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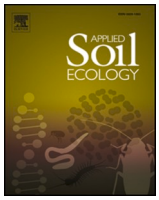

Research paper

Cold-based glaciers and underlying permafrost: A possible new habitat for fungi

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ABSTRACT

Recent studies have increasingly focused on cold-adapted microbial communities in polar and sub-polar habitats, including the effects of global warming. Among them, much research has concentrated on bacterial communities, while fungi, which are essential for carbon and nitrogen cycling, remain sometimes little considered. This study investigates how the structure of fungal communities (including yeast and filamentous life forms) in an Antarctic coastal cold-based glacier and in the underlying permafrost (which is an understudied habitat exhibiting challenging conditions for fungal life) is driven by both the key abiotic drivers and by the putative presence of ecological cooperation among dominant taxa.

Results revealed a total of 337 fungal OTUs across the different layers (ice, brine, and permafrost): overall, yeasts dominated over filamentous life forms. *Meyerozyma* and *Taphrina* were found as the dominant genera in the deepest layers, suggesting the existence of a potential ecological cooperation finalized in a mutual support for survival in such extreme conditions.

Beta-diversity analysis showed significant ($P < 0.05$) differences between fungal communities in glacier ice and permafrost, with brine as a transitional layer. Some abiotic parameters (i.e., Ca^{2+}, Cl^{-}, EC, K^{+}, Mg^{2+}, Na^{+}, NO_3^{-}, SO_4^{2-}, and total nitrogen) were the key factors affecting the structure of fungal communities in the permafrost samples.

This research offers new insights into fungal biodiversity between Antarctic cold-based glaciers and underlying permafrost, suggesting ecological equilibria affected by abiotic parameters. The dominance of *Meyerozyma* and *Taphrina* OTUs provides evidence of relict fungal taxa capable of long-term survival under extreme conditions over time.

1. Introduction

Recent studies underscore the microbiological importance of cold-based glaciers. Basal ice layers, even in their frozen state with limited nutrients, provide unique habitats supporting active microbial life through heterotrophic respiration, even at temperatures around −15 °C (Montross et al., 2014). Microbiome studies revealed the presence of distinct microbial assemblages in basal ice types, indicating that physicochemical factors and ice composition can shape microbial diversity (Montross et al., 2014). Research on cold-adapted yeasts from Asian cold-based glaciers reveals a high degree of taxonomic and ecological diversity (Luo et al., 2019). As an example from previous studies, debris-rich basal ice from Taylor Glacier, a cold-based glacier located in the McMurdo Dry Valleys of Antarctica, has been shown to host potentially active microbial communities, as indicated by high ATP concentrations, isotopic gas signatures, and high 16S rRNA/rDNA ratios (Doyle and Christner, 2022). This finding suggests the presence of metabolically active bacterial taxa such as *Paenisporosarcina* spp., *Desulfocapsa* spp., *Syntrophus* spp., and *Desulfosporosinus* spp., which play ecological roles in subglacial environments including sulfate reduction, syntrophic

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interatcions, and sporulation-based survival. This example highlights how extreme glacial habitats can support diverse and functionally relevant microbial life.

Microbiological studies on permafrost were generally focused on the active layer because it represents a unique and globally significant component of some terrestrial ecosystems (i.e. polar and sub-polar areas) (Jones et al., 2023). Studies on the biology of the active layer of permafrost have significantly increased due to growing concerns about the impact of global warming and the resulting thawing. In these cold environments, fungi play a crucial role as decomposers, contributing to the turnover of organic matter and the recycling of nutrients, even under subzero conditions. Their ability to remain viable and metabolically active in frozen or intermittently thawed substrates makes them key players in shaping microbial community dynamics and biogeochemical processes in glacier and permafrost ecosystems (Tiquia-Arashiro and Grube, 2019; da Silva et al., 2020). This phenomenon has the potential to amplify global climate change due to the release of soil organic carbon (Biskaborn et al., 2019), which can activate indigenous microorganisms (Margesin and Collins, 2019; Jiang et al., 2023; Wood et al., 2024). Despite its harsh conditions (e.g. oligotrophic state, low water availability, and frozen state) the permafrost can harbor some forms of extremophilic microbial life (Gilichinsky et al., 2008; Mackelprang et al., 2016; de Menezes et al., 2024; Martin et al., 2024). Right now, most microbiological studies on permafrost are specifically focused on bacterial communities, while fungal life forms (including yeasts) have often been understudied, despite their pivotal role as key organic matter decomposers, nutrient recyclers, and CO_2 producers (Peralta et al., 2017; Vero et al., 2019; Sannino et al., 2020; Sannino et al., 2023).

Deeper Antarctic permafrost cores can provide insights into the glaciological and geological history of the continent, helping in the reconstruction of past climate conditions and prediction of future changes in response to ongoing global warming (French, 1999; Forte et al., 2025). Permafrost underlying glaciers remains significantly understudied (Montross et al., 2014; Luo et al., 2019; Doyle and Christner, 2022) largely due to the technical and logistical challenges associated with its inaccessibility. In general, deep permafrost cores (≥10 m) have been collected and analyzed in only a limited number of Arctic sites, and such efforts are even scarcer in Antarctica (Kochkina et al., 2012).

In this framework, this study is focused on the fungal diversity of a permafrost core under a coastal cold-based glacier collected in Antarctica in comparison with the above glacier core, with the following scientific questions: i) are there significant differences between the fungal diversity found in the overlying glacier and that in the underlying permafrost? ii) What are the abiotic parameters putatively driving these communities? iii) Are there co-occurrences among the most abundant fungal genera found in the different layers? iv) Is the underlying permafrost dominated by specific fungal taxa? Answering these questions would provide an important contribution to understanding the structure of fungal communities on permafrost under a coastal cold-based glacier, which could reveal the presence of fungal taxa that have survived under extreme conditions, offering valuable information

