterogeneity for the fungal communities inhabiting the soil niche in these two regions.

Indices of biodiversity, including Chao1 richness and Shannon indices (Fig. 2C), were generally similar between soil and rock fungal communities across all regions except for the samples collected in Antarctica. Indeed, in Antarctic McMurdo Dry Valleys the edaphic fungal communities supported a significantly higher (Wilcoxon test, $P > 0.05$) diversity than their lithic counterparts; on the contrary, in the Antarctic Maritime, rocks harbor higher fungal diversity. Similar trends were observed for the Simpson index (Fig. S9, Supporting Information).

Distinctive fungal ecological lifestyles sustain rock communities in global drylands

Machine learning algorithms were used to test whether the rock or soil communities can be predicted from the composition of their fungal functional traits. The RF classification algorithm revealed that sample type could be predicted on the basis of fungal composition for rock and soil communities; we identified eight lifestyles (lichenized, ectomycorrhizal fungi, plant pathogens, RIF, soil, litter, wood and unspecified saprotrophs; Fig. S10, Supporting Information) that highly (mean decrease accuracy > 15) and significantly ($P < 0.05$; 999 permutations) contributed to explain the differences in composition between rocks and soil. Changes in relative abundance of those fungal lifestyles showed a similar trend in both cold (McMurdo Dry Valleys, Svalbard) and hot (Mojave Desert) dry regions. At these locations, we observed consistent increases in the relative abundance of lichenized fungi in the rock substrate, while increases in relative abundance of soil, litter and unspecified saprotrophs were observed in soil communities ($P < 0.05$; Fig. 2A). Similarly, we found a higher abundance of lichenized fungi in the lithic environment of the Mojave hot desert, while at this location ectomycorrhizal fungi and plant pathogens had higher abundance in soil ($P < 0.05$; Fig. 2A). In Maritime Antarctica, we found significant differences in abundance for soil plant pathogens only ($P < 0.05$; Fig. 2A). Rock-inhabiting fungi (RIF) had higher abundance in the rocks of the Antarctic McMurdo Dry Valleys exclusively ($P < 0.05$; Fig. 2A). The overall diverse distribution of fungal lifestyles to substrate type (rock vs soil) was further confirmed by a CCA plot (Fig. 2B), where lichenized fungi and RIF tended to occur predominantly in association with rock samples, while saprotrophs, pathogens and ectomycorrhizal fungi were largely associated with soil-derived samples.

Lichenized and black fungi as predictors of rock-inhabiting communities

An indicator species analysis identified a total of 57 genera uniquely and significantly ($P < 0.05$) associated with the rock substrate (Fig. 3, panel on the left; Table S1, Sheet 9, Supporting Information). Lichenized ascomycetes from the class Lecanoromycetes were the fungal group most consistently represented in each region, contributing to ~50% of the total indicator taxa (Fig. 3, right panel). However, at the genus level, members of this fungal group

