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Exchangeable cations and pH drive diversity and functionality of fungal communities in biological soil crusts from coastal sites of Victoria Land, Antarctica

Fabiana Canini [a, b, *], József Geml [b, c], Luigi Paolo D'Acqui [d], Laura Selbmann [a, e], Silvano Onofri [a], Stefano Ventura [d, f], Laura Zucconi [a]

[a] Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy
[b] Naturalis Biodiversity Center, Leiden, the Netherlands
[c] Faculty of Science, Leiden University, Leiden, the Netherlands
[d] Terrestrial Ecosystems Research Institute, National Research Council of Italy (IRET-CNR), Sesto Fiorentino, Italy
[e] Italian National Antarctic Museum (MNA), Mycological Section, Genoa, Italy
[f] The Italian Embassy in Israel, Tel Aviv, Israel

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ABSTRACT

Ice-free regions in coastal areas of Victoria Land, Antarctica, are patchily distributed, limited in extent and characterized by a simple vegetation of lichens and mosses, growing only for a short period during the austral summer. These organisms are associated with soil particles and microorganisms (e.g., algae, microfungi and bacteria) to make up biological soil crusts (BSCs), found worldwide in cold and/or arid and semi-arid regions, where plant growth is impaired. Despite BSCs being among the most widespread ecosystems throughout coastal ice-free areas of continental Antarctica, fungal components of these communities have received little focus. Through ITS1 DNA metabarcoding of samples from 17 sites of six different localities from 73 to 77°S, in a distance scale from 29 to 411 km among different sites, we provide insights into the diversity, community composition, and functionality of fungal communities of these peculiar ecosystems, deepening our knowledge on how they are related to different edaphic variables (i.e. chemical properties and texture). Although fungal richness was low (59 ± 27 OTUs per sample), we found numerous previously unsequenced, putatively unknown fungal species representing a great part of the sampled communities. Community composition was spatially auto-correlated and appeared to be driven by site-specific differences in environmental conditions, particularly edaphic factors, such as exchangeable cations and pH. These results are of particular interest, as they give a wide characterization of the parameters determining soil colonization in a such limiting environment, especially in the light of global changes that are expected to deeply modify the conditions of this environment.

1. Introduction

Antarctica is the coldest, windiest and driest continent, with the highest mean elevation on the planet and nearly all its surface is covered by ice, with an average thickness of about 2400 m on the central polar plateau of East Antarctica. Victoria Land, on the western side of the Ross Sea and the Ross Ice Shelf, along a latitudinal gradient spanning 8° from Cape Adare (71°S) to Darwin Glacier (79°S), comprises multiple ecosystems with a great diversity of soil types and minimal human perturbations. McMurdo Dry Valleys, west of McMurdo Sound, in Southern Victoria Land, form the largest ice-free area in Antarctica, while in Northern Victoria Land ice-free regions mainly occur during the warmer summer months, are limited in extent and patchily distributed, and are mostly confined in coastal regions on the fringe of the continent or in isolated nunataks and mountain peaks (Barrett et al., 2006). Microorganisms are the prevalent life forms in the Antarctic soil ecosystems, more than in other continents, with species well adapted to survive and thrive in one of the most hostile environments on Earth (Nienow and

* Corresponding author. Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy.
E-mail address: canini.fabiana@unitus.it (F. Canini).

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Friedmann, 1993) and sometimes endemic, due to the long-time geographic isolation of Antarctica (Vyverman et al., 2010). The simple structure of these communities makes it easier to study the biotic and abiotic interactions with the surrounding environment, than in lower latitudes.

Along the coastal sites of Northern Victoria Land, the vegetation, composed of lichens and mosses (Ochyra et al., 2008), has only a short growth period during the austral summer largely because of the ephemeral water availability (Zucconi et al., 2016). These ice-free areas have been defined "Antarctic oases" (Pickard, 1986) for the presence of mosses, lichens and algal associations growing isolated from each other as islands in the glaciated landscape and hosting distinct groups of organisms. The intimate associations of lichens and bryophytes with soil particles and other microorganisms, such as free-living bacteria, cyanobacteria, algae and microfungi, composes the biological soil crusts (BSCs), that are found worldwide in cold and/or arid and semi-arid regions, where plant growth is impaired and they become the only stable vegetal coverage (Belnap et al., 2001, 2003; Pointing et al., 2015). BSCs, after their establishment, undergo long successional processes that lead to the creation of complex topological and functional relationships among their mineral and biological components. This type of succession has been described in continental Antarctica since 1970, when Cameron and Devaney reported a soil colonization starting with cyanobacteria and algae, followed by lichens and ending with mosses. The photoautotrophic components, namely bryophytes, and free-living and lichenized cyanobacteria and algae, support the trophic hierarchy of the community by providing a food resource for heterotrophs and by leaching some fixed carbon compounds into the soil (Belnap and Lange, 2001; Belnap et al., 2001; Yoshitake et al., 2010). Mosses, for example, are known to contribute considerably to the total carbon (C) pools in high arctic desert soils (Arndal et al., 2009). In addition, some cyanobacterial components of BSCs can fix atmospheric nitrogen (Stewart et al., 2011) in an amount that is known to be adequate to support the needs of mosses and the associated community (Dickson, 2000; Breen and Levesque, 2008). Moreover, the interplay of species growing in filaments or sheaths with others excreting extracellular polymeric substances, leads to the stabilization of the soil and prevents erosion and cryoturbation (Evans and Johansen, 1999; Belnap et al., 2001). Moreover, it is well-known how BSCs are able to increase soil temperature in dry and cold conditions (Xiao et al., 2016), mainly due to their dark colour and increased surface roughness, that decrease surface albedo and increase absorption of solar radiation (Kidron and Tal, 2012). For these reasons, BSCs are often regarded as ecosystem engineers, because their establishment can lead to the development of protosoils, increasing the amount of organic matter (OM) and moisture retention (Bowker et al., 2006; Pointing and Belnap, 2012). Recently, the potential applications of BSCs in controlling soil and water content, e.g., in reversing desertification processes and in restoration of polluted soils (Xiao et al., 2015; Guan et al., 2018), are attracting considerable attention.

Despite being among the most widespread biological communities throughout ice-free areas of continental Antarctica, very few studies have been carried out on BSCs composition in Antarctica. Usually, studies focused on the identification of organisms present in Antarctic soils, without taking into consideration whether they might form specific associations or even biocoenoses (reviewed in Büdel and Colesie, 2014). Green and Broady (2001) provided a first review of BSCs in Antarctica, highlighting how these communities have lower biodiversity, biomass and growth rates compared to analogous ecosystems at lower latitudes. This review and more recent works (Colesie et al., 2014a,b and 2016; Pushkareva et al., 2018) focused mainly on the description of the photosynthetic components and their activity.