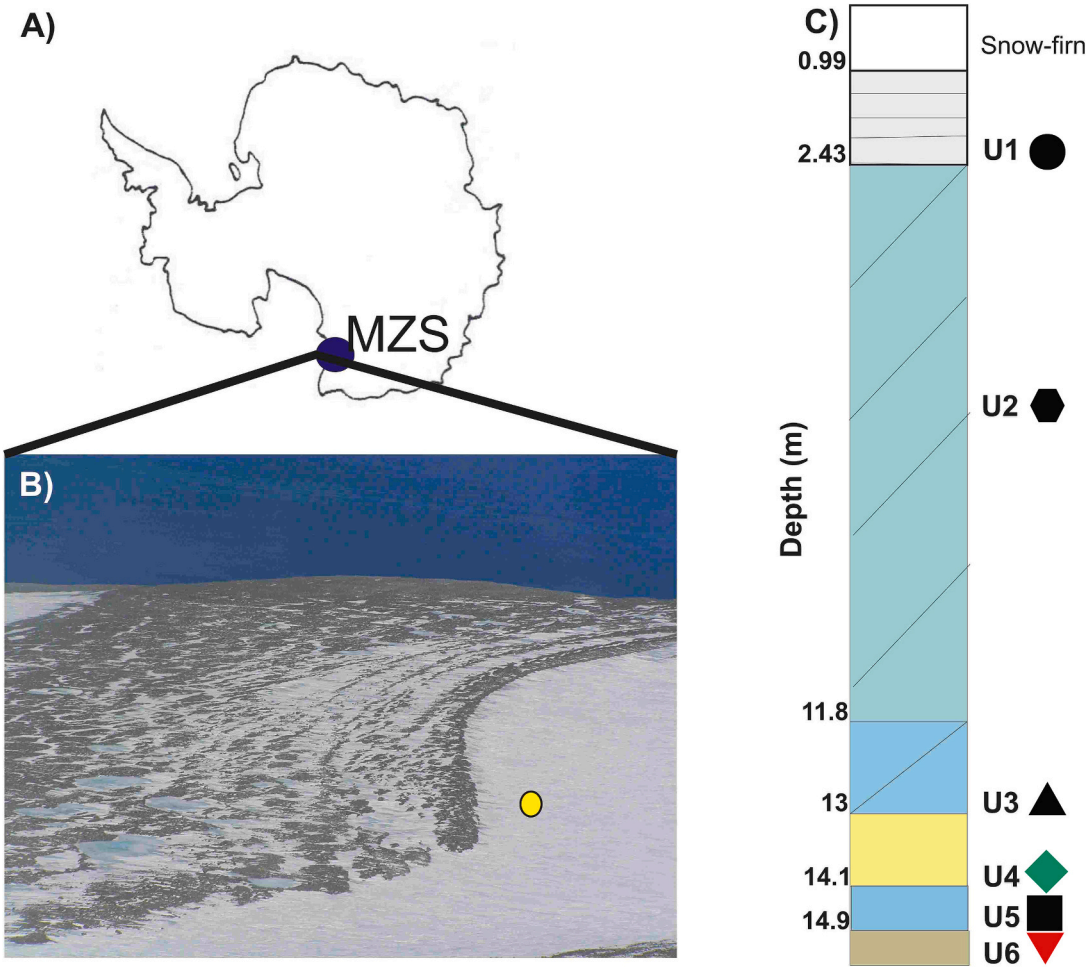

Fig. 1. Study site and ice and permafrost core: A) location of Mario Zucchelli Station in continental Antractica; B) View from south of the Boulder Clay glacier and its till coverage on the left (the yellow dot indicates the location of the core); C) Ice and permafrost core (see the text for the description of the units. (credit Mauro Guglielmin).

for research on extremophilic life forms.

2. Material and methods

2.1. Sampling area and sample collection

An ice and permafrost core, reaching a depth of 15.6 m was collected from the Boulder Clay Glacier in Antarctica (74.44 S 164.01 E), just a few kilometers from the Mario Zucchelli Italian Station (MZS). The core was divided into six units (U) according to their glaciological and cryological characteristics recorded in the field that takes account of the density and the types of bubbles and debris embedded in the glacier ice and the ice content in the permafrost (Fig. 1). A variable number of samples (for chemical, physical, and microbiological studies) have been taken for each unit in dependence of: i) the length of the unit and ii) the heterogeneity of the layer (different types and content of ice). The samples were immediately transferred to the laboratories of MZS and stored at −20 °C, and subsequently shipped to the University of Varese, maintaining the −20 °C temperature throughout the entire transport process.

The U1, from 0.99 to 2.435 m depth (number of samples = 3) underlies below 98 cm of snow and firn. It is characterized by bubbly ice with a few sparse clasts, with almost horizontal stratification, and according to Forte et al. (2025) was dated as recent ice that should be younger than 900–989 calibrated years before present (years cal BP). The U2, from 2.435 to 11.8 m depth (number of replicates = 5) is characterized mainly by bubbly ice with sparse clasts but with quite steep stratification and with a layer between 5.45 and 6.58 m of clean ice and with the deepest part (below 8.93 m) in which the bubbles are almost always elongated. At a depth of 11.8 m, some stems of mosses were found (Forte et al., 2025), dated 1060 ca. years cal BP and therefore a bit older of the U1 (U2 was classified as old ice). The U3, from 11.8 to 13.0 m depth (number of replicates = 5) is characterized by bubbly ice with a few clasts and in its uppermost part some bubbles are elongated, and was classified as deep old ice. The U4, from 13.0 to 14.1 m depth (number of replicates = 5) was classified as brine slush because it was composed by salty ice partially melted, observed at the time of drilling. The U5, from 14.7 to 14.9 m depth (number of replicates = 3), is characterized by clean ice with a chemical composition intermediate between permafrost and brine slush, or similar to the latter, and is classified as basal ice. The U6, from 15.0 to 15.6 m depth (number of replicates = 9), is classified as permafrost and composed of frozen till with a sandy-silty matrix. The frozen till of Unit 6 is reasonably dated older than 32 kyear cal BP, based on the age of shells embedded within (Guglielmin, unpublished data).

