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## – Grassland Fungi (and Flora) eDNA



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Report by:

Andy Karran

Gwent Wildlife Trust

Evidence Manager



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## Grassland Fungi (and Flora) – eDNA

<b>Client:</b>	Monmouthshire County Council – LEADER Funding
<b>Surveyor(s):</b>	Andy Karran MSc, BSc, MIEEM
<b>Author(s):</b>	Andy Karran MSc, BSc, MIEEM
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## 1.0 INTRODUCTION

The overarching purpose of this eDNA study is to seek cost effective, innovative solutions to make rapid assessments of biodiversity with potential future applications in Gwent Wildlife Trust's work, and more widely in that of NRW. This will allow the quicker assessment of protected site conditions so more sites can be surveyed with limited resources and for the Sustainable Farming Scheme to gather baseline and post intervention information.

We have 400+ Local Wildlife Sites (LWS) within Monmouthshire, some 80+ of these were recognized during previous LEADER funding in 2012-2014. For the hundreds of sites our survey work has been almost exclusively in the spring/summer focusing on the flora. These sites, particularly the grasslands may well have important fungi communities at other times of the year (principally autumn) which we have never had the opportunity to investigate. Even if we were to survey at the appropriate time of the year, the fungi are unpredictable in when they appear and ephemeral making survey work problematic. The visible toadstools are just the fruiting bodies with their "invisible" mycorrhiza being present all year round. To remedy this we took soil samples from 30 sites (refer to Figure 1.0 and Figures 2.1-2.30 for site locations) and sent these off for eDNA analysis to get a far better picture of the fungi communities present and their ecological value.

From this we can re-engage landowners with the value of their sites, better inform the sites management and educate the landowners.

Also for some of the sites we already had a good idea of the grassland fungi community from more conventional surveys and this allowed us to compare results and assess the effectiveness of the eDNA.

Additionally, the eDNA returned floral as well as fungal results so that we were to assess if there was a good correlation between known floral diversity of fields from conventional field surveys and that gained from eDNA. This allowed us to draw conclusions as to whether it was a valid technique to assess value of fields quickly and perhaps ultimately cheaper and out of season, or at least earmark which fields warrant further survey work.



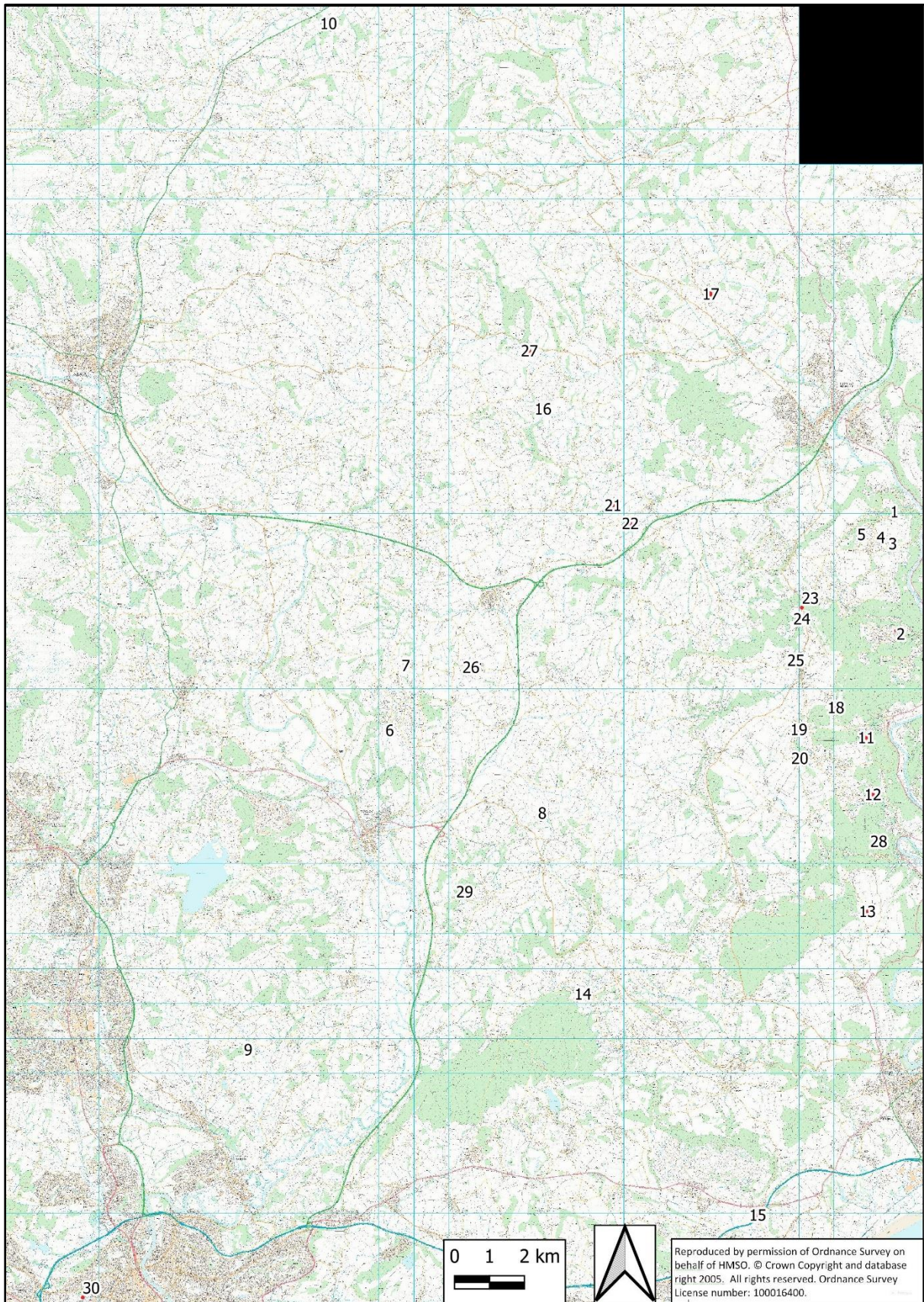


Figure 1.0 – Site Locations

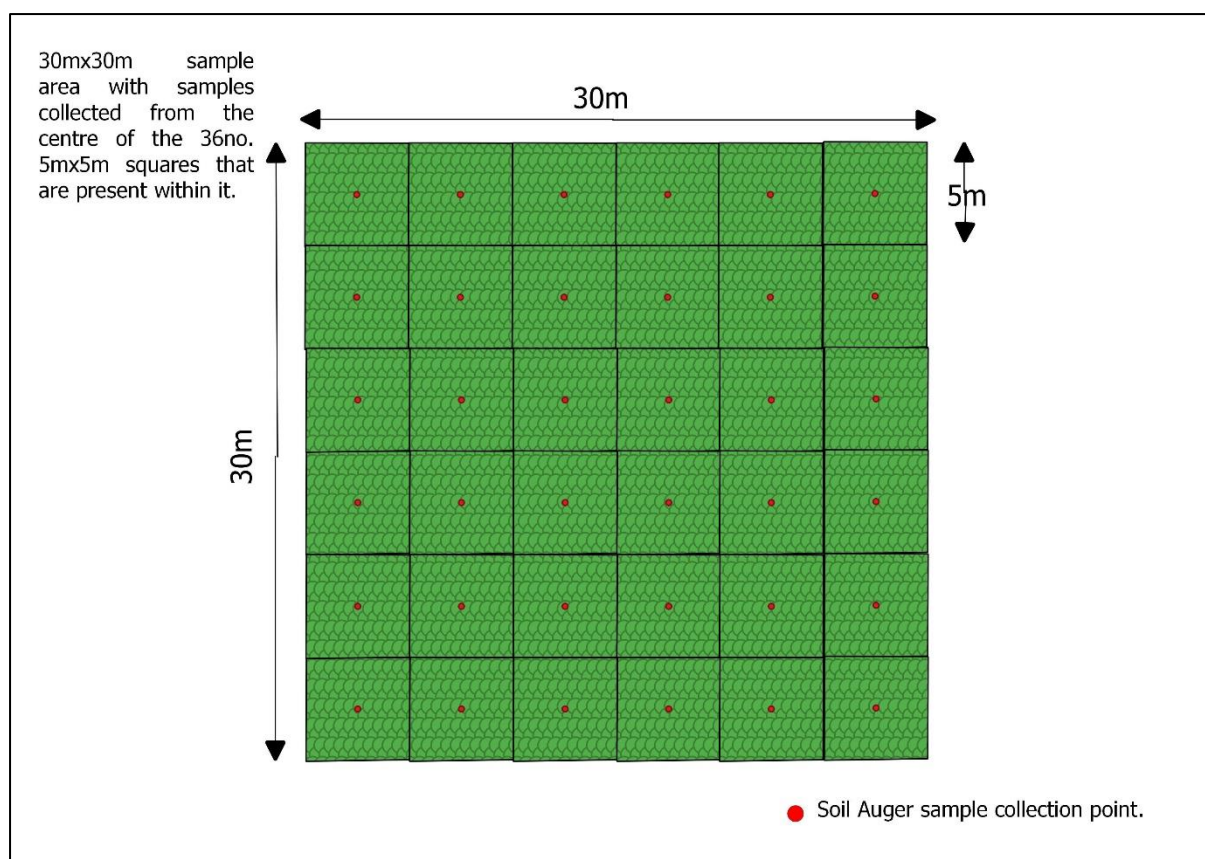


## 2.0 METHODOLOGY

### 2.1 Sampling Methodology

In March/April efforts were made to contact landowners with known grassland fungi communities on their land and also publicise the project to find further landowners we were currently unaware of. A total of 43 Landowners were engaged with, and from these 30 suitable sites (belonging to 29 different landowners), were selected and permission to sample given. The sampling was conducted during the period of the 25<sup>th</sup> April to the 11<sup>th</sup> May 2022.

At each site a 30mx30m area was identified, either through landowners advising the area where fungal presence was best, or in the absence of this a valued judgement taken by the surveyors to find the best location. The 30mx30m area was marked out using measuring tapes. The grid reference of the corners of the square were recorded so that these polygons could be drawn in GIS Software (these polygons are shown within the results in Figures 2.1-2.30, with the centre point of the polygon recorded). The area was further divided into 36no. 5mx5m squares. A single soil sample was taken from the centre of each of the 5mx5m squares using a 15mm soil auger to a depth of 10cm. The 36 soil cores were collected together in to a single ziplock bag taking care not handle the soil samples.



Each ziplock bag was labelled with site details and refrigerated as soon as the surveyors returned home and remained refrigerated until picked up by courier the next day and then delivered to Aberystwyth University for eDNA analysis the day after this. This meant that samples were only two days between collection and arriving at the laboratory and kept cold for as much of this time as possible to preserve the eDNA.

Once received by Aberystwyth University the samples were suitably stored until the eDNA analysis could be undertaken. The results of the analysis together with interpretation were then provided to Gwent Wildlife Trust to be incorporated within this report.

## 2.2 Background to eDNA methodology

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/Pseudotracholoma*) are of conservation concern. The definition of the species included in the 'CHEGD' group are described by Griffith et al. (2013). 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 new 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 internal transcribed spacer (ITS) and Large SubUnit (LSU) and regions. We have opted for the former (i.e. ITS2), which is the primary barcoding locus for fungi and thus has better coverage of species. Good species coverage across all fungi and plants is ensured by use of the mix of primers suggested by Tedersoo et al. (2014).

We occasionally use the LSU locus for metabarcoding; whilst overall species resolution is poorer, it is effective at species separation for CHEGD (but less good for other groups (e.g. Polyporales -wood-decay fungi). However, LSU provides better quantification of relative abundance for different species. The reason for this is that the LSU amplicon size is very consistent across all fungi (ca. 220bp), whereas amplicons for ITS2 vary in size from 280-540bp, which ITS2 in basidiomycete fungi generally being longer. A consequence of this is that the relative abundance of basidiomycetes (including most CHEGD fungi apart from Geoglossaceae) is under-estimated, relative to ascomycetes.

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 the (thick-walled) spores of some species is less efficient than from actively-growing mycelia. Additionally, the rRNA operon is a multicopy operon and it is estimated that ca. 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, as recently identified by Lofgren et al. (2019) 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 ca. 900 m<sup>2</sup> 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 quadrats, the 36 cores taken on a grid pattern weigh ca. 700-1000 g, suitable for convenient freeze 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. 50,000 per sample) can provide not only identification but also relative abundance information. However, as noted above, the alignment of 'genetic' and morphological species concepts is still not complete and the taxonomy of some fungal families examined here is currently in flux. We also do not yet know the extent to which fungal biomass 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 bp 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.

### **2.3 eDNA Methodology**

Sample preparation: On receipt of each sample the soil was weighed and immediately frozen at -80°C. After 24 hours the soils were freeze dried to remove the water without it entering the liquid phase hence suppressing biological activity during drying, which could affect the fungal community profile. Dried soil samples were finely ground by passing through a 2 mm wire sieve. After thorough mixing, a 50 g subsample was further ground through a 0.5 mm sieve. The moisture content of the samples was in the range 14-34% (mean 23%; Table1), slightly drier than we generally receive from the more westerly parts of the UK, likely due to the dry spring in 2022. Compared to the majority of the samples we have analysed, the samples provided in this survey were larger than we usually receive (generally ca. 1600g), due to the use of an 18 vs 15 mm auger but it is unlikely that this had a significant effect on the fungi detected. Following grinding, 150 mg of soil was taken for DNA extraction using the Qiagen *Powersoil* Soil DNA extraction kit.

Genetic analysis: PCR amplification of a 3-400 bp portion of the ITS2 region of the ribosomal RNA locus was amplified with the primer mix devised by Tedersoo et al. (2014). These primers are specific but also amplify Oomycetes. In order to allow several samples to be sequenced in a single sequencing run, a second round PCR amplification was undertaken to add unique 10bp identifier tags to sequences from each quadrat. Following PCR amplification, PCR products were quantified using a Qubit fluorometer (Invitrogen) and pooled in equimolar concentrations. The pooled library was purified using AMPure XP beads (Beckman Coulter) and the library checked and quantified with a Bioanalyzer High Sensitivity DNA analysis (Agilent). The pooled sample DNA was sequenced using an Illumina MiSeq High Throughput DNA sequencer employing the MiSeq Reagent Kit v3 (600-cycle) to give 2x300 bp paired end reads. The full method for DNA extraction, PCR amplification and bioinformatics analyses are published in Detheridge et al. (2016; 2018).

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 a total of 3,944,912 DNA sequences. Sequences were clustered to group identical sequences, and clusters containing a single sequence (219,103 'singletons') were discarded. Inclusion of a small amount of the plant forward PCR primer (Chen et al., 2010) allowed some amplification of plant DNA barcodes (476,919 sequences) with the remainder being fungi (3,248,890).

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## 3.0 RESULTS

### Fungi

The results returned by Aberystwyth University highlighted the presence of fungi that are used in the CHEGD\* assessment of fungal value of grassland sites.

\*CHEGD stands for the first letter of the 5 key fungi groups involved in this assessment and is a standard, recognised way to assess the value of Grassland Fungi sites:

**C**lavarioids (spindles, club and coral fungi)

**H**ygrocye (although recent DNA work has split this up) genus (Waxcaps)

**E**ntoloma (pinkgills)

**G**eoglossum (earthtongues and relatives)

**D**ermoloma (Crazed caps and relatives)

A total of 100 species were identified across the 30 sites in the following numbers:

**C**lavarioids = 28 species

**H**ygrocye = 30 species

**E**ntoloma = 26 species

**G**eoglossum = 14 species

**D**ermoloma = 2 species

The full list of these is show within Table 1.1 is as follows:

Scientific Name	English Name
C1 Camarophyllopsis atrovelutina	
C2 Camarophyllopsis schulzeri	
C3 Clavaria amoenoides	
C4 Clavaria appendiculata	
C5 Clavaria californica	
C6 Clavaria falcata	
C7 Clavaria flavipes	Straw Club
C8 Clavaria flavostellifera	
C9 Clavaria fragilis	White Spindles
C10 Clavaria fumosa	Smoky Spindles
C11 Clavaria griseobrunnea	
C12 Clavaria messapica	
C13 Clavaria pullei	
C14 Clavaria redolealii	
C15 Clavaria tenuipes	
C16 Clavaria vermiculata	
C17 Clavulinopsis corniculata	Meadow Coral
C18 Clavulinopsis helvola	Yellow Club
C19 Clavulinopsis hisingeri	
C20 Clavulinopsis laeticolor	Handsome Club
C21 Clavulinopsis luteoalba	Apricot Club
C22 Clavulinopsis luteonana	Dwarf Spindles
C23 Clavulinopsis umbrinella	Beige Coral
C24 Hodophilus micaceus	

C25 <i>Lamelloclavaria petersenii</i>	
C26 <i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.
C27 <i>Ramariopsis crocea</i>	a coral fungus sp.
C28 <i>Ramariopsis flavescens</i>	a coral fungus sp.
UNIDENTIFIED FAIRY CLUBS	
H1 <i>Cuphophyllus canescens</i>	a Waxcap sp.
H2 <i>Cuphophyllus flavipes</i>	Yellow Foot Waxcap
H3 <i>Cuphophyllus flavipesoides</i>	a Waxcap sp.
H4 <i>Cuphophyllus fornicatus</i>	Earthy Waxcap
H5 <i>Cuphophyllus pratensis</i>	Meadow Waxcap
H6 <i>Cuphophyllus russocoriaceus</i>	Cedarwood Waxcap
H7 <i>Cuphophyllus virgineus</i>	Snowy Waxcap
H8 <i>Gliophorus europerplexus</i>	Butterscotch Waxcap
H9 <i>Gliophorus irrigatus</i>	Slimy Waxcap
H10 <i>Gliophorus psittacinus</i>	Parrot Waxcap
H11 <i>Hygrocybe cantharellus</i>	Goblet Waxcap
H12 <i>Hygrocybe ceracea</i>	Butter Waxcap
H13 <i>Hygrocybe chlorophana</i>	Golden Waxcap
H14 <i>Hygrocybe citrinovirens</i>	Citrine Waxcap
H15 <i>Hygrocybe coccinea</i>	Scarlet Waxcap
H16 <i>Hygrocybe conica</i>	Blackening Waxcap
H17 <i>Hygrocybe glutinipes</i>	Glutinous Waxcap
H18 <i>Hygrocybe helobia</i>	Garlic Waxcap
H19 <i>Hygrocybe insipida</i>	Spangle Waxcap
H20 <i>Hygrocybe intermedia</i>	Fibrous Waxcap
H21 <i>Hygrocybe miniata</i>	Vermillion Waxcap
H22 <i>Hygrocybe phaeococcinea</i>	Shadowed Waxcap
H23 <i>Hygrocybe punicea</i>	Crimson Waxcap
H24 <i>Hygrocybe quieta</i>	Oily Waxcap
H25 <i>Hygrocybe reidii</i>	Honey Waxcap
H26 <i>Hygrocybe subpapillata</i>	Papillate Waxcap
H27 <i>Hygrocybe substrangulata</i>	a Waxcap sp.
H28 <i>Neohygrocybe ingrata</i>	Dingy Waxcap
H29 <i>Neohygrocybe ovina</i>	Blushing Waxcap
H30 <i>Porpolomopsis calyptriformis</i>	Pink (Ballerina) Waxcap
UNIDENTIFIED WAXCAPS	
E1 <i>Clitopilus baronii</i>	
E2 <i>Entoloma ameides</i>	a pinkgill sp.
E3 <i>Entoloma asprellum</i>	a pinkgill sp.
E4 <i>Entoloma asprellum</i>	a pinkgill sp.
E5 <i>Entoloma atrocoeruleum</i>	a pinkgill sp.
E6 <i>Entoloma calongei</i>	a pinkgill sp.
E7 <i>Entoloma chalybaeum</i>	Indigo Pinkgill
E8 <i>Entoloma clandestinum</i>	a pinkgill sp.
E9 <i>Entoloma conferendum</i>	a pinkgill sp.
E10 <i>Entoloma dysthales</i>	a pinkgill sp.
E11 <i>Entoloma exile</i>	a pinkgill sp.



E12 Entoloma griseocyaneum	Felted Pinkgill
E13 Entoloma henricii	a pinkgill sp.
E14 Entoloma infula	a pinkgill sp.
E15 Entoloma lampropus	a pinkgill sp.
E16 Entoloma longistriatum	a pinkgill sp.
E17 Entoloma neglectum	a pinkgill sp.
E18 Entoloma ochreoprunuloides	a pinkgill sp.
E19 Entoloma pleopodium	Aromatic Pinkgill
E20 Entoloma poliopus	a pinkgill sp.
E21 Entoloma proterum	a pinkgill sp.
E22 Entoloma prunuloides	Mealy Pinkgill
E23 Entoloma pseudocoelestinum	a pinkgill sp.
E24 Entoloma rhombisporum	a pinkgill sp.
E25 Entoloma sepium	a pinkgill sp.
E26 Entoloma sericeum	Silky Pinkgill
UNIDENTIFIED PINK GILLS	
G1 Geoglossum fallax	Deceptive Earthtongue
G2 Geoglossum nigratum	an Earthtongue sp.
G3 Geoglossum aff simile	an Earthtongue sp.
G4 Geoglossum umbratile	Plain Earthtongue
G5 Glutinoglossum heptaseptatum	an Earthtongue sp.
G6 Glutinoglossum pseudoglutinosum	an Earthtongue sp.
G7 Hemileucoglossum aff alveolatum	an Earthtongue sp.
G8 Trichoglossum hirsutum	Hairy Earthtongue
G9 Trichoglossum octopartitum	an Earthtongue sp.
G10 Trichoglossum aff. variabile	an Earthtongue sp.
G11 Trichoglossum walteri	Short-spored Earthtongue
G12 Microglossum olivaceum	Olive Earthtongue
G13 Microglossum nudipes aff	an Earthtongue sp.
G14 Microglossum parvisporum	an Earthtongue sp.
UNIDENTIFIED EARTHTONGUES	
D1 Dermoloma magicum	Black Magic
D2 Dermoloma cuneifolium	Crazed Cap Mushroom

Table 1.1 – Full list of CHEGD Fungi detected across all 30 Sites

The relative abundance of CHEGD fungi ranged from 5.8% to 39.8% of all the fungi present (mean 20.1%), with the Hygrophoraceae (waxcap family) being the most abundant of the CHEGD fungi in most quadrats (0.2-33.1%; mean 10.5%), followed by Clavariaceae (fairy clubs; 1.9-13.7%; mean 5.6%), with Entolomataceae and Geoglossaceae being less abundant (mean .3% and 1.9%), as is generally found when this eDNA metabarcoding method is applied to ancient grassland soils.

Numbers of CHEGD fungal species detected at the 30 sites ranged from 13 to 52 (mean 33), though for Clavariaceae and Entolomataceae and Geoglossaceae, a high proportion of sequences could not be linked to named species (38%, 27.3% and 16.7% respectively), the reason for this being the lack of DNA barcodes for many species in these group and the taxonomic instability of these families.

The number of waxcap species in each quadrat ranged from 2-15 (mean 8.0), with the number of species detected correlating with the relative abundance of sequences of this family in the soil. More

detailed analysis of the Hygrophoraceae revealed the presence of 30 species across the 30 and this included 8 species assessed as globally vulnerable (VU) by IUCN and a further 5 species assessed as VU but not yet formally published. Six other CHEGD fungi assessed at VU were also detected, as were three species of Green earthtongue (*Microglossum* spp) which are listed in Section 7 of the Environment (Wales) Act 2016. It is interesting that the very rare species *Dermoloma magicum* was detected at 16 sites. Other studies have detected a similar pattern at many other grassland sites. This species appears to be far more widespread and common than fruitbody records would suggest, likely because it fruits only very infrequently.

For five sites, fruitbody (Traditional) survey data (for whole site rather than just the quadrat) had previously been undertaken so comparisons could be made. Apart from Mill Bank (Site 1) all five sites had very diverse CHEDG fungal populations as assessed by either method but overall species counts were higher for total CHEGD fungi using eDNA (33 vs 21), though for waxcaps Traditional surveys reported a mean of 14.5 spp across the 6 sites vs 10.5 for eDNA. The latter is due to the fact that the areas of the sites were all much bigger than 30mx30m (0.09ha).

There were good results returned from all 30 sites, the following headlines can be pulled out.

- Of particular significance were the results from Site 22 (Dingestow Court Meadow). This was the least diverse site, however it is immediately adjacent a known site of high diversity. It was demonstrated that despite 40 years of suitable management and a ready and diverse spore source immediately adjacent the speed of colonisation for Grassland Fungi is very slow. This reinforces the importance of identifying and protecting existing sites as new sites are both difficult and very long-term to create.
- When comparisons were made between Traditional Surveys and eDNA, the eDNA performed very favourably, generally recording a greater number of species, particularly relating to the smaller/more obscure/difficult to identify Clavarioids, Entolomas, Geoglossums.
- For Waxcaps the total was generally reduced with eDNA to some degree (as these are easier to identify traditionally in the field and in some cases the main focus of surveys). However, the eDNA still returned impressively high numbers, particularly bearing in mind it just focussed on a 30mx30m part of the field.
- The number of Hygrocybe (Waxcap) species recorded on each site ranged from 2 to 15.
- Many great Grassland Fungi sites were surveyed, quite a few not previously known. Some of these sites came about by sightings of just one or two fungi by landowners, or surveyors outside the "Fungi Season". However when surveyed a huge diversity was uncovered.
- For 18 of the 30 sites the eDNA results would be sufficient for a site to be recognised as a Local Wildlife Site (LWS) based on the fungi community, from either having 8 or more Hygrocybe species or a Section 7 (Species of Principal Conservation Concern) present. With a further 3 almost at this level.

The results from each of the 30 individual sites are shown individually later in the report, within which the following is detailed:

- A map and grid reference showing survey location so it can be repeated in the future.
- A list of Fungal species for the site.
- A comparison of the Fungi eDNA results with more traditional survey methods if available.
- Text giving the results some context, particularly how they can be used to evaluate the ecological value of the site.

The individual site information was extracted from this report and sent to each of the relevant landowners together with advice regarding future management of the sites with aim of enthusing the landowners to their sites importance and providing guidance as to how to preserve/enhance it.

## Flora

The results returned by Aberystwyth University, in addition to the Fungi records, also returned the presence of any flora. Of particular interest are species that are used in the assessment of Grassland Ecological quality. Those species that are Indicator Species of Neutral Grassland in “The Guidelines for the Assessment of Wildlife Sites in South Wales, 2004” were particularly focused upon. The guidelines state that if a site has 8 or more of these Neutral Grassland Indicator Species it should be considered for recognition as a Local Wildlife Site (LWS).

A total of 100 species were identified across the 30 sites in the following numbers:

Herbs = 77 species

Grasses/Sedges = 23 species

Using this data an analysis of how well the eDNA picked up these Indicator Species was undertaken and whether the eDNA would allow assessments to be made of whether a site was Local Wildlife Site quality, this analysis is shown within Table 1.2 as follows:

		Number of Neutral Grassland Indicator Species Recorded				
		Traditional Survey	eDNA			
Site 01	<b>Mill Bank</b>	10	5.5	55.00	Y	N
Site 02	<b>The Beeches</b>	19	9.5	50.00	Y	Y
Site 03	<b>Yew Tree Cottage</b>		15.5		Y (SSSI)	Y
Site 04	<b>Pentwyn</b>		15		Y (SSSI)	Y
Site 05	<b>Moorcroft Cottage</b>	16	10.5	65.63	Y	Y
Site 06	<b>Ty Mynydd Fields</b>	9	5.5	61.11	Y	N
Site 07	<b>Cefn Maen</b>	12	8.5	70.83	Y	Y
Site 08	<b>Rockfield Farm</b>		13.5		Y (BUT ISN'T)	Y
Site 09	<b>Llansor Mill</b>	8	5.5	68.75	Y	N
Site 10	<b>Great Goytre Farm</b>	10	8.5	85.00	Y	Y
Site 11	<b>Cleddon Fields</b>	10	3.5	35.00	Y	N
Site 12	<b>Holly Tree Cottage</b>	17	13.5	79.41	Y	Y
Site 13	<b>S. of Penterry Church</b>	14	11.5	82.14	Y	Y
Site 14	<b>Wentwood Mill</b>	16	9.5	59.38	Y	Y
Site 15	<b>Crick Community Meadow</b>	12	5.5	45.83	Y	N
Site 16	<b>Upper Red House</b>	11	4.5	40.91	Y	N
Site 17	<b>Woodside House</b>		5.5		Y	N
Site 18	<b>Old Park Nursery</b>		4.5		N	N
Site 19	<b>The Elms</b>	10	2.5	25.00	Y	N
Site 20	<b>Sherrington</b>	12	11.5	95.83	Y	Y
Site 21	<b>Fishpool Farm</b>		3		?	N
Site 22	<b>Dingestow Court Meadow</b>		2.5		N	N
Site 23	<b>New Grove Farm</b>	23	9.5	41.30	Y	Y
Site 24	<b>New Grove Meadows</b>		15.5		Y	Y

Site 25	<b>Wet Meadows</b>	19	9.5	50.00	Y	Y
Site 26	<b>Twyn Sheriff Farm</b>		3		N	N
Site 27	<b>Whitehouse Farm</b>	16	8.5	53.13	Y	Y
Site 28	<b>Halewood Cottage</b>	8	9.5	118.75	Y	Y
Site 29	<b>Llanllowell House</b>	23	14.5	63.04	Y	Y
Site 30	<b>St. Woolos Cemetery</b>		5.5		Y	N
	<b>Average</b>	13.75	8.35	62.30		

Table 1.2 Floral analysis using Indicator species picked up by both Traditional methods and eDNA and whether sites could be recognised as LWS based on this.

From looking at Table 1.2 the following headlines can be pulled out.

- eDNA results for Indicator Species ranged from 25% to 118.75% of that obtained by Traditional methods.
- On average the eDNA returned 62.30% of Indicator Species records compared to Traditional Methods.
- For 17 of the 27 sites that were considered to be LWS quality by Traditional Methods, the eDNA was able to return enough Indicator Species to make a similar conclusion.
- For 9 of the 27 sites that were considered to be LWS quality by Traditional Methods, the eDNA didn't return enough Indicator Species to make a similar conclusion, however it did return enough to highlight likely ecological quality and would warrant further investigation.
- The remaining 4 sites that didn't produce enough Indicator species using eDNA to be considered Local Wildlife Site quality, it is considered that this is a true reflection as they were not LWS quality.

The results from each of the 30 individual sites are shown individually later in the report, within which the following is detailed:

- A map and grid reference showing survey location so it can be repeated in the future.
- A comparison of the Flora eDNA results with more traditional survey methods if available.
- A list of Floral species for the site.
- Text giving the results some context, particularly how they can be used to evaluate the ecological value of the site.

The individual site information was extracted from this report and sent to each of the relevant landowners together with advice regarding future management of the sites with aim of enthusing the landowners to their sites importance and providing guidance as to how to preserve/enhance it.



**Site Number/Name:** **Site 1 – Millbank**

**Date Surveyed:** **25<sup>th</sup> April 2022**

**British National Grid (centre):** **SO52740 10060**



Figure 2.1 – Millbank Site Location

The site was located within a small field on steepish South facing slopes. The field is grazed by sheep for part of the year but is left during spring/summer for the flora to flourish. It is floristically species-rich and is recognised as part of a Local Wildlife Site because of this. The field is also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.

