A COMPARISON OF FRUITBODY AND E-DNA SURVEY APPROACHES FOR ASSESSING THE DISTRIBUTION MYCELIA OF MACROFUNGI IN THE GRASSLAND AND HEATHLAND OF LUNDY

by

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ABSTRACT

Results of surveys of species of fungi in selected areas of Lundy which used fruitbodies to assess distribution and abundance are presented in comparison to data derived from identification of taxa from DNA sequences in extracts of soils (eDNA) from six sites on the island. Two sites were within acid grassland, four from heathland areas of *Calluna vulgaris* (Heather). The apparent restriction of *Cuphophyllus (=Hygrocybe) lacmus* (Grey Waxcap) to the Maritime heath on the North End as determined by past annual fruitbody surveys was confirmed by the abundance of its DNA in the two soil samples from the North End. *C. lacmus* DNA was absent in the soil samples from the two acid grassland sites and in one of the 'southern' *Calluna* sites, though it was present in the soil sample from the other 'southern' *Calluna* site, perhaps indicating a wider distribution on Lundy than that found by fruitbody surveys. The DNA sequence data for the soil sample from acid grassland on the Airfield are also compared with the fruitbody counts made over the past eleven years for this site and shows that for some taxa fruitbody abundance is reflected in the proportion of their DNA in the samples, for others it is not. In addition, some taxa found in the DNA profiles have yet to be found in any survey and may represent new records for Lundy. CHEGD scores derived from the DNA profiles and fruitbody surveys are compared with those of high diversity grassland sites on the mainland and confirm the high conservation status of Lundy grasslands.

Keywords: *Lundy, Macrofungi, eDNA in soil, CHEGD scores, Cuphophyllus lacmus, heathland, grassland, mycorrhizas, endophytes*

INTRODUCTION

Annual, usually week-long, surveys of the fungi have been carried out by ourselves and members of the Lundy Field Society (LFS) since 2003, usually in October and/or November, and the accreted records have been published (Hedger & George, 2004, Hedger *et al*. 2007, Hedger, J.N. 2016) and are also available on the Lundy Field Society website (www.lundy.org.uk>About Lundy>Wildlife on the island>Fungi). The recent

publication of an account of the fungi of Lundy by Hedger & George (2018) lists 573 species but the total continues to rise, given that there are many common mainland fungi yet to be recorded on Lundy. These surveys indicated that distribution patterns appeared to exist for some species of fungi which may relate to Lundy habitats.

Using fruitbodies of macrofungi to assess the presence of a species, although easy to do, is widely acknowledged to be flawed, since the mycelium may be present and active, for example within the soil or wood, but may fruit rarely. Even abundant fruitings are often ephemeral and can be missed unless a site is visited frequently over a period of years. This problem has been addressed by eDNA-based survey methods in which sampling of soil or wood is used to identify the presence of mycelia of species of fungi and even to give some estimates of their relative abundance. The approach is dependent upon the existence of DNA barcodes (short tracts of DNA sequence from a specific locus obtained from identified reference fungarium samples) for the taxa. The genes used as DNA barcodes for fungi differ from those used for animals and plants. For fungi it is the ribosomal RNA locus that is used, notably the Large SubUnit (LSU) and internal transcribed spacer (ITS) regions. DNA barcodes for the waxcaps are readily available, since they have been used to determine presence of CHEGD (**C**lavariaceae, **H**ygrophoraceae, **E**ntolomataceae, **G**eoglossaceae, **D**ermoloma/Porpoloma) species in soil samples. These selected grassland fungi are used as indicators to estimate the conservation status of sites both by fruitbody surveys and by DNA profiling of soil samples (Griffith *et al.*, 2004, 2013).

Plate 1: *Cuphophyllus lacmus* (Grey Waxcap) fruiting on peaty soil with *Calluna vulgaris* (Heather) and the Lichen *Cladonia* cf. *arbuscula* on the North End of Lundy

In particular, large numbers of fruitbodies of the Grey Waxcap (*Cuphophyllus lacmus*) occur in late autumn (November and early December) on the peaty soil in the *Calluna vulgaris* (Heather) and *Cladonia arbuscula* (Antler Lichen) dominated Maritime Heath (NVC H13. jncc.defra.gov.uk) on the North End. The example shown in Plate 1 was fruiting within short Heather and the lichen close to John O'Groat's House. The ecology of this

waxcap, which is found in both Europe and North America seems to be poorly understood. It is not common and is considered by some to be a grassland or even woodland species (Boertmann, 2010), whilst other field guide authors place it in poor meadows and acid heaths and moors, sometimes mentioning Heather. The strong habitat preference it appears to exhibit on Lundy thus gives the opportunity to better define the autecology of this species, especially its relationship to the Maritime Heath plant community.

The study aimed to compare the fruit body distribution of fungi on Lundy, especially that of the Grey Waxcap, with the results of fungal DNA profiling of soil samples. Six sample sites were selected: two in Maritime Heath areas dominated by *Calluna vulgaris* (Heather) at the North End, where fruitbodies of the Grey Waxcap can be abundant; two under Heather elsewhere on Lundy where it seems to be absent; two in acid grassland sites where it had also not been seen but other waxcaps were frequent. In addition at two sites, the North End and the Airfield, more detailed annual counts of fruitbodies had been made in the past, so enabling comparison with the DNA based species profiles of the soil samples from these sites.

METHODS

Field Surveys of Fruit Bodies

North End

Surveys of the Maritime Heath were usually carried out in the first week of November. Recorders using tally counters walked in a line separated by a gap of 5-10 m. The first sweep was along the west side north from St Peter's Stream to the North Light steps. This sweep was then repeated to the east across the north coast passing over John O'Groat's House. Finally, a third sweep was made down the east side south to Gannets' Coombe. Data were amalgamated as total numbers of fruit bodies recorded, nearly all of them the Grey Waxcap.

Airfield

This acid grassland site, also surveyed in the first week of November, offered the useful feature of the lines of white-washed stones on either side of the 60m wide mown landing strip. These were used to position a series of ten 6m wide transects beginning in the SW corner (Grid Reference SS132684476) and progressing along the runway for 60m, creating a sample quadrat of 60×60m (3600m^2). For each transect a centre line was walked and the species of fungi and numbers of fruit bodies recorded for approximately 3m on each side. The data were used to calculate the fruit body total for each species for the entire quadrat area.

Soil Sampling

Locations of the sampling sites

Soil sampling was conducted on 15, 16 and 17 February 2016. Air temperature was 2-5°C with no rain. Six sites were selected: two in the Maritime Heath at the North End, south of John O'Groat's House, LU2, and near Squire's View, LU3; two in apparently similar Heather dominated areas in the south of Lundy, north of Old (Quarry) Hospital, LU4, and south of Rocket Pole Pond, LU5; two from acid grassland areas, on the western end of the Airfield, LU1, within the 60×60m annual fruitbody survey quadrat, and on Castle Hill, LU6. The positions of the sites on Lundy are shown in Figure 1 and the National Grid References and longitude and latitude are given in Table 1.

