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ORIGINAL PAPER

Ecological succession of fungal and bacterial communities in Antarctic mosses affected by a fairy ring disease

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Abstract

We evaluated fungal and bacterial diversity in an established moss carpet on King George Island, Antarctica, affected by 'fairy ring' disease using metabarcoding. A total of 127 fungal and 706 bacterial taxa were assigned. *Ascomycota* dominated the fungal assemblages, followed by *Basidiomycota*, *Rozellomycota*, *Chytridiomycota*, *Mortierellomycota* and *Monoblepharomycota*. The fungal community displayed high indices of diversity, richness and dominance, which increased from healthy through infected to dead moss samples. A range of fungal taxa were more abundant in dead rather than healthy or fairy ring moss samples. Bacterial diversity and richness were greatest in healthy moss and least within the infected fairy ring. The dominant prokaryotic phyla were *Actinobacteriota*, *Proteobacteria*, *Bacteroidota* and *Cyanobacteria*. *Cyanophyceae* sp., whilst consistently dominant, were less abundant in fairy ring samples. Our data confirmed the presence and abundance of a range of plant pathogenic fungi, supporting the hypothesis that the disease is linked with multiple fungal taxa. Further studies are required to characterise the interactions between plant pathogenic fungi and their host Antarctic mosses. Monitoring the dynamics of mutualist, phytopathogenic and decomposer microorganisms associated with moss carpets may provide bioindicators of moss health.

Keywords Antarctica · Climate change · Environmental DNA · Metabarcoding · Plant diseases

Introduction

Antarctic vegetation is dominated by bryophytes, with 116 species currently recognised representing cosmopolitan, endemic and bipolar taxa (Ochyra et al. 2008; Câmara et al. 2019). Mosses may form extensive carpets in some parts of Antarctica, particularly in the maritime Antarctic, contributing to the greatest development of 'fellfield' communities globally and providing habitats and ameliorating Antarctica's extreme environmental conditions for contained microbial and invertebrate communities (Smith 1984; de Carvalho et al. 2019; Prather et al. 2019). Well established Antarctic moss carpets may act as "sentinels" sensitive to environmental changes, particularly in temperature and hydration, across the Antarctic Peninsula region (Prather et al. 2019). Moss carpet health has been a subject of research attention since the early years of Antarctic terrestrial research (Robinson et al. 2018). One of the most frequently reported concerns relating to moss health is that of attack by initially unidentified organism(s) resulting in the formation of a concentric ring ('fairy ring') visible on the surface of the carpet which eventually results in the death of the moss (Wilson 1951; Racovitza 1959; Hawksworth 1973; Longton 1973; Fenton 1983; Ochyra et al. 2008; Tojo et al. 2012; Pawłowska et al. 2017). Most recently, Rosa et al. (2020a) recorded the development of fairy rings on previously unreported moss

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species from new locations in the western Antarctic Peninsula region, suggesting that the disease is more widespread in maritime Antarctica than previously believed and may be increasing in prevalence.

The majority of studies Antarctic moss fairy rings have considered that fungi are the cause of the disease. However, there is no consensus about which species is/are the phytopathogenic agent(s) causing the disease. Fenton et al. (1983) was the first to propose *Coleroa turfosorum*, *Bryosphaeria megaspora* and *Epibryon chorisodontii* (*Ascomycota*), recovered from infected mosses on Signy Island, as the causative agent. Tojo et al. (2012) proposed *Pythium polare* (*Oomycota*) to be the species affecting *Sanionia uncinata* on King George Island. Pawłowska et al. (2017) recovered and proposed *Psychronectria hyperantarctica* as the phytopathogenic fungus causing fairy rings, in line with previous work by Putzke and Pereira (2012). Most recently, Rosa et al. (2020a) reported that fairy rings host multiple fungal taxa, which might therefore act in consortium in causing the disease.

Despite the continent's typically extreme conditions, Antarctic fungi represent a diverse eukaryote microbial group, including symbionts, decomposers and opportunistic taxa, amongst which are phytopathogenic taxa (Rosa et al. 2019). Globally, approximately 300 species from 80 genera of *Ascomycota* are known to parasitize mosses or liverworts (Döbbeler 1997). Earlier studies of the cause of fairy ring disease relied on culturing approaches and direct morphological identification. Rosa et al. (2020a) was the first study to use molecular tools to identify fungi potentially involved. DNA metabarcoding using high-throughput sequencing (HTS) is increasingly recognised as an important tool in investigating fungal diversity in various Antarctic ecosystems (Rosa et al. 2020b, c; Rosa et al. 2021). Therefore, in the present study, we evaluated fungal and bacterial diversity associated with different stages of the development of fairy ring disease in well established moss carpets on King George Island, South Shetland Islands, maritime Antarctic.

Methods

Moss carpet sampling and identification

Fungal and bacterial occurrence and diversity were investigated across different fairy ring disease stages in a well-established moss carpet on the Keller Peninsula, King George Island, South Shetland Islands (maritime Antarctica; Fig. 1) during the austral summer of 2019/20. Three moss samples (each approximately 4 cm diameter) from each visible stage of the disease, defined as healthy, fairy ring (infected) and dead (Fig. 2), were obtained. The samples were immediately stored in sterilized whirl pack bags and frozen at −20 °C until further use. The moss carpet was formed by the species *Sanionia uncinata* (Hedw.) Loeske, with identification confirmed based on macro- and micromorphological characteristics with reference to Ochyra et al. (2008). All moss specimens are deposited in the University of Brasília Herbarium (UB).

