GLORIOUS GRASSLAND FUNGI
FINAL REPORT, 2022

The Grassland Fungi of the South West Peak
2017 to 2022
with funding from the Esmée Fairbairn Foundation

South West Peak
Landscape Partnership Scheme

Esmée Fairbairn FOUNDATION

SOUTH WEST PEAK
LANDSCAPE AT A CROSSROADS

Made possible with Heritage Fund

SUMMARY

The Glorious Grasslands project was part of the South West Peak Landscape Partnership Scheme, funded through the National Lottery Heritage Fund. The project was led by the Peak District National Park Authority, working with Cheshire Wildlife Trust, and ran from 2017 to 2022.

The project aimed to improve outcomes for grasslands in the South West Peak and to support managers by providing advice and information about the importance and management of species-rich grasslands, with a main focus on hay meadows. The secondary focus was on grasslands rich in fungi, often called 'waxcap grasslands' or CHEGD grasslands after the five groups of grassland fungi of conservation importance: Fairy clubs and corals (Clavariaceae – C), Waxcaps (Hygrophoraceae – H), Pinkgills (Entolomataceae – E), Earthtongues (Geoglossaceae – G), Crazed caps (Dermoloma spp and allies – D).

Fungi conservation is a relatively new concept and the ecological requirements of waxcap grasslands are not widely understood. The project aimed to survey 150ha of waxcap grasslands and provide information on their management to farmers and conservationists.

Thanks to funding from the Esmée Fairbairn Foundation, the project was able to extend and develop a stronger focus on survey and management advice and deepen our understanding of 'waxcap grasslands' between 2019 and 2022.

Field surveys by experienced mycologists Jeanette Maddy and Neil Barden were commissioned in 2017, 2018 (Jeanette) and 2019 and 2020 (Neil). Alongside this, training and information sessions were delivered for staff, volunteers, children and farmers by Jeanette, Neil and Rob Foster.

Fungal surveys are rather challenging, and can only be done during the fungal fruiting season, typically between September and December. Fruiting is very variable, heavily affected by the weather, and doesn't occur every year. The autumn of 2018 followed a hot dry summer and fungal fruiting was seriously affected. Weather-wise, 2019 was a more normal year and the results of our surveys in 2019 identified a number of sites of regional and national importance. 2020, however, was an even better year, the ideal weather providing the best year for fungi in the Peak District in the last two decades.

To complete field surveys, suitable habitat on each site was thoroughly surveyed on between two and four visits during the prime fungal fruiting season. Species records were made using a GPS in the field, photographs were taken, and specimens requiring further analysis were collected for identification under the microscope. A full colour illustrated report was produced for the owners of each site describing the interest and providing management guidance.

Given our experience of a poor fungal fruiting season in 2018, in 2021 we expanded the project remit to explore the question: "how do you know a grassland could be important for fungi when they are not fruiting?" This question applies not only to the autumn season in a poor fruiting year but also for the remainder of the year between January and August when other surveys would be taking place to inform management decisions and potential changes in management, such as tree planting.

The next logical step was to seek to understand more about the parameters affecting CHEGD fungi, guide management recommendations and help develop a process for determining the potential value of 'unsurveyed' sites for CHEGD fungi. To this end, we teamed up with Aberystwyth University to deliver NextGen DNA sequencing of soil samples and soil chemistry analysis, alongside field survey and the collation of management information. Multiple correlation analysis was performed to look for statistically significant relationships between the ranges of chemical, physical, biotic and management factors.

KEY FINDINGS

1. **The project has surveyed a total of 1420ha of grasslands, of which approximately 500ha is of fungal interest.**
2. **Field survey and soil sampling for laboratory eDNA analysis has provided data on 54 sites across the South West Peak.**
3. **Of these, field survey and eDNA analysis identified 24 sites of international importance, 12 of national importance, 13 of regional importance, 3 of local importance and 2 of no importance for waxcaps. In addition, 28 of these sites are of national importance for one or more of the other groups of fungi – fairy clubs and corals, pinkgills, earthtongues and crazed-caps.**
4. **Of the 137 CHEGD species identified using laboratory analysis, 19 have hitherto been found in Europe but not the UK, and 6 only outside of Europe. Four potential species new to science were detected.**
5. **Amongst the named CHEGD fungi identified by DNA analysis one is categorised as Endangered on the IUCN Global Red List (same level of extinction threat as blue whale and mountain gorilla) and 31 as Vulnerable (same level of extinction threat as giant panda and snow leopard).**
6. **32 of the surveyed sites could merit notification as Sites of Special Scientific Interest (SSSI) for their waxcaps or for the other fungi groups.**
7. **Grasslands grazed by sheep or sheep and horses supported more diverse fungal communities than those grazed by cattle or those managed as hay meadows.**
8. **Citizen science, field survey and laboratory DNA analysis combined represent an excellent means of raising awareness and identifying sites of importance.**

SUMMARY STATISTICS (Field Survey 2017-19)

In the early stages of the grassland fungi work a small number of sites were surveyed in 2017, 2018 and 2019 by Jeanette Maddy.

Survey Season: **25-27 October 2017, 26 October – 5 November 2018, 5 September – 8 November 2019**

Number of Survey Days: **11 days**

Number of Data Entry and Report Writing Days: **3 days**

Number of Sites Surveyed: **5**

Survey Area: **56.8 ha**

Area of Fungal Interest: **20.9 ha**

Priority Habitat Types: **Lowland hay meadow, Lowland dry acid grassland**

Other Habitats: **Semi-improved acid grassland, neutral grassland, rough grazing, rush pasture**

Total No. Fungi Species: **n/a**

Total No. of CHEGD Species: **36**

Total No. of Conservation Importance: **n/a**

Total No. Fairy clubs (C): **4**

Total No. Waxcaps (H): **23**

Total No. Pinkgills (E): **9**

Total No. Earthtongues (G): **0**

Total No. Dermoloma & allies (D): **0**

Subsequently, with funding from the Esmée Fairbairn Foundation, we were able to significantly expand our survey work and take on a second contractor, Neil Barden. The remainder of this report focuses on the survey and analysis of data from Neil's work from 2019 to 2022.

PLANNED PROJECT OUTCOMES/INDICATORS

The following were the planned outcomes and indicators for this project, agreed with Esmée Fairbairn Foundation:

1. **Greater understanding of the distribution and significance of waxcap grasslands in the South West Peak District.**

   i) 30 sites scoped for potential and suitability for waxcap fungi.

   ii) 15 sites with full surveys completed.

   iii) Assessment of significance made for all sites surveyed.

2. **Land managers gain a greater appreciation of waxcap grasslands and their role in their management and protection.**

   i) Soil analysis of surveyed waxcap grassland sites is completed and used to inform management recommendations.

   ii) Management recommendations are written for all of the sites surveyed which are of regional, national or international significance; these will be shared and discussed with land managers.

   iii) Information is shared locally with Natural England and other local partners (e.g. the Wildlife Trusts, RSPB, National Trust).

3. **More people understand and support the importance of conservation management for waxcap grasslands.**

   i) 30 adults plus 15 farmers, 6 apprentices and 20 secondary school-age children attend fungi training/information activities.

   ii) A simple pictorial guide to identification and management of waxcap grasslands is produced and disseminated.

   iii) Information is shared with Natural England, other National Park Authorities across the UK, other relevant NLHF-funded projects and programmes.

OUTCOME 1: Greater Understanding

Greater understanding of the distribution and significance of waxcap grasslands in the South West Peak District.

i) 30 sites scoped for potential and suitability for waxcap fungi

Over the course of the project we were able to **scope a total of 35 sites** for their suitability to support assemblages of CHEGD groups of fungi. This was made possible through the local knowledge of project officers, volunteers, landowners, mycologists and colleagues at the Peak District National Park Authority and Cheshire Wildlife Trust.

ii) 15 sites with full surveys completed

Of the 35 sites scoped, **31 were surveyed**, receiving between one and four full survey visits in 2019 and/or in 2020. 25 of these sites had not previously been surveyed for grassland fungi, thus this project has significantly added to our understanding of the fungal interest across the area.

iii) Assessment of significance made for all sites surveyed

The methodology used to survey these sites was adapted from several suggested methodologies which have been employed over recent decades. In order to make the best use of time and surveyor effort a method of plotless transects was used, whereby the most suitable habitats were first identified and located on the landscape, with intensive searches of the most likely habitats and less intensive searches made of least likely habitats. In this way, all suitable habitat is searched with survey effort concentrated in fungal 'hotspots'.

