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Research Article

Positive fungal interactions are key drivers in Antarctic endolithic microcosms at the boundaries for life sustainability

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Abstract

In the ice-free areas of Victoria Land in continental Antarctica, where the conditions reach the limits for life sustainability, highly adapted and extreme-tolerant microbial communities exploit the last habitable niches inside porous rocks (i.e. cryptoendolithic communities). These guilds host the main standing biomass and principal, if not sole, contributors to environmental/biogeochemical cycles, driving ecosystem processes and functionality in these otherwise dead lands. Although knowledge advances on their composition, ecology, genomic and metabolic features, a large-scale perspective of occurring interactions and interconnections within and between endolithic fungal assemblages is still lacking to date. Unravelling the tight relational network among functional guilds in the Antarctic cryptoendolithic communities may represent a main task. Aiming to fill this knowledge gap, we performed a correlation-network analysis based on amplicon-sequencing data of 74 endolithic microbiomes collected throughout Victoria Land. Endolithic communities' compositional pattern was largely dominated by Lichenized fungi group (83.5%), mainly represented by *Lecanorales* and *Lecideales*, followed by Saprotrophs (14.2%) and RIF+BY (2.4%) guilds led by *Tremellales* and *Capnodiales* respectively. Our findings highlighted that fungal functional guilds' relational spectrum was dominated by cooperative interactions led by lichenised and black fungi, deeply engaged in community trophic sustain and protection, respectively. On the other hand, a few negative correlations found may help in preserving niche boundaries between microbes living in such strict spatial association.

Keywords: antarctica, cryptoendolithic communities, fungal interactions, global warming, ITS metabarcoding, network analysis

Introduction

The ice-free areas of Victoria Land in continental Antarctica include the mountain peaks and Nunataks along the Transantarctic mountain range and the widest ice-free portion of the continent, the McMurdo Dry Valleys. The permanently harsh environmental conditions (i.e. dryness, low and strongly fluctuating temperatures, high solar UV irradiation, and virtually total absence of nutrients) make these settings the closest Martian counterpart on Earth (Friedmann 1986, McKay et al. 2017, Lee et al. 2022). There, naked rocks offer the main opportunity for both prokaryotic and eukaryotic microbial life settlement, giving rise to edging biomes for terrestrial life in these otherwise sterile lands. The environmental conditions are, anyway, incompatible with active life on rock surface, therefore, highly adapted and extreme-tolerant microbial communities exploit the last habitable niches creeping inside the air-spaces, fissures and cracks within rock (Walker et al. 2005, Meslier et al. 2019). Indeed, the endolithic niche supplies more buffered environmental conditions to these microbial assemblages which establish self-sustaining complex aggregations of phototrophic and heterotrophic microorganisms, including bacteria, algae, and both free-living and lichen-forming fungi (Friedmann 1982, Coleine et al. 2018a). Among different types of endolithic colonisation identified to date, the lichen-dominated cryptoendolithic communities are the most complex and widespread, prevailing across sandstone outcrops featuring these places (Nienow and Friedmann 1993). The distinctive biostratification, visible in colonised rocky substrates, reflects a specific functional organisation according to fungal biological requirements. The microbial community's functionality was energetically supported by a dominant group of lichenized fungi in association with photobiont algal components (as primary producers), while black fungi (due to thick, highly melanized cell walls) protected the entire community from extreme external conditions and, in combination with other saprotrophic fungi, ensured degradation of organic matter and nutrients cycling (Coleine et al 2022). Despite occupying a niche of a few millimetres below the rock surface, these microbial communities represent the main biomass load in these areas and the main actors in key geochemical/bioenergetic processes and in maintaining the whole ice-free area ecosystem biologically active (Wierzchos et al. 2015, 2018, Coleine et al. 2020a).

Over the past decade, multidisciplinary studies on these endolithic microbiomes shed light on their structure, spatial organisation, influence of environmental factors (i.e. altitude and sun exposure) and genomic and metabolic features driving adaptation and resistance strategies to stress (Selbmann et al. 2017, 2021, Coleine et al. 2020a,b,c, Fanelli et al. 2021).

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Although these knowledge advances, a large-scale perspective of occurring interactions and interconnections within and between fungal assemblages is still lacking to date. Also, functional community structure and local taxa segregation/mixing within rock would be defined by microbial interactions (Cordero and Datta 2016). Therefore, unravelling the tight network of functional guild interactions (cooperative or competitive) in the Antarctic cryptoendolithic communities may significantly boost our knowledge on the dynamics driving structure and functionality of these rock-encased microcosms. In a spatial confinement context like the endolithic lifestyle, assessing how the combined effects of ecological interactions can influence community diversity and stability would present a crucial task. In this perspective, an extensive correlation network analysis, based on high throughput molecular data, was performed for assessing, for the first time, the structure of interactions occurring within and between the fungal guilds in the Antarctic cryptoendolithic communities, and their role in modulating the functioning of these dominant and crucial assemblages of Antarctic ecosystems.