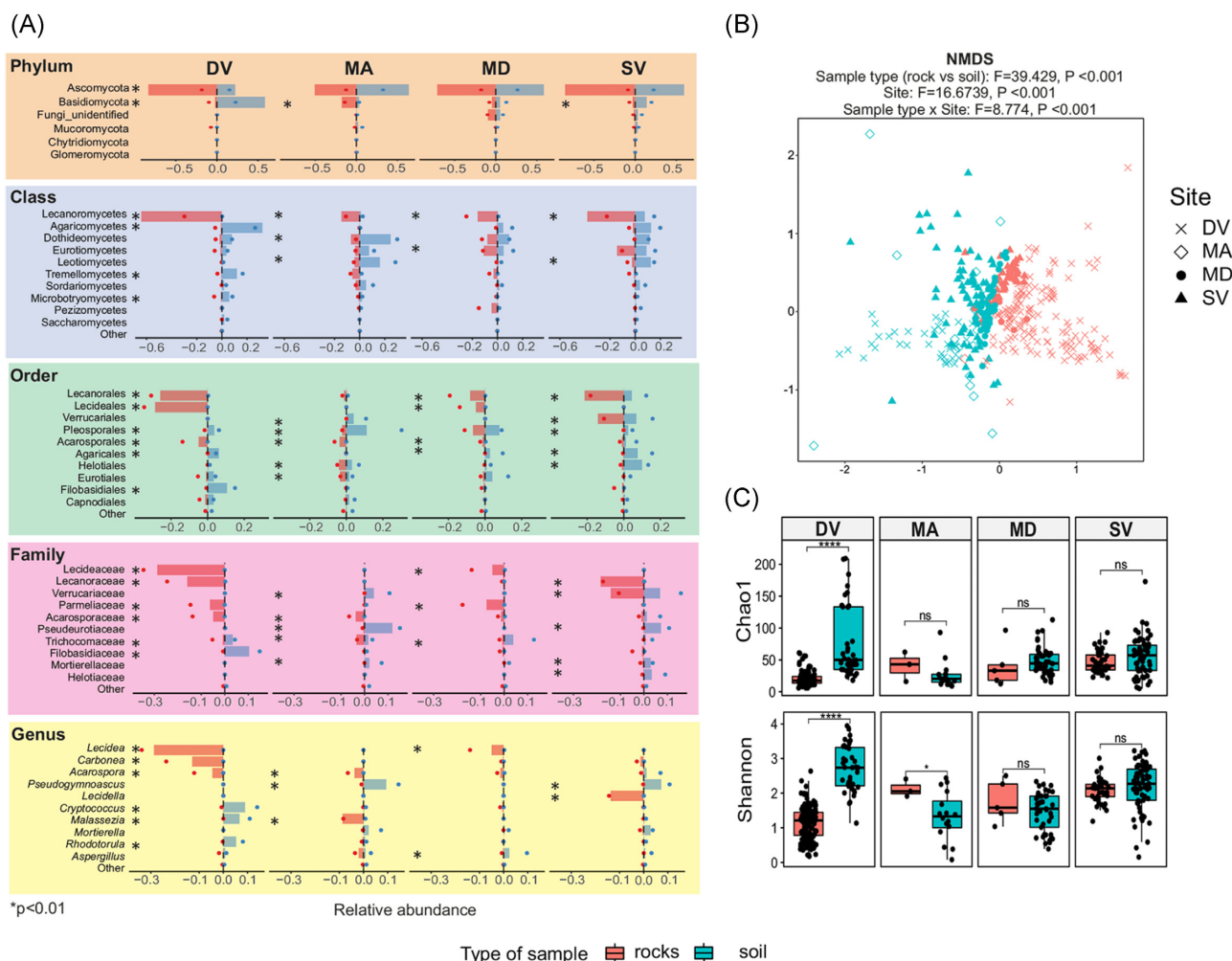


Figure 1. Taxonomic and community composition of rock vs soil samples, red and light blue in all panels, respectively. **(A)** Dominant phyla, classes, orders, families and genera (all taxa are reported in Table S1, Sheet 2, Supporting Information). The bars show the mean relative abundances expressed as percentages, while the dots show standard deviations. Significant differences ($P < 0.01$, Wilcoxon test) are reported using an asterisk. **(B)** NMDS plot of fungal communities (genus level) colored by type of sample (rock and soil) based on the Bray-Curtis index (see also Table S1, Sheets 5 and 6, Supporting Information and Figs S6 and S7, Supporting Information). Sites are represented by different shapes. Global PERMANOVA statistics are provided on top of the plot. **(C)** Box plots illustrating alpha diversity indices (Chao1 and Shannon index) of fungal communities (genus level) from rock and soil samples for each site. Individual data points, median values and interquartile ranges are shown. ns = $P > 0.05$; * $P < 0.05$; **** $P < 0.001$ (Wilcoxon test). Sites are abbreviated as follows: DV, Antarctic Dry Valleys; MA, Maritime Antarctica; MD, Mojave Desert; and SV, Svalbard.

exhibited clear differences in site preferences, with different genera being uniquely associated with sites, and only three genera (*Carbonea*: tax_gen_1418; *Lecanoromycetes* sp.: tax_gen_69; and *Paramiliaceae* sp.: tax_gen_1096) being shared among at least three drylands. The two most extreme regions, in terms of aridity, Mojave Desert (hot arid) and Antarctic McMurdo Dry Valleys (cold hyperarid) shared the highest number of lichenized indicators, for a total of six taxa, including members of the genera *Acarospora*, *Carbonea* and *Lecidea*, as well as the soil saprotroph *Saitoella*. Most of the other fungal lifestyles were uniquely represented across locations. Three genera of ectomycorrhizal fungi (*Wilcoxina*, *Rhizopogon* and *Tylospora*) and two saprotrophs (*Phlebiella* and *Oidiodendron*) appeared among the indicators in the Svalbard, although Maritime Antarctica had the highest representation of saprotrophs (litter saprotrophs *Diosgezia* and *Phaeosphaera*, soil saprotroph *Malassezia*, unspecified saprotroph *Penicillium*). The only indicator RIF was *Friedmanniomyces* in the Antarctic McMurdo Dry Valleys.

