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An assessment of the fungal conservation value of Hardcastle Crags (Hebden Bridge, West Yorkshire) using NextGen DNA sequencing of soil samples

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Foreword

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

Background

DNA based applications have the potential to significantly change how we monitor and assess biodiversity. These techniques may provide cheaper alternatives to existing species monitoring or an ability to detect species that we cannot currently detect reliably.

Natural England has been supporting the development of DNA techniques for a number of years and has funded exploratory projects looking at different taxonomic groups in a range of different ecosystems and habitats.

This report presents the results of a survey of fungi of conservation importance at Hardcastle Crags, West Yorkshire, using DNA based methods. These results are compared to fruiting body surveys which had been carried out in the preceding two years.

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Further information
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An assessment of the fungal conservation value of Hardcastle Crags (Hebden Bridge, West Yorkshire) using NextGen DNA sequencing of soil samples

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Summary

Soil samples were collected from 14 grassland areas (each ca. 900 m^2) in the Hardcastle Crags area, near Hebden Bridge, West Yorkshire (Hollin Hall, Crimsworth Dene and along the Widdop road sub-areas). After freeze-drying, grinding, DNA extraction and PCR (polymerase chain reaction) amplification of the ribosomal RNA fungal DNA barcode region, high throughput DNA sequencing was conducted using an Ion Torrent PGM sequencer. This yielded approximately 331,000 fungal DNA sequences (mean 23,686 sequences per sample [range 10,887-- 40,487]), allowing the fungi present in these soils to be both identified and quantified. Basidiomycete fungi dominated all samples (63-92% of all sequences) with the Hygrophoraceae (waxcap family) being most abundant (mean 32% of all sequences), followed by the coral fungi (Clavariaceae; mean 23%). More detailed analysis of the Hygrophoraceae revealed the presence of 25 species, of which six species had not been observed to fruit at the site, during recent autumn surveys. However, three Hygrophoraceae species which had been previously recorded fruiting at the site were not detected in soil DNA. Several species present at the site were found in different areas from those where they had been detected in fruitbody surveys. The fluid taxonomic status of many members of the other CHEGD taxa (e.g. Clavariaceae, Entolomataceae and to some extent Geoglossaceae and *Dermoloma* spp.) make it difficult to provide exact species counts for these. Based on comparison of the eDNA and data from past fruitbody surveys, these areas have high conservation value for grassland fungi and merit consideration for designation as a Site of Special Scientific Interest (SSSI).

Introduction

The aim of this study was to investigate soil fungal populations and thereby assess the conservation importance of the various grassland sites in the Hardcastle Crags area, West Yorkshire (Figure 1) using NextGen sequencing. Additionally, this study allowed Natural England to evaluate the potential of DNA metabarcoding of soil eDNA as a method for the assessment of biodiversity of fungi (and potentially other soil organisms).

Three grassland areas were investigated in three sub-areas: Hollin Hall (code HH: four sheep-grazed fields owned by the National Trust, west of the Bridge Clough stream), Crimsworth Dene (code CD: five ungrazed fields on a steep slope on the opposite side of the valley) and four fields along the Widdop Road (code WID: one haymeadow, one sheep-grazed and two sheep-grazed fields at higher elevation). Choice of these areas was guided by previous field surveys in 2015 and 2016 at HH and WID.

Most of our knowledge of the distribution of fungi is based on the occurrence of their reproductive structures (basidiocarps [mushrooms], ascocarps etc.) which occur only ephemerally and in a highly season and weather-dependent manner. Thus, establishing which fungi are present at a given site requires detailed and time-consuming field surveys.

We have adapted new developments in DNA sequencing technology (often called NextGen sequencing) to devise a method whereby extraction of DNA from soil samples can be used to assess which fungi are present.

Specifically we are developing the use of this technology to elucidate the distributions of grassland macrofungi, many of which (notably the waxcaps but also including other 'CHEGD' fungi [coral fungi-Clavariaceae, earth tongues-Geoglossaceae, pink gills-Entolomataceae, cracked cap-*Dermoloma*/*Porpoloma*]) are of conservation concern. The definition of the species included in the 'CHEGD' group are described by Griffith et al (2013) with the relevant part of the paper supplied here as Appendix 1. It is important to note that there has been a taxonomic reappraisal by Lodge et al (2014) of the Hygrophoraceae family (which contains the waxcaps but also some other lichenised fungi and ectomycorrhizal species). This has resulted in the creation of some name changes (e.g. *H. calyptriformis* [pink waxcap] is now *Porpolomopsis calyptriformis*; and what were formerly known as *Hygrocybe* spp. now in the genera *Chromosera*, *Cuphophyllus*, *Gliophorus*, *Gloioxanthomyces*, *Humidicutis*, *Neohygrocybe*). However, the specific names are preserved.

This DNA based survey method is dependent upon the existence of genetic information (DNA barcodes) relating to each of the species of interest. The genes used as DNA barcodes for fungi differ from those used for animals and plants. For fungi it is the ribosomal RNA genes that are used, notably the Large SubUnit (LSU) and internal transcribed spacer (ITS) regions. We have opted for the former (i.e. LSU), since the coverage for some fungal groups (esp. Clavariaceae) is better with LSU. The primers used for our LSU amplification are more conserved across the range of fungal phyla than those used for ITS and the amplicons from different fungal groups are more conserved in length, so there is less bias in PCR amplification (due to primer mismatch or selection against longer [mostly basidiomycete] amplicons.

The disadvantage of LSU is that for some taxa (e.g. Polyporales -wood-decay fungi), there is poor resolution at species level but for the CHEGD fungi, there is good resolution (though see note below re '*Dermoloma*'). For habitats where LSU is not suitable, we use the ITS2 locus and the mix of primers suggested by Tedersoo et al (2014). An additional factor that could cause bias is differential extraction of DNA from different fungal tissues. For example, it is likely that extraction of DNA from spores is less efficient than from actively-growing mycelia. Additionally, the rRNA operon is a multicopy operon and it is estimated that 200 copies of this operon are present in each fungal nucleus (as tandem repeats, visible as the nucleolus in microscopy). Large differences in rRNA copy number could also cause bias but to establish copy number for different species is not a simple matter.

The last factor (often not sufficiently accounted for in many peer-reviewed publications) is the sampling strategy. We have adopted a 900 m^2 quadrat. This is a moderately large area, which will fit into most grassland field plots; these are conveniently compatible with permanent quadrats which we established across Wales in 2003-4 for fruitbody surveying (Griffith et al, 2006) and also our main reference field site (Brignant long-term experiment; https://www.ecologicalcontinuitytrust.org/brignant/; Detheridge et al., 2018). Within these large quadrats, 36 cores are taken on a grid pattern to provide a total sample weighing ca. 500-700 g.

DNA barcodes are available for most of the CHEGD fungi found in semi-natural grasslands, though some of the current barcodes relate to specimens from non-UK locations (but which are likely to differ only slightly in DNA sequence). Other groups of fungi are less well-studied and thus fewer barcodes are available. As a result it is sometimes only possible to identify DNA sequences to genus or family. These 'mystery' barcodes may represent undiscovered species or alternatively known species for which no DNA barcodes have been established.

Analysis of the huge numbers of sequences from NextGen sequencing (typically ca. 20,000 per sample) can provide not only identification but also relative abundance information. However, as noted above, the alignment of 'genetic' and morphological species is still not complete and the taxonomy of several fungal families is currently in flux. We also do not yet know the extent to which fungal biomass naturally fluctuates on an annual basis but it is known that the grassland fungi of conservation interest are long-lived organisms fruiting in the same locations each year and thus very likely to be present at similar relative abundance throughout the year.

The issue of how quantitative DNA metabarcoding is (i.e. how much reliance can be placed on read abundance) has been much discussed. As noted above, primer mismatches and taxon-related differences in amplicon length may cause bias. However, for the primers we use (Detheridge et al, 2016), the primer binding sequences are identical for all the CHEGD fungi (and well conserved across all the fungal phyla, with to our knowledge only a few exceptions). Furthermore, the amplicon length varies by only ca. 10 base pairs across all the fungi, so is very unlikely to lead to bias against the longer sequences. This contrasts with the more widely used ITS2 barcode locus (Tedersoo et al., 2014) where there is significant length polymorphism (<100 bp), which can cause bias against basidiomycete fungi which have longer ITS sequences.

Methodology

Field sampling. Details of the fields surveyed are given in Table 1, with field numbers corresponding to those listed in McLay (2016). The CD fields were not surveyed by McLay (2016) but were sampled because they were believed to be the fields where a high diversity of waxcap fungi had been reported from surveys undertaken in the mid-20th century by Roy Watling and others. Most of the five quadrats grouped here as WID were also surveyed by McLay (2016) and noted as New High Laithe Farm (exception is W1 which is ca. 1 km to the south).

The sampling was conducted on 23rd October 2017. A 15 mm soil auger was used to take soil cores to a depth of ca. 10cm in each of the 14 grassland areas across a 30 m x 30 m quadrat (see Figure 1) following an approximate grid pattern with a spacing of ca. 5 m between cores (ca. 30-50 cores per quadrat). Cores for each quadrat were pooled in a plastic bag (fresh weight of ca. 500-900 g/sample) and stored in a cool box until frozen (ca, 8-10 hrs after start of sampling). The positions of the corners of the quadrats were recorded with GPS, photographs and other nearby landmarks (field boundaries, large rocks etc.).

Sample preparation: Soil samples were freeze-dried and finely ground by passing through a 0.5 mm wire sieve. The moisture content of the samples varied from 32-45%. Following grinding, samples were thoroughly mixed and stones and larger fragments of plant material were removed. A subsample (250 mg) was taken for DNA extraction using the *Powersoil*

Soil DNA extraction kit. Details of the sample codes, weight, moisture content, quadrat area etc. are shown in Table 1.

Genetic analysis: PCR amplification of a 230 bp portion of the Large Ribosomal Subunit (LSU) of ribosomal RNA locus was amplified with the primers GBD1-F2 and GBD1-NLC2-AF. 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 15 nM amplified using emulsion PCR, followed by loading onto a 316 Ion Torrent chip. All the steps from emulsion PCR onwards followed carefully the instructions provided with the Ion Torrent PGM (Personal Genome Machine). The full method for DNA extraction, PCR amplification and bioinformatics analyses are published in Detheridge et al. (2016 and 2018).

Results

Following the sequencing run, the quality of sequences was assessed and short reads not covering the whole barcode region or sequences of poor quality were removed, leaving 331,605 sequences. These sequences were then split using the 10 bp identifier tag to separate the 14 samples. The number of reads varied from 10887 to 40487 per sample after quality control (removal of incomplete sequences and singletons [unique sequences found only once]).

Analysis of the sequence data showed that the waxcap fungi (family Hygrophoraceae) were the dominant fungi in most samples (mean 32% of total fungal DNA; range 6-67%; Table. 1). Second most abundant were the coral fungi (family Clavariaceae; mean 23%; range 11-45%). As found at other sites the other CHEGD fungi (Entolomataceae, Geoglossaceae) were present only at lower abundance (0.1-3.4% and 0-5.0% respectively). In three quadrats (CD5, HH3, HH4) *Dermoloma cuneifolium* was found to be abundant (11.9-21.2%) and at WID4/5, *Lepista* sp., a non-CHEGD taxon (species not resolved) was highly abundant (30.8-38.7%). The most abundant 50 taxa over all quadrats are shown in Table 2.

Data were analysed in two ways: (1) to examine the whole fungal community in comparison to other grassland soils we have analysed (mostly from Wales) and (2) to determine the presence of certain target species. These analyses are reliant on the existence of DNA barcodes which are mostly publicly available (for example http://www.ncbi.nlm.nih.gov/nuccore/EF537888.1). For the CHEGD fungi (waxcaps and allies) we have previously undertaken extensive DNA barcoding from reference samples (i.e. fruitbodies identified microscopically) in addition to the reference DNA sequence available on GenBank. For some other taxonomic groups, there are fewer DNA barcode sequences available and this results in less accurate identification (i.e. only to family or order level).