DNA extraction, data analyses and fungal and bacterial identification

Three samples of each of healthy, infected and dead mosses were processed separately to recover the total fungal and bacterial DNA. Total DNA was extracted using the QIAGEN DNeasy PowerLyzer PowerSoil Kit, following the manufacturer's instructions. Extracted DNA was used as template for generating PCR-amplicons. For fungi, the internal transcribed spacer 2 (ITS2) of the nuclear ribosomal DNA was used as a DNA barcode for molecular species identification (Chen et al. 2010; Richardson et al. 2015). PCR-amplicons were generated using the universal primers ITS3 (5′-GCA TCGATGAAGAACGCAGC-3′) and ITS4 (5′-TCCTCC GCTTATTGATATGC-3′) for fungi (White et al. 1990). For bacteria, we used the 16S rRNA gene V3-V4 region and primers341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) that produce an amplicon of ~460 bp (Herlemann et al. 2011; Klindworth et al. 2013). These amplicons were subjected to high-throughput sequencing at Macrogen Inc. (South Korea) on an Illumina MiSeq sequencer (3 × 300 bp), using the MiSeq Reagent Kit v3 (600-cycle) following the manufacturer's protocol.

Raw fastq files were filtered using BBDuk version 38.34 (BBMap—Bushnell B.—sourceforge.net/projects/bbmap/) to remove Illumina adapters, known Illumina artefacts and the PhiX Control v3 Library. Quality read filtering was carried out using Sickle version 1.33-q 30-l 50 (Joshi and Fass 2011), to trim 3′ or 5′ ends with low Phred quality score, and sequences shorter than 50 bp were also discarded. The remaining sequences were imported to QIIME2 version 2019 for bioinformatics analyses (Bolyen et al. 2019). For fungi, the qiime2-dada2 plugin is a complete pipeline that was used for filtering, dereplication, turn paired-end fastq files into merged and remove chimeras (Callahan et al. 2016). Taxonomic assignments were determined for amplicon sequence variants (ASVs) using the qiime2-feature-classifier (Bokulich et al. 2018) classify-sklearn against the UNITE fungal ITS database version 8.2 (Abarenkov et al. 2020) trained with Naive Bayes classifier and a confidence threshold of 98.5%. For bacteria, sequences were quality filtered using "quality-flter q-score-joined" plugin to improve diversity (Bokulich et al. 2013). Sequences were denoised using deblur (Amir et al. 2017) with-p-trim-length parameter of 300 and were taxonomically assigned

