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

Luiz Henrique Rosa (✉ lhrosa@icb.ufmg.br)

Universidade Federal de Minas Gerais

Otávio Henrique Bezerra Pinto

Universidade de Brasília

Lívia Costa Coelho

Universidade Federal de Minas Gerais

Peter Convey

British Antarctic Survey, NERC

Micheline Carvalho-Silva

Universidade de Brasília

Carlos Augusto Rosa

Universidade Federal de Minas Gerais

Paulo EAS Câmara

Universidade de Brasília

Research Article

Keywords: Antarctica, climate change, environmental DNA, metabarcoding plant diseases

Posted Date: April 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-393340/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

We evaluated fungal and bacterial diversity in an established moss carpet on King George Island, Antarctica, affected by 'fairy ring' disease using metabarcoding. These microbial communities were assessed through the main stages of the disease. A total of 127 fungal and 706 bacterial taxa were assigned. The phylum *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. Bacterial diversity and richness were greatest in healthy moss and least within the infected fairy ring. *Chalara* sp. 1, *Alpinaria* sp., *Helotiaceae* sp. 2, *Chaetothyriales* sp. 1, *Ascomycota* sp. 1, *Rozellomycota* sp. and *Fungi* sp. were most abundant within the fairy ring samples. A range of fungal taxa were more abundant in dead rather than healthy or fairy ring moss samples. The dominant prokaryotic phyla were *Actinobacteriota*, *Proteobacteria*, *Bacteroidota* and *Cyanobacteria*. The taxon *Cyanobacteriia* sp., whilst consistently dominant, were less abundant in fairy ring samples. *Microbacteriaceae* sp. and *Chloroflexi* sp. were the most abundant taxa within the fairy rings. 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.

Introduction

Antarctic vegetation is dominated by bryophytes, with 116 species currently recognised representing cosmopolitan, endemic and bipolar taxa^{1,2}. Antarctic 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^{3,4,5}. Well established Antarctic moss carpets may act as "sentinels" sensitive to environmental changes, particularly in temperature and hydration, across the Antarctic Peninsula region⁵. Moss carpet health has been a subject of research attention since the early years of Antarctic terrestrial research⁶. 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^{1,7,8,9,10,11,12,13}. Most recently, Rosa *et al.*¹⁴ recorded the development of fairy rings on previously unreported moss 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 the phytopathogenic agent causing the disease. Fenton *et al.*¹¹ 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.*¹² proposed *Pythium polare* (*Oomycota*) to be the species affecting *Sanionia uncinata* on King George Island. Pawłowska *et al.*¹³ recovered and proposed *Psychonectria hyperantarctica* as the phytopathogenic fungus causing fairy rings, in line with previous work by Putzke and Pereira¹⁵. Most recently, Rosa *et al.*¹⁴ 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 taxa¹⁶. Globally, approximately 300 species from 80 genera of *Ascomycota* are known to parasitize mosses or liverworts¹⁷. Earlier studies of the cause of fairy ring disease relied on culturing approaches and direct morphological identification. Rosa *et al.*¹⁴ 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^{18,19,20}. 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 was investigated across different fairy ring disease stages in a well-established moss carpet on 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. All sampled mosses were identified based on their macro- and micromorphological characteristics, using dissecting and optical microscopes with slides prepared using Hoyer's solution. The moss carpet was formed by the species *Sanionia uncinata* (Hedw.) Loeske identified by the Dr. Paulo EAS Câmara from University of Brasília based on macro- and micromorphological characteristics with reference to Ochyra *et al.*¹. All moss specimens are deposited in the University of Brasília Herbarium (UB) under the code UB 210175. The licences to collect and study the plants in Antarctic Peninsula were authorized by the Secretariat of the Antarctic Treaty, Brazilian Antarctic Program and Ministry of Environment of Brazil, according to the international Antarctic Treaty rules. The study was conducted in accordance with relevant guidelines.