Despite the well-known role of fungi in soil ecosystems in recycling C sources, especially in oligotrophic environments and in mutualistic symbioses, and their high resistance to desiccation and UV radiations, their role in Antarctic BSCs has never been investigated, with some exception to lichenized fungi (Smykla et al., 2011; Pérez-Ortega et al., 2012). Free-living fungi associated with continental Antarctic mosses have been assessed only recently through an isolation approach (Hirose et al., 2016). Even in other environments, the description of free-living fungi associated with BSCs is limited (for example, Bates et al., 2010, 2012; Steven et al., 2015; Maier et al., 2016; Zhang et al., 2016) and all the works carried out are mostly descriptive of taxonomic composition, without taking into account the environmental factors determining the diversity and distribution of these microorganisms.

Moreover, in spite of the well-known resilience of BSC ecosystems to stress, climate change is expected to alter their composition and abundance (Evans and Lange, 2001; Johnson et al., 2012; Zelikova et al., 2012). Furthermore, especially in polar regions, increased temperatures and altered precipitation patterns are expected to induce an invasion of foreign species (Chown et al., 2012; Pushkareva et al., 2016; Lee et al., 2017). It is still unclear how Antarctic communities will respond to potential expansion of suitable habitats (deglaciation) and changes in biotic interactions linked to ongoing climate change (Convey, 2013; Chown et al., 2015). Hence, the investigation and base-line assessment of these peculiar ecosystems and the description of environmental factors determining their establishment and development is essential to better understand climate-driven changes in BSC communities.

To this extent, we analysed the total fungal community (both symbiotic and free-living fungi) associated with biological soil crusts from 17 sites of six localities in Victoria Land, where different stages of crust development have been identified. All sampled localities are located along the coast, ranging from 73 to 77°S, in a distance scale from 29 to 411 km among different sites. The main aims were to provide a first extensive taxonomic and functional characterization of the fungal communities of these peculiar ecosystems and to understand how diversity, composition and functionality of soil fungal communities are related to nutrient availability and physicochemical variables, in order to give some insights into the landscape-level dynamics of BSC communities in relation to the extreme environment to which they are adapted.

2. Material and methods

2.1. Sampling

Samples were collected in 17 sites of six coastal localities of Victoria Land, Antarctica, along a latitudinal gradient ranging from 73°31′S (Apostrophe Island) to 77°00′S (Botany Bay) (Fig. 1). All localities are in Northern Victoria Land, except for Botany Bay, located in Southern Victoria Land. Being north facing and well protected from winds, Botany Bay harbours BSC communities (Seppelt et al., 2010), physiognomically similar to the ones of northern coastal localities. This site and Edmonson Point provide exceptional locations for research on BSC communities, and for this unique ecological status they have been designated as ASPA (Antarctic Specially Protected Areas, N. 154 and N. 165, respectively). Soil samples at Edmonson Point were collected at about 500 m far from the penguin rockery, to limit its influence. In each locality, two to four sampling sites (for a total number of 17 sampling sites) were selected, depending on the presence of different stages of BSC colonization and development, in order to give the widest possible description of these ecosystems (Fig. 1). Three soil samples representative of each selected area were collected. The top soil (0–5 cm depth) after BCS removal was sampled, resulting in a total of 50

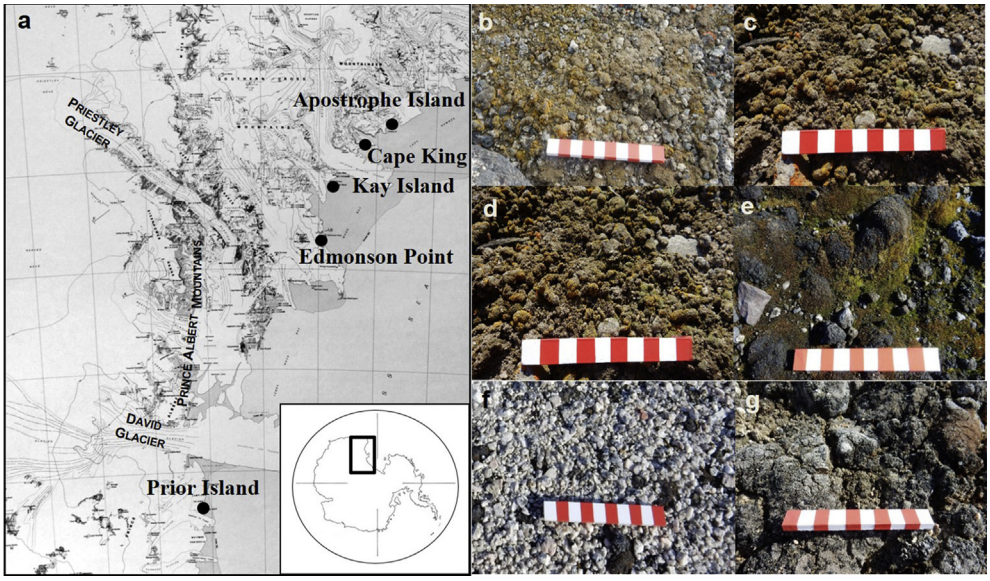

Fig. 1. Map of the sampling sites (points) in Northern Victoria Land, with the exclusion of Botany Bay located in Southern Victoria Land (a); details of some samples collected: Apostrophe Island site 2 (b); Cape King site 1 (c); Kay Island, site 2 (d); Edmonson Point, site 4 (e); Prior Island, site 1 (f); Botany Bay, site 2 (g). Scale bar 1 cm.

samples. Samples for molecular analyses were stored at −20 °C in sterile bags. The list of the samples, their geographic coordinates and a description of each sampling site are listed in Table S1.

2.2. Soil physicochemical parameters

Soil samples were air dried and <2 mm sieved. Total soil organic C and N were measured by combustion with an elemental analyzer NA 1500 CHNS (Carlo Erba, Milan, Italy). Particle size distribution, cation exchange capacity (CEC) and pH in a 1:2.5 soil:water suspension were determined according to the SISS (Società Italiana della Scienza del Suolo) methods (Colombo and Miano, 2015). Soil particle size distribution was characterized by sand (0.5–2 mm), coarse silt (0.02–0.05 mm), fine silt (0.002–0.02 mm) and clay (<0.002 mm). Soil exchangeable bases (Na^+, Ca^{2+}, K^+ and Mg^{2+}) were determined by flame atomic absorption spectrometry (ASS PerkinElmer 1100B).

2.3. DNA extraction, amplification and sequencing

For each sample, metagenomic DNA was extracted from 0.5 g of soil using DNEasy Powersoil kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. The ITS1 region was amplified using ITS1F (Gardes and Bruns, 1993) and ITS2 (White et al., 1990) primers, following the protocol described in Smith and Peay (2014). An equimolar pool of uniquely barcoded amplicons was paired-end sequenced (2 × 300 bp) on an Illumina MiSeq platform, at the Vincent J. Coates Genomics Sequencing Laboratory at University of California, Berkeley.