For the initial analyses, data 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, it can classify sequences to higher taxonomic orders. Since our focus at Aberystwyth is grassland fungi, we have modified this database to include more representatives of fungi likely to be found in these habitats. We have modified the RDP database to include our in-house reference DNA barcodes and where we find reliable differences between species, we have separated genera to allow classification of individual species. For CHEGD fungi there is sufficient variation at the LSU D1 locus to allow this to be undertaken for all the species of CHEGD (except Entolomataceae) for which we have data, thus allowing better identification than the online database. Analysis of the whole fungal community using RDP revealed the presence of ca. 850 taxa across all the samples (range of 161-461 OTUs per sample). The top 50 genera based on overall abundance are shown in Table 2.

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. Initially the 14 samples were compared (Figure 2). The HH and CD quadrats clustered together with the five WID quadrats being distinct but with the two haymeadows (WID4/WID5) and the two rough pasture quadrats (WID2/WID3) being more distinct from all other quadrats.

The second analysis conducted was using BLAST (Basic Local Alignment Search Tool; http://blast.ncbi.nlm.nih.gov/Blast.cgi) a widely used method for DNA sequencing analysis. The NextGen sequences were used to interrogate a bespoke database of grassland macrofungi (including all the known waxcap, fairy club and earth tongue sequences) to ascertain how many sequences from each DNA were closely matched to our verified DNA barcodes. A cut-off of value of E^{-70} was used in these analyses. A list of species identified from the Hardcastle Crags quadrats based on BLAST analyses and consistent with the RDP classification is shown in Table 3.

Waxcap populations

As noted above, following publication of Lodge et al (2014; Appendix 2), the taxonomy of the Hygrophoraceae is now quite stable such that there is good agreement between morphological and phylogenetic concepts of most of the species. However, for the other groups of CHEGD fungi (e.g. Clavariaceae, Entolomataceae) the situation is less clear, so the analysis here of exactly which species were present focuses on the waxcaps.

For some samples and taxa only low numbers of sequences were recovered. A cautious interpretation is used here counting only species (bottom of Table 3) where >50 sequences were recovered. Small numbers of sequences may indicate for example the presence of only very small mycelial systems not capable (yet) of forming fruitbodies or potentially ungerminated spores etc. Based on this, a total of 25 species, of which six had not been observed to fruit at the site during recent autumn surveys (highlighted in green on Table 3) were identified.

Of these species, the rarest is *Neohygrocybe ingrata*, found in the WID1 quadrat at high abundance (>1000 sequences); re-survey of this quadrat in autumn 2018 would likely lead to the discovery of basidiocarps of this species. *Arrhenia* spp. (here most likely *A. lobata*) are not historically considered as waxcaps but have been found to belong to

Hygrophoraceae; they form small basidiocarps, often lacking a stipe amongst mosses. One clade which we call *Hygrocybe conica* AFF (AFF=affinity) is related to the *H. conica* group of species but taxonomically quite distinct and close to *H. acutoconica*. It is not yet formally named but based on our eDNA analyses appears to be widely distributed in Britain. The other three species (*H. constrictospora*, *H. glutinipes*, *H. phaeococcinea*, are small often reddish-orange species, which tend to be difficult to identify without careful microscopy and may be confused with other species such as *H. insipida*, *H. helobia* or *H. mucronella*.

Three Hygrophoraceae species whose basidiocarps were previously recorded at the site (at 1 or 2 quadrats) were not detected in soil DNA (*C. russocoriaceus, G. vitellina*, *H. aurantiosplendens*; indicated by **** in Table 3). If these three species were present as mycelial systems of limited distribution, then it is possible that soil containing these species was not cored during the sampling. It is possible that *C. russocoriaceus* could be misidentified morphologically as *C. virgineus* (differing most obviously in the cedarwood odour of the former). *G. vitellina* and *H. aurantiosplendens* are fairly rare in the UK and potentially misidentified - for this reason we suggest that the retention of dried basidiocarp samples, which can potentially be DNA barcoded at a later stage, should be routinely done for site surveys where there is doubt about morphological determination.

With regard to the relative locations of eDNA and basidiocarp records, several species showed good association (e.g. *C. flavipes*, *C. pratensis*, *H. coccinea*, *H. quieta*), whereas for others the association was poor (e.g. *H. citrinovirens*, *H. punicea*, *P. calyptriformis*). It could be, as above, that their mycelial systems were present within the quadrat but not cored or alternatively that they were present outside the quadrat area.

Populations of other CHEGD fungi

We have recently updated our database of reference sequences for CHEGD fungi, now including more sequences from *Dermoloma*/*Porpoloma* spp. and also Entolomataceae. During initial analysis of the data, there seemed to be a large number of sequences attributed to the very rare species *Dermoloma magicum*, for which only five British Isles records exist (up to 2013; Griffith et al (2013), see Appendix 1). These sequences were detected in several quadrats (CD5, HH3/HH4 and WID4/WID5) and in some cases comprised up to 30% of total fungal sequences in that quadrat. We have investigated this strange finding and concluded that this is a misidentification; the LSU DNA barcode sequence of *D. magicum* is very similar to that of members of the closely related genus *Lepista*. *Lepista* spp. (blewits) are commonplace in grassland habitats, often forming large fairy rings. We had not previously concentrated on generating new DNA barcodes for Lepista spp. relying on sequences generated from non-UK samples. We aim to correct this omission and we are also working with collaborators in Slovakia (Slavomir Adamcik) to improve the taxonomy of the genus *Dermoloma* spp. which is also poorly defined.

Nine earthtongue species were detected by eDNA. One of these, *Geoglossum dunense,* is new to the UK, having been found only recently at the Leasowes site (Oct 2017; from fruitbody) and the species itself was only described recently (Loizides et al, 2015) from Cyprus. *Trichoglossum walteri* was found by both eDNA and a morphological sample (near HH4 quadrat) during the field visit. The identity of the fruitbody was confirmed by microscopy and also DNA barcoding of the dried sample.

Microglossum spp. are only distantly related to Geoglossaceae but generally counted as earth-tongues. One of these, *M. olivaceum*, is a section 41 species but recent phylogenetic

analyses have revealed the presence of several distinct *Microglossum* spp. (Kucera et al 2017; Harries et al, 2018). Here three distinct *Microglossum* spp. were found but for two of these (incl. *M. olivaceum* in quadrat CD5) the numbers of sequences recovered was low.

Despite the existence of two detailed and comprehensive monographs by Noordeloos (1992, 2004), Entolomataceae are the most difficult of the grassland fungi to identify morphologically. Additionally, there has been no taxon-wide phylogenetic analysis so relatively few reference DNA barcodes exist. Here we have included for the first time an attempt to classify the *Entoloma* spp. present. *Entoloma conferendum* is the most frequently recorded of this genus in the British Isles (Appendix 1) and here was found to be the most widely distributed in the eDNA (detected in all quadrats). Two other distinctive species, *Entoloma prunuloides* (part of the *bloxamii*/*prunuloides* complex; see below) and *E. porphyrophaeum* were also identified from both fruitbody surveys and eDNA.

As can be seen from Table 3, Clavariaceae were abundant in all quadrats and dominant in some (e.g. CD2). As with some of the other grassland fungal taxa other than waxcaps, the taxonomy of some of the genera/species is in a fluid state but several recent taxonomic monographs are clarifying the situation and we are in the process of applying these more robust species concepts to UK Clavariaceae.

Clavariaceae are often inconspicuous and tend to be under-recorded in field surveys and here all the field records (highlighted in pink on Table 3) were confirmed by the presence of eDNA. In particular *C. zollingeri* a rare and distinctive coral fungus (section 7 species in Wales) was present within the eDNA in two quadrats. The presence of eDNA in the WID1 quadrat may useful guide 2018 field surveying.

As found in other eDNA surveys, the bright yellow coral fungi (*Clavulinopsis corniculatus*, *Cp. helvola*, *Cp. laeticolor* and *Cp. luteoalba*) were most widespread and abundant across all quadrats. Similarly the white coral fungi (*Clavaria acuta*, *Cl. fragilis*) were also widespread and present at high abundance. Several of the less common species were also detected (e.g. *C. guilleminii*) as well as some unexpected taxa whose distribution in the UK is at present poorly understood (*C. atroumbrina*, *C. australiana* AFF).

The agaricoid genus *Camarophyllopsis* was recently recognised as a member of Clavariacaeae (Birkebak, 2013) and some of these were reclassified into the new genus *Hodophilus*. Members of both genera were widespread at Hardcastle Crags. We have previously observed these fungi to be abundant in ‘waxcap grassland’ sites but frequently under-recorded.

Conclusions

The approach used here has only recently been developed and is thus undergoing continued improvement, most importantly in the accuracy with which species are identified. For the Hygrophoraceae, there are reference barcodes for nearly all the UK species (*H. turunda* is an exception). Thus we are now able to confidently identify DNA sequences to species level, and that the five waxcaps (excl. *Arrhenia* sp.) detected at the site only by their DNA are correctly identified. The fact that one of these appears to be a new species (“*H. conica* AFF”; commonly detected in other UK grasslands) illustrates a common problem in mycological surveying in that recent phylogenetic analyses are providing many examples of possible cryptic speciation within marcofungal taxa. A cautious approach is needed when considering the introduction of new names; unless the new taxa are easily

distinguished from each other morphologically, then there is scope for taxonomic confusion, with surveyors unable to attribute names reliably without recourse to genetic analyses. The recent revision of the *Entoloma bloxamii* species complex (Ainsworth et al., 2018) provides a good example of how the problem of cryptic speciation may be resolved. In the case of the *H. conica* species complex, we are undertaking an extensive investigation of samples from across Europe and combining genetic analyses, with investigation of $^{15}N/^{13}C$ isotopic profiles and basidium/spore morphology.

It is important to note the contribution of David Boertmann's 1995 book (and subsequent 2010 edition) 'The Genus *Hygrocybe'* which provided clear and pragmatic concepts for (nearly) all the species found in Europe, and more recently for phylogenetic analyses (Lodge et al, 2014; Ainsworth et al, 2013). Confirmation of the occurrence of these species may be obtained by targeted surveying, though the rarity of some fungal species is attributed to their infrequent fruiting which may be the case here.

Even with access to high quality field guides and expertise in microscopy, some species identifications are still difficult. The fact that there were discrepancies between eDNA data and field survey data for these species is consistent with this, as is the recent discovery that a moderate number of samples deposited at Royal Botanic Gardens Kew were originally misidentified. For sites of high value in terms of fungal biodiversity and which may be candidate for subsequent designation, we suggest that where appropriate dried fruiting body specimens of notable species are retained. Such collections are not bulky and if kept in a plastic box with desiccant (after freezing for 24 h to kill eggs of any insects present), it will be possible to extract DNA for future DNA barcoding (and potentially other scientific uses) for many years.

The recent field surveys in 2015 and 2016 (McLay, 2016) were in part triggered by the occurrence of Crimsworth Dene on a list of the top UK waxcap sites (Appendix 3) where it is reported to be home to 25 waxcap species. It seems that this site has been surveyed on various occasions in the past 50 years. Of note is a report by Bramley (1965) of a British Mycological Society foray at Hebden Bridge in September (25-29th) 1964 (Appendix 4), where eight waxcap species are reported. The report also states:

"*Sunday was mostly devoted to Crimsworth Dene. The area is mostly acid and it was interesting to find a number of Hygrophorus and allied genera in a newly re-seeded grass field and to learn that it had been limed the previous year, an indication of the effect of a small application of lime to the total volume of the topsoil. It also raises the question of how these species got there, especially as many of them had not been recorded previously. Was mycelium present and awaiting more alkaline conditions before it could fruit, or was it a new colonisation by wind-blown spores?*"

Discussion with Professor Roy Watling who attended this foray and has some recollections of where the re-seeded grass field was located suggests that it was upstream (ca. 800-1000 m; approx. 53.7710,-2.0180) of the Crimsworth Dene sites in our study. However, more recent records on FRDBI by MW Sykes (e.g. 16/10/1997- *H. calyptriformis* at SD989291 ["CD 1, Crimsworth Dean, Hebden Bridge"]) are more ambiguous, with the grid references provided referring to sites closer to the carparks at Midgehole. It would be useful to standardise the names of fields in this area, so avoid potential ambiguity, possibly using IACS field numbers.