*\*It should be noted that this is the only site where a full 30mx30m area couldn't be sampled as the field was smaller than this*

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 2.1

<b>Table 2.1 - Site No.1 Millbank</b>				
<b>Scientific Name</b>	<b>English Name</b>	<b>IUCN Status</b>	<b>No. of the 30 sites it was recorded at</b>	<b>% of DNA</b>
Camarophylloopsis_atrovelutina			24	0.15%
Clavulinopsis_corniculata	Meadow Coral		22	0.95%
Clavulinopsis_helvola	Yellow Club		25	0.63%
Camarophylloopsis_schulzeri		[VU]	17	0.30%

Clavulinopsis_laeticolor	Handsome Club		21	0.83%
Clavulinopsis_luteoalba	Apricot Club		18	0.14%
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.03%
Ramariopsis_crocea	a coral fungus sp.		23	0.13%
Ramariopsis_flavescens	a coral fungus sp.		16	0.03%
Clavaria_amoenoides			4	0.04%
Clavaria_falcata			30	0.07%
Clavaria_flavipes	Straw Club		29	0.03%
Clavaria_fragilis	White Spindles		3	0.02%
Hygrocybe_conica	Blackening Waxcap		25	1.09%
Hygrocybe_insipida	Spangle Waxcap		19	0.69%
Cuphophyllus_virginus	Snowy Waxcap		21	0.55%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.07%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.08%
Trichoglossum_walteri	Short-spored Earthtongue	<b>VU</b>	21	1.54%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.15%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.05%
Trichoglossum_hirsutum	Hairy Earthtongue		4	0.24%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.06%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.47%

<b>SPECIES COUNT</b>			
Clavariaceae			13
Hygrophoraceae			3
Entolomataceae			2
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			24
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			8
Hygrophoraceae			3
Entolomataceae			0
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			17

**IUCN** (International Union for Conservation of Nature) – **VU** = **Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild*

A comparison of the eDNA Results with Traditional Survey methods is shown within Table 2.2

	Species	eDNA	Traditional Survey	Combined
C1	Camarophyllopsis_atrovelutina	0.15%		
C2	Camarophyllopsis_schulzeri	0.30%		
C3	Clavaria_amoenoides	0.04%		
C6	Clavaria_falcata	0.07%		
C7	Clavaria_flavipes	0.03%		
C9	Clavaria_fragilis	0.02%		

C17	Clavulinopsis_corniculata	0.95%		
C18	Clavulinopsis_helvola	0.63%		
C20	Clavulinopsis_laeticolor	0.83%		
C21	Clavulinopsis_luteoalba	0.14%		
C26	Ramariopsis_avellaneo-inversa	0.03%		
C27	Ramariopsis_crocea	0.13%		
C28	Ramariopsis_flavescens	0.03%		
	<b>Cuphophyllus_colemanniana</b>		<b>YES</b>	
H5	Cuphophyllus_pratensis		<b>YES</b>	
H7	Cuphophyllus_virgineus	0.55%	<b>YES</b>	
H10	Gliophorus_psittacinus		<b>YES</b>	
	<b>Hygrocybe_aurantiosplendens</b>		<b>YES</b>	
	<b>Hygrocybe_calciphila</b>		<b>YES</b>	
H13	Hygrocybe_chlorophana		<b>YES</b>	
H15	Hygrocybe_coccinea		<b>YES</b>	
H16	Hygrocybe_conica	1.09%	<b>YES</b>	
H17	Hygrocybe_glutinipes		<b>YES</b>	
H19	Hygrocybe_insipida	0.69%	<b>YES</b>	
H20	Hygrocybe_intermedia			
	<b>Hygrocybe_mucronella</b>		<b>YES</b>	
E9	Entoloma_conferendum	0.01%	<b>YES</b>	
	<b>Entoloma_hebes</b>		<b>YES</b>	
E26	Entoloma_sericeum	0.01%		
	<b>Entoloma_serrulatum</b>		<b>YES</b>	
	<b>Geoglossum_cookeanum</b>		<b>YES</b>	
G6	Glutinoglossum_pseudoglutinosum	0.15%		
G7	Hemileucoglossum_aff_alveolatum	0.05%		
G8	Trichoglossum_hirsutum	0.24%		
G10	Trichoglossum aff. variabile	0.08%		
G11	Trichoglossum_walteri	1.54%		
D2	Dermoloma_cuneifolium	1.47%	<b>YES</b>	

SPECIES COUNT			
	<b>13</b>	<b>0</b>	<b>13</b>
	<b>3</b>	<b>12</b>	<b>13</b>
	<b>2</b>	<b>3</b>	<b>4</b>
	<b>5</b>	<b>1</b>	<b>6</b>
	<b>1</b>	<b>1</b>	<b>1</b>
	<b>24</b>	<b>17</b>	<b>37</b>

The results show that the eDNA has picked up more CHEGD species than Traditional methods, particularly for Clavarioids and also Geoglossum, these species are smaller and harder to identify in the field however. Very surprisingly, the number of Waxcap species is much lower in the eDNA survey, this did not occur with the other sites where direct comparisons could be made. As this was

the one site where the survey area covered virtually the entire site, it would be expected that most species present would be picked up. **Aberystwth University have offered to reanalyse this site for free if a fresh soil sample can be collected to work out whether there is a genuine reduction in Waxcap numbers on site or some glitch with the eDNA survey.**

The fact that 2 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.

Overall new species have been recognised for this site and if the results of the two surveys are combined it reveals a CHEGD score of 37 making it a significant site for Grassland Fungi.

## Flora

The following two tables (2.3 and 2.4) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

SITE NAME: Millbank		FIELD NUMBER: 1		DATE: 16/06/2016	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
		<b>Achillea millefolium (yarrow)</b>	O		
		<b>Centaurea nigra (common knapweed)</b>	R		
		<b>Crepis capillaris (smooth hawks-beard)</b>	R		
<b>Holcus lanatus (yorkshire fog)</b>	F	<b>Digitalis purpurea (Foxglove)</b>	R		
<b>Lolium perenne (perennial rye grass)</b>	O	<b>Glechoma hederacea (ground ivy)</b>	LF		
<b>Luzula campestris (field wood rush)</b>	O	<b>Hypochaeris radicata (common cats ear)</b>	O		
<b>Pteridium aquilinum (bracken)</b>	O	<b>Lathyrus pratensis (meadow vetchling)</b>	R		
		<b>Scorzoneroides autumnalis (autumn hawkbit)</b>	O		
		<b>Lotus corniculatus (birds-foot trefoil)</b>	LA		
		<b>Mentha arvensis (Field Mint)</b>	O/VL F		
		<b>Plantago lanceolata (ribwort plantain)</b>	O		
		<b>Potentilla erecta (tormentil)</b>	VLF		
		<b>Potentilla sterilis (barren strawberry)</b>	O		
		<b>Prunella vulgaris (self-heal)</b>	O		
-		<b>Ranunculus acris (meadow buttercup)</b>	O		
-		<b>Ranunculus bulbosus (bulbous buttercup)</b>	O		
-		<b>Ranunculus repens (creeping buttercup)</b>	O		
		<b>Rumex acetosa (common sorrel)</b>	O		
		<b>Sagina procumbens (Procumbent Pearlwort)</b>	VLF		
		<b>Stellaria graminea (lesser stichwort)</b>	LF		
		<b>Trifolium pratense (red clover)</b>	O		



		Trifolium repens (white clover)	F		
		Urtica dioica (Stinging Nettle)	VO		
		Veronica chamaedrys (gemdr. speedwell)	LF		
		Veronica serpyllifolia (Thyme-leaved Speedwell)	R		
		Viola riviniana (common dog violet)	O		

**Table 2.4 - eDNA Survey**

SITE NAME: Millbank		FIELD NUMBER: 1		DATE: 25/04/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	2.26%	Centaurea_nigra	4.63%	Fraxinus_excelsior	1.13%
Anthoxanthum_odoratum	2.45%	Cerastium_fontanum	0.02%	Malus_domestica	0.03%
Dactylis_glomerata	0.05%	Crepis_capillaris	3.96%	Quercus_petraea_robur	0.02%
Festuca_rubra	0.48%	Ficaria_verna	1.70%		
Holcus_lanatus	2.00%	Glechoma_hederacea	2.83%		
Lolium_perr_mult	0.17%	Hypericum_maculatum_perforatum	0.01%		
Luzula_campestris	0.02%	Lotus_corniculatus	0.99%		
Poa_prat_calc_parv	0.05%	Lotus_pedunculatus	3.32%		
Poa_trivialis	1.59%	Mentha_arvensis	1.15%		
		Plantago_lanceolata	0.01%		
		Ranunculus_acris_occid	0.04%		
		Ranunculus_bulb_repe	0.41%		
		Rumex_acetosa	0.73%		
		Scorzoneroides_autumnalis	6.09%		
-		Stellaria_alsine-graminea	2.47%		
-		Trifolium_pratense	0.08%		
-		Trifolium_rep_occi_nigr	38.79%		
		Veronica_chamaedrys	17.26%		
		Veronica_praecoxAFF-OTU 2976	0.10%	-	

It can be seen that the eDNA survey didn't return as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges where certain other species may be located, so a full species list is not realistic.

The original survey recorded 10 Indicator Species, the eDNA method recorded 6/7 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). Whilst this wouldn't be enough to recognise the site as a LWS, it is not far off and would give a good indication that the site is of ecological value and worth further survey work.

Note the presence of Veronica praecox (Breckland Speedwell), a species not recorded in Gwent and highly likely an eDNA recognition error with a very similar species.

<b>Site Number/Name:</b>	<b>Site 2 – The Beeches</b>
<b>Date Surveyed:</b>	<b>25<sup>th</sup> April 2022</b>
<b>British National Grid (centre):</b>	<b>S052921 06565 &amp; S052775 06661</b>

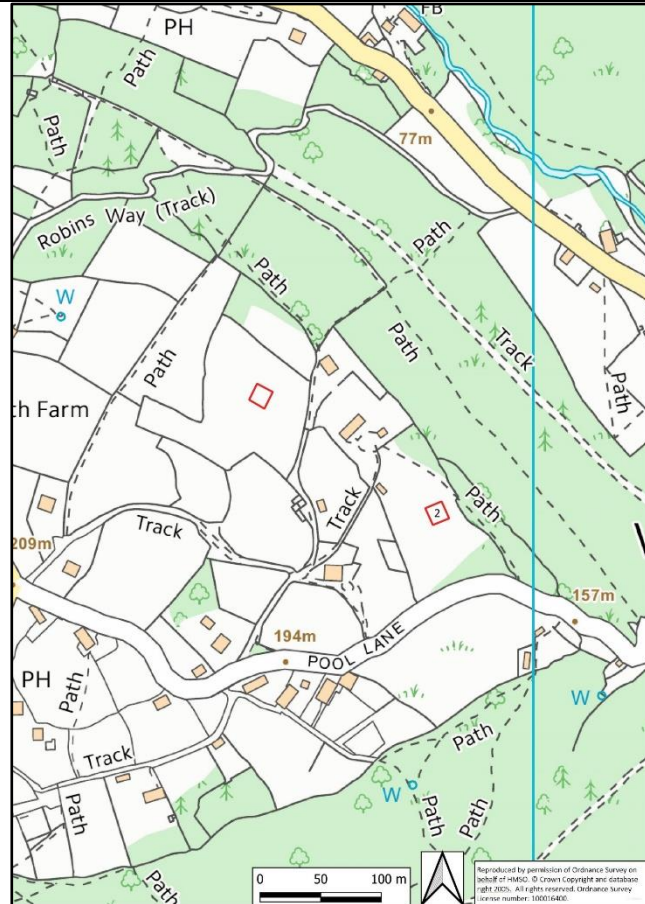


Figure 2.2 – The Beeches Site Location

The site was divided between two fields, both of which were on relatively gently sloping north-east facing slopes. The fields are managed as Hay Meadows with aftermath grazing by sheep. Both are floristically species-rich and are recognised as part of a Local Wildlife Site because of this. The fields are also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.

*\*It should be noted that this is the only site where the 30mx30m survey area was split between two smaller areas in separate fields.*

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 3.1

**Table 3.1 - Site No.2 The Beeches**

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.05%
<i>Camarophylloopsis schulzeri</i>		[VU]	17	1.14%
<i>Clavaria amoenoides</i>			4	0.03%
<i>Clavaria falcata</i>			30	0.06%
<i>Clavaria flavipes</i>	Straw Club		29	0.42%
<i>Clavaria griseobrunnea</i>			4	0.02%
<i>Clavaria messapica</i>			8	0.09%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.36%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.56%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.01%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.05%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.05%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.04%
<i>Ramariopsis flavescens</i>	a coral fungus sp.		16	0.02%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.55%
<i>Cuphophyllus flavipes</i>	Yellow Foot Waxcap	[VU]	10	1.21%
<i>Cuphophyllus pratensis</i>	Meadow Waxcap		22	3.85%
<i>Cuphophyllus virgineus</i>	Snowy Waxcap		21	0.70%
<i>Gliophorus psittacinus</i>	Parrot Waxcap		12	4.02%
<i>Hygrocybe cantharellus</i>	Goblet Waxcap		6	0.07%
<i>Hygrocybe chlorophana</i>	Golden Waxcap		16	4.04%
<i>Hygrocybe citrinovirens</i>	Citrine Waxcap	VU	11	6.46%
<i>Hygrocybe coccinea</i>	Scarlet Waxcap		9	0.53%
<i>Hygrocybe conica</i>	Blackening Waxcap		25	0.03%
<i>Hygrocybe glutinipes</i>	Glutinous Waxcap		22	2.38%
<i>Hygrocybe intermedia</i>	Fibrous Waxcap	VU	12	3.05%
<i>Entoloma ameides</i>	a pinkgill sp.		15	0.01%
<i>Entoloma asprellum</i>	a pinkgill sp.		18	0.01%
<i>Entoloma conferendum</i>	a pinkgill sp.		25	0.02%
<i>Entoloma henricii</i>	a pinkgill sp.	[VU]	12	0.01%
<i>Entoloma sericeum</i>	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
<i>Geoglossum aff simile</i>	an Earthtongue sp.		19	0.24%
<i>Glutinoglossum pseudoglutinosum</i>	an Earthtongue sp.		21	0.11%
<i>Hemileucoglossum aff alveolatum</i>	an Earthtongue sp.		25	0.08%
<i>Trichoglossum aff. variabile</i>	an Earthtongue sp.		19	0.01%
<i>Trichoglossum walteri</i>	Short-spored Earthtongue	VU	21	3.43%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.35%
<i>Dermoloma magicum</i>	Black Magic	[VU]	16	1.10%
<i>Dermoloma cuneifolium</i>	Crazed Cap Mushroom		22	1.56%

SPECIES COUNT			
Clavariaceae			14
Hygrophoraceae			11
Entolomataceae			6
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			38
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			9

Hygrophoraceae			10
Entolomataceae			0
Geoglossomycetes			4
Dermoloma			2
CHEGD Score			25

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

A comparison of the eDNA Results with Traditional Survey methods is shown within Table 3.2

	<b>Species</b>	<b>eDNA</b>	<b>Traditional Survey</b>	<b>Combined</b>
C1	Camarophyllopsis_atrovelutina	0.05%		
C2	Camarophyllopsis_schulzeri	1.14%		
C3	Clavaria_amoenoides	0.03%		
C6	Clavaria_falcata	0.06%	<b>YES</b>	
C7	Clavaria_flavipes	0.42%		
C9	Clavaria_fragilis		<b>YES</b>	
C11	Clavaria_griseobrunnea	0.02%		
C12	Clavaria_messapica	0.09%		
C17	Clavulinopsis_corniculata	0.36%	<b>YES</b>	
C18	Clavulinopsis_helvola	0.56%	<b>YES</b>	
C20	Clavulinopsis_laeticolor	0.01%		
C21	Clavulinopsis_luteoalba	0.05%	<b>YES</b>	
C26	Ramariopsis_avellaneo-inversa	0.05%		
C27	Ramariopsis_crocea	0.04%		
C28	Ramariopsis_flavescens	0.02%		
H2	Cuphophyllus_flavipes	1.21%	<b>YES</b>	
H5	Cuphophyllus_pratensis	3.85%	<b>YES</b>	
H7	Cuphophyllus_virgineus	0.70%	<b>YES</b>	
H9	Gliophorus_irrigatus		<b>YES</b>	
H10	Gliophorus_psittacinus	4.02%	<b>YES</b>	
	<b>Hygrocybe_acutoconica</b>		<b>YES</b>	
H11	Hygrocybe_cantharellus	0.07%	<b>Yes</b>	
H13	Hygrocybe_chlorophana	4.04%	<b>YES</b>	
H14	Hygrocybe_citrinovirens	6.46%	<b>YES</b>	
H15	Hygrocybe_coccinea	0.53%	<b>YES</b>	
H16	Hygrocybe_conica	0.03%	<b>YES</b>	
H17	Hygrocybe_glutinipes	2.38%	<b>YES</b>	
H19	Hygrocybe_insipida		<b>YES</b>	
H20	Hygrocybe_intermedia	3.05%	<b>YES</b>	
H25	Hygrocybe_reidii		<b>YES</b>	
	<b>Hygrocybe_splendidissima</b>		<b>YES</b>	



H28	Neohygrocybe_ingrata		YES	
H30	Porpolomopsis_calyptiformis		YES	
E2	Entoloma_ameides	0.01%		
E4	Entoloma_asprellum	0.01%		
E9	Entoloma_conferendum	0.02%		
E13	Entoloma_henricii	0.01%		
E26	Entoloma_sericeum	0.01%		
G3	Geoglossum_aff_simile	0.24%		
G6	Glutinoglossum_pseudoglutinosum	0.11%		
G7	Hemileucoglossum_aff_alveolatum	0.08%		
G10	Trichoglossum aff. variabile	0.01%		
G11	Trichoglossum_walteri	3.43%		
D1	Dermoloma_magicum	1.10%		
D2	Dermoloma_cuneifolium	1.56%		

SPECIES COUNT (ALL SEQUENCES)			
	14	5	15
	11	18	18
	5	0	5
	5	0	5
	2	0	2
	37	22	45

The results show that the eDNA has picked up more CHEGD species than Traditional methods. This is the case for Clavarioids, Entelomas, Geoglossum and Dermolomas. The number of Waxcaps is reduced however. This is to be expected as the owner is an expert and specialises in Waxcaps so that the site is very well recorded over a number of years. The 30mx30m was only a fraction of the overall field so to have returned such a high number of fungi species draws very favourable comparisons with the Traditional methods, particularly considered this was a one off survey rather than the result of numerous observations.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 7 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.

Overall new species have been recognised for this site and if the results of the two surveys are combined it reveals a CHEGD score of 45 making it a highly significant site for Grassland Fungi. It should be noted that the overall CHEGD score taking in to account other fields (not included in the eDNA survey and subsequent analysis) at The Beeches totals a huge impressive 73 of which 20 of these were new, added by the eDNA.

## Flora

The following two tables (3.3 and 3.4) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 3.3 ORIGINAL SURVEY				
SITE NAME: The Beeches		FIELD NUMBER: 1 & 3		DATE: 2012
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species
<i>Agrostis capillaris</i> (Common Bent)		<i>Achillea millefolium</i> (Yarrow)		
<i>Poa annua</i> (Annual Meadow-grass)		<i>Ajuga reptans</i> ( <b>Bugle</b> )		
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)		<i>Alchemilla filicaulis</i> subs. <b>Vestita</b> ( <b>Hairy Ladies Mantle</b> )		
<i>Arrhenatherum elatius</i> (False Oat-grass)		<i>Allium ursinum</i> (Ramsons)		
<i>Cynosurus cristatus</i> (Crested Dogstail)		<i>Anemone nemorosa</i> (Wood Anemone)		
<i>Dactylis glomerata</i> (Cocksfoot)		<i>Anthriscus sylvestris</i> (Cow Parsley)		
<i>Festuca rubra</i> (Red Fescue)		<b><i>Centaurea nigra</i> (Common Knapweed)</b>		
<i>Holcus lanatus</i> (Yorkshire Fog)		<i>Cerastium fontanum</i> (Common Mouse-ear)		
<i>Poa trivialis</i> (Rough Meadow-grass)		<i>Cirsium palustre</i> (Marsh Thistle)		
<b><i>Luzula campestris</i> (Field Wood-rush)</b>		<i>Cirsium repens</i> (Creeping Thistle)		
<i>Lolium perenne</i> (Perennial Reygrass)		<i>Cirsium vulgare</i> (Spear Thistle)		
<i>Juncus</i> sp. (Rush sp.)		<b><i>Conopodium majus</i> (Pignut)</b>		
		<b><i>Dactylorhiza fuchsii</i> (Common Spotted Orchid)</b>		
		<i>Digitalis purpurea</i> (Foxglove)		
		<i>Ficaria verna</i> (Lesser Celandine)		
		<i>Fragaria vesca</i> (Wild Strawberry)		
		<i>Galium aparine</i> (Cleavers)		
		<i>Geranium robertianum</i> (Herb Robert)		
		<i>Geum urbanum</i> (Wood Avens)		
		<i>Glechoma hederacea</i> (Ground Ivy)		
		<i>Heracleum spondylium</i> (Hogweed)		
		<i>Hyacinthoides non-scripta</i> (Bluebell)		
		<b><i>Hypochaeris radicata</i> (Catsear)</b>		
		<i>Lamium galeobdolon</i> (Yellow Archangel)		
		<b><i>Lathyrus liniifolius</i> (Bitter Vetchling)</b>		
		<b><i>Lathyrus pratensis</i> (Meadow Vetchling)</b>		
		<b><i>Leontodon hispidus</i> (Rough Hawkbit)</b>		
		<b><i>Leucanthemum vulgare</i> (Ox-eye Daisy)</b>		
		<b><i>Lotus corniculatus</i> (Birdsfoot Trefoil)</b>		
		<i>Lysimachia nemorum</i> (Yellow Pimpernel)		

	<i>Myosotis sylvatica</i> (Wood Forget-me-not)		
	<i>Oxalis acetosella</i> (Wood Sorrel)		
	<i>Pedicularis sylvatica</i> (Lousewort)		
	<i>Plantago lanceolata</i> (Ribwort Plantain)		
	<i>Potentilla erecta</i> (Tormentil)		
	<i>Potentilla hybrid</i> (Hybrid Tormentil)		
	<i>Potentilla sterilis</i> (Barren Strawberry)		
	<i>Primula veris</i> (Cowslip)		
	<i>Primula veris x vulgaris</i> (False Ox-lip)		
	<i>Primula vulgaris</i> (Primrose)		
	<i>Prunella vulgaris</i> (Selfheal)		
	<i>Ranunculus acris</i> (Meadow Buttercup)		
	<b><i>Ranunculus bulbosus</i> (Bulbous Buttercup)</b>		
	<i>Ranunculus repens</i> (Creeping Buttercup)		
	<i>Rumex acetosa</i> (Common Sorrel)		
	<i>Rumex obtusifolius</i> (Broad-leaved Dock)		
	<i>Stellaria graminea</i> (Lesser Stitchwort)		
	<i>Taraxacum officinale</i> (Dandelion)		
	<i>Trifolium dubium</i> (Lesser Trefoil)		
	<b><i>Trifolium pratense</i> (Red Clover)</b>		
	<i>Trifolium repens</i> (White Clover)		
	<i>Urtica dioica</i> (Stinging Nettles)		
	<i>Veronica chamaedrys</i> (Germander Speedwell)		
	<i>Veronica persica</i> (Slender Speedwell)		
	<i>Veronica serpyllifolia</i> (Thyme-leaved Speedwell)		
	<i>Vicia sepium</i> (Bush Vetch)		
	<i>Viola riviniana</i> (Common Dog-violet)		

<b>Table 3.4 - eDNA SURVEY</b>					
<b>SITE NAME: The Beeches</b>		<b>FIELD NUMBER: 1 &amp; 3</b>		<b>DATE: 26/4/22</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>		<b>Herbs</b>		<b>Woody Species</b>	
	<b>%</b>		<b>%</b>		<b>%</b>
<i>Agrostis cap_gig</i>	0.48 %	<i>Cerastium_fontanum</i>	0.01 %	<i>Fagus_sylvatica</i>	0.02 %
<i>Anthoxanthum_odoratum</i>	2.55 %	<i>Centaurea_nigra</i>	<b>0.07</b> %		
<b><i>Carex_caryophyllea</i></b>	<b>0.04</b> %	<i>Conopodium_majus</i>	<b>1.62</b> %		
<i>Dactylis_glomerata</i>	0.01 %	<i>Crepis_capillaris</i>	0.04 %		
<i>Festuca_rubra</i>	1.10 %	<i>Cucumis_sativus</i>	0.03 %		
<i>Holcus_lanatus</i>	0.05 %	<b><i>Euphrasia_agg</i></b>	<b>0.06</b> %		
<i>Poa_trivialis</i>	0.07 %	<i>Ficaria_verna</i>	0.09 %		
		<i>Hyacinthoides_hispanica_scillanonsc ripta</i>	1.13 %		
		<i>Leontodon_hispidus</i>	<b>0.10</b> %		
		<i>Lotus_corniculatus</i>	<b>3.82</b> %		
		<i>Lotus_pedunculatus</i>	1.34 %		
		<i>Melampyrum_cristatum</i>	0.01 %		
		<i>Plantago_lanceolata</i>	16.52 %		

		Ranunculus_acris_occid	2.79 %		
		<b>Ranunculus_bulb_repe</b>	<b>0.43</b> %		
		<b>Rhinanthus_minor</b>	<b>58.74</b> %		
		Rumex_acetosa	0.25 %		
		<b>Stellaria_alsine-graminea</b>	<b>0.20</b> %		
		Taraxacum_officinale_agg.	2.10 %		
		Trifolium_rep_occ_nigr	0.54 %		
		Veronica_chamaedrys	1.10 %		
		<b>Viola_riviniana</b>	<b>0.01</b> %		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey" (this site is very thoroughly recorded), it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges where certain other species may be located, so a full species list is not realistic.

The original survey recorded 19 Indicator Species, the eDNA method recorded 9/10 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would still be enough to recognise the site as a Local Wildlife Site.

Note the presence of Melampyrum cristatus (Crested Cow-wheat), a species not recorded in Gwent, it is highly likely an eDNA recognition error, perhaps confusing with Melampyrum pratense (Common Cow-wheat).



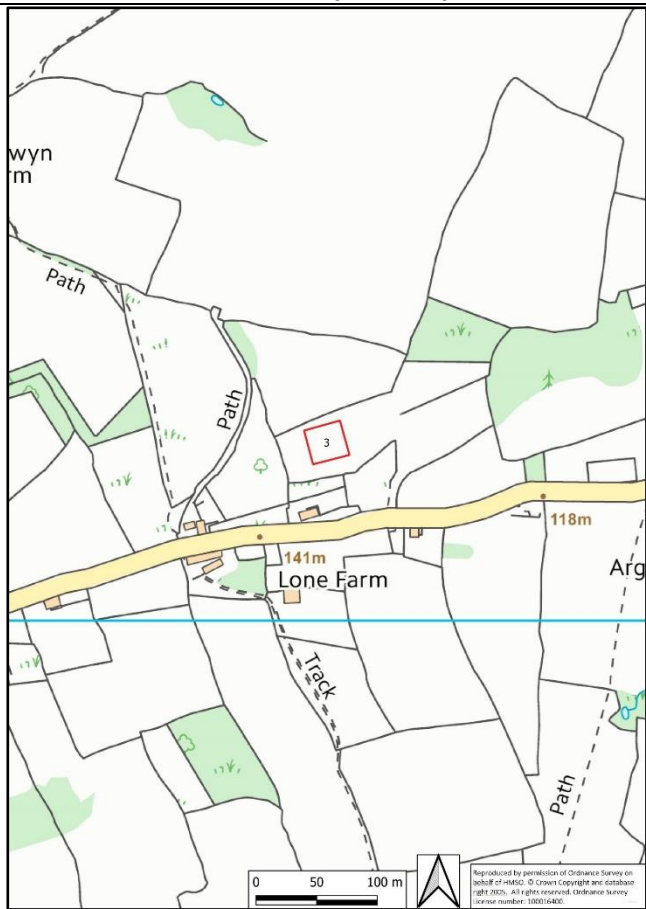
<b>Site Number/Name:</b>	<b>Site 3 – Yewtree Cottage</b>
<b>Date Surveyed:</b>	<b>25<sup>th</sup> April 2022</b>
<b>British National Grid (centre):</b>	<b>SO52692 09147</b>
	<p>The site is located on a relatively steep sloping south facing slope. The field is grazed for part of the year but is left during spring/summer for the flora to flourish. The field is known to be floristically species-rich and as such forms part of Pentwyn Farm SSSI because of this. The fields are also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.</p>

Figure 2.3 – Yewtree Cottage Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 4.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.01%
<i>Camarophyllopsis schulzeri</i>		[VU]	17	2.08%
<i>Clavaria falcata</i>			30	0.03%
<i>Clavaria flavipes</i>	Straw Club		29	0.01%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	1.79%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.10%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.01%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.05%
<i>Clavulinopsis luteonana</i>	Dwarf Spindles		2	0.01%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.09%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.02%

Ramariopsis_flavescens	a coral fungus sp.		16	0.02%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	0.99%
Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	0.28%
Cuphophyllus_pratensis	Meadow Waxcap		22	0.17%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.15%
Gliophorus_psittacinus	Parrot Waxcap		12	1.05%
Hygrocybe_chlorophana	Golden Waxcap		16	2.72%
Hygrocybe_coccinea	Scarlet Waxcap		9	0.55%
Hygrocybe_conica	Blackening Waxcap		25	0.21%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.12%
Hygrocybe_insipida	Spangle Waxcap		19	0.02%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	10.62%
Hygrocybe_phaeococcinea	Shadowed Waxcap	[VU]	3	0.04%
Hygrocybe_punicea	Crimson Waxcap	VU	7	0.37%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	0.98%
Neohygrocybe_ingrata	Dingy Waxcap	VU	4	2.24%
Neohygrocybe_ovina	Blushing Waxcap	VU	3	0.64%
Entoloma_conferendum	a pinkgill sp.		25	0.02%
Entoloma_exile	a pinkgill sp.		12	0.01%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.01%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.02%
Entoloma_longistriatum	a pinkgill sp.		6	0.04%
Entoloma_neglectum	a pinkgill sp.		5	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.19%
Geoglossum_fallax	Deceptive Earthtongue		16	0.10%
Geoglossum_nigritum	an Earthtongue sp.		15	0.34%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.03%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.03%
Microglossum_parvisporum	an Earthtongue sp.	Sect 7	5	0.04%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.15%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.30%

<b>SPECIES COUNT</b>			
Clavariaceae			12
Hygrophoraceae			15
Entolomataceae			7
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			40
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			7
Hygrophoraceae			13
Entolomataceae			0
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			23

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**Sect7** = A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.

A comparison of the eDNA Results with Traditional Survey methods is shown within Table 4.2

Table 4.2 - GWG03-Yew Tree Cottage				
	Species	eDNA	Traditional Survey	Combined
C1	Camarophylloopsis_atrovelutina	0.01%		
C2	Camarophylloopsis_schulzeri	2.08%		
C6	Clavaria_falcata	0.03%		
C7	Clavaria_flavipes	0.01%		
C9	Clavaria_fragilis		YES	
C10	Clavaria_fumosa		YES	
C17	Clavulinopsis_corniculata	1.79%	YES	
C18	Clavulinopsis_helvola	0.10%		
C20	Clavulinopsis_laeticolor	0.01%		
C21	Clavulinopsis_luteoalba	0.05%	YES	
C22	Clavulinopsis_luteonana	0.01%		
C26	Ramariopsis_avellaneo-inversa	0.09%		
C27	Ramariopsis_crocea	0.02%		
C28	Ramariopsis_flavescens	0.02%		
H2	Cuphophyllus_flavipes	0.28%		
H5	Cuphophyllus_pratensis	0.17%	YES	
H7	Cuphophyllus_virgineus	0.15%	YES	
H9	Gliophorus_irrigatus		YES	
H10	Gliophorus_psittacinus	1.05%	YES	
H13	Hygrocybe_chlorophana	2.72%		
H14	Hygrocybe_citrinovirens		YES	
H15	Hygrocybe_coccinea	0.55%	YES	
H16	Hygrocybe_conica	0.21%	YES	
H17	Hygrocybe_glutinipes	0.12%		
H19	Hygrocybe_insipida	0.02%	YES	
H20	Hygrocybe_intermedia	10.62%	YES	
H22	Hygrocybe_phaeococcinea	0.04%		
H23	Hygrocybe_punicea	0.37%		
H24	Hygrocybe_quieta	0.98%	YES	
H28	Neohygrocybe_ingrata	2.24%		
H29	Neohygrocybe_ovina	0.64%	YES	
	<b>Entoloma_araneosum</b>		YES	
	<b>Entoloma_bloxamii</b>		YES	
E9	Entoloma_conferendum	0.02%	YES	
E11	Entoloma_exile	0.01%		
E12	Entoloma_griseocyaneum	0.01%		
E13	Entoloma_henricii	0.02%		
E14	Entoloma_infula		YES	
E16	Entoloma_longistriatum	0.04%		

E17	Entoloma_neglectum	0.01%		
E22	Entoloma_prunuloides		YES	
E26	Entoloma_sericeum	0.01%	YES	
	<b>Entoloma_serrulatum</b>		YES	
G1	Geoglossum_fallax	0.10%		
G2	Geoglossum_nigratum	0.34%		
G3	Geoglossum_aff_simile	0.03%		
G7	Hemileucoglossum_aff_alveolatum	0.03%		
"G14"	Microglossum_parvisporum	0.04%		
D2	Dermoloma_cuneifolium	1.30%	YES	

SPECIES COUNT				
		12	4	14
		15	11	17
		7	7	12
		5	0	5
		1	0	1
		40	22	49

The results show that the eDNA has picked up more CHEGD species than traditional methods. This is the case for Clavarioids, Hygrocybes, Geoglossum and Dermolomas, with the same number of Enteloma found. This is an exceptionally well recorded site so that to be finding new species with the eDNA even though it only covered 30mx30m of a considerably larger field is impressive and that this was just from one survey visit. It should be noted that there were quite few species the eDNA did not pick up, this would be expected with just part of the field covered.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 10 Vulnerable species and a Section 7 species were identified by eDNA further reinforces its value and the importance of preserving this.

Overall new species have been recognised for this site and if the results of the two surveys are combined it reveals a CHEGD score of 49 making it a highly significant site for Grassland Fungi.