2.2. Chemical and physical analyses

All ice, brine, and permafrost samples were melted and centrifuged at 3000 rpm for 10 min. The liquid fractions were analyzed after filtration through a PTFE membrane with a pore size of 0.45 μm. Electrical Conductivity and pH measurements were carried out using a pH/Conductivity Meter (Hanna Instruments – HI 2210). Anions (F^-, Fluoride ion; Cl^-, Chloride ion; NO_3^-, Nitrate ion; and SO_4^{2-}, Sulphate ion) were analyzed using ion chromatography (Metrohm 761 Compact IC Chromatography) equipped with an Anion Supp/4 column (particle size 5 μm; eluent: HCO_3^-/CO_3^{2-} buffer 1.7/1.8 mM) IC-Ag cartridges (Metrohm) were used to remove the halides (mainly chlorides) to obtain a better estimation of the anions present at lower concentrations. For cation analyses (Na^+, Sodium ion; K^+, Potassium ion; Mg^{2+}, Magnesium ion; and Ca^{2+}, Calcium ion), a Cation 1–2 column (particle size 7 μm; eluent: HNO_3 3 mM) was used. When necessary, the samples were properly diluted (1:100 or 1:1000) in ultrapure water to fit the calibration range. Five blanks of ultrapure water were analyzed to correct the final concentration according to the dilution factor. Dissolved organic (DOC) and inorganic (DIC) carbon, and total nitrogen (TN) were analyzed without dilution using a Total Organic Carbon Analyzer (Model TOC-L Shimadzu 5050 A) following the instructions provided by the manufacturer.

2.3. Fungal isolation, DNA extraction, and NGS sequencing

Before microbiological analyses, ice and permafrost samples were surface-decontaminated in the laboratory to remove any external microorganisms that may have been introduced during drilling procedures using sodium hypochlorite, followed by multiple rinses with sterile water according to Rogers et al. (2004). Ice samples (i.e., U1, U2, U3, and U5) and brine samples (U4) were aseptically filtered to collect microbial biomass on sterile cellulose acetate filters (cut-off: pore size 0.2 μm, Sartorius Stedim, Biotech, Gottingen, Germany).

To assess the presence of viable fungal communities, a culture-based isolation method was employed. Approximately 1 mL of filtered ice and brine, and 1 g of permafrost were inoculated onto RBA (Rose Bengal Agar) medium supplemented with chloramphenicol (100 mg/L) to inhibit bacterial growth and selectively promote fungal development. Plates were incubated at 4 °C and 15 °C for more than 30 days. Emerging colonies were monitored periodically. To obtain pure cultures, individual colonies were isolated and subsequently subcultured at least twice on fresh RBA plates under the same incubation conditions.

Total DNA from ice and brine samples was extracted from the filters using the Power Water DNA Isolation Kit (Qiagen, Hilden, Germany). For permafrost samples (U6), DNA was extracted from 0.25 g (wet weight) using the Power Soil Pro DNA Isolation Kit (Qiagen, Hilden, Germany). DNA concentrations were measured using a Qubit 3.0 Fluorometer Assay (Life Technologies Corporation, Carlsbad, CA, USA). For the samples with a concentration of DNA < 1 ng/μL, two separate extractions were performed, and the DNA was pooled before amplification.

Amplicons of the fungal internal transcribed spacer region 2 (ITS2) were obtained using the primers IlluAdp_ITS3_NeXTf 5'-CATCGATGAAGAACGCAG-3′ and IlluAdp_ITS4_NeXTr 5'-TCCTCCGCTTATTGATATGC-3′ (Tedersoo et al., 2015). PCR products were sequenced using the Illumina MiSeq platforms, following the standard protocols provided by Biofab Research s.r.l. (Rome, Italy).

2.4. Bioinformatics and statistical analyses

Raw data qualities were assessed using FastQC (Andrews, 2010). Sequence data were pre-processed, quality filtered, trimmed, de-noised, merged, modelled, and analyzed using DADA2 within QIIME2 (Bolyen et al., 2019). Chimeras were discarded using the 'consensus' method (Callahan et al., 2016). Sequence variants were clustered into operational taxonomic units (OTUs) using VSEARCH with a cut-off of 97 % (Rognes et al., 2016). UNITE+INSD (version 9.0) was used as the reference database for the taxonomy annotations against the representative sequences (Nilsson et al., 2019). Sequences were archived in the NCBI SRA database linked to BioProject accession number PRJNA1170930.

Statistical analyses were performed by the open-source software R version 4.03 (R Core Team, 2021). Graphical representations were generated with the R package ggplot2. Differences among chemical and physical parameters, as well as fungal alpha diversity (Richness and Shannon-H indexes) were assessed using ANOVA followed by Tukey's post hoc pairwise multiple comparison procedure. Fungal beta diversity was calculated using the permutational multivariate analysis of variance (PERMANOVA) by the Adonis function in the vegan package in R. The Envfit function was used to identify the chemical and physical parameters affecting fungal community composition. The Linear discriminant analysis Effect Size (LEfSe) algorithm (LDA score ≥ 2 and p value <0.05) was used to identify fungal genera driving differences between ice and permafrost samples (Segata et al., 2011).

Fungal functional assignments were determined by FunGuild (Nguyen et al., 2016).

Relationships between fungal communities and abiotic parameters were analyzed using Pearson correlation supported by the Mantel test in R. Co-occurrences between fungal genera (with a relative abundance >1 %) were analyzed separately for ice and soil sol habitat (permafrost and slush) using Pearson correlation coefficient and visualized in Cytoscape (Shannon et al., 2003). Only significant correlations (p value <0.05) were considered.

3. Results

3.1. Chemical and physical analyses

The analysis of chemical and physical parameters exhibited significant ($P < 0.05$) differences among the six units (U1-U6) of the core studied. No significant differences were observed among units U1, U2, and U3 for EC, DOC, DIC, TN, F^-, SO_4^{2-}, Na^+, and Ca^{2+}. Several parameters (i.e. EC, Cl^-, SO_4^{2-}, Na^+, K^+, Mg^{2+}, and Ca^{2+}) increased with depth, reaching significantly ($P < 0.05$) higher values in U6. Conversely, a significantly ($P < 0.05$) highest values of DOC and DIC were observed in U5, while U3 exhibited the most alkaline conditions. TN and NO_3^- followed a similar trend: units U1, U2, and U3 were significantly ($P < 0.05$) different from units U4, U5, and U6 (Supplementary Table 1).

3.2. Fungal isolation

To investigate the presence of viable fungal communities within the ancient Antarctic permafrost and associated ice and brine units, culture-based isolation experiments were performed. This approach enables the direct observation of fungal growth under controlled conditions, providing unequivocal evidence of the presence of microbial viability in cryogenic environments (Supplementary Fig. 1). An extremely slow growth rates were observed, and a total of ten isolates were stored for future studies.