Our contracted mycologist, Neil Barden, produced a full report for each site visited, recording the area of the sites surveyed, which fields supported populations of important grassland fungi and which fields were fungal hotspots containing high abundance, high species diversity and/or rare species.

Assessment of the significance of a site is typically made in reference to methodology developed by Rald (1985) and amended by Vesterholt et al (1999) as below:

Conservation value	**Single visit *Hygrocybe* taxa**	**Total number of *Hygrocybe* taxa**
Of international importance	15(?)+	22+
Of national importance (I)	11 -14	17- 21
Of regional importance (II)	6 -10	9 - 16
Of local importance (III)	3 - 5	4 - 8
Of no importance (IV)	1 - 2	1 - 3

Table 1. Measure of site significance for waxcaps alone

This method, however, only considers the Hygrocybe (waxcaps). So Rotheroe (1999 & 2001) developed the CHEG profile in which a grassland's fungal conservation value is evaluated using four fungi groups:

1. Clavaroid fungi - the fairy clubs
2. Hygrocybes - the waxcaps
3. Entolomas - the pinkgills
4. Geoglossaceae - the earth tongues

Each species from one of these groups counted towards a numerical score for each grassland site. This scoring system is known as the CHEG profile and takes its name from the initials of the 4 groups of fungi listed above. It enables one to compare grassland sites for their relative conservation value. Evans et al (2002) considered important grassland sites would have a CHEG profile of: C8 H17 E15 G4.

Griffith et al. (2013) modified the CHEG criteria by separating out the Dermoloma giving the now widely used **'CHEGD'** notation. In terms of Dermoloma species, the JNCC suggested a D (Dermoloma and allied species) value of 5 when considering the importance of sites.

Bosanquet et al (2018) advise that sites that reach or exceed one or more of the thresholds below should be considered for SSSI notification.

Conservation value	**C *Clavaroid* Fairyclubs & Corals**	**H *Hygrocybe* Waxcaps (single visit)**	**H *Hygrocybe* Waxcaps (total no.)**	**E *Entoloma* Pinkgills**	**G *Geoglossum* Earthtongues**	**D *Dermoloma* & allies**
SSSI quality	7	19 (specific taxa)	19 (specific taxa)	15	5	3
Of international importance	n/a	15(?)+	22+	n/a	n/a	n/a
Of national importance	8	11 -14	17- 21	15	4	3
Of regional importance	n/a	6 -10	9 - 16	n/a	n/a	n/a
Of local importance	n/a	3 - 5	4 - 8	n/a	n/a	n/a
Of no importance	n/a	1 - 2	1 - 3	n/a	n/a	n/a

Table 2. Measure of site significance for all groups, and distinct thresholds for SSSI notification

Within each report we used the measures of importance as shown above to indicate whether the farm was of local, regional, national or international significance for grassland fungi and also whether it would meet the criteria for SSSI designation. One of the landowners subsequently approached us requesting to discuss with Natural England whether their site could potentially be designated a SSSI.

SUMMARY STATISTICS (Field Survey 2019,2020)

Survey Season: **14 September to 6 December 2019, 5 September to 27 November 2020**

Number of Survey Days: **85 days**

Number of Data Entry and Report Writing Days: **40 days**

Number of Sites Surveyed: **31**

Distance Travelled: **3925**

Survey Area: **1220 ha**

Area of Fungal Interest: **329.12 ha**

Priority Habitat Types: **Lowland hay meadow, Lowland dry acid grassland**

Other Habitats: **Semi-improved acid grassland, neutral grassland, rough grazing, rush pasture**

Total No. of CHEGD Species: **139**

Total No. of Conservation Importance: **64**

Total No. Fairy clubs (C): **17**

Total No. Waxcaps (H): **51**

Total No. Pinkgills (E): **52**

Total No. Earthtongues (G): **14**

Total No. Dermoloma & allies (D): **5**

Photographs of: crested coral (Clavulina coralloides) above, indigo pinkgill (Entoloma chalybaeum) below left, yellow-legged fanvault (Camarophyllopsis micacea) below right

SITE SUMMARY (field survey)

The table below summarises the results of the 2019 and 2020 field surveys. Giving the level of importance for fairy clubs (C), waxcaps (H), pinkgills (E), earthtongues (G), and crazed caps (D) at each site, the total number of CHEGD species recorded, and the number of species of conservation concern.

Site	2019 CHEGD	Level of importance 2019	2020 CHEGD	Level of importance 2020	Total CHEGD species	Species of conservation concern
Back o' th' Brook	n/a	n/a	C2 H12 E6 G0 D0	Regional (H)	20	6
Ball Bank House Farm	n/a	n/a	C4 H12 E2 G1 D0	National (H)	19	3
Boothlow Hayes	n/a	n/a	C3 H18 E4 G1 D1	National (H)	27	13
BL Farm	n/a	n/a	C9 H17 E18 G3 D0	International (H) National (C,E)	47	17
Far Brook Farm	C2 H12 E0 G1 D1	National (H)	C5 H18 E9 G3 D1	International (H)	36	14
F Farm	n/a	n/a	C7 H21 E10 G3 D0	International (H)	41	13
Franklin's Farm	n/a	n/a	C7 H23 E15 G2 D0	International (H) National (E)	47	17
Gleadtail	C4 H8 E1 G0 D0	Regional (H)	C4 H13 E5 G1 D0	Regional (H)	23	7
Greenlands	C2 H12 E5 G0 D0	National (H)	C5 H20 E18 G0 D0	International (H) National (E)	43	13
Hazelhurst Farm	C0 H13 E0 G0 D0	National (H)	C4 H21 E15 G3 D0	International (H) National (E)	43	17
Herbage Farm	C2 H6 E0 G0 D1	Regional (H)	C2 H13 E6 G0 D1	National (H)	22	5
High Ash Farm	n/a	n/a	C3 H20 E10 G3 D0	International (H)	36	13
Higher Minnend	C5 H16 E4 G0 D1	National (H)	n/a	n/a	26	6
Hurdlow Farm	C4 H19 E6 G1 D1	International (H)	C10 H25 E18 G5 D3	International (H) National (C,E,G,D)	61	20
Kisswood Farm	C5 H19 E2 G0 D0	International (H)	C5 H19 E3 G1 D0	International (H)	28	7
Lower Elkstone	C5 H5 E1 G0 D0	Local (H)	C5 H19 E11 G2 D0	International (H)	37	12
Lower Fleetgreen	C7 H17 E6 G3 D0	National (H)	n/a	n/a	33	13
Lower Wigginstall	n/a	n/a	C4 H12 E3 G1 D0	Regional (H)	20	7
Lumbs Farm	n/a	n/a	C3 H11 E2 G1 D0	Regional (H)	17	3
Mill Lane Farm	n/a	n/a	C1 H6 E0 G2 D0	Local (H)	8	4
Mixon Grange	C2 H12 E4 G0 D0	National (H)	C2 H13 E4 G0 D0	National (H)	19	6
Noon Sun Farm	C3 H5 E0 G0 D0	Local (H)	C6 H17 E11 G5 D1	National (H,G)	40	14
Oils Heath	n/a	n/a	C3 H17 E3 G0 D0	International (H)	23	7
Orchard Farm	n/a	n/a	C2 H18 E4 G3 D1	International (H)	28	13

Swainsmoor	C6 H22 E9 G4 D1	International (H) National (G)	n/a	n/a	42	12
Under Whitle	n/a	n/a	C5 H17 E1 G0 D0	National (H)	23	5
Upper Whitle	n/a	n/a	C1 H13 E2 G1 D0	National (H)	16	7
Waggonshaw Brow	n/a	n/a	C6 H19 E3 G6 D0	International (H) National (G)	34	13
Waterfall Farm	n/a	n/a	C0 H1 E0 G0 D0	None	1	0
Waterfall Low	n/a	n/a	C1 H5 E1 G0 D0	Local (H)	7	2
Wilshaw Farm	n/a	n/a	C1 H13 E3 G1 D0	National (H)	18	5

Table 3. Summary of field survey data

It should be noted that survey effort varied across sites and across years. In 2019, all sites received a single visit between mid-September and late November. In 2020, most sites had 2, 3 or 4 visits between early September and late November. All sites with increased survey effort in 2020 yielded increased CHEGD scores, notably Lower Elkstones which increased from local importance for Hygrocybe to international importance for Hygrocybe and near national importance for Entoloma; similarly Noon Sun increased from local importance for Hygrocybe to national importance for Hygrocybe and national importance for Geoglossum.