## Flora

The following table (4.3) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as the available species list covered a number of fields.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 4.2 - eDNA SURVEY**

SITE NAME: Yewtree Cottage		FIELD NUMBER:		DATE:	26/04/2022
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	1.43%	Achillea_millefolium	9.11%		
Anthoxanthum_odoratum	2.07%	<b>Callianthemum_anemonoides</b>	<b>0.01%</b>		
Arrhenatherum_elatius	0.14%	<b>Centaurea_nigra</b>	36.05%		
<b>Carex_caryophylla</b>	0.18%	Cerastium_fontanum	0.10%		
Dactylis_glomerata	0.10%	<b>Cucumis_sativus</b>	<b>0.02%</b>		
Festuca_rubra	0.28%	<b>Euphrasia_agg</b>	0.26%		
Holcus_lanatus	0.25%	Ficaria_verna	1.20%		
Lolium_perr_mult	0.11%	Hyacinthoides_hispanica_scillanonscripta	0.21%		
Poa_prat_calc_parv	0.02%	<b>Lathyrus_pratensis</b>	0.55%		
Poa_trivialis	0.69%	<b>Leontodon_hispidus</b>	6.20%		
		<b>Leontodon_saxatilis</b>	0.15%		
		<b>Lotus_corniculatus</b>	8.29%		
		Lotus_pedunculatus	1.78%		
		<b>Luzula_campestris</b>	0.01%		
		Plantago_lanceolata	6.94%		
		<b>Potentilla_erecta</b>	0.04%		
		Potentilla_reptans	0.04%		
		Potentilla_sterilis	2.57%		
		Primula_vulgaris	0.01%		
		Ranunculus_acris_occid	1.73%		
		<b>Ranunculus_bulb_repe</b>	3.33%		
		Rumex_acetosa	7.14%		
		Scorzoneroides_autumnalis	0.12%		
		Solanum_lycopersicum	0.01%		
		<b>Stellaria_alsine-graminea</b>	0.56%		
		Taraxacum_officinale_agg.	1.20%		
		<b>Trifolium_pratense</b>	0.25%		
		Trifolium_rep_occi_nigr	0.92%		
		<b>Veronica_praecox</b>	<b>0.01%</b>		
		Veronica_chamaedrys	3.14%		
		<b>Viola_riviniana</b>	0.56%		

		<b>Stellaria alsine-graminea</b>	0.56%		
		Taraxacum_officinale_agg.	1.20%		
		<b>Trifolium pratense</b>	0.25%		
		Trifolium_rep_occ_nigr	0.92%		
		Veronica_chamaedrys	3.14%		
		<b>Viola riviniana</b>	0.56%		

The eDNA survey returned a good number of species but likely well short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 15/16 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would still be enough to recognise the site as a Local Wildlife Site (if it wasn't already a SSSI).

Note the presence of a number of species highlighted in **RED** that are likely an eDNA recognition error.




<b>Site Number/Name:</b>	<b>Site 4 – Pentwyn GWT Reserve</b>
<b>Date Surveyed:</b>	<b>25<sup>th</sup> April 2022</b>
<b>British National Grid (centre):</b>	<b>SO52353 09323</b>
	<p>The site is located on a relatively gently sloping east facing slope. The fields are managed as Hay Meadows with aftermath grazing by sheep/cattle. It is known to be floristically species-rich and as such forms part of Pentwyn Farm SSSI because of this. The fields are also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.</p>

Figure 2.4 – Pentwyn GWT Reserve Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 5.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_atrovelutina</i>			24	0.11%
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	1.39%
<i>Clavaria_falcata</i>			30	0.24%
<i>Clavaria_flavipes</i>	Straw Club		29	0.22%
<i>Clavaria_messapica</i>			8	0.02%
<i>Clavulinopsis_corniculata</i>	Meadow Coral		22	0.18%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.09%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.02%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.25%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.02%
<i>Ramariopsis_flavescens</i>	a coral fungus sp.		16	0.01%

<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.70%
Cuphophyllus_pratensis	Meadow Waxcap		22	2.65%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.05%
Gliophorus_irrigatus	Slimy Waxcap		7	0.34%
Hygrocybe_chlorophana	Golden Waxcap		16	0.92%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	9.23%
Hygrocybe_coccinea	Scarlet Waxcap		9	0.09%
Hygrocybe_conica	Blackening Waxcap		25	0.35%
Hygrocybe_insipida	Spangle Waxcap		19	0.15%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	2.09%
Neohygrocybe_ingrata	Dingy Waxcap	VU	4	1.21%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.26%
Entoloma_ameides	a pinkgill sp.		15	0.03%
Entoloma_asprellum	a pinkgill sp.		18	0.03%
Entoloma_clandestinum	a pinkgill sp.		13	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.02%
Entoloma_exile	a pinkgill sp.		12	0.01%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.03%
Entoloma_infula	a pinkgill sp.		12	0.02%
Entoloma_ochreoprunuloides	a pinkgill sp.		2	0.01%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.01%
Entoloma_rhombisporum	a pinkgill sp.		4	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.07%
Geoglossum_fallax	Deceptive Earthtongue		16	0.26%
Geoglossum_nigritum	an Earthtongue sp.		15	0.19%
Geoglossum_umbratile	Plain Earthtongue		4	0.01%
Glutinoglossum_pseudoglutinosu m	an Earthtongue sp.		21	0.07%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.06%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.17%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			11
Entolomataceae			11
Geoglossomycetes			5
Dermoloma			0
CHEGD Score			38
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			7
Hygrophoraceae			11
Entolomataceae			0
Geoglossomycetes			4
Dermoloma			0
CHEGD Score			22

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

A comparison of the eDNA Results with Traditional Survey methods is shown within Table 5.2

Table 5.2 - GWG04-Pentwyn				
	Species	eDNA	Traditional Survey	Combined
C1	Camarophyllopsis_atrovelutina	0.11%		
C2	Camarophyllopsis_schulzeri	1.39%		
C6	Clavaria_falcata	0.24%		
C7	Clavaria_flavipes	0.22%		
C12	Clavaria_messapica	0.02%		
C17	Clavulinopsis_corniculata	0.18%	YES	
C18	Clavulinopsis_helvola	0.09%		
C21	Clavulinopsis_luteoalba	0.02%		
C26	Ramariopsis_avellaneo-inversa	0.25%		
C27	Ramariopsis_crocea	0.02%		
C28	Ramariopsis_flavescens	0.01%		
H5	Cuphophyllus_pratensis	2.65%	YES	
H7	Cuphophyllus_virgineus	0.05%	YES	
H9	Gliophorus_irrigatus	0.34%	YES	
H10	Gliophorus_psittacinus		YES	
	<b>Hygrocybe_acutoconica</b>		YES	
	<b>Hygrocybe_aurantiosplendens</b>		YES	
H13	Hygrocybe_chlorophana	0.92%	YES	
H14	Hygrocybe_citrinovirens	9.23%	YES	
H15	Hygrocybe_coccinea	0.09%	YES	
H16	Hygrocybe_conica	0.35%	YES	
H17	Hygrocybe_glutinipes		YES	
H19	Hygrocybe_insipida	0.15%	YES	
H23	Hygrocybe_punicea		YES	
H24	Hygrocybe_quieta		YES	
H28	Neohygrocybe_ingrata	1.21%		
H30	Porpolomopsis_calyptiformis	0.26%	YES	
E2	Entoloma_ameides	0.03%		
E4	Entoloma_asprellum	0.03%		
E8	Entoloma_clandestinum	0.01%		
E9	Entoloma_conferendum	0.02%		
E11	Entoloma_exile	0.01%		
E12	Entoloma_griseocyaneum	0.03%		
E14	Entoloma_infula	0.02%		
E18	Entoloma_ochreoprunuloides	0.01%		
	<b>Entoloma_papillatum</b>		YES	
E20	Entoloma_poliopus	0.01%		
	<b>Entoloma_porphyrophaeum</b>		YES	

E23	Entoloma_pseudocoelestinum	0.01%		
E24	Entoloma_rhombisporum	0.01%		
G1	Geoglossum_fallax	0.26%		
G2	Geoglossum_nigritum	0.19%		
G4	Geoglossum_umbratile	0.01%		
G6	Glutinoglossum_pseudoglutinosum	0.07%		
G7	Hemileucoglossum_aff_alveolatum	0.06%		
G11	Trichoglossum_walteri	0.17%		

SPECIES COUNT (ALL SEQUENCES)			
	11	1	11
	11	15	16
	11	2	13
	6	0	6
	0	0	0
	39	18	46

The results show that the eDNA has picked up more CHEGD species than traditional methods. This is the case for Clavarioids, Enteloma, Geoglossum and Dermolomas, with a somewhat reduced number of Hygrocybe found. This is a well recorded site, however it would appear previous surveys have been focussed on the Hygrocybe (Waxcaps) which would explain the results. Never the less the results are impressive, particularly with a new Waxcap species being detected, even though the survey only covered 30mx30m of multi field site and this was just from one survey visit. It should be noted that there were a few Waxcap species the eDNA did not pick up, this would be expected with just part of one field covered.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 7 Vulnerable species were identified further reinforces its value and the importance of preserving this.

Overall new species have been recognised for this site and if the results of the two surveys are combined it reveals a CHEGD score of 46 making it a highly significant site for Grassland Fungi.

## Flora

The following table (5.3) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as the available species list covered a number of fields.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 5.3 - eDNA SURVEY**

SITE NAME: Pentwyn Farm (GWT)		FIELD NUMBER:		DATE: 26/04/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	0.76%	<b>Anacamptis_pyramidalis</b>	0.17%	Fraxinus_excelsior	0.38%
Anthoxanthum_odoratum	0.86%	<b>Callianthemum_anemonoides</b>	0.02%		
Arrhenatherum_elatius	0.37%	<b>Centaurea_nigra</b>	1.12%		
Dactylis_glomerata	0.78%	Cerastium_fontanum	0.67%		
Festuca_rubra	0.31%	<b>Conopodium_majus</b>	7.32%		
Holcus_lanatus	0.53%	Crepis_capillaris	0.15%		
<b>Luzula_campestris</b>	0.01%	<b>Dactylorhiza_maculata</b>	0.02%		
Poa_trivialis	0.12%	<b>Euphrasia_agg</b>	0.04%		
<b>Vulpia_bromoides</b>	0.05%	Heracleum_sphondylium	4.94%		
		Hyacinthoides_hispanica_scillanonscripta	0.20%		
		<b>Lathyrus_pratensis</b>	0.40%		
		<b>Leontodon_hispidus</b>	16.39%		
		<b>Leontodon_saxatilis</b>	0.66%		
		<b>Lotus_corniculatus</b>	13.16%		
		Lotus_pedunculatus	0.11%		
		Plantago_lanceolata	1.45%		
		Ranunculus_acris_occid	0.16%		
		<b>Ranunculus_bulb_repe</b>	23.27%		
		<b>Rhinanthus_minor</b>	0.17%		
		Rumex_acetosa	6.97%		
		Scorzoneroides_autumnalis	1.43%		
		<b>Stellaria_alsine-graminea</b>	0.06%		
		Taraxacum_officinale_agg.	0.51%		
		<b>Trifolium_pratense</b>	10.31%		
		Trifolium_rep_occi_nigr	2.88%		
		Veronica_chamaedryas	0.63%		

The eDNA survey returned a good number of species but likely well short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 15 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would still be enough to recognise the site as a Local Wildlife Site (if it wasn't already a SSSI).

The presence of *Anacamptis pyramidalis* (Pyramidal Orchid), is of particular interest as this species hasn't been recorded on site previously. Perhaps it has fairly recently colonised but has yet to flower?

Note the presence of a number of species highlighted in RED that are likely an eDNA recognition error.



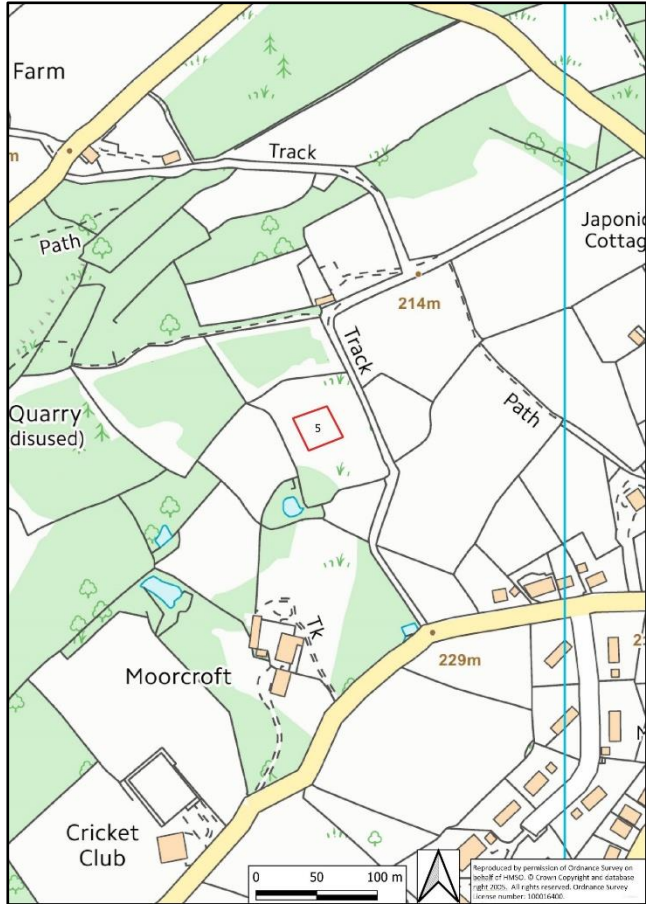
<b>Site Number/Name:</b>	<b>Site 5 – Moorcroft Meadows</b>
<b>Date Surveyed:</b>	<b>25<sup>th</sup> April 2022</b>
<b>British National Grid (centre):</b>	<b>SO51797 09413</b>
	<p>The site is located on a very gently sloping north-east facing slope. The field is managed as a Hay Meadow. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site. The fungal diversity of the field is unknown, however the owner had reported fungi in the autumn that were thought to be Waxcaps. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.5 – Moorcroft Meadows Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 6.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis_atrovelutina</i>			24	0.05%
<i>Camarophyllopsis_schulzeri</i>		[VU]	17	3.26%
<i>Clavaria_falcata</i>			30	0.05%
<i>Clavaria_flavipes</i>	Straw Club		29	1.08%
<i>Clavaria_griseobrunnea</i>			4	0.04%
<i>Clavaria_messapica</i>			8	0.02%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.04%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.12%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.23%
<i>Cuphophyllus_canescens</i>	a Waxcap sp.	VU	1	0.02%
<i>Cuphophyllus_pratensis</i>	Meadow Waxcap		22	1.45%

Hygrocybe_chlorophana	Golden Waxcap		16	10.24%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	1.14%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.11%
Hygrocybe_insipida	Spangle Waxcap		19	0.10%
Hygrocybe_subpapillata	Papillate Waxcap	[VU]	1	2.00%
Entoloma_asprellum	a pinkgill sp.		8	0.02%
Entoloma_atrocoeruleum	a pinkgill sp.		5	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.04%
Entoloma_exile	a pinkgill sp.		12	0.01%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.02%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.02%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.05%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.01%
Geoglossum_fallax	Deceptive Earthtongue		16	0.04%
Glutinoglossum_pseudoglutinosu m	an Earthtongue sp.		21	0.04%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.05%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	1.42%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.01%
Dermoloma_magicum	Black Magic	[VU]	16	6.46%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.23%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			7
Entolomataceae			6
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			28
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			6
Entolomataceae			0
Geoglossomycetes			2
Dermoloma			2
CHEGD Score			15

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with the number of Hygrocybe (Waxcaps) being particularly present and clearly demonstrate this site is of significant value for its grassland fungi as well as the already recognised floristic value. Indeed the results of the eDNA Survey would almost be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 7 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (6.3 and 6.4) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 6.3 - ORIGINAL SURVEY					
SITE NAME: Moorcroft Meadows		FIELD NUMBER: 4		DATE: 23/6/17	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	F	Achillea millefolium (yarrow)	VL	Acer pseudoplatanus (Sycamore) seedling	R
Agrostis stolonifera (creeping bent)	O	<b>Ajuga reptans (bugle)</b>	O	Populus tremula (Aspen) seedling	VLF
Anthoxanthum odoratum (sweet vnl grass)	A	<b>Centaurea nigra (common knapweed)</b>	FLA		
Cynosurus cristatus (crested dog's tail)	LF	Cerastium holosteoides (cmn. mouse-ear)	O		
Festuca rubra (red fescue)	F	Chamerion angustifolium (Rosebay Willowherb)	VL		
Holcus lanatus (yorkshire fog)	F	Cirsium palustre (marsh thistle)	VO		
<b>Luzula campestris (field wood rush)</b>	F	<b>Conopodium majus (pignut)</b>	F		
Poa annua (annual meadow grass)	VO	<b>Dactylorhiza fuchsii (c. spotted orchid)</b>	LF		
		Epilobium sp. (Willowherb sp.)	R		
		<b>Euphrasia officinalis agg. (eyebright)</b>	F		
		Geranium robertianum (herb robert)	VL		
		Hyacinthoides non-scripta (bluebell)	VO		
		<b>Hypochaeris radicata (common cats ear)</b>	F		
		<b>Leontodon hispidus (rough hawkbit)</b>	LF		
		<b>Lotus corniculatus (birds-foot trefoil)</b>	LF		
		Lotus uliginosus (greater birds-foot trefoil)	LF		
		Plantago lanceolata (ribwort plantain)	F		
		<b>Polygala vulgaris (common milkwort)</b>	O/LF		
		<b>Potentilla erecta (tormentil)</b>	F/LA		
-		Prunella vulgaris (self-heal)	O		
-		Ranunculus acris (meadow buttercup)	F		
		<b>Ranunculus bulbosus (bulbous buttercup)</b>	F		
		Ranunculus repens (creeping buttercup)	VL		
		<b>Rhinanthus minor (yellow rattle)</b>	F/A		
		Rubus fruticosus (bramble)	VL		
		Rumex acetosa (common sorrel)	O		
		Scrophularia nodosa (figwort)	R		
		Stachys sylvatica (hedge woundwort)	VL		
		<b>Stellaria graminea (lesser stichwort)</b>	O/VLF		
		Taraxacum officinale (dandelion)	O		

		Trifolium pratense (red clover)	Y		
		Trifolium repens (white clover)	VL		
		Valeriana officinalis (common valerian)	VLF		
		Viola riviniana (common dog violet)	VL		

**Table 6.4 - eDNA SURVEY**

SITE NAME: Moorcroft Meadows		FIELD NUMBER: 4		DATE: 25/4/22	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	0.89 %	Achillea_millefolium	1.68 %	Fraxinus_excelsior	0.07 %
Anthoxanthum_odoratum	0.76 %	Centaurea_nigra	15.87 %		
Cynosurus_cristatus	0.28 %	Cerastium_fontanum	0.16 %		
Festuca_rubra	0.34 %	Conopodium_majus	14.88 %		
Holcus_lanatus	1.06 %	Euphrasia_agg	0.60 %		
		Hyacinthoides_hispanica_scillanonscripta	0.01 %		
		Hypochaeris_radicata	3.19 %		
		Leontodon_hispidus	18.26 %		
		Leontodon_saxatilis	0.05 %		
		Lotus_corniculatus	0.66 %		
		Lotus_pedunculatus	1.77 %		
		Plantago_lanceolata	2.57 %		
		Potentilla_erecta	2.74 %		
		Potentilla_reptans	0.53 %		
		Prunella_vulgaris	0.03 %		
		Ranunculus_acris_occid	6.43 %		
		Ranunculus_bulb_repe	4.55 %		
		Rhinanthus_minor	14.51 %		
		Rumex_acetosa	0.03 %		
-		Stellaria_alsine-graminea	1.22 %		
-		Taraxacum_officinale_agg.	0.08 %		
		Trifolium_rep_occ_i_nigr	4.82 %		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 16 Indicator Species, the eDNA method recorded 10/11 (It can't separate Creeping and Bulbous Buttercup, the latter being an Indicator). This would still be enough to recognise the site as a LWS.

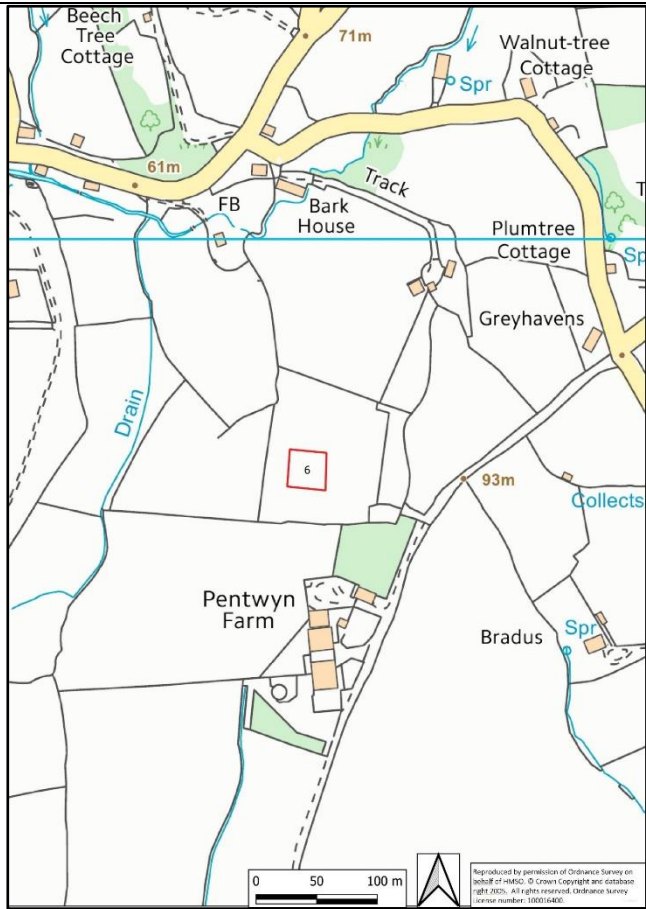
<b>Site Number/Name:</b>	<b>Site 6 – Ty Mynydd Fields</b>
<b>Date Surveyed:</b>	<b>3rd May 2022</b>
<b>British National Grid (centre):</b>	<b>SO38304 03810</b>
	<p>The site is located on a relatively gently sloping west facing slope. The field is a turn-out field and as such is grazed intermittently by horses. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site. The fungal diversity of the field is unknown, however the owner had reported a number of unidentified Waxcaps to be present in the autumn. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.6 – Ty Mynydd Fields Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 7.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.22%
<i>Clavaria falcata</i>			30	0.11%
<i>Clavaria flavipes</i>	Straw Club		29	0.04%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.08%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.08%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.11%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.16%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.70%
<i>Cuphophyllus pratensis</i>	Meadow Waxcap		22	0.05%
<i>Cuphophyllus virgineus</i>	Snowy Waxcap		21	0.05%
<i>Hygrocybe citrinovirens</i>	Citrine Waxcap	<b>VU</b>	11	0.05%
<i>Hygrocybe glutinipes</i>	Glutinous Waxcap		22	2.87%

Entoloma_asprellum	a pinkgill sp.		18	0.03%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.07%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.64%
Trichoglossum_walteri	Short-spored Earthtongue	<b>VU</b>	21	1.20%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.28%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.07%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	1.25%
Dermoloma_magicum	Black Magic	<b>[VU]</b>	16	0.58%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.37%

<b>SPECIES COUNT</b>			
Clavariaceae			7
Hygrophoraceae			4
Entolomataceae			2
Geoglossomycetes			4
Dermoloma			2
CHEGD Score			19
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			4
Entolomataceae			0
Geoglossomycetes			4
Dermoloma			2
CHEGD Score			16

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with a number of Hygrocybe (Waxcaps) being recorded and clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The fact that 3 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.



## Flora

The following two tables (7.2 and 7.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 7.2 - ORIGINAL SURVEY					
SITE NAME: Ty Mynydd Fields		FIELD NUMBER: Turn Out Field		DATE:	15/06/2020
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	LF	<i>Anthriscus sylvestris</i> (Cow Parsley)	R		
<i>Alopecurus pratensis</i> (Meadow Foxtail)	VO	<b>Conopodium majus</b> (Pignut)	R		
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	LF	<i>Epilobium parviflorum</i> (Hoary Willowherb)	VL		
<i>Cynosurus cristatus</i> (Crested Dogstail)	O	<i>Epilobium</i> sp. (Willowherb sp.)	VL		
<i>Equisetum arvense</i> (Field Horsetail)	R	<i>Galium palustre</i> (Marsh-bedstraw)	VL		
<i>Festuca rubra</i> (Red Fescue)	Y	<i>Glechoma hederacea</i> (Ground Ivy)	VL		
<i>Glyceria fluitans</i> (Floating Sweet-grass)	VL	<i>Hyacinthoides non-scripta</i> (Bluebell)	R		
<i>Holcus lanatus</i> (Yorkshire Fog)	F	<i>Hypericum humifusum</i> (Trailing St. John's Wort)	R		
<i>Juncus effusus</i> (Soft Rush)	VLF	<b>Hypochaeris radicata</b> (Common Catsear)	VO		
<i>Lolium perenne</i> (Perennial Rye-grass)	O/VL F	<b>Lotus corniculatus</b> (Birds-foot Trefoil)	O/LF		
<i>Luzula campestris</i> (Field Wood-rush)	VO	<i>Lotus uliginosus</i> (Greater Birds-foot Trefoil)	VLF		
<i>Poa annua</i> (Annual Meadow-grass)	VL	<i>Malva moschata</i> (Musk Mallow)	R		
<i>Poa trivialis</i> (Rough Meadow-grass)	O	<i>Mentha aquatica</i> (Water Mint)	VLF		
<i>Pteridium aquilinum</i> (Bracken)	O/VL F	<i>Persicaria polygonum</i> (Redleg)	VL		
		<i>Plantago major</i> (Greater Plantain)	VO		
		<i>Polygonum aviculare</i> (Knotgrass)	R		
		<i>Prunella vulgaris</i> (Self-heal)	O		
		<i>Ranunculus acris</i> (Meadow Buttercup)	O/LF		
-		<b>Ranunculus bulbosus</b> (Bulbous Buttercup)	O		
-		<i>Ranunculus flammula</i> (Lesser Spearwort)	Y		
-		<i>Ranunculus repens</i> (Creeping Buttercup)	O/LF/VL A		
		<b>Rhinanthus minor</b> (Yellow Rattle)	VL		
		<i>Rubus fruticosus</i> sp. agg. (Bramble)	VLA		
		<i>Rumex acetosa</i> (Common Sorrel)	O		
		<i>Sagina procumbens</i> (Procumbent Pearlwort)	R		
		<i>Stellaria alba</i> (Bog Stitchwort)	VL		
		<b>Stellaria graminea</b> (Lesser Stitchwort)	O/VLF		

		<i>Trifolium pratense</i> (Red Clover)	O/VLF	
		<i>Trifolium repens</i> (White Clover)	F/LA	
		<i>Tripleurospermum inodorum</i> (Scentless Mayweed)	R	
		<i>Veronica beccabunga</i> (Broomrape)	LF	
		<i>Veronica chamaedrys</i> (Germander Speedwell)	VL	
		<i>Veronica serpyllifolia</i> (Thyme-leaved Speedwell)	O	
		<i>Viola riviniana</i> (Common Dog-violet)	VLF	

Table 7.3 - eDNA SURVEY					
SITE NAME: Ty Mynydd Fields		FIELD NUMBER: Turn		DATE: 03/05/2022	
Grasses, Sedges, Rushes & Ferns		Herbs		Woody Species	
	D		D		D
<i>Agrostis_cap_gig</i>	3.89 %	<i>Achillea_millefolium</i>	13.31 %		
<i>Anthoxanthum_odoratum</i>	0.68 %	<i>Cerastium_fontanum</i>	0.20%		
<i>Cynosurus_cristatus</i>	0.13 %	<i>Hypochaeris_radicata</i>	0.07%		
<i>Festuca_rubra</i>	0.55 %	<i>Leontodon_saxatilis</i>	2.98%		
<i>Holcus_lanatus</i>	1.61 %	<i>Lotus_corniculatus</i>	0.10%		
<i>Lolium_perr_mult</i>	0.15 %	<i>Lotus_pedunculatus</i>	0.06%		
<i>Poa_trivialis</i>	0.09 %	<i>Montia_fontana</i>	0.04%		
		<i>Plantago_lanceolata</i>	0.31%		
		<i>Plantago_major</i>	1.70%		
		<i>Potentilla_erecta</i>	6.65%		
		<i>Potentilla_reptans</i>	4.45%		
		<i>Prunella_vulgaris</i>	11.68 %		
		<i>Ranunculus_acris_occid</i>	10.70 %		
		<i>Ranunculus_bulb_repe</i>	1.96%		
		<i>Rumex_acetosa</i>	0.83%		
		<i>Stellaria_alsine-graminea</i>	1.37%		
		<i>Taraxacum_officinale_agg.</i>	0.50%		
		<i>Trifolium_pratense</i>	12.31 %		
		<i>Trifolium_rep_occ_i_nigr</i>	13.45 %		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 9 Indicator Species, the eDNA method recorded 5/6 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). Whilst this wouldn't be enough to recognise the site as a LWS it is not far off and would give a good indication that the site is of ecological value and worth further survey work.

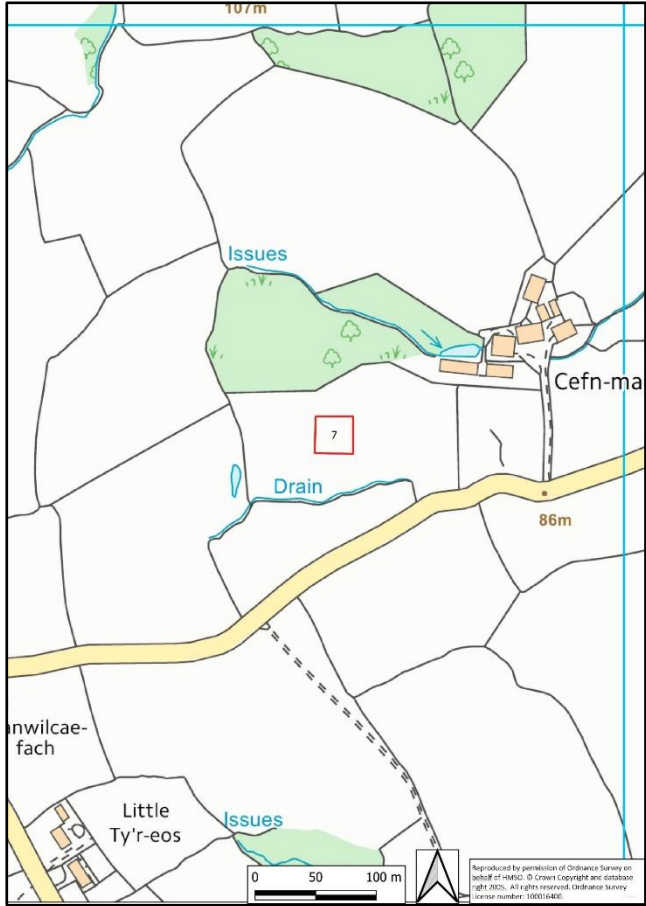
<b>Site Number/Name:</b>	<b>Site 7 – Cefn Maen Grasslands</b>
<b>Date Surveyed:</b>	<b>3rd May 2022</b>
<b>British National Grid (centre):</b>	<b>SO38762 05662</b>
	<p>The site is located on an east facing slope. The fields are managed as Hay Meadows with aftermath grazing by a variety of livestock. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner had reported a number of unidentified grassland fungi (including Waxcaps) to be present in the autumn. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.7 – Cefn Maen Grasslands Site Location

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 8.1

<b>Table 8.1 - Site No.7 Cefn Maen Grasslands</b>				
<b>Scientific Name</b>	<b>English Name</b>	<b>IUCN Status</b>	<b>No. of the 30 sites it was recorded at</b>	<b>% of DNA</b>
<i>Camarophylloopsis atrovelutina</i>			24	0.06%
<i>Clavaria appendiculata</i>			1	0.03%
<i>Clavaria falcata</i>			30	0.15%
<i>Clavaria flavipes</i>	Straw Club		29	0.24%
<i>Clavaria flavostellifera</i>			3	0.08%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.36%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.01%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.03%

Lamelloclavaria_petersenii			4	0.02%
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.09%
Ramariopsis_flavescens	a coral fungus sp.		16	0.10%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	3.50%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.22%
Hygrocybe_conica	Blackening Waxcap		25	0.23%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.14%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	0.13%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	0.39%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.03%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.05%
Entoloma_sericeum	Silky Pinkgill		24	0.02%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.20%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.12%
Geoglossum_fallax	Deceptive Earthtongue		16	0.08%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.04%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.15%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.38%
Trichoglossum_hirsutum	Hairy Earthtongue		4	1.19%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	1.08%
Dermoloma_magicum	Black Magic	[VU]	16	0.25%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			4
Entolomataceae			5
Geoglossomycetes			6
Dermoloma			1
CHEGD Score			27
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			7
Hygrophoraceae			4
Entolomataceae			1
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			18

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with a number of Hygrocybe (Waxcaps) being recored and clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The fact that 3 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.