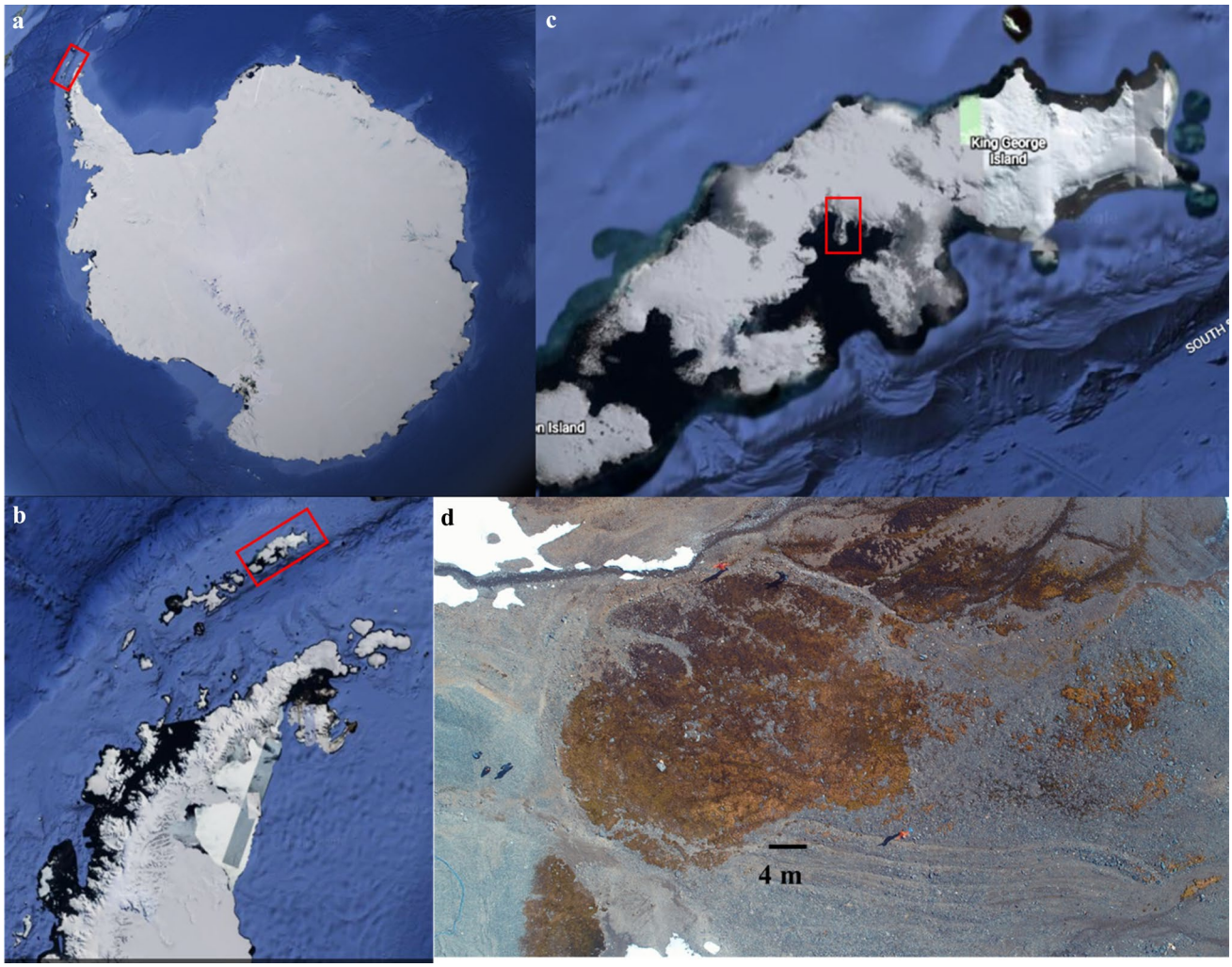

Fig. 1 Satellite images **a**, **b** and **c** (obtained in Google Earth Pro, 2019) indicating where the moss samples were obtained. **a** Antarctic continent with the north-west Antarctic Peninsula and South Shetland Islands inside the red rectangle, **b** Antarctic Peninsula with King George Island inside the red rectangle, **c** King George Island with the Keller Peninsula inside the red rectangle, **d** aerial view of the well established moss carpet (total area 530 m^2) from which samples were obtained on the Keller Peninsula, close to the Brazilian Antarctic Station Comandante Ferraz (62°5′12.869″ S; 58°23′42.312″ W). Photo L.H. Rosa

to sub-operational-taxonomic-units (sOTU) against the Silva 138 Ref NR 99 database pre-trained with Naive Bayes classifier using the "feature-classifier classify-sklearn" plugin.

Many factors, including extraction, PCR and primer bias, can affect the number of reads obtained (Medinger et al. 2010) and thus lead to misinterpretation of absolute abundance (Weber and Pawlowski 2013). However, Giner et al. (2016) concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance (Deiner et al. 2017). Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance. Fungal classification followed Kirk et al. (2011), Tedersoo et al. (2018), MycoBank (http://www.mycobank.org) and the Index Fungorum (http://www.indexfungorum.org).

Diversity, distribution and ecological analysis

To quantify species diversity, richness and dominance, we used the following indices: (i) Fisher's α, (ii) Margalef's and (iii) Simpson's, respectively, to assess alpha diversity. In addition, the Sorensen and Bray–Curtis similarity indices were used to assess beta diversity among the fungal and bacterial assemblages present in the mosses representing the different disease stages. The relative abundance of the OTUs was used

Fig. 2 Different stages of the fairy ring disease. **a** Healthy moss carpet, **b** moss carpet showing fairy rings, **c** dead moss within carpet, **d** a larger area of the moss carpet undergoing decomposition. The yellow lines in **b** outline the fairy ring and in **c** and **d** areas of decomposition. Photos L.H. Rosa

to quantify the taxa present in the different disease stages as described by Rosa et al. (2021), where OTUs with relative abundance > 10% were considered dominant, those with relative abundance of 1–10% intermediate and those with < 1% minor (rare) components of the microbial community. All of the results were obtained with 95% confidence and bootstrap values were calculated from 1,000 iterations. Taxon accumulation curves were obtained using the Mao Tao index. All diversity index calculations and t tests were performed using PAST, version 1.90 (Hammer et al. 2001). To prepare Kronar charts, QIIME2 taxonomy classifications and the table of taxa abundance were converted to tsv and biom format, respectively. The table of fungal abundance was converted to tsv by using biom convert and combined with taxonomy classification with a custom script krona_qiime.py (https://github.com/lokeshbio/Amplicon_course/blob/master/krona_qiime.py). The Krona Tools (v. 2.7.1) (Ondov et al. 2011) program, ktImportText.pl, was used to provide interactive visualization of identified fungi species. Venn analysis to compare the fungal diversity obtained from the different sampling locations was carried out using the program available at http://bioinformatics.psb.ugent.be/webtools/Venn/.

Results

Taxonomy and diversity

We obtained 55,312 fungal DNA reads representing 127 OTUs and 69,755 bacterial DNA reads representing 706 OTUs (Suppl. Table 1). The Mao Tao rarefaction curves did not reach a plateau in all samples, indicating that the total number of fungal and bacterial taxa may be greater than that detected (Suppl. Figure 1). The Krona charts (Fig. 3) illustrate the changes in the fungal and bacterial assemblages with progression through the three disease stages.

