EDITOR'S NOTE

Further studies by eDNA metabarcoding of the ecology of the Grey Waxcap (Cuphophyllus lacmus) and other fungi on Lundy by Ruben Mole, John N. Hedger, Alan Rowland, Andrew P. Detheridge and Gareth W. Griffith…follows this note.

In view of the 'information dense' nature of their account I felt it might be useful to explain some of the technical terms used, listed in the table below, most of which will be unfamiliar to many Lundy Field Society members. The project, which arose from previous field work done by LFS members, was supported by LFS grants to the lead author, Ruben Mole and to Professor Gareth Griffith.

Plate 1: Fruit bodies of the Grey Waxcap (*Cuphophyllus lacmus*) in Heather at the North End © David George.

Term	Meaning
ASV	Actual Sequence Variant: DNA sequence that represents a true biological sequence after correcting for errors
basidiolichen	Lichen made up of a basidiomycete fungus (e.g gill fungi)and an alga
biotrophic fungus	A fungus only able to grow in association with a living plant
CHEGD	Acronym of the names of fungi whose presence in grasslands indicates a high conservation value (Clavariaceae ,Hygropohraceae, Entolomataceae, Geoglossaceae & Dermoloma).
clade	A natural grouping of organisms that is composed of a common ancestor and all of its descendants.
eDNA	Environmental DNA (DNA extracted from the natural environment- e.g. water, soil, air)
Metabarcoding	Large-scale sequencing of DNA barcode regions obtained from eDNA samples (for fungi/plants ITS2 locus is generally used)
Endophytic fungus	A fungus living inside a leaves,stems etc of a plant with little or no effect on the host
epitypification	Designation of a 'type' for a species where the original material , e.g a dried specimen used to name name a specie, no longer exists or was a painting
GenBank/NCBI	Public database of DNA and protein sequences run by the National Center for Biotechnology Information, Bethesda USA (**https://www.ncbi.nlm.nih.gov/**)
Isotopic data	Relative abundance in the sample of (non-radioactive) isotopes (e.g 1% of nitrogen in air is the heavier 15N isotope [with 99% being the more 14N). Similarly for 13C and 12C carbon
ITS2 region	Barcode region for metabarcoding of Fungi. Part of the ribosomal RNA (rRNA) locus. These genes encode components of the ribosome
mycorrhiza	Mutual (symbiotic) association of plant roots with fungi
NVC Classification	System developed by John Rodwell for classificiation of plant communities across UK
ordination	Method of visualising complex (multivariate) data in simpler 2-dimensional (XY) plot. Where only the two most important components of variance across the samples are presented.
OTU	Operational taxonomic unit. Cluster of closely related sequences (typically 97% or more identical). Could represent the range of sequence of a given gene which occur in a single species
PCoA	Principle Coordinates Ordination -an ordination method
PCR	Polymerase Chain Reaction:a laboratory technique for rapidly copying specific segments of DNA, creating millions of copies from a tiny sample for analysis
phylogenetic reconstruction	Creating of a phylogenetic tree to reflect the evolution of a group of related organisms based on DNA sequences
Primer	Short synthetic DNA sequence used in PCR reactions
RA	Relative Abudance (here as % of all the fungal sequences in a sample)
Read/reads	A single sequence from the metabarcoding process
saprotrophic fungus	A decomposer fungus which derives its nutrition from dead organic matter
Sequence/sequencing	Determining the order (sequence) of nucleotides in a DNA molecule i.e. the DNA ‘code’.
SIMPER analysis	SIMPER (SIMilarity PERcentages) analysis is a statistical method used in ecology to identify which species contribute the most to the differences between groups of samples
Taxon/taxa	usually species e.g of fungi
Type specimen	original specimens used to first describe a species, usually based on the dried example kept in a herbarium (fungarium) but can be a painting (18th & 19th century taxonomy).
UNITE data base	an international data base for the molecular identification of fungi and other organisms
UNITE species hypothesis (SH)	Species Hypothesis code obtained from UNITE. Many but not all of these SH codes are linked to Latin binomial names for known organisms

FURTHER STUDIES BY eDNA METABARCODING OF THE ECOLOGY OF THE GREY WAXCAP (*CUPHOPHYLLUS LACMUS*) AND OTHER FUNGI ON LUNDY

by

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ABSTRACT

The metabarcoding of fungal environmental DNA (eDNA) was used to study the fungal community of ten 0.09 ha quadrats on Lundy dominated by Heather (*Calluna vulgaris*). At each site samples of soil, Heather roots and lichen (*Cladonia* species) were removed, and following storage and transport off the island were assayed by eDNA extraction and metabarcoding at Aberystwyth University. 1331 unique fungal OTUs (operational taxonomic units, approximating to species numbers) were detected in soil, lichen and roots. The northerly distribution of the Grey Waxcap (*Cuphophyllus lacmus*) on Lundy, previously established by fruit body surveys, was confirmed via eDNA. The significant presence of *C. lacmus* in Heather roots suggests it may be mycorrhizal (mutualistic), consistent with isotopic data and the general lack of saprotrophic ability amongst Hygrophoraceae. However, another agaric fungus, *Mycena galopus* was regularly the most abundant basidiomycete species detected in Heather roots, likely as a latent decomposer of senescent roots. A number of the other species of fungi found during the study represent new records for Lundy and data suggest an undescribed species of Waxcap may be present at the North End.

INTRODUCTION

As of 2023 a total of 833 different species or forms of true fungi, excluding the Slime Moulds and the Chromista, have been recorded on Lundy (Lundy.org.uk 2024) . The records are all based on morphology via identification of fruiting bodies of the macrofungi from field collections, as well as by microscopy of microfungi on living and dead plants and insects. Griffith *et al* (2020) were the first to study the mycoflora of Lundy by analysis of fungal eDNA of profiles in soil samples. eDNA is DNA released from an organism into the environment (Niemiller *et al.*, 2017) and is a useful tool to assess biodiversity (Bellemain *et al.*, 2010) by comparing sequences to data held online

Griffith *et al* (2020) analysed fungal eDNA in soil samples across six selected sites on Lundy in 2016. The aim was to compare the results of fruiting body and eDNA surveys of grassland fungi, especially the CHEGD species : **C**lavariaceae (Club Fungi), **H**ygrophoraceae (Waxcaps), **E**ntolomataceae (Pink Gills) , **G**eoglossaceae (Earth Tongues), **D**ermoloma/Porpoloma). Particular emphasis was placed on one species of Waxcap *Cuphophyllus lacmus* (Grey Waxcap). Yearly surveys in November by members of the Lundy Field Society since 2006 had shown that fruit bodies of this fungus seemed to be restricted to the heath of short Heather(*Calluna vulgaris*)and lichens (*Cladonia* spp.) on the north end of the island, none being found south of a line from Gannett's Coombe to St James stream on the west side, even on Heather dominated areas. Of the six sites studied, the samples from the two at the north end (one near John O'Groats and the other near Squires View), where fruit bodies of *C.lacmus* were regularly recorded, had significant presence of eDNA of this species (73% and 17 % respectively of the total fungal DNA) In contrast samples from the two grassland sites (Airfield and Castle Hill) had little or no *C.lacmus* eDNA .Of the two southerly Heather-dominated sample sites only one, below Rocket Pole, had significant presence of *C.lacmus* DNA but it was only 5% of the total fungal DNA in soil extracts. No fruit bodies of *C.lacmus* have ever been found here nor in the other sample site, above Quarry Pond.

The Grey Waxcap thus seems to be largely restricted to the species-poor Heather/ lichen community at the north end of Lundy. The challenge is to determine the reason for this association. It is unlikely that the fungus acts as a decomposer saprotroph of Heather leaf and root litter in the soil, as *Cuphophyllus* and other Waxcap genera have yet to be grown in axenic culture (Halbwachs *et al.*, 2013a) and are not decomposer fungi in the usual sense, as they lack the depolymerase enzymes necessary to utilise lignocellulose from plant litter. A more likely hypothesis suggested by studies in grassland by Halbwachs *et al.* (2018), is that Waxcaps are biotrophic partners in mycorrhizal relationships with the roots of the higher plants. In the case of *C. lacmus* on Lundy, Heather is the most likely partner, being the only higher plant in the North End heathlands where its fruit bodies are found and where the soil samples also contained Grey Waxcap eDNA. Since the family Hygrophoraceae contains several genera of basidiolichens, notably *Lichenomphalia* e.g the Heath Navel (*L.ericetorum*) on Lundy, it is possible that *C. lacmus* may form an association with Chlorophyte algae (Oberwinkler, 2012).

We are also unaware of any similar observations of *C. lacmus* fruiting in such profusion amongst Heather from any other part of the British Isles and beyond. Lundy North End is very unusual from a vegetation perspective (Figure 1). From NVC classification it would be classified most closely to '*Calluna vulgaris-Cladonia arbuscula* heath' (NVC class H13a; (Rodwell, 1991)), a habitat mainly restricted to the Cairngorm region of Scotland. Unusually, the dominant lichen at the North End is *C. portentosa*, rather than *C. arbuscula* and *C. rangifera*, which more typically dominate H13a habitats. The unexpected presence of such a community at such low altitude and latitude, is likely to be due to the thin soils

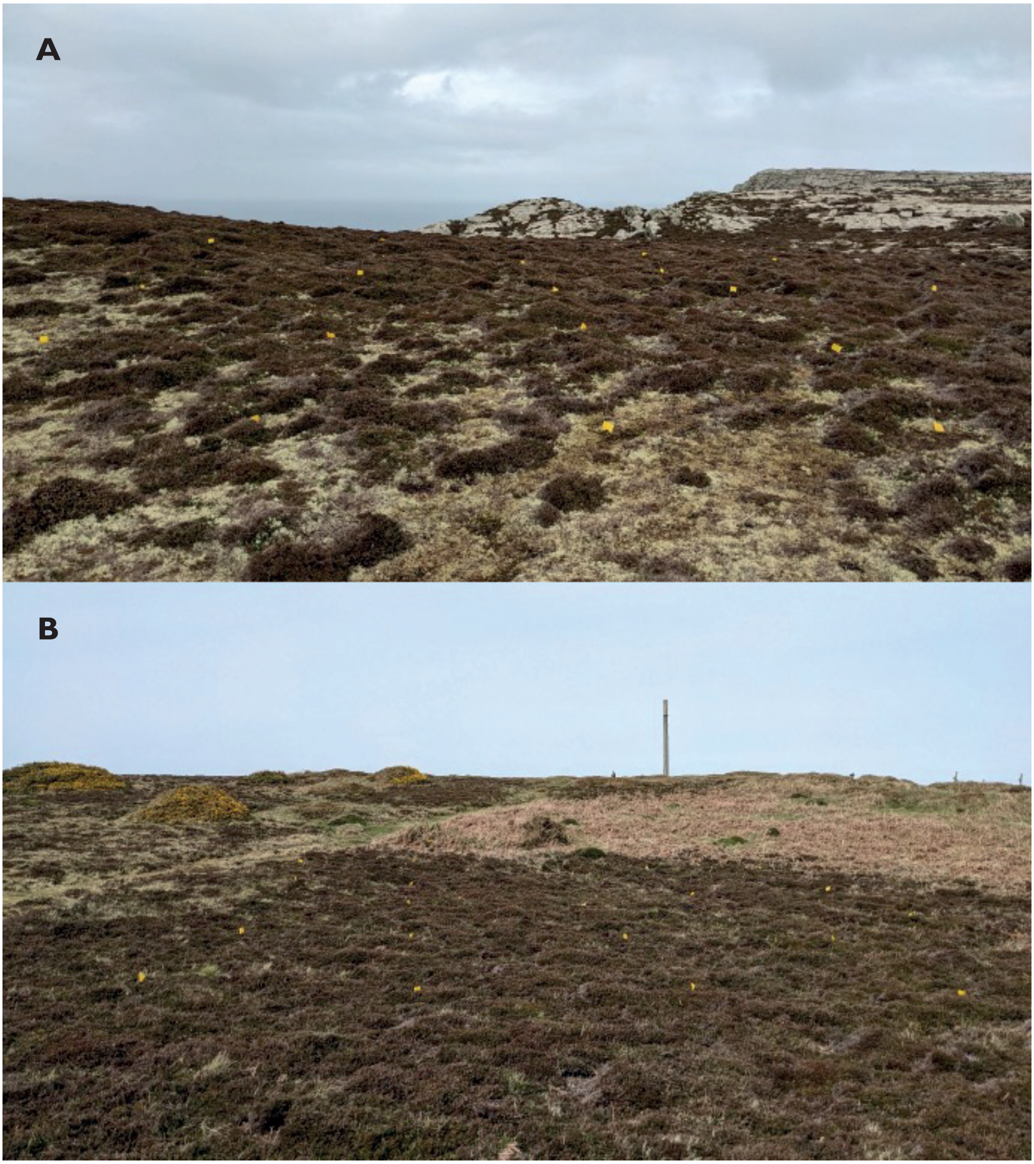


Figure 1. Heather vegetation on Lundy. A: Long Roost North (site NA1), viewed from the south, with high cover of *Cladonia* spp. (foreground) and heather; B: Rocket Pole West (site SC9), viewed from the south, with higher heather cover and little lichen. Yellow flags show the locations of the sampling positions.

on granitic rock, combined with the high wind exposure and salt spray, as well as the low levels of nitrogen deposition, to which the site is subject (Tripp *et al.*, 2012). However, it may be that the severe fire at the North End in the 1930s (Langham 1992), which destroyed much of the vegetation cover, may also be a causal factor.