2.4. Bioinformatic analyses

Bcl files were converted to FASTQ files, were demultiplexed and primers were removed using bcl2fastq (v 2.18). Dual-matched 8 bp indexes were used to eliminate the occurrence of “barcode bleed” (or tag-switching) between samples.

Demultiplexed sequences were then processed with the Amplicon ToolKit (AMPtk) for NGS data (formally UFITS) v.1.2.1 (Palmer et al., 2018). 7,172,088 starting reads were subjected to quality trimming and PhiX screening using USEARCH with default parameters (v. 9.2.64; Edgar, 2010). Reads with less than 100 bp were removed, ones longer than 300 bp were trimmed and paired-end reads were merged in one step, obtaining 3,583,937 contigs. Individual sample sequence files were merged into a single file and clustered with a 97% identity threshold into Operational Taxonomic Units (OTUs) using VSEARCH v 2.7.0 (Rognes et al., 2016), simultaneously removing putative chimeras. A total of 3,351,306 (93.5%) reads were mapped in 1476 OTUs. Taxonomy was assigned to OTUs based on the curated UNITE + INSD reference database dynamic Species Hypotheses (UTAX release of October 10, 2017) using USEARCH (v. 9.2.64; Edgar, 2010). Singletons (namely, OTUs with only one read in the dataset) and OTUs with less than 70% identity to a fungal SH (Species Hypothesis) were excluded for the following analyses. Representative sequences of each OTU were submitted to GenBank (**MK536607** - **MK537296**). The OTU table was normalized for subsequent statistical analyses by rarefying the number of sequences per sample to the smallest library size (15,921 reads) using the *rrarefy* function implemented in the *vegan* R package v. 2.5–2 (Oksanen et al., 2015), resulting in 690 OTUs retained. The initial functional assignments were made by FunGuild (Nguyen et al., 2016) and then manually checked based on ecological metadata of the corresponding SHs in UNITE, for genera that are known to comprise species with diverse functions. OTUs with more than 90% similarity to a fungal SH with known ecological function were assigned to one of two functional groups: lichenized fungi and saprotrophs. The assignment lead to the identification of some elements, known to act as animal pathogens, ectomycorrhizal (ECM) fungi, mycoparasites and plant pathogens, that were not considered in subsequent analyses for the low number of OTUs.

2.5. Statistical analyses

Unless otherwise specified, all the analyses were carried out with the *vegan* package (Oksanen et al., 2015) in R (R Core Team, 2018). Linear regression analyses were used to examine relationships between edaphic physicochemical parameters listed before and the richness and relative abundance of the two functional groups and of different taxonomic groups of fungi, with 45 degrees of freedom. Bonferroni adjustment has been applied to obtained p-values.

We ran non-metric multidimensional scaling (NMDS) on the Hellinger-transformed OTU table. Ordinations were run separately for the total community, the functional groups, and the two most abundant phyla, following specifications: distance measure = Bray-Curtis, dimensions = 2, initial configurations = 100, model = global, maximum number of iterations = 200, convergence ratio for stress = 0.999999. We used the *envfit* R function to fit edaphic physico-chemical parameters listed above and the latitude values onto the NMDS ordinations. In addition, we tested whether fungal communities were statistically different among different localities using the Multi-Response Permutation Procedure (MRPP).

Partial Mantel tests (Smouse et al., 1986) were carried out in PC-ORD v. 6.0 (McCune et al., 2002), to differentiate the effects of spatial distance (in terms of geographical coordinates) and soil parameters on community structure.

Permutational multivariate analysis of variance (PerMANOVA; Anderson, 2001) was carried out on Bray-Curtis distance matrices obtained of Hellinger-transformed OTU tables with 9999 permutations, with the *adonis* function, in order to determine the effect of each soil parameter on the observed variance of the total community, the two functional groups, and the three dominant phyla. Finally, to account for correlations among environmental variables, we performed a forward selection of parameters, based on the previous results, including only significant environmental variables in the final models.

3. Results

The metabarcoding analysis led to the construction of a dataset consisting of 690 quality-filtered and rarefied OTUs, with at least 70% identity with known fungal SH. 304 OTUs out of 690 were present in only one sample, and only 13 in at least 50% of the samples, indicating that the composition of each sample is highly localized. Fungal richness was generally low (59 ± 27 OTUs per sample; Table S2) and varied greatly among samples, often even within a plot, ranging from 15 in a poorly developed BSC from Prior Island, to 196 for site 2 in Edmonson Point (Table S2).

With respect to fungal phyla, 363 OTUs (52.6% of the total) were assigned to Ascomycota, 114 (16.5%) to Basidiomycota, and 36 (5.3%) to Chytridiomycota; other less representative phyla were Glomeromycota (3 OTUs), Kickxellomycota (1 OTU), Mortierellomycota (20 OTUs), Mucoromycota (7 OTUs) and Rozellomycota (9 OTUs). 137 OTUs (19.8%) matched fungal reference sequences that were not referred to a phylum. Regarding the relative abundance of the different phyla, Ascomycota (mean abundance per sample 62.0 ± 24.8% of the total reads) were the dominant group in all samples, followed by Basidiomycota (mean abundance per sample 14.4 ± 13.3% of the total reads) and Chytridiomycota (mean abundance per sample: 3.9 ± 8.0% of the total reads). In six samples of different localities, more than 50% of the reads belonged to OTUs not identified at phylum level.

At class level, 192 OTUs (28% of the total) remained unidentified, while seven classes represented 75% of identified OTUs. These classes were Lecanoromycetes (79 OTUs), Eurotiomycetes (63 OTUs), Dothideomycetes (62 OTUs), Leotiomycetes (51 OTUs), Agaricomycetes (50 OTUs), Sordariomycetes (36 OTUs) and Tremellomycetes (34 OTUs). In many of the samples more than 50% of the reads belonged to OTUs not identified at class level.

383 OTUs (55.5% of the total), with identity to known SH higher than 90%, were assigned to fungal functional groups: 261 OTUs as saprotrophs and 53 as lichenized fungi. Additionally, 15 OTUs were assigned to fungal pathogens, 23 to mycorrhizal fungi, 11 to mycoparasites and 20 to plant pathogens or other undefined plant-associated fungi; these groups were not analysed statistically because of their low abundance and sporadic presence in different samples.

3.1. Correlation between fungal richness/abundance and edaphic parameters

The C/N rate in soil samples was highly variable, ranging from 3.3 in Edmonson Point site 2, showing an apparent very primitive colonization, to values close to 30 in some samples (Kay Islands sites 1 and 2 and Apostrophe Island site 1) collected in areas with abundant moss coverage (Table S3). The pH ranged from slightly acidic to neutral values (minimum 5.1 for Apostrophe Island and maximum 7.4 for Edmonson Point; Table S3). Regarding soil texture, almost all the sites were dominated by sand (from 50% to 98%), with a relatively small amount of clay (Table S3). However, fine and coarse silt showed a varying trend.