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^{21,22}. PCR-amplicons were generated using the universal primers ITS3 and ITS4 for fungi²³. For bacteria we used the 16S rRNA gene V3-V4 region^{24,25}. 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 Sickel version 1.33 -q 30 -l 50²⁶, 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 analyses²⁷. 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 chimeras²⁸. Taxonomic assignments were determined for amplicon sequence variants (ASVs) using the qiime2-feature-classifier²⁹ classify-sklearn against the UNITE fungal ITS database version 8.2³⁰ trained with Naive Bayes classifier and a confidence threshold of 98.5%. For bacteria, sequences were quality filtered using "quality-filter q-score-joined" plugin to improve diversity³¹. Sequences were denoised using deblur³² with-p-trim-length parameter of 300, and were taxonomically assigned 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 obtained³³, and thus lead to misinterpretation of absolute abundance³⁴. However, Giner *et al.*³⁵ concluded that such biases did not affect the proportionality between reads and cell abundance, implying that more reads are linked with higher abundance^{36,37}. Therefore, for comparative purposes, we used the number of reads as a proxy for relative abundance. Fungal classification followed Kirk *et al.*³⁸, Tedersoo *et al.*³⁹, 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 to quantify the taxa present in the different disease stages as described by Rosa *et al.*²⁰, 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 1.90⁴⁰. 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)⁴¹ 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 detected 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. 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 *Cyanobacteriia* 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.

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	121.3	22.48	108.6	27.37	112.6	30.07	111.1
Margalef	7.61	63.26	8.05	57.96	8.91	59.71	12.45	64.11
Simpson	0.68	0.98	0.83	0.98	0.90	0.97	0.91	0.98
All of the results were obtained with 95% confidence and bootstrap values were calculated from 1,000 iterations.								

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 peltigerae*, *Ascomycota* sp. 2, *Mortierella fimbricystis*, *Lamprospora* sp., *Melanommataceae* sp., *Pseudogymnoascus* sp. 1 and *Platyglloeaceae* 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 bacterial 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 prokaryote phyla, *Cyanobacteriia* 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 abundant in dead moss. *Cyanobacteriia* sp. and *Haliangium* sp. (*Myxococcota*) were the most abundant taxa detected in dead moss.

Discussion

Fungal diversity

Mosses are the dominant flora in Antarctica, providing habitat for multiple microbial and invertebrate taxa and communities^{1,14}. Endophytic and epiphytic fungi and bacteria are considered to be the dominant microorganisms present in these habitats, known as the bryosphere^{42,43,44}.

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 recently¹⁴ 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.*¹⁴, only representatives of the genera *Alpinaria*, *Helotiales*, *Cladosporium*, *Cadophora*, *Pseudogymnoascus*, *Glarea*, *Chalara*, *Ophiocordycipitaceae*, *Juncaceicola* and the species *Mortierella fimbricystis* and *Gyoerffyyella 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*)⁴⁵. It has recently been reported on mosses affected by fairy ring disease in maritime Antarctica¹⁴. The family *Melanommataceae* includes plant pathogenic species such as *Gemmamyces piceae*⁴⁶ and, according to the FunGuild database⁴⁷, *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 species⁴⁸. Plant pathogens in the genus include *C. fusiformis* on leaves of *Rhododendron*⁴⁹, *C. eucalypti* and *C. eucalyptorum* on *Eucalyptus*⁵⁰, *C.*

gevuinae on *Gevuina*⁵¹, *C. empetri* on *Vaccinium*⁵² and *C. proteae* on *Protea caffra*⁵³. *Rozellomycota* species are common in temperate, sub-Arctic and Antarctic environments¹⁸. According to Grossart *et al.*⁵⁴, 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 disease⁵⁵.

The taxa *Chaetothyriales* sp. 1, *Serendipita* sp., *Agaricomycetes* sp., *Sebacinales* sp. and *Knufia peltigerae* are notable due 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 Antarctic moss carpet. The order *Chaetothyriales* (*Ascomycota*) includes species with multiple ecological roles, including soil saprophytes, human and animal opportunistic pathogens and plant epi- and/or endophytes⁵⁶. In addition, some representatives of *Chaetothyriales* are known to colonize extreme environments characterized by drought, oligotrophic conditions, extreme temperatures and high UV-radiation exposure⁵⁷. Some species are known phytopathogens⁵⁸.

Serendipita is a genus with eight known species³⁸, including *S. indica*, formerly known as *Piriformospora indica*⁵⁹, an endophytic fungus detected in low-nutrient desert soil in Rajasthan, India⁶⁰, which acts to increase nutrient uptake and utilization in its host^{61,62}. *Serendipita* has been reported as an endophyte of bryophytes⁶³. *Agaricomycetes* is a class of *Basidiomycota* that includes almost 21,000 described species³⁸ whose members play different ecologicals role such as decomposers, pathogens and mutualists in different environments⁶⁴.