Materials and methods

Study area

All 74 sandstone samples were collected throughout Victoria Land (continental Antarctica) (Fig. 1), along a latitudinal transect ranging from 71°47'36''S (Helliwell Hills, Northern Victoria Land) to 77°54'37''S (Knobhead, Southern Victoria Land). Samples have been collected in the frame of Italian National Antarctic Research Program (PNRA) projects, during Italian Antarctic Expeditions XXVI (2010–2011), XXXII (2015–2016) and the XI (2015–2016) Ganovex Expedition (Dutch Antarctic Expedition of the German Antarctic North Victoria Land Expedition project). Sampling permits have been obtained for activity in Special Managed Areas (ASMA) and Special Protected Areas (ASPA) in compliance with the 'Protocol on Environmental Protection to the Antarctic Treaty' Annex V, art.7. The presence of endolithic colonisation was assessed by direct in situ observation using magnification lenses. Sampling activities were performed under rigorous maintenance of sterile conditions. Rocks were excised using a sterile chisel, placed in cooled sterile bags and shipped at University of Tuscia (Italy) under sterile conditions, where they have been preserved at -20 °C in the Mycological Section of the Italian Antarctic National Museum (MNA), until processing. Further information on investigated localities are provided in Supplementary Table S1.

Metagenomics DNA extraction and sequencing

All sandstone samples were powdered in sterile conditions; metagenomic DNA was extracted from 0.5 g of rocks using Qiagen DNeasy PowerSoil Kit (Carlsbad, CA, USA). The Internal Transcribed Sequence 1 ribosomal region (ITS1) was targeted to assess fungal community composition. The ITS1 region was amplified using barcoded primers ITS1F/ITS2, suitable for shorter read length (Smith and Peay 2014). PCR was performed with 1 μL of each primer, 12.5 μL of Taq DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA), 9.5 μL of nuclease-free water (Sigma–Aldrich, St. Louis, MO, USA) and 5 ng of DNA template by an automated thermal cycler (BioRad, Hercules, CA, USA), for a total volume of 25 μL. The ITS1 locus was amplified following initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 52°C for 30 s, extension at 68°C for 90 s, followed by a final extension at 68°C for 7 min. Amplicons were quantified and pooled by a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA), and with Qiagen PCR CleanUp kit (Macherey-Nagel, Hoerdt, France). Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform. All protocols and technical specifications follow-up what previously performed in Coleine et al. (2021b).

Sequences were deposited on NCBI Sequence Read Archive database under BioProject Accession Numbers PRJNA453198; PRJNA379160; PRJNA675815.

Bioinformatic processing

Demultiplexed ITS sequence data set was processed using AMPtk (Palmer et al. 2018) v.1.5.4 software. Briefly, reads were merged using VSEARCH 2.21.1 (Rognes et al. 2016) and barcodes/indexes and primer sequences were removed from raw data; reads were then subjected to quality trimming to a maximum of 250 bp and discarding reads less than 100 bp in length, and sequencing artefacts were dropped by using USEARCH v.9.1.13 with default parameters (Edgar et al. 2010). Sequence quality filtering was performed with the expected error parameter of 0.9 (Edgar et al. 2015) and the cleaned reads were clustered at 99% similarity using DADA2 denoising algorithm (Callahan et al. 2016) that uses a statistical error model to correct sequencing errors to infer the Amplicon Sequence Variants (ASVs). Global singletons and rare taxa (<5 reads) were skipped as likely false positives due to sequencing errors, following Lindahl et al. (2013). Finally, taxonomic identification was performed with a sequence identity of 97% as threshold, using hybrid database Global Alignment and SINTAX algorithm with an ≥80% probability threshold (Edgar et al. 2010) on reference databases UNITE 8.2.

The ecological guild of fungal ASVs was assessed using the FungalTraits database (Flores-Moreno et al. 2019) for a first general classification. Thereafter, a more accurate functional assignment for Antarctic endolithic lifestyle was primarily provided by research in literature and, where appropriate, alternative guild assignments were chosen depending on our own experience. The functional organisation of endolithic fungal community addressed in this work, according to Coleine et al (2018b), was organised from 3 key ecological roles: (i) primary producers, labelled as Lichenised fungi group, taking into account the mutualistic relationship with algal component forming the (lichens) photoautotrophic assemblages; (ii) consumers/saprotrophs bound to Saprotrophs guild and (iii) protection of microbial community, related to Rock-Inhabiting Fungi and Black yeasts (RIF+BY) guild. Total reads distribution and relative abundance for each functional guild was processed and plotted by the R package 'Phyloseq' (McMurdie et al. 2013).

Correlation network analysis

The relationships driving fungal functional groups composition were investigated using the software MICtools (Albanese et al. 2018), which implements a multistep analysis to identify relevant associations among a large number of variables, assess their statistical significance, and rank them according to the strength of the relationship. Briefly, the procedure consists into 4 steps, starting from a matrix of variables pairs (Xi-Yi) measured in n samples: (i) estimating the empirical TICe null distribution by permutations; (ii) computing TICe statistics and the empirical p-value for each variable pair; (iii) applying a multiple testing correction strategy for family-wise-error rate (FWER) or the FDR; (iv) using MICe to estimate the strength of the significant relationships. In this