## Flora

The following two tables (8.2 and 8.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 8.2 - ORIGINAL SURVEY					
SITE NAME: Cefn Maen Grasslands		FIELD NUMBER: Quarry		DATE: 15/07/2020	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	F/L A	<i>Achillea millefolium</i> (Yarrow)	VL	<i>Prunus spinosa</i> (Blackthorn) sucker	VL
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	F/L A	<i>Achillea ptarmica</i> (Sneezewort)	R	<i>Quercus</i> sp. (Oak sp.) seedling	R
<i>Carex flacca</i> (Glaucous Sedge)	VLA	<b><i>Centaurea nigra</i> (Common Knapweed)</b>	LF	<i>Salix</i> sp. (Willow sp.) seedling	R
<i>Cynosurus cristatus</i> (Crested Dogtail)	O	<i>Cerastium holosteoides</i> (Common Mouse-ear)	O		
<i>Dactylis glomerata</i> (Cock's foot)	O/L F	<i>Chenopodium album</i> (Fat Hen)	R		
<i>Festuca rubra</i> (Red Fescue)	O/V LF	<i>Cirsium arvense</i> (Creeping Thistle)	O/V LF		
<i>Holcus lanatus</i> (Yorkshire Fog)	F	<i>Cirsium palustre</i> (Marsh Thistle)	VO		
<i>Holcus mollis</i> (Creeping Soft-grass)	VLF	<i>Dactylorhiza fuchsii</i> (Cinnamon Spotted Orchid)	R		
<i>Juncus acutiflorus</i> (Sharp-flowered Rush)	VLF	<i>Digitalis purpurea</i> (Foxglove)	R		
<i>Juncus conglomeratus</i> (Compact Rush)	VLF	<i>Epilobium</i> sp. (Willowherb sp.)	VL		
<i>Juncus effusus</i> (Soft Rush)	VLF	<i>Galium aparine</i> (Cleavers)	VL		
<i>Luzula campestris</i> (Field Wood-rush)	Y	<i>Galium palustre</i> (Marsh-bedstraw)	VL		
<i>Phleum pratense</i> (Timothy Grass)	VL	<b><i>Hypochaeris radicata</i> (Common Catsear)</b>	LF		
<i>Poa trivialis</i> (Rough Meadow-grass)	O	<b><i>Lathyrus pratensis</i> (Meadow Vetchling)</b>	O/V LA		
		<b><i>Lotus corniculatus</i> (Birds-foot Trefoil)</b>	F/LA		
		<i>Lotus uliginosus</i> (Greater Birds-foot Trefoil)	LF		
		<i>Oenanthe crocata</i> (Henlock Water-dropwort)	R		
		<i>Plantago lanceolata</i> (Ribwort Plantain)	F/LA		
		<i>Potentilla erecta</i> (Tormentil)	VLF		
		<i>Potentilla reptans</i> (Creeping Cinquefoil)	VL		
		<i>Prunella vulgaris</i> (Self-heal)	O		
		<i>Ranunculus acris</i> (Meadow Buttercup)	LF		
		<b><i>Ranunculus bulbosus</i> (Bulbous Buttercup)</b>	LF		
-		<i>Ranunculus repens</i> (Creeping Buttercup)	O/V LA		
-		<i>Rubus fruticosus</i> sp. agg. (Bramble)	VL		
-		<i>Rumex acetosa</i> (Common Sorrel)	O		
		<i>Rumex conglomeratus</i> (Clustered Dock)	R		
		<i>Rumex obtusifolius</i> (Broad-leaved Dock)	O		
		<i>Rumex sanguineus</i> (Wood Dock)	R		

		<i>Senecio jacobea</i> (Ragwort)	R		
		<i>Silene dioica</i> (Red Champion)	R		
		<i>Solanum dulcamara</i> (Bittersweet)	VL		
		<i>Sonchus asper</i> (Prickly Sow-thistle)	R		
		<i>Stachys sylvatica</i> (Hedge Woundwort)	VL		
		<i>Stellaria graminea</i> (Lesser Stitchwort)	F		
		<i>Trifolium dubium</i> (Lesser Trefoil)	O		
		<i>Trifolium pratense</i> (Red Clover)	F/A		
		<i>Trifolium repens</i> (White Clover)	F		
		<i>Urtica dioica</i> (Stinging Nettle)	VLF		
		<i>Vicia sativa</i> (Common Vetch)	R		

Table 8.3 - eDNA SURVEY					
SITE NAME: Cefn Maen Grasslands		FIELD NUMBER: Quarry		DATE: 03/05/2022	
Grasses, Sedges, Rushes & Ferns		Herbs		Woody Species	
	%		%		%
<i>Agrostis cap_gig</i>	1.32%	<i>Achillea millefolium</i>	0.24%	<i>Salix purpurea</i>	0.03%
<i>Anthoxanthum odoratum</i>	1.21%	<i>Cardamine prat_flex</i>	0.32%		
<i>Cynosurus cristatus</i>	0.79%	<i>Centaurea nigra</i>	0.04%		
<i>Dactylis glomerata</i>	0.07%	<i>Cerastium fontanum</i>	0.37%		
<i>Festuca rubra</i>	0.04%	<i>Cucumis sativus</i>	0.01%		
<i>Holcus lanatus</i>	0.14%	<i>Hypochaeris radicata</i>	9.19%		
<i>Lolium perr_mult</i>	0.08%	<i>Lathyrus pratensis</i>	0.16%		
<i>Poa trivialis</i>	0.12%	<i>Lotus corniculatus</i>	4.36%		
		<i>Lotus pedunculatus</i>	0.71%		
			12.83%		
		<i>Plantago lanceolata</i>	%		
		<i>Potentilla erecta</i>	1.17%		
		<i>Potentilla reptans</i>	0.70%		
		<i>Prunella vulgaris</i>	2.36%		
		<i>Ranunculus acris occid</i>	4.77%		
		<i>Ranunculus bulb_repe</i>	2.86%		
		<i>Rumex acetosa</i>	1.36%		
		<i>Stellaria alsine-graminea</i>	0.46%		
		<i>Taraxacum officinale_agg.</i>	4.41%		
		<i>Trifolium dubium</i>	0.10%		
		<i>Trifolium pratense</i>	34.16%		
		<i>Trifolium rep_occi_nigr</i>	11.10%		
		<i>Veronica serpyllifolia</i>	0.21%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.



The original survey recorded 12 Indicator Species, the eDNA method recorded 8/9 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator. This would be be enough to recognise the site as a LWS.

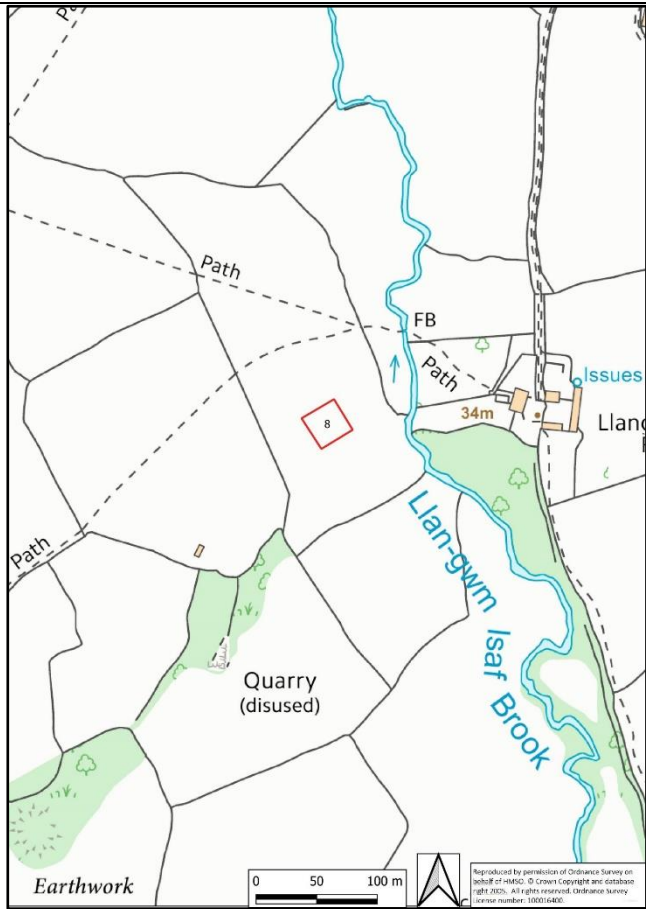
<b>Site Number/Name:</b>	<b>Site 8 – Rockfield Farm</b>
<b>Date Surveyed:</b>	<b>3rd May 2022</b>
<b>British National Grid (centre):</b>	<b>SO42667 01443</b>
	<p>The site is located on quite a steep north-east facing slope, this is the only sloping part of an otherwise flat field. The fields are grazed throughout the year by sheep. It has not been formally surveyed botanically, however notes made during the collection of soil samples revealed a number of Species-rich Grasslands Indicator Species to be present. The fungal diversity of the field is unknown, however the locals have reported a number of Waxcaps to be present in the autumn. A more limited list of floral Indicator Species and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.8 – Rockfield Farm Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 9.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_atrovelutina</i>			24	0.06%
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	0.71%
<i>Clavaria_falcata</i>			30	0.20%
<i>Clavaria_flavipes</i>	Straw Club		29	0.08%
<i>Clavaria_messapica</i>			8	0.16%
<i>Clavaria_tenuipes</i>			3	0.02%
<i>Clavulinopsis_corniculata</i>	Meadow Coral		22	2.16%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.11%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.16%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.04%
<i>Clavulinopsis_umbrinella</i>	Beige Coral		4	0.55%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.26%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.07%
<i>Ramariopsis_flavescens</i>	a coral fungus sp.		16	0.27%

<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.73%
Cuphophyllus_pratensis	Meadow Waxcap		22	5.44%
Cuphophyllus_russocoriaceus	Cedarwood Waxcap		4	0.88%
Cuphophyllus_virginus	Snowy Waxcap		21	2.13%
Gliophorus_irrigatus	Slimy Waxcap		7	0.09%
Gliophorus_psittacinus	Parrot Waxcap		12	0.53%
Hygrocybe_chlorophana	Golden Waxcap		16	0.85%
Hygrocybe_conica	Blackening Waxcap		25	2.72%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.42%
Hygrocybe_inspida	Spangle Waxcap		19	0.18%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	3.02%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.31%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_exile	a pinkgill sp.		12	0.04%
Entoloma_infula	a pinkgill sp.		12	0.01%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.01%
Entoloma_asprellum	a pinkgill sp.		8	0.02%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_clandestinum	a pinkgill sp.		13	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.07%
Geoglossum_fallax	Deceptive Earthtongue		16	0.19%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.17%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.01%
Geoglossum_nigritum	an Earthtongue sp.		15	0.15%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.06%
Glutinoglossum_pseudoglutinosu m	an Earthtongue sp.		21	0.11%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.01%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.68%
Dermoloma_magicum	Black Magic	[VU]	16	0.46%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.98%

<b>SPECIES COUNT</b>			
Clavariaceae			14
Hygrophoraceae			11
Entolomataceae			8
Geoglossomycetes			7
Dermoloma			2
CHEGD Score			42
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			12
Hygrophoraceae			11
Entolomataceae			0
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			30

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a particularly valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (*Hygrocybe* spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 5 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following table (9.3) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Dubious ID from eDNA

SITE NAME: Rockfield Farm		FIELD NUMBER:		DATE:	03/05/2022
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
<i>Agrostis_cap_gig</i>	1.39%	<i>Achillea_millefolium</i>	3.96%		
<i>Alopecurus_pratensis</i>	0.15%	<b><i>Centaurea_nigra</i></b>	6.95%		
<i>Anthoxanthum_odoratum</i>	1.69%	<i>Cerastium_fontanum</i>	0.25%		
<i>Bromus_hordeaceus</i>	0.02%	<b><i>Conopodium_majus</i></b>	0.45%		
<b><i>Carex_caryophylla</i></b>	0.03%	<b><i>Cucumis_sativus</i></b>	0.01%		
<i>Cynosurus_cristatus</i>	0.29%	<b><i>Hypochaeris_radicata</i></b>	0.04%		
<i>Festuca_rubra</i>	0.62%	<b><i>Lathyrus_pratensis</i></b>	0.86%		
<i>Holcus_lanatus</i>	0.36%	<b><i>Leontodon_hispidus</i></b>	4.91%		
<i>Lolium_perr_mult</i>	0.07%	<b><i>Leontodon_saxatilis</i></b>	0.03%		
<i>Poa_trivialis</i>	0.12%	<b><i>Lotus_corniculatus</i></b>	25.72%		
		<b><i>Pimpinella_saxifraga</i></b>	0.14%		
		<i>Plantago_lanceolata</i>	1.75%		
		<b><i>Potentilla_erecta</i></b>	0.05%		
		<i>Ranunculus_acris_occid</i>	3.55%		
		<b><i>Ranunculus_bulb_repe</i></b>	12.61%		
		<b><i>Rhinanthus_minor</i></b>	12.03%		
		<i>Rumex_acetosa</i>	0.22%		
		<b><i>Succisa_pratensis</i></b>	0.05%		
		<i>Taraxacum_officinale_agg.</i>	5.43%		
		<b><i>Trifolium_pratense</i></b>	11.21%		
		<i>Trifolium_rep_occ_nigr</i>	2.37%		

The eDNA survey returned a good number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 13/14 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a Local Wildlife Site

Note the presence of a number of species highlighted in **RED** that are likely an eDNA recognition error.

**Site Number/Name:**

**Site 9 – Llansor Mill**

**Date Surveyed:** 3rd May 2022

**British National Grid (centre):** ST34254 94677

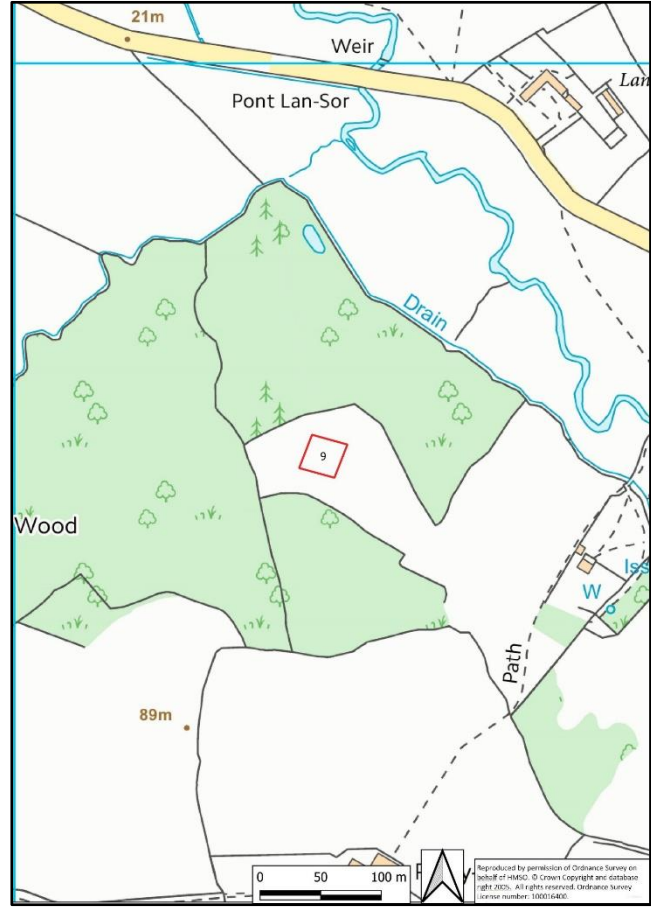


Figure 2.9 – Llansor Mill

The site is located on relatively a north facing slope. The fields are grazed intermittently, but at quite a low level throughout the year by sheep. The botanical diversity of the field was surveyed this year and shown to be quite species-rich although lacking in some expected species. The fungal diversity of the field is unknown, and it was not clear whether any grassland fungi would be present, however the sloping relatively floral species-rich grazed grassland with much moss in the sward appeared very suitable. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 10.1

Table 10.1 - Site No.9 Llansor Mill				
Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.10%
<i>Clavaria falcata</i>			30	0.16%
<i>Clavaria flavipes</i>	Straw Club		29	0.61%
<i>Clavaria flavostellifera</i>			3	0.01%
<i>Clavaria griseobrunnea</i>			4	0.08%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.04%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.15%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.05%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.44%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.03%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.06%

Ramariopsis_flavescens	a coral fungus sp.		16	0.07%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.58%
Hygrocybe_chlorophana	Golden Waxcap		16	0.52%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	0.82%
Hygrocybe_conica	Blackening Waxcap		25	0.14%
Hygrocybe_glutinipes	Glutinous Waxcap		22	1.91%
Hygrocybe_insipida	Spangle Waxcap		19	0.07%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	1.65%
Cuphophyllus_pratensis	Meadow Waxcap		22	0.37%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.44%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.01%
Entoloma_poliopus	a pinkgill sp.		14	0.04%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.04%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
Trichoglossum aff. variabile	an Earthtongue sp.		19	1.00%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.73%
Geoglossum_nigritum	an Earthtongue sp.		15	0.08%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.23%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.19%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.04%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.19%
Dermoloma_magicum	Black Magic	[VU]	16	1.33%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.64%

<b>SPECIES COUNT</b>			
Clavariaceae			12
Hygrophoraceae			8
Entolomataceae			5
Geoglossomycetes			6
Dermoloma			2
CHEGD Score			32
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			9
Hygrophoraceae			8
Entolomataceae			0
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			23

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)



- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 5 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (10.2 and 10.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 10.2 - ORIGINAL SURVEY					
SITE NAME: Llansor Mill		FIELD NUMBER: 3		DATE: 13/6/2022	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	Y	<i>Achillea millefolium</i> (Yarrow)	VO	<i>Fraxinus excelsior</i> (Ash) seedling	O
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	F	<i>Cardamine pratensis</i> (Cuckoo Flower)	R		
<i>Cynosurus cristatus</i> (Crested Dogstail)	LF	<i>Cerastium holosteoides</i> (Common Mouse-ear)	O		
<i>Dactylis glomerata</i> (Cock's foot)	O	<i>Cirsium arvense</i> (Creeping Thistle)	VO		
<i>Festuca rubra</i> (Red Fescue)	F	<i>Cirsium palustre</i> (Marsh Thistle)	VO		
<i>Holcus lanatus</i> (Yorkshire Fog)	F	<i>Conopodium majus</i> (Pignut)	VLF		
<i>Juncus effusus</i> (Soft Rush)	VO	<i>Ficaria verna</i> (Lesser Celandine)	Y		
<i>Lolium perenne</i> (Perennial Rye-grass)	O	<i>Galium aparine</i> (Cleavers)	VL		
<i>Luzula campestris</i> (Field Wood-rush)	LF	<i>Lathyrus pratensis</i> (Meadow Vetchling)	O/VL F		
<i>Poa trivialis</i> (Rough Meadow-grass)	VLF	<i>Lotus corniculatus</i> (Birds-foot Trefoil)	LF		
<i>Pteridium aquilinum</i> (Bracken)	LF/L D	<i>Lotus pedunculatus</i> (Greater Birds-foot Trefoil)	VO		
		<i>Plantago lanceolata</i> (Ribwort Plantain)	O		
		<i>Potentilla erecta</i> (Tormentil)	LF		
		<i>Potentilla reptans</i> (Creeping Cinquefoil)	O		
		<i>Potentilla sterilis</i> (Barren Strawberry)	O		
		<i>Ranunculus acris</i> (Meadow Buttercup)	VO		
		<i>Ranunculus repens</i> (Creeping Buttercup)	VO		
		<i>Stellaria graminea</i> (Lesser Stitchwort)	LF		
		<i>Trifolium pratense</i> (Red Clover)	VO		
		<i>Urtica dioica</i> (Stinging Nettle)	VLF		
		<i>Veronica chamaedrys</i> (Germander Speedwell)	O		
		<i>Viola riviniana</i> (Common Dog-violet)	O		

Table 10.3 - eDNA SURVEY

SITE NAME: Llansor Mill		FIELD NUMBER: 3		DATE: 3/5/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	2.24%	Achillea_millefolium	0.03%	Fraxinus_excelsior	0.22%
Anthoxanthum_odoratum	4.39%	Cerastium_fontanum	0.12%		
Festuca_rubra	0.91%	Cucumis_sativus	0.02%		
Holcus_lanatus	0.69%	Ficaria_verna	21.58%		
Lolium_perr_mult	0.22%	Lathyrus_pratensis	1.68%		
Poa_trivialis	0.39%	Lotus_corniculatus	0.23%		
		Lotus_pedunculatus	2.97%		
		Plantago_lanceolata	7.04%		
		Potentilla_erecta	0.46%		
		Potentilla_reptans	0.09%		
		Ranunculus_acris_occid	0.04%		
		Ranunculus_bulb_repe	4.48%		
		Rumex_acetosa	0.44%		
		Scorzoneroideides_autumnalis	0.03%		
		Stellaria_alsine-graminea	0.20%		
		Taraxacum_officinale_agg.	0.53%		
		Trifolium_pratense	28.01%		
		Trifolium_rep_occ_i_nigr	13.09%		
		Veronica_chamaedrys	1.22%		

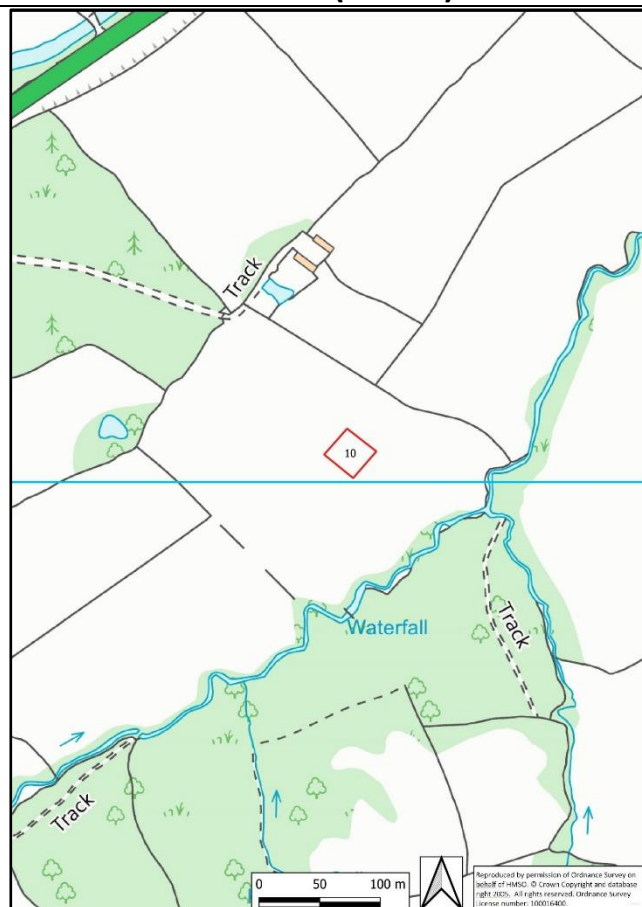
It can be seen that the eDNA survey didn't return as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges where certain other species may be located so a full species list is not realistic.

The original survey recorded 8 Indicator Species, the eDNA method recorded 5/6 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). Whilst this wouldn't be enough to recognise the site as a LWS it is not far off and would give a good indication that the site is of ecological value and worth further survey work.

**Site Number/Name:** Site 10 – Great Goytre Field

**Date Surveyed:** 3rd May 2022

**British National Grid (centre):** ST36553 24024



The site is located on quite steep south-east facing slope. The field is managed as low intensity cattle grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however during surveys in summer (outside of the peak Fungi season) 2007, the surveyor noted some yellow/orange Waxcaps to be present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.

Figure 2.10 – Great Goytre Field

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 11.1

<b>Table 11.1 - Site No.10 Great Goytre Farm</b>				
Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.11%
Ramariopsis_crocea	a coral fungus sp.		23	0.01%
Clavulinopsis_luteoalba	Apricot Club		18	0.02%
Clavulinopsis_laeticolor	Handsome Club		21	0.11%
Clavulinopsis_corniculata	Meadow Coral		22	3.17%
Clavaria_flavipes	Straw Club		29	0.02%
Clavulinopsis_helvola	Yellow Club		25	0.03%
Camarophylloopsis_atrovelutina			24	0.03%
Clavaria_pullei			1	0.02%
Lamelloclavaria_petersenii			4	0.01%
Clavaria_falcata			30	0.16%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.72%

Hygrocybe_conica	Blackening Waxcap		25	0.99%
Cuphophyllus_russocoriaceus	Cedarwood Waxcap		4	0.87%
Hygrocybe_glutinipes	Glutinous Waxcap		22	2.31%
Hygrocybe_chlorophana	Golden Waxcap		16	0.06%
Cuphophyllus_virgineus	Snowy Waxcap		21	2.07%
Entoloma_dysthales	a pinkgill sp.		10	0.01%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.02%
Entoloma_rhombisporum	a pinkgill sp.		4	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.12%
Entoloma_clandestinum	a pinkgill sp.		13	0.02%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.18%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.05%
Geoglossum_nigritum	an Earthtongue sp.		15	0.53%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.59%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.88%
Microglossum_parvisporum	an Earthtongue sp.	Sect7	5	0.01%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	1.06%
Dermoloma_magicum	Black Magic	[VU]	16	0.91%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.74%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			5
Entolomataceae			7
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			30
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			4
Hygrophoraceae			5
Entolomataceae			1
Geoglossomycetes			4
Dermoloma			2
CHEGD Score			16

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**Sect7** = *A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.*

The results showed a good variety of Fungi to be present with the a good number of Hygrocybe (Waxcaps) being recorded and particularly good diversity of Clavarioids (Fairy Clubs) and Geoglossum (Earthtongues) clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The results of the eDNA Survey and more specifically the presence of the Section 7 species *Geoglossum pseudoglutinosum* would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)

- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species
- The fact that 3 Vulnerable species and a Section 7 species were identified by eDNA further reinforces its value and the importance of preserving this.

## Flora

The following two tables (11.2 and 11.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 11.2 - ORIGINAL SURVEY					
SITE NAME: Great Goytre Fields		FIELD NUMBER: 1		DATE:	19/06/20 07
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis stolonifera</i> (Creeping Bent)	LF	<i>Achillea millefolium</i> (Yarrow)	LF		
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	F	<i>Bellis perennis</i> (Daisy)	O		
<i>Beiza media</i> (Quaking Grass)	LF	<i>Cerastium glomeratum</i> (Sticky Mouse-ear)	LO		
<i>Carex caryophylla</i> (Spring Sedge)	LO	<i>Cerastium holosteoides</i> (Common Mouse-ear)	F/O		
<i>Carex panicea</i> (Carnation Sedge)	LF	<i>Cirsium arvense</i> (Creeping Thistle)	O		
<i>Carex sp.</i> (Sedge sp.)	R/L O	<i>Cirsium palustre</i> (Marsh Thistle)	O		
<i>Cynosurus cristatus</i> (Crested Dogstail)	F	<i>Hypericum humifusum</i> (Trailing St. John's Wort)	R		
<i>Danthonia decumbens</i> (Heath Grass)	L/O R	<i>Hypochaeris radicata</i> (Common Catsear)	L O		
<i>Festuca rubra</i> (Red Fescue)	F	<i>Leontodon hispidus</i> (Rough Hawkbit)	A		
<i>Holcus lanatus</i> (Yorkshire Fog)	R	<i>Lotus corniculatus</i> (Birds-foot Trefoil)	LF		
<i>Juncus effusus</i> (Soft Rush)	LF	<i>Plantago lanceolata</i> (Ribwort Plantain)	O		
<i>Juncus inflexus</i> (Hard Rush)	LF	<i>Potentilla erecta</i> (Tormentil)	LF		
<i>Luzula campestris</i> (Field Wood-rush)	LO	<i>Prunella vulgaris</i> (Self-heal)	LF		
<i>Poa sp.</i> (Meadow Grass sp.)	LO	<i>Ranunculus acris</i> (Meadow Buttercup)	R/ O		
<i>Pteridium aquilinum</i> (Bracken)	LF	<i>Ranunculus repens</i> (Creeping Buttercup)	O		
		<i>Rumex acetosa</i> (Common Sorrel)	O/ R		
		<i>Taraxicum officinale</i> sp. Agg. (Dandelion)	LF		
		<i>Trifolium pratense</i> (Red Clover)	L O		
		<i>Trifolium repens</i> (White Clover)	A		
		<i>Trifolium sp.</i> (Clover sp.)	LF		

Table 11.3 - eDNA SURVEY					
SITE NAME: Great Goytre Fields		FIELD NUMBER: 1		DATE:	03/05/2022
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	2.16%	Achillea_millefolium	0.12%	Fraxinus_excelsior	0.03%
Anthoxanthum_odoratum	1.89%	Centaurea_nigra	2.06%	Quercus_petraea_r	0.03%
Briza_media	0.08%	Cerastium_fontanum	0.14%		
Carex_caryophylla	0.02%	Cirsium_palustre	3.81%		
Carex_flacca	0.12%	Leontodon_saxatilis	0.19%		
Cynosurus_cristatus	0.03%	Lotus_corniculatus	20.79%		
Festuca_rubra	0.57%	Lotus_pedunculatus	0.04%		
Holcus_lanatus	0.38%	Plantago_lanceolata	5.22%		
Lolium_perr_mult	2.65%	Potentilla_erecta	1.27%		
Poa_trivialis	0.07%	Potentilla_reptans	1.83%		
		Prunella_vulgaris	0.48%		
		Ranunculus_acris_occid	6.43%		
		Ranunculus_bulb_repe	3.00%		
		Rumex_acetosa	0.64%		
		Scorzoneroideis_autumnalis	0.98%		
		Taraxacum_officinale_agg.	2.28%		
		Trifolium_pratense	16.30%		
		Trifolium_rep_occi_nigr	19.70%		

It can be seen that the eDNA survey didn't return as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the large field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 10 Indicator Species, the eDNA method recorded 8/9 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a LWS.

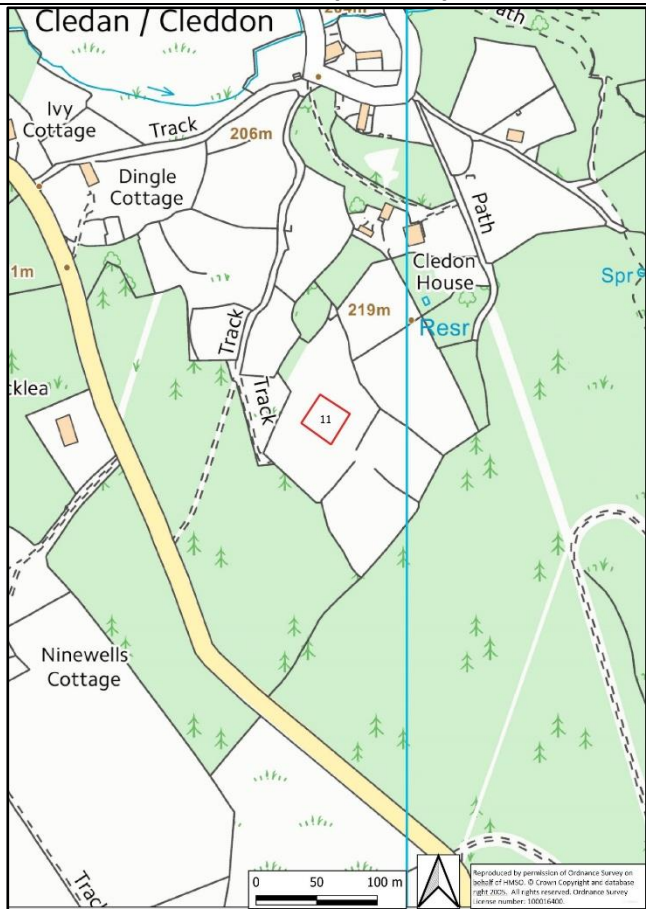
<b>Site Number/Name:</b>	<b>Site 11 – Cleddon Fields</b>
<b>Date Surveyed:</b>	<b>7<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO51933 03600</b>
	<p>The site is located on a quite gentle north-west facing slope. The field was managed as very low intensity cattle grazing, but now also receives a Hay Cut. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner did report seeing “spindles”. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.11 – Cleddon Fields Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 12.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.17%
<i>Clavaria falcata</i>			30	0.25%
<i>Clavaria flavipes</i>	Straw Club		29	0.18%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.40%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.76%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.76%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.11%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.29%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.01%
<i>Ramariopsis flavescens</i>	a coral fungus sp.		16	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	5.80%



Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	1.02%
Cuphophyllus_pratensis	Meadow Waxcap		22	1.16%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.26%
Gliophorus_psittacinus	Parrot Waxcap		12	2.80%
Hygrocybe_cantharellus	Goblet Waxcap		6	0.01%
Hygrocybe_ceracea	Butter Waxcap		3	0.04%
Hygrocybe_conica	Blackening Waxcap		25	1.28%
Hygrocybe_insipida	Spangle Waxcap		19	0.11%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.03%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.06%
Entoloma_infula	a pinkgill sp.		12	0.01%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.11%
Entoloma_sericeum	Silky Pinkgill		24	0.03%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.17%
Geoglossum_fallax	Deceptive Earthtongue		16	0.18%
Geoglossum_nigritum	an Earthtongue sp.		15	0.05%
Glutinoglossum_heptaseptatum	an Earthtongue sp.		4	0.01%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.47%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.08%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.63%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.39%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.09%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.31%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			8
Entolomataceae			5
Geoglossomycetes			7
Dermoloma			1
CHEGD Score			29
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			6
Entolomataceae			2
Geoglossomycetes			6
Dermoloma			1
CHEGD Score			21

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a valuable Grassland Fungi site with a good CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 3 Vulnerable species were identified further reinforces its value and the importance of preserving this.

This site was highlighted as a possible survey site by the owner due to the sighting of some spindles in part of one field, this demonstrates that an indicator such as that can turn out to a sign of a site of far greater significance.