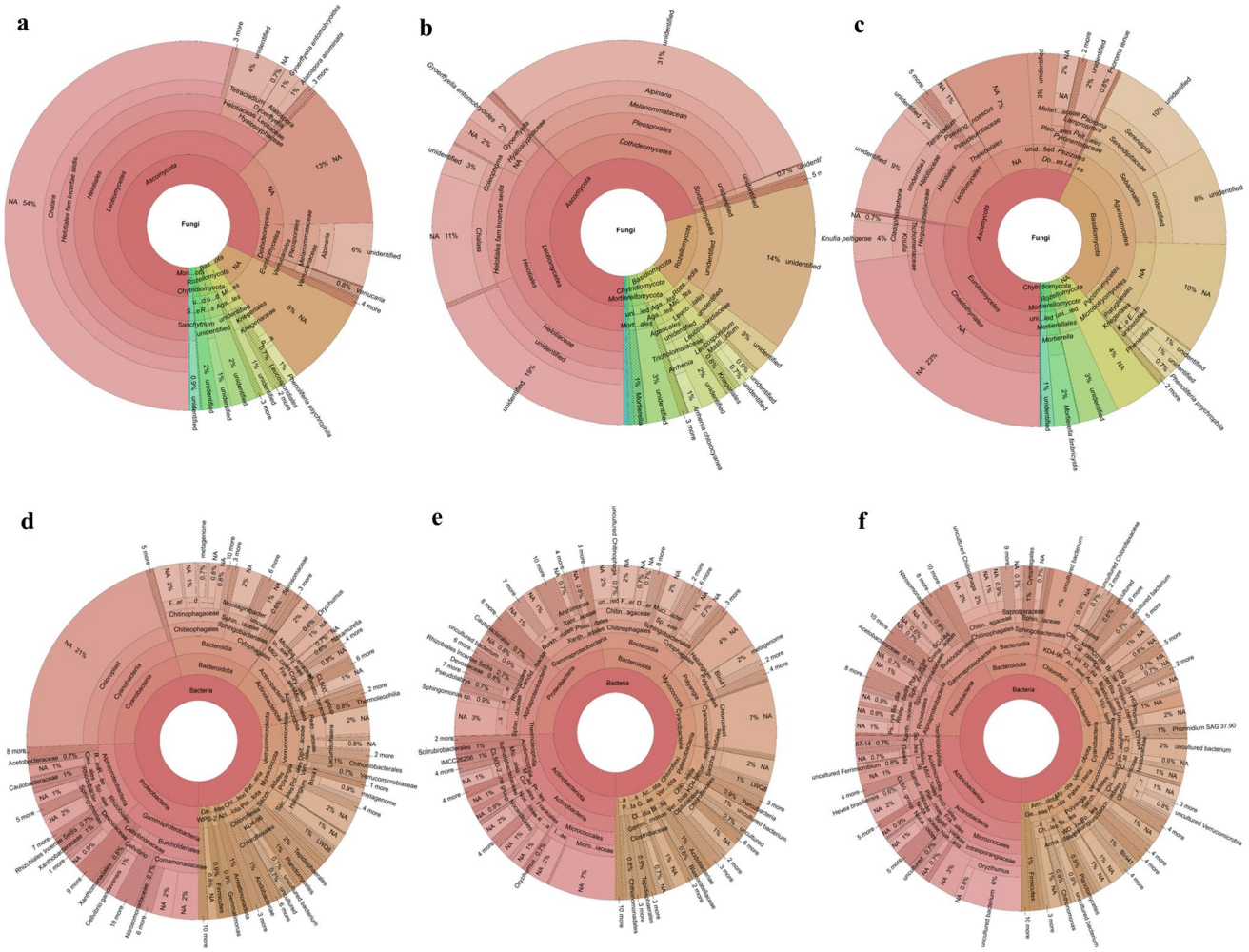

Fig. 3 Krona charts of the different fungal and bacterial assemblages detected. Fungi: **a** healthy moss carpet, **b** moss carpet with fairy ring symptoms and **c** dead moss carpet. Bacteria: **d** healthy moss carpet, **e** moss carpet with fairy ring symptoms and **f** dead moss carpet

Table 1 Diversity indices of fungal assemblages detected in the different stages of fairy ring infection of established moss carpet

Diversity indices	Healthy		Fairy ring		Dead		Total	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
Number of taxa	78	636	83	584	80	602	127	716
Number of reads	12,321.32	22,864.33	24,404.31	23,364.01	18,585.98	23,527.09	55,311.63	69,755.39
Fisher α	20.24 ± 1.07[a]	121.3 ± 15.39	22.48 ± 0.83	108.6 ± 4.86	27.37 ± 1.42	112.6 ± 1.65	30.07 ± 1.10	111.1 ± 61.75
Margalef	7.61 ± 0.70	63.26 ± 7.22	8.05 ± 0.61	57.96 ± 2.34	8.91 ± 1.02	59.71 ± 0.70	12.45 ± 0.77	64.11 ± 3.42
Simpson	0.68 ± 0.15	0.98 ± 0.02	0.83 ± 0.06	0.98 ± 0.02	0.90 ± 0.05	0.97 ± 0.04	0.91 ± 0.08	0.98 ± 0.03

All of the results were obtained with 95% confidence and bootstrap values were calculated from 1000 iterations

[a]Standard error value

The fungal phylum *Ascomycota* dominated the assemblages in all samples, followed by *Basidiomycota*, *Rozellomycota*, *Chytridiomycota*, *Mortierellomycota* and *Monoblepharomycota*. *Chalara* sp. 1, *Alpinaria* sp., *Helotiaceae* sp. 2, *Chaetothyriales* sp. 1, *Ascomycota* sp. 1 (all *Ascomycota*), *Rozellomycota* sp. and Fungi sp. generally displayed relative abundance > 10% and were the dominant taxa, followed by 15 taxa characterized as intermediate and 109 as minor (rare) components of the total fungal community. The dominant bacterial phylum was *Actinobacteriota*, followed by

Proteobacteria, *Bacteroidota* and *Cyanobacteria*, in rank. The class level taxon *Cyanophyceae* sp. (*Cyanobacteria*) represented the dominant prokaryotic taxa. Seventy taxa were characterized as intermediate and 643 as minor components of the bacterial community.