By far the most outstanding site was Hurdlow which was found to be internationally important for waxcaps (H) and nationally important for the other four groups of fungi of conservation interest – fairy clubs (C), pinkgills (E), earthtongues (G) and crazed caps (D), placing this among the top five known sites in the country. Of significance here, is that all fungal interest was found in one large field on the holding which is under management prescriptions targeted at upland breeding waders, as the current agri--environment schemes do not have prescriptions relating to grassland fungi.

One of the most diverse sites for grassland fungi

OUTCOME 2: Greater Appreciation

Land managers gain a greater appreciation of waxcap grasslands and their role in their management and protection.

i) Soil analysis of surveyed waxcap grassland sites is completed and used to inform management recommendations.

Our initial intention was to explore soil compaction and its effect on fungal resilience and fruiting together with soil chemistry analysis. However, based on our experience of limited field survey in 2018 (a year in which we experienced a very hot, dry summer which badly affected fungal fruiting) followed by two good seasons of field survey in 2019 and 2020, we decided, thanks to additional funding from Esmée Fairbairn Foundation, to expand our project scope in its final year. Further detail on this is provided later in this report.

ii) Management recommendations are written for all of the sites surveyed which are of regional, national or international significance; these will be shared and discussed with land managers.

Each field survey was accompanied by a site report explaining what was found on each site/farm, which fields were important, noting rare species and those of particular conservation concern. Together with a description of the fungal interest, management recommendations were provided, including general guidance applicable to all sites with additional recommendations specific to the site in question. The management recommendations focused on: the effect of additional fertilisation, the effect of over-grazing, the effect of under-grazing, the effect of improving drainage, control of bracken and scrub, the effect of liming, and the impact from stock trampling, poaching and vehicle compaction/sward damage.

iii) Information is shared locally with Natural England and other local partners (e.g. the Wildlife Trusts, RSPB, National Trust).

Feedback on overall survey findings was shared with the project steering group, including representatives from Peak District National Park Authority, Natural England, Cheshire Wildlife Trust and Nature Peak District and this was used to inform further survey work and conversations with land managers.

OUTCOME 3: Greater Support

More people understand and support the importance of conservation management for waxcap grasslands.

i) 30 adults plus 15 farmers, 6 apprentices and 20 secondary school-age children attend fungi training/information activities.

Over the course of the project we delivered 7 grassland fungi training and identification sessions in 5 different locations, attended by 104 people (97 unique individuals). Of these, 27 were farmers/land owners, 3 were apprentices and 19 were children.

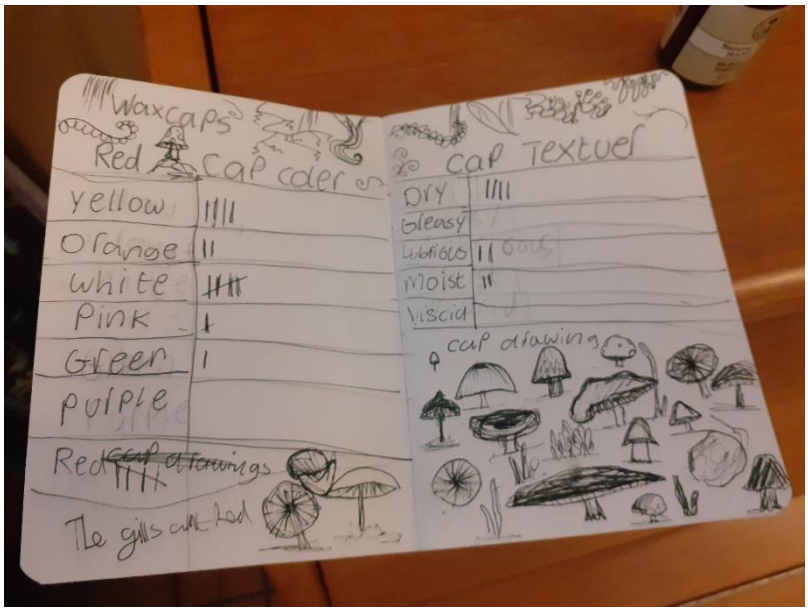
One attendee at the 2019 training event created a gallery of images which can be viewed here: Grassland Waxcap Fungi (rakm.co.uk)

In 2021, our contractor Neil gave an evening talk for farmers and land mangers focusing on the range of fungi they could find on their land, why they were of such importance and how to manage land appropriately. The talk was attended by 27 local farmers, from 15 different farms. A number of those

who attended had been involved in the project since surveys in 2019 and at least three had often contacted members of the team sending photographs of fungi they had found on their land.

In November 2021, our Youth Engagement Officer ran 2 field sessions, one near Buxton with the Buxton Junior Rangers which was attended by 5 young people; and a second open to all Peak District Junior Rangers on a site owned by the National Trust just on the edge of the South West Peak, which was attended by 14 young people.

The Junior Rangers were encouraged to count, photograph and draw the fungi they saw, and to evaluate the site using a simple guide and scoring methodology based on cap colour.



Here are some of their comments:

"I really enjoyed it, especially the part where we found all the fungi and wrote down all about it so we could find out what they were. I also liked it when we scored the area, ours had a high score"

"I enjoyed learning how to identify the different mushroom categories"

"I enjoyed walking around looking at the different species of fungi and looking at all the different kinds there were"

"I like the puffballs, they puff when you touch them!"

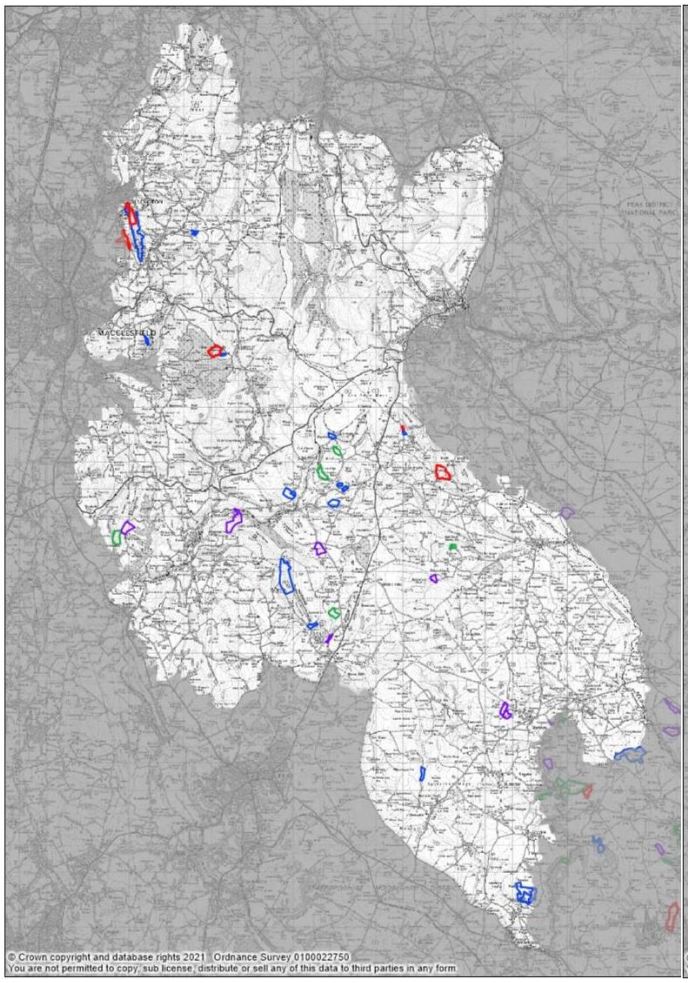
ii) A simple pictorial guide to identification and management of waxcap grasslands is produced and disseminated.