## Flora

The following two tables (12.2 and 12.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 12.2 - ORIGINAL SURVEY					
SITE NAME: Cleddon Fields		FIELD NUMBER: 8		DATE: 7/07/2017	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis tenuis (common bent)	F	<b>Conopodium majus (pignut)</b>	VL	Acer pseudoplatanus (Sycamore ) seedling	V L
Anthoxanthum odoratum(sweet vnl grass)	A	<b>Centaurea nigra (common knapweed)</b>	O/LF	Fagus sylvatica (Beech ) seedling	V L
Cynosurus cristatus (crested dog's tail)	O	Cerastium holosteoides(cmn. mouse-ear)	O	Fraxinus excelsior (Ash) seedling	V L
Dactylis glomerata (cock's foot)	F	Cirsium arvense (creeping thistle)	VO		
Festuca rubra (red fescue)	LF	<b>Hypochaeris radicata (common cats ear)</b>	F		
Holcus lanatus (yorkshire fog)	F	<b>Lathyrus pratensis (meadow vetchling)</b>	VLF		
Lolium perenne (perennial rye grass)	O	<b>Lotus corniculatus (birds-foot trefoil)</b>	O/VL F		
<b>Luzula campestris (field wood rush)</b>	F	Lotus uliginosus(greater birds-foot trefoil)	O/LF		
Pteridium aquilinum (bracken)	VL A	Plantago lanceolata (ribwort plantain)	F		
		<b>Potentilla erecta (tormentil)</b>	R		
		Ranunculus acris (meadow buttercup)	F		
		Ranunculus repens (creeping buttercup)	O/VLF		
		<b>Rhinanthus minor (yellow rattle)</b>	LF		
		Rumex acetosa (common sorrel)	O		
		<b>Stellaria graminea (lesser stichwort)</b>	O/VL F		
		Taraxacum officinale (dandelion)	O		
		Trifolium dubium (Lesser Trefoil)	O		
		<b>Trifolium pratense (red clover)</b>	F		
		Trifolium repens (white clover)	F		
		Urtica dioica (Stinging Nettle)	VLF		
		Veronica chamaedrys (gемdr. speedwell)	O		
		Vicia sepium (bush vetch)	VL		

<b>Table 12.3 - eDNA SURVEY</b>					
<b>SITE NAME: Cleddon Fields</b>		<b>FIELD NUMBER: 8</b>		<b>DATE: 7/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>D</b>	<b>Herbs</b>	<b>D</b>	<b>Woody Species</b>	<b>D</b>
Agrostis_cap_gig	3.09 %	Cerastium_fontanum	0.27 %	Fagus_sylvatica	0.31 %
Anthoxanthum_odoratum	3.67 %	Hyacinthoides_hispanica_scillanons cripta	0.31 %		
Cynosurus_cristatus	1.33 %	<b>Hypochaeris_radicata</b>	<b>26.97</b> %		
Festuca_rubra	0.88 %	<b>Lotus_corniculatus</b>	<b>3.89</b> %		
Holcus_lanatus	0.80 %	Plantago_lanceolata	0.44 %		
Lolium_perr_mult	0.27 %	<b>Prunella_vulgaris</b>	<b>0.40</b> %		
		Ranunculus_acris_occid	21.66 %		
		<b>Ranunculus_bulb_repe</b>	<b>5.92</b> %		
		Rumex_acetosa	2.74 %		
		Taraxacum_officinale_agg.	3.36 %		
		<b>Trifolium_pratense</b>	<b>5.13</b> %		
		Trifolium_rep_occ_i_nigr	2.08 %		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 10 Indicator Species, the eDNA method recorded 3/4 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator. Whilst this wouldn't be enough to recognise the site as a LWS it would give some indication that the site is of ecological value and worth further survey work.

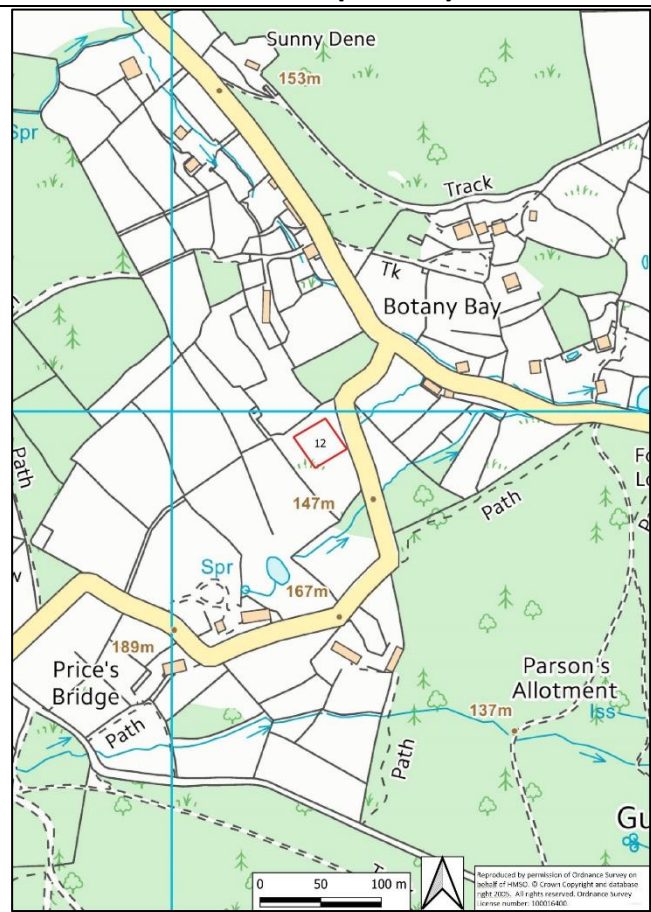
<b>Site Number/Name:</b>	<b>Site 12 – Hollytree Cottage</b>
<b>Date Surveyed:</b>	<b>7<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO52122 01974</b>
	<p>The site is located on a steep north-east facing slope. The field is grazed for part of the year but is left during spring/summer for the flora to flourish. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown and the owners report never having seen any Grassland Fungi It was surveyed however as it seemed very suitable being steeply, sloping, grazed, very floristically rich, and with much moss in the sward. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.12 – Hollytree Cottage Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 13.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.01%
<i>Camarophylloopsis schulzeri</i>		[VU]	17	0.91%
<i>Clavaria falcata</i>			30	0.14%
<i>Clavaria flavipes</i>	Straw Club		29	0.11%
<i>Clavaria griseobrunnea</i>			4	0.02%
<i>Clavaria redolealii</i>			2	0.01%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.05%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.01%
<i>Ramariopsis flavescens</i>	a coral fungus sp.		16	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.43%
<i>Cuphophyllus fornicatus</i>	Earthy Waxcap		1	1.33%
<i>Cuphophyllus pratensis</i>	Meadow Waxcap		22	1.13%

Cuphophyllus_virgineus	Snowy Waxcap		21	0.02%
Gliophorus_psittacinus	Parrot Waxcap		12	0.65%
Hygrocybe_cantharellus	Goblet Waxcap		6	0.03%
Hygrocybe_chlorophana	Golden Waxcap		16	0.36%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	2.33%
Hygrocybe_conica	Blackening Waxcap		25	0.65%
Hygrocybe_glutinipes	Glutinous Waxcap		22	1.27%
Hygrocybe_insipida	Spangle Waxcap		19	0.08%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	0.05%
Hygrocybe_reidii	Honey Waxcap	DD	6	1.49%
Neohygrocybe_ingrata	Dingy Waxcap	VU	4	2.58%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.16%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_atrocoeruleum	a pinkgill sp.		5	0.03%
Entoloma_conferendum	a pinkgill sp.		25	0.03%
Entoloma_exile	a pinkgill sp.		12	0.02%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.02%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.14%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_prunuloides	Mealy Pinkgill	VU	6	0.01%
Entoloma_sepium	a pinkgill sp.		1	0.16%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.15%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.05%
Geoglossum_nigritum	an Earthtongue sp.		15	0.04%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.07%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.03%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.40%
Trichoglossum_hirsutum	Hairy Earthtongue		4	0.40%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.56%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.03%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.13%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			14
Entolomataceae			9
Geoglossomycetes			7
Dermoloma			1
<b>CHEGD Score</b>			<b>40</b>
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			4
Hygrophoraceae			12
Entolomataceae			2
Geoglossomycetes			5
Dermoloma			1
<b>CHEGD Score</b>			<b>24</b>

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN** (International Union for Conservation of Nature) – **DD = Data Deficient** – *Not enough data available to make a conclusion.*

This is a highly valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 9 Vulnerable species were identified further reinforces its value and the importance of preserving this.

No fungi had been noted at this site previously, this demonstrates how it can be easy to overlook even a fantastic site as this as the visible fruiting bodies are so ephemeral and present when wildflower rich grasslands aren't often being visited.

## Flora

The following two tables (13.2 and 13.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 13.2 - ORIGINAL SURVEY					
SITE NAME: Holly Tree Cottage		FIELD NUMBER: 4		DATE: 26/06/2015	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis tenuis (common bent)	F	Achillea millefolium (yarrow)	O	Acer pseudoplatanus (Sycamore)	R
Anthoxanthum odoratum (sweet vnl grass)	F	Ajuga reptans (bugle)	O/V LF	Alnus glutinosa (Alder)	R
Briza media (quaking grass)	O	Conopodium majus (pignut)	O	Crataegus monogyna (Hawthorn)	O
Carex caryophylla (spring sedge)	L F	Centaurea nigra (common knapweed)	F	Fraxinus sylvatica (Ash)	R
Cynosurus cristatus (crested dog's tail)	F	Cerastium holosteoides (cmn. mouse-ear)	O	Malus sylvestris (Crab Apple)	O
Dactylis glomerata (cock's foot)	V O	Crepis capillaris (smooth hawks-beard)	R		
Festuca rubra (red fescue)	F	Dactylorhiza fuchsii (Common spotted orchid)	O		
Holcus lanatus (yorkshire fog)	F	Geranium robertianum (herb robert)	VL		
Juncus effusus (soft rush)	O	Hyacinthoides non-scripta (bluebell)	O		
Lolium perenne (perennial rye grass)	O	Hypochaeris radicata (common cats ear)	LF		
Poa trivialis (rough meadow grass)	O	Leontodon hispidus (rough hawkbit)	O		
Pteridium aquilinum (bracken)	O	Leucanthemum vulgare (oxeye daisy)	F		
		Lotus corniculatus (birds-foot trefoil)	VL		

	Lotus uliginosus(greater birds-foot trefoil)	O	
	Lysimachia nemorum (Yellow Pimpernel)	Y	
	Oxalis acetosella (Wood Sorrel)	VL	
	<b>Pedicularis sylvatica (Lousewort)</b>	<b>O</b>	
	<b>Pilosella officinarum (msec-ear hawkweed)</b>	<b>O</b>	
	Plantago lanceolata (ribwort plantain)	F	
	<b>Potentilla erecta (tormentil)</b>	<b>LF</b>	
	Potentilla sterilis (barren strawberry)	O	
-	Primula vulgaris (Primrose)	VLF	
-	Prunella vulgaris (self-heal)	O/LF	
-	Ranunculus acris (meadow buttercup)	O	
	Ranunculus repens (creeping buttercup)	O/LF	
	Rubus fruticosus (bramble)	O	
	Rumex acetosa (common sorrel)	O	
	Rumex obtusifolius (Broad-leaved Dock)	R	
	<b>Stellaria graminea (lesser stichwort)</b>	<b>O/V LF</b>	
	<b>Succisa pratensis (devils bit-scabious)</b>	<b>LF</b>	
	Tamus communis (Black Bryony)	R	
	<b>Trifolium pratense (red clover)</b>	<b>LF</b>	
	Trifolium repens (white clover)	O	
	Veronica chamaedrys (Germander speedwell)	O	
	Vicia sepium (bush vetch)	R	
	<b>Viola riviniana (common dog violet)</b>	<b>R</b>	

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 17 Indicator Species, the eDNA method recorded 13/14 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a LWS.



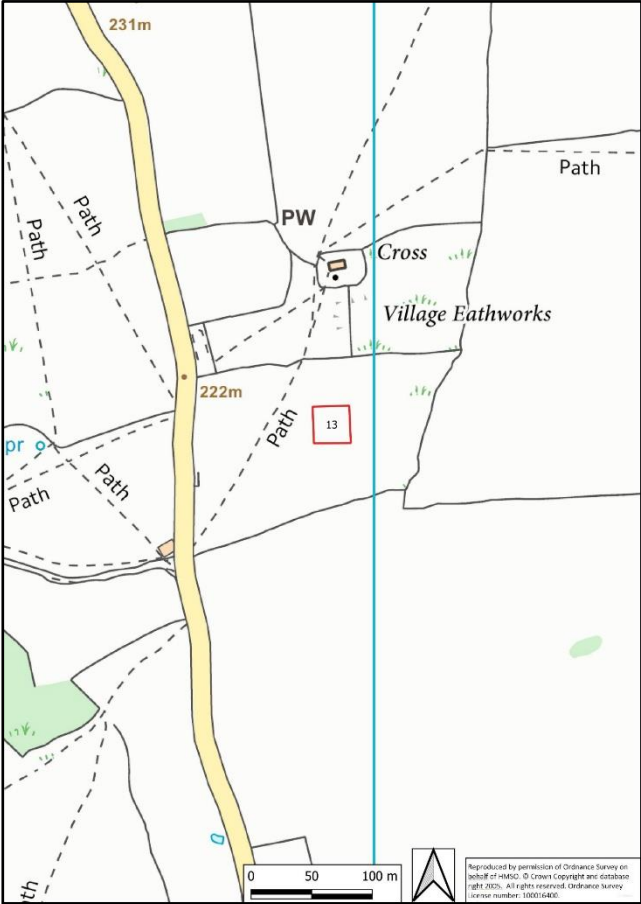
<b>Site Number/Name:</b>	<b>Site 13 – Penterry Fields (Field S. of Penterry Church)</b>
<b>Date Surveyed:</b>	<b>7<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>ST51965 98631</b>
	<p>The site is located on a gentle east facing slope. The fields are managed as Hay Meadows with aftermath grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner had reported a number of unidentified grassland fungi to be present in the autumn. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.13 – Penterry Fields (Field S. of Penterry Church) Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 14.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_atrovelutina</i>			24	0.04%
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	0.63%
<i>Clavaria_falcata</i>			30	0.16%
<i>Clavaria_flavipes</i>	Straw Club		29	0.33%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.01%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.21%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.23%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.93%
<i>Cuphophyllus_flavipes</i>	Yellow Foot Waxcap	[VU]	10	2.36%

Cuphophyllus_pratensis	Meadow Waxcap		22	0.32%
Cuphophyllus_virginicus	Snowy Waxcap		21	0.02%
Gliophorus_europeperplexus	Butterscotch Waxcap	VU	1	0.09%
Gliophorus_psittacinus	Parrot Waxcap		12	0.56%
Hygrocybe_ceracea	Butter Waxcap		3	0.41%
Hygrocybe_chlorophana	Golden Waxcap		16	0.85%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	0.73%
Hygrocybe_coccinea	Scarlet Waxcap		9	0.18%
Hygrocybe_conica	Blackening Waxcap		25	0.07%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.07%
Hygrocybe_insipida	Spangle Waxcap		19	0.19%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_asprellum	a pinkgill sp.		8	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.03%
Entoloma_atrocoeruleum	a pinkgill sp.		5	0.01%
Entoloma_clandestinum	a pinkgill sp.		13	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.03%
Entoloma_exile	a pinkgill sp.		12	0.03%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.04%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.01%
Entoloma_infula	a pinkgill sp.		12	0.02%
Entoloma_poliopus	a pinkgill sp.		14	0.02%
Entoloma_proterum	a pinkgill sp.		3	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.08%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.09%
Geoglossum_nigritum	an Earthtongue sp.		15	0.41%
Geoglossum_umbratile	Plain Earthtongue		4	0.01%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.07%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.34%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.39%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	1.48%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.02%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.11%

<b>SPECIES COUNT</b>			
Clavariaceae			7
Hygrophoraceae			12
Entolomataceae			13
Geoglossomycetes			7
Dermoloma			1
CHEGD Score			40
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			11
Entolomataceae			0
Geoglossomycetes			6
Dermoloma			1
CHEGD Score			23

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 7 Vulnerable species were identified further reinforces its value and the importance of preserving this.

This site was highlighted as a possible survey site by the owner due to the sighting of a few unidentified fungi field, this demonstrates that an indicator such as that can turn out to a sign of a site of far greater significance.

## Flora

The following two tables (14.2 and 14.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

SITE NAME: S. of Penterry Church (Penterry Fields)		FIELD NUMBER: 1		DATE: 22/06/2015	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (Common Bent)		Calystegia sepium (Hedge Bindweed)		Corylus avellana (Hazel)	
Alopecurus pratensis (Meadow Foxtail)		<b>Cardamine pratensis (Cuckoo Flower)</b>			
Anthoxanthum odoratum (Sweet Vernal Grass)		<b>Centaurea nigra (Common Knapweed)</b>			
Carex ovalis (Oval Sedge)		Cerastium holosteoides (Common Mouse-ear)			
Cynosurus cristatus (Crested Dog's-tail)		<b>Conopodium majus (Pignut)</b>			
Festuca rubra (Red Fescue)		Crepis capillaris (Smooth Hawks-beard)			
Holcus lanatus (Yorkshire Fog)		<b>Dactylorhiza fuchsii (Common Spotted Orchid)</b>			
Lolium perenne (Perennial Rye-grass)		Galium aparine (Cleavers)			
<b>Luzula campestris (Field Wood-rush)</b>		Heracleum sphondylium (Hogweed)			
Poa trivialis (Rough Meadow-grass)		<b>Hypochaeris radicata (Common Catsear)</b>			
Pteridium aquilinum (Bracken)		<b>Lathyrus pratensis (Meadow Vetchling)</b>			
		<b>Lotus corniculatus (Birds-foot Trefoil)</b>			
		Lotus pedunculatus (Greater Birds-foot Trefoil)			
		Plantago lanceolata (Ribwort Plantain)			

		Potentilla erecta (Tormentil)		
		Potentilla reptans (Creeping Cinquefoil)		
		Prunella vulgaris (Selfheal)		
		Ranunculus acris (Meadow Buttercup)		
		Ranunculus repens (Creeping Buttercup)		
		Rhinanthus minor (Yellow Rattle)		
		Rubus fruticosus (Bramble)		
-		Rumex acetosa (Common Sorrel)		
-		Rumex obtusifolius (Broad-leaved Dock)		
-		Scorzoneroide autumnalis (Autumn Hawkbit)		
		Stellaria graminea (Lesser Stitchwort)		
		Trifolium pratense (Red Clover)		
		Trifolium repens (White Clover)		
		Urtica dioica (Stinging Nettle)		
		Vicia cracca (Tufted Vetch)		

**Table 14.3 - eDNA SURVEY**

SITE NAME: S. of Penterry Church (Penterry Fields)		FIELD NUMBER: 1		DATE: 7/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	1.61 %	Achillea_millefolium	3.78 %		
Anthoxanthum_odoratum	1.80 %	Callianthemum_anemonoides	0.02 %		
Avenula_pubescens	0.08 %	Centaurea_nigra	2.26 %		
Carex_caryophylla	0.06 %	Cerastium_fontanum	0.20 %		
Dactylis_glomerata	0.43 %	Conopodium_majus	17.83 %		
Festuca_rubra	1.11 %	Cucumis_sativus	0.03 %		
Holcus_lanatus	0.16 %	Heracleum_sphondylium	0.07 %		
		Hyacinthoides_hispanica_scillan onscripta	1.69 %		
		Hypochaeris_radicata	1.96 %		
		Leontodon_hispidus	0.06 %		
-		Lotus_corniculatus	15.21 %		
		Lotus_pedunculatus	0.20 %		
		Plantago_lanceolata	13.68 %		
		Potentilla_erecta	0.16 %		
		Ranunculus_acris_occid	0.45 %		
		Ranunculus_bulb_repe	0.07 %		
		Rhinanthus_minor	2.95 %		
		Rumex_acetosa	9.58 %		
		Scorzoneroide_autumnalis	0.06 %		
		Stellaria_alsine-graminea	0.06 %		
		Taraxacum_officinale_agg.	15.15 %		
-		Veronica_chamaedrys	1.54 %		
-		Viola_riviniana	0.09 %		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 14 Indicator Species, the eDNA method recorded 11/12 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator. This would be be enough to recognise the site as a LWS.

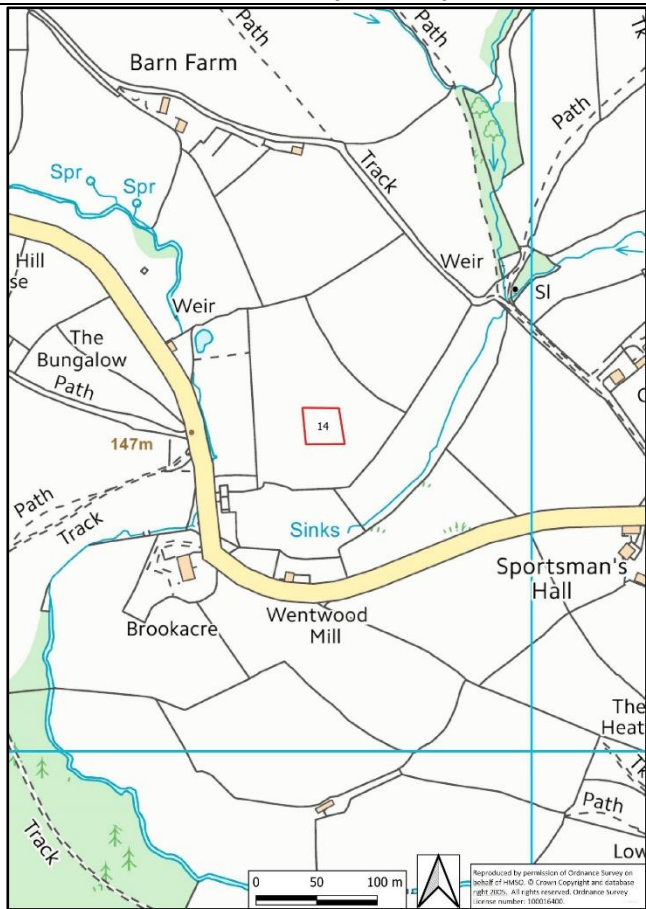
<b>Site Number/Name:</b>	<b>Site 14 – Wentwood Mill Fields</b>
<b>Date Surveyed:</b>	<b>7<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>ST43828 96268</b>
	<p>The site is located on a relatively gentle south facing slope. The fields are managed as Hay Meadows with aftermath grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however during surveys in July (outside of the peak Fungi season) 2006, the surveyor noted some yellow Waxcaps to be present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.14 – Wentwood Mill Fields Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 15.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis_atrovelutina</i>			24	0.04%
<i>Camarophyllopsis_schulzeri</i>		[VU]	17	0.58%
<i>Clavaria_falcata</i>			30	0.29%
<i>Clavaria_flavipes</i>	Straw Club		29	0.39%
<i>Clavaria_fumosa</i>	Smoky Spindles		3	0.29%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.29%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.26%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.05%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.02%
<i>Ramariopsis_flavescens</i>	a coral fungus sp.		16	0.04%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.22%

Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	3.11%
Cuphophyllus_pratensis	Meadow Waxcap		22	0.92%
Cuphophyllus_russocoriaceus	Cedarwood Waxcap		4	0.05%
Cuphophyllus_virginicus	Snowy Waxcap		21	0.83%
Gliophorus_irrigatus	Slimy Waxcap		7	0.80%
Hygrocybe_coccinea	Scarlet Waxcap		9	4.89%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.16%
Hygrocybe_helobia	Garlic Waxcap	NT	1	0.30%
Hygrocybe_insipida	Spangle Waxcap		19	0.36%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	2.21%
Hygrocybe_miniata	Vermillion Waxcap		1	2.50%
Hygrocybe_punicea	Crimson Waxcap	VU	7	3.70%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	0.15%
Hygrocybe_reidii	Honey Waxcap	DD	6	1.53%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.30%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.04%
Entoloma_asprellum	a pinkgill sp.		8	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.03%
Entoloma_exile	a pinkgill sp.		12	0.01%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.02%
Entoloma_infula	a pinkgill sp.		12	0.01%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_prunuloides	Mealy Pinkgill	VU	6	0.58%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.02%
Geoglossum_fallax	Deceptive Earthtongue		16	0.04%
Geoglossum_nigritum	an Earthtongue sp.		15	0.12%
Glutinoglossum_pseudoglutinosu m	an Earthtongue sp.		21	0.02%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.03%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	1.84%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.01%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.01%

<b>SPECIES COUNT</b>			
Clavariaceae			10
Hygrophoraceae			15
Entolomataceae			8
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			39
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			7
Hygrophoraceae			15
Entolomataceae			1
Geoglossomycetes			2
Dermoloma			0
CHEGD Score			25

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN NT = Near Threatened** – *IUCN Criteria suggest that whilst not currently at high risk of extinction it is considered this may be the case in the near future.*



**IUCN – DD = Data Deficient** – Not enough data available to make a conclusion.

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 9 Vulnerable species were identified further reinforces its value and the importance of preserving this.

This site was highlighted as a possible survey site by reviewing old floral surveys of the fields that noted a few yellow Waxacaps being present in the summer (outside of “Fungi Season”), this demonstrates that an indicator such as that can turn out to a sign of a site of far greater significance.

## Flora

The following two tables (15.2 and 15.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 15.2 - ORIGINAL SURVEY					
SITE NAME: Wentwood Mill		FIELD NUMBER: 2		DATE: 21/06/2016	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	LF	Achillea millefolium (yarrow)	O	Quercus sp. (Oak sp.) seedling	R
Anthoxanthum odoratum (sweet vnl grass)	F	<b>Centaurea nigra (common knapweed)</b>	O/LA		
<b>Carex caryophylla (spring sedge)</b>	LF	Cerastium holosteoides (cmn. mouse-ear)	O		
Festuca ovina (sheep's fescue)	VL	<b>Dactylorhiza fuchsii (c. spotted orchid)</b>	VO		
Festuca rubra (red fescue)	F	<b>Hypochaeris radicata (common cats ear)</b>	F/LA		
Holcus lanatus (yorkshire fog)	F	<b>Lathyrus pratensis (meadow vetchling)</b>	R		
<b>Luzula campestris (field wood rush)</b>	O	<b>Leontodon hispidus (rough hawkbit)</b>	F/LA		
Pteridium aquilinum (bracken)	LD	<b>Leucanthemum vulgare (oxeye daisy)</b>	LA		
<b>Trisetum flavescens (yellow oat grass)</b>	VL F	<b>Lotus corniculatus (birds-foot trefoil)</b>	F/A		
		Lotus uliginosus (greater birds-foot trefoil)	O		
		Plantago lanceolata (ribwort plantain)	F		

		Potentilla erecta (tormentil)	VL		
		Ranunculus acris (meadow buttercup)	VO		
		Ranunculus bulbosus (bulbous buttercup)	O		
		Rhinanthus minor (yellow rattle)	O/VL F		
		Rumex acetosa (common sorrel)	O		
		Senecio jacobea (ragwort)	R		
-		Stellaria graminea (lesser stichwort)	O/VL F		
-		Trifolium dubium (Lesser Trefoil)	LF		
-		Trifolium pratense (red clover)	F		
		Trifolium repens (white clover)	O		
		Veronica chamaedrys (gendr. speedwell)	O		
		Vicia cracca (tufted vetch)	VLF		

**Table 15.3 - eDNA SURVEY**

SITE NAME: Wentwood Mill		FIELD NUMBER: 2		DATE: 7/5/22	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	1.08%	Achillea_millefolium	5.08%		
Anthoxanthum_odoratum	2.38%	Centaurea_nigra	27.89%		
Carex_caryophylla	0.32%	Conopodium_majus	1.08%		
Festuca_rubra	1.51%	Cucumis_sativus	0.43%		
Holcus_lanatus	0.32%	Hypochaeris_radicata	0.65%		
		Leontodon_hispidus	41.08%		
		Leontodon_saxatilis	1.08%		
		Lotus_corniculatus	2.59%		
		Ranunculus_bulb_repe	3.57%		
		Rumex_acetosa	2.92%		
		Scorzonerooides_autumnalis	0.54%		
		Taraxacum_officinale_agg.	0.86%		
		Trifolium_pratense	0.65%		
		Vicia_cracca	1.62%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 16 Indicator Species, the eDNA method recorded 9/10 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator. This would be be enough to recognise the site as a LWS.

<b>Site Number/Name:</b>	<b>Site 15 – Crick Community Meadow</b>
<b>Date Surveyed:</b>	<b>7<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>ST48828 89950</b>

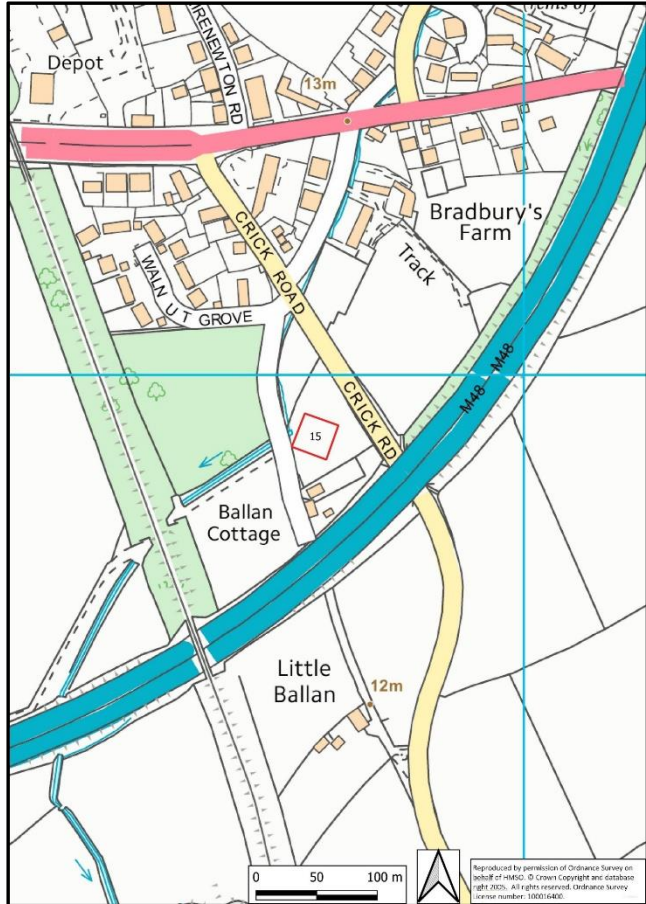


Figure 2.15 – Crick Community Meadow

The site is located on a relatively gentle north facing slope. The field is managed as a Hay Meadow, although as recently as 10 years ago it was very rank with clumps of Cocksfoot etc. and limited floristic diversity. It is now known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field recently became apparent when GWT were provided with photos of a number of different Waxcaps (White, Yellow, greenish, Orange & Red) known to be present, these haven't been formally identified however. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to (although Snowy and Parrot are very likely present + others).

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 16.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.01%
<i>Clavaria falcata</i>			30	0.17%
<i>Clavaria flavipes</i>	Straw Club		29	0.17%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	3.27%

Clavulinopsis_helvola	Yellow Club		25	4.11%
Clavulinopsis_laeticolor	Handsome Club		21	2.36%
Clavulinopsis_luteoalba	Apricot Club		18	0.01%
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.01%
Ramariopsis_crocea	a coral fungus sp.		23	0.15%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	3.37%
Cuphophyllus_virginus	Snowy Waxcap		21	2.50%
Hygrocybe_conica	Blackening Waxcap		25	0.47%
Hygrocybe_glutinipes	Glutinous Waxcap		22	1.62%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	1.22%
Entoloma_conferendum	a pinkgill sp.		25	0.10%
Entoloma_sericeum	Silky Pinkgill		24	0.05%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.13%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.05%
Glutinoglossum_pseudoglutinatum	an Earthtongue sp.		21	0.04%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.33%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.24%
Trichoglossum_octopartitum	an Earthtongue sp.		1	0.05%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	3.54%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.24%
Dermoloma_magicum	Black Magic	[VU]	16	3.97%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			4
Entolomataceae			2
Geoglossomycetes			6
Dermoloma			1
CHEGD Score			22
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			4
Entolomataceae			2
Geoglossomycetes			5
Dermoloma			1
CHEGD Score			18

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The White waxcaps that were reported from the site will be the Snowy Waxcaps. The red/orange ones could be any one of Blackening, Glutinous or Fibrous. It is interesting to note that the eDNA results didn't pick up Parrot Waxcap which is thought to be present (although Glutinous Waxcap which is similar was picked up) or any "yellow" species which are also known to be present. There are clearly additional species present that weren't picked up by the eDNA, it must be borne in mind that the eDNA only sampled from a 30mx30m part of the site.

Overall the results showed a good variety of Fungi to be present with a number of Hygrocybe (Waxcaps) being recorded and clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The fact that 3 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.

It is interesting to note that in contrast to other sites surveyed this site was previously quite floristically species-poor and had become rank. This shows that despite becoming rank and losing a lot of the flora, the fungi must have hung on and given the opportunity are starting the flourish and fruit again.