AIMS

In order to test the hypothesis that the Grey Waxcap has a mycorrhizal association with either Heather or Lichen, more detailed island-wide sampling was needed. Consequently, sampling was carried out in April 2024 to compare its presence via eDNA extraction in soil, Heather roots and lichens at 10 sites north to south on Lundy

METHODS

a) *Sample Collection*

Sample collection at each site was based on the fungal eDNA sampling protocol set out by Natural England (Detheridge and Griffith, 2021). At each of the 10 sites (Figure 2), a 10 m x 10 m grid was laid out. Within each grid, 25 soil samples were taken with a T-bar gauge auger which had an 18 mm inner diameter and pooled into a sample bag; only the top 5cm of soil was taken. Four *Cladonia* thalli (each ca. 3x3 cm) were picked from a point 2 m from the edge of the grid and pooled as a single sample. It should be noted that no lichen samples were obtained from the two southern sites below Rocket Pole (SC9/SP10) due to their absence (Figure 2).

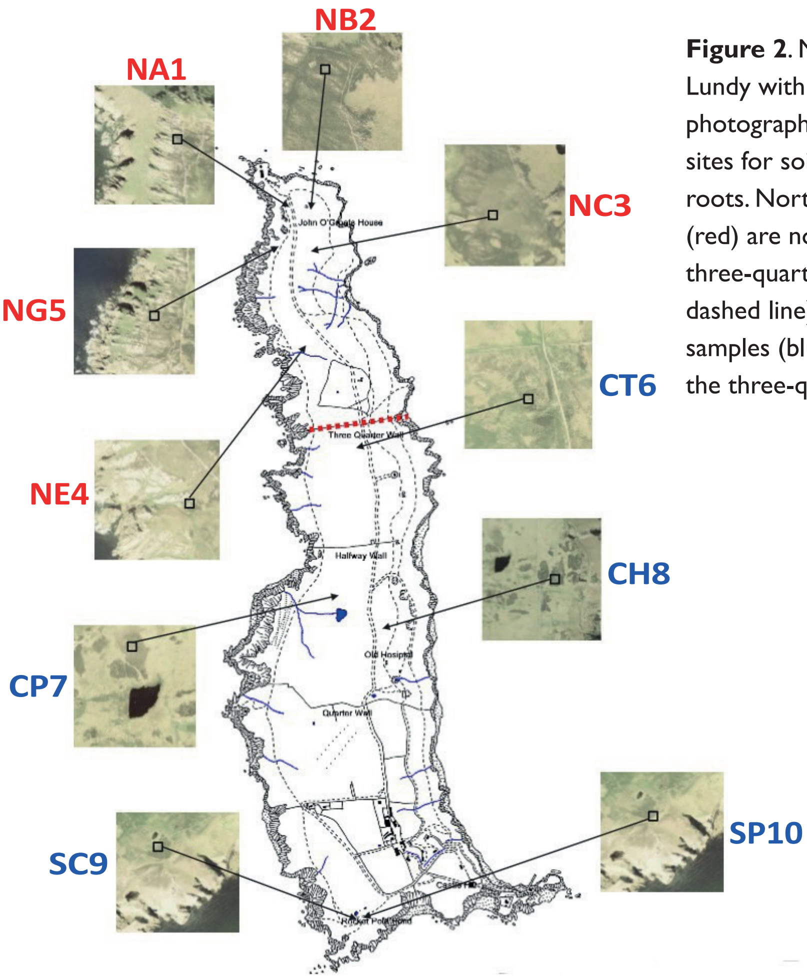


Figure 2. Map of Lundy with inserts of photographs of sampling sites for soil, lichen and roots. North samples (red) are north of the three-quarter wall (red dashed line). Southern samples (blue) are below the three-quarter wall.

Table 1. Sample and sample site details

Site code	Site name	La/Long	Soil cores FW (g)	Heather root FW (g)	Lichen thallus FW (g)
NA1	Long Roost North	51.199845, -4.674841	195	1.3	0.5
NB2	John O'Groat's House	51.199699, -4.673115	200	1.1	0.5
NC3	Gannets' Coombe	51.197597, -4.673056	188	0.7	0.3
NE4	St Peter's Stream	51.191669, -4.672654	193	0.9	0.7
NG5	Long Roost South	51.196979, -4.676416	200	1.3	0.5
CT6	Middle Park (Quarter Wall)	51.190889, -4.668675	203	0.8	0.5
CP7	Pondsbury	51.179143, -4.670674	210	0.5	0.7
CH8	Old Hospital	51.176913, -4.666633	213	1.2	1.1
SC9	Rocket Pole Pond West	51.161304, -4.668930	190	0.7	NA
SP10	Rocket Pole Pond East	51.161253, -4.668541	195	0.7	NA

Four Heather root samples were also taken 2 metres from the edge of the grid by cutting out a 10 cm x 10 cm x 5 cm (L x W x depth) soil block, which was then stored in a sample bag. On return to the Bird Observatory Laboratory in the Village, each root sample was soaked in water for at least 2 hours, after which the roots free of soil were removed with forceps, and washed again under water until 2 g of clean roots had been obtained; these were then stored in labelled sealed plastic bags. All sampling was undertaken on 5th April 2024 and samples were refrigerated within 6 hours of collecting. All equipment was scrupulously cleaned with soapy water between use on each site.

Since the site is protected under Section 28(E)(1)(a) of the Wildlife and Countryside Act 1981, sampling consent was obtained from Natural England (Charlotte Selway, Date: 4th March 2024)

b) *DNA Extraction*

Samples were sent to Aberystwyth University by overnight courier and stored at -80°C until the DNA extraction protocol had begun. For all stages, samples from different sample locations and sample types (soil, Heather roots and lichen) were kept separate, and thorough cleaning of equipment and lab space was conducted between handling of different sample locations and when switching between sample types. For the first step of this protocol, all samples were freeze-dried. The soil was freeze-dried for 1 week, and Heather root and lichen material for 24 hours. The soil samples were then sieved twice, first through a 2 mm sieve and then a 0.5 mm sieve. A 50 mg sub-sample of the fraction which had passed the 0.5mm sieve was stored in 2 ml test tubes. Lichen samples were crushed in bags using a pestle and mortar, and subsamples weighing between 10 mg and 19.5 mg were stored in 2 ml test tubes. Heather root samples were crushed in individual pestles and mortars, and 142 mg and 365 mg sub-samples were placed in 2 ml tubes. All samples were stored at -20°C until DNA extraction.

The DNA extraction of soil was conducted using the DNeasy PowerSoil Pro Kit, as described by the manufacturer's instructions, alongside two blanks, with the exception that

50 mg of freeze-dried soil was used for each location due to the hypothesised organic nature of the soil. The DNA extraction of roots was conducted using CTAB PVP lysis buffer (2% cetyltrimethylammonium bromide, 2% polyvinylpyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA) alongside two blanks. The DNA extraction of the lichen alongside two blanks was conducted using CTAB DNA extraction as above, but without the PVP (2% cetyltrimethylammonium bromide, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA).

A polymerase chain reaction PCR was then conducted on the DNA extracted from the samples using primers fITS7 and ITS4 (to isolate the ITS 2 region), which were supplemented at the 5' end with adaptors for 2nd round PCR. The 2nd round PCR products were quantified using the Qubit v2 Fluorometer with the broad range dsDNA kit and pooled in equal concentrations. The pooled library was cleaned using Ampure XP beads (Beckman Coulter) and quality checked using a Bioanalyser 2100 with high sensitivity chips (Agilent) and sequenced on the Illumina MiSeq platform (2x300 bp paired end reads).

c) *Bioinformatics*

The Illumina 2x300reads were paired using PEAR (Zhang *et al.*, 2014). Primer sequences were trimmed and the sequences quality checked with short sequences (<150bp) discarded using a Python script. Sequences were then clustered to actual sequence variants (ASV) using the UNoise3 algorithm (Edgar, 2016), singleton sequences and clusters of 5 or fewer sequences were rejected as likely sequence errors. Taxonomy of ASVs was assigned using the naïve Bayesian classifier (Wang *et al.*, 2007) against a data base created from UNITE v10 (downloaded February 2024) (Abarenkov *et al.*, 2024), with species hypotheses clustered at 98.5%.

The UNITE Species Hypothesis (SH) allows for identifying and communicating fungal species using DNA ITS sequences. Both described and undescribed fungal species are assigned a unique SH based on different levels of sequence identity (99.5% – 97%), here we use the numbers assigned to clusters at 98.5% identity. The SH is then assigned to a species name when those sequences are unambiguously named but can be left as a number if the species name is uncertain. This approach helps address the challenge of identifying fungal species, especially when actual species identification is absent but giving higher level taxonomic information.

RESULTS AND DISCUSSION

eDNA sequences recovered

From the soil and root samples taken from the five North and five South quadrats (see Figure 2 and Table 1 for a description of their locations), a total of 3,864,753 sequence reads were obtained. Following removal of singletons (sequences found only once) and low-quality sequences (76,868), as well as non-fungal sequences (254,048), there was a mean of 176,692 sequences per sample (range108,618-263,435). Similar sequences (>98.5% identical) were grouped into unique 'Species Hypothesis' (SH) clusters using the UNITE database, where they were linked to taxon names. Most SHs are linked to named species but for others, identification was only possible to higher taxonomic levels, since a high proportion of fungi are not yet sequenced and some also do not yet have reference barcodes. Within the whole dataset of soil and root samples, 1333 unique fungal ASV's were detected.

eDNA metabarcoding and distribution of 50 species of fungi in soil and root samples

eDNA metabarcoding analysis of the soil and root samples from the ten Heather quadrats is shown in Table 2. Relative abundance (as % of all fungal DNA sequences) of the 50 most common fungal taxa detected is given for soil (left hand side as two columns, *Soil north and soil south* and Heather roots (right hand side as two columns, *Root north and root south*). Also shown in the second to last column are the average abundance of these species in the Heather root samples. The ratio of abundance of the taxa in soil vs. root samples is shown in the last column. The Grey Waxcap,*Cuphophyllus lacmus* and Milking Bonnet, *Mycena galopus* are indicated in blue and red font respectively. Lower rows of the table indicate the cumulative abundance of the major families/orders found in the soil and roots.

Even with meticulous washing of roots, it not possible to be certain that all the adherent soil and mycelia have been removed (and some root fragments will be present in bulk soil). Equally it is likely that some of the roots sampled were in early stages of senescence. However, given the high disparity in relative abundance of different fungal species in roots vs soil (Table 2), it is likely that most have some endophytic capability and several may be mycorrhizal.

Communities of fungi detected in the soil samples

Analysis of the fungal communities present in the ten samples at class level (Figure 3) reveals the dominance of classes Eurotiomycetes and Leotiomycetes. Orders Chaetothyriales (12 of the 50 most abundant taxa; notably family Herpotrichiellaceae) and Helotiales (12 of the 50 most abundant taxa; notably family Helotiaceae) were dominant across all samples (mean RA 35% and 21%), thus dominating the fungal community (Table 2).

It is known that many species within these taxa are associated with the roots of plants but they are taxonomically poorly known, so most are only identifiable to family level. However, the most abundant species in the soil was identified to species level: *Pezoloma ericae,*

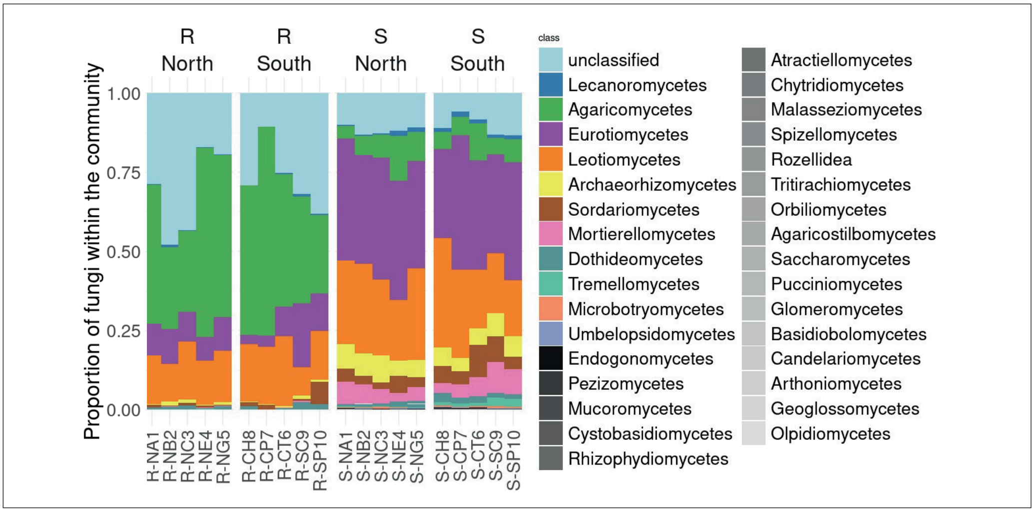


Figure 3. Stacked barchart, showing the more abundant classes of fungi detected by eDNA extraction in samples of soil and Heather roots from the North End sample sites.

Table 2. A list of the 50 most abundant species of fungi detected in soil and root eDNA at the 10 sampling sites.