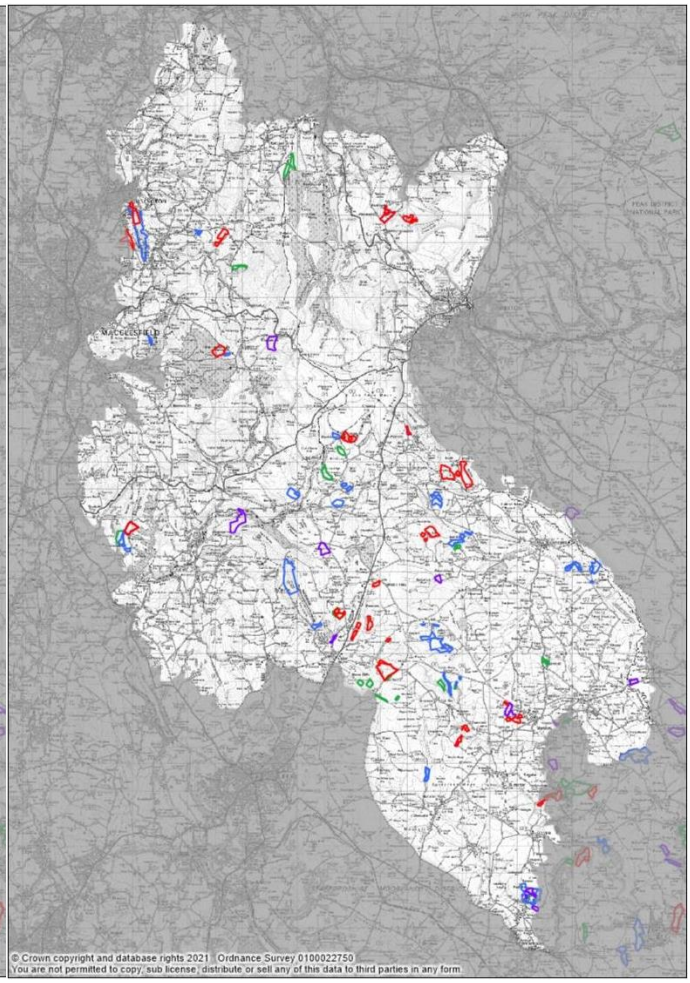
To support volunteers in identifying grassland fungi we uploaded labelled photographs of some of the key species on our website: Waxcap Identification Guide: South West Peak

Using the data we had gathered from field surveys and soil analysis we have put together a decision key to help land managers determine whether their grasslands are likely to be important for grassland fungi of conservation interest or CHEGD fungi. This will soon be available on our website: Glorious Grasslands: South West Peak

iii) Information is shared with Natural England, other National Park Authorities across the UK, other relevant NLHF-funded projects and programmes.

We have provided up to date site records to Natural England to include on their Priority Habitat Inventory. The two maps below show the coverage of waxcap surveys in the South West Peak Landscape Partnership Scheme area before (left) and after (right) this project. Level of importance: Red = International, Blue = National, Green = Regional, Purple = Local


MACCLESFIELD
© Crown copyright and database rights 2021 Ordnance Survey 0100022750
You are not permitted to copy, sub license, distribute or sell any of this data to third parties in any form


MACCLESFIELD
© Crown copyright and database rights 2021 Ordnance Survey 0100022750
You are not permitted to copy, sub license, distribute or sell any of this data to third parties in any form

SOIL eDNA ANALYSIS

A number of points of interest arose from the Glorious Grasslands fungi survey work and from collating data from previous surveys in the area:

- Field surveys for CHEGD fungi are time consuming, season-specific, impacted by weather conditions and require specialist knowledge and skills.
- Field survey has been extremely rewarding and has engendered a great deal of interest in CHEGD fungi amongst staff, volunteers and land managers.
- Sites in the South West Peak have been shown to support a diverse range of CHEGD species and also support a number of rarities.
- A proportion of surveyed sites included grassland managed as hay meadow, yielding good results for waxcaps and pinkgills, indicating that hay meadows deserve more attention with regard to their fungal communities.
- Some sites appear to have deteriorated in fungal diversity over the years since surveys in 2012 and 2013.
- Floristic value/diversity of a site is not always a good indicator of fungal value/diversity, sites of importance for grassland fungi are often species poor.
- Grassland surveys carried out to inform agri-environment schemes could have been conducted at any time of the year and will tend to have focussed more on floristic interest and (in the South West Peak) suitability as habitats for breeding/feeding waders. Scheme application deadlines are a factor too, with tightly defined windows for new Countryside Stewardship agreements. Fungal interest is therefore seldom recorded (in part due to timing of survey, in part due to inexperience or lack of knowledge).
- There is an increasing interest in, and Government funding for, tree planting which poses a potential threat to marginal grasslands. Typical locations with potential for tree planting are cloughs, bracken covered slopes, moorland fringes, unproductive grassland and grassland of low floristic interest. Aside from bracken slopes these other areas could hold significant fungal interest, but without appropriate survey effort this value remains largely unknown.
- There is increasing interest in the importance of soil health and in carbon sequestration. Work is underway in the Peak District to understand and promote the value of unimproved and species-rich grassland for carbon sequestration and storage; however, any connection between soil health and CHEGD fungi is not widely understood amongst conservationists and land managers.

In 2020 we began discussions with experts in fungal DNA and compared the relative merits of field survey versus laboratory analysis:

- Field survey requires time and timing. Several visits may be required to assess the full suite of fungi species present, and these need to take place when fruiting is occurring. In a good year, a single well-timed survey visit can yield excellent results, but in a poor year a visit to the same site will give a very different result.
- Soil samples can be taken at any time of year and environmental DNA extracted and analysed to provide data on fungal diversity and abundance. Little seasonal or annual variation has been detected at test sites. An adequate number of well-placed soil cores must be taken to find the full suite of species on any site, so a level of understanding of suitable habitat types is required.
- It may be that both methods need to be used to complement one another. Decisions over whether to conduct field survey or eDNA analysis may depend on what the data are needed for. If a site is at risk of damage or loss and information is needed rapidly, then eDNA may be the most appropriate approach, particularly if assessment is needed out of fruiting season.

In 2021, we entered into a contract with Aberystwyth University to conduct environmental DNA analysis (or eDNA) on soil samples taken from a range of sites. Employing the same contractor who conducted field survey, we agreed a methodology which was practical within our time and resources:

Soil sampling protocol

- The standardised methodology developed by Aberystwyth university researchers requires a 30m x 30m quadrat (900m2) with a standard size soil core (15mm diameter, 100mm depth) taken every 5m in a grid pattern to produce a total of 36 cores, which are then pooled into a single sample weighing between 500 and 700g.
- Most sites support heterogeneous habitat so 'quadrat' positioning is key to ensure that soil samples are taken from the most suitable locations within a site. A quadrat does not have to be a 30m x 30m square, but can be linear or divided into sub-quadrats to ensure that samples are suitably located in areas likely to support fungal communities.
- Samples can be taken by a competent fieldworker with a sufficient amount of training and ability to identify suitable habitat areas.
- Soil samples are transferred on site into a plastic bag and labelled with a unique code identifying the site, field number and quadrat number.
- Soil samples must be chilled (using a refrigerator or cool box with ice blocks), wrapped in layers of bubble wrap, boxed up and sent by next day courier to the laboratory. Once received, they are freeze dried until such time as they can be analysed against a series of barcodes.
- Soil samples were analysed to provide data on the following: all fungi species, CHEGD fungi species, higher plants, moisture content, pH, fungi to bacteria ratio, organic matter, nitrogen, carbon, total and available potassium, total and available phosphorus.

Site selection

In order to understand more about the factors affecting CHEGD fungi in the South West Peak, we selected sites which were categorised in the following way:

- Highest ranked sites (international importance for Hygrocybe, national importance for other groups, these formed 'control' sites comparing field survey results with laboratory analysis results)
- Hay meadows (typically neutral, dry to damp grasslands managed for hay making)
- Mediocre or degraded sites (change in results between survey years)
- Unknown sites (sites not previously included in field mycology survey but with vegetation data indicating unimproved or good semi-improved acid/neutral grassland, or relevant NVC classifications)
- At risk sites (sites under consideration from tree planting, but where there could be potential fungal interest)

The number of soil samples to be collected per site was determined by the area of fungal interest on the site (where this is known from previous survey) and the heterogeneity of the habitat. The smallest, most homogenous site required just one quadrat. For 'unknown sites' with no previous fungal survey data, the number of samples was determined by the approximate area of likely suitable habitat.