## Flora

The following two tables (16.2 and 16.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table – 16.2 ORIGINAL SURVEY					
SITE NAME: Crick Community Meadow		FIELD NUMBER: 1		DATE: 26/5/2022	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	O	<i>Achillea millefolium</i> (Yarrow)	O		
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	F	<i>Ajuga reptans</i> (Bugle)	O		
<i>Cynosurus cristatus</i> (Crested Dogstail)	O	<b><i>Centaurea nigra</i> (Common Knapweed)</b>	O/ F		
<i>Dactylis glomerata</i> (Cock's foot)	O	<i>Cerastium holosteoides</i> (Common Mouse-ear)	O		
<i>Festuca rubra</i> (Red Fescue)	F	<i>Cirsium arvense</i> (Creeping Thistle)	O		
<i>Holcus lanatus</i> (Yorkshire Fog)	F	<i>Crepis capillaris</i> (Smooth Hawks-beard)	R		
		<i>Crepis versicaria</i> (Beaked Hawksbeard)	R		
		<i>Daucus carota</i> (Wild Carrot)	O		
		<b><i>Euphrasia officinalis</i> agg. (Eyebright)</b>	?		
		<i>Geranium dissectum</i> (Cut-leaved Cranesbill)	R		
		<i>Glechoma hederacea</i> (Ground Ivy)	O		
		<i>Heracleum sphondylium</i> (Hogweed)	O		
		<b><i>Hypochaeris radicata</i> (Common Catsear)</b>	O		
		<i>Lathyrus pratensis</i> (Meadow Vetchling)	O		
		<b><i>Leucanthemum vulgare</i> (Oxeye Daisy)</b>	LF		
		<i>Lotus corniculatus</i> (Birds-foot Trefoil)	O		
		<i>Myosotis arvensis</i> (Field Forget-me-not)	O		
		<i>Pilosella aurantiaca</i> (Fox & Cubs)	R		
		<i>Plantago lanceolata</i> (Ribwort Plantain)	F		
		<i>Potentilla anserina</i> (Silverweed)	R		
		<i>Potentilla sterilis</i> (Barren Strawberry)	VL		
-		<i>Ranunculus acris</i> (Meadow Buttercup)	F		
-		<b><i>Ranunculus bulbosus</i> (Bulbous Buttercup)</b>	O		

-	<i>Ranunculus repens</i> (Creeping Buttercup)	A	
	<i>Rhinanthus minor</i> (Yellow Rattle)	F/A	
	<i>Rumex acetosa</i> (Common Sorrel)	O	
	<i>Rumex obtusifolius</i> (Broad-leaved Dock)	O	
	<i>Senecio jacobea</i> (Ragwort)	O	
	<i>Stellaria graminea</i> (Lesser Stitchwort)	O	
	<i>Succisa pratensis</i> (Devil's Bit Scabious)	R	
	<i>Taraxacum officinale</i> sp. agg. (Dandelion)	O	
	<i>Trifolium dubium</i> (Lesser Trefoil)	O	
	<i>Trifolium pratense</i> (Red Clover)	O/F	
	<i>Trifolium repens</i> (White Clover)	O	
	<i>Veronica chamaedrys</i> (Germander Speedwell)	LF	
	<i>Vicia sativa</i> (Common Vetch)	O	

Table 16.3 - eDNA SURVEY					
SITE NAME: Crick Community Meadow		FIELD NUMBER: 1		DATE: 7/5/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
<i>Agrostis cap_gig</i>	2.31%	<i>Centaurea nigra</i>	0.08%	<i>Quercus petraea_robur</i>	0.02%
<i>Anthoxanthum odoratum</i>	0.89%	<i>Cerastium fontanum</i>	0.16%	<i>Salix purpurea</i>	0.04%
<i>Dactylis glomerata</i>	0.04%	<i>Cucumis sativus</i>	0.02%		
<i>Festuca rubra</i>	0.03%	<i>Hypochaeris radicata</i>	0.32%		
<i>Holcus lanatus</i>	0.21%	<i>Leucanthemum vulgare</i>	0.71%		
<i>Poa trivialis</i>	0.51%	<i>Plantago lanceolata</i>	9.86%		
		<i>Ranunculus acris_occid</i>	0.83%		
		<i>Ranunculus bulb_repe</i>	0.77%		
		<i>Rhinanthus minor</i>	46.90%		
		<i>Rumex acetosa</i>	2.97%		
		<i>Trifolium pratense</i>	0.60%		
		<i>Trifolium rep_occi_nigr</i>	25.03%		
		<i>Veronica chamaedrys</i>	0.05%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 12 Indicator Species, the eDNA method recorded 5/6 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator. Whilst this wouldn't be enough to recognise the site as a LWS it would give a good indication that the site is of ecological value and worth further survey work.

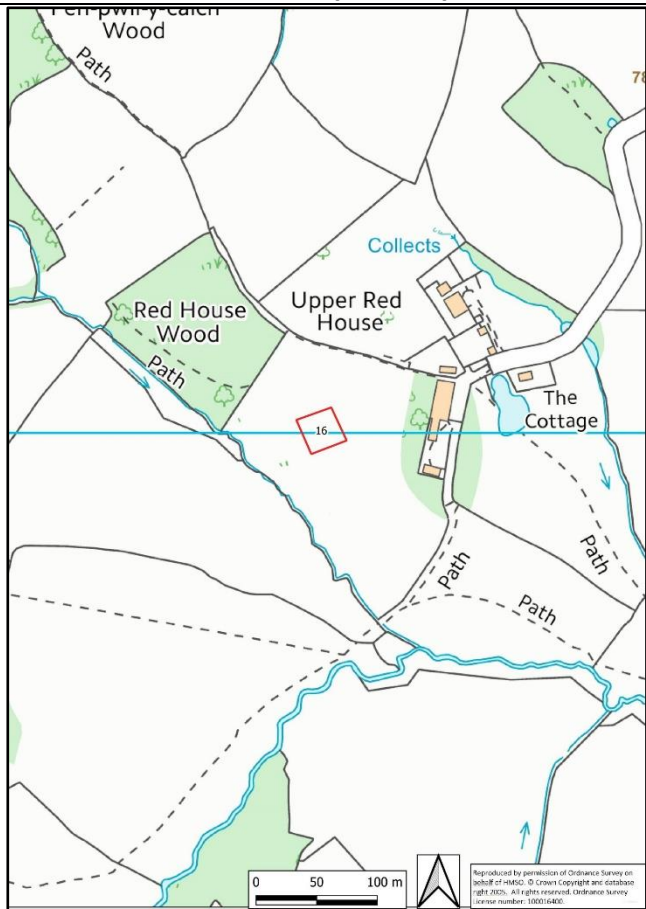
<b>Site Number/Name:</b>	<b>Site 16 – Upper Redhouse Farm</b>
<b>Date Surveyed:</b>	<b>8<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO42687 13002</b>
 <p>The map shows the site location at Upper Redhouse Farm. Key features include 'Red House Wood', 'Upper Red House', 'The Cottage', and several paths. A red box labeled '16' indicates the specific site location. A scale bar shows 0, 50, and 100 meters. A north arrow is also present. Small text at the bottom of the map reads: 'Reproduced by permission of Ordnance Survey on behalf of HMISO. © Crown Copyright and database right (2022). All rights reserved. Ordnance Survey license number: 100014840.'</p>	<p>The site is located on a relatively steeply sloping west facing slope. The field is notable for the presence of many Yellow Meadow Ant Hills. The field is managed by grazing, with the grazing excluded in spring/summer to allow the flowers to flourish. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The owner of the field reported a number of Waxcaps to be present, these haven't been formally identified however. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.16 – Upper Redhouse Farm Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 17.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_atrovelutina</i>			24	0.05%
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	0.31%
<i>Clavaria_falcata</i>			30	0.25%
<i>Clavaria_flavipes</i>	Straw Club		29	0.10%
<i>Clavulinopsis_corniculata</i>	Meadow Coral		22	0.49%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.51%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.17%
<i>Hodophilus_micaceus</i>			4	0.02%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.06%



Ramariopsis_crocea	a coral fungus sp.		23	0.02%
Ramariopsis_flavescens	a coral fungus sp.		16	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.47%
Cuphophyllus_virgineus	Snowy Waxcap		21	1.47%
Hygrocybe_chlorophana	Golden Waxcap		16	0.51%
Hygrocybe_conica	Blackening Waxcap		25	1.39%
Hygrocybe_glutinipes	Glutinous Waxcap		22	1.88%
Hygrocybe_insipida	Spangle Waxcap		19	0.24%
Entoloma_asprellum	a pinkgill sp.		18	0.02%
Entoloma_conferendum	a pinkgill sp.		25	0.23%
Entoloma_dysthales	a pinkgill sp.		10	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.06%
Geoglossum_fallax	Deceptive Earthtongue		16	0.41%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.02%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.36%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.04%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.20%
Dermoloma_magicum	Black Magic	[VU]	16	1.44%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.69%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			5
Entolomataceae			4
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			27
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			8
Hygrophoraceae			5
Entolomataceae			1
Geoglossomycetes			3
Dermoloma			2
CHEGD Score			19

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with a number of Hygrocybe (Waxcaps) being recorded and clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The fact that 2 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.



## Flora

The following two tables (17.2 and 17.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

SITE NAME: Upper Red House Farm		FIELD NUMBER: 1	DATE: 10/06/2016		
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis tenuis (common bent)	LF	Achillea millefolium (yarrow)	O	Alnus glutinosa (Alder)	V L
Alopecurus pratensis (meadow foxtail)	VL F	Bellis perennis (Daisy)	R	Betula sp. (Birch)	V L
Anthoxanthum odoratum (sweet vnl grass)	F	<b>Cardamine pratensis (cuckoo flower)</b>	O	Sambucus nigra (Elder)	V L
Bromujs hordaceus (Soft Brome)	O/L F	<b>Centaurea nigra (common knapweed)</b>	O/V LF		
Carex hirta (Hairy Sedge)	VL F	Digitalis purpurea (Foxglove)	R		
Dactylis glomerata (cock's foot)	O	Galium palustre (marsh-bedstraw)	VL		
Festuca rubra (red fescue)	LF	Glechoma hederacea (ground ivy)	O		
Holcus lanatus (yorkshire fog)	F/A	<b>Hypochaeris radicata (common cats ear)</b>	O		
Juncus acutiflorus (sharp-flowered rush)	VL A	Lathyrus pratensis (meadow vetchling)	O		
Juncus inflexus (hard rush)	VL A	<b>Lotus corniculatus (birds-foot trefoil)</b>	F/LA		
Lolium perenne (perennial rye grass)	LF	Lotus uliginosus (greater birds-foot trefoil)	O/LF		
Luzula campestris (field wood rush)	O	<b>Ononis repens (common restharrow)</b>	VLA		
Phleum pratense (timothy grass)	R	Prunella vulgaris (self-heal)	VO		
Poa trivialis (rough meadow grass)	O/L F	Pulicaria dysenterica (fleabane)	VL		
<b>Vulpia bromoides (Squirrel Tail Fescue)</b>	R	Ranunculus acris (meadow buttercup)	F		
		<b>Ranunculus bulbosus (bulbous buttercup)</b>	O		
		Ranunculus repens (creeping buttercup)	O		
		Rubus fruticosus (bramble)	VL		
-		Rumex acetosa (common sorrel)	O		
-		Rumex obtusifolius (Broad-leaved Dock)	O		
-		<b>Stellaria graminea (lesser stichwort)</b>	LF		
		Trifolium dubium (Lesser Trefoil)	O		
		<b>Trifolium pratense (red clover)</b>	O		
		Trifolium repens (white clover)	O		
		Urtica dioica (Stinging Nettles)	O		
		Veronica chamaedrys (gemdr. speedwell)	O		
		Veronica serpyllifolia (Thyme-leaved Speedwell)	R		

<b>Table 17.3 - eDNA SURVEY</b>					
<b>SITE NAME: Upper Red House Farm</b>		<b>FIELD NUMBER: 1</b>		<b>DATE: 8/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Woody Species</b>	<b>%</b>
Agrostis_cap_gig	2.92%	Cerastium_fontanum	0.13%		
Anthoxanthum_odoratum	2.97%	Cirsium_palustre	0.22%		
Cynosurus_cristatus	0.06%	Cucumis_sativus	0.05%		
Festuca_rubra	1.28%	Ficaria_verna	0.60%		
Holcus_lanatus	0.57%	Lotus_corniculatus	8.41%		
Lolium_perr_mult	1.66%	Plantago_lanceolata	0.17%		
Poa_trivialis	0.65%	Potentilla_erecta	0.05%		
		Ranunculus_bulb_repe	1.93%		
		Rumex_acetosa	1.49%		
		Stellaria_alsine-graminea	4.27%		
		Taraxacum_officinale_agg.	0.62%		
		Trifolium_pratense	30.43%		
		Trifolium_rep_occi_nigr	25.19%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 11 Indicator Species, the eDNA method recorded 4/5 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). Whilst this wouldn't be enough to recognise the site as a LWS it would give good indication that the site is of ecological value and worth further survey work.

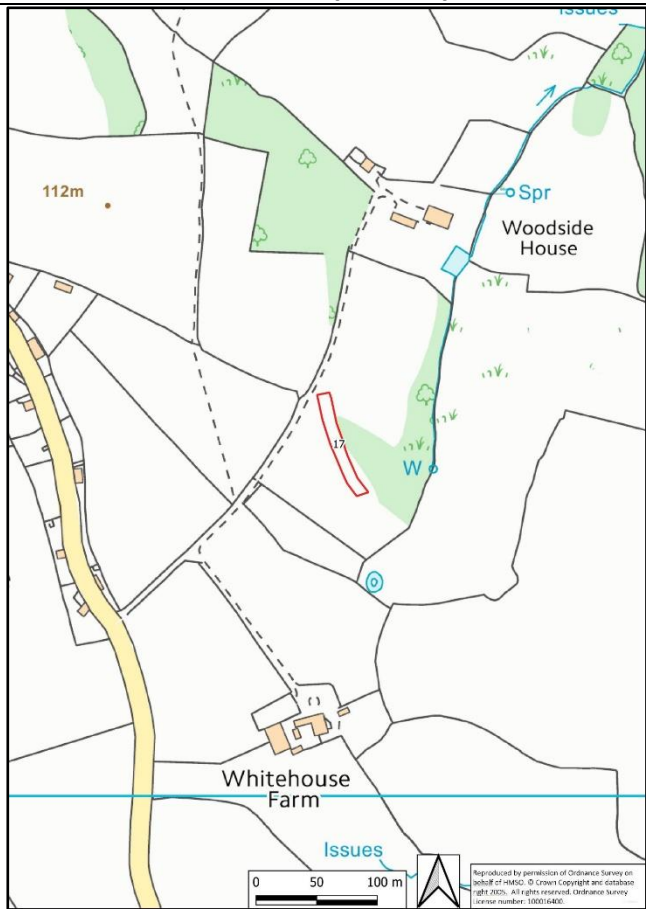
<b>Site Number/Name:</b>	<b>Site 17 – Woodside House, Maypole</b>
<b>Date Surveyed:</b>	<b>8<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO47485 16290</b>
	<p>The site was located within a narrow steep north facing slope. It is floristically species-rich and is recognised as part of a Local Wildlife Site because of this. The owners reported a number of grassland fungi to be present. Both the eDNA Fungal and Floral results are shown below, however there are no direct previous results to compare these to.</p> <p><i>*It should be noted that this is the only site where a square 30mx30m area wasn't used, because the area was too narrow to accommodate this. A 90mx10m strip was utilised instead.</i></p>

Figure 2.17 – Woodside House, Maypole Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 18.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
Clavaria_californica			6	0.03%
Clavaria_falcata			30	0.17%
Clavaria_flavipes	Straw Club		29	0.05%
Clavaria_fragilis	White Spindles		3	0.09%
Clavaria_tenuipes			3	0.03%
Clavulinopsis_corniculata	Meadow Coral		22	0.01%
Clavulinopsis_laeticolor	Handsome Club		21	0.26%
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.11%
Ramariopsis_crocea	a coral fungus sp.		23	0.02%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.79%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.11%

Hygrocybe_conica	Blackening Waxcap		25	2.28%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.01%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	1.13%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.02%
Entoloma_infula	a pinkgill sp.		12	0.02%
Entoloma_neglectum	a pinkgill sp.		5	0.05%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.05%
Entoloma_sericeum	Silky Pinkgill		24	0.03%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.11%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.57%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.01%
Trichoglossum aff. variabile	an Earthtongue sp.		19	2.10%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.49%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.37%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			4
Entolomataceae			6
Geoglossomycetes			3
Dermoloma			1
CHEGD Score			23
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			3
Entolomataceae			2
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			13

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with the a number of Hygrocybe (Waxcaps) being recored and clearly demonstrates this site has significant value for its grassland fungi as well as the already recognised floristic value.

The fact that a Vulnerable species was identified by eDNA further reinforces its value and the importance of preserving this.

## Flora

The following table (18.3) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 18.3 - eDNA SURVEY**

SITE NAME: Woodside House		FIELD NUMBER:		DATE: 08/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	0.81%	Achillea_millefolium	3.41%	Crataegus_monogyna	0.02%
Alopecurus_pratensis	0.14%	<b>Callianthemum_anemonoides</b>	<b>0.04%</b>	Salix_purpurea	0.02%
Anthoxanthum_odoratum	0.50%	<b>Centaurea_nigra</b>	0.12%		
Arrhenatherum_elatius	0.08%	Cerastium_fontanum	1.47%		
Cynosurus_cristatus	0.34%	Cirsium_arvense	3.37%		
Dactylis_glomerata	0.11%	Heracleum_sphondylium	0.04%		
Elymus_repens	0.05%	<b>Hypochaeris_radicata</b>	0.17%		
Festuca_rubra	0.19%	<b>Lathyrus_pratensis</b>	0.17%		
Holcus_lanatus	0.83%	Plantago_lanceolata	1.58%		
Lolium_perr_mult	1.03%	<b>Potentilla_erecta</b>	2.34%		
Phleum_pratense	0.12%	Potentilla_reptans	1.33%		
Poa_trivialis	0.52%	Prunella_vulgaris	0.03%		
		Ranunculus_acris_occid	7.89%		
		<b>Ranunculus_bulb_repe</b>	5.48%		
		Rumex_acetosa	17.35%		
		Taraxacum_officinale_agg.	6.61%		
		<b>Trifolium_pratense</b>	2.62%		
		Trifolium_rep_occi_nigr	30.64%		
-		Vicia_sativa	2.47%		

The eDNA survey returned a good number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 5/6 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This wouldn't be enough to recognise the site as a Local Wildlife Site, however it would give a good idea of potential value and warrant further survey work.

Note the presence of a number of species highlighted in **RED** that are likely an eDNA recognition error.

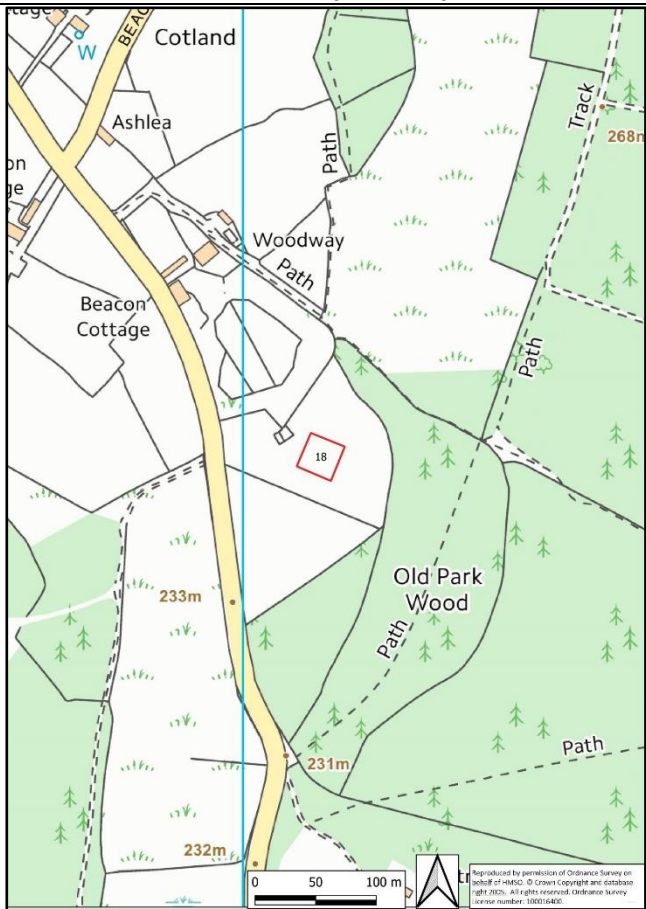
<b>Site Number/Name:</b>	<b>Site 18 – Old Park Nursery Field</b>
<b>Date Surveyed:</b>	<b>8<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO51064 04460</b>
	<p>The site is located on gently sloping, south facing slope. The fields are grazed by horses but weren't at the time of survey. It has not been formally surveyed botanically, however notes made during the collection of soil samples revealed a number of Species-rich Grasslands Indicator Species to be present. The fungal diversity of the field is unknown, however the tenants reported a number of grassland fungi to be present in the autumn. A more limited list of floral Indicator Species and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.18 – Old Park Nursery Field Site Location

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 19.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	0.03%
<i>Clavaria_falcata</i>			30	0.53%
<i>Clavaria_flavipes</i>	Straw Club		29	0.24%
<i>Clavaria_fragilis</i>	White Spindles		3	2.45%
<i>Clavulinopsis_corniculata</i>	Meadow Coral		22	0.15%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.23%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.89%
<i>Clavulinopsis_umbrinella</i>	Beige Coral		4	3.61%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.10%

Ramariopsis_crocea	a coral fungus sp.		23	0.01%
Ramariopsis_flavescens	a coral fungus sp.		16	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.39%
Cuphophyllus_pratensis	Meadow Waxcap		22	3.23%
Cuphophyllus_virgineus	Snowy Waxcap		21	0.02%
Hygrocybe_conica	Blackening Waxcap		25	0.29%
Hygrocybe_insipida	Spangle Waxcap		19	0.05%
Hygrocybe_phaeococcinea	Shadowed Waxcap	[VU]	3	0.03%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.02%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.01%
Entoloma_pleopodium	Aromatic Pinkgill		1	0.02%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.03%
Entoloma_sericeum	Silky Pinkgill		24	0.02%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.11%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.33%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.10%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.47%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.65%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	1.78%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.45%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			5
Entolomataceae			7
Geoglossomycetes			5
Dermoloma			0
CHEGD Score			28
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			8
Hygrophoraceae			3
Entolomataceae			7
Geoglossomycetes			5
Dermoloma			0
CHEGD Score			23

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with the a number of Hygrocybe (Waxcaps) being recorded and clearly demonstrates this site has significant value for its grassland fungi.

The fact that 4 Vulnerable species were identified by eDNA further reinforces its value and the importance of preserving this.

## Flora

The following table (19.2) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 19.2 - eDNA SURVEY**

SITE NAME: Old Park Nursery		FIELD NUMBER:		DATE: 08/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	0.59%	Achillea_millefolium	3.27%		
Anthoxanthum_odoratum	0.28%	<b>Centaurea_nigra</b>	52.31%		
Festuca_rubra	0.17%	Cerastium_fontanum	0.13%		
Holcus_lanatus	0.28%	<b>Hypochaeris_radicata</b>	11.45%		
		Lotus_pedunculatus	0.71%		
		Plantago_lanceolata	0.11%		
		Ranunculus_acris_occid	0.45%		
		<b>Ranunculus_bulb_repe</b>	0.39%		
		<b>Rhinanthus_minor</b>	0.23%		
		Rumex_acetosa	0.56%		
		Taraxacum_officinale_agg.	10.09%		
		<b>Trifolium_pratense</b>	14.82%		
		Trifolium_rep_occid_nigr	0.54%		

The eDNA survey returned a reasonable number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 4/5 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This wouldn't be enough to recognise the site as a Local Wildlife Site, however it would give a good idea of potential value and warrant further survey work.

Site Number/Name:

Site 19 – The Elms



Date Surveyed:

8<sup>th</sup> May 2022

British National Grid (centre):

SO50005 03834

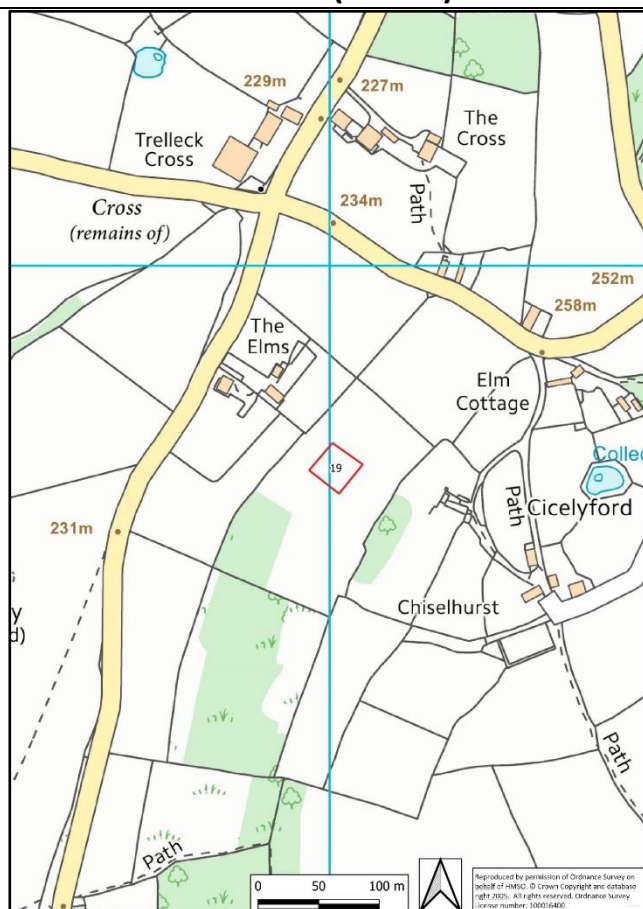


Figure 2.19 – The Elms Site Location

The site is located on a quite steep, north-west facing slope. The field is managed as low intensity cattle grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field recently became apparent when GWT were provided with photos of a number of different Waxcaps (White, Yellow, Red, & Pink) known to be present, these haven't been formally identified however. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to (although Snowy and Pink are very likely present + others).

### Results and comparison of these between conventional and eDNA.

#### Fungi

The results of the eDNA Survey are shown within Table 20.1

Table 20.1 - Site No.19 The Elms

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.11%
<i>Camarophyllopsis schulzeri</i>		[VU]	17	1.29%
<i>Clavaria falcata</i>			30	0.23%
<i>Clavaria flavipes</i>	Straw Club		29	0.50%
<i>Clavaria messapica</i>			8	0.09%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.02%
<i>Clavulinopsis helvola</i>	Yellow Club		25	1.00%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.21%
<i>Clavulinopsis umbrinella</i>	Beige Coral		4	0.03%
<i>Lamelloclavaria petersenii</i>			4	0.01%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.22%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.06%

Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	0.94%
Cuphophyllus_pratensis	Meadow Waxcap		22	2.81%
Gliophorus_irrigatus	Slimy Waxcap		7	0.41%
Hygrocybe_chlorophana	Golden Waxcap		16	3.88%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	0.99%
Hygrocybe_inspida	Spangle Waxcap		19	0.06%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	1.99%
Hygrocybe_punicea	Crimson Waxcap	VU	7	4.50%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	0.13%
Hygrocybe_reidii	Honey Waxcap	DD	6	0.03%
Neohygrocybe_ovina	Blushing Waxcap	VU	3	0.05%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.32%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.26%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.02%
Geoglossum_nigritum	an Earthtongue sp.		15	0.04%
Microglossum_parvisporum	an Earthtongue sp.	Sect7	5	1.59%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.02%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	4.78%

<b>SPECIES COUNT</b>			
Clavariaceae			11
Hygrophoraceae			12
Entolomataceae			4
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			30
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			8
Hygrophoraceae			11
Entolomataceae			1
Geoglossomycetes			1
Dermoloma			1
CHEGD Score			22

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN** (International Union for Conservation of Nature) – **DD = Data Deficient** – *Not enough data available to make a conclusion.*

**Sect7** = *A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.*

The red ones reported may well be Spangle Waxcap. The yellow ones reported could well be Golden Waxcap. The reported Pink Waxcap was also picked up by the eDNA. It is interesting to note that the eDNA results didn't pick up Snowy Waxcap which is known to be present. There are clearly additional species present that weren't picked up by the eDNA, it must be borne in mind that the eDNA only sampled from a 30mx30m part of the site.

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 8 Vulnerable species and a Section 7 species were identified further reinforces its value and the importance of preserving this.

This site was highlighted as a possible survey site after receiving a number of photos of different coloured Waxcaps. This demonstrates the noted presence of a few species, particularly if of a number of different colours can be a sign of a significant site.

## Flora

The following two tables (20.2 and 21.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

ORIGINAL SURVEY					
SITE NAME: The Elms		FIELD NUMBER: 7		DATE: 14/6/17	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	F	Achillea millefolium (yarrow)	VO	Crataegus monogyna (Hawthorn)	VO
Anthoxanthum odoratum (sweet vnl grass)	F	<b>Centaurea nigra (common knapweed)</b>	<b>O/L F</b>	Fraxinus excelsior (Ash)	VO
Dactylis glomerata (cock's foot)	VO	Cerastium holosteoides (cmn. mouse-ear)	O	Quercus sp. (Oak sp.) seedling	O
Festuca rubra (red fescue)	F	Cirsium arvense (creeping thistle)	VO	Sambucus nigra (Elder)	VL
Holcus lanatus (yorkshire fog)	F	Cirsium palustre (marsh thistle)	O/L F	Ulex europaeus (Gorse)	VL
<b>Luzula campestris (field wood rush)</b>	F	Galium saxatile (heath bedstraw)	VL		
Poa trivialis (rough meadow grass)	LF	Geranium robertianum (herb robert)	VL		
Pteridium aquilinum (bracken)	VL	Hedera helix (Ivy)	VL		
		<b>Hypochaeris radicata (common cats ear)</b>	O		
		<b>Leontodon hispidus (rough hawkbit)</b>	VLF		
		<b>Lotus corniculatus (birds-foot trefoil)</b>	F/L A		
		<b>Pilosella officinarum (mse-ear hawkweed)</b>	VLF		

	Plantago lanceolata (ribwort plantain)	F	
	<b>Potentilla erecta (tormentil)</b>	<b>VLF</b>	
	Prunella vulgaris (self-heal)	F	
	Ranunculus acris (meadow buttercup)	O	
	<b>Ranunculus bulbosus (bulbous buttercup)</b>	<b>F</b>	
	Ranunculus repens (creeping buttercup)	VL	
-	Rubus fruticosus (bramble)	VL	
-	Rumex acetosa (common sorrel)	LF	
-	Rumex acetosella (Sheeps Sorrel)	VL	
	Rumex crispus (Curled Dock)	R	
	Rumex obtusifolius (Broad-leaved Dock)	O	
	Senecio jacobea (ragwort)	R	
	<b>Stellaria graminea (lesser stichwort)</b>	<b>VL</b>	
	<b>Trifolium pratense (red clover)</b>	<b>O</b>	
	Trifolium repens (white clover)	O	
	Urtica Dioica (Stinging Nettles)	VLF	
	Veronica chamaedrys (gemdr. speedwell)	VO	

**Table 20.3 - eDNA SURVEY**

SITE NAME: The Elms		FIELD NUMBER: 7		DATE: 8/5/22	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	3.59%	Cerastium_fontanum	1.05%		
Anthoxanthum_odoratum	7.78%	Hieracium_agg.	14.97%		
Festuca_rubra	1.95%	<b>Lotus_corniculatus</b>	<b>4.79%</b>		
Holcus_lanatus	1.65%	Lotus_pedunculatus	0.75%		
		Plantago_lanceolata	0.45%		
		<b>Potentilla_erecta</b>	<b>22.46%</b>		
		<b>Ranunculus_bulb_repe</b>	<b>6.74%</b>		
		Rumex_acetosa	1.50%		
		Trifolium_rep_occ_i_nigr	2.40%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 10 Indicator Species, the eDNA method recorded 2/3 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). Whilst this wouldn't be enough to recognise the site as a LWS it may give some indication that the site is of ecological value and worth further survey work, particularly with Tormentil being picked up as sites rarely have this species and not have many other Indicator species as well. It was this site that returned the least number of Indicator species with the eDNA, in contrast it had a really good Fungal diversity with the eDNA results.