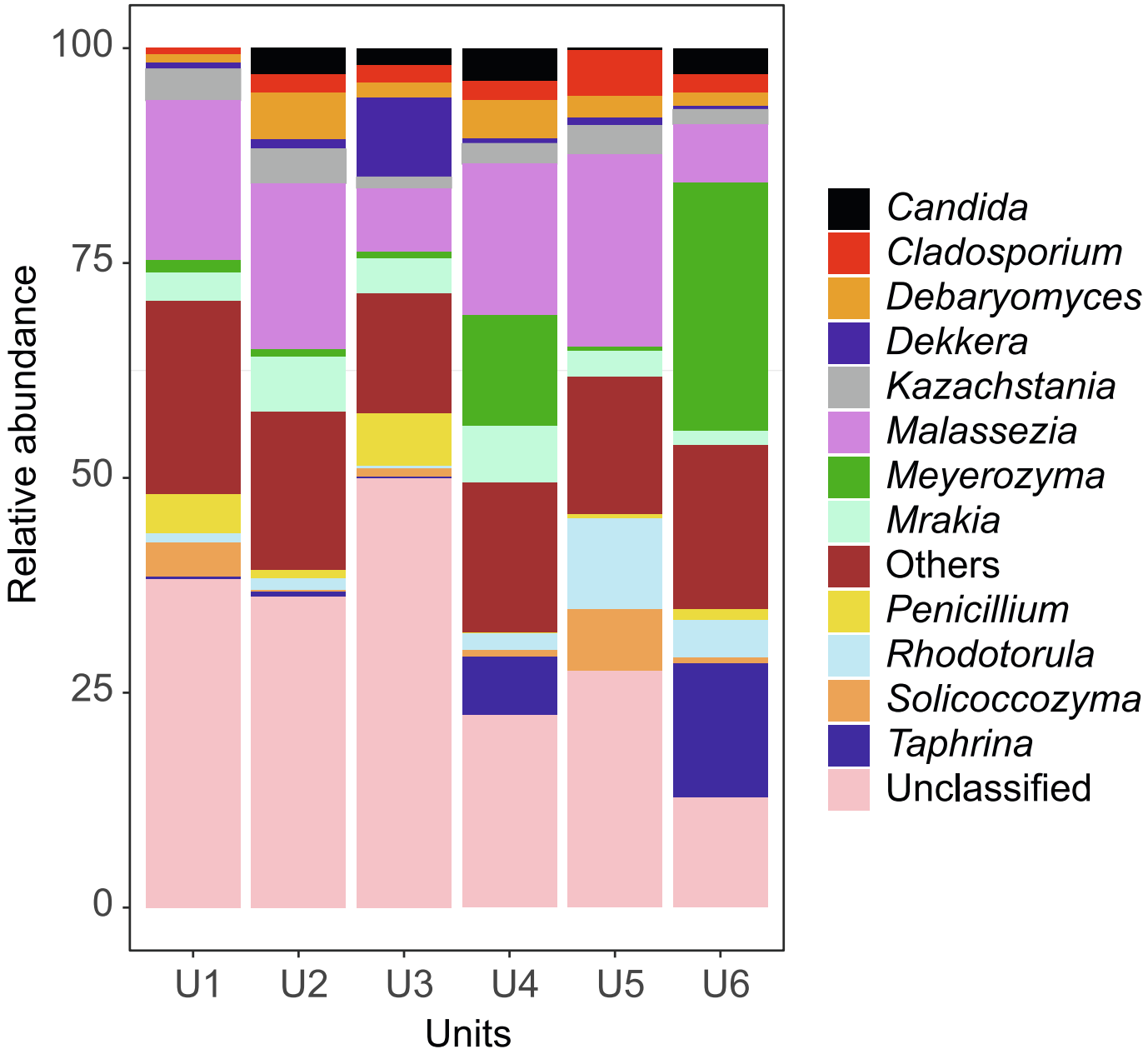

Fig. 2. Fungal diversity at the genus level in the six units (U1-U6) of the ice and permafrost core. Only genera with a relative abundance >2 % are reported.

3.3. Fungal communities and environmental associations

3.3.1. Fungal taxonomic structure

The bioinformatic analyses reported 309,720 fungal reads (10,324 fungal reads for each sample after rarefaction) organized into 337 fungal OTUs. These OTUs were analyzed by considering fungal growth morphology splitting the OTUs between filamentous and yeast lifestyles. Following this subdivision, it was observed that fungal communities were dominated by yeast life forms (Supplementary Fig. 2 A). At the phylum level, the abundance of Ascomycota and Basidiomycota varied across the six units. Ascomycota dominated in U3 and U6, while Basidiomycota dominated in U1, U2, U4, and U5. (Supplementary Fig. 2B). The ratio between Ascomycota and Basidiomycota for both filamentous fungal and yeast taxa is reported in Supplementary Figs. 2C and 2D, respectively. In both cases, Ascomycota were more abundant in U6.

At the genus level, *Malassezia* (present in all units), *Meyerozyma*, and *Taphrina* (notably in U4 and U6) were the most abundant. The combined OTUs assigned to *Meyerozyma* and *Taphrina* accounted for 12.9 and 6.8 % in U4, and 28.9 and 15.6 % in U6, respectively (Fig. 2). Other genera with a relative abundance greater than 2 % included: *Candida* (in U2, U4, and U6), *Cladosporium* (in U5), *Debaryomyces* (in U2 and U4), *Dekkera* (in U3), *Kazachstania* (in U1 and U2) and *Penicillium* (in U1 and U3) among Ascomycota, and *Mrakia* (in U1, U2, U3, and U4), *Rhodotorula* (in U5 and U6) and *Solicoccozyma* (in U1 and U5) among Basidiomycota. All genera with a relative abundance of less than 2 % were included in "Others" (Fig. 2).

The distribution of trophic modes in the six units (U1 to U6) reveals the dominance of pathotroph-saprotrophs (in U1), saprotrophs (in U1, U2, U4, U5, and U6), pathotroph-saprotroph-symbiotrophs (in U2, U3, U4, U5, and U6), and pathotroph (in U6) (Supplementary Fig. 3).