Materials and Equipment

- Site map, contact details
- Recording forms
- GPS unit
- Soil auger
- pH and moisture meter
- Plastic ziplock bags to hold pooled soil samples for each quadrat
- Waterproof pen to label soil sample bags

- Cool box and ice blocks to keep samples chilled
- Bubble wrap and boxes for posting samples
- Printer to print courier labels

Collecting soil cores using an auger and transferring these to a plastic bag before chilling

Measuring soil pH using a probe

SUMMARY STATISTICS (Soil Sample Analysis)

Survey Season: **5 August to 2 November 2021**

Number of Survey Days: **38.25 days**

Number of Data Entry Days: **5 days**

Number of Sites Sampled: **25**

Number of Quadrats: **116**

Number of Soil Samples Collected: **4176**

Number of Fungi DNA Sequences: **4,349,165**

Average Number of Fungi DNA Sequences per Quadrat: **37,493**

Total No. Higher Plant Species: **91**

Total No. of CHEGD Species: **137**

Total No. of Conservation Importance: **32 species on the IUCN red list as either Vulnerable or Endangered**

Total No. Fairy clubs (C): **34**

Total No. Waxcaps (H): **41**

Total No. Pinkgills (E): **37**

Total No. Earthtongues (G): **21**

Total No. Dermoloma & allies (D): **4**

SITE SUMMARY (soil analysis)

The table below summarises the results of the 2021 soil analysis work giving CHEGD results from eDNA analysis of soil samples; the level of importance* for fairy clubs and corals (C), waxcaps (H), pinkgills (E), earthtongues (G), and crazed caps (D); the total number of CHEGD species, and the number of species of conservation concern recorded by eDNA analysis at each site. *Field records from survey or casual observation for just the fields included in the soil sampling are also given for interest.*

Site	eDNA CHEGD	* Level of importance 2021	Total CHEGD species	Species of conservation concern	*Field records CHEGD*
Badger's Croft	C15 H10 E9 G8 D1	Regional (H) National (C,G)	43	3	*C3 H3 E3 G0 D0*
BF Anon 1	C18 H20 E15 G10 D2	International (H) National (C,E,G)	65	14	*C9 H18 E21 G3 D0*
Brownsetts	C21 H26 E24 G10 D4	International (H) National (C,E,G)	83	17	*C5 H18 E8 G2 D0*
Cut-Thorn	C13 H18 E13 G7 D1	International (H) National (C,G)	52	7	*C3 H10 E4 G0 D0*
F Anon 4	C19 H13 E9 G10 D3	National (C,H,G,D)	54	7	*C7 H23 E7 G3 D0*
Franklin's Farm	C19 H18 E24 G12 D1	International (H) National (C,E,G)	74	10	*C7 H23 E15 G2 D0*
Gag Aye	C12 H20 E10 G6 D1	International (H) National (C,G)	49	7	*C3 H13 E5 G1 D0*
Greenland	C16 H14 E18 G8 D1	National (C,H,E,G)	57	12	*C5 H20 E18 G0 D0*
Howe Green	C18 H17 E14 G8 D0	National (C,H,G)	57	6	*C2 H11 E4 G2 D0*
Knotbury	C13 H10 E18 G7 D0	Regional (H) National (E,G)	48	6	*n/a*
Knotbury Common	C15 H15 E14 G8 D1	International (H) National (C,G)	53	8	*n/a*
Leycote	C20 H27 E22 G10 D2	International (H) National (C,E,G)	81	16	*C8 H31 E18 G4 D0*
Lower Fleetgreen 2	C21 H23 E22 G8 D1	International (H) National (C,E,G)	75	12	*C0 H5 E3 G0 D0*
Oils Heath	C11 H17 E10 G9 D2	International (H) National (C,G)	49	9	*C3 H17 E3 G0 D0*
Pyeclough	C19 H18 E19 G8 D1	International (H) National (C,E,G)	65	8	*C0 H2 E1 G0 D0*
RK Anon 2	C13 H9 E7 G10 D2	Regional (H) National (G)	41	6	*C0 H2 E2 G0 D0*
SH Anon 3	C20 H21 E13 G10 D2	International (H) National (C,G)	66	10	*n/a*
Shawbottom	C11 H8 E6 G8 D0	Regional (H) National (G)	33	5	*C3 H10 E6 G1 D0*
Steps	C5 H6 E0 G5 D1	Local (H)	17	1	*C0 H1 E0 G0 D0*
Summerclose	C19 H21 E19 G8 D2	International (H) National (C,E,G)	69	11	*C2 H10 E9 G0 D1*
Tunstead farm	C15 H13 E10 G7 D1	Regional (H) National (G)	46	8	*C7 H15 E6 G0 D0*
Under Whitle	C14 H9 E6 G10 D3	Regional (H) National (G,D)	42	4	*C5 H16 E0 G0 D0*
Upper Brownhills	C14 H8 E9 G8 D3	Regional (H) National (C,D)	42	5	*C0 H2 E0 G0 D0*

Waterfall Farm	C20 H10 E7 G10 D4	Regional (H) National (C,G,D)	51	7	*C0 H1 E1 G0 D0*
Waterfall Low	C26 H19 E18 G15 D4	International (H) National (C,E,G,D)	82	15	*C1 H5 E1 G0 D0*

Table 4. Summary of data from eDNA analysis of soil samples.

***** There is currently no recognised threshold to measure site importance using eDNA analysis, thus the thresholds used for field survey have been used here as a guide. Species identified by their DNA also include immature individuals which may not be found by field survey.

Discussion on soil analysis data

From sites where we have comparable field survey (i.e. at least three full survey visits - CHEGD scores are shown in bold text) and soil analysis data, the soil analysis method has identified much higher numbers of fairy clubs and corals (C) pinkgills (E) and earthtongues (G) than were found through field survey; whereas similar numbers of waxcaps (H) have been identified by field survey (in a good year) and soil analysis. There may be a number of reasons for this:

- The waxcaps as a group are better described, better studied, easier to differentiate in the field and the taxonomy is more stable. For many of the other groups the taxonomy is still very fluid with some species being split into several different species in recent years due to more detailed taxonomic and DNA study.
- As the majority of the fungal body (the mycelium) is present in the soil with only the fruiting body becoming apparent above ground, soil eDNA analysis will identify immature individuals (i.e. those that are not mature enough to produce fruiting bodies) which field surveys cannot possibly locate.
- Surveyor knowledge, skill and experience can affect the location and identification of species in the field. In this instance, we are confident that this is not a confounding factor due to our surveyor having two decades of survey experience.
- The fruiting season for the earthtongues in particular is highly affected by weather conditions as they tend to fruit late in the season after a hard frost, so can be missed if survey effort is insufficient or poorly timed.

Of the 137 CHEGD species identified using laboratory analysis, 19 have hitherto been found in Europe but not the UK, and 6 only outside of Europe. Four potential species new to science were detected. Amongst the named CHEGD fungi, one (Gloioxanthomyces vitellinus) is categorised as Endangered on the IUCN Global Red List (equivalent conservation status to blue whale and mountain gorilla) and 31 as Vulnerable. Several of these species are also listed in Section 41 of the NERC Act (2006). Gloioxanthomyces vitellinus (pictured below) had previously been recorded in the field by Neil Barden at only 4 sites in the South West Peak, this work has identified a further 2 sites.

Results of note:
Environmental DNA analysis of soil samples has indicated much higher fungal diversity at Waterfall Farm and Waterfall Low compared to previous field surveys. These sites received two survey visits in September and October of 2020 (a year when most sites in the South West Peak were fruiting prolifically) but yielded poor results, despite the surveyors note that these sites appeared to have suitable habitat. This can be interpreted in a number of ways: perhaps the surveyor was unlucky to visit at the wrong time; there is sufficient fungal mycelium in the soil to show diversity through eDNA analysis but insufficient to produce fruiting bodies; management of the site is hampering fungal fruiting. Further study of these sites is warranted to explore the reasons for this finding.

A diverse array of grassland fungi, including those of conservation concern, was found at almost all sites sampled, including pastures and hay meadows. All four of the sites where tree planting had been proposed were found to be of significant conservation value for grassland fungi indicating that such a change in management would be highly damaging to the conservation interest of the sites.

Greenland Farm, one of the smallest sites surveyed and sampled (comprising just three fields, each less than 0.5ha in size), is of high conservation value for both its hay meadow species and its grassland fungi. The site is not in an agri-environment scheme or designated as SSSI but the owners manage it for conservation, thereby protecting its interest.

These results have added to our understanding of the value of some traditionally managed hay meadows for both botanical and fungal interest, and suggest that this warrants further exploration. Whilst the hay meadows in this study generally supported a lower diversity of grassland fungi than grazed pastures, their value for both flora and fungi increases the importance of securing appropriate long-term management for this fragile resource.

The eDNA analysis of sites previously thought to have been mediocre or degraded in terms of their fungal interest has in fact shown that two of the four ranked in the top five sites for fungal diversity. This illustrates the importance of both sufficient field survey and supplementary investigation such as the DNA work to give a true picture of site importance.

CONCLUSIONS

The information below is summarised from the full report on eDNA analysis conducted in 2021 (Griffith et al 2022) added to our findings from field survey.