Temperature and precipitation as major environmental drivers

To explore the contribution of UV radiation, climatic variables and spatial factors in shaping the composition of rock-dwelling fungi, those taxa that were consistently more abundant in rock samples across all sites were first identified and then further characterized using a variation partitioning analysis (VPA) approach. This rock-associated subcommunity (*rock cluster*; Table S1, Sheets 10 and 11, Supporting Information) harbored one unidentified basidiomycete and 14 ascomycetes, which included 10 lichenized fungi (genera *Lecidea*, *Lecidella*, *Lecanora*, *Carbonea*, and other unidentified members of Lecanorales), in addition to one soil saprotroph (genus *Saitoella*) and one member of the RIF group (genus *Friedmanniomyces*). Twelve of these taxa also belonged to the rock community indicators (Fig. 3). VPA revealed that sample type (rock vs soil) and climate (MAT, MAP, PSEA, diurnal temperature range, temperature seasonality) alone contributed significantly to explain 30% of the abundant rock cluster variation, while spatial distance

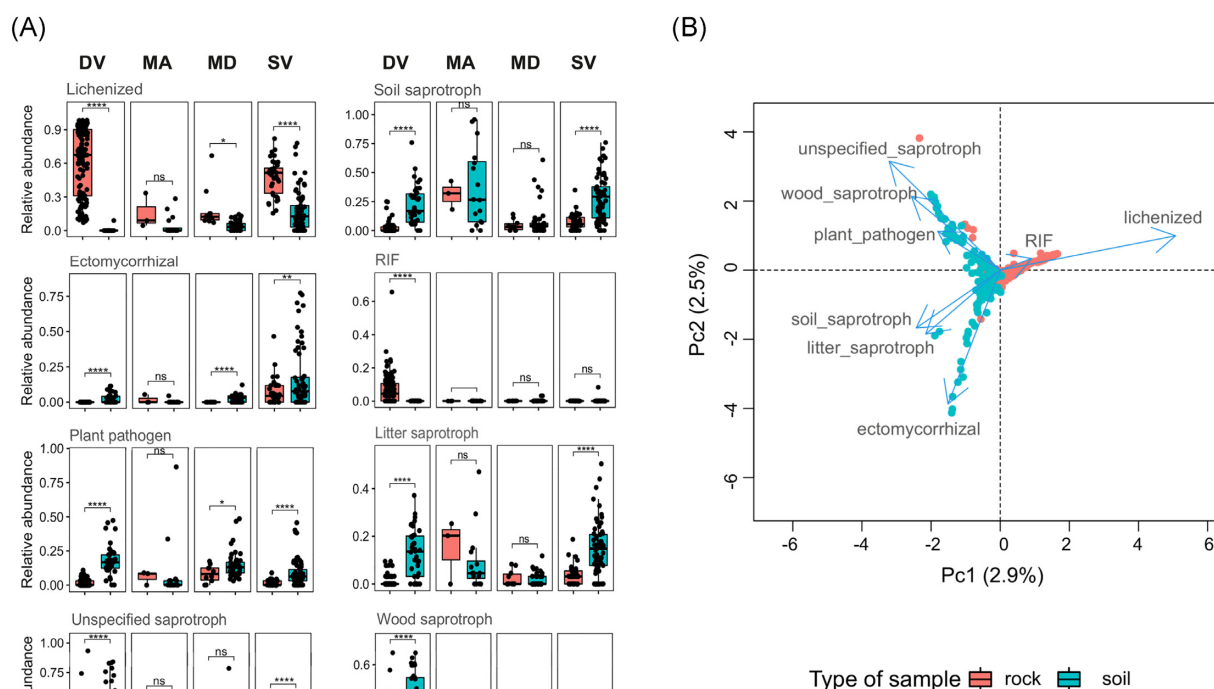


Figure 2. Most relevant fungal lifestyles in rock vs soil samples. **(A)** Box plots illustrate changes in relative abundance of fungal lifestyles associated with rock and soil samples for each site (see also Table S1, Sheet 8, Supporting Information and Fig. S9, Supporting Information). Individual data points, median values and interquartile ranges are shown. ns = $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; **** $P < 0.001$ (Wilcoxon test). **(B)** Plots of the first and second principal components (PC1 and PC2) from principal coordinate analysis (PCoA) of fungal (ITS) data within fungal lifestyles and across types of samples. The numbers in brackets refer to the variance explained by the PCoA axes. Sites are abbreviated as follows: DV, Antarctic Dry Valleys; MA, Maritime Antarctica; MD, Mojave Desert; and SV, Svalbard.

contributed to explain 10% of the cluster variation; only a small portion of the variability was explained by UV irradiation (<3%; Fig. 4A). Among the climatic variables, MAT and MAP had the highest correlation with the rock sub community abundance (Pearson product-moment correlation $r = -2.97$ and -1.91 , $P < 0.01$, respectively; Fig. 4B; Table S1, Sheet 12, Supporting Information). Similar patterns were observed when correlating fungal lifestyles with climatic variables (Table S1, Sheet 13, Supporting Information).

Discussion