We found no significant correlations between total fungal richness and the measured soil parameters (Figs. S1 and S2). On the other hand, the richness of lichenized fungi was positively correlated with CEC (slope = 0.9794 and r^2 = 0.2658; Fig. 2) and negatively correlated with both Na^+ (slope = −5.0553 and r^2 = 0.1188, marginally significant; Figs. S1) and K^+ content (slope = −14.510 and r^2 = 0.2376; Fig. 2 and Fig. S1). The relative abundance of this group was positively correlated with CEC (slope = 1.3056 and r^2 = 0.1109, marginally significant), but not correlated with Na^+ and K^+ content (Fig. 3 and Fig. S3). Finally, an increase of pH from acidic to neutral values lead to a significant decrease in richness (slope = −3.9355 and r^2 = 0.3522; Fig. 2), and a not significant decrease in abundance (Fig. 3).

As for the total community, neither richness nor abundance of saprotrophic fungi, the dominant functional category, showed

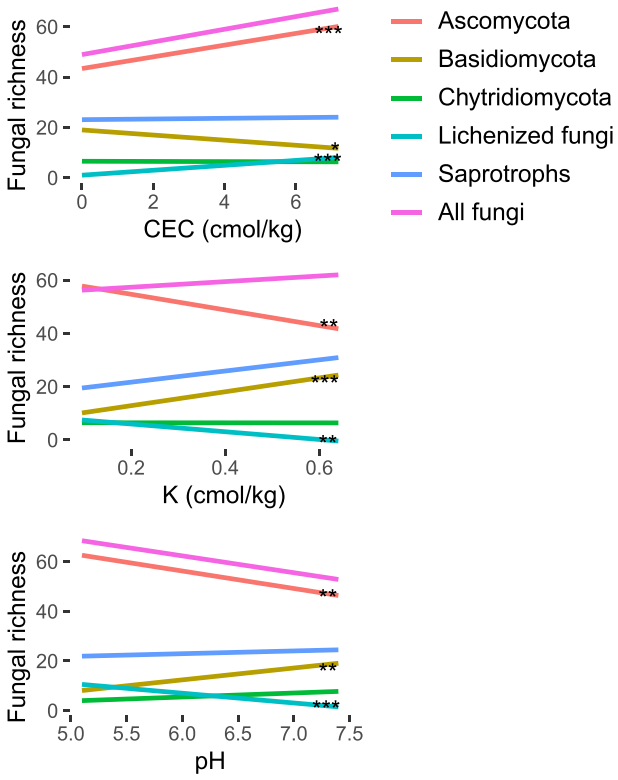

Fig. 2. Regression lines for the variation of richness (y-axis) of the total fungal community, the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla (for the phyla the relative richness has been plotted), in response to soil chemical parameters: CEC, exchangeable K^+, and pH (x-axis). The significance of the regressions is indicated as ***p < 0.001, **p < 0.01. Single graphs of the regressions with the points corresponding to all the samples are in Fig. S1.

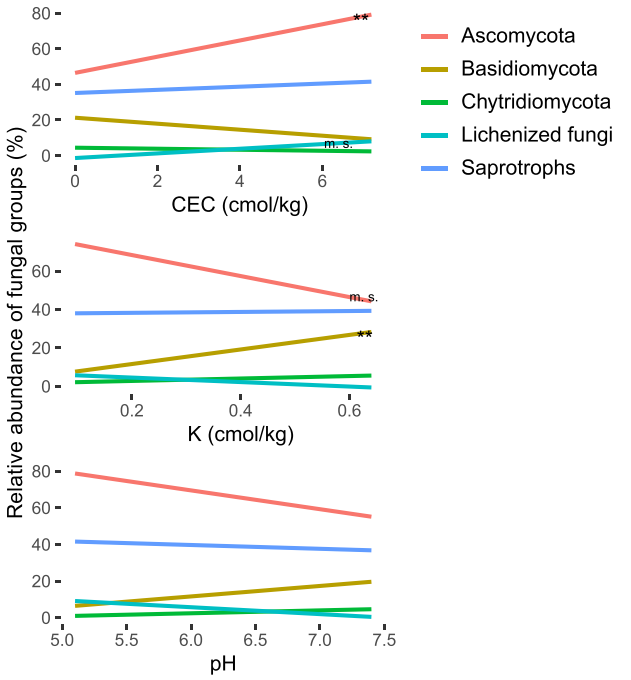

Fig. 3. Regression lines for the variation of relative abundance (y-axis) of the two functional groups of saprotrophic and lichenized fungi, and the three dominant phyla, in response to soil chemical parameters: CEC, exchangeable K^+, and pH (x-axis). The significance of the regressions is indicated as **$p < 0.01$ and m. s. (marginally significant) $p < 0.1$. Single graphs of the regressions with the points corresponding to all the samples are in Fig. S3.

significant correlations with measured edaphic parameters (Figs. 2 and 3, Fig. S1 and S3).

Neither N, nor C content correlated with ascomycete richness and abundance (Figs. S1 and S3), and with the richness and abundance of the classes belonging to this phylum (Table 1). Both richness (slope = 0.3992 and $r^2 = 0.1299$) and abundance (slope = 0.9287 and $r^2 = 0.01337$) of Ascomycota were positively correlated with C/N ratio (Fig. S1 and S3), and a similar trend was observed for the richness of Eurotiomycetes (Table 1).

Regarding cation availability, the CEC had a strong positive relationship with Ascomycota richness (slope = 2.3280 and $r^2 = 0.3466$; Fig. 2) and abundance (slope = 4.554 and $r^2 = 0.2028$; Fig. 3), as did Mg^{2+} for richness (slope = 4.661 and $r^2 = 0.1679$) but not significantly for abundance (Figs. S1 and S3, respectively). On the contrary, Na^+ and K^+ showed strong negative correlations with both richness (Na^+: slope = −15.558 and $r^2 = 0.2805$, Fig. S1; K^+: slope = −29.415 and $r^2 = 0.2198$, Fig. 2) and abundance (Na^+: slope = −44.228 and $r^2 = 0.3681$, Fig. S3; K^+: slope = −54.712 and $r^2 = 0.1113$, marginally significant; Fig. 3). These trends were observed in several classes (Table 1). Finally, pH was negatively correlated with Ascomycota richness (slope = −7.038 and $r^2 = 0.2492$, Fig. 2), but not significantly with abundance (Fig. 3). A negative correlation was also reported for Dothideomycetes abundance and Lecanoromycetes richness (Table 1).