Take home messages

1) Afforestation / management changes

There is a nationwide drive towards more tree planting as a carbon sequestration tool; however, concerns remain about the impact that such a change of management would have on other interest features, notably grassland fungi. As the timing of field survey and specialist knowledge of the surveyor is so crucial to gaining an understanding of the fungal value of a site, we would recommend additional data are gathered before afforestation is considered.

In our eDNA study, all of the four sites threatened with afforestation host diverse grassland fungal populations and should be protected from destruction by tree-planting. It is not clear how quickly the grassland fungi would decline if trees were planted, likely slowly (e.g. over 10 year timescale). From our current knowledge of the ecology of these fungi, the likely driver of loss will be the proliferation of ectomycorrhizal fungi associated with the trees (Sepp et al., 2021).

Similarly changes in consistency of management such as relaxing management or re-wilding are likely to have detrimental impacts on fungi and the cost versus benefit should be carefully considered.

2) Additional conservation actions

Several of the sites host exceptionally diverse grassland fungal populations and 32 would merit notification as SSSIs (Bosanquet et al 2018). Only eight of our 54 surveyed/studies sites are within the Leek Moors SSSI or Hamps and Manifold Valleys SSSI, but grassland fungi are not a notified interest feature of those sites. 33 are currently managed (or have been until recently) in some form of agri-environment scheme, although not necessarily under prescriptions designed to benefit grassland fungi. Negotiation of appropriate management agreements and/or notification as SSSI could offer owners further management guidance, financial benefit and site protection.

3) Proxies for identification of sites/quadrats with diverse grassland fungal populations

Whilst areas with diverse higher plant populations will likely also host diverse populations of CHEGD fungi due to lack of past disturbance via ploughing or fertiliser application, heavy grazing can also result in loss of plant diversity yet leave the CHEGD fungal populations undamaged. Thus, an apparently uninteresting, heavily grazed site can yield surprising results for fungi.

Our data have not shown any clear correlation between vegetation species/diversity and fungal diversity or abundance; however, it should be noted that the vegetation data gathered from soil eDNA cannot be used as a direct reference to actual species presence, due to seasonality. Annual species were poorly represented in soil samples collected in the autumn. Mosses (bryophytes) were also poorly represented in the soil eDNA as they have rhizoids (simple hair-like projections which anchor the plant in the substrate) rather than roots, so it is unlikely that they would form an association with soil fungi; however, the two are often found together and indicate low nutrient grasslands.

Levels of total and available phosphorus (P) and potassium (K), and total nitrogen (N) showed a predominantly negative relationship with species richness of CHEGD fungi, which is consistent with the negative effects of synthetic (i.e. NPK) fertiliser addition. P is a highly immobile and insoluble nutrient, remaining within the soil for long periods, so soil P levels provide a good indicator of past fertiliser

application which may explain the observed negative relationship, even on sites which are now managed less intensively.

Simple measurements such as pH and soil moisture, which have shown correlations with fungal diversity could be used to provide useful information on the suitability of sites to support grassland fungi (i.e. sites with pH greater than 4.5 and soil moisture in late summer/autumn of 25-35%).

4) Soil organic matter levels

A key driver for tree planting is to enhance carbon sequestration but there is low awareness beyond the scientific community that most carbon is sequestered below ground, rather than in visible vegetation. All the soils in this study had moderate C levels in the top 10cm sampled (mean 9.6%C = ca. 96 T/ha) and likely at least twice as much in deeper soil horizons (i.e. 200-340 T/ha in total). For comparison, the trees in a 50 year old Sitka spruce plantation could contain ca. 100 T/ha C in above-ground vegetation depending on their planting density and growth rates, with 73-120T/ha below ground (Anderson, 2021).

Aside from questions as to how well-sequestered the harvest wood is (depends on intended use), on organo-mineral soils, there is invariably more C in the soil than visible in vegetation. To our knowledge, there are no studies showing net gain of soil C following tree planting on undisturbed grasslands but there are many that demonstrate loss of soil C following tree planting, for example, Upson et al. (2016) and Poeplau et al. (2011).

5) Recommendations for management of waxcap grasslands

The longstanding recommendations for 'waxcap grasslands' (avoidance of synthetic fertiliser, slurry and manure) should be followed (Griffith et al., 2004; Halbwachs et al., 2018). The effects of periodic lime addition are less clear; very high levels can have a detrimental effect on fruiting (Halbwachs et al., 2018) but the longer term effects of a normal application rate (5T/ha at 5-10 yr intervals) could be beneficial in some cases, since it prevents excessively low (<4.5) soil pH levels. However, these grasslands would support other botanical interest, so management change is not advocated.

Some positive correlations between particular sward management regimes and CHEGD diversity/abundance were observed, with pasture management and sheep grazing being beneficial to CHEGD fungi. Sward height is known to affect fruiting of CHEGD fungi (Griffith et al., 2012) but there is no evidence that it influences levels of mycelium in the soil. CHEGD fungi are long-lived so do not need to reproduce annually and even absence of any grazing for several years would not necessarily lead to decline. Ultimately failure to reproduce would lead to decline on decade/century timescales. Similarly, long term absence of grazing would lead to successional changes in plant communities.

It is important to recognise that the correlations between sward management and CHEGD fungi observed in our eDNA study do not imply causality. For example, some upland pastures are less likely to be disturbed due to inaccessibility and for the same reasons less likely to be managed as hay meadows or grazed by cattle. However, since CHEGD-rich hay meadows are much rarer than CHEGD -rich sheep-grazed pastures, their conservation value is greater, for example, Greenland and Howe Green.

6) Taxonomic considerations

The approach to eDNA analysis of grassland fungi as 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 (waxcaps), there are reference barcodes for nearly all the UK species. A cautious approach is needed when considering the introduction of new names; unless the new taxa are not 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.

7) Need for fruit body surveys and novel approaches

This project has highlighted the importance and value of both dedicated field survey and eDNA analysis to fully understand the conservation value of sites. An experienced and knowledgeable field surveyor can cover a large area (given sufficient time, ideally three visits per year for more than one year) and identify fruit bodies across several hectares of a site, provided conditions are suitable. Soil sampling for eDNA analysis following the methodology in this project can only assess a relatively small area but only a single sampling visit is required, and can take place at any time of year.

eDNA provides accurate identification and evidence that a particular species is present, however, whether the mycelia detected represent mature individuals is more difficult to assess via eDNA. Therefore, confirmation of the occurrence of (especially) rarer species should be obtained by targeted field surveying of fruit bodies. The apparent rarity of some fungal species may be attributable to their infrequent fruiting. For example, Dermoloma magicum, though considered to be rare in the UK and globally, with only 5 UK records listed in GBIF (the Global Biodiversity Information Facility) was found in 56/116 of our soil quadrats, often in abundance. This species is clearly more widespread and common than fruit body surveys would lead us to believe (the same pattern has been observed at many other sites), likely because it fruits only very rarely.

Cost comparison between field survey and eDNA analysis is not absolute as it depends on many variable costs including staff time for coordination and gaining access permissions from landowners, survey/sampling visits, laboratory analysis and report writing. Field surveys can be scaled as required whereas eDNA analysis is currently not cost effective for a small number of samples.

8) The importance of retaining voucher specimens from field surveys

Even with access to high quality field guides and expertise in microscopy, some species identifications are still difficult. The fact that there are often discrepancies between eDNA data and field survey data 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 higher value in terms of fungal biodiversity and which may be candidates for subsequent designation, we suggest that dried specimens are routinely retained from field surveys. Such collections are not bulky and if kept in a plastic box with desiccant, it will be possible to extract DNA for future DNA barcoding for many years.

Future work

A) Awareness raising

Increasing awareness and understanding of the conservation value of grassland fungi will help in identifying and managing sites. The broader priority across the UK is to raise awareness that tree-planting is not necessarily a beneficial activity even if generous subsidies are available. It needs to be communicated that this is for two reasons, first that grassland habitats containing valuable biodiversity at a global level may be destroyed, and second that 'marginal' grasslands (less productive agriculturally and thus where the business case for afforestation is stronger) are already high in soil carbon (mean 9.6% carbon here in the upper 10cm), so the potential for firstly, the loss of this carbon through soil disturbance in the planting process and secondly, any increase in soil C sequestration from the trees is low. The deeper cycling of nutrients with ecologically different plants (trees as opposed to adapted acid grassland plants and lots of mosses) changes the soils and results in faster turnover rates of litter and dead material, hence recycling carbon more into the air than combining it in to the soil.