With regard to the conservation value of the areas examined here, all are of high value in

different ways for their grassland fungal populations but each representing different types of undisturbed grassland. Most precious, since such habitats are rare, would be the two haymeadows (WID4/5). The Crimsworth Dene fields would benefit from higher levels of grazing in autumn - this would increase the visibility of grassland fungi when fruiting, though such management would not be expected in the short term to influence the fungal populations in the soil.

The CHEGD score across the whole site is summarised in Table 4. When evaluated against the current SSSI selection guidelines (Bosanquet et al. 2018), only species recorded by fruitbody identification can be included, resulting in a whole-site CHEGD score of 13:25:9:5:3. A site should be considered for notification if the total taxa count for each CHEGD group (as defined in the guidelines and tables therein) reaches or exceeds the following thresholds: 7:19:15:5:3. Therefore the site as a whole meets the SSSI selection requirements on four counts: clavarioid fungi, *Hygrocybe* s.l., geoglossoid fungi, and the *Dermoloma* etc. group. For the remaining group, *Entoloma* s.l., the site scores 9 against a selection threshold of 15. Note, however, that an additional 5 *Entoloma* species were detected through molecular survey, bringing the total to just below the threshold and suggests that fruitbody surveys in future years may be rewarding.

Although the SSSI guidelines (Bosanquet et al. 2018) explicitly exclude DNA-based data for site selection, the authors acknowledged that fruitbody recording only provides a partial picture of fungal distribution and that some fungi rarely if ever fruit. Nevertheless eDNA species records can be seen as supportive evidence and help to inform boundary setting (section 5.2 in the Guidelines). An important caveat is that only 8 of the 14 quadrats sampled for eDNA [4 at HH and 4 at WID] were within fields also surveyed for fruitbodies; and that an additional five fields at HH and seven at WID were surveyed for fruitbodies but not sampled for eDNA).

The eDNA data indicates where future field surveys should focus in order to discover the 19 species (CHEGD-3:5:5:1:1) not yet discovered by FB surveys. Additional soil sampling and eDNA analysis in other fields would very likely reveal the presence of additional species. As noted above it is important to preserve voucher specimens from FB surveys alongside detailed location information for potential confirmation of identification by DNA barcoding.

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Figures and Tables

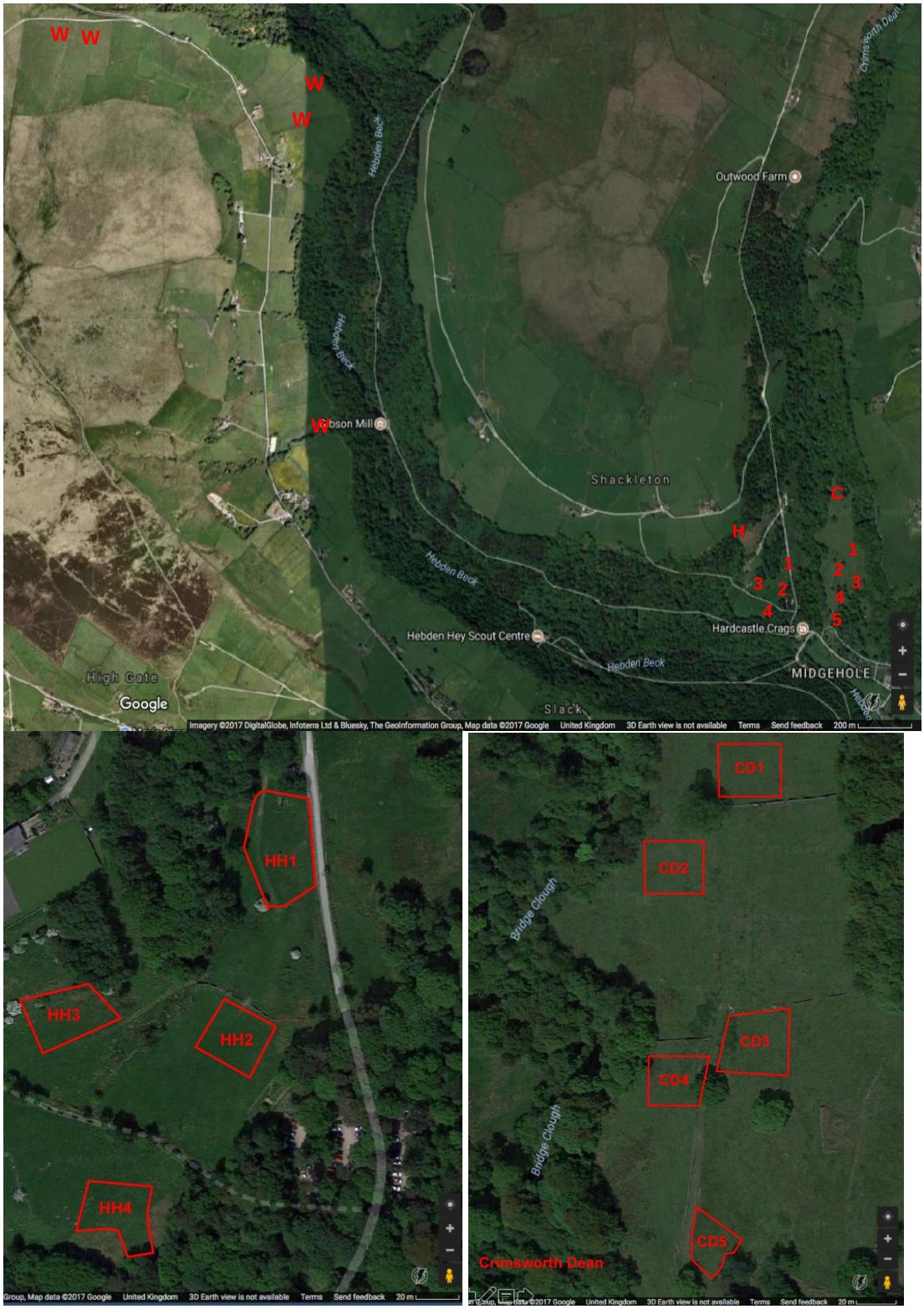


Figure 1 Aerial photographs showing locations of (A) all quadrats in the Hardcastle Crags area, (B) Hollin Hall quadrats and (C) Crimsworth Dene quadrats. Precise locations of Widdip quadrats and in Appendix 5.

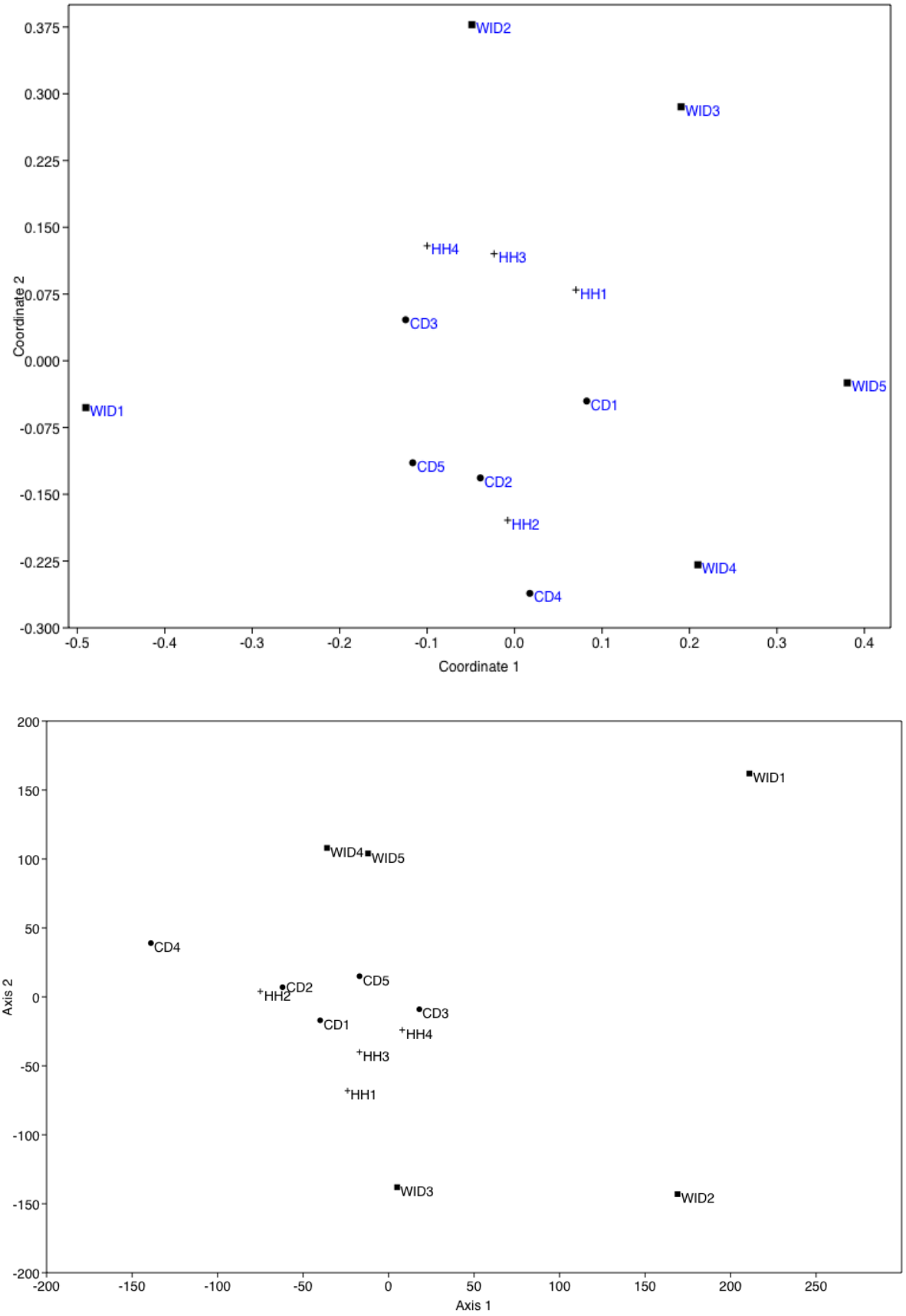


Figure 2 A) Non-metric multidimensional scaling (NMDS) and B) Detrended correspondence analysis (DCA) ordination plots of the fungal communities in the 14 quadrats in the Hardcastle Crags area.

Table 1 Quadrat locations in the Hardcastle Crags area

	Code	Informal name	Collected by	Gridref	Wet weight (g)	Dry weight (g)	Moisture (%)	No. Fungal Sequences	OTU count	% CLAV	% HYG	% ENT	% GEOG	% DERM	**% CHEGD**
1	CD1	Above path, North	GWG/ TW	53.7622, -2.0168	946.50	603.64	36%	16682	403	20.7%	26.1%	1.1%	3.8%	1.8%	**53.5%**
2	CD2	Below path, North	GWG/ TW	53.7619, -2.0173	902.70	540.46	40%	25299	366	45.2%	14.7%	0.5%	2.7%	6.8%	**69.8%**
3	CD3	Above path, Mid	GWG/ TW	53.7610, -2.0167	1068.50	677.08	37%	28874	365	30.8%	27.7%	3.1%	1.5%	1.8%	**64.8%**
4	CD4	Below path, Mid	GWG/ TW	53.7608, -2.0173	1021.10	651.46	36%	40475	461	26.7%	10.6%	0.5%	4.1%	0.0%	**41.9%**
5	CD5	Corner lower field	GWG/ TW	53.7601, -2.0171	854.80	534.37	38%	18742	343	22.3%	31.1%	2.5%	2.6%	11.9%	**70.3%**
6	HH1	Electric substation	GWG/ TW	53.7606, -2.0198	1191.40	702.30	41%	29469	457	17.2%	44.2%	2.6%	1.9%	0.3%	**66.1%**
7	HH2	Nettle field	GWG/ TW	53.7599, -2.0204	1002.10	659.89	35%	31148	421	42.7%	13.6%	0.7%	5.0%	0.0%	**62.0%**
8	HH3	Steep bank, rocky field	GWG/ TW	53.7600, -2.0214	928.60	571.15	38%	29270	384	25.6%	27.8%	2.8%	0.6%	16.9%	**73.7%**
9	HH4	Rocky, sloping field	GWG/ TW	53.7593, -2.0211	1291.30	812.13	37%	23270	270	19.4%	38.7%	0.4%	0.5%	21.2%	**80.1%**
10	WID1	Narrow sheep field	GWG	53.7650, -2.0456	857.90	584.07	32%	15465	207	11.9%	47.1%	1.8%	0.0%	0.0%	**60.8%**
11	WID2	Big field by gate	GWG	53.7768, -2.0589	875.20	514.26	41%	18688	220	10.8%	66.5%	1.6%	0.4%	0.0%	**79.2%**
12	WID3	Next field by Squam	AJ/AM	53.7769, -2.0606	803.90	438.26	45%	19759	237	16.4%	59.6%	3.4%	0.3%	0.0%	**79.7%**
13	WID4	Haymeadow good	AJ/AM	53.7742, -2.0470	972.00	587.88	40%	23439	353	19.5%	6.4%	0.4%	2.2%	0.0%	**28.4%**
14	WID5	Haymeadow poor	GWG	53.7753, -2.0462	982.30	552.69	44%	10887	161	16.2%	32.1%	0.1%	0.4%	1.7%	**50.5%**

Table 2A List of the top 50 most abundant fungal taxa across all the 14 quadrats. Key groups are highlighted (left side in yellow (fairy club), orange (waxcap), grey (earthtongue, pink (pink gill).