Basidiomycota, compared to Ascomycota, displayed opposite trends with respect to the impact of many of the parameters considered, with negative correlations between richness and CEC (slope = −1.0148 and $r^2 = 0.129$), and positive correlations between richness and both K^+ (slope = 26.088 and $r^2 = 0.390$) and pH (slope = 4.773 and $r^2 = 0.248$) (Fig. 2). Relative abundance of Basidiomycota positively correlated with K^+ (slope = 37.901 and $r^2 = 0.195$) (Fig. 3). The two Basidiomycota classes considered (Agaricomycetes and Tremellomycetes) showed similar trends and were also negatively correlated with Ca^{2+} and Mg^{2+} content (Table 1).

Finally, Chytridiomycota richness was only positively correlated with Ca^{2+} content (slope = 1.4972 and $r^2 = 0.166$; Fig. S1).

The soil texture did not show any significant trend with many of the groups considered (Fig. S2), except for the negative correlation between coarse silt and relative richness of Basidiomycota (slope = −0.3057 and $r^2 = 0.158$) and Chytridiomycota (slope = −0.26648 and $r^2 = 0.1290$). Basidiomycota richness positively correlated with sand content (slope = 0.18235 and $r^2 = 0.156$) and negatively with fine silt (slope = −0.4353 and $r^2 = 0.130$). Finally, the richness of Chytridiomycota had a positive relationship with clay content (slope = 0.9277 and $r^2 = 0.323$).

3.2. Fungal communities composition

Total fungal community composition differed significantly among the six localities, as indicated by the MRPP analysis ($p = 0.001$, A = 0.1409) and illustrated by the two-dimensional NMDS ordination (Fig. 4a). Among the soil chemical parameters measured, pH showed the strongest correlation with fungal community structure, followed by Na^+ and CEC (Table S4). All four soil texture classes correlated significantly with fungal community composition, in order of decreasing importance: coarse silt, sand, clay, and fine silt content (Table S4).

The NMDS ordinations revealed different structuring of fungal communities at the level of functional groups (Fig. 4 b and c) and phyla (Fig. 4 d and e). The ordinations resulted in a 3-dimensional solution (stress: 0.156) for saprotrophic fungi and in 2-dimensional solution (stress: 0.155) for lichenized fungi, Ascomycota (0.180), and Basidiomycota (0.183). As for the total fungal community, MRPP revealed significant structuring among the sampling sites for saprotrophs (A = 0.1692), lichenized fungi (A = 0.1678), Ascomycota (A = 0.1485) and Basidiomycota (A = 0.167), all with $p = 0.001$ significance. The CEC and the pH were the only edaphic parameters that showed significant correlation with the community structure in all groups (Table S4). Also latitude was significant for all the groups (Table S4).

3.3. Effects of environmental parameters on fungal community composition

Partial Mantel tests indicated that soil physicochemical parameters had a strongly significant effect on community structure ($r = 0.245$, $p < 0.001$) when the geographic distance was accounted for (control matrix), while the effect of the geographic distance was substantially weaker, though still significant ($r = 0.155$, $p = 0.013$), when soil parameters matrix was controlled.

PerMANOVA analysis was used to quantify the degree to which edaphic physicochemical parameters could explain the distances in community composition among samples. First, variables were considered individually for the total community, the two functional groups, and the three phyla. For the total community, the K^+ content explained the greatest amount of variance, followed by the Mg^{2+} content and the pH (Table 2). Three out of the four soil texture classes (sand, coarse silt, and fine silt) explained a significant portion of variance, while the effect of clay content was not significant. When considering the functional groups and the three phyla separately, the percentages of variance explained for each soil parameter were lower, but in many cases they remained significant for shaping the structure of the communities.

When the environmental variables were combined to account for correlation among them, most of the edaphic parameters still contributed significantly to explain proportions of variance in the combined model in all fungal groups (Table 3). For the total community, K^+ content explained more than 53% of the observed

Table 1
Regression slopes for the variation of relative richness (S) and abundance of seven dominant classes in response to edaphic parameters (significant for $p < 0.05$; §marginally significant, $p < 0.1$; n. s. non-significant).

		Dothideomycetes		Eurotiomycetes		Lecanoromycetes		Leotiomycetes		Sordariomycetes		Agaricomycetes		Tremellomycetes	
		S	Abundance	S	Abundance	S	Abundance	S	Abundance	S	Abundance	S	Abundance	S	Abundance
Chemical parameters	C	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	N	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	C/N ratio	n. s.	n. s.	0.163	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−0.121§	n. s.	n. s.	n. s.
	CEC	n. s.	2.912	n. s.	n. s.	1.500	1.587	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−1.7579
	Na^+	n. s.	n. s.	−4.953	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	K^+	n. s.	n. s.	n. s.	n. s.	−26.944	n. s.	13.246	n. s.	n. s.	n. s.	9.034	n. s.	n. s.	33.532
	Ca^{2+}	n. s.	n. s.	1.256	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−0.981§	−0.888§	−0.912§	n. s.
	Mg^{2+}	3.231	n. s.	1.437§	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−1.354	−1.441	−1.357	n. s.
	pH	n. s.	−9.272	n. s.	n. s.	−6.989	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
Physical parameters	Sand	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	0.159	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	Coarse silt	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−0.334	n. s.	0.1598§	0.5231	n. s.	n. s.	n. s.	n. s.
	Fine silt	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	−0.265	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
	Clay	n. s.	n. s.	0.590	2.293	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.

variance in composition, Mg^{2+} content explained an additional 14%, followed by sand content (7.43%) and CEC (6.48%), while the remaining variables explained less than 5% each in the combined model. For saprotrophic fungi, pH, CEC and different exchangeable cations explained most of the variation in community composition, followed by clay and coarse silt content. At phylum level, pH and soil texture (in particular, silt and clay) explained the most variance, although the contribution of individual variables to the combined model differed among the phyla (Table 3).

4. Discussion

Our study presents a first deep molecular investigation of both symbiotic and free-living fungi associated with BSCs in continental Antarctica. The relatively low fungal richness values recorded can be compared to the ones of cyanobacterial components of Antarctic BSCs (Pushkareva et al., 2018) and are similar to values recorded in other Antarctic fungal community surveys, as those associated with cryptoendolithic lichen-dominated communities (Coleine et al., 2018a). The low biodiversity of Antarctic ecosystems is mainly due to the low temperature and water availability (Green and Broady, 2001), and complies with the trends reported globally for fungi, i.e., decreasing their richness from mid-latitudes to the poles (Tedersoo et al., 2014; Bahram et al., 2018), even though these studies did not include Antarctic samples.

This study also reveals the occurrence of several sequences that could not be identified even to phylum level, many of which could play important roles in these communities. Additionally, more than 40% of the OTUs found were present in only one out of 50 samples processed, indicating the high degree of patchiness of fungi, often seen in other biomes as well, and the need to carry out more BSC studies in these environments.

Most organisms in the analysed mycobiomes, both in terms of abundance and biodiversity, belong to Ascomycota, followed by Basidiomycota, as previously reported from both molecular and culture-dependent studies for soils of the Antarctic peninsula (Cox et al., 2016; Ji et al., 2016) and the McMurdo Dry Valleys (Wei et al., 2016), for rock communities in Victoria Land (Coleine et al., 2018a) and soils under mosses in ice-free coastal areas of the same region (Hirose et al., 2016).