The order *Sebacinales* (*Agaricomycetes*, *Basidiomycota*) includes species recognized to show diverse interactions with plants, which range from mutualistic root endophytes (obligate biotrophs, mycorrhizae) to saprophytes⁵⁹. 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*⁶⁵.

The genus *Knufa* comprises black fungi and has six known species⁶⁶. *Knufia peltigerae* is a lichenicolous fungus⁵⁸ which, according to Lawrey & Diederich⁶⁷, represents an important ecological group that form obligate associations with lichens. The ascomata of *K. peltigerae* (originally reported as *Capronia peltigerae*) was first described on thalli of the lichen *Peltigera rufescens*^{68,69}. *Peltigera rufescens* is a cosmopolitan lichen that occurs on sub-Antarctic South Georgia, the South Orkney Islands and in various locations along the Antarctic Pensinsula (both east and west coasts, including James Ross and Alexander Islands)⁷⁰. Possibly analogous to the bleaching effect of fairy rings on mosses, Untereiner *et al.*⁶⁹ 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.*⁷¹ 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 promoters⁶⁰. Bridge & Newsham⁷² 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 environments⁶⁴.

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 functions³⁸. 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^{73,74}.

Previous studies have concluded that the causative agent of the fairy ring disease in Antarctica is *Psychonectria hyperantarctica*, identified using classical morphological techniques from its fruiting body^{9,13}. 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.*¹⁴ 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 symptoms, 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.*⁶ 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.*⁷⁵ 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 Antarctica. However, the overall dominance of the phyla *Actinobacteriota*, *Proteobacteria*, *Bacteroidota* and *Cyanobacteria* documented here are consistent with studies such as those of Holland-Moritz *et al.*⁷⁶ and Wang *et al.*⁷⁷, 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.*⁷⁸ reported *Proteobacteria*, *Cyanobacteria* and *Firmicutes* as dominant groups. Park *et al.*⁷⁵ and Câmara *et al.*⁷⁹ reported highest relative abundances of sequences representing the phylum *Actinobacteria* in a transplanted *S. uncinata* carpet moss in a study also carried out on Keller Peninsula. Raymond⁸⁰ reported *Actinobacteria* (genera *Conexibacter*, *Rhodococcus*, *Marmoricola*, *Micromonospora* and *Streptomyces*) and *Bacteroidetes* (genera *Flavobacterium*, *Segetibacterium*, *Epilithonimonas* and *Pedobacter*) from *Bryum argenteum* leaves, further suggesting that they may offer the moss some freezing protection.

The dominance of *Microbacteriaceae* sp. (*Actinobacteriota*) in the assemblage of fairy ring affected moss may be notable. Representatives of *Actinobacteria* are among the most common prokaryotic organisms in Antarctic terrestrial environments⁸¹. They are also known as prolific producers of bioactive natural products⁸², including some able to suppress plant diseases⁸³. Gu *et al.*⁸⁴ 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.*⁸⁴ in fairy ring infected moss, which may suggest that the abundance of *Microbacteriaceae* sp. is linked with the presence of pathogenic fungi causing the fairy ring symptoms.

The presence of members of the phylum *Chloroflexi* may indicate involvement with organic matter degradation⁸⁵. Suominen *et al.*⁸⁶ reported *Chloroflexi* as important agents of decomposition in the Black Sea sulphidic zone. Colatriano *et al.*⁸⁷ reported that the genomes of *Chloroflexi* from the Arctic Ocean include several aromatic compound degradation genes.

Cyanobacteriia 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 ecosystems⁸⁸ and represent the greatest accumulation of biomass the benthic habitats of lakes and ponds⁸⁹. Pandey *et al.*⁹⁰ 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 matter⁹¹. These bacteria are strictly aerobic and usually live in the surface layers of the soil, but can also be found in decaying plant material⁹². 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 reported 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 mutualistics, phytopathogens 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 sequence presence, and does not confirm the viability or functional activity of the species 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.