Since their discovery in the harshest desert of Earth, rock-inhabiting communities have stimulated our imagination, proving that microorganisms can still adapt and reproduce under conditions typically incompatible with active life. Fungi in particular, either in symbiotic associations or as free-living organisms, are dominant members in these peculiar microbial consortia that are able to spread throughout the most extreme environments on Earth. Yet, the current knowledge on the compositions of rock fungal communities primarily comes from investigations limited to single rock samples or from a single location, and only recently a few larger scale surveys have been undertaken (Qu *et al.* 2019, Choe *et al.* 2021). Therefore, information on global distribution and ecology of these fascinating organisms is still patchy and incomplete. Yet, this knowledge would represent a major advancement for unraveling those processes that ensure balance and functionality even in the driest ecosystems. Fungi, indeed, play irreplaceable roles for ecosystem functioning (Chen *et al.* 2000, Heckman *et al.* 2001, Waring *et al.* 2013, Coleine *et al.* 2022) and global biogeo-

chemical processes, which include organic and inorganic transformations and element cycling, bioweathering and mycogenic mineral formation (Gorbushina 2007, Gadd 2017) even in the far extremes. As such, understanding patterns and drivers of these keystone organisms is important to predict how global drylands may respond to ongoing environmental changes.

In this work, we provide for the first time a synthesis of the biodiversity and environmental preferences of rock fungal abundance and distribution. Our meta-analysis included >350 rock and soil samples, encompassing 700 genera of fungi across four main biogeographic regions of the world, collectively representing the most comprehensive census of fungal entities from dryland lithic substrates available so far.

We found that the lithic substrates support a peculiar and consistent biodiversity of fungi that is largely not shared with their surrounding soils across contrasting Earth's regions, confirming that rocks and soils are reservoir of different fungal communities (Yung *et al.* 2014, Garrido-Benavent *et al.* 2020). These major differences in fungal composition and structure between rocks and soils were mirrored by overall similar levels of alpha diversity, measured as species dominance, evenness and richness. One exception was the Antarctic McMurdo Dry Valleys, hosting a higher diversity of fungi in soils than in rocks. This area represents the farthest and most extreme environment in our dataset, where rocks are exposed to very high UV radiation intensity, low temperatures and high aridity (Cary *et al.* 2010). As such, the lower alpha diversity levels of rock fungi at this location may be due to a combination of both extremely prohibitive conditions, and geographic and genetic isolation, leading to evolution of a restricted group