Lecanoromycetes, Eurotiomycetes and Dothideomycetes are the prevalent classes in the BSCs analysed. Lecanoromycetes and Dothideomycetes were already counted as the dominant classes within the Antarctic endolithic microbial communities, known to include some of the most resistant and extremotolerant organisms (Coleine et al., 2018a).

Ascomycota comprise lichenized, saprotrophic and parasitic fungi (Dahlberg and Bültmann, 2013), which are the main functional groups in our dataset. Lichenized fungi are fundamental components of BSCs, while saprotrophic fungi are important for OM decomposition and nutrient recycling and can utilize the organic compounds released from the photosynthetic component of BSCs. These two functional groups have also been reported as the major colonizers of Antarctic lichen-dominated cryptoendolithic communities (Coleine et al., 2018b). Other groups with lower incidence and abundance in the samples, such as plant pathogens on mosses, and fungal and animal parasites, could be metabolically active in these ecosystems, while fungi known to be associated with vascular plants (mycorrhizal fungi) likely occur in these soils as dormant spores dispersed from other continents. It is well known that DNA metabarcoding also detects fungi that are present only as spores in the given sample. This has frequently been addressed in culture-based studies on Antarctic fungal communities, because of the ability of many fungi to remain viable in a dormant state for extended periods. Therefore, the detection of a particular fungal species in soil or other substrates is not considered a definitive evidence that the species could have an ecological role in these ecosystems (Arenz et al., 2014). The presence of cosmopolitan species of genera such as *Alternaria*, *Penicillium*, *Aspergillus*, also quite common in our dataset, are often attributed to their wide dispersal potential and even to the ubiquitous association with human structures and materials (Ruisi et al., 2007). Metabarcoding of DNA does not distinguish active organisms from relic DNA, which may represent a greater proportion of total DNA in low biomass soils (Carini et al., 2017). This effect could be exacerbated by the preservation of relic DNA in cold Antarctic soils (Willerslev et al., 2004). A recent study on maritime Antarctic soils showed that DNA-based studies may be biased towards cosmopolitan fungi, perhaps due to a higher proportion of inactive molecules for these fungi (Cox et al., 2019).

The simple Antarctic ecosystems, characterized by limiting harsh environmental conditions and presumably lower levels of competition than at lower latitudes, offer an ideal setting to study the role of edaphic parameters in shaping the diversity and functionality of fungal communities. Richness did not statistically differ among the sampling sites and localities and it was not correlated

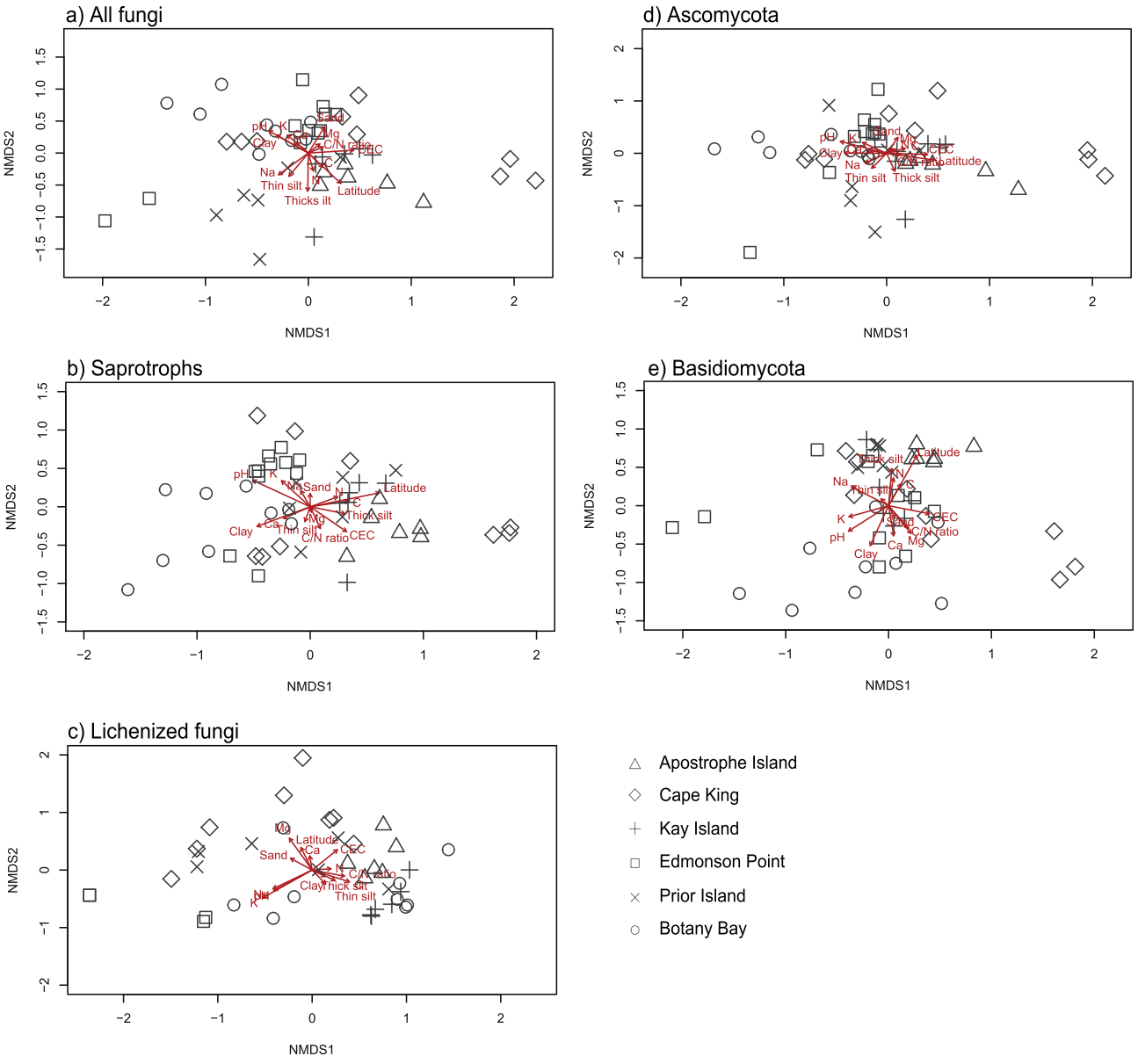

Fig. 4. Nonmetric multidimensional scaling (NMDS) ordinations of the differences (Bray–Curtis distance) in fungal communities composition (Hellinger transformed OTUs abundances) in the different localities for: the total fungal community (a), the two functional groups of saprotrophic and lichenized fungi (b, c) and the two dominant phyla (d, e). The significance and the strength of the correlation of all the variables in the figure are reported in Table S4.

with the apparent complexity of BSCs at the sampling sites either. Rather, fungal community composition differed significantly among localities and even among sites within the same locality. As shown via partial Mantel tests, the geographic distance had a significant effect on community composition, even if weaker than the edaphic parameters tested. These patterns are in agreement with certain levels of regional endemism previously reported for non-fungal Antarctic microorganisms, such as diatoms, green algae, cyanobacteria and other bacteria (Vyverman et al., 2010), and are likely driven by the environmental filtering due to differences in mesoclimatic and edaphic parameters both among and within localities.