Fungal and bacterial alpha diversity indices across the samples are given in Table 1. The fungal communities displayed high diversity (Fisher α), richness (Margalef) and dominance (Simpson) indices, which increased progressively from the healthy to the dead moss carpet samples. For the bacterial communities the Fisher (diversity) and Margalef (richness) indices were greatest in the the healthy moss, decreasing in the fairy ring infected moss and partially recovering in the dead moss sample. The Simpson (dominance) index did not differ across all samples.

The beta diversity of the fungal and bacterial assemblages varied across the different samples (Suppl. Figure 2). Both presence-absence-based Sorensen and the abundance-related Bray–Curtis similarity indices showed that the fungal assemblages detected in the healthy and fairy ring samples were the most similar, while those of the dead moss were the most different, mainly due the dominance of *Basidiomycota* taxa. In contrast, the bacterial assemblages detected in the healthy and dead moss carpet samples were the most similar and those present in the fairy ring formed a separate group.

The fungal and bacterial community composition varied through the different stages of the disease (Suppl. Figure 3). For fungi (Suppl. Figure 3a), 42 taxa occurred in all samples, but each disease stage displayed different composition and 20 taxa occurred only in fairy ring and dead samples. For the bacterial communities (Suppl. Figure 3b) the largest proportion of the taxa (254) were present in all samples. Few taxa were in common between healthy/fairy ring, fairy ring/dead and healthy/dead samples.

Comparison of the dominant and intermediate fungal and bacterial communities identified different patterns of composition at the different stages of the infection (Fig. 4; Suppl. Table 2). Amongst the fungi, *Chalara* sp. 1, *Ascomycota* sp. 1, *Tetracladium* sp. 2 and Fungi sp. dominated the healthy moss carpet and decreased in abundance in the fairy ring infected and dead moss. In contrast, *Alpinaria* sp., *Helotiaceae* sp. 2, *Helotiales* sp. 1 and *Rozellomycota* sp. increased considerably in relative abundance between the healthy and the infected moss samples, but decreased in the dead moss. Finally, *Chaetothyriales* sp. 1, *Serendipita* sp., *Agaricomycetes* sp., *Sebacinales* sp., *Knufia*