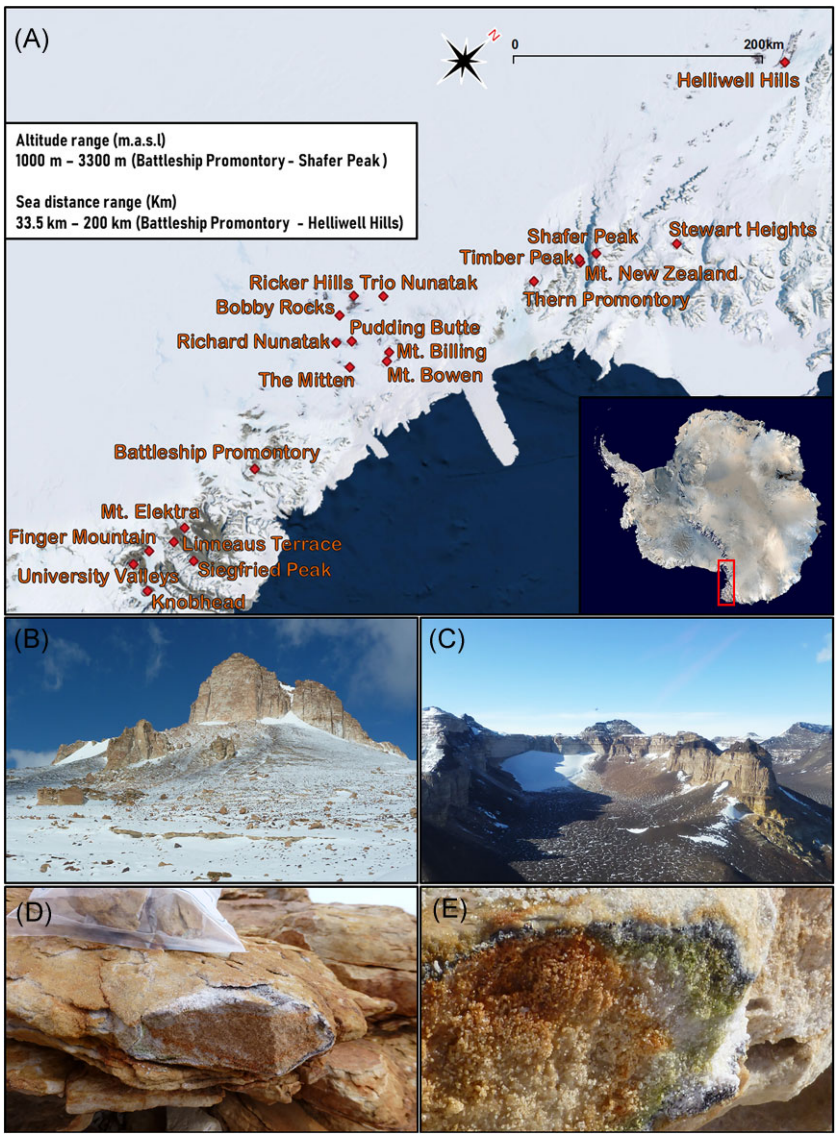

Figure 1. Map of Victoria Land (Continental Antarctica) with locations of sites surveyed **(A)**; Examples of landscapes in sampling locations: Linnaeus Terrace **(B)**; University Valley **(C)**; colonised sandstone bedrock **(D)** and magnification of colonised rock with its typical cryptoendolithic microbial community stratification **(E)**.

study, ASVs matched against themselves to investigate the compositional relationships occurring within and between functional guilds. We then implemented Spearman's rank correlation coefficient (ρ) to set up a subsequent network analysis to highlight and describe the degree of any possible relationship detected. In order to obtain a consistent and representative network of relationships, only interactions with absolute value of Spearman's rank correlation coefficient ≥ 0.3 were addressed. Subsequently, relationships were classified by sign (positive and negative) and strength as follows: strong positive and negative, in a range of 0.5–1.0 (±S) and weak among values of 0.3–0.49 (±W). All filtered correlations within and between functional guilds were shown graphically using a Network-Graph (Supp X). A summary Network-graph (Fig. 4) was assembled for clearer representation and interpretation of obtained results. All ASVs were clustered under respective order for each functional guild, filtered from self-loops and increasing network-edges overlap. The graphics of network-analysis were obtained using the open-source software Cytoscape v. 3.8.0 (available at https://cytoscape.org) (Shannon et al. 2003).

Results

Bioinformatics and guilds assignment

The ITS raw data set counted 8791561 sequence reads, resulting in 7 849 888 quality-filtered reads mapped in 563 ASVs (out of 885 ASVs) after chimera sequences, singletons and rare taxa (<5 reads) removal. Fungal ASVs identified at least at order level were included in the downstream analysis, resulting in a total of 244 ASVs (see Supplementary Table S2).

Ecological guild assignment performed by FungalTraits database (Flores-Moreno et al. 2019) and reviewed in context of Antarctic endolithic fungal communities (Coleine et al. 2018b), resulted in 187 functional ASVs validated (7319248 total reads) and clustered in 3 functional groups (see Supplementary Table S3), named as follows: (i) Lichenized fungi group, which included

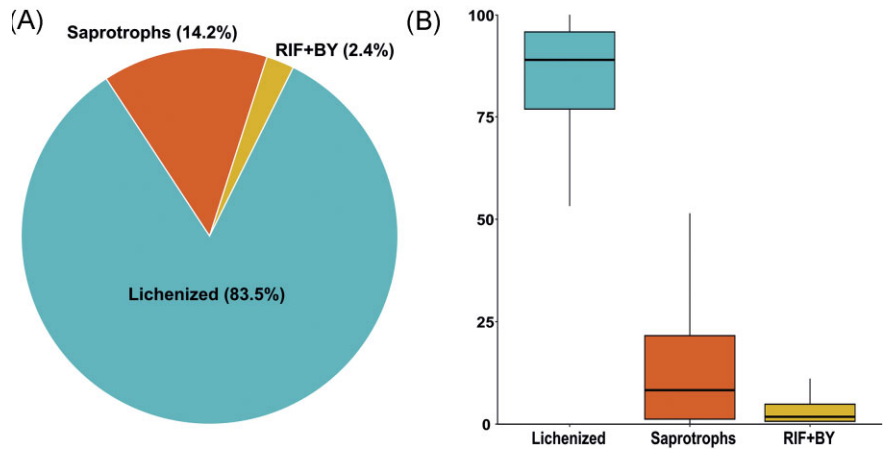

Figure 2. Overall composition **(A)** and relative abundance distribution **(B)** for fungal functional guilds along the Antarctic endolithic microbial community.