3.3.2. Alpha and beta-diversity

The Richness and Shannon-H indexes indicated no significant differences in alpha diversity among the six units of the permafrost core (Supplementary Table 2). In contrast, beta diversity analysis revealed a significant dichotomy ($P < 0.05$) between permafrost and ice samples, while brines appeared to serve as a transitional layer between ice and permafrost, showing no significant differences (Fig. 3 and Supplementary Table 3). LEfSe analysis identified two sets of genera whose relative abundance is responsible for significant ($p < 0.05$) dichotomy observed between permafrost and ice samples (listed in decreasing order of LDA score): i) *Meyerozyma*, *Taphrina*, *Rhodotorula*, *Cryptococcus*, *Candida*, *Pichia*, *Lachancea*, *Filobasidium*, *Penicillium*, and *Vishniacozyma* in permafrost samples; and ii) *Malassezia*, *Aspergillus*, *Kazachstania*, *Issatchenkia*, *Dekkera*, and *Mrakia* in ice samples (Fig. 4).

3.3.3. Environmental drivers affecting fungal communities

Envfit analysis identified several abiotic parameters, namely EC, Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}, NO_3^{-}, SO_4^{2-}, and TN as key factors affecting the structure of fungal communities in the permafrost samples (Fig. 3 and Supplementary Table 4). To further validate these associations, correlation analyses (based on Pearson's coefficient and supported by the Mantel test) were performed between the most abundant genera and abiotic parameters. The mantel test results revealed several statistically significant associations between environmental parameters and fungal community composition across different genera. Considering the highly

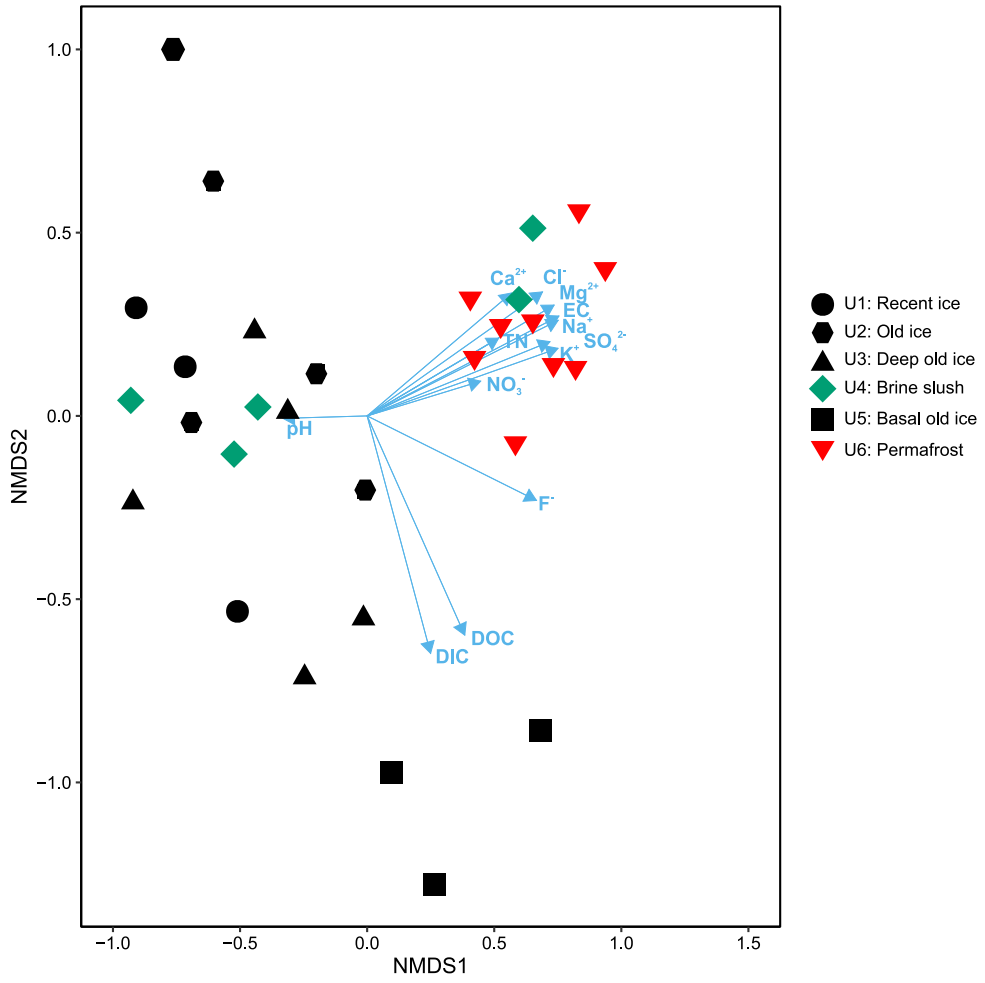

Fig. 3. Non-Metrical Dimension Scaling (NMDS) ordination and Envfit function reporting the influence of chemical/physical parameters on fungal communities.

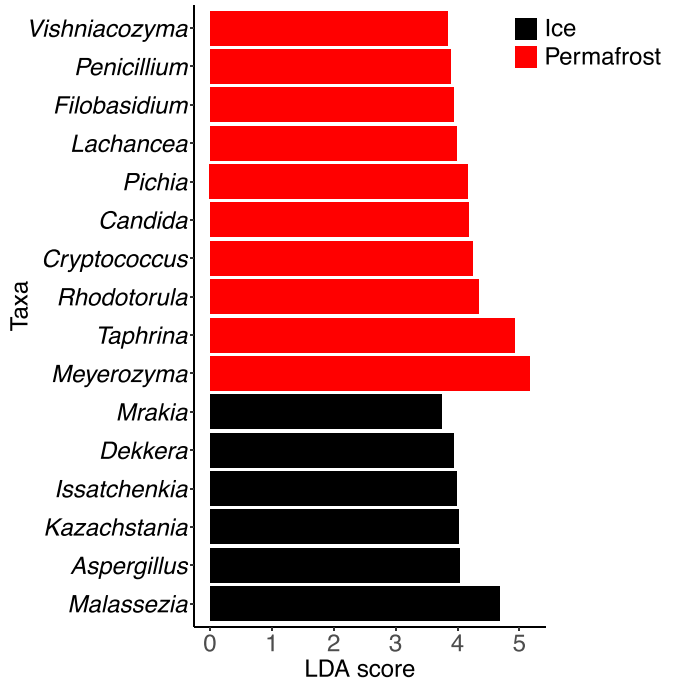

Fig. 4. Linear discriminant analysis Effect Size (LEfSe) analysis reporting the fungal genera characterizing the differences between ice and permafrost samples.