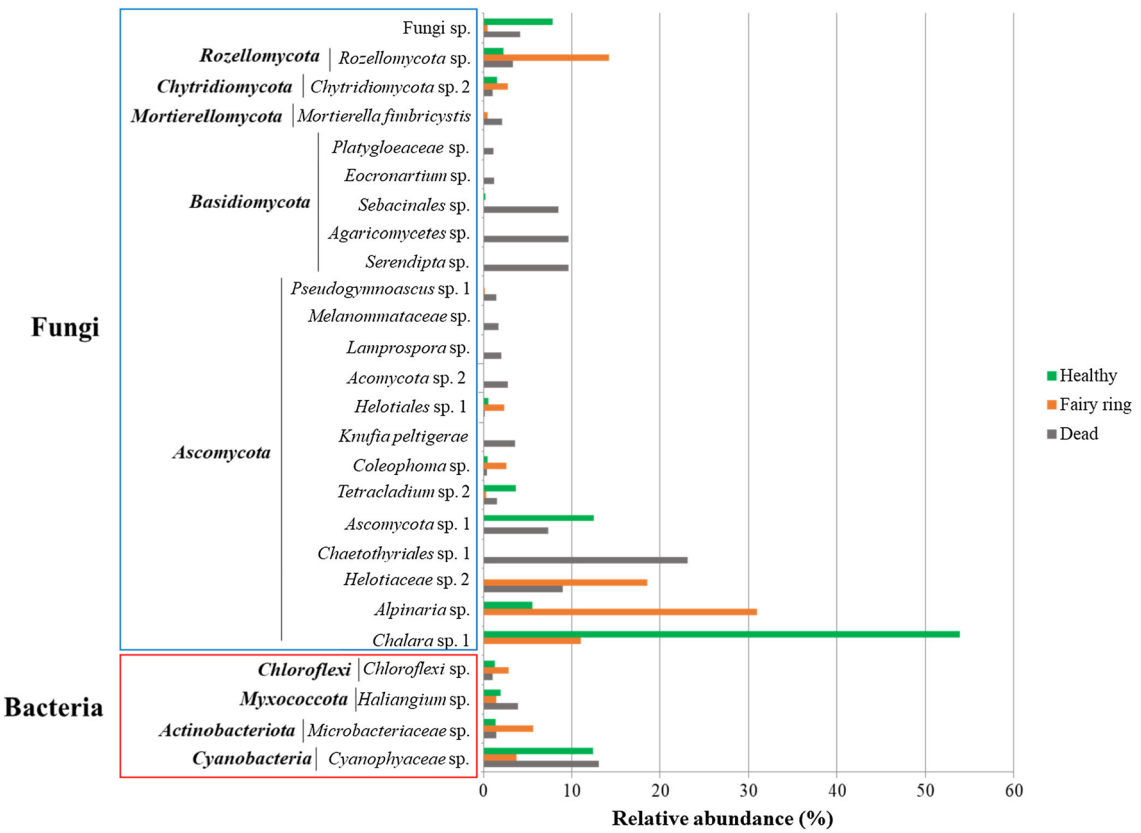

Fig. 4 Comparison of the median relative abundance of fungal and bacterial assemblages in moss samples collected from an established moss carpet on the Keller Peninsula, King George Island, South Shetland Islands, at the three stages of infection (healthy, infected fairy ring and dead moss)

peltigerae, *Ascomycota* sp. 2, *Mortierella fimbricystis*, *Lamprospora* sp., *Melanommataceae* sp., *Pseudogymnoascus* sp. 1 and *Platygloeaceae* sp. were the most abundant fungi in the dead moss but had low relative abundance in the healthy and infected moss.

The relative abundance of the four prokaryote phyla varied across the three infection stages. *Actinobacteriota* displayed moderate abundance in healthy moss carpet, increasing to dominate the bacterial community in the infected moss and decreasing in the dead moss. In contrast, *Proteobacteria*, *Bacteroidota* and *Cyanobacteria* displayed moderate abundance in healthy moss, which decreased in the infected moss and increased again in dead moss. Among these phyla, *Cyanophyceae* sp. (*Cyanobacteria*) was the dominant taxon, although only reaching intermediate abundance in the infected moss (3.74%) and dominance in healthy (12.39%) and dead moss (13.05%) samples. *Microbacteriaceae* sp. (*Actinobacteriota*) and *Chloroflexi* sp. (*Chloroflexi*) occurred at intermediate abundance in all moss samples and were the most abundant taxa in the infected moss samples and least adundant in dead moss. *Cyanophyceae* sp. and *Haliangium* sp. (*Myxococcota*) were the most abundant taxa detected in dead moss.

Discussion

Fungal diversity

Mosses are the dominant flora in Antartica, providing habitat for multiple microbial and invertebrate taxa and communities (Ochyra et al. 2008; de Carvalho et al. 2019). Endophytic and epiphytic fungi and bacteria are considered to be the dominant microorganisms present in these habitats, known as the bryosphere (Möller and Dreyfuss 1996; Tosi et al. 2002; de Carvalho et al. 2020).

A range of Antarctic moss species have been documented to be vulnerable to infection by 'fairy ring disease'. In the current study comparing the microbial communities present in healthy, visibly infected (within the ring) and dead moss from the same moss carpet, we detected complex and diverse fungal and bacterial communities using a metabarcoding approach. Overall, the fungal community was richer (3.2 times greater) than that reported recently (Rosa et al. 2020a) in a study using culture methods which detected 40 taxa in eight moss species sampled from different locations in the north-west Antarctic Peninsula region. Among the taxa reported by Rosa et al. (2020a), only representatives of the genera *Alpinaria*, *Helotiales*, *Cladosporium*, *Cadophora*, *Pseudogymnoascus*, *Glarea*, *Chalara*, *Ophiocordycipitaceae*, *Juncaceicola* and the species *Mortierella fimbricystis* and *Gyoerffyella entomobryoides* were shared with the current study.

In the current study, relative abundance of the fungal taxa *Alpinaria* sp., *Helotiaceae* sp. 2, *Coleophoma* sp., *Helotiales* sp. 1, *Chytridiomycota* sp. 2, *Rozellomycota* sp. and Fungi sp. increased between healthy and infected moss samples, but was lower in dead moss. The genus *Alpinaria* (*Melanommataceae*) includes only a single described species (*A. rhododendri*) and seems to be common in the subalpine to alpine zone worldwide on twigs or buds of *Rhododendron* spp. (Ericaceae) (Hashimoto et al. 2017). It has recently been reported on mosses affected by fairy ring disease in maritime Antarctica (Rosa et al. 2020a). The family *Melanommataceae* includes plant pathogenic species such as *Gemmamyces piceae* (Jaklitsch and Voglmayr 2017) and, according to the FunGuild database (Nguyen et al. 2016), *A. rhododendri* is considered a probable plant pathogenic and/or wood saprotrophic species.

The genus *Coleophoma* includes species reported as plant pathogenic, saprophytic or endophytic on different plant species (Crous and Groenewald 2016). Plant pathogens in the genus include *C. fusiformis* on leaves of *Rhododendron* (Sutton 1980), *C. eucalypti* and *C. eucalyptorum* on *Eucalyptus* (Yuan 1996), *C. gevuinae* on *Gevuina* (Bianchinotti and Rajchenberg 2004), *C. empetri* on *Vaccinium* (Polashock et al. 2009) and *C. proteae* on *Protea caffra* (Crous et al. 2012). *Rozellomycota* species are common in temperate, sub-Arctic and Antarctic environments (Rosa et al. 2020b). According to Grossart et al. (2016), all known *Rozellomycota* taxa are obligate pathogens of eukaryotes, including amoebae, fungi and algae. However, there are no reports of *Rozellomycota* acting as plant pathogens. *Chytridiomycota*, known as chytrids, primarily includes free-living saprophytic taxa present in aquatic and terrestrial environments. However, some species are reconized as plant pathogens, such as *Synchytrium endobioticum* that causes potato wart disease (van de Vossenberg et al. 2019).