				Soil NORTH					Soil SOUTH						Root NORTH					Root SOUTH						Soil:Root
	Phylum	Class	Species	NA1	NB2	NC3	NE4	NG5	CT6	CP7	CH8	SC9	SP10	Soil AVE	R-NA1	R-NB2	R-NC3	R-NE4	R-NG5	R-CT6	R-CP7	R-CH8	R-SC9	R-SP10	Root AVE	Ratio
1	Ascomycota	Leotiomycetes	Pezoloma_ericae:SH0897049.10FU	13.64%	18.63%	14.18%	6.041%	18.09%	5.436%	10.63%	13.14%	1.905%	0.742%	**10.24%**	1.685%	4.846%	6.480%	1.103%	2.980%	0.490%	3.783%	1.957%	0.254%	0.067%	**2.36%**	4.3
2	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828226.10FU	6.164%	3.881%	3.985%	7.500%	8.226%	9.446%	10.37%	8.292%	7.359%	6.182%	**7.14%**	5.94%	3.53%	2.86%	2.13%	3.69%	5.79%	1.83%	1.26%	10.87%	3.24%	**4.12%**	1.7
3	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828602.10FU	10.32%	9.054%	9.015%	6.980%	6.467%	5.976%	9.434%	3.914%	2.931%	3.615%	**6.77%**	0.412%	1.040%	0.958%	0.359%	0.678%	0.303%	0.194%	0.084%	0.344%	0.331%	**0.47%**	14.4
4	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0827748.10FU	2.952%	3.723%	5.977%	4.925%	6.522%	3.419%	6.769%	7.615%	4.084%	4.791%	**5.08%**	0.475%	1.752%	1.188%	0.514%	1.079%	0.779%	0.468%	0.487%	2.901%	0.293%	**0.99%**	5.1
5	Ascomycota	Eurotiomycetes	Sclerococcum_sp:SH0855981.10FU	4.354%	7.878%	7.177%	4.862%	2.997%	4.052%	1.023%	5.290%	5.562%	5.701%	**4.89%**	8.19%	21.36%	15.64%	2.63%	4.42%	5.47%	0.39%	4.78%	8.56%	5.41%	**7.69%**	0.6
6	Ascomycota	Archaeorhizomycetes	Archaeorhizomyces_sp:SH0764667.10FU	5.602%	4.858%	4.557%	3.459%	3.847%	3.696%	3.975%	5.662%	4.555%	4.942%	**4.52%**	0.368%	1.034%	0.522%	0.245%	0.661%	0.272%	0.129%	0.136%	0.443%	0.629%	**0.44%**	10.2
7	Mortierellomycota	Mortierellomycetes	Podila_humilis:SH0879619.10FU	4.037%	1.963%	2.135%	1.789%	1.554%	3.846%	2.153%	2.046%	6.056%	5.557%	**3.11%**	0.029%	0.024%	0.035%	0.054%	0.044%	0.061%	0.006%	0.017%	0.188%	0.065%	**0.05%**	59.6
8	Ascomycota	Eurotiomycetes	Cladophialophora_sp:SH0827868.10FU	2.066%	2.038%	2.518%	2.594%	3.272%	3.952%	3.136%	1.993%	2.368%	2.789%	**2.67%**	0.696%	0.242%	0.425%	1.024%	1.358%	1.215%	0.419%	0.099%	1.808%	1.107%	**0.84%**	3.2
9	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:OTU 18	2.221%	3.311%	2.027%	3.376%	1.331%	2.102%	3.872%	1.779%	2.607%	3.779%	**2.64%**	0.276%	1.056%	0.746%	0.292%	0.746%	0.522%	0.103%	0.342%	1.786%	1.019%	**0.69%**	3.8
10	Ascomycota	Eurotiomycetes	Eurotiomycetes_sp:OTU 48	3.952%	1.283%	2.536%	1.458%	0.348%	2.348%	3.595%	1.784%	1.384%	3.588%	**2.23%**	0.109%	0.079%	0.046%	0.055%	0.004%	0.115%	0.036%	0.010%	0.113%	0.393%	**0.10%**	23.2
11	Ascomycota	Eurotiomycetes	Herpotrichiellaceae_sp:SH0828653.10FU	3.019%	5.266%	4.464%	3.934%	2.875%	2.350%			0.067%	0.100%	**2.21%**	0.631%	1.415%	1.042%	0.552%	0.797%	0.226%			0.016%	0.041%	**0.47%**	4.7
12	Ascomycota		Ascomycota_sp:SH0945193.10FU	2.019%	3.064%	1.564%	2.947%	1.604%	0.780%	1.784%	2.915%	1.719%	1.383%	**1.98%**	0.072%	0.093%	0.062%	0.112%	0.146%	0.047%	0.018%	0.026%	0.022%	0.077%	**0.07%**	29.4
13	Basidiomycota	Agaricomycetes	Cuphophyllus_lacmus:SH0952050.10FU	0.590%	2.981%	2.913%	9.170%	3.259%	0.010%			0.002%	0.804%	**1.97%**			2.976%	2.492%	1.509%					0.006%	**0.70%**	2.8
14	Ascomycota	Leotiomycetes	Helotiales_sp:SH0741605.10FU	0.757%	0.961%	0.765%	1.827%	0.768%	2.470%	0.204%	3.208%	3.567%	3.148%	**1.77%**	0.052%	0.214%	0.052%	0.054%	0.091%	0.159%		0.858%	1.209%	0.229%	**0.29%**	6.1
15	Ascomycota	Eurotiomycetes	Capronia_sp:SH0828621.10FU	2.073%	0.444%	1.966%	2.013%	1.088%	1.810%	3.131%	0.796%	1.311%	1.373%	**1.60%**	0.016%	0.031%	1.030%	0.728%	0.178%	0.012%	0.267%	0.200%	0.489%	0.647%	**0.36%**	4.4
16	Ascomycota	Leotiomycetes	Leotiomycetes_sp:SH0741574.10FU	1.849%	1.637%	1.342%	0.727%	0.711%	1.542%	1.039%	2.051%	2.206%	2.149%	**1.53%**	0.052%	0.148%	0.136%	0.015%	0.048%	0.234%	0.023%	0.138%	0.267%	0.182%	**0.12%**	12.3
17	Ascomycota	Leotiomycetes	Helotiales:_UNKsp:OTU 52	2.157%	0.364%	0.197%	1.289%	2.138%	0.002%	5.212%	2.999%			**1.44%**	0.128%	0.003%	0.043%	0.054%	0.143%		0.622%	0.045%			**0.10%**	13.8
18	Ascomycota	Leotiomycetes	Helotiales_sp:SH0741401.10FU	1.454%	2.101%	1.222%	1.031%	0.558%	2.037%	1.415%	1.722%	0.030%	1.715%	**1.33%**	0.096%	0.786%	0.321%	0.008%	0.435%	0.044%	0.026%	0.077%	0.005%	0.200%	**0.20%**	6.7
19	Ascomycota	Leotiomycetes	Hyaloscypha_sp:SH0897600.10FU	2.324%	0.570%	1.996%	1.407%	1.240%	0.826%	2.086%	0.773%	0.828%	1.179%	**1.32%**	3.527%	0.321%	4.970%	2.337%	5.504%	1.594%	0.469%	2.947%	0.432%	0.638%	**2.27%**	0.6
20	Ascomycota	Eurotiomycetes	Sclerococcum_sp:SH0931721.10FU	0.714%	0.956%	1.939%	0.513%	3.299%	0.326%	0.007%	0.005%	1.777%	2.539%	**1.21%**		1.695%	2.821%	0.004%	0.871%	0.002%		0.002%	0.020%	0.252%	**0.57%**	2.1
21	Ascomycota	Archaeorhizomycetes	Archaeorhizomyces_sp:SH0783911.10FU	1.918%	2.183%	3.579%	0.661%	1.041%	1.232%	0.078%	0.178%		0.038%	**1.09%**	0.005%	0.162%	0.333%	0.015%	0.099%	0.031%					**0.06%**	16.9
22	Ascomycota	Eurotiomycetes	Capronia_sp:SH0827807.10FU	2.933%	0.606%	0.924%	0.179%	1.077%	0.745%	0.135%	0.148%	1.512%	2.503%	**1.08%**	0.190%	0.264%	0.629%	0.189%	0.307%	0.001%			0.029%	2.204%	**0.38%**	2.8
23	Ascomycota	Eurotiomycetes	Herpotrichiellaceae_UNKsp:OTU 89	1.432%	2.800%	3.278%	0.620%	0.272%	1.035%	0.069%		0.313%	0.720%	**1.05%**	0.011%	0.092%	0.049%	0.010%	0.019%	0.015%	0.004%		0.033%	0.030%	**0.03%**	40.0
24	Ascomycota	Sordariomycetes	Hypocreales_sp:SH0940997.10FU	0.256%	0.058%	0.153%	1.265%	0.723%	3.873%	0.078%	0.175%	3.424%	0.314%	**1.03%**			0.076%		0.021%	0.032%		0.001%	0.033%	0.197%	**0.04%**	28.7
25	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828164.10FU	0.339%	0.625%	0.303%	1.536%	0.310%	0.274%	0.749%	0.865%	1.249%	2.657%	**0.89%**	0.319%	1.469%	0.292%	0.436%	1.224%	0.250%	0.077%	0.357%	0.014%	0.003%	**0.44%**	2.0
26	Mortierellomycota	Mortierellomycetes	Mortierella_turficola:SH0867680.10FU	1.007%	1.378%	1.281%	0.357%	0.776%	1.103%	0.477%	0.242%	0.645%	0.463%	**0.77%**	0.019%	0.011%	0.008%	0.003%	0.022%	0.010%	0.009%		0.027%	0.005%	**0.01%**	68.5
27	Ascomycota	unclassified_Ascomyc	Ascomycota_sp:SH0857096.10FU	0.790%	0.223%	0.417%	0.932%	0.568%	0.981%	0.650%	0.534%	1.075%	0.667%	**0.68%**	0.237%	0.072%	0.002%	0.303%	0.023%	0.030%		0.027%	**0.075%**	0.003%	**0.08%**	8.9
28	Ascomycota	Lecanoromycetes	Micropeltidaceae_sp:SH0735777.10FU	0.231%	0.124%	0.236%	0.994%	0.533%	0.945%	1.251%	0.980%	0.544%	0.757%	**0.66%**						0.002%		0.024%	0.005%		**0.00%**	206.1
29	Ascomycota		Ascomycota_sp; OTU 42	0.781%	0.171%	0.365%	1.096%	0.611%	0.293%	0.551%	0.502%	0.501%	1.203%	**0.61%**	1.402%	1.139%	0.569%	0.606%	0.818%	0.539%	0.247%	0.192%	0.424%	0.276%	**0.62%**	1.0
30	Ascomycota	Leotiomycetes	Leotiomycetes_sp:SH0741719.10FU	0.020%	0.716%	0.825%	0.047%		0.286%		0.112%	2.454%	1.153%	**0.56%**			0.219%	0.038%	0.021%				0.048%	0.182%	**0.05%**	11.0
31	Ascomycota	Leotiomycetes	Meliniomyces_sp:SH0897224.10FU	0.323%		0.080%	1.421%	0.170%	0.313%	0.484%	1.922%	0.030%	0.399%	**0.51%**	1.868%		0.004%	0.033%		0.028%	4.506%	4.182%	0.002%	0.027%	**1.06%**	0.5
32	Ascomycota	Leotiomycetes	Ascocorticium_sp:SH0887512.10FU	0.243%	0.208%	0.472%	0.404%	0.343%	1.281%	1.292%	0.728%		0.103%	**0.51%**	0.142%	0.594%	0.187%	0.071%	0.205%	0.320%	0.572%	0.114%			**0.22%**	2.3
33	Ascomycota	Leotiomycetes	Hyaloscyphaceae_UNKsp:OTU 33	0.759%			0.298%	0.036%	0.510%	1.663%	1.410%	0.305%	0.084%	**0.51%**	1.185%			0.025%		1.240%	4.700%	3.131%	0.131%	0.034%	**1.04%**	0.5
34	Ascomycota	Sordariomycetes	Tolypocladium_album:SH0848502.10FU	0.633%	0.899%	0.218%	0.464%	0.248%	1.031%	0.093%	0.952%	0.272%	0.147%	**0.50%**	0.039%	0.009%	0.027%	0.011%	0.030%	0.002%	0.002%	0.024%		0.006%	**0.02%**	33.0
35	Basidiomycota	Agaricomycetes	Serendipitaceae_sp:SH0734281.10FU	0.165%	0.045%	0.031%	0.547%	0.693%	0.404%	0.544%	0.903%	0.534%	1.083%	**0.49%**	1.046%	0.027%	0.016%		0.034%	0.034%	0.015%	0.153%	0.464%	0.010%	**0.18%**	2.8
36	Ascomycota	Leotiomycetes	Hyaloscypha_gryndleri:SH0897609.10FU	0.537%	0.007%	0.168%	0.634%	0.119%	0.776%	0.467%		1.373%	0.830%	**0.49%**	1.620%	0.505%	0.191%	0.389%	0.328%	1.448%			3.406%	1.191%	**0.91%**	0.5
37	Basidiomycota	Agaricomycetes	Mycena_galopus:SH0748945.10FU/OTU835	0.827%	0.366%	0.139%	0.279%	0.536%	0.256%	1.290%	0.505%	0.307%	0.190%	**0.47%**	38.96%	20.35%	18.88%	49.16%	46.99%	22.84%	33.81%	40.92%	23.71%	20.22%	**31.58%**	**0.015**
38	Ascomycota	Leotiomycetes	Oidiodendron_majus:SH0945817.10FU	0.392%	0.268%	0.394%	0.672%	0.586%	0.271%	0.210%	0.410%	0.828%	0.595%	**0.46%**	1.225%	1.439%	0.847%	0.705%	1.476%	0.227%	0.202%	0.214%	1.085%	0.429%	**0.78%**	0.6
39	Ascomycota	Archaeorhizomycetes	Archaeorhizomyces_sp:SH0783903.10FU	0.163%	0.087%		0.291%	0.460%	0.079%			2.337%	0.768%	**0.42%**			0.295%	0.064%	0.011%				0.503%	0.018%	**0.09%**	4.7
40	Mortierellomycota	Mortierellomycetes	Mortierella_rishikesha:SH0960692.10FU	0.276%	0.012%	0.067%	0.037%	0.684%	0.176%	0.234%	0.117%	1.466%	0.929%	**0.40%**	0.038%		0.006%	0.002%	0.016%				0.067%	0.018%	**0.01%**	27.4
41	Mortierellomycota	Mortierellomycetes	Entomortierella_parvispora:SH0867675.10FU	0.554%	0.187%	0.148%	0.312%	0.629%	0.560%	0.979%	0.260%	0.093%	0.218%	**0.39%**	0.013%	0.009%		0.009%	0.003%	0.002%	0.004%	0.003%	0.006%	0.003%	**0.01%**	76.8
42	Basidiomycota	Agaricomycetes	Hyaloriaceae_sp:SH0749083.10FU	0.170%	0.096%	0.273%	0.741%	0.444%	0.708%	0.395%	0.296%	0.305%	0.260%	**0.37%**	1.131%	2.099%	3.252%	2.076%	0.982%	1.753%	0.013%	0.104%	1.287%	0.111%	**1.28%**	0.3
43	Basidiomycota	Agaricomycetes	Agaricomycetes_sp:SH0848226.10FU	0.124%	0.003%	0.102%	0.503%	0.504%	1.440%	0.099%	0.003%	0.337%	0.297%	**0.34%**	0.064%	0.034%		0.027%	0.013%	0.268%			0.337%	0.094%	**0.08%**	4.1
44	Ascomycota	Sordariomycetes	Neopestalotiopsis_foedans:SH0911533.10FU	0.202%	0.103%	0.150%	0.240%	0.385%	0.605%	0.419%	0.598%	0.260%	0.216%	**0.32%**	0.006%		0.007%	0.007%	0.026%	0.004%	0.006%				**0.01%**	57.2
45	Basidiomycota	Agaricomycetes	Hymenochaetales_sp:OTU 138	0.361%	0.884%	0.861%	0.193%	0.461%	0.076%	0.219%	0.113%			**0.32%**		0.047%	0.031%	0.008%	0.066%	0.002%	0.030%				**0.02%**	17.2
46	Ascomycota	Eurotiomycetes	Capronia_sp:SH0828033.10FU	0.006%			0.561%	0.018%		0.072%		1.705%	0.788%	**0.31%**				0.017%			0.007%		0.011%	0.060%	**0.01%**	33.0
47	Ascomycota	Sordariomycetes	Tolypocladium_inflatum:SH0940809.10FU	0.164%	0.006%	0.176%	0.257%	0.083%	0.003%	0.405%	0.560%	0.804%	0.684%	**0.31%**	0.005%		0.005%	0.013%	0.007%		0.005%	0.009%	0.025%	0.023%	**0.01%**	34.4
48	Ascomycota	Leotiomycetes	Helotiales_sp:SH0741541.10FU	0.347%	0.504%	0.554%	0.253%	0.393%	0.004%	0.085%	0.776%	0.011%	0.011%	**0.29%**	0.004%	0.003%	0.245%		0.106%			0.114%		0.050%	**0.05%**	5.6
49	Ascomycota	Leotiomycetes	Hyaloscyphaceae_sp:SH0908101.10FU	0.212%	0.188%	0.225%	0.259%	0.405%	0.298%	0.123%	0.176%	0.272%	0.401%	**0.26%**		0.006%	0.004%		0.003%	0.001%					**0.00%**	192.9
50	Basidiomycota	Agaricomycetes	Clavaria_sphagnicola:SH0737317.10FU	0.419%	0.473%	1.529%	0.017%	0.034%	0.003%			0.017%	0.040%	**0.25%**		0.016%	0.046%		0.004%						**0.01%**	38.6
			Ascomycota/Basidiomycota Ratio	17.83	12.89	10.48	5.55	8.78	5.82	11.73	12.67	8.99	7.92	**10.27**	0.94	2.06	2.08	0.50	0.75	0.93	0.42	0.74	1.27	1.75	**1.14**	9.0
	Basidiomycota		**Clavariaceae**	0.42%	0.47%	1.53%	0.02%	0.03%	0.01%			0.76%	0.78%	**0.40%**		0.02%	0.05%		0.00%					0.06%	**0.0001**	31.5
	Basidiomycota		**Entolomataceae**	0.03%	0.00%	0.01%	0.02%	0.02%	0.00%		0.01%	0.32%	0.29%	**0.07%**											n/a	n/a
	Basidiomycota		**Hygrophoraceae**	0.59%	3.08%	2.99%	9.17%	3.33%	0.01%			0.00%	0.80%	**2.00%**		0.02%	3.02%	2.49%	1.51%				0.003%	0.01%	**0.01**	2.8
	Basidiomycota		**Mycenaceae**	0.87%	0.41%	0.18%	0.54%	0.68%	0.79%	1.93%	0.88%	0.61%	0.51%	**0.74%**	40.65%	20.47%	18.88%	49.26%	48.05%	22.84%	65.83%	46.77%	24.07%	20.28%	**0.36**	**0.021**
	Ascomycota		Chaetothyriales	38.17%	34.21%	38.29%	37.59%	33.37%	34.37%	41.98%	27.65%	30.58%	36.72%	**35.29%**	9.53%	11.04%	9.44%	7.43%	10.43%	9.33%	3.49%	2.86%	19.99%	11.77%	**0.10**	3.7
	Ascomycota		**Archaeorhizomycetes**	7.71%	7.14%	8.42%	4.78%	5.42%	5.24%	4.05%	5.89%	7.21%	6.54%	**6.24%**	0.37%	1.20%	1.17%	0.34%	0.77%	0.32%	0.13%	0.14%	1.16%	0.73%	**0.01**	9.9
	Ascomycota		**Helotiales**	24.41%	25.69%	21.67%	17.70%	27.70%	16.25%	26.05%	31.61%	12.26%	13.31%	**21.67%**	14.59%	11.13%	17.33%	12.96%	14.93%	6.30%	17.46%	17.61%	8.22%	14.79%	**0.14**	1.6
	Ascomycota		**Lecanoromycetes (lichenised fungi)**	0.37%	0.36%	0.45%	1.74%	1.40%	1.29%	1.61%	1.27%	0.87%	1.34%	**1.07%**	0.15%	0.83%	0.13%	0.05%	0.20%	0.43%	0.05%	0.08%	0.70%	0.39%	**0.00**	3.5