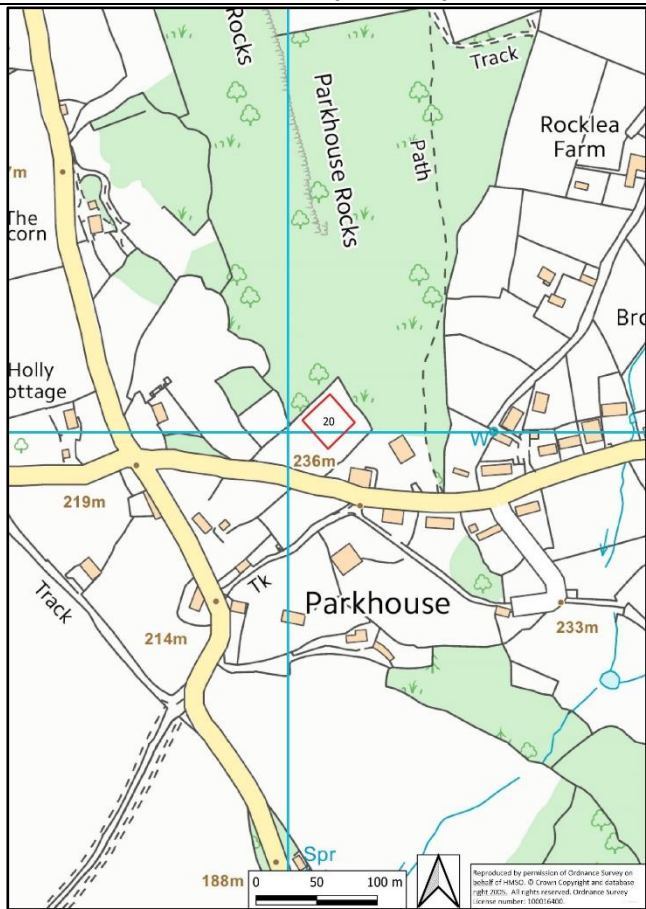
<b>Site Number/Name:</b>	<b>Site 20 – Parkhouse (Sherrington)</b>
<b>Date Surveyed:</b>	<b>8<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO50033 03009</b>
	<p>The site is located on a south-west facing slope, bordered closely by woodland. The fields are managed as Hay Meadows with aftermath grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner did report grassland fungi present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.20 – Parkhouse (Sherrington) Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 21.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis_schulzeri</i>		[VU]	17	2.47%
<i>Clavaria_amoenoides</i>			4	0.01%
<i>Clavaria_falcata</i>			30	0.08%
<i>Clavaria_flavipes</i>	Straw Club		29	0.62%
<i>Clavulinopsis_corniculata</i>	Meadow Coral		22	0.09%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.08%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.02%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.06%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.03%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.25%
<i>Cuphophyllus_flavipes</i>	Yellow Foot Waxcap	[VU]	10	2.24%

Cuphophyllus_virgineus	Snowy Waxcap		21	0.02%
Hygrocybe_cantharellus	Goblet Waxcap		6	0.04%
Hygrocybe_coccinea	Scarlet Waxcap		9	1.26%
Hygrocybe_conica	Blackening Waxcap		25	0.13%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.83%
Hygrocybe_inspida	Spangle Waxcap		19	0.47%
Hygrocybe_phaeococcinea	Shadowed Waxcap	[VU]	3	0.40%
Hygrocybe_quieta	Oily Waxcap	[VU]	6	0.07%
Hygrocybe_reidii	Honey Waxcap	DD	6	0.92%
Entoloma_asprellum	a pinkgill sp.		8	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.05%
Entoloma_infula	a pinkgill sp.		12	0.01%
Entoloma_poliopus	a pinkgill sp.		14	0.02%
Entoloma_prunuloides	Mealy Pinkgill	VU	6	0.29%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.01%
Geoglossum_fallax	Deceptive Earthtongue		16	0.14%
Geoglossum_nigritum	an Earthtongue sp.		15	0.01%
Geoglossum_umbratile	Plain Earthtongue		4	0.11%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.01%
Microglossum_olivaceum	Olive Earthtongue	Sect7	3	2.56%
Microglossum_parvisporum	an Earthtongue sp.	Sect7	5	0.25%
Trichoglossum_hirsutum	Hairy Earthtongue		4	0.06%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.55%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			10
Entolomataceae			8
Geoglossomycetes			6
Dermoloma			1
CHEGD Score			34
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			8
Entolomataceae			2
Geoglossomycetes			4
Dermoloma			1
CHEGD Score			21

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN** (International Union for Conservation of Nature) – **DD = Data Deficient** – *Not enough data available to make a conclusion.*

**Sect7** = *A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.*

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (*Hygrocybe* spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 5 Vulnerable species and 2 Section 7 species were identified further reinforces its value and the importance of preserving this.

This site was highlighted as a possible survey site after receiving reports of a few fungi in the grassland. This demonstrates the noted presence of a few grassland fungi can be a sign of a significant site.

## Flora

The following two tables (21.1 and 21.2) show the results of the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

SITE NAME: Sherrington (Parkhouse)		FIELD NUMBER: 1		DATE: 29/05/2012	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	F	<i>Achillea millefolium</i> (Yarrow)	O/L F		
<i>Alopecurus pratensis</i> (Meadow Foxtail)	O	<b><i>Ajuga reptans</i> (Bugle)</b>	O		
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	Y	<i>Calluna vulgaris</i> (Ling)	R		
<i>Arrhenatherum elatius</i> (False Oat-grass)	Y	<b><i>Centaurea nigra</i> (Common Knapweed)</b>	F		
<i>Cynosurus cristatus</i> (Crested Dogtail)	O	<i>Cerastium fontanum</i> (Common Mouse-ear)	F		
<i>Dactylis glomerata</i> (Cocksfoot)	Y	<i>Cirsium repens</i> (Creeping Thistle)	O		
<i>Festuca rubra</i> (Red Fescue)	F	<i>Cirsium vulgare</i> (Spear Thistle)	R		
<i>Holcus lanatus</i> (Yorkshire Fog)	O/ F	<b><i>Conopodium majus</i> (Pignut)</b>	O		
<i>Poa trivialis</i> (Rough Meadow-grass)	F	<i>Digitalis purpurea</i> (Foxglove)	O		
<b><i>Luzula campestris</i> (Field Wood-rush)</b>	F	<i>Erica cinerea</i> (Bell Heather)	VL		
		<i>Galium saxatile</i> (Heath Bedstraw)	VL		
		<i>Galium aparine</i> (Cleavers)	VL		
		<i>Geranium robertianum</i> (Herb Robert)	VL		
		<i>Glechoma hederacea</i> (Ground Ivy)	O/V LF		
		<i>Heracleum spondylium</i> (Hogweed)	O		
		<b><i>Hypochaeris radicata</i> (Catsear)</b>	F/A		
		<i>Lathyrus pratensis</i> (Meadow Vetchling)	LF		
		<b><i>Leucanthemum vulgare</i> (Ox-eye Daisy)</b>	O		



		<i>Lotus corniculatus</i> (Birdsfoot Trefoil)	O/L F		
		<i>Plantago lanceolata</i> (Ribwort Plantain)	F		
		<i>Primula veris</i> (Cowslip)	O		
		<i>Ranunculus ficaria</i> (Lesser Celandine)	Y		
		<i>Ranunculus repens</i> (Creeping Buttercup)	F		
		<i>Ranunculus acris</i> (Meadow Buttercup)	F		
		<i>Ranunculus bulbosus</i> (Bulbous Buttercup)	Y		
		<i>Rubus fruticosus</i> sp. Agg. (Bramble)	Y		
		<i>Rumex acetosa</i> (Common Sorrel)	F		
		<i>Rumex obtusifolius</i> (Broad-leaved Dock)	O		
		<i>Senecio jacobaea</i> (Ragwort)	O		
		<i>Stachys sylvatica</i> (Hedge Woundwort)	VL		
		<i>Stellaria graminea</i> (Lesser Stitchwort)	O		
		<i>Taraxacum officinale</i> (Dandelion)	F		
		<i>Trifolium pratense</i> (Red Clover)	F		
		<i>Trifolium repens</i> (White Clover)	R		
		<i>Urtica dioica</i> (Stinging Nettles)	O		
		<i>Veronica chamaedrys</i> (Germander Speedwell)	O/L A		
		<i>Vicia sepium</i> (Bush Vetch)	O		

<b>Table 21.2 ORIGINAL SURVEY</b>					
<b>SITE NAME: Sherrington (Parkhouse)</b>		<b>FIELD NUMBER: 1</b>		<b>DATE: 08/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Woody Species</b>	<b>%</b>
<i>Agrostis_cap_gig</i>	0.25%	<i>Centaurea_nigra</i>	6.24%	<i>Fagus_sylvatica</i>	0.01%
<i>Alopecurus_pratensis</i>	0.02%	<i>Conopodium_majus</i>	0.01%	<i>Quercus_petraea_robur</i>	0.31%
<i>Anthoxanthum_odoratum</i>	0.59%	<i>Euphrasia_agg</i>	6.14%		
<i>Arrhenatherum_elatius</i>	1.28%	<i>Heracleum_sphondylium</i>	0.03%		
<i>Dactylis_glomerata</i>	0.09%	<i>Hypochaeris_radicata</i>	4.53%		
<i>Festuca_rubra</i>	0.30%	<i>Lathyrus_pratensis</i>	0.33%		
<i>Holcus_lanatus</i>	0.19%	<i>Leontodon_hispidus</i>	37.36%		
<i>Poa_trivialis</i>	0.01%	<i>Leontodon_saxatilis</i>	0.01%		
		<i>Lotus_corniculatus</i>	5.19%		
		<i>Plantago_lanceolata</i>	0.52%		
		<i>Ranunculus_acris_occid</i>	3.65%		
		<i>Ranunculus_bulb_repe</i>	0.78%		
		<i>Rhinanthus_minor</i>	17.65%		
		<i>Rumex_acetosa</i>	1.10%		
		<i>Trifolium_pratense</i>	0.46%		
		<i>Trifolium_rep_occi_nigr</i>	7.96%		
		<i>Veronica_chamaedrys</i>	0.15%		
		<i>Veronica_officinalis</i>	1.09%		

It can be seen that the eDNA survey didn't return as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a



number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 12 Indicator Species, the eDNA method recorded 11/12 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be be enough to recognise the site as a LWS.

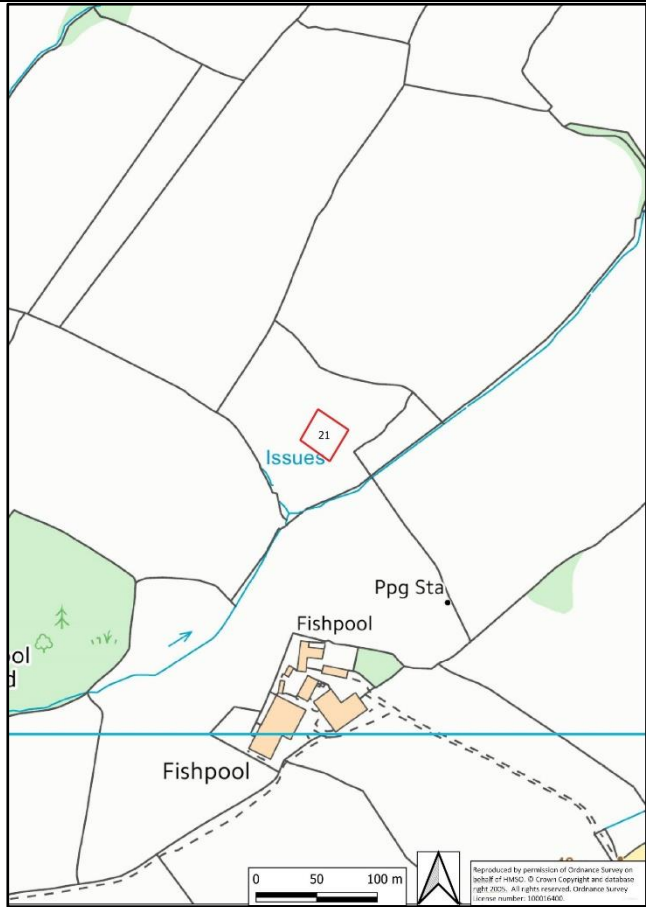
<b>Site Number/Name:</b>	<b>Site 21 – Fishpool Farm</b>
<b>Date Surveyed:</b>	<b>9<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO44691 10247</b>
	<p>The site is located on steepish, south-east facing slope The field has ant-hills present and the grass was becoming relatively heavy in places. The field is cattle grazed after June. It has not been formally surveyed botanically, however notes made during the collection of soil samples revealed a number of Species-rich Grasslands Indicator Species to be present. The fungal diversity of the field is unknown, however the owners reported a number of grassland fungi to be present in the autumn. A more limited list of floral Indicator Species and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown.</p>

Figure 2.21 – Fishpool Farm Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 22.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Clavaria falcata</i>			30	0.01%
<i>Clavaria messapica</i>			8	0.03%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.22%
<i>Clavulinopsis helvola</i>	Yellow Club		25	2.36%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.01%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	1.58%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.04%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.03%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.31%
<i>Cuphophyllus pratensis</i>	Meadow Waxcap		22	0.74%
<i>Cuphophyllus virgineus</i>	Snowy Waxcap		21	1.15%
<i>Gliophorus irrigatus</i>	Slimy Waxcap		7	0.16%

Hygrocybe_ceracea	Butter Waxcap		3	0.77%
Hygrocybe_chlorophana	Golden Waxcap		16	4.48%
Hygrocybe_coccinea	Scarlet Waxcap		9	0.24%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.22%
Entoloma_conferendum	a pinkgill sp.		25	0.03%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
Geoglossum_fallax	Deceptive Earthtongue		16	0.17%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.08%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.03%
Trichoglossum_walteri	Short-spored Earthtongue	<b>VU</b>	21	0.54%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.02%
Dermoloma_magicum	Black Magic	<b>[VU]</b>	16	0.24%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.98%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			7
Entolomataceae			2
Geoglossomycetes			4
Dermoloma			2
<b>CHEGD Score</b>			<b>23</b>
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			3
Hygrophoraceae			7
Entolomataceae			0
Geoglossomycetes			3
Dermoloma			2
<b>CHEGD Score</b>			<b>15</b>

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

The results showed a good variety of Fungi to be present with the number of Hygrocybe (Waxcaps) being particularly present and clearly demonstrate this site is of significant value for its grassland fungi. Indeed the results of the eDNA Survey would almost be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 2 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following table (22.2) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 22.2 - DNA SURVEY**

SITE NAME: Fishpool Farm		FIELD NUMBER:		DATE: 09/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Mosses	%
<i>Agrostis_cap_gig</i>	6.45%	<i>Achillea_millefolium</i>	0.10%	<i>Brachythecium_rivulare</i>	0.10%
<i>Anthoxanthum_odoratum</i>	2.08%	<b><i>Callianthemum_anemonoides</i></b>	<b>0.06%</b>	<i>Calliergonella_lindbergii</i>	0.62%
<i>Arrhenatherum_elatius</i>	1.24%	<b><i>Cucumis_sativus</i></b>	<b>0.06%</b>	<i>Pseudoscleropodium_purum</i>	0.26%
<i>Dactylis_glomerata</i>	0.28%	<b><i>Lotus_corniculatus</i></b>	1.74%		
<i>Festuca_rubra</i>	2.32%	<b><i>Potentilla_erecta</i></b>	0.08%		
<i>Holcus_lanatus</i>	4.00%	<i>Potentilla_reptans</i>	0.06%		
<i>Poa_prat_calc_parv</i>	0.36%	<i>Ranunculus_acris_occid</i>	3.54%		
<i>Poa_trivialis</i>	1.24%	<i>Rumex_acetosa</i>	42.05%		
		<b><i>Stellaria_alsine-graminea</i></b>	16.16%		
		<i>Trifolium_rep_occi_nigr</i>	8.99%		

The eDNA survey returned a reasonable number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 3 Indicator Species. This wouldn't be enough to recognise the site as a Local Wildlife Site, however it would give a good idea of potential value and warrant further survey work, particularly with Tormentil being picked up as sites rarely have this species and not have many other Indicator species as well.

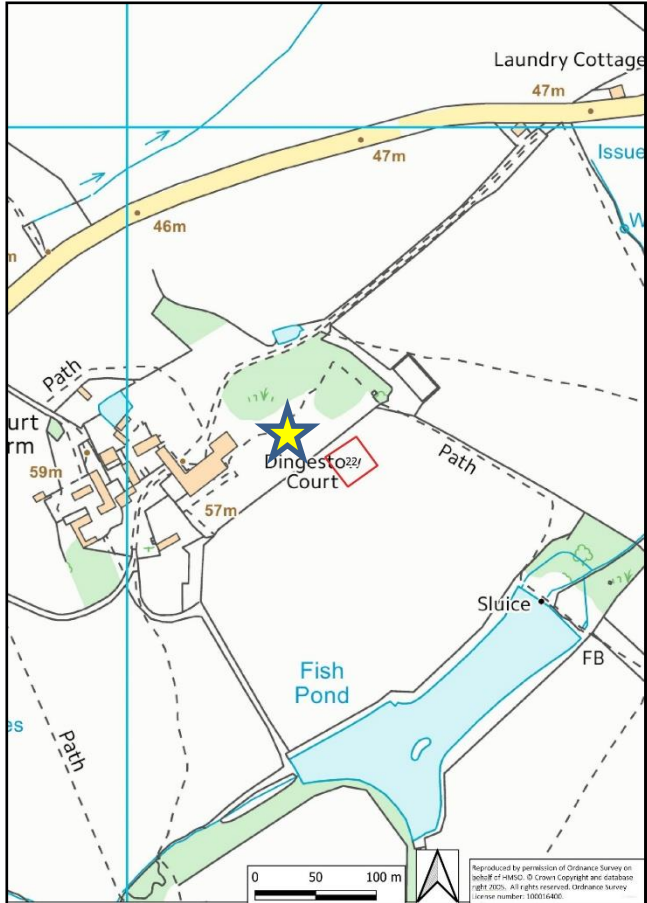
<b>Site Number/Name:</b>	<b>Site 22 – Dingestow Court Meadow</b>
<b>Date Surveyed:</b>	<b>9<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO45184 09725</b>
	<p>The site is located on a flat meadow immediately adjacent to an area of lawn at Dingestow Court that is known to have high fungal diversity (yellow star). The meadow (from traditional surveys) has surprisingly low fungal diversity considering its close presence to the high diversity site. It is managed as a Hay Meadow with aftermath grazing. It has not been formally surveyed botanically, however notes made during the collection of soil samples revealed a number of Species-rich Grasslands Indicator Species to be present, although the species mix present included quite a lot of clovers from previous “improvement” 40 years ago. The fungal diversity of the field is thought to be low, despite the presence of a high diversity site immediately adjacent. A more limited list of floral Indicator Species and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown.</p>

Figure 2.22 – Dingestow Court Lawn Meadow Site Location

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 23.1.1, these can be contrasted with those from the immediately adjacent site within Table 23.1.2 (yellow star in Figure 2.22) which was surveyed in 2018 also using eDNA.

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis atrovelutina</i>			24	0.10%
<i>Clavaria falcata</i>			30	0.06%
<i>Clavaria flavipes</i>	Straw Club		29	0.04%
<i>Clavaria vermiculata</i>			4	0.01%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.96%

Clavulinopsis_helvola	Yellow Club		25	1.21%
Clavulinopsis_laeticolor	Handsome Club		21	6.44%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	2.45%
Hygrocybe_conica	Blackening Waxcap		25	0.01%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.22%
Entoloma_sericeum	Silky Pinkgill		24	0.03%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.05%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.02%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.71%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.56%

<b>SPECIES COUNT</b>			
Clavariaceae			7
Hygrophoraceae			2
Entolomataceae			1
Geoglossomycetes			2
Dermoloma			
CHEGD Score			12
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			1
Entolomataceae			1
Geoglossomycetes			1
Dermoloma			
CHEGD Score			8

**Table 23.1.2 – Immediately adjacent Site 22 at Dingestow Court Lawn (South)**

Scientific Name	English Name	Also present in Site 22?
Camarophylloopsis_atrovelutina		Y
Clavaria acuta		
Clavaria argillacea		
Clavaria flavipes		Y
Clavaria fragilis		
Clavaria incarnata		
Clavulinopsis_corniculata		Y
Clavulinopsis_helvola		Y
Clavulinopsis_laeticolor		Y
Clavulinopsis_luteoalba		
Hodophilus_variabilipes		
Ramariopsis crocea		
Ramariopsis kunzei		
Hygrocybe colemanniana		
Hygrocybe fornicate		
Hygrocybe russocoriacea		
Hygrocybe virginea		
Hygrocybe citrinovirens		
Hygrocybe conica		Y
Hygrocybe glutinipes		Y
Hygrocybe insipida		
Hygrocybe reidii		
Hygrocybe calyptriformis		

Entoloma_conferendum		
Glutinoglossum_pseudoglutinatum		
Trichoglossum_hirsutum		
Dermoloma_cuneifolium		

SPECIES COUNT	
Clavariaceae	13
Hygrophoraceae	10
Entolomataceae	1
Geoglossomycetes	2
Dermoloma	1
CHEGD Score	27

Whilst not without value, the number of CHEGD Fungi is quite low at this site. This was expected as the landowner didn't think the diversity was great. What is of interest is that this site is immediately adjacent (other side of fence (see Figure 2.22)) with a diverse Grassland Fungi site (species list in Table 23.1.2) and despite the close proximity very few species have colonised despite management being conducive. The CHEGD score is greatly less and only two of the 10 Waxcap species have managed to colonise in 40 years despite such close proximity. This demonstrates how long it can take Grassland Fungi to colonise a site and why it is so important to preserve the existing site and indeed identify these so they can be protected.

## Flora

The following table (23.2) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

Table 23.2 - eDNA SURVEY					
<b>SITE NAME: Dingestow Court Meadow</b>		<b>FIELD NUMBER:</b>		<b>DATE:</b> 09/05/2022	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Mosses</b>	<b>%</b>
Agrostis_cap_gig	2.18%	Achillea_millefolium	1.55%	Brachythecium_rivulare	0.09%
Anthoxanthum_odoratum	0.74%	Cerastium_fontanum	3.78%	Kindbergia_praelonga	0.11%
Cynosurus_cristatus	0.56%	Cirsium_arvense	0.01%		
Holcus_lanatus	2.43%	<b>Cucumis_sativus</b>	<b>0.01%</b>		
Lolium_perr_mult	1.69%	<b>Hypochoeris_radicata</b>	0.13%		
Phleum_pratense	0.05%	Ranunculus_acris_occid	19.84%		
Poa_annua	0.05%	<b>Ranunculus_bulb_repe</b>	9.89%		
Poa_prat_calc_parv	0.07%	Taraxacum_officinale_agg.	6.22%		
Poa_trivialis	0.37%	Trifolium_dubium	18.57%		
		<b>Trifolium_pratense</b>	12.78%		
		Trifolium_rep_occid_nigr	15.04%		

The eDNA survey returned quite a small number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 2/3 Indicator Species (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This wouldn't be enough to recognise the site as a Local Wildlife Site, however it may give a clue to warrant further survey work. This was the joint lowest number of Indicator Species recorded on any of the 30 sites, and also the one of only two sites that were considered not to be LWS quality so it offered a good correlation.



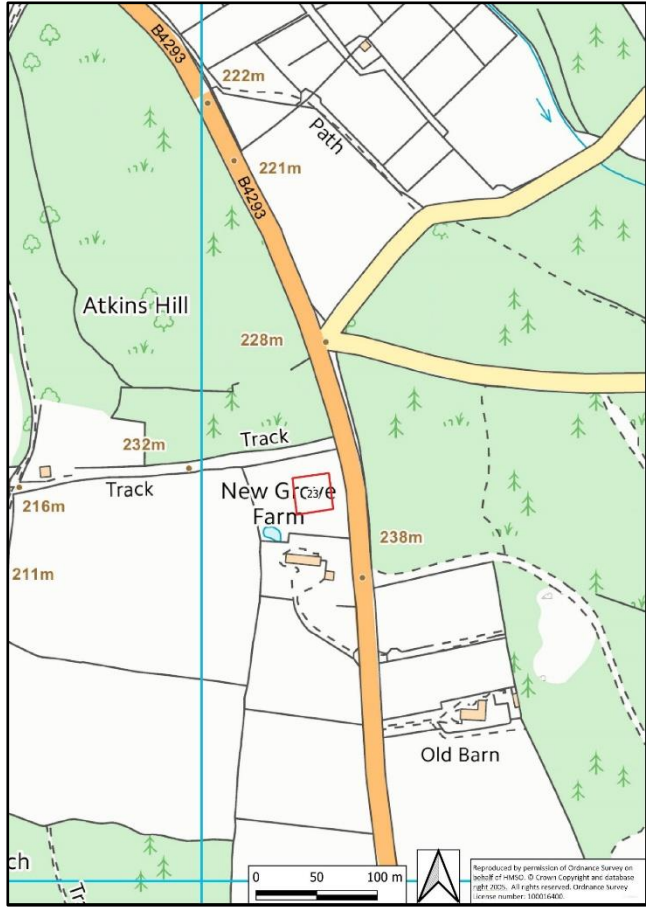
<b>Site Number/Name:</b>	<b>Site 23 – Newgrove Farm</b>
<b>Date Surveyed:</b>	<b>9<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO50091 07319</b>
 <p>The map shows the site location at Newgrove Farm. Key features include Atkins Hill to the west, New Grove Farm in the center, and an Old Barn to the south. A road labeled B4293 runs north-south through the site. Various tracks and paths are shown, along with elevation markers such as 222m, 221m, 228m, 232m, 216m, 211m, and 238m. A scale bar indicates 0, 50, and 100 meters. A north arrow is also present.</p>	<p>The site is located on an area of flat ground. The fields are managed as Hay Meadows with aftermath grazing by sheep. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fields are also known to contain a rich grassland fungi diversity similar to nearby Newgrove GWT Reserve. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, with a comparison with results obtained from “Traditional” Surveys.</p>

Figure 2.23 – Newgrove Farm Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 24.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis_atrovelutina</i>			24	0.21%
<i>Camarophyllopsis_schulzeri</i>		[VU]	17	2.11%
<i>Clavaria_falcata</i>			30	0.28%
<i>Clavaria_flavipes</i>	Straw Club		29	0.20%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.03%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.44%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.31%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.94%
<i>Cuphophyllus_pratensis</i>	Meadow Waxcap		22	0.70%
<i>Gliophorus_irrigatus</i>	Slimy Waxcap		7	0.68%

Gliophorus_psittacinus	Parrot Waxcap		12	1.17%
Hygrocybe_cantharellus	Goblet Waxcap		6	0.24%
Hygrocybe_chlorophana	Golden Waxcap		16	2.64%
Hygrocybe_conica	Blackening Waxcap		25	0.27%
Hygrocybe_insipida	Spangle Waxcap		19	0.26%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	3.67%
Hygrocybe_punicea	Crimson Waxcap	VU	7	21.74%
Hygrocybe_reidii	Honey Waxcap	DD	6	0.53%
Neohygrocybe_ovina	Blushing Waxcap	VU	3	0.01%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.01%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_asprellum	a pinkgill sp.		8	0.01%
Entoloma_clandestinum	a pinkgill sp.		13	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.02%
Entoloma_exile	a pinkgill sp.		12	0.02%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.10%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.06%
Entoloma_longistriatum	a pinkgill sp.		6	0.01%
Entoloma_ochreoprunuloides	a pinkgill sp.		2	0.92%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.01%
Geoglossum_nigritum	an Earthtongue sp.		15	0.11%
Microglossum_parvisporum	an Earthtongue sp.	Sect7	5	0.12%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.98%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			12
Entolomataceae			8
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			31
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			10
Entolomataceae			3
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			22

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN** (International Union for Conservation of Nature) – **DD = Data Deficient** – *Not enough data available to make a conclusion.*

**Sect7** = *A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.*

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)

- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 8 Vulnerable species and a Section 7 species were identified further reinforces its value and the importance of preserving this.

This site is adjacent to the Newgrove Meadows GWT Reserve (Site 24), and is managed similarly so there is little surprise that they are both highly valuable.

## Flora

The following two tables (24.2 and 24.5) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 24.2 - ORIGINAL SURVEY					
SITE NAME: Newgrove Farm Meadow		FIELD NUMBER: 2		DATE: 06/07/2015	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	F	<b>Conopodium majus (pignut)</b>	LF		
Anthoxanthum odoratum (sweet vnl grass)	F	<b>Centaurea nigra (common knapweed)</b>	F		
Arrhenatherum elatius (false oat grass)	VL	Cerastium holosteoides (cmn. mouse-ear)	VL		
<b>Briza media (quaking grass)</b>	O	Chamerion angustifolium (Rosebay Willowherb)	VL		
<b>Carex caryophyllea (spring sedge)</b>	LF	Cirsium palustre (marsh thistle)	R		
<b>Carex flacca (glaucous sedge)</b>	LF	<b>Dactylorhiza fuchsii (c. spotted orchid)</b>	LF		
Cynosurus cristatus (crested dog's tail)	O/V LF	<b>Euphrasia officinalis agg. (eyebright)</b>	LF		
Dactylis glomerata (cock's foot)	VO	Galium aparine (Cleavers)	VL		
<b>Danthonia decumbens (heath grass)</b>	LF	Geranium robertianum (herb robert)	VL		
Festuca rubra (red fescue)	F	Geum urbanum (Wood Avens)	R		
Holcus lanatus (yorkshire fog)	O/L A	Heracleum sphondylium (hogweed)	O		
Juncus conglomeratus (compact rush)	R	<b>Hypochaeris radicata (common cats ear)</b>	O		
Lolium perenne (perennial rye grass)	VL	<b>Lathyrus pratensis (meadow vetchling)</b>	VL		
<b>Luzula campestris (field wood rush)</b>	O	<b>Leontodon hispidus (rough hawkbit)</b>	O/F		
Poa annua (annual meadow grass)	VL	<b>Leucanthemum vulgare (oxeye daisy)</b>	VLF		
Poa trivialis (rough meadow grass)	VL	<b>Lotus corniculatus (birds-foot trefoil)</b>	F		
Pteridium aquilinum (bracken)	LF	Lotus uliginosus (greater birds-foot trefoil)	VL		
		<b>Orchis morio (green-winged orchid)</b>	Y		
		<b>Pilosella officinarum (mse-ear hawkweed)</b>	R		
		Plantago lanceolata (ribwort plantain)	F		

		<b>Polygala vulgaris (common milkwort)</b>	<b>O/V LF</b>		
		<b>Potentilla erecta (tormentil)</b>	<b>LA/F</b>		
		<b>Primula veris (cowslip)</b>	<b>O</b>		
		Prunella vulgaris (self-heal)	O		
		Ranunculus acris (meadow buttercup)	O		
		Ranunculus repens (creeping buttercup)	O		
		<b>Rhinanthus minor (yellow rattle)</b>	<b>F/LA</b>		
		Rubus fruticosus (bramble)	VL		
		Rumex acetosa (common sorrel)	O		
		Rumex obtusifolius (Broad-leaved Dock)	O/VL F		
		<b>Stellaria graminea (lesser stichwort)</b>	<b>O/V LF</b>		
		<b>Trifolium pratense (red clover)</b>	<b>O</b>		
		Trifolium repens (white clover)	O/VL F		
		Urtica dioica (Stinging Nettle)	VL		
		<b>Viola riviniana (common dog violet)</b>	<b>VL</b>		

<b>Table 24.3 - eDNA SURVEY</b>					
<b>SITE NAME: Newgrove Farm Meadow</b>		<b>FIELD NUMBER: 2</b>		<b>DATE: 09/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Woody Species</b>	<b>%</b>
Agrostis_cap_gig	2.93 %	Achillea_millefolium	0.66%	Fagus_sylvatica	0.31 %
Anthoxanthum_odoratum	11.69 %	<b>Centaurea_nigra</b>	<b>0.07%</b>	Quercus_petraea_robur	0.49 %
Festuca_rubra	0.95 %	Cerastium_fontanum	0.23%		
Holcus_lanatus	0.22 %	Crepis_capillaris	0.04%		
<b>Luzula_campestris</b>	<b>0.07 %</b>	<b>Cucumis_sativus</b>	<b>0.03%</b>		
Poa_trivialis	1.38 %	<b>Euphrasia_agg</b>	<b>0.09%</b>		
		<b>Hypochoeris_radicata</b>	<b>4.37%</b>		
		Jacobaea_vulgaris	0.08%		
		<b>Leucanthemum_vulgare</b>	<b>0.51%</b>		
		<b>Lotus_corniculatus</b>	<b>0.06%</b>		
		Lotus_pedunculatus	15.66 %		
		Plantago_lanceolata	16.84 %		
		Prunella_vulgaris	0.10%		
		<b>Ranunculus_bulb_repe</b>	<b>0.55%</b>		
		<b>Rhinanthus_minor</b>	<b>8.18%</b>		
		Rumex_acetosa	1.19%		
		Rumex_acetosella	2.83%		
		<b>Stellaria_alsine-graminea</b>	<b>0.70%</b>		
		Taraxacum_officinale_agg.	2.11%		
		<b>Trifolium_pratense</b>	<b>0.72%</b>		
		Trifolium_rep_occ_nigr	6.76%		
		Veronica_chamaedrys	1.44%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 23 Indicator Species, the eDNA method recorded 9/10 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be be enough to recognise the site as a LWS.