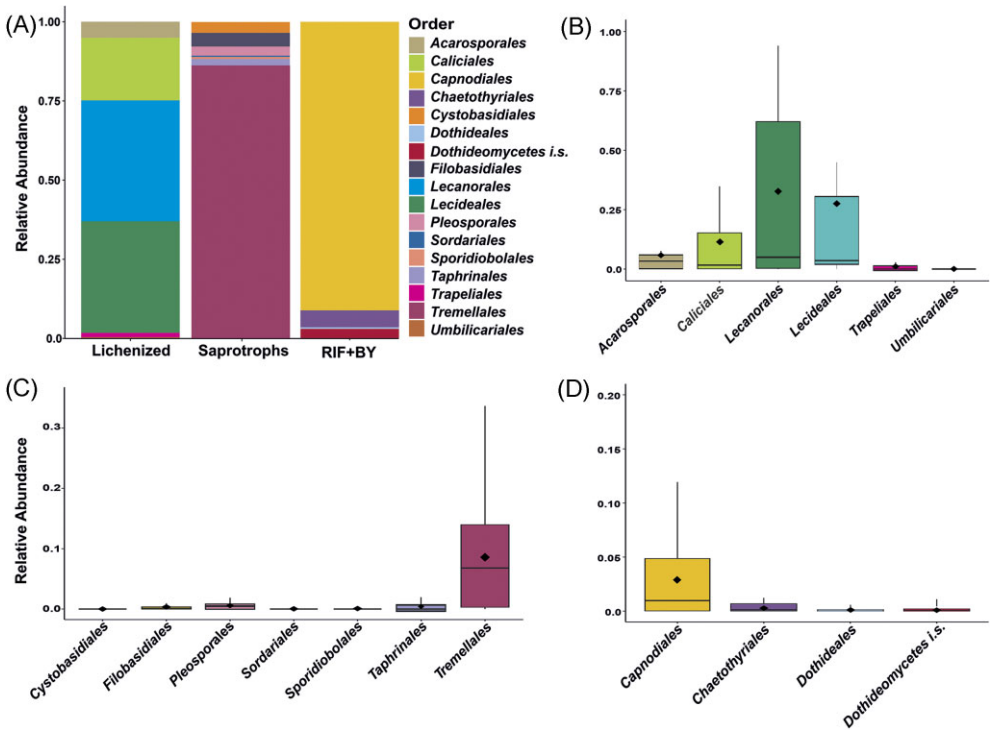

Figure 3. The stacked-bar chart **(A)** shows taxonomic composition of investigated fungal functional guilds grouped at order level. Box-plots show relative abundance and distribution throughout the dataset for each fungal functional guild: Lichenized fungi **(B)**, Saprotrophs **(C)**, Rock-Inhabiting Fungi and Black Yeasts (RIF+BY) **(D)**.

90 ASVs across *Acarosporales*, *Caliciales*, *Lecanorales*, *Lecideales*, *Trapeliales* and *Umbilicariales* orders; (ii) Saprotrophs guild counted 54 ASVs spreaded on 7 orders, such as *Tremellales*, followed by *Filobasidiales*, *Cystobasidiales*, *Pleosporales*, *Sordariales*, *Sporidiobolales* and *Taphrinales* mainly characterised by the ascomycetous yeast genus *Taphrina*; (iii) Rock-Inhabiting Fungi and Black Yeasts (RIF+BY) group was mainly featured by *Capnodiales* order, followed by *Chaetothyriales*, *Dothideales* and *Dothideomycetes incertae sedis* (genus *Cryomyces*) for a total of 43 ASVs reported. Some ASVs dominated by members of *Hypocreales* order, reported as Plant Pathogens, were not addressed during the downstream analyses due to their low abundance recorded throughout the dataset. Finally, to mitigate differences in sequencing depth the final dataset was rarefied without replacement to 6 912 total reads.

Fungal community composition

Fig. 2(A, B) showed mean and distribution of relative abundance throughout 74 sites surveyed for each functional guild: Lichenized fungi, Saprotrophs, Rock Inhabiting Fungi and Black Yeast (RIF+BY).

Lichenized fungi consistently dominated across the dataset (421126 reads and 90 ASVs in total) representing an average of 83.5% of total fungal component, and ranging from 77% to over 90%. Saprotrophs group with 71 449 total reads distributed in 55 ASVs and RIF+BY guild with 12001 reads mapped in 43 ASVs, represented the 14.2% and 2.4% of total fungal abundance respectively. Although RIF+BY was the lowest abundant component, it showed a homogeneous distribution (from 1% to 6% circa) along