a known mycorrhizal symbiont of Heather (>10% mean relative abundance across all the samples). Also abundant were three species of the genus *Archaeorhizomyces*, a recently discovered group of abundant soil dwelling fungi (Rosling *et al.*, 2011), which are associated with ericoid hosts (Baba and Hirose, 2024). Several species of *Mortierella*, a genus found in higher abundance in Arctic/alpine soils were also abundant (Telagathoti *et al.*, 2021).

Ordination of the whole fungal community present in the soils showed that the fungal communities in soils from the North End were significantly different (PERMANOVA pseudo-P=0.0159) from those further south (Figure 4). SIMPER analysis revealed that the five taxa contributing most to this difference and explaining 26% of the total variance were: *Pezoloma ericae*, *Cuphophyllus lacmus* and three unidentified Chaetothyriales species.

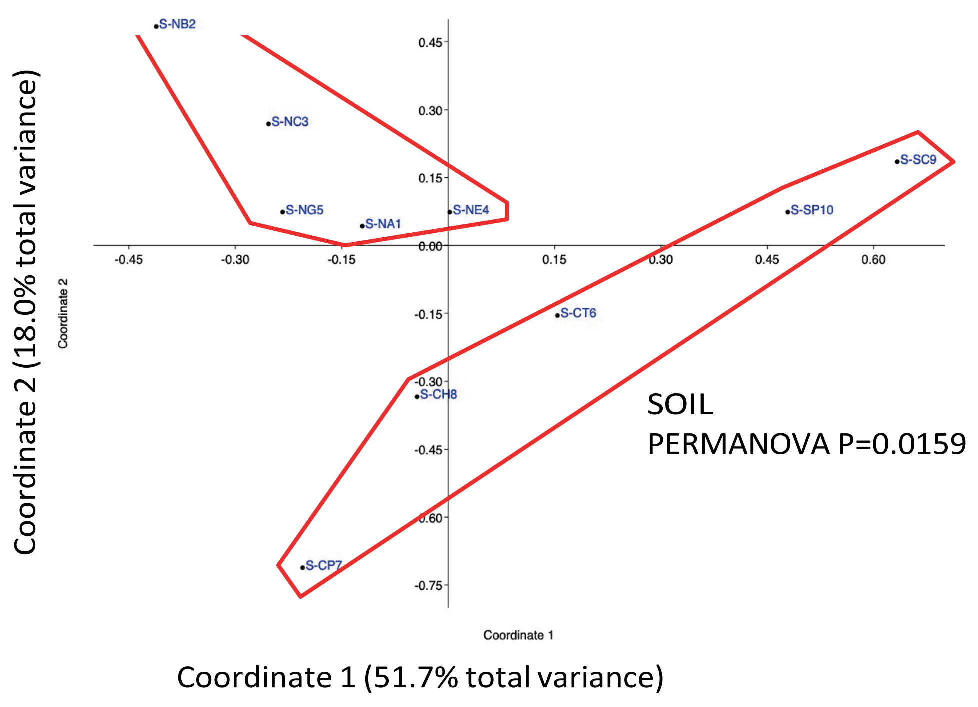


Figure 4. Ordination of soil fungal communities using Principal Coordinates Ordination analysis (PCoA). The communities from quadrats at the north end and south end of Lundy were significantly different (PERMANOVA pseudo-P = 0.0159).

CHEGD species of fungi found in the soil samples

In the **C**lavariaceae (Club and Coral Fungi), the most abundant was *Clavaria sphagnicola*, not yet recorded from fruit bodies on Lundy, which was present in 8/10 soil samples, including all the North End samples. As well as the Grey Waxcap, three other Waxcaps (**H**ygrophoraceae) were detected. Two were identifiable to species level (Goblet Waxcap, *Hygrocybe cantharellus* and Glutinous Waxcap, *Hygrocybe glutinipes*), with a fourth, detected in 3/5 of the North End soil samples, not matching any known species but closest to the Cedarwood Waxcap *Cuphophyllus russocoriaceus*. The ITS2 sequence was only 93% identical to *C. russocoriaceus*, so may represents a new species. The basidiolichen Lichenomphalia *umbellifera* (not strictly a Waxcap but closely related and belonging to family Hygrophoraceae) was also detected in the soil from two quadrats.

Five **E**ntolomas(Pink Gills,family Entolomataceae) were identifiable to species level *Entoloma elodes, E. jubatum* (Sepia Pinkgill) ; *E. conferendum* (Star Pinkgill); *E. turbidum* (Yellowfoot Pinkgill) *and E. ventricosum*. *E.elodes* and *E.ventricosum* have not yet been recorded for Lundy. The other species are widespread on Lundy though the Yellow Foot PInkgill is recorded most frequently from the North End with Heather. No **G**eoglossaceae nor ***D****ermoloma* spp. were detected in soil samples. Many of the Clavariaceae and Entolomataceae sequences detected (37.2% and 8.1% respectively) could not be identified to species level, due to lack of reference DNA barcode or potentially the species being unknown to science, as are >95% of all fungi (Hawksworth and Lücking, 2017).

Grey Waxcap (*Cuphophyllus lacmus).*

By far the most abundant CHEGD species in the eDNA from the soil samples was *C.lacmus* (1.97% mean relative abundance [RA] of all fungal sequences) (summarised in Figure 5). It was detected in all the North quadrats (mean RA 3.78%; maximum RA was 9.2% in quadrat NE4) but also present in three of the South quadrats though at lower RA (0.16%). These data are consistent with annual autumn monitoring of Lundy-wide fruiting of this species by Lundy Field Society members since 2006 (summarised in Griffith *et al.*2020), the north end being the only place it has been found. The *C. lacmus* sequences detected from soil samples in the present study confirmed the northern distribution of the fungus on Lundy, though the detection of *C. lacmus* eDNA in soil at site SP10 (below Rocket Pole and above the Devil's Limekiln), a finding also made by Griffith *et al.* (2020), means that the species is present in more southerly quadrats but only as a minor component of the soil mycota. Data from GBIF (https://www.gbif.org/species/2538616) do not indicate that *C. lacmus* is particularly associated with heathland vegetation but for most of the >2000 global records, no habitat description is provided. However, several UK mycologists report that they encounter this species commonly in heathland habitats (Emma Williams and David Mitchel, personal communications, 2025).

Our earlier eDNA analysis (Griffith *et al.*, 2020) included two heathland quadrats (Lu2/Lu3) from the north end of the island. The metabarcoding approach used was slightly different (LSU S1 barcode locus which provides inferior taxonomic resolution compared to ITS2) but, as in the present study, *C. lacmus* was the dominant Waxcap in both quadrats. Whilst it is risky to make direct comparison of the RA (relative abundance) of *C. lacmus*, it is noteworthy that the RA of *C. lacmus* from the heathland quadrats was higher (74% and 17%) than found in the present study. This may relate to the dates of sampling, February 2016 in the 2020 report, vs April in the present study, where decomposition of roots which had died over the winter period could have resulted in a decline in *C. lacmus* RA in the April samples.