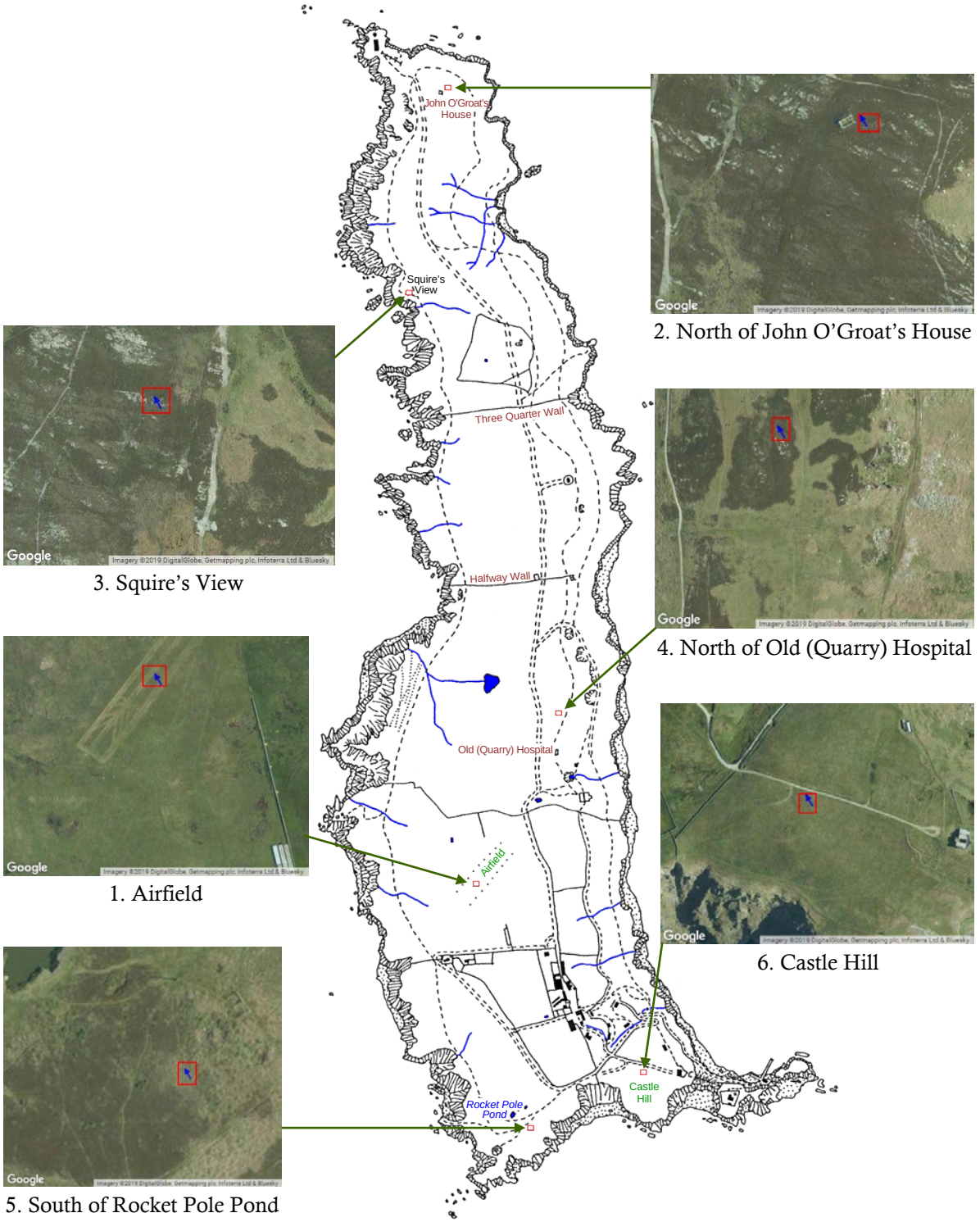

Figure 1: Map of Lundy with inset photographs of the soil sampling sites

Table 1: Details of quadrat locations and sampling dates

Site	Location	Habitat	Sample Date	UK Grid Ref	Lat.	Long.
LU1	Airfield	Acid Grassland	15/02/2016	SS1328844569	51.1698	-4.6715
LU2	North of John O'Groat's House	Maritime Heath	16/02/2016	SS1333447895	51.1997	-4.6731
LU3	Squire's View	Maritime Heath	16/02/2016	SS1317947589	51.1969	-4.6753
LU4	North of Old (Quarry) Hospital	Maritime Heath	16/02/2016	SS1375045422	51.1776	-4.6660
LU5	South of Rocket Pole Pond	Maritime Heath	17/02/2016	SS1351743624	51.1614	-4.6684
LU6	Castle Hill	Acid Grassland	17/02/2016	SS1397343830	51.1634	-4.6620

Soil Coring

Apple corers (20mm diameter) were used to take soil cores to a depth of c.5cm across a 30m×30m quadrat (900m^2) laid out using a 30m tape following a grid pattern with a spacing of c.5m between cores (see Figure 2), giving a total of 36 cores per quadrat. The size of the quadrat and pattern of sampling were as previously used in similar studies of acid grassland sites in Wales and England (Griffith *et al.*, 2018). The positions of the corners of the quadrats were recorded with GPS, photographs and other nearby landmarks. Cores from each quadrat were pooled in a plastic bag (fresh weight of c.300-500g/sample) and placed in a freezer 2-6 hours later. They were transported off Lundy in a cold box and re-stored in a freezer on the mainland prior to transport to Aberystwyth in a cold box where they were kept at -80°C before processing by freeze-drying and finely grinding by passing through a 0.5mm wire sieve. The moisture content of the samples varied from 44-72%. Following grinding, samples were thoroughly mixed and stones and larger fragments of plant material were removed. A subsample (250mg) was taken for DNA extraction using a Qiagen DNeasy PowerSoil Kit, according to the manufacturer's instructions.

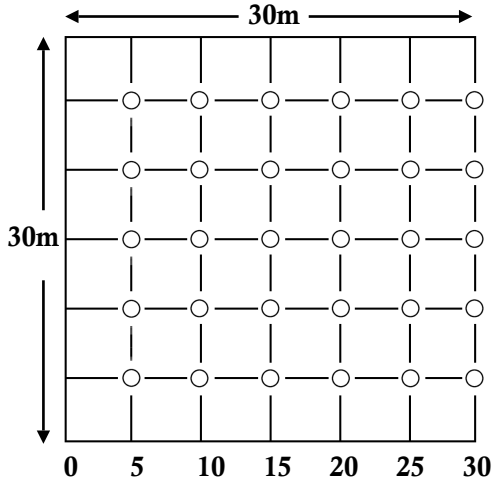

Figure 2: Grid layout of soil sampling points in the 30×30m quadrat

Plate 2: Soil sampling within a 30×30m quadrat near John O'Groat's House (photograph by Sandra Rowland)

Laboratory Methods

Genetic analysis

A 230bp portion of the Large Ribosomal Subunit (LSU) of ribosomal RNA locus was amplified with the primers GBD1-F2 and GBD1-NLC2-AF (Detheridge *et al.,* 2016, 2018). These primers are specific to fungi and bind to highly conserved regions which flank the D1 variable region of the LSU. In order to allow several samples to be sequenced in a single sequencing run, the GBD1-F2 primer contained a 10bp identifier tag. Following PCR amplification, PCR products were cleaned using Spin Column PCR Purification kit (NBS Biologicals) and the yield of DNA was quantified (Nanodrop). The samples were then pooled to give equimolar concentrations. Agarose gel electrophoresis (E-gel) was used to further purify the samples and remove any non-full length PCR products and then quantified once more using an Agilent Bioanalyser. The pooled sample DNA was then diluted to a concentration of 15nM amplified using emulsion PCR, followed by loading onto a 316 Ion Torrent chip. All the steps from emulsion PCR onwards carefully followed the instructions provided with the Ion Torrent PGM (Personal Genome Machine). The full method for DNA extraction, PCR amplification and bioinformatics analyses is published in Detheridge *et al*. (2016 and 2018).

Bioinformatic Methods

Following the sequencing run, the quality of sequences was assessed and short reads not covering the whole barcode region, sequences of poor quality, singletons/doubletons (unique sequences found only once or twice) and non-fungal sequences were removed. These sequences were then split using the 10bp identifier index tag to separate the six samples. Examination of the fungal communities (all the fungi detected) was undertaken with two ordination methods, detrended correspondence analysis (DCA)

and also Non-metric Multi-Dimensional Scaling (NMDS) using the PAST3 program (http://folk.uio.no/ohammer/past/). These methods are widely used in ecology, for instance to analyse plant communities based on quadrat data, with points closer together being more closely related.