Declarations

Acknowledgements

We acknowledge financial support from PROANTAR CNPq (442258/2018-6), INCT Criosfera, FAPEMIG, CAPES, FNDCT and Pró-Reitoria de Pesquisa (PRPq) of the Universidade Federal de Minas Gerais. P. Convey is supported by NERC core funding to the British Antarctic Survey's 'Biodiversity, Evolution and Adaptation' Team. We also thank congresswoman Jô Moraes and the Biological Sciences Institute of the University of Brasília.

Author contributions

LHR, LCC and PEASC conceived the study. LCC and LHR performed fungal DNA extraction from mosses samples. LHR, PEASC, LCC, OHBZ, PC, MCS and CAR analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Data Availability Statement

All raw sequences have been deposited in the NCBI database under the code PRJNA715819.

References

1. Ochyra, R., Lewis-Smith, R.I., Bednarek-Ochyra, H. The illustrated moss flora of Antarctica. Cambridge University Press, Cambridge, 685p. (2008).
2. Câmara, P.E.A.S. *et al.* New insights into the species diversity of *Bartramia* Hedw. (Bryophyta) in Antarctica from a morpho-molecular approach. *Antarct. Sci.* **31**, 208–215 (2019).
3. Smith, R.I.L. Terrestrial plant biology of the sub-Antarctic and Antarctic, in: Laws, R.M. (ed.) *Antarctic Ecology, vol. 1*. London, Academic Press, pp. 61-162 (1984).
4. Robinson, S.A. *et al.* Rapid change in East Antarctic terrestrial vegetation in response to regional drying. *Nat. Clim. Chang.* **8**, 879–884 (2018).
5. Wilson, J.W. Observations on concentric 'fairy rings' in Arctic moss mat. *J. Ecol.* **39**, 407-416 (1951).
6. Racovitza, A. Étude systématique et biologique des champignons bryophiles. *Mém. Mus. Natl. hist., Sér. B Bot.* **10**, 1-288 (1959).
7. Hawksworth, D.L. *Thyronectria antarctica* (Speg.) Seeler var. *hyperantarctica* D. Hawksw. var. nov. *Brit. Antarct. Sur. Bull.* **32**, 51-53 (1973).
8. Longton, R.E. The occurrence of radial infection patterns in colonies of polar bryophytes. *Brit. Antarct. Sur. Bull.* **32**, 41-49 (1973).
9. Fenton, J.H.C. Concentric fungal rings in Antarctic moss communities. *Trans. Br. Mycol. Soc.* **80**, 415-420 (1983).
10. Tojo, M. *et al.* *Pythium polare*, a new heterothallic oomycete causing brown discolouration of *Sanionia uncinata* in the Arctic and Antarctic. *Fungal Biol.* **116**, 756-768 (2012).