significant associations (Mantel's $P < 0.01$), the genera *Aspergillus* ($r = 0.34$, $P = 0.006$), *Debaryomyces* ($r = 0.24$, $P = 0.027$), and *Issatchenkia* ($r = 0.27$, $P = 0.025$) showed positive correlations with pH. *Cladosporium* was positively associated with DOC ($r = 0.285$, $P = 0.034$) and DIC ($r = 0.309$, $P = 0.033$). *Meyerozyma* displayed strong correlations with EC ($r = 0.69$, $P = 0.001$), TN ($r = 0.39$, $P = 0.001$), F^- ($r = 0.23$, $P = 0.005$), Cl^- ($r = 0.69$, $P = 0.001$), NO_3^- ($r = 0.29$, $P = 0.006$), SO_4^{2-} ($r = 0.57$, $P = 0.001$), Na^+ ($r = 0.67$, $P = 0.001$), K^+ ($r = 0.58$, $P = 0.001$), Mg^{2+} ($r = 0.70$, $P = 0.001$), and Ca^{2+} ($r = 0.55$, $P = 0.001$). *Pichia* was significantly associated with Mg^{2+} ($r = 0.27$, $P = 0.012$), EC ($r = 0.21$, $P = 0.017$), Ca^{2+} ($r = 0.36$, $P = 0.040$), and Cl^- ($r = 0.20$, $P = 0.048$). *Rhodotorula* correlated positively with DOC ($r = 0.44$, $P = 0.045$). *Taphrina* exhibited strong positive correlations with EC ($r = 0.65$, $P = 0.001$), Cl^- ($r = 0.65$, $P = 0.001$), SO_4^{2-} ($r = 0.51$, $P = 0.001$), Na^+ ($r = 0.61$, $P = 0.001$), K^+ ($r = 0.52$, $P = 0.001$), Mg^{2+} ($r = 0.66$, $P = 0.001$), and Ca^{2+} ($r = 0.55$, $P = 0.001$) (Fig. 5 and Supplementary Table 5).

3.3.4. Correlations analysis among fungal genera

Significant correlations ($P < 0.05$), in the form of specific co-occurrences, were identified among fungal genera with a relative abundance greater than 2 % separately from ice and soil habitats (permafrost and brine slush) of the core. Most of the correlations involved yeast genera, with almost all being yeast vs. yeast correlations. In the ice samples only positive significant ($P < 0.05$) correlations were found. These correlations included: i) *Mrakia* vs. *Debaryomyces*; ii) *Solicoccozyma* vs. *Rhodotorula*; iii) *Dekkera* vs. *Cladosporium* and *Pichia*, and *Pichia* vs. *Penicillium*; iv) *Issatchenkia* vs. *Aspergillus* and *Kazachstania* (Supplementary Fig. 4 A and Supplementary Table 6).

In the soil habitats (permafrost and brine slush) the yeast genera *Meyerozyma* and *Taphrina* exhibited a significant ($P < 0.05$) positive correlation (strength = 0.96) (Supplementary Fig. 4B and Supplementary Table 6). Additional positive correlations were observed between i) *Meyerozyma* vs. *Rhodotorula*; ii) *Malassezia* vs. *Aspergillus* and *Mrakia*; iii) *Mrakia* vs. *Aspergillus*; iv) *Taphrina* vs. *Rhodotorula*; v) *Issatchenkia* vs. *Penicillium*; (Supplementary Fig. 4B and Supplementary Table 6). Negative correlations included: i) *Malassezia* vs. *Meyerozyma*, and *Taphrina*; and ii) *Mrakia* vs. *Taphrina*, *Rhodotorula* and *Meyerozyma*; iii) *Aspergillus* vs. *Taphrina* and *Meyerozyma* (Supplementary Fig. 4B and Supplementary Table 6).

4. Discussion

The results reported in the present study emphasize the current view

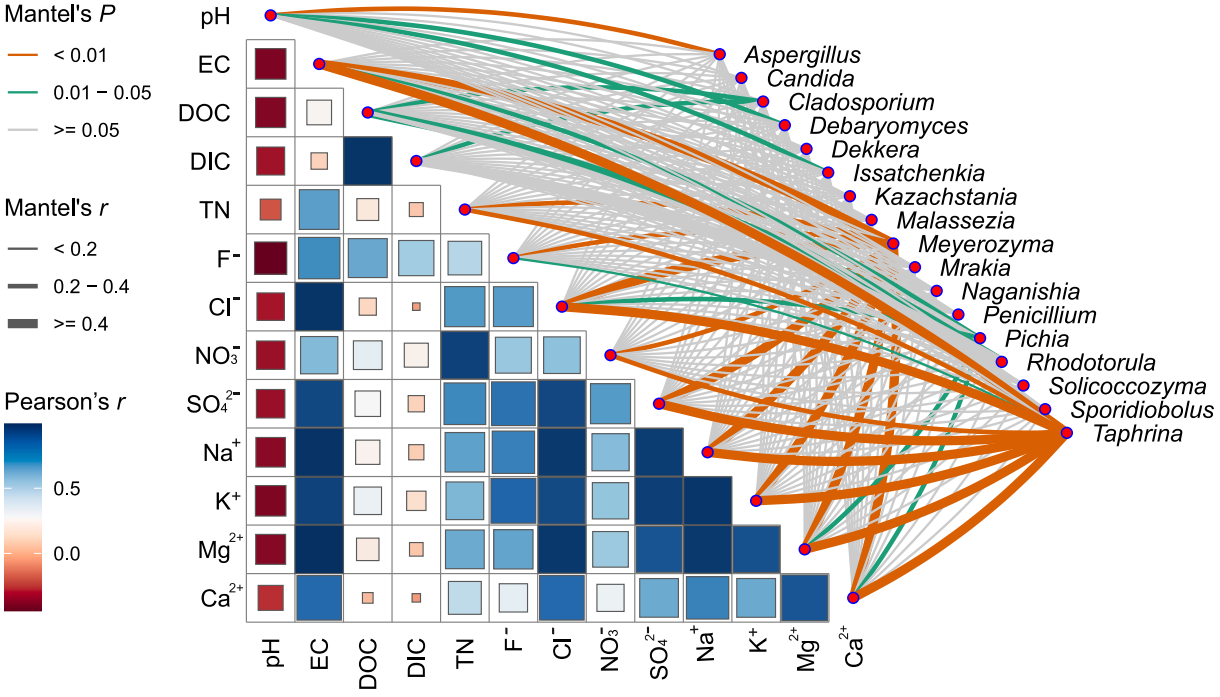

Fig. 5. Correlation matrix between fungal community composition and environmental variables. The color intensity of the squares indicates the Pearson's correlation coefficient (r) between pairs of variables (range 0.0–0.5). Mantel test results are overlaid, with significant correlations categorized according to Mantel's r (<0.2, 0.2–0.4, ≥0.4) and *p*-values (<0.01, 0.01–0.05, ≥0.05). Stronger positive associations are represented by darker squares, while lighter shades indicate weaker.