RESULTS

Fruitbody Surveys

Quantitative annual surveys of fruitbodies of species of fungi were only carried out in two of the areas where soil-sample sites were located, the North End and the Airfield. However, some qualitative data for the other sampling sites was also available, based on species location lists made over the whole island during annual visits, and is used in the discussion of the DNA profiles.

North End Survey

Large numbers of *Cuphophyllus lacmus* (Grey Waxcap) fruitbodies were found in the autumn surveys of the North End from 2013 to 2018 (Figure 3) emphasising its abundance in the Maritime Heath habitat. The size of the 'flush' of fruitbodies during the November survey week varied, most likely due to the amount of rainfall, with high numbers in damp Novembers such as 2015 and very low numbers in dry ones such as 2018. The one set of figures for December (2016) also shows that flushes can occur very late in the autumn, perhaps delayed by a dry November. However, fruitbodies of this species were never found south of St Peter's Stream on the west coast or further south than the north slopes of Gannets' Coombe on the east side, even in apparently similar Heather areas such as above the Quarries on the East Side, the slopes below Rocket Pole and above the West Coast cliffs in Middle Park.

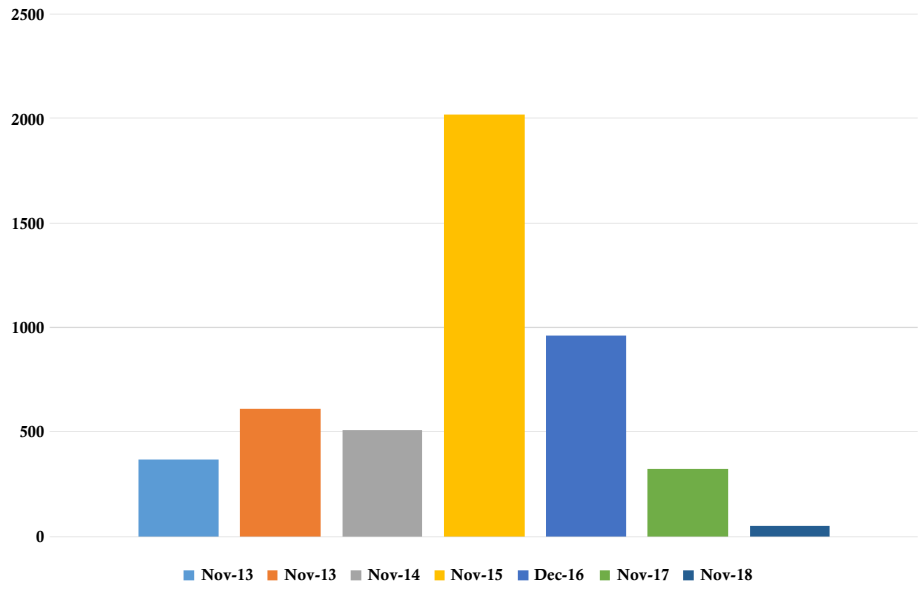

Figure 3: Totals of *Cuphophyllus lacmus* (Grey Waxcap) fruitbodies recorded in surveys of the North End from 2013 to 2018

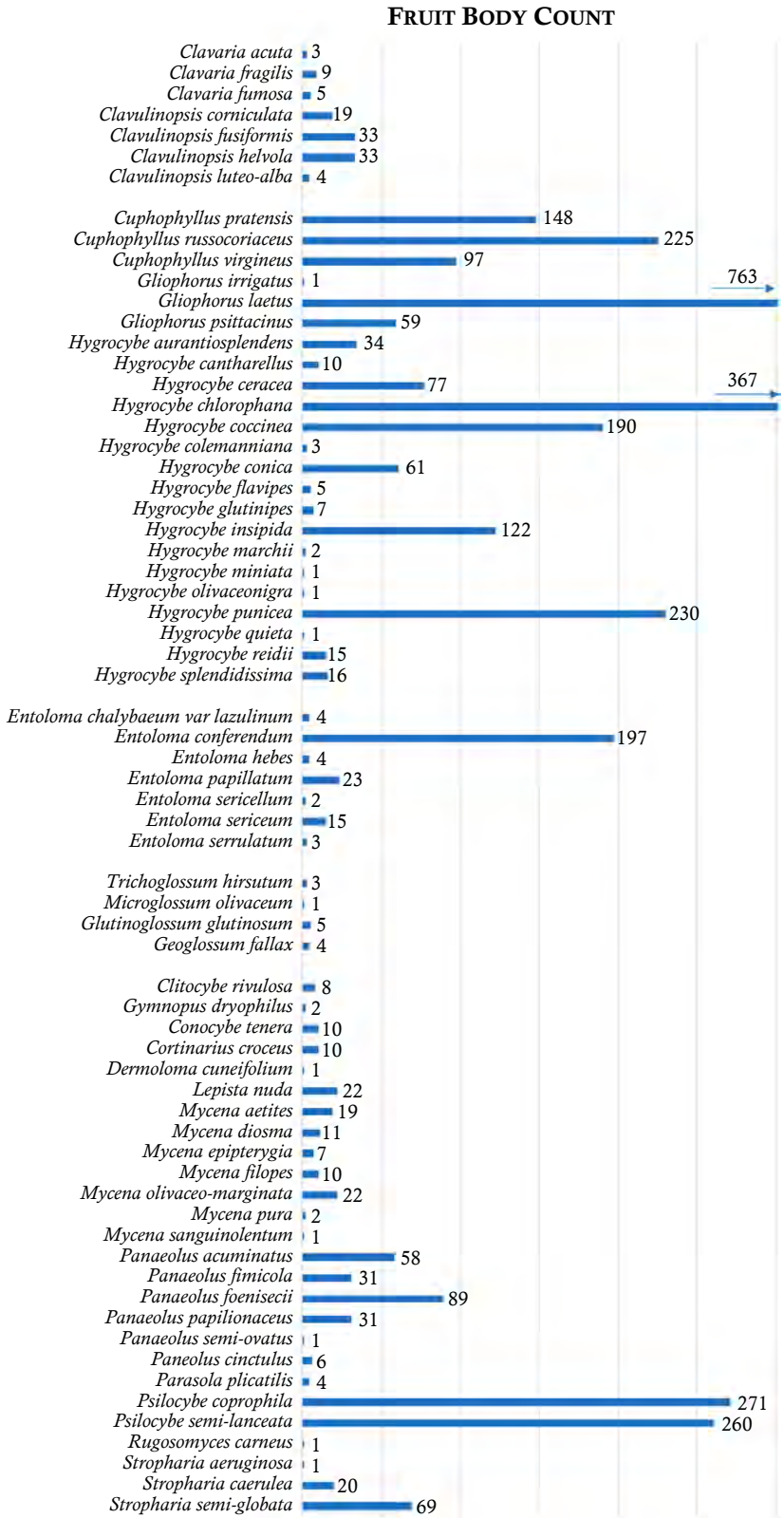

Figure 4: Numbers of fruitbodies of species of macrofungi recorded on the Airfield quadrat 2009-2018

Airfield Survey

The histogram (Figure 4) shows the totals of fruitbodies recorded by the survey on the Airfield quadrat from 2009 to 2018. The species are grouped from top to bottom in the same order as the CHEGD system: **C**lavariaceae; **H**ygrophoraceae; **E**ntolomataceae; **G**eoglossaceae; ***D****ermoloma/Porpoloma* followed by a group of 'non-CHEGD' gilled fungi.