11. Pawłowska, J. *et al.* *Psychronectria hyperantarctica*, gen. nov., comb. nov., epitypification and phylogenetic position of an Antarctic bryophilous ascomycete. *Mycologia* **109**, 601-607 (2017).
12. Rosa, L.H. *et al.* Opportunistic fungal assemblages present on fairy rings spread on different moss species in the Antarctic Peninsula. *Polar Biol.* **43**, 587-596 (2020a).
13. Putzke, J., Pereira, A.B. Fungos muscícolas na Ilha Elefante-Antártica. *Cad. Pesq. Biol.* **24**, 155–164 (2012).
14. Rosa, L.H. *et al.* Fungi in Antarctica: Diversity, Ecology, Effects of Climate Change, and Bioprospection for Bioactive Compounds, in: Rosa, L.H. (ed) *Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications*, 1st edn. Springer, Switzerland, pp 1-18 (2019).
15. Döbbeler, P. Biodiversity of bryophilous ascomycetes. *Biodivers. Conserv.* **6**, 721–738 (1997).
16. Rosa, L.H. *et al.* DNA metabarcoding uncovers fungal diversity in soils of protected and non-protected areas on Deception Island, Antarctica. *Sci. Rep.* **10**, 1-9 (2020b).
17. Rosa, L.H. *et al.* DNA metabarcoding of fungal diversity in air and snow of Livingston Island, South Shetland Islands, Antarctica. *Sci. Rep.* **10**, 1-11 (2020c).
18. Rosa, L.H. *et al.* DNA Metabarcoding to Assess the Diversity of Airborne Fungi Present over Keller Peninsula, King George Island, Antarctica. *Microb. Ecol.* doi: 10.1007/s00248-020-01627-1 (2020d).
19. Chen, S. *et al.* Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PloS One* **5**, e8613 (2010).
20. Richardson, R.T. *et al.* Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. *Appl. Plant Sci.* **3**, 1400066. (2015).
21. White, T.J. *et al.* Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (eds) *PCR Protocols: a guide to methods and applications*, 1st edn. Academic Press, New York, pp 315–322 (1990).
22. Herlemann, D.P. *et al.* Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME J.* **5**, 1571-1579 (2011).
23. Klindworth, A. *et al.* Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **41**, e1-e1 (2013).
24. Joshi, N.A., Fass, J.N. Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files. (Version 1.33) [Software] <https://github.com/najoshi/sickle> (2011).
25. Bolyen, E. *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852-857 (2019).
26. Callahan, B.J. *et al.* DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581-583 (2016).
27. Bokulich, N.A. *et al.* Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome* **6**, 90 (2018).
28. Abarenkov, K. UNITE QIIME release for eukaryotes. Version 04.02. 2020. UNITE Community (2020).
29. Bokulich, N.A. *et al.* Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nat. methods* **10**, 57-59 (2013).
30. Amir, A. *et al.* Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *mSystems* **2**, e00191-16 (2017).
31. Medinger, R. *et al.* Diversity in a hidden world: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. *Mol. Ecol.* **19**, 32-40 (2010).
32. Weber, A.A., Pawłowski, J. Can abundance of protists be inferred from sequence data: a case study of Foraminifera. *PloS One* **8**, e56739 (2013).
33. Giner, C.R. *et al.* Environmental sequencing provides reasonable estimates of the relative abundance of specific picoeukaryotes. *Appl. Environ. Microbiol.* **82**, 4757-4766 (2016).
34. Deiner, K. *et al.* Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Mol. Ecol.* **26**, 5872-5895 (2017).