B) Ensuring that regulations are enforced

In Wales, the current 'light touch' assessment of afforestation programmes results in inadequate biodiversity assessment (i.e. no consideration of grassland fungal populations). Often no on-site biodiversity (EIA) assessment is done, and for smaller sites (<2 hectares in Wales), no EIA assessment is

required. In England there is also a threshold of 2 hectares (ha), either in one block or as a total on one holding, below which an EIA screening decision is not required; although Natural England will consider proposals that affect land of a smaller area that is semi-natural, has heritage features, and/or has special landscape features, e.g. historic parkland. Planting or natural regeneration of less than 0.5 ha is not considered afforestation under the forestry EIA regulations unless it is adjacent to another afforestation project completed in the preceding five years. There is a presumption against there being a likely significant effect from planting between 0.5 and 2ha within a National Park unless within a defined 'sensitive area', however the Forestry Commission must 'provide an opinion'. Forestry Commission England EIA scoping guidance advises describing communities of plants and animals but not fungal populations. On the basis of the evidence presented here, this stance needs to change.

C) Rapid assessment of waxcap grasslands

With incomplete habitat data, inadequate regulation and poor enforcement of existing regulations, there is a high likelihood that many biodiverse grasslands will be lost to afforestation, or other changes in management. Rapid assessment of grassland habitats, for example with citizen scientists using novice waxcap keys ((Griffith et al., 2004) and the Plantlife leaflet based on this (Harries and Lamacraft, 2014)) would cover many sites at low cost. However, the issue of permission to access private land presents a problem. Promising sites could be later assessed via eDNA and field survey.

The use of field-based pH/moisture meters is potentially helpful but perhaps a more useful approach would be to directly measure moisture from small soil samples (i.e. wet/dry weight measurement) which can be undertaken outside a laboratory. Caution must be used here as precipitation levels will affect measurements. Similarly, measurement of pH off-site from soil slurry would provide more reliable measurement than field-deployed meters. Direct assessment of soil carbon requires laboratory analysis but some methods have been developed for colour-based assessment of soil organic matter which can be conducted in the field (Aitkenhead et al., 2015). In addition, loss on ignition to identify the organic matter in the upper horizon is a standard soil measure, and several studies have shown about 58% of this is carbon, this could then be used as a proxy measure.

D) Methodological improvements

Whilst the cost of high-throughput sequencing has fallen substantially, the limiting factor cost-wise is person-time for the collection of soil samples and preparation of eDNA for sequencing. At sufficient scale, the cost of the latter could be reduced. Since eDNA analysis, as conducted here, is a direct measurement of fungal diversity, it is not likely that there will be radical technological advances in the short-term (<5yrs). From an ecological perspective, economical/reliable quantification of microbial biomass in soil is a priority. At the same time, the differences in the results obtained by field assessment and eDNA need to be addressed. The evaluation of the sites (for SSSI notification) is geared currently to field assessment, but there needs to be some calibration between the eDNA results and the field surveys in order to evaluate sites consistently.

E) Recording and sharing of survey data

Of the 54 sites included in this project, only 7 appear completely, and 13 in part, on Natural England's Priority Habitat Inventory which is currently used in targeting for agri-environment schemes and woodland creation. The reasons for this are twofold: firstly, 'waxcap grassland' is not in itself a priority habitat type, rather an assemblage of fungi species which can inhabit a range of priority to non-priority grassland types; secondly there has been less focus on surveying and recording this species assemblage, predominantly because it is not a priority habitat type, and due to insufficient numbers of skilled surveyors. It is a priority to increase the amount of survey work conducted and share the data with Natural England and others, and for these data to be added to targeting layers in order to conserve many sites, and individual fungi species, of international and national significance.

OVERALL SITE SUMMARY

The table below summarises the results of all field survey and laboratory analysis during this project, giving the level of importance for fairy clubs (C), waxcaps (H), pinkgills (E), earthtongues (G), and crazed caps (D) at each site. Note that only waxcaps (H) have recognised thresholds for all levels of importance from local to international. The number of visits is combined for all survey years.

Site name	Survey year(s)	Number of visits	Field survey CHEGD	Level of importance (from field)	eDNA CHEGD	Level of importance (from eDNA)*
Back of the brook	2020	2	C2 H12 E6 G0 D0	Regional (H)	n/a	n/a
Badger's Croft	2021	#	C3 H3 E1 G0 D0	Local (H)	C15 H10 E9 G8 D1	Regional (H) National (C,G)
Ball Bank House	2020	1	C4 H12 E2 G1 D0	National (H)	n/a	n/a
Blackhill Gate Farm	2017, 2018, 2019	5	C3 H16 E8 G2 D2	Regional (H)	n/a	n/a
Boothlow Hayes	2020	2	C23 H18 E4 G1 D1	National (H)	n/a	n/a
BF Anon 1	2020	4	C9 H17 E18 G3 D0	International (H) National (C,E)	C18 H20 E15 G10 D2	International (H), National (C,E,G)
Brownsetts	2021	#	C5 H18 E8 G2 D0	National (H)	C21 H26 E24 G10 D2	International (H), National (C,E,G)
Cut-thorn	2021	#	C3 H10 E4 G0 D0	Regional (H)	C13 H18 E13 G7 D1	International (H), National (C,G)
Far Brook Farm	2019, 2020	4	C5 H18 E9 G3 D1	International (H)	n/a	n/a
F Anon 4	2020	3	C7 H21 E10 G3 D0	International (H)	C19 H13 E8 G10 D3	National (C,H,G,D)
Franklins Farm (Bluehills)	2020	3	C7 H23 E15 G2 D0	International (H) National (E)	C18 H18 E24 G12 D1	International (H), National (C,E,G)
Gag Aye	2021	#	C3 H13 E5 G1 D0	National (H)	C12 H20 E10 G6 D1	International (H), National (C,G)
Gleadtail	2019, 2020	4	C4 H13 E5 G1 D0	Regional (H)	n/a	n/a
Greenlands	2019, 2020	4	C5 H20 E18 G0 D0	International (H) National (E)	C16 H14 E18 G8 D1	National (C,H,E,G)
Hazelhurst	2019, 2020	5	C4 H21 E15 G3 D0	International (H) National (E)	n/a	n/a
Hedgerow Farm	2017	1	C2 H12 E2 G0 D0	Regional (H)	n/a	n/a
Herbage	2019, 2020	3	C2 H13 E6 G0 D1	National (H)	n/a	n/a
High Ash Farm	2020	5	C3 H20 E10 G3 D0	International (H)	n/a	n/a
Higher Minnend Farm	2019	1	C5 H16 E4 G0 D1	National (H)	n/a	n/a
Howe Green	2021	#	C2 H11 E4 G2 D0	Regional (H)	C18 H17 E14 G8 D0	National (C,H,G)

Hurdlow Farm - main holding	2019, 2020	4	C10 H25 E18 G5 D3	International (H) National (C,E,G,D)	n/a	n/a
Kisswood Farm	2019, 2020	2	C5 H19 E3 G1 D0	International (H)	n/a	n/a
Knotbury	2021	#	n/a	n/a	C13 H10 E18 G7 D0	Regional (H) National (E,G)
Knotbury Common	2021	#	n/a	n/a	C15 H15 E14 G8 D1	International (H), National (C,G)
Lamaload Clough	2018, 2019	3	C0 H13 E5 G0 D0	Regional (H)	n/a	n/a
Leycote	2021	#	C8 H31 E18 G4 D0	International (H)	C20 H27 E22 G10 D2	International (H), National (C,E,G)
Lower Elkstones	2019, 2020	4	C5 H19 E11 G2 D0	International (H)	n/a	n/a
Lower Fleetgreen	2019	1	C7 H17 E6 G3 D0	National (H)	n/a	n/a
Lower Fleetgreen Outliers	2021	#	C0 H5 E3 G0 D0	Local (H)	C21 H23 E22 G8 D1	International (H), National (C,E,G)
Lower Whitle	2020	1	C0 H0 E0 G0 D0	None	n/a	n/a
Lower Wigginstall	2020	2	C4 H12 E3 G1 D0	Regional (H)	n/a	n/a
Lumbs Farm	2020	2	C3 H11 E2 G1 D0	Regional (H)	n/a	n/a
Mill Lane Farm	2020	1	C1 H6 E0 G2 D0	Local (H)	n/a	n/a
Mixon Grange	2019, 2020	2	C2 H13 E4 G0 D0	National (H)	n/a	n/a
Noon Sun	2019, 2020	4	C6 H17 E11 G5 D1	National (H,G)	n/a	n/a
Oils Heath	2020	2	C3 H17 E3 G0 D0	International (H)	C11 H17 E10 G9 D2	International (H), National (C,G)
Orchard Farm	2020	3	C2 H19 E4 G3 D1	International (H)	n/a	n/a
Pyeclough	2021	#	C0 H2 E1 G0 D0	None	C19 H18 E19 G8 D1	International (H), National (C,E,G)
RK Anon 2	2021	#	C0 H2 E2 G0 D0	None	C13 H9 E7 G10 D2	Regional (H), National (G)
SH Anon 3	2021	#	n/a	n/a	C20 H21 E13 G10 D2	International (H), National (C,G)
Shawbottom	2021	#	C3 H10 E6 G1 D0	Regional (H)	C11 H8 E6 G8 D0	Regional (H), National (G)
Steps	2021	#	C0 H1 E0 G0 D0	None	C5 H6 E0 G5 D1	Local (H)
Summerclose	2021	#	C2 H10 E9 G0 D1	Regional (H)	C19 H21 E19 G8 D2	International (H), National (C,E,G)
Sutton End Farm	2018	1	C0 H1 E0 G0 D0	None	n/a	n/a
Swainsmoor	2019	1	C6 H22 E9 G4 D1	International (H)	n/a	n/a
Torgate Farm	2019	1	C1 H7 E0 G0 D0	Local (H)	n/a	n/a
Tunstead	2021	#	C7 H15 E6 G0 D0	National (H)	C15 H13 E10 G7 D1	Regional (H), National (G)