Phylum	Class	Order	Family	Genus/species	Ecology	Count	CumTotal	Mean	Median	Max	Min
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus1 pratensis		12	128.24%	10.69%	9.59%	31.52%	0.13%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis CPCO	CHEGD	11	64.32%	5.36%	3.43%	26.50%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	LepistaAFF	CHEGD	10	78.61%	6.55%	1.34%	38.69%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Dermoloma cuneifolium	CHEGD	7	53.78%	4.48%	0.13%	21.21%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgPS1 coccinea	CHEGD	7	44.57%	3.71%	0.29%	17.15%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis CPLA	CHEGD	12	35.98%	3.00%	2.74%	8.48%	0.52%
Fungi incertae sedis	Mortierellomycotina	Mortierellales	Mortierellaceae	Mortierella	SAP	12	35.28%	2.94%	2.59%	5.53%	0.93%
Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	Sorocybe	MR DSE	12	29.51%	2.46%	1.90%	5.79%	0.21%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria CVAR	CHEGD	12	25.96%	2.16%	1.76%	5.06%	0.51%
Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	Solicoccozyma		12	29.80%	2.48%	2.07%	8.12%	0.62%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgPS2 quieta	CHEGD	6	33.23%	2.77%	0.01%	20.80%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Camarophyllopsis atrovelutina	CHEGD	12	28.12%	2.34%	2.06%	5.50%	0.01%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgH3 noninqAffBrignant	CHEGD	9	19.34%	1.61%	0.73%	5.83%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Cuphophyllus4 virgineus	CHEGD	10	23.63%	1.97%	0.11%	17.15%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	Cortinarius	MR EM	4	21.62%	1.80%	0.00%	21.54%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgH1 chlorophana	CHEGD	4	21.09%	1.76%	0.00%	11.40%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgH5 glutinipes	CHEGD	5	15.97%	1.33%	0.00%	10.32%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus psittacinusA	CHEGD	9	11.04%	0.92%	0.48%	4.15%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Ramariopsis RMKU	CHEGD	12	12.98%	1.08%	1.06%	1.65%	0.32%

Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgPS2 reidii	CHEGD	5	14.90%	1.24%	0.00%	11.48%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus psittacinusB	CHEGD	3	14.86%	1.24%	0.00%	14.39%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria CVAC	CHEGD	12	11.38%	0.95%	0.73%	2.12%	0.12%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria CVZO	CHEGD	9	14.00%	1.17%	0.68%	2.80%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavulinopsis CPFU	CHEGD	12	11.70%	0.97%	0.78%	4.49%	0.03%
Ascomycota	Leotiomycetes	Helotiales	X	X		12	9.09%	0.76%	0.63%	2.13%	0.14%
Ascomycota	Geoglossomycetes	Geoglossales	Geoglossaceae	Trichoglossum hirsutum	MR CHEG	10	9.12%	0.76%	0.11%	4.19%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus irrigatus	CHEGD	7	12.10%	1.01%	0.30%	6.64%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgPS1 punicea	CHEGD	1	11.92%	0.99%	0.00%	11.92%	0.00%
Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Glomus 3	MR AM	12	9.19%	0.77%	0.75%	1.54%	0.24%
Ascomycota	X	X	X	X		8	8.92%	0.74%	0.16%	3.87%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Camarophyllopsis schulzeri	CHEGD	7	9.57%	0.80%	0.01%	4.68%	0.00%
Basidiomycota	Tremellomycetes	Tremellales	Trimorphomycetaceae	Saitozyma	PARA FUNGI	12	8.69%	0.72%	0.42%	3.97%	0.13%
Basidiomycota	Agaricomycetes	Trechisporales	Trechisporaceae	Trechispora	SAP SOIL	12	7.44%	0.62%	0.50%	1.47%	0.05%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavariaceae sp. 2 CRJH-20	CHEGD	7	4.92%	0.41%	0.02%	2.47%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Neohygrocybe ingrata	CHEGD	1	8.56%	0.71%	0.00%	8.56%	0.00%
Basidiomycota	Agaricomycetes	Boletales	Stephanosporaceae	Stephanospora	MR EM	9	5.93%	0.49%	0.09%	2.98%	0.00%
Basidiomycota	Agaricomycetes	X	X	X		3	8.09%	0.67%	0.00%	6.01%	0.00%
X	X	X	X	X		12	6.85%	0.57%	0.42%	1.61%	0.02%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria CVFL	CHEGD	11	4.84%	0.40%	0.30%	1.65%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	Mycena	SAP SOIL	12	5.74%	0.48%	0.36%	1.37%	0.03%
Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	Entoloma bloxamii	CHEGD	4	7.04%	0.59%	0.00%	2.21%	0.00%
Ascomycota	Leotiomycetes	Thelebolales	Thelebolaceae	Thelebolus	SAP DUNG	12	6.01%	0.50%	0.24%	2.83%	0.13%
Ascomycota	Geoglossomycetes	Geoglossales	Geoglossaceae	Glutinoglossum sp.	MR CHEG	10	5.69%	0.47%	0.17%	2.35%	0.00%

Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgH2 conicaAFFX	CHEGD	10	2.45%	0.20%	0.08%	1.55%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Gliophorus laetus	CHEGD	3	6.26%	0.52%	0.00%	2.86%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Hygrophoraceae	Hygrocybe sgPS1 coccineaX	CHEGD	4	4.21%	0.35%	0.00%	2.33%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Clavaria CVX2	CHEGD	12	3.20%	0.27%	0.17%	0.73%	0.02%
Basidiomycota	Agaricomycetes	Agaricales	X	X		6	4.53%	0.38%	0.01%	1.79%	0.00%
Basidiomycota	Agaricomycetes	X	X	X		4	5.08%	0.42%	0.00%	3.87%	0.00%
Basidiomycota	Agaricomycetes	Agaricales	Clavariaceae	Ramariopsis RMPU	CHEGD	12	4.45%	0.37%	0.28%	1.26%	0.02%

Table 2B List of the top 50 most abundant fungal taxa across all the 14 quadrats. Key groups are highlighted (left side in yellow (fairy club), orange (waxcap), grey (earthtongue, pink (pink gill). Taxa with abundance of >5% or <0.001% in a given quadrat are highlighted in green/red respectively (on right side of table).

Family	Genus/species	CD1	CD2	CD3	CD4	CD5	HH1	HH2	HH3	HH4	WID1	WID2	WID3	WID4	WID5
Hygrophoraceae	Cuphophyllus1 pratensis	9.07%	1.27%	9.43%	2.78%	0.13%	31.51%	3.49%	13.80%	9.75%	1.11%	12.13%	31.52%	0.76%	11.83%
Clavariaceae	Clavulinopsis CPCO	0.89%	23.74%	12.26%	3.50%	3.36%	5.30%	26.50%	4.50%	5.15%	1.32%	0.02%	0.86%	1.54%	0.00%
Tricholomataceae	LepistaAFF	0.00%	0.00%	1.52%	0.03%	2.14%	1.50%	0.00%	0.04%	2.66%	1.18%	0.03%	0.00%	30.84%	38.69%
Tricholomataceae	Dermoloma cuneifolium	1.80%	6.77%	1.81%	0.01%	11.86%	0.25%	0.00%	16.92%	21.21%	0.00%	0.00%	0.00%	0.00%	1.73%
Hygrophoraceae	Hygrocybe sgPS1 coccinea	0.00%	0.00%	11.35%	0.21%	6.76%	0.38%	0.00%	0.00%	0.37%	8.34%	17.15%	0.00%	0.00%	0.00%
Clavariaceae	Clavulinopsis CPLA	1.66%	5.61%	4.35%	3.14%	1.66%	4.53%	1.13%	8.48%	3.72%	1.90%	1.06%	0.52%	2.70%	2.78%
Mortierellaceae	Mortierella	3.95%	3.08%	1.10%	4.07%	3.58%	3.42%	5.23%	1.28%	1.43%	5.39%	1.76%	1.56%	5.53%	0.93%
Herpotrichiellaceae	Sorocybe	4.84%	3.73%	2.21%	5.79%	2.04%	1.76%	3.87%	2.80%	1.62%	0.21%	1.36%	1.03%	5.15%	1.67%
Clavariaceae	Clavaria CVAR	4.38%	5.45%	1.75%	4.56%	3.36%	1.78%	5.06%	0.77%	1.88%	0.62%	0.51%	0.89%	3.43%	1.34%
Piskurozymaceae	Solicoccozyma	1.68%	2.51%	0.86%	0.81%	3.36%	1.46%	2.34%	2.55%	1.31%	8.12%	2.53%	1.79%	4.04%	0.62%
Hygrophoraceae	Hygrocybe sgPS2 quieta	0.00%	0.00%	0.00%	0.00%	0.00%	4.97%	0.02%	3.29%	3.11%	0.00%	1.05%	20.80%	0.00%	0.00%
Clavariaceae	Camarophyllopsis atrovelutina	0.31%	3.75%	1.55%	5.50%	5.41%	0.02%	4.07%	0.01%	0.02%	1.49%	0.05%	4.47%	2.97%	2.56%
Hygrophoraceae	Hygrocybe sgH3 noninqAffBrignant	2.66%	5.23%	0.05%	2.01%	1.54%	4.06%	5.83%	1.28%	4.38%	0.03%	0.00%	0.00%	0.17%	0.00%
Hygrophoraceae	Cuphophyllus4 virgineus	0.00%	0.00%	0.00%	0.47%	17.15%	0.01%	0.13%	0.40%	1.20%	0.10%	0.06%	0.00%	0.06%	4.04%
Cortinariaceae	Cortinarius	0.00%	0.03%	0.00%	21.54%	0.03%	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.03%	0.00%
Hygrophoraceae	Hygrocybe sgH1 chlorophana	0.00%	0.00%	3.00%	0.00%	0.00%	0.00%	0.00%	0.00%	11.40%	6.58%	0.00%	0.00%	0.00%	0.10%
Hygrophoraceae	Hygrocybe sgH5 glutinipes	4.49%	0.02%	0.02%	0.00%	0.00%	0.18%	0.00%	0.00%	0.00%	0.00%	3.82%	0.00%	1.63%	10.32%
Hygrophoraceae	Gliophorus	2.76%	5.58%	0.68%	4.15%	0.44%	0.07%	3.55%	0.62%	0.27%	0.00%	0.00%	0.00%	0.74%	0.53%