35. Hering, D. *et al.* Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Res.* **138**, 192–205 (2018).
36. Kirk, P.M. *et al.* Dictionary of the Fungi. CAB International, Wallingford (2011).
37. Tedersoo, L. *et al.* High-level classification of the Fungi and a tool for evolutionary ecological analyses. *Fungal Div.* **90**, 135-159 (2018).
38. de Carvalho C.R. *et al.* (2019) Fungi associated with plants and lichens of Antarctica, in: Rosa, L.H. (ed) *Fungi of Antarctica: Diversity, Ecology and Biotechnological Applications*, 1st edn. Springer, Switzerland, pp 165-199 (2019).
39. Hammer, Ø., Harper, D.A.T., Ryan, P.D. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol. Electron.* **4**, 1-9 (2001).
40. Ondov, B.D., Bergman, N.H., Phillippy, A.M. Interactive metagenomic visualization in a Web browser. *BMC Bioinform.* **12**, 385 (2011).
41. Möller, C., Dreyfuss, M.M. Microfungi from Antarctic lichens, mosses and vascular plants. *Mycologia* **88**, 922-933 (1996).
42. Tosi, S., Casado, B., Gerdol, R. Fungi isolated From Antarctic mosses. *Polar Biol.* **25**, 262-268 (2002).
43. de Carvalho, C.R. *et al.* Cultivable fungi associated with bryosphere of bipolar mosses *Polytrichastrum alpinum* and *Polytrichum juniperinum* in King George Island, South Shetland Islands, Maritime Antarctica. *Polar Biol.* **43**, 545-553 (2020).
44. Hashimoto, A. *et al.* *Pseudodidymellaceae* fam. nov.: phylogenetic affiliations of mycopappus-like genera in *Dothideomycetes*. *Stud. Mycol.* **87**, 187–206 (2017).
45. Jaklitsch, W.M., Voglmayr, H. Three former taxa of *Cucurbitaria* and considerations on *Petrakia* in the *Melanommataceae*. *Sydowia* **69**, 81-95 (2017).
46. Nguyen, N.H. *et al.* FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **20**, 241-248 (2016).
47. Crous, P.W., Groenewald, J.Z. They seldom occur alone. *Fungal Biol.* **120**, 1392-1415 (2016).
48. Sutton, B.C. The *Coelomycetes*. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. Commonwealth Mycological Institute, Kew, Surrey, England, 696p. (1980).
49. Prather, H.M., *et al.* Species-specific effects of passive warming in an Antarctic moss system. *Royal Soc. Open Sci.* **6**, 190744 (2019).
50. Yuan, Z.Q. Fungi and associated tree diseases in Melville Island, Northern Territory, Australia. *Aust. Syst. Bot.* **9**, 337e360 (1996).
51. Bianchinotti, M. , Rajchenberg, M. *Coleophoma gevuinae* comb. nov., a foliar pathogen on *Gevuina avellana* (Proteaceae). *Sydowia-Horn* **56**, 1-5 (2004).
52. Polashock, J.J. *et al.* The North American cranberry fruit rot fungal community: a systematic overview using morphological and phylogenetic affinities. *Plant Pathol.* **58**, 1116-1127 (2009).
53. Crous, P.W. *et al.* Fungal planet description sheets: 107-127. *Persoonia* **28**, 138-182 (2012).
54. Grossart, H.P. *et al.* Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. *Fungal Ecol.* **19**, 28–38 (2016).
55. van de Vossenbergh, B.T.L.H. *et al.* Comparative genomics of chytrid fungi reveal insights into the obligate biotrophic and pathogenic lifestyle of *Synchytrium endobioticum*. *Sci. Rep.* **9**, 8672 (2019).
56. Madrid, H. *et al.* New and interesting chaetothyrialean fungi from Spain. *Mycol. Progr.* **15**, 1179-1201 (2016).
57. Tsuneda, A., Hambleton, S., Currah, R.S. The anamorph genus *Knufia* and its phylogenetically allied species in *Coniosporium*, *Sarcinomyces* and *Phaeococcomyces*. *Botany* **89**, 523–536 (2011).
58. Gueidan, C. *et al.* A reappraisal of orders and families within the subclass *Chaetothyriomycetidae* (Eurotiomycetes, Ascomycota). *Mycol. Prog.* **13**, 1027–1039 (2014).
59. Weiß, M. *et al.* *Sebacinales* - one thousand and one interactions with land plants. *New Phytol.* **211**, 20–40 (2016).
60. Verma, A. *et al.* *Piriformospora indica*, gen. et sp. nov., a new root colonizing fungus. *Mycologia* **90**, 896-903 (1998).
61. Yadav, V. *et al.* A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant. *J. Biol. Chem.* **285**, 26532–26544 (2010).