Seven members of the Clavariaceae (Fairy Clubs) were recorded, of which *Clavulinopsis fusiformis* (Golden Spindles) and *C. helvola* (Yellow Club) were the commonest. In practice these two are difficult to distinguish without microscopy so the field distinction made on the survey may not be reliable. A detailed re-survey in November 2019 in fact showed that *C. helvola* fruit bodies were likely to have made up most of the records made in the survey. The family Hygrophoraceae has recently undergone taxonomic revision (Lodge *et al.*, 2014) and several waxcap species formerly placed in the genus *Hygrocybe* now reside in different genera, for example *Cuphophyllus* and *Gliophorus*, both of which are well-represented in the data. A total of 23 species in the Hygrophoraceae were found during the survey. The most abundant fruitbodies were of *Gliophorus laetus* (Heath Waxcap) followed by *Hygrocybe chlorophana* (Golden Waxcap) and *H. punicea* (Crimson Waxcap), all three running into hundreds over the survey period. Other species were comparatively rare, with only one or two fruitbodies found, for example *Hygrocybe miniata* (Vermilion Waxcap) and *H. quieta* (Oily Waxcap). Of the Entolomataceae (Pink Gills) *Entoloma conferendum* (Star Pinkgill) was by far the commonest species, the other six much less frequent. The Geoglossaceae were infrequently found, the four species only amounting to a total of 11 fruitbodies found over 11 years of search. *Dermoloma cuneifolium* (Crazed Cap), was only found once.

The most abundant components of the final grouping of 'non CHEGD' gill fungi in Figure 4 are species which are associated with or grow on the sheep dung on the Airfield such as *Psilocybe semilanceata* (Liberty Cap), *P. coprophila* and species of *Panaeolus*, *Panaeolina* and *Stropharia*. Grassland fungi, often found in meadow surveys in association with the CHEGD fungi, include the seven species of *Mycena* (Bonnets), *Lepista nuda* (Wood Blewit) and *Clitocybe rivulosa* (Fool's Funnel).

Analysis of Sequence Data

A total of 251,436 sequence reads for the LSU D1 locus were obtained across the six quadrat samples (range 29,971 to 71,805 per sample). For the initial analyses, sequences were classified to genus level using the RDP database (Ribosomal Database Project; http://rdp.cme.msu.edu/classifier/). The RDP analysis uses a Naïve Bayesian Classifier to classify sequences to genus level but where suitable matching DNA barcodes are absent, sequences are classified to higher taxonomic orders (Table 2). Green highlighting means the sequence is >5% of the fungal DNA, a white background 0.1-5.0% and a pink background <0.1%. The CHEGD taxa make up a high proportion of the sequences. Clavariaceae are highlighted in yellow, Hygrophoraceae in orange, Entolomataceae in purple. Geoglossaceae are present but none were ranked higher than 64th, so do not appear on Table 2.

Phylum	Class	Order	Family	Genus	Ecology	Count	Cumul. Total	Mean	Median	Max	Min	Lu1	Lu2	Lu3	Lu4	Lu5	Lu6
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus4_lacmus	MR CHEG	5	96.85%	16.14%	2.83%	73.76%	0.00%	0.00%	73.76%	17.41%	0.05%	5.61%	0.02%
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	Rhizoscyphus ericae	MR DSE	5	55.67%	9.28%	3.58%	26.62%	0.00%	0.00%	6.05%	26.62%	21.88%	1.11%	0.01%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe_sgPS1_punicea	MR CHEG	2	47.08%	7.85%	0.00%	47.06%	0.00%	47.06%	0.03%	0.00%	0.00%	0.00%	0.00%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	OTU 6: unknown	MR DSE	6	41.53%	6.92%	7.33%	12.79%	1.09%	1.09%	4.22%	12.79%	10.43%	10.69%	2.31%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis_CPFU	MR CHEG	4	30.84%	5.14%	3.44%	16.10%	0.00%	6.88%	0.00%	0.00%	0.01%	16.10%	7.85%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Dermoloma_cuneifolium	MR CHEG	1	22.68%	3.78%	0.00%	22.68%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	22.68%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	SAP SOIL	6	15.00%	2.50%	1.89%	5.36%	0.63%	0.63%	1.12%	1.51%	5.36%	4.12%	2.27%
Basidiomycota	Agaricomycetes	Trechisporales	Trechisporaceae	OTU 11: unknown	SAP SOIL	6	12.03%	2.00%	1.46%	5.07%	0.86%	1.13%	0.86%	2.05%	1.48%	1.43%	5.07%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus1_pratensis	MR CHEG	5	11.46%	1.91%	0.12%	7.74%	0.00%	3.46%	0.00%	0.11%	0.03%	0.13%	7.74%
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	OTU 12: unknown	MR DSE	4	11.39%	1.90%	0.06%	9.18%	0.00%	0.00%	0.02%	2.09%	9.18%	0.11%	0.00%
Ascomycota	Eurotiomycetes	Chaetothyriales	Cyphellophoraceae	OTU 10: unknown	MR DSE	6	10.91%	1.82%	1.76%	3.76%	0.01%	0.01%	2.26%	3.76%	1.25%	3.62%	0.01%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe_sgPS2_reidii	MR CHEG	1	9.31%	1.55%	0.00%	9.31%	0.00%	9.31%	0.00%	0.00%	0.00%	0.00%	0.00%
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	OTU 9: unknown	MR DSE	5	9.07%	1.51%	1.22%	4.70%	0.00%	0.01%	0.56%	1.91%	4.70%	1.89%	0.00%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	OTU 13: unknown	MR DSE	2	9.02%	1.50%	0.00%	7.86%	0.00%	0.00%	1.16%	0.00%	7.86%	0.00%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis_CPCO	MR CHEG	4	8.35%	1.39%	0.06%	6.65%	0.00%	6.65%	0.00%	0.11%	0.00%	0.01%	1.58%
Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	OTU 23: unknown		6	8.14%	1.36%	0.83%	3.75%	0.01%	0.03%	0.92%	2.68%	3.75%	0.75%	0.01%
Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	Hyaloscypha	MR DSE	4	7.25%	1.21%	0.97%	2.89%	0.00%	0.00%	0.47%	1.47%	2.89%	2.42%	0.00%
Basidiomycota	Agaricomycetes	Trechisporales	Trechisporaceae	Trechispora	SAP SOIL	6	7.11%	1.18%	0.60%	4.50%	0.05%	0.20%	0.11%	1.01%	0.05%	4.50%	1.24%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	OTU 310	MR CHEG	6	6.54%	1.09%	0.01%	6.45%	0.00%	0.01%	0.00%	0.01%	0.01%	0.04%	6.45%
Ascomycota	Leotiomycetes	Helotiales	X	OTU 16	MR DSE	5	6.26%	1.04%	0.72%	3.65%	0.00%	0.03%	0.54%	1.15%	3.65%	0.90%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis_CPLA	MR CHEG	6	5.54%	0.92%	0.14%	3.41%	0.01%	1.83%	0.01%	0.12%	0.01%	0.16%	3.41%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria_CVAR	MR CHEG	6	5.36%	0.89%	0.40%	3.70%	0.04%	0.71%	0.24%	0.55%	0.04%	3.70%	0.12%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	OTU 22	MR CHEG	3	5.29%	0.88%	0.00%	5.27%	0.00%	5.27%	0.02%	0.01%	0.00%	0.00%	0.00%
Ascomycota	X	X	X	OTU 18		5	5.24%	0.87%	1.02%	1.76%	0.00%	0.00%	0.65%	1.39%	1.76%	1.39%	0.05%
Ascomycota	Leotiomycetes	X	X	OTU 17		4	5.14%	0.86%	0.47%	3.22%	0.00%	0.00%	0.46%	0.48%	0.97%	3.22%	0.00%
Ascomycota	X	X	X	OTU 37		5	4.87%	0.81%	0.85%	1.80%	0.00%	0.05%	0.50%	1.21%	1.80%	1.31%	0.00%
Ascomycota	Leotiomycetes	Helotiales	X	OTU 26	MR DSE	4	4.61%	0.77%	0.14%	3.74%	0.00%	0.00%	0.02%	0.27%	3.74%	0.58%	0.00%
Fungi incertae sedis	Mortierellomycotina	Mortierellales	Mortierellaceae	Mortierella	SAP	6	4.12%	0.69%	0.54%	1.95%	0.11%	0.58%	0.11%	0.34%	0.50%	0.64%	1.95%
Ascomycota	Archaeorhizomycetes	Archaeorhizomycetales	Archaeorhizomycetes	Archaeorhizomyces	SAP SOIL	5	3.99%	0.66%	0.27%	2.58%	0.00%	0.03%	0.13%	2.58%	0.83%	0.41%	0.00%
Ascomycota	Agaricomycetes	X	X	OTU 20		4	3.75%	0.62%	0.43%	1.65%	0.00%	0.00%	0.76%	1.23%	1.65%	0.10%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe_sgHS_glutinipes	MR CHEG	4	3.58%	0.60%	0.04%	3.31%	0.00%	0.19%	0.00%	0.07%	0.00%	3.31%	0.01%
Basidiomycota	Agaricomycetes	Trechisporales	X	OTU 32		3	3.51%	0.58%	0.09%	3.12%	0.00%	0.20%	0.00%	0.00%	0.00%	0.18%	3.12%
Basidiomycota	X	X	X	OTU 21		2	3.19%	0.53%	0.00%	3.15%	0.00%	0.04%	0.00%	0.00%	0.00%	0.00%	3.15%
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	OTU 36	MR CHEG	5	2.66%	0.44%	0.12%	2.11%	0.00%	0.22%	0.00%	0.02%	0.02%	2.11%	0.29%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	OTU 44		5	2.52%	0.42%	0.20%	1.60%	0.00%	0.51%	0.00%	0.03%	0.01%	0.36%	1.60%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis_CPCR	MR CHEG	3	2.50%	0.42%	0.04%	2.01%	0.00%	0.40%	0.00%	0.00%	0.00%	2.01%	0.08%
Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	Thelebolus	SAP DUNG	4	2.25%	0.37%	0.05%	2.03%	0.00%	0.08%	0.00%	0.00%	0.02%	0.11%	2.03%
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma		6	2.18%	0.36%	0.26%	0.94%	0.01%	0.43%	0.01%	0.09%	0.09%	0.63%	0.94%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Ramariopsis_RMKU	MR CHEG	6	1.82%	0.30%	0.07%	0.91%	0.01%	0.74%	0.01%	0.03%	0.02%	0.91%	0.11%
Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	Cudoniella	MR DSE	6	1.82%	0.30%	0.30%	0.60%	0.09%	0.09%	0.14%	0.34%	0.26%	0.38%	0.60%
Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	Penicillium	SAP SOIL	6	1.73%	0.29%	0.18%	0.62%	0.05%	0.05%	0.14%	0.56%	0.62%	0.13%	0.23%
Ascomycota	Leotiomycetes	Helotiales	Sclerotiniaceae	Torrendiella	MR DSE	6	1.62%	0.27%	0.22%	0.52%	0.04%	0.04%	0.15%	0.46%	0.29%	0.52%	0.16%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria_CVAC	MR CHEG	4	1.45%	0.24%	0.04%	0.69%	0.00%	0.69%	0.00%	0.04%	0.00%	0.69%	0.03%
Ascomycota	Leotiomycetes	X	X	OTU 51		4	1.45%	0.24%	0.10%	1.12%	0.00%	0.00%	0.10%	0.09%	0.14%	1.12%	0.00%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	OTU 40	MR DSE	5	1.40%	0.23%	0.08%	1.06%	0.00%	0.15%	0.00%	0.01%	0.02%	0.16%	1.06%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	OTU 291		6	1.34%	0.22%	0.09%	0.59%	0.02%	0.06%	0.05%	0.11%	0.02%	0.59%	0.51%
Ascomycota	Leotiomycetes	Helotiales	X	OTU 62	MR DSE	5	1.31%	0.22%	0.09%	0.62%	0.00%	0.14%	0.00%	0.04%	0.00%	0.62%	0.50%
Fungi incertae sedis	Mortierellomycotina	Mortierellales	Mortierellaceae	OTU 323		6	1.30%	0.22%	0.19%	0.55%	0.05%	0.05%	0.06%	0.10%	0.27%	0.27%	0.55%
Ascomycota	X	X	X	OTU 347		5	1.24%	0.21%	0.04%	1.04%	0.00%	0.04%	0.03%	0.04%	0.08%	1.04%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus3_roseascens	MR CHEG	1	1.19%	0.20%	0.00%	1.19%	0.00%	0.00%	0.00%	0.00%	0.00%	1.19%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe_sgPS4_cantharellus	MR CHEG	2	1.14%	0.19%	0.00%	1.13%	0.00%	1.13%	0.00%	0.00%	0.00%	0.01%	0.00%
Basidiomycota	Agaricomycetes	Sebacinales	X	OTU 45		5	1.12%	0.19%	0.11%	0.50%	0.00%	0.00%	0.17%	0.37%	0.50%	0.06%	0.02%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Ramariopsis_RMPU	MR CHEG	3	1.10%	0.18%	0.06%	0.68%	0.00%	0.30%	0.00%	0.00%	0.00%	0.12%	0.68%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis_CPX1	MR CHEG	4	1.10%	0.18%	0.18%	0.42%	0.00%	0.26%	0.00%	0.11%	0.00%	0.42%	0.31%