	psittacinusA														
Clavariaceae	Ramariopsis RMKU	1.25%	1.37%	0.98%	1.09%	1.33%	1.60%	1.37%	0.80%	1.02%	0.58%	0.59%	1.65%	1.64%	0.32%
Hygrophoraceae	Hygrocybe sgPS2 reidii	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.08%	1.61%	0.55%	11.48%	0.19%	0.00%	0.00%
Hygrophoraceae	Gliophorus psittacinusB	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.05%	0.41%	14.39%	0.00%	0.00%	0.00%
Clavariaceae	Clavaria CVAC	2.46%	0.72%	0.59%	1.64%	1.50%	1.29%	1.81%	0.23%	0.34%	0.12%	0.80%	2.12%	0.67%	0.26%
Clavariaceae	Clavaria CVZO	0.02%	0.00%	0.46%	0.00%	0.66%	0.00%	0.70%	2.12%	2.60%	1.72%	2.53%	0.41%	0.00%	2.80%
Clavariaceae	Clavulinopsis CPFU	1.22%	0.73%	1.53%	0.57%	0.80%	0.35%	1.00%	4.49%	0.91%	0.05%	0.76%	0.87%	0.34%	0.03%
X	X	3.40%	1.13%	2.13%	0.52%	0.74%	1.16%	0.80%	0.79%	0.49%	0.27%	0.49%	1.27%	0.30%	0.14%
Geoglossaceae	Trichoglossum hirsutum	2.42%	1.84%	0.33%	1.01%	1.89%	1.36%	4.19%	0.13%	0.08%	0.00%	0.06%	0.00%	0.01%	0.05%
Hygrophoraceae	Gliophorus irrigatus	0.00%	1.10%	0.58%	0.67%	0.00%	0.00%	0.00%	1.07%	0.02%	6.64%	0.00%	0.00%	2.16%	0.96%
Hygrophoraceae	Hygrocybe sgPS1 punicea	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	11.92%	0.00%	0.00%	0.00%	0.00%
Glomeraceae	Glomus 3	1.08%	0.94%	0.49%	0.60%	0.75%	0.46%	1.10%	0.76%	0.42%	0.93%	0.95%	1.54%	0.96%	0.24%
X	X	0.92%	0.52%	0.23%	2.35%	0.43%	1.07%	3.87%	0.00%	0.02%	0.00%	0.00%	0.00%	0.86%	0.09%
Clavariaceae	Camarophyllopsis schulzeri	0.00%	0.00%	4.68%	0.01%	0.00%	0.00%	0.01%	0.00%	0.21%	2.64%	0.00%	0.00%	0.76%	1.26%
Trimorphomycetaceae	Saitozyma	0.13%	0.59%	0.36%	0.48%	0.72%	0.20%	0.68%	0.83%	0.27%	3.97%	0.13%	0.15%	0.74%	0.16%
Trechisporaceae	Trechispora	0.95%	0.72%	0.23%	0.32%	0.10%	0.88%	0.99%	0.97%	1.41%	0.05%	0.69%	1.47%	0.22%	0.11%
Clavariaceae	Clavariaceae sp. 2 CRJH-20	1.98%	1.73%	0.03%	0.20%	0.76%	0.47%	0.00%	0.00%	0.02%	0.97%	0.00%	0.00%	0.00%	2.47%
Hygrophoraceae	Neohygrocybe ingrata	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	8.56%	0.00%	0.00%	0.00%	0.00%
Stephanosporaceae	Stephanospora	2.15%	0.06%	0.19%	0.00%	0.22%	0.10%	0.00%	0.03%	0.31%	0.09%	2.98%	1.97%	0.04%	0.00%
X	X	0.02%	0.00%	6.01%	0.00%	0.00%	0.00%	0.00%	0.75%	1.33%	0.00%	0.00%	0.00%	0.00%	0.00%
X	X	0.21%	0.67%	0.02%	0.26%	0.62%	0.14%	0.38%	1.61%	0.63%	0.20%	0.46%	0.14%	1.57%	0.81%
Clavariaceae	Clavaria CVFL	1.64%	0.95%	0.45%	1.65%	0.59%	0.04%	0.41%	0.18%	0.16%	0.08%	0.08%	0.00%	0.77%	0.43%
Tricholomataceae	Mycena	0.79%	0.69%	0.64%	0.10%	0.03%	1.16%	0.33%	1.37%	0.40%	0.54%	0.59%	0.13%	0.14%	0.32%
Entolomataceae	Entoloma bloxamii	0.02%	0.00%	2.21%	0.00%	1.38%	0.00%	0.00%	2.20%	0.00%	0.00%	0.00%	1.25%	0.00%	0.00%

Thelebolaceae	Thelebolus	0.28%	0.39%	0.47%	0.22%	0.77%	0.24%	0.23%	0.32%	0.21%	2.83%	0.13%	0.17%	0.29%	0.13%
Geoglossaceae	Glutinoglossum sp.	0.53%	0.21%	0.62%	2.35%	0.35%	0.23%	0.47%	0.11%	0.08%	0.00%	0.08%	0.00%	1.37%	0.04%
Hygrophoraceae	Hygrocybe sgH2 conicaAFFX	3.89%	0.02%	0.02%	0.05%	1.55%	0.11%	0.18%	0.03%	0.20%	0.00%	0.10%	0.20%	0.02%	0.00%
Hygrophoraceae	Gliophorus laetus	0.00%	0.00%	0.00%	0.00%	1.96%	0.00%	0.00%	2.86%	0.00%	0.00%	0.00%	1.44%	0.00%	0.00%
Hygrophoraceae	Hygrocybe sgPS1 coccineaX	1.44%	0.00%	0.00%	0.00%	0.00%	1.74%	0.04%	0.00%	0.00%	0.00%	0.00%	2.33%	0.00%	0.11%
Clavariaceae	Clavaria CVX2	2.10%	0.20%	0.27%	0.09%	0.31%	0.49%	0.07%	0.02%	0.05%	0.16%	0.73%	0.72%	0.19%	0.09%
X	X	0.20%	0.43%	0.00%	0.00%	0.35%	1.11%	0.03%	0.00%	0.00%	0.00%	1.79%	1.23%	0.00%	0.03%
X	X	0.00%	0.06%	3.87%	0.06%	0.00%	0.10%	0.00%	0.00%	1.04%	0.00%	0.00%	0.00%	0.00%	0.00%
Clavariaceae	Ramariopsis RMPU	0.49%	0.18%	0.35%	0.31%	0.23%	0.34%	0.26%	0.20%	0.19%	0.02%	0.60%	0.09%	0.61%	1.26%

Table 3 List of CHEGD species detected in soil eDNA from the 14 Hardcastle Crags quadrats. Numbers indicate the number of sequences of each species detected (normalised to 20000 per sample. For the totals of CHEGD species only instances of >50 sequences per sample are counted. Species indicated by **** were discovered in fruiting body surveys only (not found in eDNA) whereas species highlighted in light green were detected on the eDNA. Cells highlighted in pink indicate where fungi have been found during fruiting body surveys.

	Species	CD1	CD2	CD3	CD4	CD5	HH1	HH2	HH3	HH4	WID1	WID2	WID3	WID4	WID5	COUNT	SUM
C1	Camarophyllopsis atrovelutina		684	3	666	407		732		38	32		80	398	253	10	3293
C2	Clavaria acuta	696	141	254	295	273	243	325	76	62	28	156	416	576	49	14	3592
C3	Clavaria argillacea	37	38	16				34	28	23		7		61		8	245
C4	Clavaria atroumbrina											36				1	36
C5	Clavaria australianaAFF	295	173	84	297	107	7	74	33	29	14	15		140	82	13	1350
C6	Clavaria fragilis	321	178	165	313	167	27	74	203	487	14	20	3	143	108	14	2223
C7	Clavaria fumosa	3		86		119		125	381	467	263	474	77		422	10	2418
C8	Clavaria guilleminii	6	4		3	4	13	6							14	7	51
C9	Clavaria incarnata	19			4	210	55	3	7	24	4	7	161		56	11	550
C10	Clavaria straminea	790	998	328	818	612	318	907	138	338	113	96	165	622	255	14	6498
C11	Clavaria zollingeri										51				110	2	161
C12	Clavulinopsis corniculata	161	4347	2299	628	611	955	4751	813	924	241	4	160	279		13	16173
C13	Clavulinopsis helvola	463	888	481	1053	410	822	310	1651	709	333	299	169	311	80	14	7978
C14	Clavulinopsis laeticolor	79	192	198		52	47	50	231	129	40	86	35	326	157	13	1623
C15	Clavulinopsis luteoalba	19	65	449	63	7	50	10	798	29	17	16	118	26	297	14	1963
C16	Hodophilus foetens		6		23	5		7	40	7		450	446	29		9	1012
C17	Ramariopsis biformis	89	30	65	56	43	60	46	58	34	4	112	17	110	239	14	962
C18	Ramariopsis kunzei	226	294	190	196	269	302	252	157	187	112	110	305	298	61	14	2958
D1	Dermoloma cuneifolium	325	1240	340	1	2155	46		3037	3801					328	9	11273
E1	Entoloma bloxamii/prunuloides	3		414		251			395				231			5	1294

E2	Entoloma chalybaeum						2		4	5						3	10
E3	Entoloma clypeatum						333									1	333
E4	Entoloma conferendum	66	53	34	46	49	38	43	60	25	177	40	36	60	19	14	747
E5	Entoloma infula						2		4							2	6
E6	Entoloma porphyrophaeum		2	8					3							3	14
E7	Entoloma subserrulatum					3	3									2	6
E8	Entoloma turci						2			9	4	36	154			5	205
E9	Unclassified: Entolomataceae	121	31	116	39	145	88	89	37	30	144	221	207	6		13	1274
G1	Geoglossum cookeanum****															0	0
G2	Geoglossum dunense	4	9	5	27		4	5						12		7	65
G3	Geoglossum fallax	23		4	32			10	4			8	19	22		8	120
G4	Geoglossum umbratile	119	109	92	68	55	41	32	7			4	43	22		11	592
G5	Glutinoglossum glutinosum	95	38	116	422	63	41	85	20	15		15		248	7	12	1164
G6	Trichoglossum hirsutum	437	336	62	181	343	244	752	23	15		12		2	9	12	2416
G7	Trichoglossum walteri					11	3		63	58		34		94	61	7	324
"G"8	Microglossum olivaceum					14										1	14
"G"9	Microglossum rufum											19				1	19
"G"10	Microglossum truncatum			99												1	99
H1	Arrhenia sp.			5	8		2	57		2			4	2		7	81
H2	Cuphophyllus russocoriaceus****															0	0
H3	Cuphophyllus virgineus				84	3118	2	23	72	216	18	11		12	768	10	4323
H4	Cuphophyllus flavipes									361						1	361
H5	Cuphophyllus fornicatus					8		11			139					3	158
H6	Cuphophyllus pratensis	1655	51	1742	466	31	5361	178	2348	1743	203	2265	5815	137	2253	14	24247
H7	Gliophorus irrigatus		202	109	120				192	3	1213			392	183	8	2414
H8	Gliophorus laetus					356			513				266			3	1135
H9	Gliophorus psittacinus	494	1021	127	742	79	12	634	111	57	76	2698		134	101	13	6287
H10	Glioxanthomyces vitellina****															0	0

H11	Hygrocybe aurantiosplendens****															0	0
H12	Hygrocybe cantharellus			15		13	101		5	71		288	90		115	8	697
H13	Hygrocybe ceracea											7				1	7
H14	Hygrocybe chlorophana			563						2046	1202				19	4	3831
H15	Hygrocybe citrinovirens	236									9					2	245
H16	Hygrocybe coccinea	259		2121	39	1224	382	7		66	1524	3200	430		21	11	9273
H17	Hygrocybe conica	700	3	3	52	298	30	32	347	392		61	200	75		12	2193
H18	Hygrocybe conicaAFF	479	951	8	361	277	727	1046	228	782	5			31		11	4895
H19	Hygrocybe contrictospora								230	24						2	254
H20	Hygrocybe glutinipes	810	4	5			32					716		295	1961	7	3822
H21	Hygrocybe insipida		118	363		96			4		5			15		6	600
H22	Hygrocybe mucronella	15	123	10	1	51	36		20	317	12	836	317	66		12	2027
H23	Hygrocybe phaeococcinea		23	38												2	61
H24	Hygrocybe punicea										2178					1	2178
H25	Hygrocybe quieta						890	3	591	557		197	3844			6	6081
H26	Hygrocybe reidii								193	289	100	2152	35			5	2769
H27	Neohygrocybe ingrata										1565					1	1565
H28	Porpolomopsis calyptriformis		6				9				360				443	4	818
		Number of species															
		CD1	CD2	CD3	CD4	CD5	HH1	HH2	HH3	HH4	WID1	WID2	WID3	WID4	WID5		
	No. Clavariaceae spp.	9	10	11	10	11	8	10	10	8	6	8	10	11	12		
	No. Hygrophoraceae spp.	7	6	6	6	8	5	4	10	12	10	9	7	6	7		
	No. Entolomataceae spp.	2	1	2	0	2	2	1	2	0	2	1	3	1	0		
	No. Geoglossaceae spp.	3	2	4	3	3	1	2	1	1	0	0	0	2	1		
	No. Dermoloma/Porpoloma spp.	1	1	1	0	1	0	0	1	1	0	0	0	0	1		
	Total number of CHEGD spp.	**22**	**20**	**24**	**19**	**25**	**16**	**17**	**24**	**22**	**18**	**18**	**20**	**20**	**21**		

Table 4 Summary of the CHEGD species found at the Hardcastle Crags grassland sites during the eDNA survey and the fruiting body survey of McLay (2016). Note there was incomplete coverage of the areas surveyed by both methods.