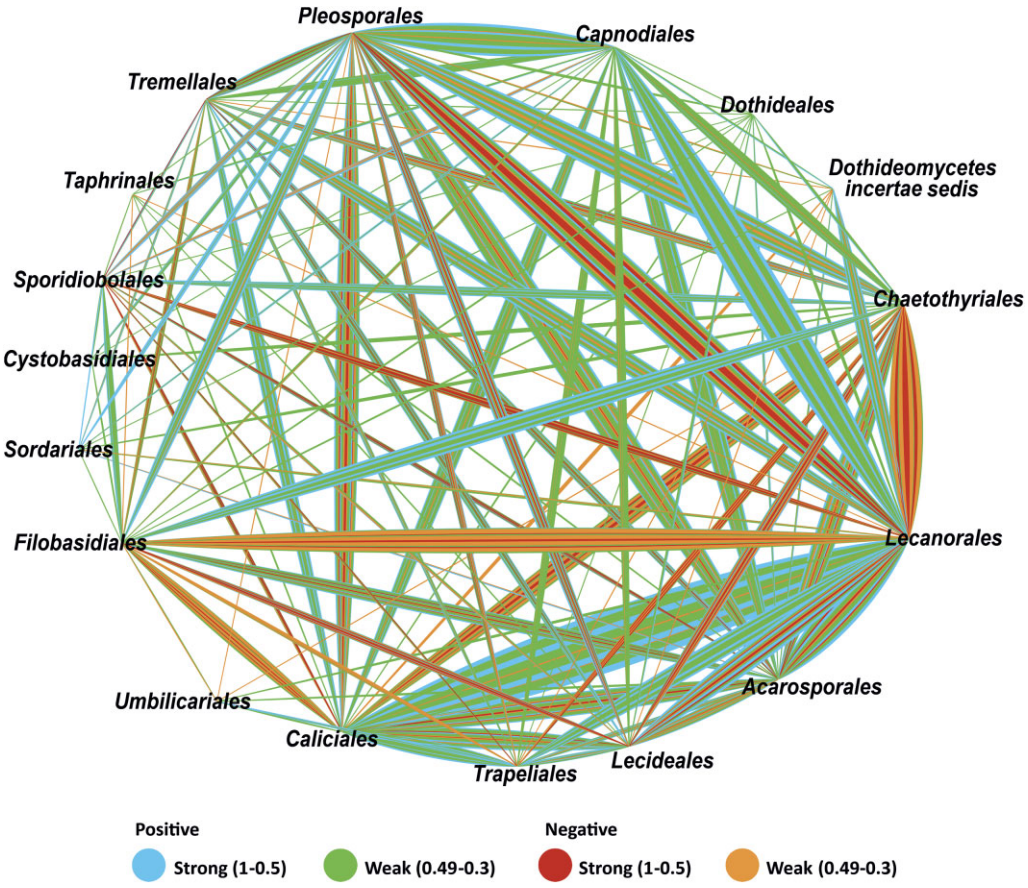

Figure 4. The summary network shows the resulting correlations between pairs of ASVs throughout the endolithic fungal community, grouped under respective order and functional guild. Sign and strength of each correlation is plotted by the colour-code according to Spearman's index range used in this work.

the dataset, while Saprotrophs relative abundance appeared more variable, ranging from 2% to 25% circa.

Lichenized fungi group (Fig. 2) were mainly represented by *Lecanorales* (38.86%), *Lecideales* (34.76%) and *Caliciales* (19.81%), which were the top 3 most abundant orders across the dataset, with 32.4%, 29% and 16.5% respectively. Moreover, this group was differently populated, by including less representative *Acarosporales* (5.1%) *Trapeliales* (1.45%) and *Umbilicariales* (0.05%) (Fig. 3). *Capnodiales* (91.7%) and *Tremellales* (85.31%) largely dominated the RIF+BY and Saprotrophs groups, respectively, holding almost total abundance (2.2% and 12.2%) of their own guilds throughout the dataset. For instance, the other 6 orders featuring the Saprotroph functional group did not exceed 2% of the entire fungal community collectively. Finally, within the RIF+BY group, members of the *Chaetothyriales* (5.4%) and *Dothideomycetes incertae sedis* (represented exclusively by the genus *Cryomyces*) (2.7%) included a small portion of the entire fungal assemblage with 0.13% and 0.07% respectively, reporting a coherent spread throughout the entire dataset.

Correlation and network analysis

A total of 5345 (3935 positive and 1410 negative) correlations between pairs of fungal ASVs were obtained, resulting in 2941 (2247 positive and 694 negative) after filtering step. Classification process by rank and degree returned 793 $^{+}$Strong, 1 454 $^{+}$Weak and 250 $^{-}$Strong, 444 $^{-}$Weak of total correlation (Fig. 4). Among the functional groups, Lichenized fungi showed the highest number of interactions 1 566 (30% $^{+}$S; 47% $^{+}$W; 9% $^{-}$S; 14% $^{-}$W), followed by Saprotrophs 784 (25% $^{+}$S; 48% $^{+}$W; 9% $^{-}$S; 14% $^{-}$W) and RIF+BY 597 (21% $^{+}$S; 56% $^{+}$W; 6% $^{-}$S; 16% $^{-}$W), counting for 53.2%, 26.5% and 20.3% of total interactions respectively. In detail, 3 orders of the Lichenized fungi group build the highest number of interactions, *Lecanorales* with 623 correlations in total, followed by *Caliciales* (356) and *Acarosporales* (285), establishing at least the 79% of positive interactions with all functional guilds. However, relationship landscape for these 3 orders also included negative interactions, mainly established with *Pleosporales* (38%), *Filobasidiales* (63%), *Sporidiobolales* (65%) for Saprotrophs functional group and *Chaetothyriales* (67%) for RIF+BY guild. Finally, the relationship patterns observed among the top-dominant lichen orders, *Lacanorales*, *Lecideales* and *Acarosporales*, were featured by over 28% of negative correlations. Within the Saprotrophs group the major amount of established relationships mainly involved *Pleosporales* (45%), *Filobasidiales* (20%) and *Tremellales* (18%) orders. Although saprotrophs' interaction web was dominated by positive interactions (70%) across the fungal component, the distribution of negative correlations displayed a different pattern according to orders engaged. While *Pleosporales* and *Filobasidiales* pointed the largest number of negative interactions (48%) toward Lichenized fungi guild, *Tremellales* established negative interactions (39%) mainly with saprophytic fungi, such as *Filobasidiales*, *Pleosporales*, *Chaetothyriales* and *Dothideomycetes incertae sedis* here represented by the endemic specie *Cryomyces antarcticus*. RIF+BY guild's relationship network was dominated by positive interactions (78%) and mainly ascribed to ASVs of *Capnodiales* and *Chaetothyriales*. Yet, if *Capnodiales* exhib-