Table 2 (opposite page): Raw output from RDP analysis of the DNA metabarcoding data for the six Lundy quadrats. Taxa are ranked by cumulative percentage abundance over all six quadrats, with the top 55 (of the 607 taxa) shown here. On the left side, key taxonomic groupings are highlighted in orange (waxcaps), yellow (fairy clubs), pink (pink gills), cracked caps (brown) and dark septate endophyes (green). On the right side, percentage abundance of each taxon (as percentage of the total fungal community) is shown with most abundant taxa highlighted in green (>5%) and least abundant (<0.1%) in red

The DNA barcode sequences present in the RDP database analyses are mostly derived from publicly available sequence data submitted to the GenBank database (http://www.ncbi.nlm.nih.gov/nuccore/EF537888.1). For the CHEGD fungi (waxcaps and associated taxa) Griffith (unpublished data) has previously undertaken extensive DNA barcoding from reference samples (i.e. fruitbodies identified to species level by microscopic analysis), in addition to the reference DNA sequence available on GenBank. These sequences have been added to our in-house version of the original RDP database and the standard generic classifications in RDP have been modified to allow classification at species level. For CHEGD fungi there is sufficient variation at the LSU D1 locus to allow this to be undertaken for all the species of CHEGD, thus allowing better identification than the original RDP database. However, for Entolomataceae (Pink Gills), limited DNA barcode data from reference samples, and various other taxonomic uncertainties relating to this group, make it difficult to link sequences to recognised species.

For some other taxonomic groups, there are fewer DNA barcode sequences available and not all distinct DNA sequences have to date been linked to named species (or possibly relate to species not yet known to science); this results in less precise identification (i.e. only to family or order level). These taxa are listed as numbered OTUs (operational taxonomic units). Analysis of the entire fungal community in the six quadrats revealed the presence of 607 taxa across all the samples (a range of 244-443 OTUs per sample).

Summary data derived from Table 2 are presented in Table 3, showing relative abundances of the various CHEGD fungi as well as abundances of major mycorrhizal groupings. CHEGD species comprise the majority of fungal biomass in the two grassland quadrats LU1 (Airfield) and LU6 (Castle Hill) but also in LU2 (north of John O'Groat's House), due to the predominance of *C. lacmus* at this site.