Under Whitle	2020	4	C5 H17 E1 G0 D0	National (H)	C14 H9 E6 G10 D3	Regional (H), National (G,D)
Upper Brownhills	2021	#	C0 H2 E0 G0 D0	None	C14 H8 E9 G8 D3	Regional (H), National (C,D)
Upper Whitle	2020	2	C1 H13 E2 G1 D0	National (H)	n/a	n/a
Waggonshaw Brow	2020	2	C6 H19 E3 G6 D0	International (H) National (G)	n/a	n/a
Waterfall	2020	2	C0 H1 E0 G0 D0	None	C20 H10 E7 G10 D4	Regional (H), National (C,G,D),
Waterfall Low	2020	2	C1 H5 E1 G0 D0	Local (H)	C26 H19 E18 G15 D4	International (H), National (C,E,G,D)
Wilshaw Farm	2020	1	C1 H13 E3 G1 D0	National (H)	n/a	n/a

Table 5. Summary of all field survey and eDNA analysis results

***** There is currently no recognised threshold to measure site importance using eDNA analysis, thus the thresholds used for field survey have been used here as a guide.

No full field surveys were conducted by experts in 2021, but casual records were made during soil sampling visits.

The table shows the level of knowledge gained about this array of sites as a result of this project and indicates the value of both field survey and laboratory analysis to assess the level of importance of sites. Undoubtedly laboratory analysis has increased our understanding of the diversity of fungi species present at each of these sites and supplements the knowledge gained from field survey. However, eDNA analysis of soil samples can only detail the presence and abundance of species within the soil, it cannot indicate whether those species are mature individuals capable of producing fruiting bodies. At present this can only be ascertained via field survey by a competent surveyor. The two methods of site assessment are complimentary and the relative cost and time for either method should be evaluated when considering the purpose of site assessment.

PHOTOGRAPHIC CREDITS

Front cover image: Pink or Ballerina waxcap (Porpolomopsis calyptriformis), photograph courtesy of Alex Hyde

Page 7: Crested coral (Clavulina coralloides), indigo pinkgill (Entoloma chalybaeum), yellow-legged fanvault (Camarophyllopsis micacea), photographs by Neil Barden

Page 9: Site of international importance for waxcaps and national importance for all other groups, photograph by Karen Shelley-Jones

Page 11: Volunteer training day (top), left - parrot waxcap (Gliophorus psittacinus), right - an earthtongue, photographs by Karen Shelley-Jones

Page 12: Junior rangers, photographs by Jackie Wragg

Page 17: Neil Barden collecting soil samples and testing for soil pH, photographs courtesy of Alex Hyde

Page 20: Gloioxanthomyces vitellinus, photograph by Neil Barden

Back cover (clockwise from top left): yellow spindles (Clavulinopsis fusiformis), violet coral (Clavaria zollingeri), parrot waxcap (Gliophorus psittacinus), star pinkgill (Entoloma conferendum), Entoloma clandestinum, a crazed-cap (Dermoloma cunefolium), olive earthtongue (Microglossum olivaceum), big blue pinkgill (Entoloma bloxamii), honey waxcap (Hygrocybe reidii), photographs by Neil Barden

REFERENCES

Aitkenhead, M., Donnelly, D., Sutherland, L., Miller, D., Coull, M., Black, H., 2015. Predicting Scottish topsoil organic matter content from colour and environmental factors. European Journal of Soil Science 66, 112-120

Anderson, P. 2021. Carbon and ecosystems: restoration and creation to capture carbon. CIEEM In Practice, issue 111.

Bosanquet, S.D.S., Ainsworth, A.M., Cooch, S.P., Genney, D.R, & Wilkins, T.C. (2018). Guidelines for the Selection of Biological SSSIs. Part 2: Detailed Guidelines for Habitats and Species Groups. Chapter 14 Non-lichenised Fungi. Joint Nature Conservation Committee, Peterborough.

Evans, S., Marren, P. & Harper, M. (2002). Important Fungus Areas: a provisional assessment of the best sites for fungi in the United Kingdom. Plantlife International, Salisbury, UK.

Griffith, G.W., Bratton, J.L., Easton, G.L., 2004. Charismatic megafungi: the conservation of waxcap grasslands. British Wildlife 15, 31-43.

Griffith, G.W., Roderick, K., Graham, A., Causton, D.R., 2012. Sward management influences fruiting of grassland basidiomycete fungi. Biological Conservation 145, 234–240.

Griffith, G.W., Camarra, J.G.P, Holden, E.M., Mitchel, D., Graham ,A., Evans, D.A., Evans, S.E., Aron, C., Moordeloos, M.E., Kirk, P.M. and Smith, S.L.N. 2013. The international conservation importance of Welsh 'waxcap' grasslands. Mycosphere 4, (5), 969-984.

Gareth W Griffith, Andrew P Detheridge and Ruby Bye, 2022. Use of eDNA analysis of soil samples to evaluate the fungal conservation value of grassland areas in the South West Peak. IBERS, Aberystwyth University. Internal report to Peak District National Park Authority.

Halbwachs, H., Easton, G.L., Bol, R., Hobbie, E.A., Garnett, M.H., Peršoh, D., Dixon, L., Ostle, N., Karasch, P., Griffith, G.W., 2018. Isotopic evidence of biotrophy and unusual nitrogen nutrition in soil-dwelling Hygrophoraceae. Environmental microbiology 20, 3573-3588.

Harries, D., Lamacraft, D., 2014. Waxcaps and grassland fungi: A guide to identification and management. https://www.plantlife.org.uk/application/files/6915/0460/9899/Waxcap_ID_guide_low_res_website.pdf Published by Plantlife Cymru.

Poeplau, C., Don, A., Vesterdal, L., Leifeld, J., Van Wesemael, B., Schumacher, J., Gensior, A., 2011. Temporal dynamics of soil organic carbon after land-use change in the temperate zone–carbon response functions as a model approach. Global change biology 17, 2415-2427.

Rald E. (1985). Vokshatte som indikatorarter for mykoloisk vaerdifulde overdrevslokaliteter - Svampe11: 1-9.

Rotheroe M, (1999). Mycological survey of selected semi-natural Grasslands in Camarthenshire. CCW Contract Science Report no. 340.

Rotheroe M (2001). A preliminary survey of Waxcap grassland indicator species in South Wales. In Fungal Conservation: Issues and Solutions. D Moore, M Nauta, S Evans and M Rotheroe eds. CUP 2001.

Sepp, S.K., Davison, J., Moora, M., Neuenkamp, L., Oja, J., Roslin, T., Vasar, M., Öpik, M., Zobel, M., 2021. Woody encroachment in grassland elicits complex changes in the functional structure of above-and belowground biota. Ecosphere 12, e03512.

Upson, M.A., Burgess, P.J., Morison, J., 2016. Soil carbon changes after establishing woodland and agroforestry trees in a grazed pasture. Geoderma 283, 10-20.

Vesterholt, J., Boertmann, D and Tranberg, H. (1999). Et usaedvanlig godt ar for overdrevssvampe. Svampe, 40 36-44.