that Antarctic habitats (long considered "abiotic") can harbor a wide diversity of psychrophilic and psychrotolerant fungi, including yeasts (Margesin et al., 2007; Buzzini et al., 2017), even where the combination of extreme abiotic parameters exhibits challenging conditions for fungal life. In the core herein presented, the observed predominance of yeast life forms over filamentous fungi may reinforce the hypothesis that unicellular lifestyles could be apparently better adapted to harsh Antarctic conditions than hyphal ones (Sannino et al., 2023). Interestingly, the high abundance of Ascomycota in U3 and U6 suggests that the previously observed higher abundance of basidiomycetous taxa found in cold environments (Buzzini et al., 2017; Sannino et al., 2023) may be the result of different combinations of extreme abiotic factors occurring in the different sites, and therefore habitat-dependent (Tripathi et al., 2018).

The most abundant genera found in the permafrost (U6) were *Meyerozyma* and *Taphrina*. The significant ($P < 0.05$) positive correlation found between both genera (as well as, with lower strength, among other genera) may suggest the existence of ecological networks aimed at establishing an ecological equilibrium inside the fungal communities sharing the ecological niche under study. The putative existence of trophic chains, together with the presence of additional mechanisms regulating the ecological balance among fungal taxa (i.e. the secretion of antifungal molecules and the extracellular release of cold-adapted enzymes), could justify their survival under the extreme permafrost conditions (Carrasco et al., 2012; Krishnan et al., 2016; Klassen et al., 2017). The existence of microbial networks could facilitate a reduced, but sustained, metabolic activity, sufficient to maintain viability even under subzero temperatures (Gunde-Cimermann et al., 2003; Rivkina et al., 2018; da Silva et al., 2019). Although psychrophilic microorganisms are generally highly specialized life forms that have developed a set of adaptive strategies to overcome the negative effect of cold (Shivaji and Prasad, 2009; Buzzini et al., 2012), their high specialization for some substrates (e.g. narrow carbon and nitrogen assimilation profiles) may suggest the existence of syntrophic relationships among some members of the permafrost microbial communities.

In this context, integrating fungal co-occurrence networks with the results of the Mantel test showed that fungal interactions are strongly affected by specific environmental variables. Several correlations are supported by significant links to chemical and physical parameters, indicating that environmental factors act as selective forces driving the structure of fungal communities (Liu et al., 2021). For instance, in soil habitats (brines and permafrost), the genus *Taphrina* shows strong positive correlations with *Meyerozyma* and *Rhodotorula*, and negative correlations with *Mrakia*, *Malassezia*, and *Aspergillus*. These relationships are consistent with Mantel test results, which indicate significant positive associations of *Taphrina* with EC, Ca^{2+}, Cl^{-}, Mg^{2+}, and K^{+}. Similarly, the genus *Meyerozyma* is significantly correlated with the same variables, suggesting potential co-selection by environmental conditions, while *Mrakia* and *Malassezia*, which are negatively correlated with *Taphrina* and *Meyerozyma*, showed no significant associations with these variables, postulating the presence of possible competitive exclusion mediated by environmental filtering (Huang et al., 2021; Ma et al., 2021). In ice samples, correlations between *Pichia* and other genera (e.g., *Dekkera*, *Penicillium*) are supported by significant associations of *Pichia* with calcium, EC, Cl^{-}, and Mg^{2+}, indicating that even in extreme environments (i.e. ice), chemical conditions can affect microbial co-occurrence (Tian et al., 2025).

The distribution of trophic modes in the six units (U1 to U6) reveals the dominance of pathotroph-saprotrophs (in U1), saprotrophs (in U1, U2, U4, U5, and U6), pathotroph-saprotroph-symbiotrophs (in U2, U3, U4, U5, and U6), and pathotroph (in U6). Although the overall pattern is similar among the units, the observed differences in the relative abundance of each trophic mode suggest the existence of subtle ecological variations across the sampled environments. The prevalence of saprotrophs is consistent with the necessity of mobilizing nutrients from recalcitrant organic matter in nutrient-limited polar habitats, highlighting their role as primary decomposers under extreme conditions (Tedersoo et al., 2014; Baldrian, 2017). On the other hand, the recurrent occurrence of mixed trophic modes (e.g., pathotroph-saprotroph-symbiotroph) indicates ecological versatility, enabling fungi to switch between nutritional strategies in response to resource scarcity or environmental stress, which is considered an adaptive advantage in cold and oligotrophic environments (Fodor, 2011; Nguyen et al., 2016). Furthermore, the presence of symbiotrophic components within mixed guilds suggests the potential existence of mutualistic or commensal interactions, supporting the idea that fungal survival in permafrost is not solely based on decomposition, but also on complex ecological networks that enhance resilience and resource acquisition (Hibbett et al., 2011; Tedersoo et al., 2014).