	Species	Present in eDNA	Missed by eDNA	FB	Missed by FB survey	FB and eDNA	FB OR eDNA	FB at HH	FB at NHLF	Common Name
C1	*Clavaria acuta*	1		1		1	1	P		Pointed Club
C2	*Clavaria argillacea*	1			1		1			
C3	*Clavaria atroumbrina*	1			1		1			
C4	*Clavaria australianaAFF*	1			1		1			
C5	*Clavaria fragilis*	1		1		1	1	P	P	White Spindles
C6	*Clavaria fumosa*	1		1		1	1	P	P	Smoky Spindles
C7	*Clavaria guilleminii*	1			1		1			
C8	*Clavaria incarnata*	1		1		1	1		P	Skinny Club
C9	*Clavaria straminea*	1		1		1	1		P	Straw Club
C10	*Clavaria zollingeri*	1		1		1	1		P	Violet Coral
C11	*Clavulinopsis corniculata*	1		1		1	1	P	P	Meadow Coral
C12	*Clavulinopsis fusiformi*		1	1			1	P	P	Golden Spindles
C13	*Clavulinopsis helvola*	1		1		1	1	P	P	Yellow Club
C14	*Clavulinopsis laeticolor*	1		1		1	1			
C15	*Clavulinopsis luteoalba*	1		1		1	1	P	P	Apricot Club
C16	*Clavulinopsis umbrinella*		1	1			1		P	Beige Coral
C17	*Ramariopsis biformis*	1			1		1			
C18	*Ramariopsis kunzei*	1		1		1	1	P		Ivory Coral
D1	*Camarophyllopsis atrovelutina*	1			1		1			
D2	*Camarophyllopsis foetens*		1	1			1	P		Stinking Fanvault

	Species	Present in eDNA	Missed by eDNA	FB	Missed by FB survey	FB and eDNA	FB OR eDNA	FB at HH	FB at NHLF	Common Name
D3	*Dermoloma cuneifolium*	1		1		1	1	P	P	Crazed Cap
D4	*Hodophilus foetens*	1		1		1	1			
E1	*Entoloma ameides*		1	1			1	P		No common name
E2	*Entoloma bloxamii/prunuloides*	1		1		1	1			
E3	*Entoloma chalybaeum*	1			1		1			
E4	*Entoloma clypeatum*	1			1		1			
E5	*Entoloma conferendum*	1		1		1	1	P	P	Star Pinkgill
E6	*Entoloma incanum*		1	1			1	P		Mousepee Pinkgill
E7	*Entoloma infula*	1			1		1			
E8	*Entoloma porphyrophaeum*	1		1		1	1		P	Lilac Pinkgill
E9	*Entoloma prunuloides*		1	1			1	P	P	Mealy Pinkgill
E10	*Entoloma sericellum*		1	1			1	P		Cream Pinkgill
E11	*Entoloma sericeum*		1	1			1	P	P	Silky Pinkgill
E12	*Entoloma serrulatum*		1	1			1	P	P	Blue-edge Pinkgill
E13	*Entoloma subserrulatum*	1			1		1			
E14	*Entoloma turci*	1			1		1			
G1	*Geoglossum cookeanum*		1	1			1			
G2	*Geoglossum dunense*	1			1		1			
G3	*Geoglossum fallax*	1		1		1	1		P	An earthtongue
G4	*Geoglossum umbratile*	1		1		1	1			
G5	*Glutinoglossum glutinosum*	1		1		1	1			
G6	*Trichoglossum hirsutum*	1			1		1			
G7	*Trichoglossum walteri*	1		1		1	1			

	Species	Present in eDNA	Missed by eDNA	FB	Missed by FB survey	FB and eDNA	FB OR eDNA	FB at HH	FB at NHLF	Common Name
"G"8	*Microglossum olivaceum*	1			1		1			
"G"9	*Microglossum rufum*	1			1		1			
"G"10	*Microglossum truncatum*	1			1		1			

	Species	Present in eDNA	Missed by eDNA	FB	Missed by FB survey	FB AND eDNA	FB OR eDNA	FB at HH	FB at NHLF	
H1	*Cuphophyllus russocoriaceus*		1	1			1		P	Cedarwood Waxcap
H2	*Cuphophyllus virgineus*	1		1		1	1	P	P	Snowy Waxcap
H3	*Cuphophyllus flavipes*	1		1		1	1	P		Yellow Foot Waxcap
H4	*Cuphophyllus fornicatus*	1		1		1	1		P	Arched Waxcap
H5	*Cuphophyllus pratensis*	1		1		1	1	P	P	Meadow Waxcap
H6	*Gliophorus irrigatus*	1		1		1	1	P	P	Slimy Waxcap
H7	*Gliophorus laetus*	1		1		1	1	P	P	Heath Waxcap
H8	*Gliophorus psittacinus*	1		1		1	1	P	P	Parrot Waxcap
H9	*Glioxanthomyces vitellina*		1	1			1		P	No common name
H10	*Hygrocybe aurantiosplendens*		1	1			1	P		Orange Waxcap
H11	*Hygrocybe cantharellus*	1		1		1	1	P	P	Goblet Waxcap
H12	*Hygrocybe ceracea*	1		1		1	1	P	P	Butter Waxcap
H13	*Hygrocybe chlorophana*	1		1		1	1	P	P	Golden Waxcap
H14	*Hygrocybe citrinovirens*	1		1		1	1	P		Citrine Waxcap
H15	*Hygrocybe coccinea*	1		1		1	1	P	P	Scarlet Waxcap

	Species	Present in eDNA	Missed by eDNA	FB	Missed by FB survey	FB AND eDNA	FB OR eDNA	FB at HH	FB at NHLF	
H16	*Hygrocybe conica*	1		1		1	1	P	P	Blackening Waxcap
H17	*Hygrocybe contrictospora*	1			1		1			
H18	*Hygrocybe glutinipes*	1		1		1	1	P	P	Glutinous Waxcap
H19	*Hygrocybe insipida*	1		1		1	1	P	P	Spangle Waxcap
H20	*Hygrocybe intermedia*		1	1			1	P	P	Fibrous Waxcap
H21	*Hygrocybe mucronella*	1		1		1	1	P		Bitter Waxcap
H22	*Hygrocybe phaeococcinea*	1			1		1			
H23	*Hygrocybe punicea*	1		1		1	1	P	P	Crimson Waxcap
H24	*Hygrocybe quieta*	1		1		1	1	P	P	Oily Waxcap
H25	*Hygrocybe reidii*	1		1		1	1	P	P	Honey Waxcap
H26	*Hygrocybe splendidissima*		1	1			1	P	P	Splendid Waxcap
H27	*Neohygrocybe ingrata*	1			1		1	P		Nitrous Waxcap
H28	*Porpolomopsis calyptriformis*	1		1		1	1	P	P	Pink Waxcap
	No. Clavariaceae spp.	16	2	13	5	11	18			
	No. Hygrophoraceae spp.	23	5	25	3	20	28			
	No. Entolomataceae spp.	8	5	8	5	3	13			
	No. Geoglossaceae spp.	9	1	5	5	4	10			
	No. Dermoloma/Porpoloma spp.	3	1	3	1	2	4			
	Total number of CHEGD spp.	**59**	**14**	**54**	**19**	**40**	**73**			

Appendix 1

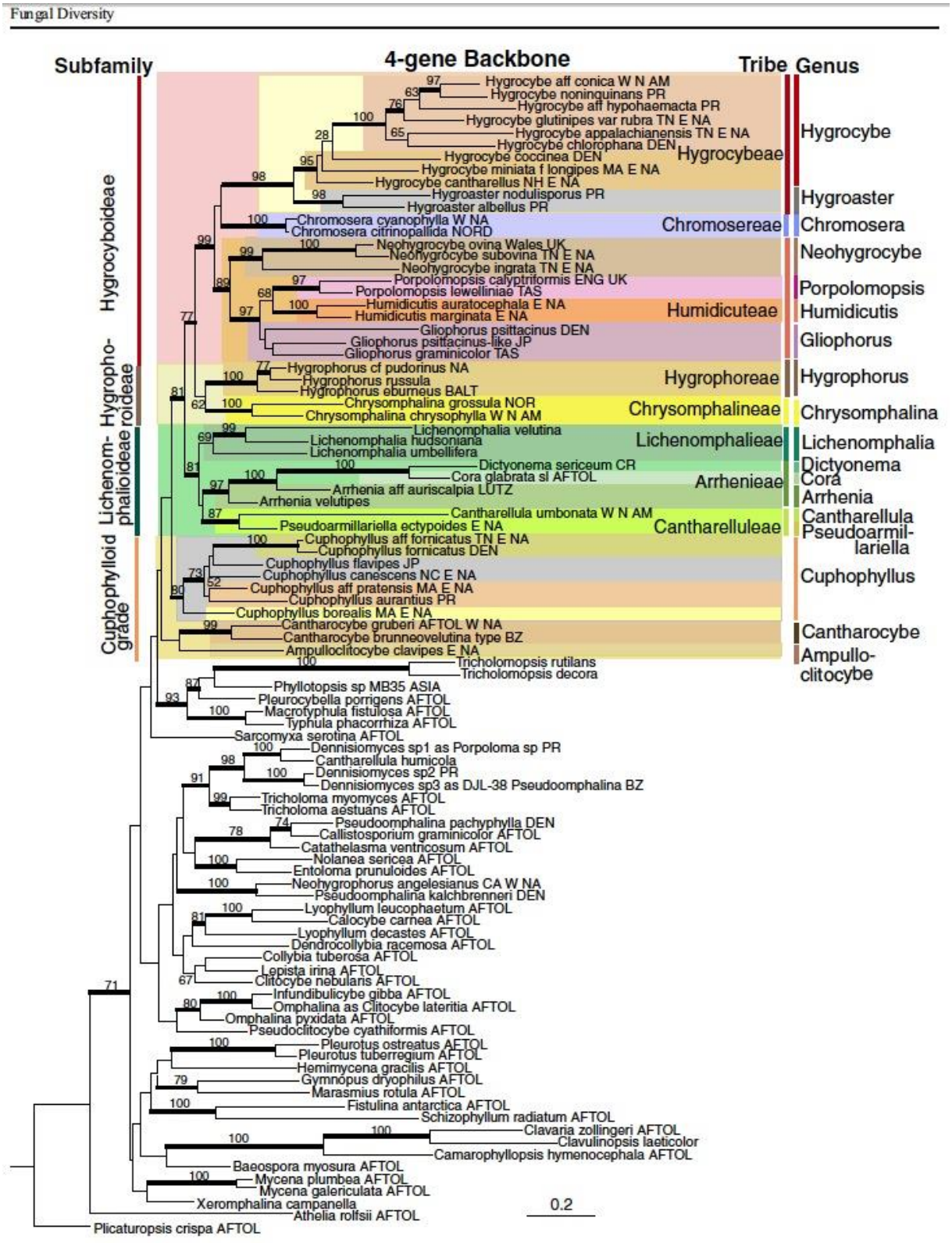
From Griffith et al (2013), listing the range of CHEGD species found in Wales at the 48 sites studied. Also shown are numbers of BMSFRD records for each species. Since publication of these data, there have been some taxonomic changes (See Appendix 2).