Table 3: Summary data for the fungal communities in the six Lundy quadrats

Site	CLAV	HYG	ENT	GEOG	DERM	Total CHEGD	AMF*	DSE**-Helotiales	DSE**-Chaetothyriales
LU1	22.0%	67.6%	0.5%	0.2%	0.0%	**90.3%**	0.21%	0.80%	1.24%
LU2	0.3%	74.0%	0.0%	0.1%	0.0%	**74.4%**	0.00%	7.80%	4.32%
LU3	1.0%	18.2%	0.1%	0.4%	0.0%	**19.7%**	0.02%	33.82%	13.77%
LU4	0.1%	0.6%	0.1%	0.4%	0.0%	**1.2%**	0.02%	45.20%	10.55%
LU5	25.8%	10.9%	2.4%	0.9%	0.0%	**40.0%**	0.16%	9.32%	11.05%
LU6	16.2%	15.1%	0.4%	0.3%	22.7%	**54.7%**	0.42%	4.09%	3.41%

* AMF=Arbuscular Mycorrhizal Fungi (Glomeromycota)
** DSE=Dark Septate Endophytes

The most numerous non-CHEGD taxa were members of the ascomycete orders Helotiales and Chaetothyriales, together known as dark septate endophytes (DSE; highlighted in green on Table 2) These fungi are commonly found associated with the roots of higher plants and several have been shown to form mycorrhizal associations with their hosts. The most abundant DSE taxon was *Rhizoscyphus ericae*, which forms ericoid mycorrhizal associations with *Calluna* and related ericaceous hosts (Read, 1983; Hambleton & Sigler, 2005). This was most abundant in the Heather-dominated quadrats. Other DSE (OTU6; OTU9; OTU10; OTU12; OTU13) were also abundant in the Heather-dominated quadrats. However, the taxonomy of these fungi remains poorly understood and these five taxa could only be classified to family level due to the absence of closely related DNA barcodes.

It is commonly stated that grassland habitats are dominated by arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota (Smith & Read, 2010). However, whilst AMF were more abundant in grassland compared to heathland habitats (see Table 3), they comprise only a small amount of the total fungal DNA (<0.5%). Despite being present at only low levels, it is possible that they are highly active and may thus 'punch above their weight' in ecological terms. DSE are also known to be associated with grasses and herbs (Wilberforce *et al.*, 2002) and thus were also present but at lower abundance in the grass-dominated quadrats (LU1/LU6 in Table 3).

Further Analysis of the Data

Further analysis sought to examine the whole fungal community in comparison to other grassland soils we have analysed (mostly from Wales) and more specifically to determine the presence of CHEGD target species in comparison to records of their fruitbodies on Lundy.

Ordination analysis

Initial analysis of the fungal communities in the six Lundy quadrats (LU1-6) by either NMDS or DCA ordination (Figure 5) was undertaken alongside similar community data from 'top quality' waxcap grassland sites in Wales (coded as CCW). In such analyses, samples which ordinate closely together have similar fungal communities. LU1 (The Airfield) is clearly close to the most diverse Welsh waxcap meadow sites. LU6 (Castle Hill), although grassland, is much more of an outlier. Of the four Lundy heathland sites, LU2 and LU3 from the North End show greatest separation from the grassland sites, with LU4 and LU5 occupying an intermediate position. LU5 (south of Rocket Pole Pond), was the closest and also had a more diverse plant community of grasses and forbs as well as Heather.

Detailed analysis of CHEGD fungi

For more detailed analysis of CHEGD fungi, BLAST analysis of the sequence data against the curated database on CHEGD sequences was undertaken (Table 4).The most abundant 55 fungal taxa across the six Lundy sites ranked by cumulative numbers of sequences are shown. Columns on the right hand side indicate the percentage of all fungal sequences for each taxon at each site. This yields similar data to the RDP analysis but taxonomic resolution is more accurate. For each CHEGD species, the number of sequences detected for each species in each quadrat is shown. In some cases, many

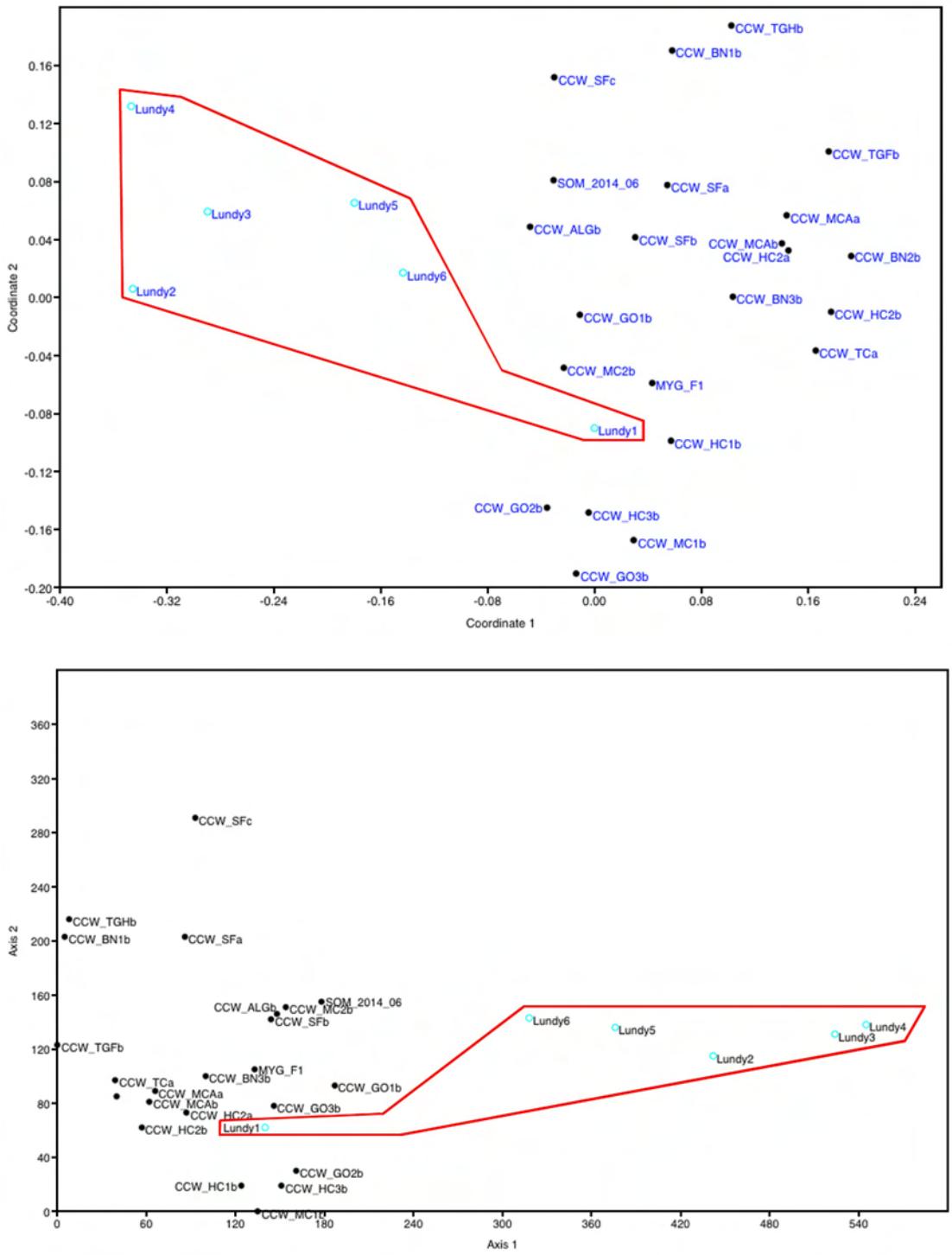

Figure 5: Non-metric MultiDimensional Scaling (NMDS; upper) and Detrended Correspondence Analysis (DCA; lower) ordination of Lundy fungal community data (encircled in red polygons) alongside data from 'top quality' waxcap grasslands in Wales. Samples ordinated more closely together have more similar fungal communities

Table 4: BLAST analysis of fungal sequence data for CHEGD fungi, showing number of sequences for each species found in each quadrat soil sample. CHEGD totals are for species with >50 eDNA sequence reads. *Cuphophyllus lacmus* (Grey Waxcap) reads are shown in bold font