When combined with co-occurrence network analysis, the LEfSe results further emphasize that some of the discriminant taxa are not only statistically enriched in either permafrost or ice samples but also occupy a central role within the interaction network. In particular, taxa such as *Meyerozyma* and *Taphrina* emerge as core microorganisms, showing high connectivity and functioning as ecological taxa supporting the overall stability and resilience of the fungal community. This suggests that both genera are critically important in maintaining ecological interactions and metabolic activity under extreme permafrost conditions. Such a role aligns with the idea that key taxa can buffer environmental stress and sustain microbial networks in harsh ecosystems. (Faust and Raes, 2012; Ma et al., 2021). Moreover, the observed co-occurrence and positive correlation between *Meyerozyma* and *Taphrina* may reflect mutualistic or syntrophic interactions, as supported by broader studies on bacterial–fungal interactions (Morris et al., 2013). Such interactions are known to enhance stress tolerance, nutrient acquisition, and metabolic efficiency, especially in oligotrophic and cryogenic ecosystems. Importantly, the ecological roles of these fungi extend beyond local survival: their metabolic activity, even at subzero temperatures, may contribute to biogeochemical cycling of key elements such as carbon and nitrogen (Bore et al., 2017). This highlights the relevance of fungal networks in regulating elemental fluxes in polar ecosystems, with potential implications for understanding climate-driven changes in global nutrient cycles (Averill et al., 2014; Treseder, 2004). The above hypotheses could raise a fundamental question: may the above postulated ecological equilibria reflect the existence in more ancient Antarctic permafrost (>32 Kyr of U6 against ca 1kyr or less in the less deep layers) under study of relict fungal taxa that have survived under extreme conditions over time? The endorsement of this hypothesis would require to assume the existence of an appreciable level of in situ fungal activity. The presumed level of a certain degree of fungal metabolic activity at subzero temperatures, long thought to be negligible, has been positively re-evaluated over the few decades: the existence of low reaction rates in Antarctic ecosystems occurring over timescales ranging from several hundred to a few thousand years has been supposed (Price, 2000; Gunde-Cimermann et al., 2003; Price and Sowers, 2004; Su et al., 2016; Buzzini et al., 2018; Rivkina et al., 2018; da Silva et al., 2019). Furthermore, more recent studies hypothesized that some ancient non-Antarctic (ancient igneous rocks in South Africa) habitats can also support microbial life (Suzuki et al., 2024), implying that microbial life could persist in extreme environments even for extremely long timescales.

These findings, together with co-occurrence network results, suggest that the persistence of ancient fungal taxa in Antarctica cannot be explained solely by historical factors. Environmental filtering is a key driver in shaping fungal communities across various ecosystems, often surpassing stochastic processes like dispersal limitation (Liu et al., 2021). Additionally, co-occurrence network analyses reveal that groups of closely interacting fungal taxa are closely connected to nutrient availability and ecosystem structure, underscoring the ecological significance of these interactions even within permafrost habitats (Ma et al., 2021; Mishra et al., 2025).

The high abundance of OTUs assigned to the genus *Meyerozyma*

found in the permafrost (U6) is consistent with similar observations reported in the deepest layers of Polar and Alpine permafrost worldwide (Sangorrín et al., 2014; Sannino et al., 2021), confirming the adaptation of this taxon to cold habitats. Its abundance was affected by the EC, especially by the concentration of specific ions (e.g. Cl^{-}, Mg^{2+}, and Na^{+}), suggesting that trace elements may shape the distribution of *Meyerozyma* spp. in the habitat under study. Moreover, some species of this genus exhibit halophilic characteristics, being capable of growing in media containing 10 % NaCl (Kurtzman et al., 2011). Likewise, the high abundance of OTUs assigned to the genus *Taphrina* in permafrost (U6) is consistent with the first (and so far, unique) isolation and description of the new species *Taphrina antarctica* (Selbmann et al., 2014) in Antarctic rock-associated habitats and confirms the adaptability of members of the genus to such extreme conditions. A permanent switch (via a temperature-induced phase transition) from their prevalent hyphal plant-parasitic habitus (Fonseca and Rodrigues, 2011) to a yeast-like asexual saprotrophic lifestyle may have determined this ability, consistently with previous observations on different fungal taxa (San-Blas and San-Blas, 1984; Newman et al., 1995; Sanna et al., 2012). Moreover, the presence of the genus *Taphrina* has been documented across a wide range of cold biomes, including cryptoendolithic habitats in Antarctica (Coleine et al., 2020), permafrost in the Alps (Pérez-Mon et al., 2022), glacial environments in Canada (Hamilton et al., 2013), and aquatic systems in the High Arctic (Zhang et al., 2017). This distribution, coupled with its dimorphic nature (Selbmann et al., 2014), reinforces the hypothesis that this yeast genus possesses ecological plasticity enabling survival and proliferation under diverse and extreme environmental conditions.

Considering the adaptation of *Meyerozyma* and *Taphrina* to cold environments (Sangorrín et al., 2014; Selbmann et al., 2014; Sannino et al., 2021), it seems reasonable to hypothesize that both taxa could be a relict (putatively metabolically active) population originating from the ancient fungal community that colonized the permafrost under study. The permafrost core studied is estimated to be around 32 kyears old (Guglielmin, unpublished data), a timeframe that suggests these fungi may have survived under extreme conditions over extremely long timescales by sustaining minimal metabolic activity.

The overlying glacier ice, being cold-based and non-erosive, could have acted as a protective barrier, maintaining the integrity of the permafrost habitat and its original microbiota. This is consistent with previous Antarctic observations, where ancient biological materials have been preserved under similar glaciological conditions (Cannone et al., 2017). Thus, the deep permafrost buried beneath cold-based glacier ice could have acted as a natural archive, preserving ancient microbial communities and offering a unique window into past ecosystems and long-term fungal survival strategies in extreme environments.

5. Conclusion

This study provides novel insights into the fungal diversity preserved in deep ancient Antarctic permafrost underlying a coastal cold-based glacier. Significant differences in fungal community composition between glacier ice and permafrost were observed, with brine acting as a transitional layer. The dominance of specific taxa such as *Meyerozyma* and *Taphrina* in the permafrost, their already confirmed occurrence in similar habitats, along with their positive correlations with the abiotic parameters of the deep permafrost under study, suggests a structured microbial community that may have persisted under such extreme conditions, through the maintenance of a reduced metabolic activity and microbial turnover, sufficient to maintain viability even under subzero temperatures, over extremely long timescales. Therefore, the fungal community embedded in the deep permafrost under study may represent a relict microbiota preserved since the time of burial.

CRediT authorship contribution statement

Gianmarco Mugnai: Writing – original draft, Formal analysis, Data curation. **Ciro Sannino:** Writing – review & editing, Supervision, Formal analysis, Data curation, Conceptualization. **Luigimaria Borruso:** Writing – review & editing, Formal analysis, Conceptualization. **Daniele Andreani:** Investigation, Formal analysis. **Dario Battistel:** Investigation, Formal analysis. **Benedetta Turchetti:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Pietro Buzzini:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization. **Mauro Guglielmin:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106529.

Data availability

Data will be made available on request.

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