ited a network almost exclusively composed of positive relationships (94%) across the fungal community, *Chaetothyriales* showed significant portion of negative interactions (39%), established mainly with the Lichenised fungi group: *Lecanorales* (38%), *Caliciales* (19%), *Lecideales* (10%), *Acarosporales* (8%) and *Trapeliales* (8%). All recorded interactions for each order are shown in supplementary Fig. S1

Discussion

The first step to reconstruct the correlations intercurring in the fungal compartment of the rock-dwelling microbial communities was the assignment to specific functional guilds of each specimen identified at the order level. The functional guild of Lichenized fungi resulted dominant in Antarctic cryptoendolithic communities; these specimens live in obligate symbiotic association (lichens) with photosynthetic component (cyanobacteria and/or eukaryotic green algae) which play the pivotal role of primary producers and sustain trophic webs. These symbiotic organisms are pioneer colonizers of lithic substrates, able to penetrate rocks and establish habitable zones accumulating nutrients in otherwise totally oligotrophic niches (Blackhurst et al. 2005, Coleine et al. 2018a). Being poikilohydric, lichens are able to cope with most extreme conditions for life, like those characterising the hyper-arid ice-free areas of Antarctica (Perez-Ortega et al. 2012). The dominant ecological success of Lichenized fungi in these extreme ecosystems was also confirmed by the wide relational network found in this study across endolithic fungal communities (more than 50% of total correlations), characterised by over 75% of positive correlations and some negative itra-guild interactions. Negative relationships (~29%) found among dominant orders of *Lecanorales*, *Lecideales* and *Acarosporales* may be the result of competition for limited air-space and sunlight inside rocks. Although lichen interspecific competition has been partially investigated from an epilithic context, it was highlighted how contrasting interactions in multispecific communities lead to the exclusion of individual species from the assemblages (Armstrong and Welch 2007).

The high number of positive correlations (over 95%) found between taxa of *Capnodiales* order (RIF+BY) and Lichenized fungi suggested the existence of mutual beneficial interaction between them. In particular, on one side RIF+BY benefits from photosynthetic products by lichens (Perez-Ortega et al. 2012, Selbmann et al. 2013) and, on the other side, they screen lichens, as well as other components of the assemblage, from the high solar and UV irradiation forming a black shield as first layer of the community stratification (Fig. 1) (Coleine et al. 2018b). Therefore, although RIF+BY covers 2.5% of the whole endolithic assemblage, this wide morpho-ecological group of highly melanized and multiple stress-tolerant and resilient fungi may be considered as key guilds for their role in community protection. There is, anyway, a clear difference both in the abundance and in the balance of positive/negative interactions established when RIF+BY and Lichenized fungi are considered. In fact, *Capnodiales* are far more abundant in the RIF+BY than *Chaetothyriales* and most of the negative interactions with Lichenized fungi are committed to the latter. This contrasting situation may be explained by considering the different origins of these two groups. Capnodiales belong to dothideomycetous black fungi; his class diversified in the past ages with drier and colder conditions than those occurring today (Silurian–Devonian era), and for this reason are particularly rich in extremotolerant cold-adapted black fungal species, highly recurrent in rocks of the Antarctic desert. The dominating positive correlation spectrum (94%) directed towards the entire fungal component emphasises their leading role in microbial community protection. On the other hand, *Chaetothyriales* originated in a period of expanding arid land masses with relatively high global temperatures (Permian–Triassic); the subsequent epilithic-lichen-associated evolution of this lineage may have become a driver towards parasitism of photobiont assemblages by managing toxins stored in the thallus (i.e. lichenic acids), coupled with a high thermotolerance capacity (Gueidan et al. 2011, Quan et al. 2020). Therefore, being originally more adapted to higher temperature they are less abundant despite still being involved in community protection, as suggested by the considerable network of positive interactions (61%). Yet, they established numerous negative correlations (39%) especially toward the Lichenized fungi guild with which they have co-evolved eventually acquiring the capacity to metabolise lichen toxicants.

Saprotrophs represented the second most abundant functional guild (14.2% total abundance) and included the largest number of different fungal orders. The organic matter degradation and nutrient recycling function assigned to this group was related to the ability of assimilating different carbon and nitrogen sources expressed by the basidiomycetous (taxa of *Tremellales*, *Cystobasidiales*, *Phylobasidiales* and *Sporoboridiales* orders) and ascomycetous (taxa of *Pleosporales*, *Sordariales* and *Taphrinales* orders). In general, the high number of positive correlations found (73%) between Saprotroph and other functional guilds highlighted their important role in the trophic web, although relationships appeared largely divergent among groups. In fact, while ASVs of *Pleosporales*, *Filobasidiales* and *Sporidiobolales* were positively correlated with all saprophytic life-style fungi (including RIF+BY) and negatively with the Lichenized fungi group, *Tremellales* exhibited the opposite trend (87% of positive correlation with Lichenized fungi).