Table 2 Frequency of occurrence of CHEGD species at sites and in quadrats, compared to number of UK records held in the FRDBI. **indicates new UK record; # indicates new Welsh record; + indicates confirmed Welsh record

Species	No. sites (/48)	No. UK records
***HYGROCYBE* (35+4)**		
H. aurantiosplendens	9	298
H. calyptriformis	22	1645
H. cantharellus	11	849
H. ceracea	33	2190
H. chlorophana	34	4001
H. citrinovirens	10	338
H. coccinea	42	3486
H. colemannia	9	413
H. conica	38	5555
H. flavipes	20	518
H. fornicata	29	690
H. glutinipes	19	732
H. helobia	8	240
H. ingrata	3	81
H. insipida	41	2088
H. intermedia	13	546
H. irrigata	29	1709
H. lacmus	2	243
H. laeta	24	1813
H. marchii	2	410
H. miniata	9	1240
H. mucronella	14	474
H. nitrata	8	401
H. ovina	5	255
H. persistens	3	1215
H. pratensis	46	4754
H. psittacina	45	4726
H. punicea	28	1965
H. quieta	44	2045
H. reidii	34	1808
H. russocoriacea	22	1343
H. spadicea	1	73
H. splendissima	14	549
H. virginea	46	5929
H. vitellina	1	269
H. pratensis v. pallida	6	518
H. psittacina v. perplexa	6	124
H. virginea v. fuscescens	8	220
H. virginea v.	15	362
***CAMAROPHYLLOPSIS* (3)**		
#Cm. atropuncta	2	87
Cm. foetens	1	94
Cm. schulzeri	3	60
***DERMOLOMA* (2)**		
D. cuneifolium	35	1065
#D. magicum	2	5
***PORPOLOMA* (1)**		
P. metapodium	1	151
***ENTOLOMA* (46+1)**		
E. ameides	4	148
+E. anatinum	9	139
E. asprellum	1	115
E. atroceruleum	5	120
E. bloxamii	7	225
E. caesiocinctum	1	183
#E. catalaunicum	2	48
E. chalybaeum	18	309
#E. clandestinum	4	70
E. conferendum	37	2817
***E. conocybecystis*	1	0
E. corvinum	8	284
***E. eryriensis sp. nov.*	1	0
E. exile	10	226
E. formosum	5	267
***E. glaucobasis*	1	0
E. griseocyaneum	6	266
E. hebes	4	343
+E. hirtipes	2	226
E. incanum	3	300
E. infula	9	205
E. iubatum	1	320
E. lamprosus	3	350
#E. langei	1	18
+E. lividocyanulum	3	110
#E. longistriatum	8	69
E. minutum	1	57
#E. nigroviolaceum	2	93
#E. ochromicaceum	1	12
E. olorinum	1	9
E. papillatum	16	598
E. poliopus	8	97
E. politum	1	134
E. porphyrophaeum	16	877
#E. pratulense	1	4
E. prunuloides	15	482
#E. pseudoturci	1	27
E. roseum	1	72
E. sericellum	21	1419
E. sericeum	16	1627
E. serrulatum	14	948
E. sodale	1	72
E. solstitiale	1	36
E. turci	3	102
E. undatum	2	156
#E. xanthochroum	4	94
E. longistriatum v. sarcitulum	1	171
***CLAVARIA* (7)**		
C. acuta	11	564
C. argillacea	1	364
C. fragilis	25	1129
C. fumosa	20	641
C. incarnata	2	102
C. straminea	11	145
C. zollingeri	5	229
***CLAVULINOPSIS* (7)**		
Cp. corniculata	34	2002
Cp. fusiformis	14	992
Cp. helvola	38	2794
Cp. laeticolor	21	427
Cp. luteoalba	27	914
#Cp. subtilis	1	111
Cp. umbrinella	6	244
RAMARIOPSIS (2)		
R. biformis	1	37
R. kunzei	4	300
***GEOGLOSSUM* (5)**		
G. atropurpureum	3	191
G. cookeanum	6	590
G. fallax	25	891
G. glutinosum	15	426
G. umbratile	7	347
***MICROGLOSSUM* (1)**		
M. olivaceum	12	342
***TRICHOGLOSSUM* (2)**		
T. hirsutum	17	645
T. walteri	3	76

Appendix 2

From Lodge et al (2014), showing the taxonomic revision of the family Hygrophoraceae. The result of this is that several new generic names are now used for species previously in the genus *Hygrocybe* (which still exists and contains the yellow/red/orange species. However, new genera now exist to contain the other species (eg *Gliophorus* for the slimy-capped spp. such as *G. psittacina* and *Cuphophyllus* for several of the plain-coloured species such as *C. pratensis*). The specific names remain unchanged.



Appendix 3

Table 5 from Griffith et al (2013)

Table 5 Top 20 Sites for *Hygrocybe* spp. and other CHEGD taxa in the British Isles.

Rank	Site Name	Location	Grid Ref	Site Area	No. visits	No. *Hygrocybe*	CHEGD	Source
1	Trawscoed	Gwynedd, Wales	SH844326	418 ha	>20	34 (21)*	78 15:34:21:3:	Andrew Graham, pers. comm. 2010
2	Mynydd Epynt	Powys, Wales	SN83,/93/94	14568 ha	>20	33 (22)*	64 10:33:12:7:	R.Woods, pers. comm.2006
3=	Longshaw Estate	Peaks, England	SK250790	280 ha	>20	30	74 11:30:26:7:	Neil Barton, pers. comm. 2006
3=	Alport	Peaks, England	SK143893	nk	>20	30	67 10:30:20:6:	Neil Barton, pers. comm. 2006
3=	Garn Ddyrys	Gwent, Wales	SO257118	5.4ha	>20	30 (19)*	57 10:30:9:6:2	Shelley Evans, pers. comm., 2006
6	Moel Tryfan	Gwynedd, Wales	SH511560	15 ha	ca. 10	29	59 11:29:11:5:	D.A. Evans, pers. comm., 2008
7	Llanishen SSSI	Glamorgan, Wales	ST186815	43.7 ha	>20	28 (15)*	43 7:28:6:1:1	RPS, 2005; D. Mitchel, pers.comm.
8=	St. Kilda (Hirta)	Highlands, Scotland	HS65	c.1500 ha	1?	27	70 7:27:32:3:1	Liz Holden, pers. comm. 2006
8=	Goodmans	Devon, England	ST260050	250ha	>20	27	60 0:27:28:0:5	FRDBI, 2008
8=	Moel y Ci	Gwynedd, Wales	SH598675	142 ha	>20	27	59 10:27:15:4:	John Harold, pers. comm., 2008
8=	Gilfach Farm SSSI	Powys, Wales	SN963716	84.7 ha	ca. 10	27	53 10:27:11:4:	Shelley Evans, pers. Comm., 2006
8=	Hafod SSSI	Ceredigion, Wales	SN756731	26.3 ha	>20	27 (10)*	45 6:27:10:1:1	(Evans and Holden 2003; Rotheroe 2002)
8=	Hopetoun House	W. Lothian, Scotland	NT090790	ca. 2ha	15	27	42 9:27:5:1:0	Holden, pers. comm. 27/2/06
14=	Kindrogan	Perthshire, Scotland	NO050620	ca 10ha	>20	26	66 6:26:27:2:3	Liz Holden, pers. comm. 2006
14=	Gilwern Hill SSSI	Gwent, Wales	SO246129	81 ha	5	26 (20)*	50 11:26:6:5:2	Evans, pers. comm, 2006
14=	Kerridge Hill	Cheshire, England	SJ942767	Nk	9	26	43 7:26:10:0	FRDBI, 2006
14=	Pal y Cwrt, Trapp SSSI	Carms, Wales	SN678182	57 ha	9	26 (9)*	40 8:26:1:5:0	(Evans and Holden 2003)
14=	Mynydd Du SSSI	Carms, Wales	SN727193	77.2 ha	5	26	37 6:26:0:4:1	Evans, pers. comm. 2006
19=	Crimsworth Dean	Yorks, England	SD980290	'large'	>20	25‡	62 5:25:28:3:1	FRDBI, 2006
19=	Blaen Nedd SSSI	Powys, Wales	SN916142	187 ha	2	25 (21)*	47 6:25:11:4:1	This study

* Numbers in brackets indicate the highest count of *Hygrocybe* spp. in a single site survey during the present study. ‡Newton et al. (2003) reported 25 *Hygrocybe* spp. during a single visit at Rassal (Highlands). Shaded rows indicate sites in Wales. Taxa are counted according to the guidelines described in the methods section.

Appendix 4

Bramley, W.G. (1965). Autumn Foray at Hebden Bridge; 25th to 29th September, 1964. *The Naturalist* Hull (Yorkshire Naturalists' Union): 107-108.

AUTUMN FORAY AT HEBDEN BRIDGE

25th to 29th September, 1964

W. G. BRAMLEY

With headquarters at Bent Head Farm, some fifteen members and friends took part in the Autumn Foray. It was a pleasure to welcome as visitors several members of local natural history societies and we should like more of these to take part in our activities even if they have no knowledge of the fungi.

The Hebden Bridge and Halifax area has had the longest continuous mycological attention in the county and most of the past and present members of the Section have collected there. This activity was started in the mid-eighteenth century by James Bolton who published the first book in English dealing with the subject, *A History of the Funguses growing about Halifax*, 1788–1791. Records then gradually accumulated until in 1904 C. Crossland brought them up to date, incorporating much that had been done by himself and James Needham, one of the old artisan naturalists of the late nineteenth and early twentieth centuries to whom local natural history owes so much. Of late years this list has been brought up to date and annotated by Mr. R. Watling. At present in manuscript, it is hoped that some day it may be published.

Visitors from eastern parts of the county were pleasantly surprised at the quantity of the larger toadstools to be found, indicating dramatically the wetter conditions of the Pennine area. More of the larger species were seen in an hour than they had been accustomed to find in a day. Three days were spent in the Hardcastle Crags valley, dividing it into three portions (1) from the entrance to Gibson Mill, (2) from Gibson Mill to the footbridge, (3) the remainder of the valley to Dean Head. Sunday was mostly devoted to Crimsworth Dene.

The area is mostly acid and it was interesting to find a number of *Hygrophorus* and allied genera in a newly re-seeded grass field and to learn that it had been limed the previous year, an indication of the effect of a small application of lime to the total volume of the topsoil. It also raises the question of how these species got there, especially as many of them had not been recorded previously. Was mycelium present and awaiting more alkaline conditions before it could fruit, or was it a new colonisation by wind-blown spores?

Dr. Webster made two collections of a species of *Massarina* which he had already proved in culture to be the perfect stage of an aquatic hyphomycete, details of which will appear later in the Transactions of the B.M.S. Two collections of a species of *Hypocrea* appear so far to be undescribed.

Helotium (*Cudoniella*) *aciculare* on wood, and *Rutstroemia luteo-virescens* on petioles of Sycamore are not often seen, but both were quite common. *Puccinia bistortae* was quite common in the teleuto-stage and it is hoped to see to which biological race it belongs.

We must thank all who have helped in the compiling of this abridged report, both by collecting and naming. Without the presence of Mr. R. Watling the list of agarics would have been greatly curtailed.

CD = Crimsworth Dene D = Dean Head (Sect. 3)
H = High Greenwood (Sect. 2) HC = Hardcastle Crags (Sect. 1)

*Not in Mason and Grainger's *Catalogue of Yorkshire Fungi* for V.C. 63
†Not in Mason and Grainger's *Catalogue of Yorkshire Fungi*
‡New record to revised Fungus Flora of Halifax

EXOASCALES
*‡*Taphrina populina* Fr. (*aurea*), on *Populus*, HC.
‡*T. tosquinetii* (West.) Magn., on *Alnus*, HC.