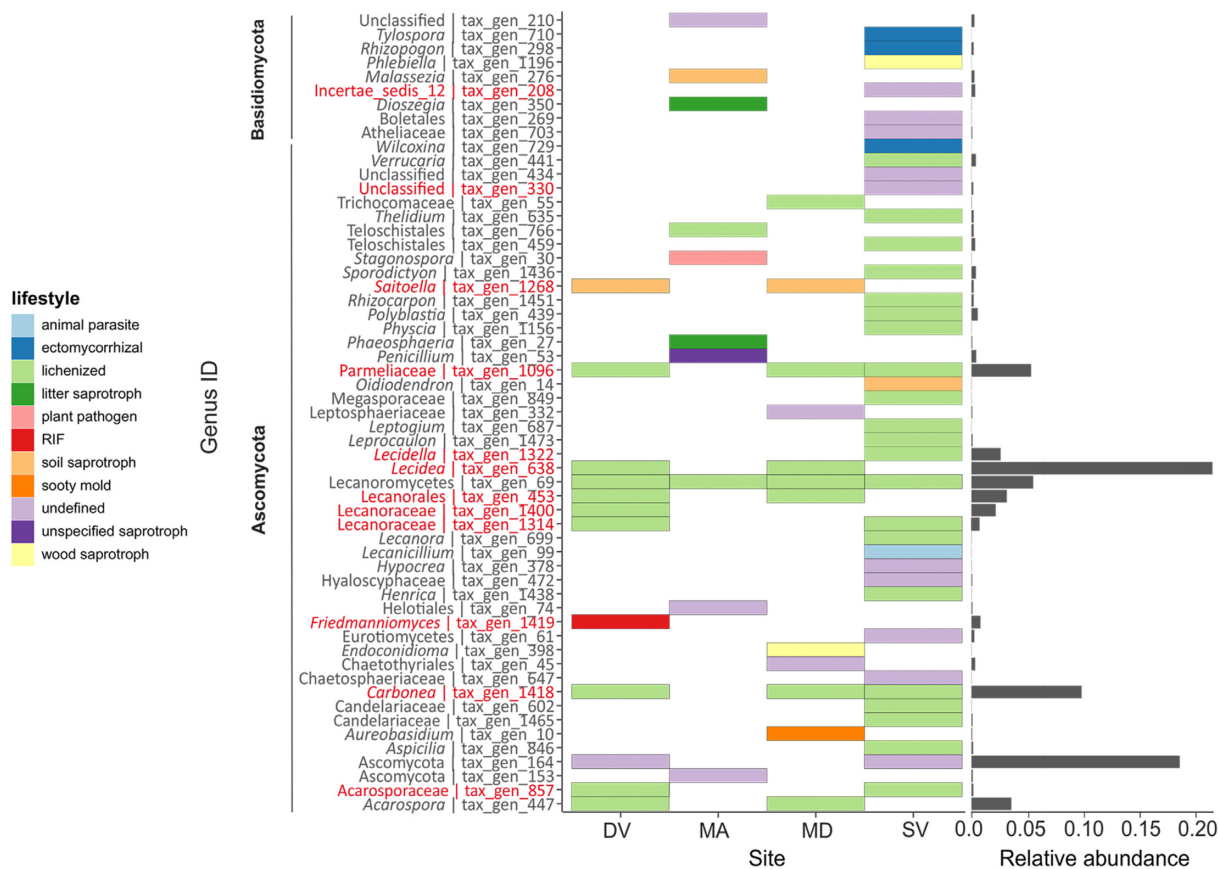


Figure 3. Dominant taxa in rock fungal communities across different sites. Indicator species analysis by site, showing differences in indicator genera and fungal lifestyles between different rock samples (Table S1, Sheet 9, Supporting Information). Each row represents a unique genus, and each column represents a site (DV, Antarctic McMurdo Dry Valleys; MA, Maritime Antarctica; MD, Mojave Desert; SV, Svalbard). Color scale represents fungal lifestyles. Left-hand labels indicate the phylum-level classification. Indicator taxa that were also identified as belonging to the rock cluster (see Fig. 4A) are highlighted in red.

of fungal taxa particularly specialized and adapted to the lithic niche (Selbmann et al. 2013, Egidi et al. 2014). Overall, our findings demonstrate, for the first time, that rocks might represent an underappreciated reservoir of fungal biodiversity, which is comparable to that found in dryland soils, especially in less prohibitive environments. This implies that rocks can harbor a unique biodiversity in global drylands and, therefore, lithic substrates should be explicitly included in global inventories of biodiversity to capture the full membership of these ecosystems.