The taxa *Chaetothyriales* sp. 1, *Serendipita* sp., *Agaricomycetes* sp., *Sebacinales* sp. and *Knufia peltigerae* are notable here due to their increase in abundance in dead relative to healthy moss carpet. They may, therefore, represent major decomposing taxa in the ecological succession following the death of the moss. The order *Chaetothyriales* (*Ascomycota*) includes species with multiple ecological roles, including soil saprophytes, human and animal opportunistic pathogens and plant epi- and/or endophytes (Madrid et al. 2016). In addition, some representatives of *Chaetothyriales* are known to colonize extreme environments characterized by drought, oligotrophic conditions, extreme temperatures and high UV-radiation exposure (Tsuneda et al. 2011). Some species are known phytopathogens (Gueidan et al. 2014).

Serendipita is a genus with eight known species (Kirk et al. 2011), including S. *indica*, formerly known as *Piriformospora indica* (Weiß et al. 2016), an endophytic fungus detected in low-nutrient desert soil in Rajasthan, India

(Verma et al. 1998), which acts to increase nutrient uptake and utilization in its host (Yadav et al. 2010; Ngwene et al. 2016). *Serendipita* has been reported as an endophyte of bryophytes (Varma et al. 2012). *Agaricomycetes* is a class of *Basidiomycota* that includes almost 21,000 described species (Kirk et al. 2011) whose members play different ecologicals role such as decomposers, pathogens and mutualists in different environments (Hibbett et al. 2014).

The order *Sebacinales* (*Agaricomycetes*, *Basidiomycota*) includes species recognized to show diverse interactions with plants, which range from mutualistic root endophytes (obligate biotrophs, mycorrhizae) to saprophytes (Weiß et al. 2016). Within the order, members of the family *Serendipitaceae* have been reported from the Antarctic Peninsula associated with the liverwort *Barbilophozia hatcheri* and the mosses *Chorisodontium aciphyllum* and *Sanionia uncinata* (Zhang et al. 2013).

The genus *Knufa* comprises black fungi and has six known species (He et al. 2013). *Knufia peltigerae* is a lichenicolous fungus (Gueidan et al. 2014) which, according to Lawrey and Diederich (2003), represents an important ecological group that forms obligate associations with lichens. The ascomata of *K. peltigerae* (originally reported as *Capronia peltigerae*) was first described on thalli of the lichen *Peltigera rufescens* (Fuckel 1874; Untereiner et al. 2011). *Peltigera rufescens* is a cosmopolitan lichen that occurs on sub-Antarctic South Georgia, the South Orkney Islands and in various locations along the Antarctic Pensinula (both east and west coasts, including James Ross and Alexander Islands) (Øvstedal and Smith 2001). Possibly analogous to the bleaching effect of fairy rings on mosses, Untereiner et al. (2011) reported the presence of *K. peltigerae* ascomata on decolourized or moribund *P. rufescens* thalli. However, it is unclear if *K. peltigerae* was responsible for the discolouration or represents an opportunistic fungus occurring on aging parts of the lichen thalli. The species has been rarely recorded taxa in Antarctica using culture approaches. de Souza et al. (2021) detected the DNA of *K. peltigerae* in cotton baits deposited in a lake at Hennequin point, King George Island, close to a moss carpet that was under attack from fairy ring disease.

The taxa *Serendipita* sp. and *Agaricomycetes* sp. occurred exclusively and were dominant in the dead moss carpet. *Serendipita* species include root fungal endophytes and arbuscular mycorrhizal fungi (AMF) known as plant growth promoters (Verma et al. 1998). Bridge and Newsham (2009) reported *Serendipita*-like Sebacinale fungi in soil at Mars Oasis, Alexander Island, in the southern maritime Antarctic. The class *Agaricomycetes* includes about 21,000 described mushroom-forming species with ecological roles such as decomposers, pathogens and mutualists in different terrestrial and aquatic environments (Hibbett et al. 2014).

Chalara sp. displayed high dominance in the healthy moss carpet, decreasing in dominance in infected moss. The genus includes 103 widespread species with multiple ecological functions (Kirk et al. 2011). Among *Chalara* species, *C. fraxinea* (teleomorph: *Hymenoscyphus pseudoalbidus*) has been reported as an emerging epidemic plant pathogen that has severely affected ash tree stands in Europe since 1990 (Kowalski 2006; Husson et al. 2011).