		Lu1	Lu2	Lu3	Lu4	Lu5	Lu6	Total Sequence Reads	Total Quadrats (/6)
C	*Camarophyllopsis atrovelutina*	1052	.	.	3	9	10	1074	4
C	*Clavaria acuta*	264	.	17	.	246	11	538	4
C	*Clavaria argillacea*	.	167	188	.	6	.	361	3
C	*Clavaria fragilis*	5	6	20	.	71	12	114	5
C	*Clavaria guilleminii*	178	.	.	3	28	.	209	3
C	*Clavaria straminea*	264	8	31	11	1095	11	1420	6
C	*Clavulinopsis corniculata*	2544	.	45	.	5	853	3447	4
C	*Clavulinopsis helvola*	170	.	.	.	.	742	912	2
C	*Clavulinopsis laeticolor*	500	7	47	3	35	391	983	6
C	*Clavulinopsis luteoalba*	2686	.	.	4	5828	2844	11362	4
C	*Ramariopsis biformis*	285	9	13	5	326	38	676	6
C	*Ramariopsis pulchella*	61	.	.	.	.	93	154	2
	Clavariaceae spp. unknown	366	.	51	.	1433	551	2401	4
H	*Cuphophyllus fornicatus*	.	48	.	.	.	.	48	1
H	*Cuphophyllus lacmus*	.	**52829**	**7079**	**15**	**2008**	7	61938	5
H	*Cuphophyllus pratensis*	1323	.	43	9	47	2687	4109	5
H	*Cuphophyllus roseascens*	.	.	.	.	426	.	426	1
H	*Cuphophyllus russocoriaceae*	5	3	6	3	16	2270	2303	6
H	*Cuphophyllus virgineus*	.	.	.	.	.	55	55	1
H	*Gliophorus irrigatus*	19	.	.	3	.	4	26	3
H	*Gliophorus psittacinus*	51	.	10	.	10	.	71	3
H	*Hygrocybe cantharellus*	431	.	.	.	4	.	435	2
H	*Hygrocybe ceracea*	3	.	.	.	.	18	21	2
H	*Hygrocybe chlorophana*	14	.	.	.	.	96	110	2
H	*Hygrocybe conica*	343	42	45	12	150	3	595	6
H	*Hygrocybe contrictospora*	.	.	.	.	.	10	10	1
H	*Hygrocybe glutinipes*	71	.	30	17	1208	4	1330	5
H	*Hygrocybe helobia*	2016	13	3	.	.	.	2032	3
H	*Hygrocybe mucronella*	.	.	.	.	.	101	101	1
H	*Hygrocybe pseudoconica*	5	.	.	.	.	.	5	1
H	*Hygrocybe punicea*	17994	20	.	.	.	.	18014	2
H	*Hygrocybe quieta*	9	.	.	9	7	.	25	3
H	*Hygrocybe reidii*	3565	.	.	.	.	.	3565	1
E	*Entoloma conferendum*	85	.	7	5	757	101	955	5
E	*Entoloma porphyrophaeum*	.	6	38	7	8	.	59	4
E	*Entoloma sp.*	.	.	.	4	75	24	103	3
E	*Entoloma serrulatum*	31	.	.	.	3	.	34	2
E	*Inocephalus sp.*	85	.	.	.	4	.	89	2
G	*Geoglossum fallax*	17	.	.	.	43	97	157	3
G	*Geoglossum umbratile*	.	.	.	.	6	.	6	1
G	*Glutinoglossum glutinosum*	46	.	9	.	72	16	143	4
G	*Glutinoglossum heptaseptatum*	.	.	.	.	18	.	18	1
G	*Glutinoglossum pseudoglutinosum*	.	.	.	.	3	.	3	1
G	*Trichoglossum hirsutum*	.	.	.	.	5	.	5	1
G	*Trichoglossum walteri*	.	.	5	.	138	.	143	2
G	*Geoglossaceae spp. unknown*	27	.	.	.	16	.	43	2
"G"	*Microglossum olivaceum*	.	3	.	.	55	.	58	2
D	*Dermoloma cuneifolium*	.	.	.	.	.	7874	7874	1
	Total no. fungal sequences	38278	71805	40754	29971	35903	34725	251436	
	Total no. CHEGD sequences	34515	53161	7687	113	14161	18923	18923	
	Clav	10	1	2	0	6	6	13	
	Hyg	8	1	1	0	4	5	14	
	Ent	2	0	0	0	2	1	4	
	Geo	0	0	0	0	3	1	4	
	Derm	0	0	0	0	0	1	1	
	CHEGD total	20	2	3	0	15	14	36	

thousands of sequences of a single species are detected in a single quadrat indicating the large amount of mycelium of these species in those particular quadrats (e.g. 17,994 sequences of *H. punicea* in the soil sample from quadrat LU1 on the Airfield). However, other species are present at only low abundance in the sequence data and could represent much smaller colonies, potentially indicative of the presence of only spores or small mycelia which might not be large enough to form fruitbodies. This issue makes it difficult to draw direct equivalence between the numbers of species found by eDNA analysis and fruitbody surveys. In deciding how many sequences should be detected in a quadrat for a particular species to be added to the CHEGD total, we have opted to use a threshold of 50 sequences (Table 4). Thus, quadrat LU1 (Airfield) had the highest CHEGD species count (20) followed by quadrats LU5 (below Rocket Pole Pond) and LU6 (Castle Hill) at 15 and 14 respectively. The high CHEGD score at LU5 would indicate that this area, in spite of its selection as a *Calluna* site for the soil survey, has strong affinities with acid grassland.

Across all the quadrats, 14 waxcap species were detected at >50 eDNA sequence reads and a further six species at lower (<50 sequence reads) abundance. Comparing the data in Table 4 for species of fungi detected in the soil sample from the Airfield site (LU1) with the species found over the Airfield fruitbody survey period (Figure 4) some patterns emerge. Firstly in terms of numbers of Hygrophoraceae, eight taxa were recorded at >50 e DNA reads and a further six at <50 reads i.e. a total of fourteen. 23 species were recorded from the annual fruitbody surveys and included twelve of the taxa appearing in the sequence data, the exception being *H. acutoconica*, recorded on Lundy as *H. persistens* (Persistent Waxcap) but not yet found on the Airfield, and *H. helobia* known only from Quarter Wall Cottages. For some species there was a good fit between abundance of fruitbodies in the survey and proportion of its DNA in the sample: *Hygrocybe punicea* (Crimson Waxcap) DNA was over 40% of sequences in the Airfield soil sample and its fruitbodies were one of the most abundant recorded in the survey (a total of 230), as well as some of the largest, so presumably having a large supporting mycelium in the soil (compare Table 3 and Figure 4). A weaker correlation is seen for *Cuphophyllus pratensis* (Meadow Waxcap) (2.8% of the DNA sequences/148 fruitbodies recorded).

For others, the fit between DNA profiles of samples and the survey data was less good. *Hygrocybe reidii* (Honey Waxcap), accounting for over 7% of the sequences in the sample, was only found 15 times and *Hygrocybe cantharellus* (Goblet Waxcap), 0.77% of the sequences, was only found ten times. On the other hand, fruitbodies of some species absent or at <0.15% of the fungal DNA, such as *Hygrocybe chlorophana* (Golden Waxcap), *Gliophorus laetus* (Heath Waxcap) and *Hygrocybe coccinea* (Scarlet Waxcap), were abundant in all years of the Airfield survey. These contradictions may be related to the smaller area of the soil sampling grid compared to the fruitbody count area, perhaps missing localised mycelia of some species and over-emphasising the presence of others.