Among the main edaphic parameters driving the composition of fungal communities, soil pH was among the most influential ones, affecting the composition of all functional and taxonomic groups studied. However, although soil pH was among the main drivers of fungal and bacterial diversity in both polar regions (Siciliano et al., 2014) and of fungal diversity and distribution at the global scale (Tedersoo et al., 2014; Bahram et al., 2018), it did not show a significant effect on the fungal richness in this study. Similarly, while soil C and N are known to affect fungal richness and C/N is a major predictor of fungal abundance and gene function on global and regional scales (Bahram et al., 2018), in this study their correlations with richness, abundance and community composition were weaker than that of several other edaphic parameters. PerMANOVA analysis highlighted that C and N content, and their ratio, even being significant for the communities structure, were not independent from the other physicochemical parameters considered. For these reasons, the distribution of fungi appears to be more linked to the mineral characteristics of the environment than to the presence of OM derived from BSCs.

Soil parameters found to be determinant for fungal community composition included the exchangeable cations, as K^{+}, explaining more than 53% of the observed variance, followed by Mg^{2+}.

Table 2
Proportion of variation in fungal community composition, at the level of the total community (all fungi), the two functional groups (saprotrophs and lichenized fungi) and the three major phyla (Ascomycota, Basidiomycota, and Chytridiomycota), explained by soil physicochemical parameters calculated independently with permutational multivariate analysis of variance, based on Hellinger-transformed fungal community matrix. Significant variables (in bold) were included in the final model for each fungal group (Table 3).

	All fungi		Saprotrophs		Lichenized fungi		Ascomycota		Basidiomycota		Chytridiomycota	
Variable	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*	Variance (%)	*p*
C	**17.94**	**0.001**	**6.40**	**0.001**	**8.81**	**0.001**	**5.79**	**0.002**	**4.36**	**0.043**	**11.96**	**0.001**
N	**9.94**	**0.017**	**4.02**	**0.028**	3.87	0.101	3.31	0.079	**4.88**	**0.026**	**10.29**	**0.001**
C/N ratio	**19.70**	**0.001**	**4.78**	**0.006**	2.67	0.425	**4.12**	**0.031**	4.12	0.051	**4.49**	**0.045**
CEC	**25.22**	**0.001**	**9.50**	**0.001**	**5.52**	**0.018**	**7.34**	**0.001**	**4.41**	**0.032**	3.79	0.085
Na^+	**16.31**	**0.003**	**7.37**	**0.001**	**5.12**	**0.029**	**5.93**	**0.004**	**5.39**	**0.014**	3.60	0.112
K^+	**53.11**	**0.001**	**7.76**	**0.001**	**8.33**	**0.001**	**7.73**	**0.001**	**6.10**	**0.004**	**9.10**	**0.001**
Ca^{2+}	**19.92**	**0.002**	**6.49**	**0.001**	**5.44**	**0.030**	**5.39**	**0.002**	**4.61**	**0.026**	3.94	0.082
Mg^{2+}	**30.57**	**0.001**	**6.38**	**0.001**	**9.36**	**0.002**	**5.66**	**0.004**	**4.85**	**0.019**	1.95	0.564
pH	**29.20**	**0.001**	**11.52**	**0.001**	**8.61**	**0.001**	**9.70**	**0.001**	**6.55**	**0.007**	**10.49**	**0.001**
Sand	**19.14**	**0.001**	**4.18**	**0.016**	4.31	0.076	**5.51**	**0.002**	**4.22**	**0.048**	**8.35**	**0.001**
Coarse silt	**15.50**	**0.004**	**6.85**	**0.001**	4.37	0.061	**6.27**	**0.001**	**8.23**	**0.001**	**16.04**	**0.001**
Fine silt	**19.19**	**0.002**	**4.07**	**0.018**	4.29	0.096	**5.41**	**0.006**	3.82	0.073	**6.61**	**0.004**
Clay	5.82	0.078	**6.89**	**0.001**	**5.06**	**0.032**	**5.13**	**0.010**	**7.07**	**0.003**	**4.59**	**0.034**

Previous reports on the effects of exchangeable cations on fungal diversity are that of Tedersoo et al. (2014) on the global diversity of fungi, and the one of Bahram et al. (2018) on global microbial diversity and function, although neither of them included samples from Antarctica. Tedersoo et al. (2014) reported Ca^{2+} as one of the most important predictors of fungal diversity at the global scale, even though it was not determinant for community composition. Ca^{2+} is important for many physiological processes in plants and microorganisms and can influence the turnover rate of soil OM (Reich et al., 2005). It had a significant influence on community composition in our dataset as well, but K^+ and Mg^{2+} had stronger effects, not reported at all at a global scale. The other cations affecting the composition or functionality of fungal communities in our study were not determinant either in Tedersoo et al. (2014), or in Bahram et al. (2018). Similar to the results of this study, a significant effect of K^+ on the diversity of fungi in maritime Antarctic soils has also been documented (Newsham et al., 2016), although other cations appeared more important in driving fungal composition in this Antarctic region.

In an earlier study carried out on different Antarctic soil environments with a culture-dependent approach, soil C and N were reported to have a direct effect on fungal community composition, while pH and salinity were thought to influence fungi indirectly (Arenz and Blanchette, 2011). Interestingly, in the same work, fungal diversity and abundance were negatively correlated with the total salinity, estimated through the electrical conductivity. Our study confirmed this trend for Na^+ and K^+ on Ascomycota, while abundance and diversity of Basidiomycota were positively influenced by both these cations. In Antarctic soils, Ca^{2+}, K^+, Mg^{2+} and Na^+ content has been shown to be less influenced by biological activity than by a suite of physical factors, including the proximity of marine sources, soil age and the degree of mineral weathering, which is extremely slow due to the scarcity of available liquid water (Campbell and Claridge, 1987). The high potential of fungi in promoting mineral weathering (reviewed in Hoffland et al., 2004) can support the nutrient needs of the total BSCs community and their symbiotic primary producers, explaining the strong connection of the fungal community with the presence of such cations.