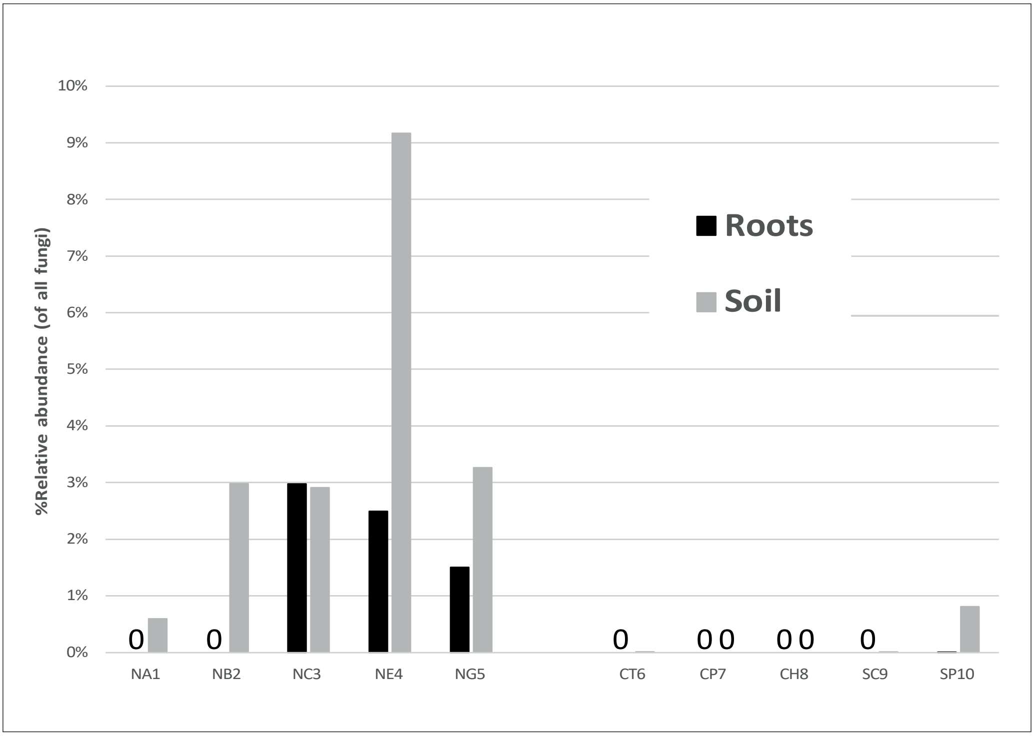
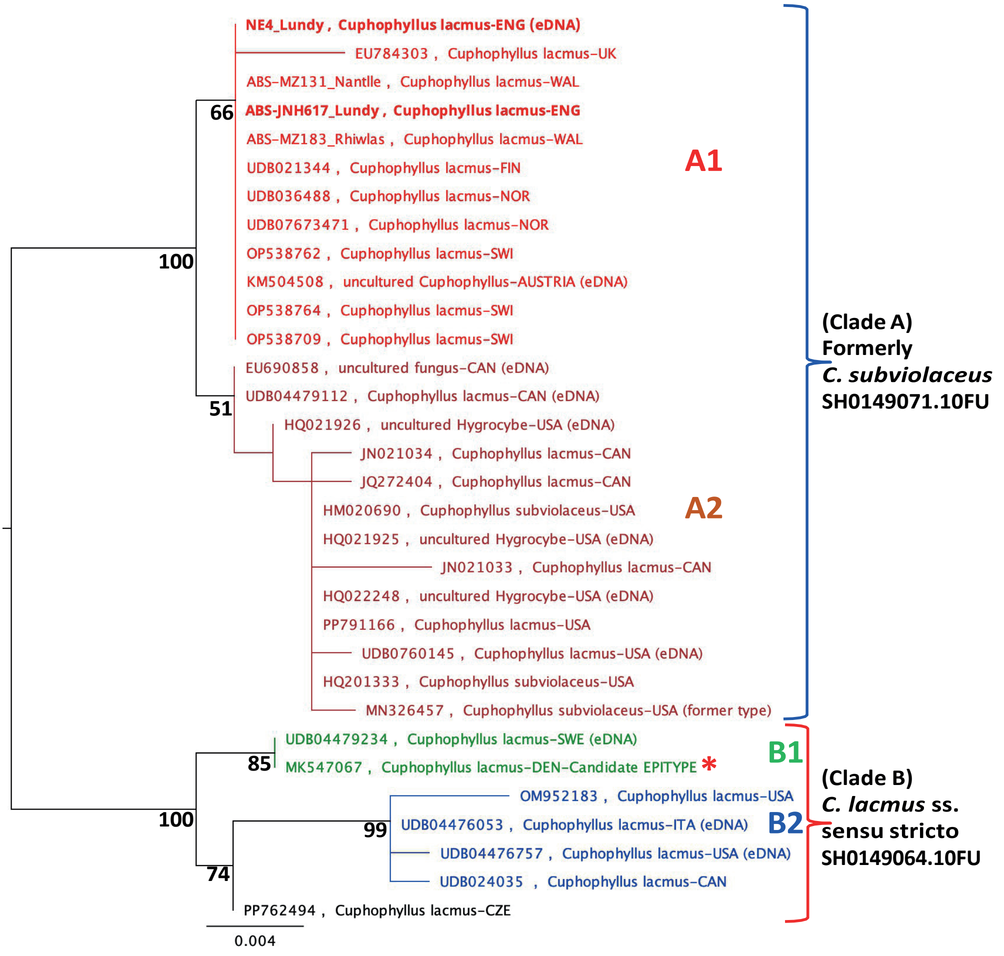


Figure 5. Percentage relative abundance of *Cuphophyllus lacmus* sequences in soil and roots from the ten quadrats.

The DNA sequence obtained from eDNA metabarcoding was identical to that obtained from a fruitbody collected at the North End in 2004. Comparison of these ITS sequences with others from the GenBank and UNITE sequence repositories and DNA barcodes from the Aberystwyth (ABS) fungarium revealed that *C. lacmus* exhibits some intraspecific variation, with two distinct clades present (Figure 6), corresponding to UNITE species hypotheses SHSH0149071.10FU and SH0149064.10FU (both at 97% clustering).

The Lundy samples and all other UK sequences sit close to a species formerly known as *C. subviolaceus* (Peck) Bon (Voitk *et al*., 2020), mostly recognised from north America. However, this species was synonymised with *C. lacmus* by Bon (1985). The data presented here support Bon's decision, since, whilst distinct, the ITS sequences of the two clades are >96% identical and members of both clades are present both in northern Europe and north America. It is interesting to note that *C. lacmus* was first named (as *Agaricus lacmus*) by Schumacher in 1803 ("*in ericetis circa*" [amongst Heather] in Birkerød, Denmark). To our knowledge, Schumacher's observation is the only other record of *C. lacmus* being found in Heather.

It is suggested that formal epitypification of *C. lacmus* is undertaken, potentially using voucher C-F-17644 (GenBank MK547067), collected in Denmark by David Boertmann (Voitk *et al*., 2020).



		ABS-JNH61...	HM020690	MK547067	UDB024035
A1: lacA EUR	ABS-JNH617_Lundy		99.055%	96.423%	97.003%
A2: lacA US	HM020690	99.055%		95.814%	96.541%
B2: lacB EUR	MK547067	96.423%	95.814%		97.196%
B1: lacB US	UDB024035	97.003%	96.541%	97.196%	

Figure 6. Phylogenetic reconstruction of the *Cuphophyllus lacmus* clade.

Communities of fungi in the Heather roots

Fungal communities within the Heather roots clearly differed from those of the bulk soil (Figure 7). Whilst Leotiomycetes (mostly Helotiales) were still present at high abundance, root communities were dominated by Agaricomycetes (Gill Fungi). Of the top 40 fungal taxa present in roots at highest mean relative abundance, only 16 were also in the 40 most abundant in soil detected in roots (Appendix 1). SIMPER analysis showed that the ten species contributing most strongly to the differences between the root and soil samples and were more abundant in roots; two were *Mycena* spp. and three were very poorly defined taxa identifiable only to phylum level or higher (Fungi_sp:SH1010170.10FU; Ascomycota_sp:SH0855981.10FU; Fungi_sp:SH0748653.10FU). Of the 40 taxa most abundant in roots, 13 were at least 25-fold more abundant in roots than soil (Appendix 1) suggesting that they may have a role either as mycorrhizas or early-stage root decomposers.

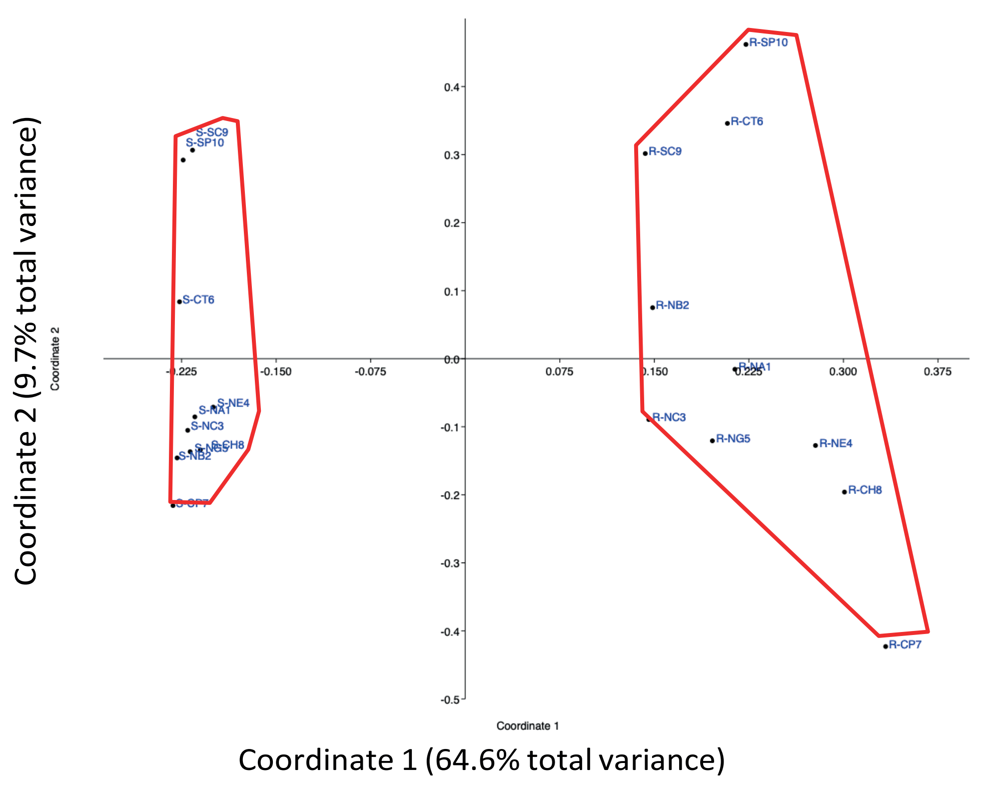


Figure 7. Ordination of soil and root fungal communities using Principal Coordinates Ordination analysis (PCoA). The communities from soil and roots were significantly different (PERMANOVA pseudo-P <0.0001).

Grey Waxcap (*Cuphophyllus lacmus)* in roots of Heather

The Grey Waxcap, *C. lacmus,* was detected in 3/5 of the northern root samples and also in roots from one of the two southern quadrats where this species had been detected in soil in the 2020 survey (Griffith *et al* 2020). In all but one of these samples, the relative abundance of *C. lacmus* was lower than in the associated soil (see figure 5). The nutrition of soil-dwelling Hygrophoraceae is not well-understood. Evidence from $\delta^{15}N$ and $\delta^{13}C$ isotopic analysis suggest that they may be mycorrhizal, since their isotopic profiles are very different from saprotrophic species, resembling those of ectomycorrhizal fungi (Seitzman *et al.*, 2011; Halbwachs *et al.*, 2018). Several other lines of evidence are also strongly suggestive of a biotrophic nutritional mode (Griffith *et al.*, 2012; Halbwachs *et al.*, 2013a; Halbwachs *et al.*, 2013b). The novel discovery of the presence of *C. lacmus* DNA in Heather roots (the only potential higher plant host present in the north Lundy heathland) is consistent with this possibility. Further microscopic analysis is needed to characterise the nature of the putative mycorrhizal interface (Halbwachs *et al.*, 2013a).

Mycena species in roots of Heather

The gill fungus *Mycena galopus* (Milking Bonnet) was present in all root samples and was particularly dominant, comprising ca. 32% of the fungal sequences detected within roots. A second species, *Mycena galericulata* (Common Bonnet) was also present in most (7/10) root samples with a mean relative abundance of 4.1% (Figure 8), and in one root sample (CP7) these two species together comprised >65% of all the fungal DNA detected.

The dominance of the Heather root mycobiome in all quadrats by *Mycena galopus* was unexpected. Whilst *Mycena* spp. are generally considered to be litter saprotrophs (Emmett *et al.*, 2008), several studies have detected *M. galopus* (and other *Mycena* spp.) within the roots of Ericaceae, including *C. vulgaris* as well as in roots of Birch, *Betula spp.* , Pine, *Pinus spp.* and several other Arctic-alpine host plants. These studies have also demonstrated a growth-promoting effect in some cases (*Vaccinium corymbosum* inoculated with *M. galopus* in plant-pot experiments), the formation of distinctive peg-like structure within roots and also some transfer of radiolabelled (^{32}P) phosphate from the fungus to *Betula* seedlings (Grelet *et al.*, 2017; Thoen *et al.*, 2020).

More detailed investigation of the possible role of *Mycena* spp in the roots of heathland plants has shown that they can invade the internal tissues of healthy roots. However, they do not display the distinctive 15N/13C isotopic profiles observed in ectomycorrhizal fungi (and Hygrophoraceae), and the prevailing view at present is that they are latent-invading root saprotrophs (Harder *et al.*, 2023).

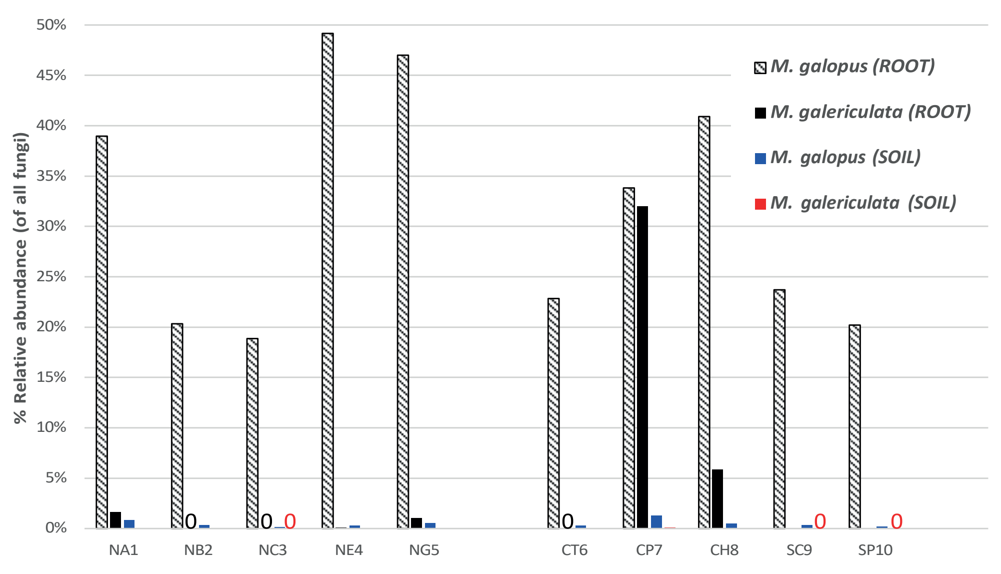


Figure 8. Percentage relative abundance of sequences of *Mycena galopus* and *Mycena galericulata* detected in Heather roots (black) and soil (blue/red) from the ten quadrats.