62. Ngwene, B. *et al.* Phosphate utilization by the fungal root endophyte *Piriformospora indica*. *Plant Soil* **405**, 231-241 (2016).
63. Varma, A. *et al.* *Piriformospora indica*: a novel plant growth-promoting mycorrhizal fungus. *Agric. Res. J.* **1**, 117–131 (2012).
64. Hibbett, D.S. *et al.* 14 *Agaricomycetes*. In *Systematics and evolution* (pp. 373-429). Springer, Berlin, Heidelberg (2014).
65. Zhang, T. *et al.* Molecular analysis of fungal diversity associated with three bryophyte species in the Fildes Region, King George Island, maritime Antarctica. *Extremophiles* **17**, 757-65 (2013).
66. He, F. *et al.* *Knufia aspidiotus* sp. nov., a new black yeast from scale insects. *Phytotaxa* **153**, 39-50 (2013).
67. Lawrey, J., Diederich, P. Lichenicolous Fungi: Interactions, Evolution, and Biodiversity. *Bryologist* **106**, 80-120 (2003).
68. Fuckel, L. Symbolae Mycologicae. Zweiter Nachtrag. *Jb. nass. Ver. Naturk.* **27**, 1–99 (1874).
69. Untereiner, W.A. *et al.* The phylogenetic position of the lichenicolous ascomycete *Capronia peltigerae*. *Fungal Div.* **49**, 225–233 (2011).
70. Øvestad, D.O., Smith, R.I.L. Lichens of Antarctica and South Georgia: A Guide to Their Identification and Ecology. Cambridge University Press, Cambridge, 452p. (2001).
71. de Souza, L.M.D. *et al.* Assessment of fungal diversity present in lakes of Maritime Antarctica using DNA metabarcoding: a temporal microcosm experiment. *Extremophiles* **25**, 77–84 (2021).
72. Bridge, P.D., Newsham, K.K. Soil fungal community composition at Mars Oasis, a southern maritime Antarctic site, assessed by PCR amplification and cloning. *Fungal Ecol.* **2**, 66-74 (2009).
73. Kowalski, T. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *For. Pathol.* **36**, 264-270 (2006).
74. Husson, C. *et al.* *Chalara fraxinea* is an invasive pathogen in France. *Eur. J. Plant Pathol.* **130**, 311-324 (2011).
75. Park, M. *et al.* Endophytic bacterial diversity of an Antarctic moss, *Sanionia uncinata*. *Antarct. Sci.* **25**, 51 (2013).
76. Holland-Moritz, H. *et al.* Novel bacterial lineages associated with boreal moss species. *Environ. Microbiol.* **20**, 2625-2638 (2018).
77. Wang, S. *et al.* Moss habitats distinctly affect their associated bacterial community structures as revealed by the high-throughput sequencing method. *World J. Microbiol. Biotechnol.* **34**, 58 (2018).
78. Nakai, R. *et al.* Microflorae of aquatic moss pillars in a freshwater lake, East Antarctica, based on fatty acid and 16S rRNA gene analyses. *Polar Biol.* **35**, 425-433 (2012).
79. Câmara, P.E.A.S. *et al.* Practical measures to minimize environmental impact associated from Antarctic station construction: the largest moss carpet transplant in Antarctica and impact on its contained cryptic biodiversity. *Extremophiles*, in review (2021).
80. Raymond, J.A. Dependence on epiphytic bacteria for freezing protection in an Antarctic moss, *Bryum argenteum*. *Environ. Microbiol. Rep.* **8**, 14-19 (2016).
81. Pearce A. *et al.* Metagenomic analysis of a southern maritime Antarctic soil. *Front. Microbiol.* **3**, 403 (2012).
82. Liu, X. *et al.* Systematics-guided bioprospecting for bioactive microbial natural products. *Antonie Van Leeuwenhoek* **101**, 55-66 (2012).
83. Palaniyandi, S.A. *et al.* Effects of actinobacteria on plant disease suppression and growth promotion. *Appl. Microbiol. Biotechnol.* **97**, 9621-9636 (2013).
84. Gu, Y. *et al.* Study on the diversity of fungal and bacterial communities in continuous cropping fields of chinese chives (*Allium tuberosum*). *BioMed Res. Int.* 3589758 (2020).
85. Speirs, L. *et al.* The Phylogeny, Biodiversity, and Ecology of the *Chloroflexi* in Activated Sludge. *Front. Microbiol.* **10**, 2015 (2019).
86. Suominen, S. *et al.* A diverse uncultivated microbial community is responsible for organic matter degradation in the Black Sea sulphidic zone. *Environ. Microbiol.* (2019). doi: 10.1111/1462-2920.14902
87. Colatrigano, D. *et al.* Genomic evidence for the degradation of terrestrial organic matter by pelagic Arctic Ocean Chloroflexi bacteria. *Communi. Biol.* **1**, 90 (2018).
88. Taton, A. *et al.* Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach. *Appl. Environ. Microbiol.* **69**, 5157–5169 (2003).

89. Vincent, W.F. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. *Antarc. Sci.***12**, 374-385 (2000).
90. Pandey, K.D. *et al.* Cyanobacteria in Antarctica: ecology, physiology and cold adaptation. *Cell. Mol. Biol. (Noisy-le-grand)***50**, 575-584 (2004).
91. Fudou, R. *et al.* *Haliangium ochraceum* gen. nov., sp. nov. and *Haliangium tepidum* sp. nov.: novel moderately halophilic myxobacteria isolated from coastal saline environments. *J. Gen. Appl. Microbiol.***48**, 109–115 (2002).
92. Reichenbach, H. The ecology of the myxobacteria. *Environ. Microbiol.***1**, 15-21 (1999).

Figures

Figure 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 the South Shetland Islands archipelago 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 m²) 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.

Figure 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.

Figure 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.

Figure 4

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

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Suppl.Fig.1.docx](#)
- [Suppl.Fig.2.docx](#)
- [Suppl.Fig.3.docx](#)
- [Suppl.Table1.docx](#)
- [Suppl.Table2.docx](#)