The broad ecological and relational success of basidiomycetous yeasts compared to the ascomycetous counterpart could depend on peculiar morphological features and/or on the relationships established with lichens. Basidiomycetous yeasts exhibit a dense cell wall often associated with an exopolysaccharidic capsule, which provide protection against environmental extremes (Coleine et al. 2021a) and could also act as a defensive barrier against lichen toxins. Furthermore, recent studies indicated how several lichenicolous fungi, including basidiomycetous yeasts of *Tremella* genus (here reported, ASV8, see Supplementary Table S2), were easily isolated or exhibited a widespread presence of yeast life forms within the lichen thalli (Muggia and Grube 2018, Tuovinen et al. 2021).

Moreover, the broad ecological performance of basidiomycetous and ascomycetous yeasts was largely reported throughout Antarctica, even in different and geographically separated bedrock and rock formations compared to the sandstones here investigated (Gonçalves et al. 2017, Alves et al. 2019, de Menezes et al. 2021), emphasising their wide array of resistance skills (e.g. psychrophilic, oligotrophic and xero-tolerant) to environmental extremes.

This study highlighted that the success of fungi in these highly specialised microbial rock-inhabiting communities at the boundaries for life sustainability rely not only to the peculiar morpho-ecological adaptations of the allies but is also promoted by a complex network of interactions, witnessing the presence of a well-structured community; positive correlations, which were found as most abundant, could be the clue of the presence of an extensive functional cooperation necessary for coping with exacerbating environmental constraints, thus suggesting that cooperation is a winning strategy of fungal communities in the far extremes.

On the other hand, negative correlations could signify the necessity of preserving niche boundaries within the confined endolithic environment, regulating partnerships between microbes living in such strict spatial association.

Although the establishment of extreme environmental conditions over time in the Antarctic desert has been the evolutionary driver toward endolithic lifestyle adaptations, nowadays the rapid shift in climatic conditions (as the consequence of global warming due to the ongoing climate change) could reshape the microbial community dynamics. Based on the observations herein reported, in a future perspective of wetter and warmer conditions in Antarctica (Lee et al. 2017, Convey et al. 2019) *Chaetothyriales*, evolved in the past ages subjected to warmer conditions, may have the opportunity to spread earning extra niches over the lichen assemblage with a consequent increasing of negative interactions. Such an event may lead in a complete alteration of the fragile balance of endolithic community and ice-free areas ecosystems.

Overall, this study represents a first step toward the understanding of compositional relations driving the functional biodiversity of Antarctic fungal endolithic assemblages. The tight network of correlations mapped between taxa of fungal guilds highlighted how ecological and evolutionary relationships could define the dominance of specific microbial traits, driving their distribution and defining their niche boundaries, especially in a spatially confined endolithic ecosystem.

The critical impact of this study is also represented by the large dataset of endolithic microbiomes analysed, resulting from an extensive survey throughout Victoria Land and characterised by environmental and microclimatic contexts among the most extreme and peculiar of the Antarctic continent.

Furthermore, the fragility and vulnerability of these communities has been highlighted, speculating on how microbial diversity shifts induced by climate change could disrupt the delicate balance of endolithic ecosystems of hyper-arid areas. Future efforts should be paid to validate and expand this correlation model, including new sampling areas, endolithic bacterial guilds and microclimate data trends, aiming to provide an innovative monitoring bio-tools for these fragile and extreme environments.

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Supplementary data

Supplementary data is available at FEMSEConline.

Conflict of interest statement. The authors have no conflicts of interest to declare.

References

Albanese D, Riccadonna S, Donati C *et al.* a practical tool for maximal information coefficient analysis. *GigaScience* 2018;**7**:giy032.

Alves IM, Gonçalves VN, Oliveira FS *et al.* The diversity, distribution, and pathogenic potential of cultivable fungi present in rocks from the South Shetlands archipelago, Maritime Antarctica. *Extremophiles* 2019;**23**:327–36.

Armstrong RA, Welch AR. Competition in lichen communities. *Symbiosis* 2007;**43**:1–12.

Blackhurst RL, Genge MJ, Kearsley AT *et al.* Cryptoendolithic alteration of Antarctic sandstones: pioneers or opportunists?. *J Geophys Res* 2005;**110**:E12.

Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**:581–3.

Coleine C, Stajich JE, de Los Ríos A *et al.* Beyond the extremes: rocks as ultimate refuge for fungi in drylands. *Mycologia* 2021a;**113**:108–33.

Coleine C, Biagioli F, de Vera JP *et al.* Endolithic microbial composition in Helliwell Hills, a newly investigated Mars-like area in Antarctica. *Environ Microbiol* 2021b;**23**:4002–16.

Coleine C, Delgado-Baquerizo M, Albanese D *et al.* Rocks support a distinctive and consistent mycobiome across contrasting dry regions of Earth. *FEMS Microbiol Ecol* 2022;**98**:fiac030.

Coleine C, Pombubpa N, Zucconi L *et al.* Endolithic fungal species markers for harshest conditions in the McMurdo Dry valleys, Antarctica. *Life* 2020a;**10**:13.

Coleine C, Gevi F, Fanelli G *et al.* Specific adaptations are selected in opposite sun exposed Antarctic cryptoendolithic communities as revealed by untargeted metabolomics. *PLoS One* 2020b;**15**:e0233805.

Coleine C, Albanese D, Onofri S *et al.* Metagenomes in the borderline ecosystems of the Antarctic cryptoendolithic communities. *Microbiol Resour Announc* 2020c;**9**:e01599–19.