Other fungi present in Heather roots.

The second most abundant taxon in roots was an unidentified *Sclerococcum* species (SH0855981.10FU; family Dactylosporaceae, Ascomycota), which was also abundant in soil. Members of this genus are often lichenicolous and abundant in tundra soils (Alstrup *et al.*, 2009; Huusko *et al.*, 2024). However, this particular species was found only at very low abundance in lichen thalli in the quadrats (see below), suggesting that the ecological niche of this particular species is very different from other members of this genus.

Another unusual, potentially mycorrhizal, species found in the root samples (>50-fold more abundant than in soil), was an unidentified ascomycete *Colpoma* species (SH0748653.10FU; ascomycete family Rhytismataceae), also abundant in ericoid roots in Bohemia (Vohník *et al.*, 2023).

Comparison of fungal communities in Heather roots: north vs south Lundy.

Ordination of the root-inhabiting fungal communities from the North and South quadrats revealed them to be significantly different (PERMANOVA pseudo-P<0.0487; Figure 9). The species primarily responsible for this difference were identified via SIMPER analysis as *Mycena galopus*, Ascomycota sp:SH0855981.10FU and Fungi_sp:SH1010170.10FU (all more abundant in northern quadrats), whereas *Mycena galericulata*, Fungi sp:SH0858794.10FU, Fungi sp:SH0897234.10FU and Trechisporales sp:SH1010152.10FU were more abundant in southern quadrats.

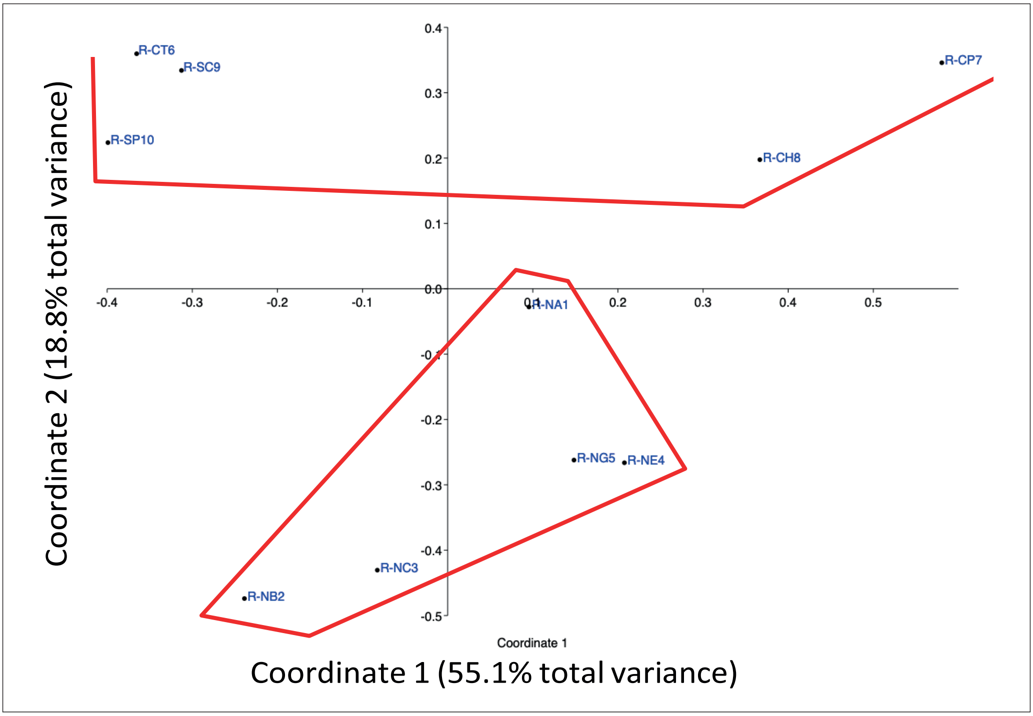


Figure 9. Ordination of soil and root fungal communities using Principal Coordinates Ordination analysis (PCoA).The root fungal communities from North and South quadrats were significantly different (PERMANOVA pseudo-P<0.0487).

Results of eDNA extraction from the lichen samples

eDNA analysis of eight lichen samples from each of the North quadrats and three of the South quadrats, found no trace of *C. lacmus*. No other CHEGD fungi were detected in any of the lichen thallus samples. As expected, the dominant fungus in the lichen thalli was *Cladonia portentosa* (SH0933093.10FU), with small amounts of other *Cladonia* species, including *C. ciliata* (SH0932955.10FU) and *C. furcata* (SH0993788.10FU) (data are summarised in Appendix 2).

Within the lichen thalli examined, 353 ASV's were detected, of which 246 and 101 were also detected in soil or roots respectively, with 98 ASV's being unique to lichen thalli. These included lichenicolous fungi, for example *Zyzygomyces bachmannii* (SH0880452.10FU; Filobasidiaceae), a well-defined lichenicolous fungus, specific to *Cladonia* spp. (Diederich *et al*., 2022). Two of the other abundant taxa, *Xenopolyscytalum* sp. (SH1012222.10FU; Pezizellaceae) and *Neopestalotiopsis (*SH0911533.10FU; Sporocadaceae), have also been reported from within lichen thalli (Park *et al*., 2015; Masumoto and Degawa, 2019) (see Appendix 2).

The eDNA metabarcoding method used here also detects members of kingdom Viridiplantae (higher plants/green algae), so it is also possible to identify the Chlorophyta photobionts associated with the lichenised fungi. The most abundant of the Viridiplantae was *Asterochloris woessiae* (SH0769029.10FU; Trebouxiaceae), comprising 71% of the non-fungal sequences. Members of genus *Asterochloris* are the dominant photobionts of *Cladonia* spp. (Pino-Bodas and Stenroos, 2021). Smaller amounts of other Trebouxiaceae (*Trebouxia suecica*:SH0769319.10FU) were also detected but only at very low abundance.

Coccomyxa viridis (SH0832661.10FU; Coccomyxaceae) and several unidentified species of the same genus were abundant in all the lichen thalli, comprising ca. 18% of the non-fungal sequences (and >50% in one case). Although *Coccomyxa* spp. are photobionts associated with *Licheomphalia* and several other basidiolichens (Oberwinkler, 2012), they are generally regarded as "non-photobiont" algae. However, it has recently been discovered that they are abundant in the internal tissues of the thalli of many lichen species globally (Tagirdzhanova *et al*., 2023), and it is possible that their significance in the lichen symbiosis (relative to Terebouxiaceae algae) has hitherto been underestimated.

The same range of *Coccomyxa* spp. were also detected in the soil eDNA samples, though the most abundant (*Coccomyxa* sp:SH0818359.10FU) was not the dominant species in the thalli. The significance of these algae was only recently discovered and their ecological significance and potential photosynthetic contribution to the lichen thallus economy is at present unclear.

CONCLUSIONS

- This study deployed eDNA analysis to further investigate the apparent restriction of fruit bodies of the Grey Waxcap, *Cuphophyllus lacmus*, to the heathland areas of north Lundy. This distribution was largely confirmed by the soil data and presence of the fungus in the roots of the Heather adds weight to the hypothesis that this species and perhaps other Waxcap fungi are mycorrhizal.
- The fact that the relative abundance of *C. lacmus* in soil samples from the present study (taken in April) was much lower than observed in our earlier 2016 sampling (February)

(Griffith *et al.*, 2020) raises the possibility that there may be a strong seasonal component. There is high turnover of Heather and other root tissues in heathland ecosystems (Aerts *et al.*, 1989; Aerts *et al.*, 1992), root mortality is known to exhibit seasonal variation (Huo *et al.*, 2022; Garthen *et al.*, 2025). Thus the putatively mycorrhizal *C. lacmus* would be expected to proliferate during periods of root growth in the summer, both inside the roots (mycorrhizal interface) and in the soil (uptake network).

- Despite the fact that *Cladonia portentosa* thalli were also much more abundant in the north end quadrats, we found no evidence of any association between *C. lacmus* and the *Cladonia* lichens.
- One surprising outcome of the study was the consistent presence of DNA *of Mycena* species in the roots of Heather across all the study sites, raising the possibilities of an undiscovered relationship between the plant and these *Mycena* species. It is hypothesised that latent colonisation of healthy roots by Mycena may accelerate decomposition of dead roots and more rapid recycling of their nutrients. Microscopic analysis of roots could elucidate the role of both *Mycena* spp. and also *C. lacmus* in Heather roots, though since both bear clamp connections, some means of staining the hyphae of one species but not the other (e.g. Fluorescent *in situ* hybridisation using sequence specific probes) would need to be devised.
- Any latently invading *Mycena* spp. would show the opposite pattern to infection by *C.lacmus*, proliferating as roots senesce/die in Winter and early Spring, thus mediating rapid decomposition of dead roots and rapid recycling of nutrients contained within them.
- Whilst the majority of fungi detected via eDNA were microfungi, and thus not easily detectable by field surveys of fungi, several species not previously known from Lundy were revealed to be present. For example, the lichenicolous *Zyzygomyces bachmannii*, which causes distinctive deformation of *Cladonia* podetia (**https://www.dorsetnature.co.uk/pages-lichen/lch-433.html**) was widely present and should be easily discoverable if sought.
- Similarly, it would be good to pay particular attention to any small white Waxcaps found in heathland areas, these may be fruitbodies of a hitherto unknown (or not yet DNA barcoded) species close to the Cedarwood Waxcap *Cuphophyllus russocoriaceus.*

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Appendix I Relative abundance (as % of all fungal sequences detected) of the 40 most abundant fungal taxa detected in Heather roots.