The CEC and the content of minerals had a profound influence on the composition of the total fungal community and of some taxonomic and functional groups studied, as well as on their richness and relative abundance. The CEC of soils varies according to the clay percentage, pH, OM amount, and nature of soil substrate (Brady and Weil, 2002). The greater the amount of clay in soil, the

Table 3
Proportion of variation in fungal community composition, at the level of the total community (all fungi), the two functional groups (saprotrophs and lichenized fungi) and the three major phyla (Ascomycota, Basidiomycota, and Chytridiomycota) explained by soil physicochemical parameters added sequentially (first to the last) in a model, depending on their independent influence in the variance, as reported in Table 2. Significant values in bold.

All fungi			Saprotrophs			Lichenized fungi			Ascomycota			Basidiomycota			Chytridiomycota		
Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*	Variable	Variance (%)	*p*
K^+	**53.12**	**0.0001**	pH	**11.52**	**0.0001**	Mg^{2+}	**9.36**	**0.0001**	pH	**9.70**	**0.0001**	Coarse silt	**8.23**	**0.0001**	Coarse silt	**16.03**	**0.0001**
Mg^{2+}	**14.28**	**0.0001**	CEC	**7.80**	**0.0001**	C	**6.50**	**0.0003**	K^+	**4.40**	**0.0003**	Clay	**6.67**	**0.0001**	C	2.60	0.0652
pH	**3.21**	**0.0001**	K^+	**4.38**	**0.0001**	pH	**6.49**	**0.0001**	CEC	**4.95**	**0.0004**	pH	**5.19**	**0.0007**	N	1.64	0.3361
CEC	**6.48**	**0.0001**	Na^+	**3.63**	**0.0011**	K^+	**6.09**	**0.0003**	Coarse silt	2.26	0.0674	K^+	**4.39**	**0.0037**	pH	**5.80**	**0.0003**
Ca^{2+}	**1.77**	**0.0002**	Clay	**4.67**	**0.0001**	CEC	**7.40**	**0.0001**	Na^+	**4.59**	**0.0001**	Na^+	**6.65**	**0.0001**	K^+	**5.28**	**0.0010**
C/N ratio	**1.60**	**0.0001**	Coarse silt	**4.54**	**0.0001**	Ca^{2+}	**3.64**	**0.0222**	C	**5.97**	**0.0001**	N	0.38	0.9751	Sand	**3.88**	**0.0082**
Fine silt	**4.41**	**0.0001**	Ca^{2+}	**5.85**	**0.0001**	Na^{2+}	**4.98**	**0.0008**	Mg^{2+}	**4.56**	**0.0002**	Mg^{2+}	**3.93**	**0.0065**	Fine silt	**4.71**	**0.0013**
Sand	**7.43**	**0.0001**	C	**3.29**	**0.0016**				Sand	**4.77**	**0.0001**	Ca^{2+}	**8.25**	**0.0001**	Clay	**6.30**	**0.0003**
C	0.24	0.1115	Mg^{2+}	**3.84**	**0.0005**				Fine silt	**4.97**	**0.0001**	CEC	**4.61**	**0.0020**	C/N ratio	2.71	0.0620
Na^{2+}	**3.58**	**0.0001**	C/N ratio	2.01	0.0909				C/N ratio	**2.96**	**0.0141**	C	0.92	0.7356			
N	0.16	0.2069	N	2.07	0.0740												

greater its ability to retain water and nutrients and provide more favourable conditions for microbial activity. Instead, sandy soils, such as the ones in our samples, are characterized by very low OM and CEC. Therefore, it could be hypothesized that in these extreme conditions the low capacity of the soil to make cations available for living organisms is likely to be a limiting factor for the colonization and survival of fungi and other microbes. In such limiting environments, even small changes in pH values, due to environmental changes, can dramatically affect the CEC and the consequent availability of exchangeable cations, strongly influencing fungal communities. On the other hand, pH, soil texture and associated soil CEC have all been shown to be influenced by the development of BSCs in arid environments (Anderson et al., 1982; Chamizo et al., 2012), which could also explain their link with fungal community composition, given the effect that they could exert on primary production and the entire BSCs community.

Regarding the functionality of the communities, even if direct reports on saprotrophic fungi are not available for Antarctic soils, there have been a few studies on the influence of environmental factors on decomposition rates. One of these studies reported that pH and C/N ratio, mainly dependent on the quality of the substratum, affected the decomposition rates in two moss communities in maritime Antarctica (Davis, 1986). In our study, the saprotrophic community was mainly influenced by abiotic parameters, particularly soil pH, followed by CEC, exchangeable cations and soil texture, while C/N ratio, although significant, was not independent from other parameters in determining community composition. Globally, saprotrophic fungal community composition is strongly influenced by pH, and richness by Ca^{2+} content (Tedersoo et al., 2014), while in our dataset Ca^{2+} content only correlated with community composition and not with richness.

For lichenized fungi, Mg^{2+} and K^{+} were the main cations driving the community composition, in agreement with previous studies (Kranner et al., 2008). In fact, taking into account the well-known drought tolerance of lichens, it has been reported that the recovery of activity after rewetting depends on the quality of the habitat, and is mainly due to the loss of K^{+} and Mg^{2+} during desiccation (Kranner et al., 2008). We hypothesize that in Antarctica, where lichens have to cope with a combination of fluctuating conditions of water availability and low CEC, K^{+} and Mg^{2+} cation availability may be further limiting factors for their development. Additionally, one further factor connected with the community composition of lichenized fungi is soil C content, that also has a positive correlation with richness. This may be connected with primary production capacity of lichens, that also affects the quality of the soil OM, resulting in a positive correlation between C/N ratio and lichenized fungal richness and, to a lesser extent, their abundance.

This study is the first comprehensive taxonomic and functional characterization of fungal communities in the continental Antarctica biological soil crust system. Although the richness values reported here are substantially lower than those reported from soils in various biomes, including the High Arctic, using the same DNA metabarcoding methodology (e.g., Geml et al., 2012; Tedersoo et al., 2014; Timling et al., 2014; Grau et al., 2017; Bahram et al., 2018; Canini et al., 2019; Geml, 2019), they are nonetheless impressive when considering the very thin soil layer, the harshness of the environmental conditions and the paucity of vegetation and other eukaryotes (Zucconi et al., 2016). Our study provides further insights into the effects of abiotic factors on fungal diversity and community composition, highlighting a stronger effect of pH and the presence of exchangeable cations, namely K^{+} and Mg^{2+}, as driving factors. This baseline characterization is of particular relevance also in the context of global warming, as temperature increase is expected to affect fungal community assemblages, mainly due to increases in the amount of available water that will affect soil conditions and to the possible invasion of more competitive alien species (Turner et al., 2014).

Author contributions

LZ and FC designed the experiment. LZ, LPD and SV collected the samples during the XXXI Italian Antarctic Expedition (2015–16). FC performed the DNA extraction. LPD performed soil analyses. FC and JG performed the data processing and analyses. FC, LZ and JG wrote the paper with inputs from LPD, LS, SO and SV.

Data accessibility

DNA sequences object of this work were submitted to NCBI gene bank MK536607 - MK537296.

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Supplementary data

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