Coleine C, Stajich JE, Zucconi L *et al.* Antarctic cryptoendolithic fungal communities are highly adapted and dominated by Lecanoromycetes and Dothideomycetes. *Frontiers in Microbiology* 2018a, **9**:1392.

Coleine C, Zucconi L, Onofri S *et al.* Sun exposure shapes functional grouping of fungi in cryptoendolithic Antarctic communities. *Life* 2018b;**8**:19.

Convey P, Peck LS. Antarctic environmental change and biological responses. *Sci Adv* 2019;**5**:eaaz0888.

Cordero OX, Datta MS. Microbial interactions and community assembly at microscales. *Curr Opin Microbiol* 2016;**31**:227–34.

de Menezes GCA, Câmara PE, Pinto OHB *et al.* Fungal diversity present on rocks from a polar desert in continental Antarctica assessed using DNA metabarcoding. *Extremophiles* 2021;**25**:193–202.

Edgar RC, Flyvbjerg H. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* 2015;**31**:3476–82.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Fanelli G, Coleine C, Gevi F *et al.* Metabolomics of dry versus reanimated antarctic lichen-dominated endolithic communities. *Life* 2021;**11**:96.

Flores-Moreno H, Treseder K, Cornwell K *et al.* fungaltraits aka funfun: a dynamic functional trait database for the world's fungi. 2019Dataset: https://github.com/traitecoevo/fungaltraits.

Friedmann EI. Endolithic microorganisms in the Antarctic cold desert. *Science* 1982;**215**:1045–53.

Friedmann EI. The Antarctic cold desert and the search for traces of life on Mars. *Adv Space Res* 1986;**6**:265–8.

Gonçalves VN, Oliveira FS, Carvalho CR *et al.* Antarctic rocks from continental Antarctica as source of potential human opportunistic fungi. *Extremophiles* 2017;**21**:851–60.

Gueidan C, Ruibal C, De Hoog GS *et al.* Rock-inhabiting fungi originated during periods of dry climate in the late Devonian and middle Triassic. *Fungal Biology* 2011;**115**:987–96.

Lee JR, Raymond B, Bracegirdle TJ *et al.* Climate change drives expansion of Antarctic ice-free habitat. *Nature* 2017;**547**:49–54.

Lee JR, Waterman MJ, Shaw JD *et al.* Islands in the ice: potential impacts of habitat transformation on Antarctic biodiversity. *Global Change Biol* 2022;**28**:5865–80.

Lindahl BD, Nilsson RH, Tedersoo L *et al.* Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytol* 2013;**199**:288–99.

McKay CP, Andersen D, Davila A. Antarctic environments as models of planetary habitats: university Valley as a model for modern Mars and Lake Untersee as a model for Enceladus and ancient Mars. *The Polar Journal* 2017;**7**:303–18.

McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 2013;**8**:e61217.

Meslier V, DiRuggiero J. Endolithic microbial communities as model systems for ecology and astrobiology. In: *Model Ecosystems in Extreme Environments*. Elsevier, London: Academic Press, 2019, 145–68.

Muggia L, Grube M. Fungal diversity in lichens: from extremotolerance to interactions with algae. *Life* 2018;**8**:15.

Nienow JA, Friedmann EI. Terrestrial lithophytic (rock) communities. In: *Antarctic Microbiology*. Friedmann EI (ed.), Wiley-Liss: New York, NY, USA, 1993,343–412.

Palmer JM, Jusino MA, Banik MT *et al.* Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. *PeerJ* 2018;**6**:e4925.

Perez-Ortega S, Ortiz-Álvarez R, Allan Green TG *et al.* Lichen myco- and photobiont diversity and their relationships at the edge of life (McMurdo Dry Valleys, Antarctica). *FEMS Microbiol Ecol* 2012;**82**:429–48.

Quan Y, Muggia L, Moreno LF *et al.* a re-evaluation of the Chaetothyriales using criteria of comparative biology. *Fungal Diversity* 2020;**103**:47–85.

Rognes T, Flouri T, Nichols B *et al.* VSEARCH: a versatile open source tool for metagenomics. *PeerJ* 2016;**4**:e2584.

Selbmann L, Grube M, Onofri S *et al.* Antarctic epilithic lichens as niches for black meristematic fungi. *Biology* 2013;**2**:784–97.

Selbmann L, Onofri S, Coleine C *et al.* Effect of environmental parameters on biodiversity of the fungal component in lithic Antarctic communities. *Extremophiles* 2017;**21**:1069–80.

Selbmann L, Stoppiello GA, Onofri S *et al.* Culture-dependent and amplicon sequencing approaches reveal diversity and distribution of black fungi in Antarctic cryptoendolithic communities. *Journal of Fungi* 2021;**7**:213.

Shannon P, Markiel A, Ozier O *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–504.

Smith DP, Peay KG. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLoS One* 2014;**9**:e90234.

Tuovinen V, Millanes AM, Freire-Rallo S *et al.* Tremella macrobasidiata and Tremella variae have abundant and widespread yeast stages in Lecanora lichens. *Environ Microbiol* 2021;**23**:2484–98.

Walker JJ, Spear JR, Pace NR. Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature* 2005;**434**:1011–4.

Wierzchos J, Casero MC, Artieda O *et al.* Endolithic microbial habitats as refuges for life in polyextreme environment of the Atacama Desert. *Curr Opin Microbiol* 2018;**43**:124–31.

Wierzchos J, DiRuggiero J, Vítek P *et al.* Adaptation strategies of endolithic chlorophototrophs to survive the hyperarid and extreme solar radiation environment of the Atacama Desert. *Front Microbiol* 2015;**6**:934.

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