				Soil NORTH					Soil SOUTH						Root NORTH					Root SOUTH						Root:Soil
SoilRank	phylum	class	species	NA1	NB2	NC3	NE4	NG5	CT6	S-CP7	CH8	SC9	SP10	Soil AVE	NA1	NB2	NC3	NE4	NG5	CT6	S-CP7	CH8	SC9	SP10	ROOTtot	R/Sratio
37	Basidiomycota	Agaricomycetes	Mycena_galopus:SH0748945.10FU	0.827%	0.366%	0.139%	0.279%	0.536%	0.256%	1.290%	0.505%	0.307%	0.190%	0.470%	38.96%	20.35%	18.88%	49.16%	46.99%	22.84%	33.81%	40.92%	23.71%	20.22%	31.584%	67.3
5	Ascomycota	Eurotiomycetes	Sclerococcum_UNKsp:SH0855981.10FU	4.354%	7.878%	7.177%	4.862%	2.997%	4.052%	1.023%	5.290%	5.562%	5.701%	4.890%	8.19%	21.36%	15.64%	2.63%	4.42%	5.47%	0.39%	4.78%	8.56%	5.41%	7.685%	1.6
136	Ascomycota	Dothideomycetes	Dothideomycetes_UNKsp:SH1010170.10FU	0.042%	0.077%	0.101%	0.072%	0.111%	0.016%		0.017%	0.075%	0.066%	0.058%	10.92%	10.41%	5.72%	5.47%	4.95%	3.23%	0.01%	1.03%	2.56%	4.96%	4.926%	85.1
108	Ascomycota	Leotiomycetes	Colpoma_UNKsp:SH0748653.10FU	0.054%	0.013%	0.044%	0.105%	0.163%	0.050%	0.060%	0.056%	0.115%	0.156%	0.082%	1.97%	5.99%	3.32%	2.00%	0.99%	10.01%	0.74%	2.62%	9.96%	4.33%	4.193%	51.4
2	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828226.10FU	6.164%	3.881%	3.985%	7.500%	8.226%	9.446%	10.371%	8.292%	7.359%	6.182%	7.141%	5.94%	3.53%	2.86%	2.13%	3.69%	5.79%	1.83%	1.26%	10.87%	3.24%	4.114%	0.6
215	Basidiomycota	Agaricomycetes	Mycena_galericulata:SH0948057.10FU	0.002%	0.022%		0.010%	0.067%	0.011%	0.103%	0.079%			0.029%	1.62%			0.10%	1.05%		31.99%	5.84%		0.05%	4.065%	138.5
145	unclass_Fungi		Fungi_sp:SH0897234.10FU	0.003%	0.007%	0.003%	0.047%		0.064%	0.108%	0.239%	0.035%	0.054%	0.056%	0.04%		9.19%				4.11%	11.62%	4.28%	2.82%	3.206%	57.2
119	Ascomycota	Leotiomycetes	MollisiaceaeiUNK_sp:SH0858794.10FU	0.007%	0.003%	0.131%	0.060%	0.012%	0.109%	0.059%	0.046%	0.108%	0.157%	0.069%	0.08%	0.26%	0.02%			5.49%	0.17%	0.21%	5.13%	18.95%	3.031%	43.7
322	Ascomycota	Dothideomycetes	Ascomycota_sp:SH0942786.10FU	0.016%	0.025%	0.016%	0.012%	0.020%	0.014%	0.021%	0.013%			0.014%	4.38%	2.51%	3.57%	3.96%	2.91%	0.09%	3.46%	4.97%	0.05%	0.27%	2.617%	192.8
95	Basidiomycota	Agaricomycetes	Trechisporales_sp:SH1010152.10FU	0.042%		0.010%	0.152%	0.006%	0.373%	0.168%		0.160%	0.079%	0.099%	0.903%	1.691%		0.187%	0.052%	15.44%	0.002%		3.417%	3.765%	2.546%	25.7
1	Ascomycota	Leotiomycetes	Pezoloma_ericae:SH0897049.10FU	13.637%	18.634%	14.176%	6.041%	18.089%	5.436%	10.632%	13.138%	1.905%	0.742%	10.243%	1.685%	4.846%	6.480%	1.103%	2.980%	0.490%	3.783%	1.957%	0.254%	0.067%	2.365%	0.2
19	Ascomycota	Leotiomycetes	Hyaloscypha_UNKsp:SH0897600.10FU	2.324%	0.570%	1.996%	1.407%	1.240%	0.826%	2.086%	0.773%	0.828%	1.179%	1.323%	3.527%	0.321%	4.970%	2.337%	5.504%	1.594%	0.469%	2.947%	0.432%	0.638%	2.274%	1.7
494	Ascomycota	Leotiomycetes	Pezicula_rhizophila:SH0948646.10FU	0.004%	0.002%	0.002%	0.004%	0.003%	0.005%	0.006%		0.010%	0.018%	0.005%	2.072%	0.215%	3.044%	0.799%	1.851%	0.078%	0.216%	0.161%	0.735%	7.660%	1.683%	317.3
633	Ascomycota	Leotiomycetes	Coccomyces_australis:SH0980036.10FU						0.029%					0.003%						15.59%			0.002%		1.559%	529.7
111	Ascomycota	Leotiomycetes	Hyphodiscus_UNKsp:SH0907918.10FU	0.008%	0.232%	0.113%	0.058%	0.297%	0.017%	0.021%	0.004%			0.075%	1.322%	4.048%	2.042%	1.825%	4.121%	0.168%	0.016%	0.138%			1.368%	18.3
42	Basidiomycota	Agaricomycetes	Hyaloriaceae_sp:SH0749083.10FU	0.170%	0.096%	0.273%	0.741%	0.444%	0.708%	0.395%	0.296%	0.305%	0.260%	0.369%	1.131%	2.099%	3.252%	2.076%	0.982%	1.753%	0.013%	0.104%	1.287%	0.111%	1.281%	3.5
31	Ascomycota	Leotiomycetes	Meliniomyces_sp:SH0897224.10FU	0.323%		0.080%	1.421%	0.170%	0.313%	0.484%	1.922%	0.030%	0.399%	0.514%	1.868%		0.004%	0.033%		0.028%	4.506%	4.182%	0.002%	0.027%	1.065%	2.1
33	Ascomycota	Leotiomycetes	Leotiomycetes_sp:OTU 33	0.759%			0.298%	0.036%	0.510%	1.663%	1.410%	0.305%	0.084%	0.507%	1.185%			0.025%		1.240%	4.700%	3.131%	0.131%	0.034%	1.045%	2.1
4	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0827748.10FU	2.952%	3.723%	5.977%	4.925%	6.522%	3.419%	6.769%	7.615%	4.084%	4.791%	5.078%	0.475%	1.752%	1.188%	0.514%	1.079%	0.779%	0.468%	0.487%	2.901%	0.293%	0.994%	0.2
36	Ascomycota	Leotiomycetes	Hyaloscypha_gryndleri:SH0897609.10FU	0.537%	0.007%	0.168%	0.634%	0.119%	0.776%	0.467%		1.373%	0.830%	0.491%	1.620%	0.505%	0.191%	0.389%	0.328%	1.448%			3.406%	1.191%	0.908%	1.9
8	Ascomycota	Eurotiomycetes	Cladophialophora_sp:SH0827868.10FU	2.066%	2.038%	2.518%	2.594%	3.272%	3.952%	3.136%	1.993%	2.368%	2.789%	2.673%	0.696%	0.242%	0.425%	1.024%	1.358%	1.215%	0.419%	0.099%	1.808%	1.107%	0.839%	0.3
38	Ascomycota	Leotiomycetes	Oidiodendron_majus:SH0945817.10FU	0.392%	0.268%	0.394%	0.672%	0.586%	0.271%	0.210%	0.410%	0.828%	0.595%	0.463%	1.225%	1.439%	0.847%	0.705%	1.476%	0.227%	0.202%	0.214%	1.085%	0.429%	0.785%	1.7
956	Ascomycota	Sordariomycetes	Diaporthe_sp:SH0754169.10FU						0.001%	0.008%				0.001%							0.399%	0.951%	0.044%	6.404%	0.780%	828.9
77	Ascomycota	Leotiomycetes	Glutinomyces_UNKsp;OTU 53		0.044%		0.162%	0.756%			0.006%	0.159%	0.187%	0.131%		0.015%		6.310%	0.518%	0.010%		0.205%			0.706%	5.4
13	Basidiomycota	Agaricomycetes	Cuphophyllus_lacmus:SH0952050.10FU	0.590%	2.981%	2.913%	9.170%	3.259%	0.010%			0.002%	0.804%	1.973%			2.976%	2.492%	1.509%					0.006%	0.698%	0.4
9	Ascomycota	Eurotiomycetes	Chaetothyriales_UNKsp:OTU 18	2.221%	3.311%	2.027%	3.376%	1.331%	2.102%	3.872%	1.779%	2.607%	3.779%	2.641%	0.276%	1.056%	0.746%	0.292%	0.746%	0.522%	0.103%	0.342%	1.786%	1.019%	0.689%	0.3
29	Ascomycota		Ascomycota_UNKsp; OTU 42	0.781%	0.171%	0.365%	1.096%	0.611%	0.293%	0.551%	0.502%	0.501%	1.203%	0.607%	1.402%	1.139%	0.569%	0.606%	0.818%	0.539%	0.247%	0.192%	0.424%	0.276%	0.621%	1.0
70	Basidiomycota	Agaricomycetes	Hydnodontaceae_UNKsp;OTU 72	0.014%	0.259%	0.099%	0.200%	0.102%	0.108%	0.225%	0.500%	0.004%	0.006%	0.152%	0.003%	1.090%	0.132%	4.554%	0.003%	0.016%			0.011%		0.581%	3.8
20	Ascomycota	Eurotiomycetes	Sclerococcum_UNKsp:SH0931721.10FU	0.714%	0.956%	1.939%	0.513%	3.299%	0.326%	0.007%	0.005%	1.777%	2.539%	1.208%		1.695%	2.821%	0.004%	0.871%	0.002%		0.002%	0.020%	0.252%	0.567%	0.5
11	Ascomycota	Eurotiomycetes	Herpotrichiellaceae_sp:SH0828653.10FU	3.019%	5.266%	4.464%	3.934%	2.875%	2.350%			0.067%	0.100%	2.208%	0.631%	1.415%	1.042%	0.552%	0.797%	0.226%			0.016%	0.041%	0.472%	0.2
3	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828602.10FU	10.322%	9.054%	9.015%	6.980%	6.467%	5.976%	9.434%	3.914%	2.931%	3.615%	6.771%	0.412%	1.040%	0.958%	0.359%	0.678%	0.303%	0.194%	0.084%	0.344%	0.331%	0.470%	0.1
25	Ascomycota	Eurotiomycetes	Chaetothyriales_sp:SH0828164.10FU	0.339%	0.625%	0.303%	1.536%	0.310%	0.274%	0.749%	0.865%	1.249%	2.657%	0.891%	0.319%	1.469%	0.292%	0.436%	1.224%	0.250%	0.077%	0.357%	0.014%	0.003%	0.444%	0.5
6	Ascomycota	Archaeorhizomyce	Archaeorhizomyces_sp:SH0764667.10FU	5.602%	4.858%	4.557%	3.459%	3.847%	3.696%	3.975%	5.662%	4.555%	4.942%	4.515%	0.368%	1.034%	0.522%	0.245%	0.661%	0.272%	0.129%	0.136%	0.443%	0.629%	0.444%	0.1
872	Ascomycota	Leotiomycetes	Helotiales_sp;OTU 67		0.003%		0.003%			0.004%	0.002%			0.001%	0.475%	0.565%	0.355%	0.794%	1.002%		0.549%	0.202%	0.283%	0.198%	0.442%	349.0
22	Ascomycota	Eurotiomycetes	Capronia_sp:SH0827807.10FU	2.933%	0.606%	0.924%	0.179%	1.077%	0.745%	0.135%	0.148%	1.512%	2.503%	1.076%	0.190%	0.264%	0.629%	0.189%	0.307%	0.001%			0.029%	2.204%	0.381%	0.4
174	Basidiomycota	Agaricomycetes	Agaricales_sp:SH0814676.10FU	0.006%			0.016%	0.056%				0.193%	0.144%	0.042%			0.036%						3.469%	0.231%	0.374%	9.0
251	unclass_Fungi		Fungi_sp:SH0846109.10FU	0.066%	0.004%	0.009%	0.005%	0.061%	0.003%	0.027%	0.045%			0.022%	0.068%		0.022%	0.116%	0.037%		1.364%	2.118%			0.373%	17.0
15	Ascomycota	Eurotiomycetes	Capronia_sp:SH0828621.10FU	2.073%	0.444%	1.966%	2.013%	1.088%	1.810%	3.131%	0.796%	1.311%	1.373%	1.601%	0.016%	0.031%	1.030%	0.728%	0.178%	0.012%	0.267%	0.200%	0.489%	0.647%	0.360%	0.2
522	Ascomycota	Dothideomycetes	Dothideomycetes_sp:SH0822430.10FU	0.002%			0.002%	0.008%	0.002%			0.020%	0.014%	0.005%	0.318%	0.081%	0.193%	0.104%	0.311%				1.645%	0.830%	0.348%	73.0
126	Ascomycota	Leotiomycetes	Lachnum_pygmaeum:SH0948769.10FU	0.036%	0.021%	0.024%	0.078%	0.043%	0.065%	0.031%	0.130%	0.073%	0.137%	0.064%	0.265%	0.256%	0.241%	0.247%	0.203%	0.325%		0.689%	0.297%	0.770%	0.329%	5.2