Our study shows that rocks support distinctive and consistent fungal taxa across contrasting Earth’s regions. The differences in community structure between lithic and edaphic niches were evidenced at coarse taxonomic levels, whereby Ascomycota were significantly more abundant in the rock global dataset, and Lecanoromycetes (primarily from the orders Lecideales and Lecanorales) were the most abundant fungal class retrieved in rocks from all the surveyed biogeographic regions, from hot to cold deserts. Members of Lecanoromycetes are typically lichen-forming fungal taxa (Miadlikowska et al. 2014), and are especially well adapted to the lithic niche, owing to their temperature tolerance, low mineral nutrient demand, and ability to withstand drought conditions and high UV irradiation (Gadd 2007, Ding and Eldridge 2020). Therefore, it is not surprising that taxonomic groups encompassing lichen-associated fungi represent rock specialists and predominant members of rock-inhabiting communities worldwide (Coleine et al. 2021). Notably, lichens are a very stress resistant and self-supporting association of a mycobiont

and a photobiont. These associations can also host numerous other microbes, including fungi, to whom they provide nutrients and protection (Hawksworth and Grube 2020). As such, lichens may easily represent a hotspot for microbial diversification, particularly under extreme conditions (Selbmann et al. 2010). In rock communities, the high abundance of lichens may have played a key role in developing the peculiar mycobiome revealed in this study.

While lichenization appears as a conserved trait in rock mycobiome at a broad taxonomic level (i.e. class to order), at finer taxonomic scales (i.e. genus level), the overall distribution of fungi across the different biomes and continents surveyed in our study was highly variable. For example, few of the same Lecanoromycetes occurred across multiple regions, with only *Carbonea*, *Acarospora* and *Lecidea* spanning both hot and cold deserts. Conversely, other lichenized and nonlichenized fungal genera were exclusive to a particular region and environmental type, such as in the case of Svalbard, the most anthropized dryland analyzed in this study; there, the detection of lichenized fungi was often associated with polluted environments (e.g. *Aspicilia*) (Puy-Alquiza et al. 2017), and saprotrophic fungi such as *Oidiodendron* and *Phlebiella* spp. may be seen as evidence of anthropogenic activity. Collectively, these findings support the new concept of ‘rock ‘core’ community’ (Coleine et al. 2021), hypothesizing that, among locally adapted fungi, a few fungal taxa can occur in rocks of drylands globally, harboring unique traits that allow them to adapt to wide ecological and environmental gradients. More sampling cov-

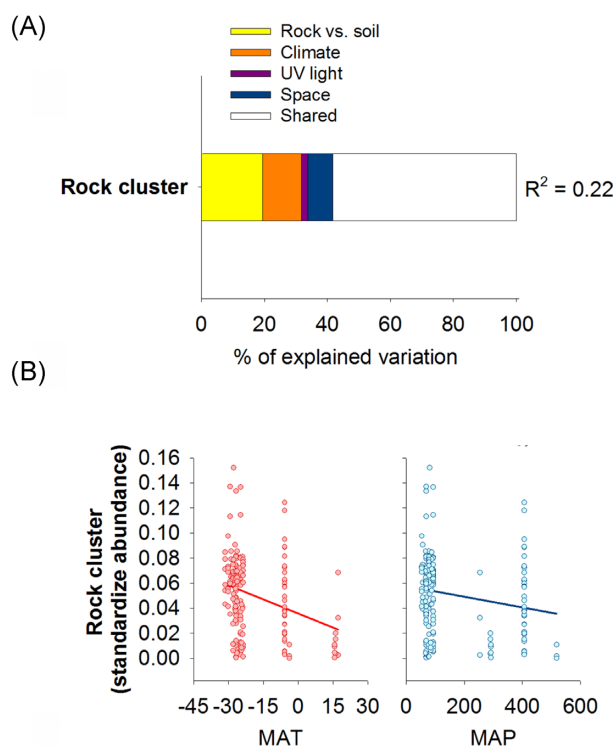


Figure 4. Environmental drivers of fungi in the rock cluster. **(A)** Relative contribution of environmental parameters in explaining changes in relative abundance of the rock cluster subcommunity. **(B)** Pearson correlation between rock subcommunity relative abundance and MAT and MAP.

ering broader climatic regions across various spatial scales will be critical to identify rock microorganisms able to colonize diverse environments and rocky substrates worldwide, to resolve to what extent global adaptation may occur in these communities, and which traits underpin global distribution of these rock specialists.