Previous studies have concluded that the causative agent of the fairy ring disease in Antarctica is *Psychronectria hyperantarctica*, identified using classical morphological techniques from its fruiting body (Hawksworth 1973; Pawłowska et al. 2017). However, despite the potentially high taxonomic resolution of the metabarcoding approach, we did not detect sequences of *P. hyperantarctica* in any samples. Rather, our data indicated the presence of several other recognized plant pathogenic fungi, supporting the suggestion of Rosa et al. (2020a) that the disease may be caused by multiple fungal infections in parallel. The fungal taxa *Alpinaria* sp., *Helotiaceae* sp. 2, *Coleophoma* sp., *Helotiales* sp., *Rozellomycota* sp. and *Chytridiomycota* sp. 2 showed high levels of dominance in infected moss showing fairy ring symtoms, which deserve further detailed taxonomic characterization and assays in vivo using plant models to confirm whether they are able to cause plant disease symptoms. Robinson et al. (2018) demonstrated that moss vegetation in the Windmill Islands, East Antarctica is changing rapidly in response to a drying climate causing declining viability in some species. It is possible that the incidence of fungal attack, evidenced by the fairy ring disease, might be connected to a decrease in moss health resulting from climatic changes in the Antarctic Peninsula region in recent decades, although no studies have specifically addressed this or directly quantified disease incidence.

Bacterial diversity

Few studies have addressed the bacterial communities associated with Antarctic mosses. Park et al. (2013) studied endophytic bacteria associated with healthy material of the moss *Sanionia uncinata*. To our knowledge, no studies have focused on the bacterial diversity present specifically in mosses affected by the fairy ring disease in Antartica. However, the overall dominance of the phyla *Actinobacteriota*, *Proteobacteria*, *Bacteroidota* and *Cyanobacteria* documented here is consistent with studies such as those of Holland-Moritz et al. (2018) and Wang et al. (2018), which reported that moss-associated bacterial communities were commonly dominated by *Proteobacteria* and *Bacteroidetes*. Using molecular phylogenetic techniques to analyse the bacterial diversity associated with aquatic moss pillars in continental Antarctic lakes, Nakai et al. (2012) reported *Proteobacteria*, *Cyanobacteria* and *Firmicutes* as dominant

groups. Park et al. (2013) and Câmara et al. (2021) reported highest relative abundances of sequences representing the phylum *Actinobacteria* in a transplanted *S. uncinata* carpet moss in a study also carried out on the Keller Peninsula. Raymond (2016) reported *Actinobacteria* (genera *Conexibacter*, *Rhodococcus*, *Marmoricola*, *Micromonospora* and *Streptomyces*) and *Bacteroidetes* (genera *Flavobacterium*, *Segetibacterium*, *Epilithonimonas* and *Pedobacter*) from *Bryum argenteum* leaves.

The dominance of *Microbacteriaceae* sp. (*Actinobacteriota*) in the bacterial assemblage of fairy ring affected moss may be notable. Representatives of *Actinobacteria* are among the most common prokaryotic organisms in Antarctic terrestrial environments (Pearce et al. 2012). They are also known as prolific producers of bioactive natural products (Liu et al. 2012), including some able to suppress plant diseases (Palaniyandi et al. 2013). Gu et al. (2020) analysed the diversity and composition of fungal and bacterial communities in continuous cropping soil from Chinese chive cultivation, reporting dominance of *Actinobacteria* in the same samples where potential phytopathogenic fungi were detected. We found a similar high *Actinobacteria* abundance pattern to that reported by Gu et al. (2020) in fairy ring infected moss.

Cyanophyceae sp. and *Haliangium* sp. (*Myxococcota*) were present in all moss samples, but were the most abundant taxa detected in dead moss. *Cyanobacteria* are the dominant phototrophs in Antarctic terrestrial and freshwater ecosystems (Taton et al. 2003) and represent the greatest accumulation of biomass the benthic habitats of lakes and ponds (Vincent 2000). Pandey et al. (2004) reported several cyanobacterial taxa in association with mosses sampled in the Schirmacher Oasis, continental Antarctica. The primary habitats of myxobacteria such as *Haliangium* are rich in organic matter (Fudou et al. 2002). These bacteria are strictly aerobic and usually live in the surface layers of the soil, but can also be found in decaying plant material (Reichenbach 1999). The dominance of these two taxa in dead moss may be due the high concentration of minerals released during the organic decomposition of the moss.

Conclusions

Previous culture-based and morphological studies have proposed fungi to be the causative agent of the 'fairy ring' disease in different Antarctic mosses species. The use of a metabarcoding approach to assess the diversity of microbial communities associated with different stages of the disease in a carpet of the moss *S. antarctica* revealed, based on sequence assignment, a greater diversity of associated mutualisticic, phytopathogenic and decomposer fungi than previously recognised, with clear community differences as the disease progressed. In contrast with the fungal community, bacterial diversity decreased in infected relative to healthy moss carpet. We recognise that the metabarcoding approach identifies sequences presence and does not confirm the viability or functional activity of the taxa detected. For these reasons, further traditional isolation studies to recover phytopathogenic fungi are necessary to understand if and how fungi may contribute to the disease and, consequently, moss death. In addition, future long-term monitoring of microbial community dynamics associated with moss carpets may provide a novel bioindicator of moss health in Antarctica.

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Data availability statement All raw sequences have been deposited in the NCBI database under the codes SAMN17612011, SAMN17612012, SAMN17612013, SAMN17612014, SAMN17612015, SAMN17612016 and SAMN17612017.

Declarations

Conflict of interest The authors declare no competing interests.

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