Appendix 2 Relative abundance of eDNA of species of fungi in lichen thalli

phylum	class	order	family	genus	species	Trait	Count	CumulTOT	Mean	Max	Min	L-NA1	L-NB2	L-NC3	L-NE4	L-NG5	L-CT6	L-CP7	L-CH8
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_portentosa:SH0933093.10FU	lichenized	8	601.75%	75.22%	86.03%	65.76%	85.08%	76.08%	65.76%	69.61%	69.01%	86.03%	68.37%	81.81%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_ciliata:SH0932955.10FU	lichenized	8	58.85%	7.36%	15.19%	0.02%	0.02%	3.28%	11.97%	8.93%	7.14%	3.63%	15.19%	8.69%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_furcata:OTU 79	lichenized	7	24.98%	3.12%	9.43%	0.00%	3.090%	0.010%	0.010%	8.520%	9.430%	0.550%	3.370%	0.000%
X	X	X	X	X	OTU 64 (Unidentified)		8	19.70%	2.46%	5.70%	0.61%	2.390%	5.510%	1.400%	0.610%	5.700%	1.540%	1.700%	0.850%
Basidiomycota	Tremellomycetes	Filobasidiales	Filobasidiaceae	Zyzygomyces	Zyzygomyces_bachmannii:SH0880452.10FU	Lichenicolous	7	15.06%	1.88%	5.19%	0.00%	3.710%	1.590%	5.190%	0.050%	0.860%	0.000%	3.260%	0.400%
Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	Sistotrema	OTU 136 (Unidentified)	litter_saprotroph	5	10.46%	1.31%	8.51%	0.00%	0.010%	0.420%	8.510%	1.510%	0.000%	0.000%	0.000%	0.010%
Ascomycota	X	X	X	X	OTU 93 (Unidentified)		8	8.780%	1.098%	3.000%	0.140%	0.880%	3.000%	0.660%	1.270%	1.230%	0.140%	0.840%	0.760%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	OTU 527 (Unidentified)	lichenized	7	7.730%	0.966%	1.730%	0.000%	0.000%	0.390%	1.480%	1.020%	0.880%	0.550%	1.730%	1.680%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	OTU 177 (Unidentified)	lichenized	7	5.660%	0.708%	1.320%	0.000%	0.000%	0.240%	1.140%	0.760%	0.640%	0.300%	1.260%	1.320%
Ascomycota	Sordariomycetes	Amphisphaeriales	Sporocadaceae	Neopestalotiopsis	Neopestalotiopsis_foedans:SH0911533.10FU	foliar_endophyte	8	2.740%	0.343%	0.870%	0.090%	0.090%	0.460%	0.110%	0.630%	0.870%	0.100%	0.100%	0.380%
Ascomycota	Leotiomycetes	Helotiales	Pezizellaceae	Xenopolyscytalum	Xenopolyscytalum_sp:SH1012222.10FU	foliar_endophyte	7	2.650%	0.331%	1.130%	0.000%	0.540%	0.210%	0.140%	0.150%	0.460%	0.000%	0.020%	1.130%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_arbuscula:OTU 170	lichenized	1	2.550%	0.319%	2.550%	0.000%	0.000%	0.000%	0.000%	2.550%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_rangiformis:SH0933060.10FU	lichenized	2	2.290%	0.286%	2.110%	0.000%	0.000%	2.110%	0.000%	0.000%	0.180%	0.000%	0.000%	0.000%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_fenestralis:SH0932988.10FU	lichenized	4	2.180%	0.273%	1.090%	0.000%	0.010%	0.000%	0.000%	0.290%	1.090%	0.000%	0.790%	0.000%
Basidiomycota	Tremellomycetes	Tremellales	Bulleribasidiaceae	Vishniacozyma	OTU 193 (Unidentified)	soil_saprotroph	3	1.810%	0.226%	1.780%	0.000%	0.010%	0.020%	1.780%	0.000%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_uncialis:SH0933004.10FU	lichenized	2	1.370%	0.171%	1.250%	0.000%	0.000%	0.000%	0.000%	0.120%	0.000%	0.000%	1.250%	0.000%
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	unclassified_Sporormiaceae	Sporormiaceae_sp:SH0967217.10FU		7	1.280%	0.160%	0.970%	0.000%	0.010%	0.120%	0.010%	0.020%	0.120%	0.970%	0.030%	0.000%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_ochrochlora:SH0932963.10FU	lichenized	1	1.070%	0.134%	1.070%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	1.070%	0.000%	0.000%
Ascomycota	Leotiomycetes	Phacidiales	Phacidiaceae	Phacidium	Phacidium_lacerum:SH0931217.10FU	foliar_endophyte	7	0.870%	0.109%	0.240%	0.000%	0.000%	0.240%	0.010%	0.150%	0.060%	0.120%	0.050%	0.240%
Ascomycota	Eurotiomycetes	Chaetothyriales	X	X	OTU 378 (Unidentified)		6	0.850%	0.106%	0.620%	0.000%	0.060%	0.620%	0.030%	0.030%	0.000%	0.080%	0.000%	0.030%
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sporormiella	Sporormiella_intermedia:SH0790049.10FU	litter_saprotroph	6	0.710%	0.089%	0.550%	0.000%	0.010%	0.000%	0.000%	0.040%	0.060%	0.550%	0.020%	0.030%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_merochlorophaea:SH0933165.10FU	lichenized	1	0.690%	0.086%	0.690%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.690%	0.000%	0.000%
Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	Venturia	Venturia_sp:SH0987965.10FU	plant_pathogen	3	0.650%	0.081%	0.550%	0.000%	0.000%	0.030%	0.000%	0.000%	0.000%	0.070%	0.000%	0.550%
Basidiomycota	Agaricomycetes	Cantharellales	Hydnaceae	Sistotrema	OTU 422 (Unidentified)	litter_saprotroph	2	0.620%	0.078%	0.600%	0.000%	0.000%	0.000%	0.020%	0.600%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Dothideomycetes	Pleosporales	unclassified_Pleosporales	unclassified_Pleosporales	Pleosporales_sp:SH0757640.10FU		1	0.560%	0.070%	0.560%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.560%	0.000%	0.000%
Ascomycota	Leotiomycetes	X	X	X	OTU 468 (Unidentified)		6	0.490%	0.061%	0.310%	0.000%	0.310%	0.030%	0.010%	0.040%	0.080%	0.020%	0.000%	0.000%
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	Mycena	Mycena_UNKsp:SH0952157.10FU	litter_saprotroph	5	0.470%	0.059%	0.230%	0.000%	0.010%	0.000%	0.000%	0.030%	0.170%	0.230%	0.000%	0.030%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	Cladonia_merochlorophaea:SH0933073.10FU	lichenized	6	0.440%	0.055%	0.140%	0.000%	0.010%	0.080%	0.010%	0.130%	0.000%	0.140%	0.070%	0.000%
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Sporormiella	Sporormiella_irregularis:SH0967100.10FU	litter_saprotroph	5	0.430%	0.054%	0.120%	0.000%	0.000%	0.120%	0.000%	0.020%	0.100%	0.110%	0.000%	0.080%
Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	Neostagonospora	Neostagonospora_caricis:SH1021722.10FU	litter_saprotroph	2	0.430%	0.054%	0.410%	0.000%	0.410%	0.000%	0.000%	0.020%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	Thelebolus	Thelebolus_globosus:SH0897423.10FU	dung_saprotroph	3	0.390%	0.049%	0.290%	0.000%	0.290%	0.000%	0.000%	0.000%	0.060%	0.040%	0.000%	0.000%
Ascomycota	Leotiomycetes	Helotiales	X	X	OTU 513 (Unidentified)		6	0.370%	0.046%	0.150%	0.000%	0.150%	0.060%	0.060%	0.080%	0.010%	0.010%	0.000%	0.000%
Ascomycota	Dothideomycetes	Myriangiales	Elsinoaceae	unclassified_Elsinoaceae	Elsinoaceae_sp:SH1015477.10FU		2	0.360%	0.045%	0.350%	0.000%	0.000%	0.350%	0.000%	0.000%	0.010%	0.000%	0.000%	0.000%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	Cladophialophora_minutissima:SH0959369.10	soil_saprotroph	5	0.370%	0.046%	0.150%	0.000%	0.110%	0.010%	0.000%	0.010%	0.000%	0.150%	0.090%	0.000%
Ascomycota	unclassified_Ascomy	unclassified_Asco	unclassified_Ascomycota	unclassified_Ascomycota	Ascomycota_sp:SH0926002.10FU		8	0.380%	0.048%	0.150%	0.010%	0.020%	0.150%	0.060%	0.010%	0.020%	0.040%	0.060%	0.020%
Ascomycota	X	X	X	X	OTU 523 (Unidentified)		4	0.350%	0.044%	0.280%	0.000%	0.000%	0.280%	0.040%	0.010%	0.000%	0.020%	0.000%	0.000%
Ascomycota	Leotiomycetes	Helotiales	Arachnopezizaceae	Arachnopeziza	Arachnopeziza_sp:SH1023313.10FU	wood_saprotroph	5	0.340%	0.043%	0.110%	0.000%	0.010%	0.000%	0.000%	0.100%	0.100%	0.000%	0.020%	0.110%
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Neocrinula	Neocrinula_xanthorrhoeae:SH0748395.10FU	litter_saprotroph	8	0.330%	0.041%	0.090%	0.010%	0.010%	0.070%	0.040%	0.010%	0.020%	0.080%	0.010%	0.090%
X	X	X	X	X	OTU 491 (Unidentified)		1	0.320%	0.040%	0.320%	0.000%	0.000%	0.000%	0.000%	0.320%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae	Cladosporium	Cladosporium_herbarum:SH0962330.10FU	foliar_endophyte	7	0.310%	0.039%	0.190%	0.000%	0.010%	0.000%	0.010%	0.050%	0.010%	0.010%	0.190%	0.030%
Ascomycota	Sordariomycetes	Sordariales	Podosporaceae	Podospora	Podospora_excentrica:SH0941777.10FU	dung_saprotroph	6	0.320%	0.040%	0.190%	0.000%	0.000%	0.000%	0.040%	0.030%	0.020%	0.190%	0.030%	0.010%
Ascomycota	Sordariomycetes	Xylariales	Microdochiaceae	Microdochium	Microdochium_phragmitis:SH0913814.10FU	foliar_endophyte	2	0.300%	0.038%	0.250%	0.000%	0.000%	0.000%	0.000%	0.250%	0.000%	0.050%	0.000%	0.000%
Ascomycota	Leotiomycetes	X	X	X	OTU 299 (Unidentified)		6	0.300%	0.038%	0.240%	0.000%	0.240%	0.000%	0.010%	0.010%	0.010%	0.010%	0.000%	0.020%
Ascomycota	Leotiomycetes	Helotiales	Myxotrichaceae	Oidiodendron	Oidiodendron_cereale:SH0946007.10FU	soil_saprotroph;ro	2	0.300%	0.038%	0.290%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.290%	0.000%	0.010%
Ascomycota	Lecanoromycetes	Lecanorales	Pilocarpaceae	Micarea	OTU 510 (Unidentified)	lichen_parasite	7	0.280%	0.035%	0.170%	0.000%	0.010%	0.170%	0.010%	0.050%	0.010%	0.020%	0.010%	0.000%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	OTU 604 (Unidentified)	soil_saprotroph	8	0.290%	0.036%	0.090%	0.020%	0.090%	0.020%	0.020%	0.020%	0.040%	0.030%	0.020%	0.050%
Ascomycota	Dothideomycetes	Mycosphaerellale	Teratosphaeriaceae	Constantinomyces	Constantinomyces_oldenburgensis:SH0938147.10FU		6	0.270%	0.034%	0.150%	0.000%	0.000%	0.150%	0.020%	0.010%	0.000%	0.040%	0.020%	0.030%
Ascomycota	Dothideomycetes	Hysteriales	Hysteriaceae	Hysterobrevium	Hysterobrevium_rosae:SH0886013.10FU	wood_saprotroph	6	0.270%	0.034%	0.100%	0.000%	0.010%	0.000%	0.100%	0.040%	0.020%	0.050%	0.050%	0.000%
Ascomycota	Sordariomycetes	Hypocreales	X	X	OTU 695 (Unidentified)		4	0.250%	0.031%	0.190%	0.000%	0.030%	0.000%	0.000%	0.020%	0.000%	0.000%	0.010%	0.190%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	Cladophialophora_sp:SH0976513.10FU	soil_saprotroph;ro	5	0.250%	0.031%	0.180%	0.000%	0.020%	0.180%	0.010%	0.000%	0.030%	0.010%	0.000%	0.000%
Ascomycota	Lecanoromycetes	Lecanorales	Cladoniaceae	Cladonia	OTU 2300 (Unidentified)	lichenized	4	0.260%	0.033%	0.100%	0.000%	0.000%	0.000%	0.000%	0.100%	0.070%	0.010%	0.080%	0.000%
unclassified_Fu	unclassified_Fungi	unclassified_Fung	unclassified_Fungi	unclassified_Fungi	Fungi_sp:SH1015224.10FU		6	0.250%	0.031%	0.060%	0.000%	0.000%	0.050%	0.060%	0.060%	0.030%	0.010%	0.040%	0.000%
Ascomycota	Dothideomycetes	Venturiales	Sympoventuriaceae	unclassified_Sympoventuriaceae	Sympoventuriaceae_sp:SH0743259.10FU		6	0.240%	0.030%	0.090%	0.000%	0.010%	0.020%	0.020%	0.050%	0.000%	0.050%	0.000%	0.090%
Ascomycota	Sordariomycetes	Amphisphaeriales	Sporocadaceae	Pestalotiopsis	Pestalotiopsis_rhododendri:SH0911739.10FU	plant_pathogen	4	0.250%	0.031%	0.140%	0.000%	0.000%	0.140%	0.000%	0.030%	0.050%	0.000%	0.000%	0.030%
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	Preussia	Preussia_dubia:SH0967183.10FU	dung_saprotroph	3	0.230%	0.029%	0.110%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.030%	0.090%	0.110%
Ascomycota	Dothideomycetes	X	X	X	OTU 281 (Unidentified)		8	0.230%	0.029%	0.080%	0.010%	0.020%	0.080%	0.010%	0.030%	0.010%	0.020%	0.030%	0.030%
Basidiomycota	Agaricomycetes	Agaricales	Mycenaceae	Mycena	Mycena_galopus:SH0748945.10FU	litter_saprotroph	1	0.220%	0.028%	0.220%	0.000%	0.220%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
Ascomycota	Leotiomycetes	Helotiales	Discinellaceae	Pezoloma	Pezoloma_ericae:SH0897049.10FU	root_endophyte	5	0.200%	0.025%	0.120%	0.000%	0.120%	0.030%	0.000%	0.000%	0.010%	0.030%	0.000%	0.010%
Ascomycota	Leotiomycetes	Helotiales	unclassified_Helotiales	unclassified_Helotiales	Helotiales_sp:SH0908128.10FU		2	0.190%	0.024%	0.130%	0.000%	0.130%	0.000%	0.000%	0.000%	0.060%	0.000%	0.000%	0.000%
Ascomycota	Dothideomycetes	Pleosporales	Sporormiaceae	unclassified_Sporormiaceae	Sporormiaceae_sp:SH0789780.10FU		3	0.200%	0.025%	0.180%	0.000%	0.000%	0.000%	0.010%	0.000%	0.010%	0.180%	0.000%	0.000%
Ascomycota	Sordariomycetes	Amphisphaeriales	X	X	OTU 808 (Unidentified)		3	0.190%	0.024%	0.110%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.010%	0.110%	0.070%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Cladophialophora	OTU 331 (Unidentified)	soil_saprotroph	6	0.190%	0.024%	0.090%	0.000%	0.020%	0.020%	0.020%	0.030%	0.000%	0.010%	0.000%	0.090%
Ascomycota	Leotiomycetes	Helotiales	Lachnaceae	Lachnum	Lachnum_pygmaeum:SH0948769.10FU	litter_saprotroph	7	0.170%	0.021%	0.060%	0.000%	0.010%	0.020%	0.060%	0.020%	0.000%	0.020%	0.020%	0.020%
Ascomycota	Lecanoromycetes	Lecanorales	Scoliciosporaceae	Scoliciosporum	Scoliciosporum_sarothamni:SH0925044.10FU	lichenized	6	0.170%	0.021%	0.070%	0.000%	0.010%	0.020%	0.000%	0.020%	0.070%	0.020%	0.030%	0.000%
Basidiomycota	Tremellomycetes	Tremellales	unclassified_Tremellales	unclassified_Tremellales	OTU 691 (Unidentified)		6	0.170%	0.021%	0.080%	0.000%	0.020%	0.010%	0.080%	0.020%	0.010%	0.030%	0.000%	0.000%
Basidiomycota	Cystobasidiomycete	Cystobasidiomyce	Microsporomycetaceae	unclassified_Microsporomycetaceae	Microsporomycetaceae_sp:SH0964623.10FU		5	0.170%	0.021%	0.120%	0.000%	0.120%	0.000%	0.000%	0.010%	0.010%	0.010%	0.020%	0.000%
Ascomycota	Dothideomycetes	Capnodiales	unclassified_Capnodiales	unclassified_Capnodiales	Capnodiales_sp:SH0950800.10FU		5	0.170%	0.021%	0.080%	0.000%	0.000%	0.080%	0.020%	0.020%	0.030%	0.000%	0.020%	0.000%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Capronia	Capronia_sp:SH0828111.10FU	soil_saprotroph	4	0.160%	0.020%	0.090%	0.000%	0.000%	0.090%	0.040%	0.020%	0.010%	0.000%	0.000%	0.000%
Ascomycota	Lecanoromycetes	X	X	X	OTU 973 (Unidentified)		3	0.150%	0.019%	0.120%	0.000%	0.000%	0.120%	0.020%	0.000%	0.000%	0.010%	0.000%	0.000%

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