DISCOMYCETALES (W. G. Bramley, J. Webster, R. Watling)
Catinella olivacea (Rabenh. ex Fr.) Boud., HC.
**Dasyscyphus diminutus* (Rob.) Sacc., on *Juncus*, HC.
†*Geoglossum fallax* Durrand, CD
Helvella lacunosa Afz. ex Fr., H.
Microglossum olivaceum (Pers. ex Fr.) Gill, CD.
†‡*Peziza praetervisa* Bres., on burnt ground, D.
†*Plicaria fulva* Schneider, on burnt *Quercus*, HC.
*‡*Rutstroemia luteo-virescens* (Rob.) White, H.D.
‡*Trichoscyphella hahniana* (Seav.) Manners., on *Larix*, H.

PYRENOMYCETES (J. Webster, W. G. Bramley)
Claviceps purpurea (Fr.) Tul., ergots on *Deschampsia*, H.
‡*Eutypa spinosa* (Pers. ex Fr.) Tul., on *Fagus*, CD.
Hypomyces aurantius (A. and S.) Tul., on *Armillaria mellea*, D.
‡*Lasiosphaeria hirsuta* (Fr.) Ces. and de Not., H.
†‡*Nectria purtonii* (Grev.) Berk., on *Alnus*, D.
**Quaternaria quaternata* (Pers.) Tul. on *Fagus*, CD.

UREDINALES (W. G. Bramley)
‡*Puccinia cirsii* Lasch., II, III, on *C. palustre*, HC.
*‡*Thecopsora vacciniorum* (D.C.) Lagerh., II on *Vaccinium*, HC.

AGARICALES (R. Watling)
Authors according to "New Check List . . .", *Trans. Brit. Mycol. Soc.* (1960).

†*Amanita citrina* var. *alba*, CD, HC.
**A. excelsa*, CD, HC.
A. inaurata, HC.
*‡*Boletus spadiceus*, H.
Cantharellus lutescens, H.
‡*Clitocybe clavipes*, HC.
†‡*C. langei*, HC.
**Collybia tesquorum*, HC.
†‡*Coprinus lagopides*, on burnt area, D.
†‡*C. miser*, on dung, CD.
C. patouillardii (=*cordisporus* Gibbs), on horse dung, CD.
†‡*C. pellucidus*, on dung, CD.
†‡*C. stellatus*, on horse dung, CD.
*‡*Cortinarius pseudosalor*, CD, HC, under *Fagus*.
†‡*C. flexipes*, HC.
‡*C. glandicolor*, HC.
†‡*C. saniosus*, HC.
†‡*Flocculina granulosa*, under *Acer*, CD.
‡*Galerina paludosa*, in boggy area, HC.
†‡*Hebeloma sacchariolens*, CD.
†‡*H. testaceum*, CD.
†‡*Hygrophorus berkeleyi*, CD, Lumb Fall.
‡*H. flavescens*, CD.
H. intermedius, CD.
†‡*H. marchii*, H.
†‡*H. quietus*, CD.
*‡*H. reai*, CD.
†‡*H. strangulatus*, HC.
†‡*H. substrangulatus*, CD.
*‡*Inocybe margaritispora*, HC.
*‡*I. napipes*, HC.
†‡*I. pusio*, HC.
†‡*I. xanthomelas*, CD.
*‡*Laccaria proxima*, H, HC.
‡*Lactarius vietus*, HC.
†‡*Mycena bulbosa*, on *Juncus effusus*, Blake Dean.
†‡*M. crispula*, CD.
†‡*M. mucor*, HC.
**Nolanea cetrata*, HC.
†*Panaeolus rickenii*, HC.
‡*Pleurotus dryinus*, on *Acer*, Blake Dean.
Porphyrellus pseudoscaber, under *Fagus*, H.
†‡*Psathyrella squamosa*, HC, CD.
†‡*Russula betularum*, H, HC.
†‡*R. claroflava*, H, HC.
†‡*R. emeticella*, HC.
R. mairei, H, HC.
†‡*R. nitida*, HC.
*‡*R. xerampelina*, CD, HC.

APHYLLOPHORALES (R. Watling)
Hydnum rufescens Pers., under *Fagus*, H.
Polyporus adiposus B. and Br., on soil on stump of blown-down *Fagus*, H.
P. stipticus (Pers.) Fr., on *Fagus*, H.
†‡*Poria rhodella* (Fr.) Sacc., on *Quercus*, HC (det. D. A. Reid).

FUNGI IMPERFECTI (W. G. Bramley, J. Webster)
‡*Sporocybe flexuosa* (Mass.) Mason, on *Quercus*, HC.
†‡*Trichoderma sporulosum* (Link) Hughes, on *Pinus*, HC.

Appendix 5

Locations of the Widdop quadrats. Aerial photographs courtesy of @Google (2018)

Widdop 1(WID1)


Widdop Rd
Widdop Rd
Widdop Rd
W3
W2
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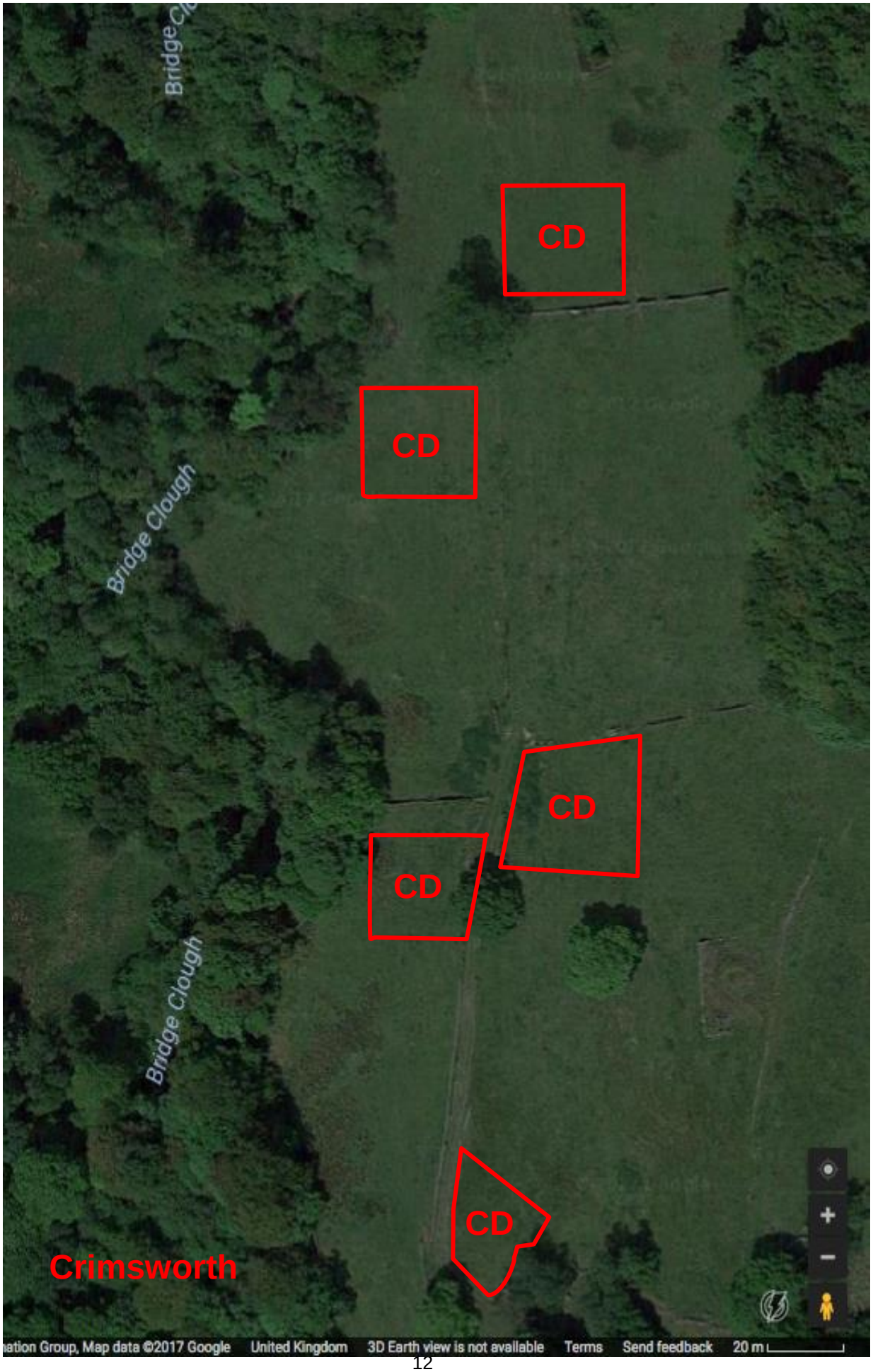
Bridge Clough
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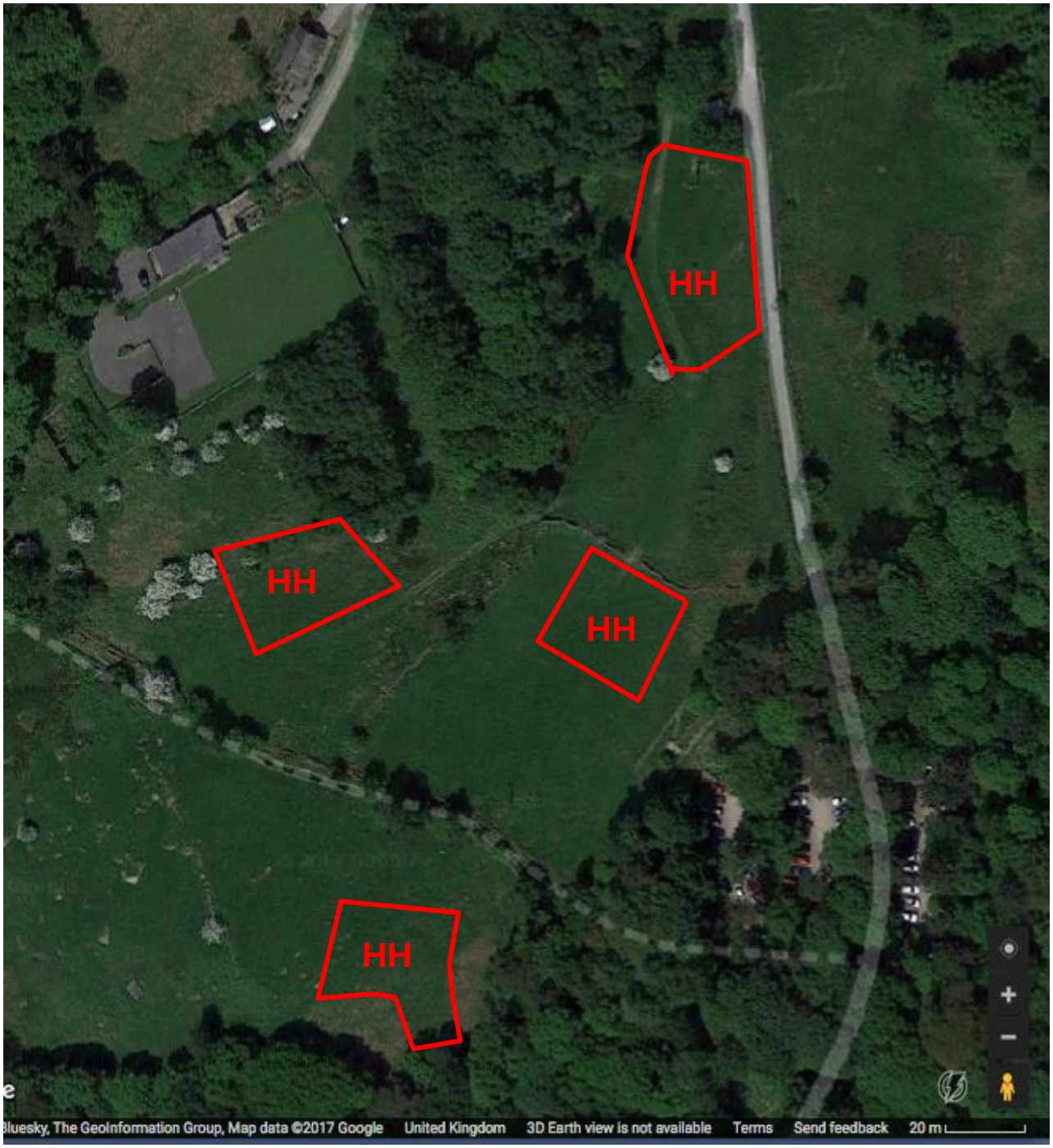
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