The second component of the CHEGD system for which we feel some confidence in the DNA barcoding data is the family Clavariaceae with nine taxa at >0.15% of the fungal DNA in the Airfield soil sample. Seven taxa were identified in the fruitbody

survey (Figure 4) but only two of these, *Clavulinopsis helvola* (Yellow Club) and *Clavulinopsis (=Ramariopsis) corniculata* (Meadow Coral), were detected from soil eDNA (Table 4). As noted earlier, field identifications of another *Clavulinopsis* species, *C. fusiformis* (Golden Spindles) do not separate it with certainty from *C. helvola*, so records made in the fruitbody surveys of the Airfield were probably of *C. helvola* not *C. fusiformis*. The taxonomy of this family is difficult so the exact status of taxa in databases remains uncertain. However the data suggest that the Airfield grassland has a number of Clavariaceae yet to be recorded as fruitbodies from Lundy.

Sequence data for Geoglossaceae (Earthtongues) are also presented in Table 4. Two species, *Geoglossum fallax* (Black Earthtongue) and *Geoglossum* (=*Glutinoglossum*) *glutinosum* (Slimy Earthtongue), were also found as fruitbodies (Figure 4) so may be more widespread on the Airfield than the low fruitbody numbers suggest.

Seven species of *Entoloma* were found in the Airfield survey, of which one, *E. conferendum* (Star Pinkgill), was abundant in most years (Figure 4) and also occurred as the greatest number of sequences in the soil extracts (Table 4). *E. serrulatum* (Blue Edge Pinkgill) fruitbodies were much less frequent but it was also detected as DNA sequences. Recovery of Entolomataceae DNA sequences from soil samples is usually much lower than for the other CHEGD components, perhaps reflecting lower mycelial biomass in the soil or low DNA content.

The other grassland site from which a soil sample was taken, Castle Hill, LU6, has not had any systematic annual counts of fruitbodies but has had brief species surveys every autumn. The tall rank grass, due to undergrazing for many years following the crash in the Rabbit population in 2005, makes it difficult to find fruitbodies of fungi. Fourteen CHEGD species sequences were detected at >50 eDNA sequence reads of the fungal DNA (Table 4). Four were Clavariaceae, all of them species of *Clavulinopsis* (=*Ramariopsis*) – *C. corniculata* (Meadow Coral), *C. helvola* (Yellow Club), *C. laeticolor* and *C. luteoalba* (Apricot Club). Of these only fruitbodies of *C. helvola* and *C. corniculata* had been found to be common in surveys of Castle Hill, together with *C. fusiformis* (Golden Spindles), which, as in the Airfield site, does not appear in the sequence data. *C. luteoalba* (Apricot Club) has been recorded for Lundy (Hedger & George, 2018), but not on Castle Hill. *C. laeticolor* would be a new record, now confirmed by the finding of fruit bodies of this species on Castle Hill in November 2019. Of the Hygrophoraceae, *Cuphophyllus pratensis* (Meadow Waxcap) was well represented in the DNA sequences and is the only waxcap regularly seen on Castle Hill, possibly because its large size makes it easier to find in the long grass. The much smaller *H. russocoriacea* (Cedarwood Waxcap) sequences have almost the same value but the fruitbodies have never been found. Of the Entolomataceae *E. conferendum* (Star Pinkgill) is the only species regularly recorded from Castle Hill and is also the only representative in the DNA profile. As with the Airfield site Geoglossaceae were a small percentage of the fungal DNA but the same two species, *Geoglossum fallax* and *G. glutinosum,* were detected though never found in the field. The one surprising feature of the Castle Hill data is the abundance of DNA of *Dermoloma cuneifoleum* (Crazed Cap), a CHEGD species not found in the profiles of any of the other five sites but fruitbodies have never been seen on Castle Hill though recorded from the nearby St John's valley.

In contrast, as expected, *Calluna* sites at the North End LU2 (John O'Groat's House) and LU3 (Squire's View) and in the south at LU4 (north of Old (Quarry) Hospital) had CHEGD species counts of 0-3 of which one component, *Cuphophyllus lacmus*, accounted for over 73% of the total fungal DNA at LU2 (John O'Groat's House) and over 17% at LU3 (Squire's View) (as bold figures in Table 4). Both quadrats are within the areas where the annual surveys found fruitbodies of this species. *C. lacmus* DNA was also detected in the soil samples from the two 'southern' *Calluna* sites. It was a trace (0.05%) at LU4 (north of Old (Quarry) Hospital) but accounted for over 5% at LU5 (below Rocket Pole Pond), although its fruitbodies have never been found in either place. It is clearly a much less important component of the soil mycota here. Its DNA was not detected in any of the grassland soil samples on Lundy, LU1 (Airfield) and LU6 (Castle Hill).

DISCUSSION

Up to now the study of the distribution of fungi on Lundy has been by assessment of presence (and absence) of fruitbodies in different areas of the island. This has enabled assignment of species to different terrestrial habitats, an approach used by Hedger and George in their 2018 account of the fungi of Lundy. The relatively new use of eDNA profiling to identify fungi in the habitat can directly prove the presence and even abundance of the mycelium of each species. To date only one study of this kind has been carried out on Lundy, by Monk *et al*. (2014) who used DNA profiling to investigate colonisation of *Rhododendron* stumps on the east side by sampling wood at different stages of decomposition. Their data were rich in sequences of wood rotting taxa, for example species in the family Polyporaceae, some of them new to Lundy.

Our study has focused on sequences of taxa likely to be found in grassland, the CHEGD species, with especially rich information on the waxcaps. It has confirmed that restriction of the fruitbodies of one these fungi, the Grey Waxcap, *Cuphophyllus lacmus*, to the Maritime Heath on the North End of Lundy reflects the real distribution of its mycelia, although it may be present at low levels in soil at other Heather sites. This habitat preference may be because of a mycorrhizal relationship with Heather and/or as a mycobiont partner. Several lines of evidence suggest that waxcaps (and likely other CHEGD) are not saprotrophs, as previously suspected, but rather mycorrhizal with grasses and/or herbs (Griffith *et al*., 2014). Halbwachs *et al*. (2018) presented isotopic evidence consistent with a mycorrhizal habit, and the hyphae of waxcap have been detected within the roots and shoots of putative plant hosts (Halbwachs *et al*., 2013; Tello *et al*., 2014). However, association with ericaceous hosts has not previously been observed. Another possibility is that *C. lacmus* forms an association with lichens such as the foliose *Cladonia arbuscula,* another major component of the Maritime Heath. Both are novel ideas, requiring further supporting evidence. More soil DNA profiling of a range of Heather sites on Lundy, together with evaluation of their plant communities and soil characteristics, is needed as well as a search for *C. lacmus* DNA in samples of Heather roots and in lichen thalli.

The CHEGD system was developed to help evaluate the conservation status of grassland sites. Of the six Lundy sample sites, two were from grassland, the Airfield and Castle Hill. The DNA profiles of the former ordinated close to data from known 'high quality' meadow sites on the mainland. The CHEGD value derived from the DNA data for the Airfield site was 20 (see Table 4), from fruitbody count it was 40 (see Figure 4); the fruitbody count must be higher both due to the eleven-year monitoring period and the much larger sampling area. CHEGD counts using fruitbody surveys on other unimproved grassland sites on Lundy since 2003 have often been in the region of 10-20 species and the island 'total CHEGD' score, derived from the current species total of fungi, is 72 (Hedger & George, 2018). These figures show that the current management of Lundy has created short turf habitats that are rich in species of grassland fungi and underlines the value of Lundy grasslands for conservation of fungal diversity.

The question as to whether DNA sequence data can be used as new records of fungal species in the absence of fruitbody identifications remains open and depends upon the reliability of the databases used. Table 4 includes six highlighted (>50 eDNA sequence reads) species of Clavariaceae (Club Fungi) not yet seen on Lundy, although we are not confident as to the exact current status of some of them. There is also a possible new waxcap record for Lundy. *Cuphophyllus roseascens*, 0.8% of the fungal sequences from the site below Rocket Pole Pond (LU5), is a small pinkish capped waxcap which was first described in Sweden in 2004 and is rare in the UK.

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