We also sought to identify the most dominant members of the rock fungal community and characterize their ecological preferences. Confirming the general patterns of widespread occurrence of lichenized fungi in rocks, several genera of lichenized fungi (e.g. *Carbonea*, *Lecidea*, *Lecidella*, *Parmeliaceae* spp., *Acarosporaceae* spp. and *Lecanorales* spp.) were also represented in the dominant rock cluster. In addition to lichenized fungi, we retrieved the endemic Antarctic fungus *Friedmanniomyces*, the fungal genus *Capnobotryella* and other ascomycetous representatives of black fungi in Arthoniomycetes, Dothideomycetes and Eurotiomycetes. This polyphyletic group of black fungi is ubiquitous in natural and anthropogenic habitats (e.g. Zakharova et al. 2013, Egidi et al. 2019, Selbmann et al. 2020 and references therein), making them among the most extreme-tolerant organisms on our planet. They have also been found as recurrent members of rock communities in drylands worldwide even if having lowest percentage compared with lichenized fungi (Choe et al. 2021, Coleine et al. 2021).

We then explored associations between environmental variables and the relative abundance of rock fungi across the globe. We found that MAT and MAP had the largest negative and significant direct association with the relative abundance of dominant fungi globally. Additional correlation analyses suggested that MAT and MAP are also related with the relative abundance of multiple genera classified as lichenized fungi, which were found to be ubiquitous in rocks across the globe (>75% of all locations), e.g. *Carbonea*. Previous studies have already highlighted that

decreases of water availability and, thus, increases in aridity negatively affect microbial diversity and abundance of microbial communities in soils of global drylands (Maestre et al. 2015, Chen et al. 2019). Moreover, major reorganization from drier to wetter conditions has also been reported in desert soil communities (Neilson et al. 2017) as well as in lithic bacterial consortia (Uritskiy et al. 2019). Our results indicate, for the first time, that changes in temperature and precipitation—as predicted under a warming climate—might have a holistic impact on the ecology of rock microorganisms, also altering the relative abundance of fungi in extreme environments such as those of drylands. Conversely, UV radiation has generally been assumed to be the most important factor for desert communities, particularly those of the Antarctic desert, where the extended duration of sunlight, the hole in the ozone layer and the light's reflection off the ice and water contributed to the high levels of UV radiation exposure (Frederick and Snell 1988). However, the most noticeable community response to higher UV in our global dataset was a slight decrease of the relative abundance of a few saprotrophs and lichenized fungi, indicating that this environmental factor is a secondary driver of global fungal community assembly. The distributional patterns associated with climate as identified in our work pave the way to untangle the future distribution of the paramount group of rock fungi on global drylands, especially in the light of climate change.

Taken together, our work provides new insights about the diversity and distribution of rock fungi in dryland ecosystems across contrasting regions of the planet, and identifies environmental preferences of dominant fungal taxa associated with rock communities across the globe. In particular, our finding that rocks support distinctive communities of fungi opens other critical yet under-explored scientific questions, related to mechanisms of adaptation and evolution, and relationships of these unique communities with surrounding habitats. Notably, we determined that climate-based environmental selection, such as changes in MAT and MAP, plays important roles in driving the distribution of rock fungi at a global scale. This implies that the impact of climate change might be disproportionate for these communities, with unknown consequences for the ecosystem-level functions they mediate. Given their exceptional functional properties, future more extensive surveys at the global scale will be critical to understand how rock fungi adapt to their niches, under conditions of dryness and oligotrophy. We also envisage that the consistent patterns in mycobiome diversity found in such different regions of the world will spark interest in explicitly considering the rock communities, still largely neglected, in global surveys of drylands. New insights into microbial succession for these fungal communities will be useful for protecting, managing and mitigating dryland resilience in an era of global warming and expanding desertification.

Supplementary data

Supplementary data are available at [FEMSEC](https://academic.oup.com/femsec/article/98/3/fac030/6550019) online.

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Authors' contributions

CC, MD-B and EE developed the original idea of the analyses presented in the manuscript. Literature selection and raw data retrieval were done by CC. Bioinformatic analyses were done by DA. Statistical analyses, environmental modeling and data interpretations were done by CC, MD-B, DA and EE. The manuscript was written by CC and EE with contributions from all the coauthors. All authors have read and agreed to the published version of the manuscript.

Data accessibility

All metadata, datasets and statistical analysis results are available at Figshare (<https://figshare.com/s/bcdf2633d9872a7eb8ca>). All other relevant data are available upon request.

Conflict of interest statement. None declared.

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