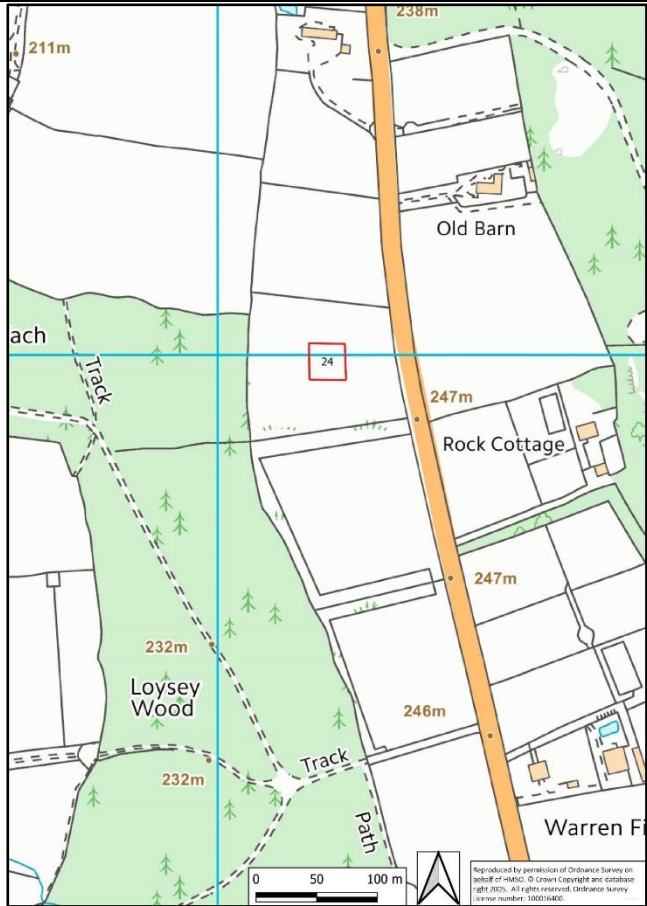
<b>Site Number/Name:</b>	<b>Site 24 – Newgrove GWT Reserve</b>
<b>Date Surveyed:</b>	<b>9<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO50090 06994</b>
	<p>The site is located on an area of west facing slope. The fields are managed as Hay Meadows with aftermath grazing by sheep. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fields are also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.</p>

Figure 2.24 – Newgrove GWT Reserve Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 25.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.24%
<i>Clavaria fumosa</i>	Smoky Spindles		3	0.75%
<i>Camarophyllopsis schulzeri</i>		[VU]	17	2.90%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.56%
<i>Clavaria falcata</i>			30	0.18%
<i>Clavaria flavipes</i>	Straw Club		29	0.03%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.08%
<i>Hygrocybe cantharellus</i>	Goblet Waxcap		6	0.24%
<i>Hygrocybe chlorophana</i>	Golden Waxcap		16	0.11%
<i>Hygrocybe citrinovirens</i>	Citrine Waxcap	VU	11	2.43%

Hygrocybe_coccinea	Scarlet Waxcap		9	0.88%
Hygrocybe_conica	Blackening Waxcap		25	0.06%
Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	4.99%
Hygrocybe_intermedia	Fibrous Waxcap	VU	12	9.99%
Hygrocybe_punicea	Crimson Waxcap	VU	7	13.47%
Hygrocybe_reidii	Honey Waxcap	DD	6	0.66%
Cuphophyllus_pratensis	Meadow Waxcap		22	0.24%
Gliophorus_irrigatus	Slimy Waxcap		7	0.02%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.04%
Entoloma_exile	a pinkgill sp.		12	0.03%
Entoloma_griseocyaneum	Felted Pinkgill	VU	10	0.02%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.07%
Entoloma_longistriatum	a pinkgill sp.		6	0.01%
Entoloma_ameides	a pinkgill sp.		15	0.03%
Entoloma_prunuloides	Mealy Pinkgill	VU	6	0.07%
Entoloma_asprellum	a pinkgill sp.		8	0.01%
Entoloma_atrocoeruleum	a pinkgill sp.		5	0.04%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.02%
Geoglossum_nigratum	an Earthtongue sp.		15	0.10%

<b>SPECIES COUNT</b>			
Clavariaceae			6
Hygrophoraceae			10
Entolomataceae			10
Geoglossomycetes			1
Dermoloma			0
CHEGD Score			27
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			9
Entolomataceae			2
Geoglossomycetes			1
Dermoloma			0
CHEGD Score			17

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**IUCN** (International Union for Conservation of Nature) – **DD = Data Deficient** – *Not enough data available to make a conclusion.*

A comparison of the eDNA Results with Traditional Survey methods is shown within Table 25.2

<b>GWG24-New Grove Meadows</b>				
	<b>Species</b>	<b>eDNA</b>	<b>Traditional Survey</b>	<b>Combined</b>
C1	Camarophyllopsis_atrovelutina	0.24%		
C2	Camarophyllopsis_schulzeri	2.90%		
C6	Clavaria_falcata	0.18%		
C7	Clavaria_flavipes	0.03%		

C9	Clavaria_fragilis		YES	
C10	Clavaria_fumosa	0.75%		
C17	Clavulinopsis_corniculata		YES	
C21	Clavulinopsis_luteoalba		YES	
C26	Ramariopsis_avellaneo-inversa	0.56%		
	<b>Cuphophyllus_colemanniana</b>		YES	
H2	Cuphophyllus_flavipes	4.99%	YES	
H5	Cuphophyllus_pratensis	0.24%	YES	
H7	Cuphophyllus_virgineus		YES	
H9	Gliophorus_irrigatus	0.02%	YES	
H10	Gliophorus_psittacinus		YES	
	<b>Hygrocybe_acutoconica</b>		YES	
	<b>Hygrocybe_aurantiosplendens</b>		YES	
H11	Hygrocybe_cantharellus	0.24%	YES	
H13	Hygrocybe_chlorophana	0.11%	YES	
H14	Hygrocybe_citrinovirens	2.43%	YES	
H15	Hygrocybe_coccinea	0.88%	YES	
H16	Hygrocybe_conica	0.06%	YES	
H17	Hygrocybe_glutinipes		YES	
H19	Hygrocybe_insipida		YES	
H20	Hygrocybe_intermedia	9.99%	YES	
H23	Hygrocybe_punicea	13.47%	YES	
H24	Hygrocybe_quieta		YES	
H25	Hygrocybe_reidii	0.66%	YES	
H29	Neohygrocybe_ovina		YES	
H30	Porpolomopsis_calyptriformis		YES	
E2	Entoloma_ameides	0.03%		
E3	Entoloma_asprellum	0.01%		
E5	Entoloma_atrocoeruleum	0.04%		
	<b>Entoloma_bloxamii</b>		YES	
	<b>Entoloma_cetratum</b>		YES	
E9	Entoloma_conferendum	0.01%	YES	
	<b>Entoloma_corvinum</b>		YES	
E11	Entoloma_exile	0.03%		
E12	Entoloma_griseocyaneum	0.02%		
E13	Entoloma_henricii	0.07%		
E16	Entoloma_longistriatum	0.01%		
	<b>Entoloma_mougeotii</b>		YES	
	<b>Entoloma_porphyrphaeum</b>		YES	
E22	Entoloma_prunuloides	0.07%	YES	
	<b>Entoloma_sericellum</b>		YES	
	<b>Entoloma_serrulatum</b>		YES	
	<b>Entoloma_tjallingiorum</b>		YES	
G2	Geoglossum_nigritum	0.10%		



SPECIES COUNT (ALL SEQUENCES)			
	6	3	6
	11	21	21
	9	10	15
	1	0	1
	0	0	0
	27	34	43

The results show that the eDNA has picked up roughly the same number of CHEGD species as traditional methods. This is the case for Enteloma, Geoglossum and Dermolomas. More Clavarioids were picked up with the eDNA however and there was a somewhat reduced number of Hygrocybe found. It is interesting to note that there were a considerable number of differences in actual species recorded for the Clavarioids and Entoloma, perhaps more than any other site. This is a well recorded site, however it would appear previous surveys have been more focussed on the Hygrocybe (Waxcaps) which would explain the results relating to Waxcaps. Never the less the results are impressive, even though the survey only covered 30mx30m of a multi field site and this was just from one survey visit. It should be noted that there were a few Waxcap species the eDNA did not pick up, this would be expected with just part of one field covered.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 8 Vulnerable species were identified further reinforces its value and the importance of preserving this.

Overall new species have been recognised for this site and if the results of the two surveys are combined it reveals a CHEGD score of 42 making it a highly significant site for Grassland Fungi.

## Flora

The following table (25.3) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as the available species list covered a number of fields.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 25.3 - eDNA SURVEY**

SITE NAME: Newgrove Meadows (GWT)		FIELD NUMBER:		DATE:	09/05/2022
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
<i>Agrostis_cap_gig</i>	0.58%	<b>Centaurea_nigra</b>	4.41%		
<i>Anthoxanthum_odoratum</i>	0.16%	<b>Cucumis_sativus</b>	0.02%		
<b>Briza_media</b>	0.08%	<i>Dactylorhiza_maculata</i>	0.19%		
<i>Carex_caryophylla</i>	0.44%	<i>Euphrasia_agg</i>	0.70%		
<b>Danthonia_decumbens</b>	3.13%	<b>Hypochaeris_radicata</b>	5.92%		
<i>Festuca_ovina</i>	0.30%	<b>Leontodon_hispidus</b>	28.57%		
<i>Festuca_rubra</i>	0.40%	<b>Leontodon_saxatilis</b>	1.90%		
		<b>Leucanthemum_vulgare</b>	0.24%		
		<b>Lotus_corniculatus</b>	25.02%		
		<i>Plantago_lanceolata</i>	11.37%		
		<b>Polygala_vulgaris</b>	0.43%		
		<i>Ranunculus_acris_occid</i>	0.49%		
		<b>Ranunculus_bulb_repe</b>	0.61%		
		<b>Rhinanthus_minor</b>	6.77%		
		<b>Trifolium_pratense</b>	3.18%		
		<b>Viola_riviniana</b>	3.23%		

The eDNA survey returned a good number of species but likely well short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 15/16 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would still be enough to recognise the site as a Local Wildlife Site.

Note the presence of a number of species highlighted in **RED** that are likely an eDNA recognition error.

<b>Site Number/Name:</b>	<b>Site 25 – Wet Meadow (MMG)</b>
<b>Date Surveyed:</b>	<b>9<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO49918 05811</b>
	<p>The site is located on an area of south-east facing slope. The fields are managed as Hay Meadows with aftermath grazing by sheep. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fields are also known to contain a rich grassland fungi diversity. The full list and comparison with both the fungi and floral eDNA is shown below.</p>

Figure 2.25 – Wet Meadow (MMG) Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 26.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophylloopsis_atrovelutina</i>			24	0.03%
<i>Camarophylloopsis_schulzeri</i>		[VU]	17	0.71%
<i>Clavaria_falcata</i>			30	0.15%
<i>Clavaria_flavipes</i>	Straw Club		29	0.25%
<i>Clavaria_fumosa</i>	Smoky Spindles		3	0.09%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.77%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.13%
<i>Clavulinopsis_umbrinella</i>	Beige Coral		4	0.01%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.03%
<i>Ramariopsis_flavescens</i>	a coral fungus sp.		16	0.01%

<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.40%
Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	0.89%
Cuphophyllus_pratensis	Meadow Waxcap		22	1.12%
Cuphophyllus_virginus	Snowy Waxcap		21	0.11%
Hygrocybe_coccinea	Scarlet Waxcap		9	1.65%
Hygrocybe_conica	Blackening Waxcap		25	0.01%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.24%
Hygrocybe_insipida	Spangle Waxcap		19	0.08%
Hygrocybe_punicea	Crimson Waxcap	VU	7	7.60%
Neohygrocybe_ingrata	Dingy Waxcap	VU	4	0.37%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_ameides	a pinkgill sp.		15	0.01%
Entoloma_asprellum	a pinkgill sp.		18	0.05%
Entoloma_clandestinum	a pinkgill sp.		13	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.02%
Entoloma_dysthales	a pinkgill sp.		10	0.01%
Entoloma_exile	a pinkgill sp.		12	0.02%
Entoloma_infula	a pinkgill sp.		12	0.04%
Entoloma_poliopus	a pinkgill sp.		14	0.05%
Entoloma_prunuloides	Mealy Pinkgill	VU	6	0.05%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.05%
Entoloma_sericeum	Silky Pinkgill		24	0.02%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.01%
Geoglossum_fallax	Deceptive Earthtongue		16	0.02%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.25%
Microglossum_olivaceum	Olive Earthtongue	Sect7	3	0.09%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.01%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	1.02%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.01%
Dermoloma_magicum	Black Magic	[VU]	16	1.03%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.79%

<b>SPECIES COUNT</b>			
Clavariaceae			10
Hygrophoraceae			9
Entolomataceae			11
Geoglossomycetes			6
Dermoloma			2
<b>CHEGD Score</b>			<b>38</b>
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			8
Entolomataceae			4
Geoglossomycetes			3
Dermoloma			2
<b>CHEGD Score</b>			<b>23</b>

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

**Sect7** = A species of "Principle Importance" for the purpose of maintain and enhancing biodiversity in relation to Wales under the Environment (Wales) Act (2016), Section 7.

This is a very valuable Grassland Fungi site with a high CHEGD score, and a particularly impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 7 Vulnerable species and Section 7 species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (26.2 and 26.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 26.2 - ORIGINAL SURVEY					
SITE NAME: Wet Meadow, Trellech		FIELD 5	NUMBER:	DATE: 13&14/6/17	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	A	Achillea millefolium (yarrow)	O	Corylus avellana (Hazel) seedling	V O
Anthoxanthum odoratum (sweet vnl grass)	F/A	Aegopodium podagraria (Ground Elder)	VL	Fraxinus excelsior (Ash) seedling	V O
Carex pallescens (Pale Sedge)	VL	Alliaria petiolata (Garlic Mustard)	R	Ligustrum vulgare (Privet) (cultivated)	R
Cynosurus cristatus (crested dog's tail)	LF	Angelica sylvestris (wild angelica)	VO	Prunus spinosa (Blackthorn suckers)	V L
Dactylis glomerata (cock's foot)	O/V LF	Arctium minus (Lesser Burdock)	R	Quercus sp. (Oak seedling)	V O
<b>Festuca pratensis (Meadow Fescue)</b>	<b>VL</b>	<b>Centaurea nigra (common knapweed)</b>	<b>O/LF</b>	Salix cinerea (Grey Willow)	V L
Festuca rubra (red fescue)	A	Cerastium holosteoides (cmn. mouse-ear)	O		
Holcus lanatus (yorkshire fog)	F/LA	Chamerion angustifolium (Rosebay Willowherb)	VL		
Holcus mollis (creeping soft grass)	VLF	Circaea lutetiana (Enchanter's Nightshade)	VL		
Juncus conglomeratus (compact rush)	VL	Cirsium arvense (creeping thistle)	VO		
Juncus effusus (soft rush)	VL	Cirsium palustre (marsh thistle)	VO		
Lolium perenne (perennial rye grass)	O	<b>Conopodium majus (pignut)</b>	<b>O</b>		
<b>Luzula campestris (field wood rush)</b>	<b>F</b>	<b>Dactylorhiza fuchsii (c. spotted orchid)</b>	<b>O/V LF</b>		
Phalaris arundinacea (Reed Canary Grass)	VL	<b>Dactylorhiza maculata (Heath Spotted Orchid)</b>	<b>R</b>		
Poa trivialis (rough meadow grass)	O	Epilobium montanum (Broad-leaved Willowherb)	VL		
Pteridium aquilinum (bracken)	LA	Filipendula ulmaria (meadowsweet)	O/VL		

	Galeopsis tetrahit (Common Hemp Nettle)	R	
	Galium aparine (Cleavers)	VO	
	Geranium robertianum (herb robert)	VL	
	Geum urbanum (Wood Avens)	VL	
	Hedera helix (Ivy)	VL	
	Heracleum sphondylium (hogweed)	VO	
	Hyacinthoides non-scripta (bluebell)	VL	
	<b>Hypericum maculatum(imp StJohns-Wort)</b>	<b>R</b>	
	Hypochaeris radicata (common cats ear)	F/LA	
	<b>Lathyrus pratensis (meadow vetchling)</b>	<b>O/V LF</b>	
-	Leontodon autumnalis (autumn hawkbit)	O	
-	<b>Leontodon hispidus (rough hawkbit)</b>	<b>VO</b>	
	<b>Lotus corniculatus (birds-foot trefoil)</b>	<b>F/A</b>	
	Lotus uliginosus(greater birds-foot trefoil)	VLF	
	Oenanthe crocata (Hemlock water dropwort).	VL	
	<b>Pimpinella saxifraga (burnet saxifrage)</b>	<b>VL</b>	
	Plantago lanceolata (ribwort plantain)	F	
	<b>Potentilla erecta (tormentil)</b>	<b>O/LF</b>	
	Potentilla reptans (creeping cinquefoil)	O	
	Ranunculus acris (meadow buttercup)	F	
	<b>Ranunculus bulbosus (bulbous buttercup)</b>	<b>F</b>	
	Ranunculus repens (creeping buttercup)	O	
	<b>Rhinanthus minor (yellow rattle)</b>	<b>O/V LF</b>	
	Rosa sp. (Rose sp.)	Y	
	Rubus fruticosus (bramble)	VLF	
	Rubus idaeus (Raspberry)	VLF	
	Rumex acetosa (common sorrel)	O/LF	
	Rumex obtusifolius (Broad-leaved Dock)	VO	
	Stachys sylvatica (hedge woundwort)	VL	
	<b>Stellaria graminea (lesser stichwort)</b>	<b>O/LF</b>	
	Stellaria holostea (greater stichwort)	VL	
	Taraxacum officinale (dandelion)	O	
	<b>Trifolium pratense (red clover)</b>	<b>F</b>	
	Trifolium repens (white clover)	Y	
	Urtica dioica (Stinging Nettles)	O/VL F	
	Veronica chamaedrys (gendr. speedwell)	VL	
	<b>Vicia cracca (tufted vetch)</b>	<b>O/V LF</b>	
	<b>Viola riviniana (common dog violet)</b>	<b>Y</b>	

<b>Table 26.3 - ORIGINAL SURVEY</b>					
<b>SITE NAME: Wet Meadow, Trellech</b>		<b>FIELD NUMBER: 5</b>		<b>DATE: 9/5/22</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>D</b>	<b>Herbs</b>	<b>D</b>	<b>Woody Species</b>	<b>D</b>
Agrostis_cap_gig	0.74 %	Achillea_millefolium	4.98%	Quercus_petraea_robur	0.03 %
Anthoxanthum_odoratum	0.44 %	<b>Centaurea_nigra</b>	<b>11.25 %</b>		
Dactylis_glomerata	0.05 %	Cerastium_fontanum	0.04%		

Festuca_rubra	0.44 %	Daucus_carota	0.01%		
Holcus_lanatus	0.14 %	Hypochaeris_radicata	0.36%		
Luzula_campestris	0.01 %	Lathyrus_pratensis	0.02%		
		Lotus_corniculatus	10.83 %		
		Lotus_pedunculatus	0.24%		
		Plantago_lanceolata	3.39%		
		Ranunculus_acris_occid	0.71%		
		Ranunculus_bulb_repe	2.95%		
		Rhinanthus_minor	49.62 %		
		Rumex_acetosa	0.09%		
		Stellaria_alsine-graminea	0.02%		
		Taraxacum_officinale_agg.	0.07%		
		Trifolium_pratense	7.95%		
		Trifolium_rep_occid_nigr	3.59%		
		Vicia_cracca	0.04%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 19 Indicator Species, the eDNA method recorded 9/10 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a LWS.

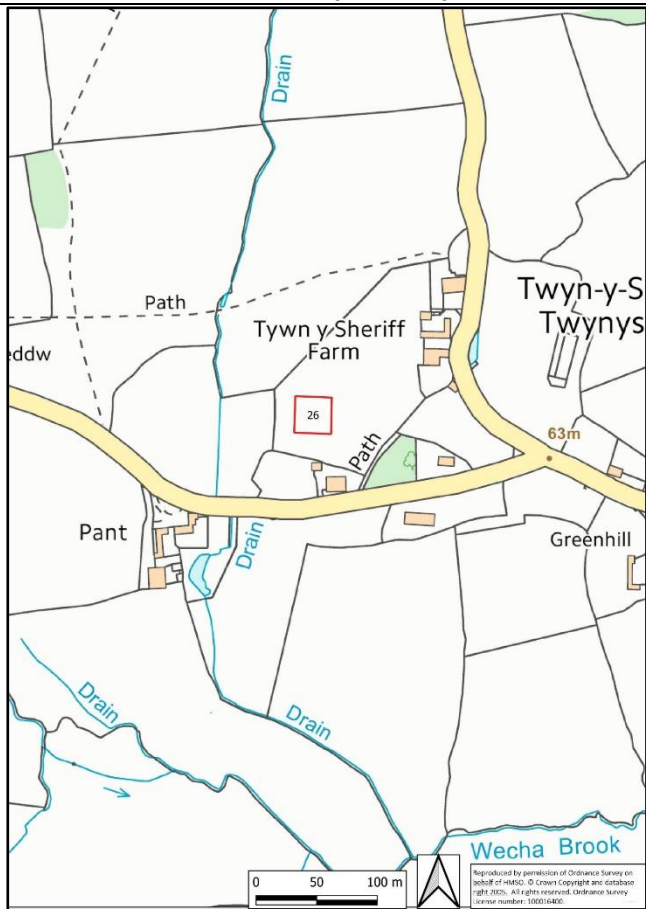
<b>Site Number/Name:</b>	<b>Site 26 – Twyn-y-Sheriff Farm, Raglan</b>
<b>Date Surveyed:</b>	<b>10<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO40628 05613</b>
	<p>The site is located on fairly steeply sloping, west facing ground. It is within an old cider orchard, of which some trees remain. There was much moss in the sward, however the grass was quite rank. It has not been formally surveyed botanically, however notes made during the collection of soil samples revealed a number of Species-rich Grasslands Indicator Species to be present. The fungal diversity of the field is unknown, however the owner reports a number of grassland fungi to be present in the autumn. A more limited list of floral Indicator Species and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.26 – Twyn-y-Sheriff Farm, Raglan Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 27.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.50%
<i>Clavaria falcata</i>			30	0.08%
<i>Clavaria flavipes</i>	Straw Club		29	0.22%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	0.04%
<i>Clavulinopsis helvola</i>	Yellow Club		25	2.23%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.98%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.32%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.02%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.59%
<i>Gliophorus psittacinus</i>	Parrot Waxcap		12	2.61%



Cuphophyllus_pratensis	Meadow Waxcap		22	0.14%
Hygrocybe_conica	Blackening Waxcap		25	0.32%
Hygrocybe_glutinipes	Glutinous Waxcap		22	2.87%
Hygrocybe_inspida	Spangle Waxcap		19	1.04%
Porpolomopsis_calyptiformis	Pink (Ballerina) Waxcap	VU	7	0.32%
Entoloma_conferendum	a pinkgill sp.		25	0.22%
Geoglossum_fallax	Deceptive Earthtongue		16	0.31%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.03%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.20%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.05%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.06%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.46%
Dermoloma_magicum	Black Magic	[VU]	16	1.96%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	0.70%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			6
Entolomataceae			1
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			23
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			6
Entolomataceae			1
Geoglossomycetes			4
Dermoloma			2
CHEGD Score			19

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a valuable Grassland Fungi site with a fairly high CHEGD score, and a quite impressive number of Hygrocybe (Waxcap) species recorded.

The results of the eDNA Survey would almost be sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 3 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following table (27.2) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 27.2 - eDNA SURVEY**

SITE NAME: Twyn Sheriff		FIELD NUMBER:		DATE: 10/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	16.20%	Ficaria_verna	17.65%		
Alopecurus_pratensis	2.85%	<b>Lathyrus_pratensis</b>	1.96%		
Anthoxanthum_odoratum	7.77%	Lotus_pedunculatus	8.16%		
Festuca_rubra	2.91%	Rumex_acetosa	0.17%		
Holcus_lanatus	6.76%	Rumex_obtusifolius	0.56%		
Lolium_perr_mult	3.18%	<b>Stellaria_alsine-graminea</b>	5.64%		
Poa_prat_calc_parv	3.30%	Trifolium_rep_occi_nigr	0.17%		
		Vicia_sativa	3.69%		
		<b>Viola_riviniana</b>	3.80%		

The eDNA survey returned quite a small number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 3 Indicator Species. This wouldn't be enough to recognise the site as a Local Wildlife Site, however it may give a clue to warrant further survey work. This was the joint lowest number of Indicator Species recorded on any of the 30 sites, and also the one of only two sites that were considered not to be LWS quality so it offered a good correlation.

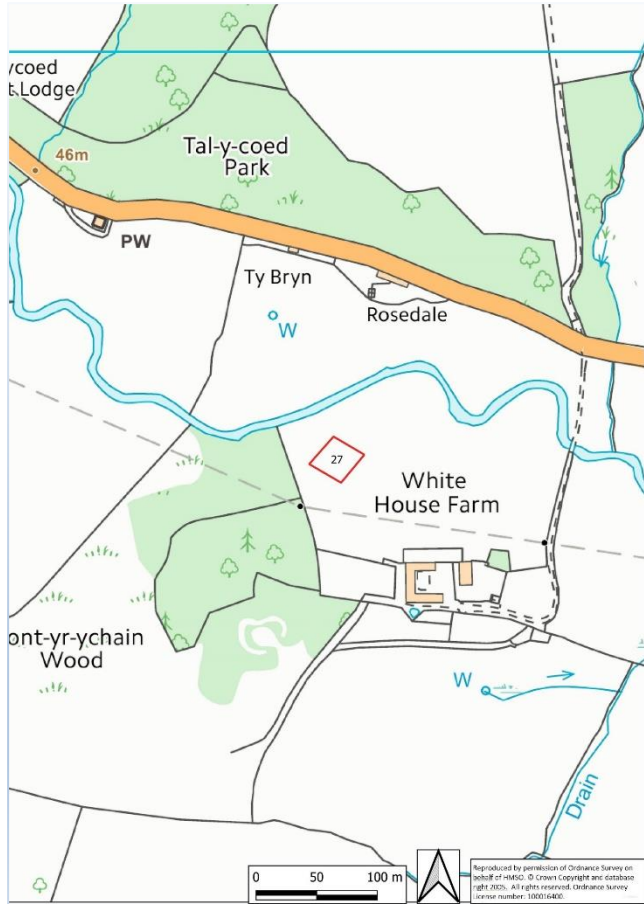
<b>Site Number/Name:</b>	<b>Site 27 – Whitehouse Farm</b>
<b>Date Surveyed:</b>	<b>10<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO42298 14664</b>
	<p>The site is located on a quite steep north facing slope. The fields are managed as Hay Meadows with aftermath grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner did report grassland fungi present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.</p>

Figure 2.27 – Whitehouse Farm Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 28.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis atrovelutina</i>			24	0.38%
<i>Clavaria falcata</i>			30	0.13%
<i>Clavaria flavipes</i>	Straw Club		29	0.16%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	1.20%
<i>Clavulinopsis helvola</i>	Yellow Club		25	1.51%
<i>Clavulinopsis laeticolor</i>	Handsome Club		21	0.15%
<i>Clavulinopsis luteoalba</i>	Apricot Club		18	0.82%
<i>Hodophilus micaceus</i>			4	0.20%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.08%
<i>Ramariopsis crocea</i>	a coral fungus sp.		23	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	3.18%

Cuphophyllus_virginus	Snowy Waxcap		21	0.05%
Gliophorus_psittacinus	Parrot Waxcap		12	0.32%
Hygrocybe_chlorophana	Golden Waxcap		16	2.97%
Hygrocybe_citrinovirens	Citrine Waxcap	VU	11	0.08%
Hygrocybe_conica	Blackening Waxcap		25	0.72%
Hygrocybe_glutinipes	Glutinous Waxcap		22	1.14%
Hygrocybe_inspida	Spangle Waxcap		19	0.22%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
Entoloma_lampropus	a pinkgill sp.		1	0.18%
Entoloma_sericeum	Silky Pinkgill		24	0.02%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.10%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.18%
Geoglossum_fallax	Deceptive Earthtongue		16	0.19%
Glutinoglossum_heptaseptatum	an Earthtongue sp.		4	0.01%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.07%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.07%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.31%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.86%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.60%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			7
Entolomataceae			3
Geoglossomycetes			7
Dermoloma			0
CHEGD Score			26
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			8
Hygrophoraceae			7
Entolomataceae			1
Geoglossomycetes			6
Dermoloma			0
CHEGD Score			22

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a valuable Grassland Fungi site with a fairly high CHEGD score, and an impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be almost sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the “Section 74 List”\* (WAG 2003). \*Now Section 7 species

The fact that 2 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (28.2 & 28.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi), however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 28.2 ORIGINAL SURVEY					
SITE NAME: Whitehouse Farm, Llanvihangel Ystern Llewern		FIELD NUMBER: 1		DATE: 19/07/2020	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis capillaris</i> (Common Bent)	F/L A	<i>Achillea millefolium</i> (Yarrow)	O	<i>Alnus glutinosa</i> (Alder)	LF (by river)
<i>Agrostis stolonifera</i> (Creeping Bent)	VL A	<i>Anemone nemorosa</i> (Wood Anemone)	O	<i>Populus tremula</i> (Aspen)	VL (copse)
<i>Alopecurus geniculatus</i> (Marsh Foxtail)	O	<i>Angelica sylvestris</i> (Wild Angelica)	O	<i>Prunus avium</i> (Wild Cherry)	VL (copse)
<i>Alopecurus myosuroides</i> (Black Grass)	R	<i>Calystegia sylvatica</i> (Large Bindweed)	V L	<i>Salix x fragilis</i> 'fragilis' (Crack Willow)	LF
<i>Alopecurus pratensis</i> (Meadow Foxtail)	O	<b><i>Centaurea nigra</i> (Common Knapweed)</b>	L F	<i>Sorbus aucuparia</i> (Rowan)	VL (copse)
<i>Anthoxanthum odoratum</i> (Sweet Vernal Grass)	F/A	<b><i>Centaury erythraea</i> (Common Centaury)</b>	R		
<i>Arrhenatherum elatius</i> (False Oat-grass)	LA	<i>Cerastium holosteoides</i> (Common Mouse-ear)	O/ F		
<i>Briza media</i> (Quaking Grass)	LA	<i>Cirsium arvense</i> (Creeping Thistle)	Y		
<b><i>Bromus commutatus</i> (Meadow Brome)</b>	R	<i>Cirsium palustre</i> (Marsh Thistle)	Y		
<i>Carex flacca</i> (Glaucous Sedge)	LO	<i>Cirsium vulgare</i> (Spear Thistle)	Y		
<i>Carex hirta</i> (Hairy Sedge)	LO	<i>Conium maculatum</i> (Hemlock)	L A		
<i>Cynosurus cristatus</i> (Crested Dogtail)	O	<i>Epilobium hirsutum</i> (Greater Willowherb)	V LF		
<i>Dactylis glomerata</i> (Cock's foot)	LF/ LA	<b><i>Euphrasia officinalis</i> agg. (Eyebright)</b>	L F		
<i>Festuca rubra</i> (Red Fescue)	F	<i>Filipendula ulmaria</i> (Meadowsweet)	LF		
<i>Glyceria fluitans</i> (Floating Sweet-grass)	VL	<i>Gnaphalium uliginosum</i> (Marsh Cudweed)	V L		
<i>Holcus lanatus</i> (Yorkshire Fog)	F/A	<i>Heracleum sphondylium</i> (Hogweed)	O/ F		
<i>Holcus mollis</i> (Creeping Soft-grass)	LF	<i>Hypericum tetrapetrum</i> (Square Stalked St. Johns-Wort)	V L O		
<i>Juncus acutiflorus</i> (Sharp-flowered Rush)	LF	<b><i>Hypochaeris radicata</i> (Common Catsear)</b>	O/ F		
<i>Juncus inflexus</i> (Hard Rush)	O	<i>Impatiens glandulifera</i> (Himalayan Balsam)	L A		
<i>Phalaris arundinacea</i> (Reed Canary-grass)	VL F	<i>Iris pseudacorus</i> (Yellow Flag Iris)	V LF		
<i>Phleum pratense</i> (Timothy Grass)	VL	<b><i>Lathyrus pratensis</i> (Meadow Vetchling)</b>	F		
<i>Poa trivialis</i> (Rough Meadow-grass)	F	<b><i>Leontodon hispidus</i> (Rough Hawkbit)</b>	L F		
<i>Sparganium erectum</i> (Branched Bur-reed)	VL F	<i>Leucanthemum vulgare</i> (Oxeye Daisy)	R		
		<b><i>Lotus corniculatus</i> (Birds-foot Trefoil)</b>	L A		
		<i>Lotus uliginosus</i> (Greater Birds-foot Trefoil)	LF		
		<i>Oenanthe crocata</i> (Hemlock Water-dropwort).	V L		

		<i>Persicaria maculosa</i> (Redshank)	V L	
		<i>Phacelia tanacetifolia</i> (Phacelia)	V L	
-		<i>Plantago lanceolata</i> (Ribwort Plantain)	LF	
-		<i>Potentilla anglica</i> (Trailing Tormentil)	O	
		<i>Potentilla erecta</i> (Tormentil)	O	
		<i>Potentilla reptans</i> (Creeping Cinquefoil)	O	
		<i>Pulicaria dysenterica</i> (Fleabane)	R	
		<i>Ranunculus acris</i> (Meadow Buttercup)	F	
		<i>Ranunculus repens</i> (Creeping Buttercup)	L A	
		<i>Rhinanthus minor</i> (Yellow Rattle)	F/ L A	
		<i>Rumex acetosa</i> (Common Sorrel)	F	-
		<i>Rumex obtusifolius</i> (Broad-leaved Dock)	O/ LF	
		<i>Sisymbrium officinale</i> (Hedge Mustard)	O	
		<i>Stachys officinalis</i> (Betony)	R	
		<i>Stachys sylvatica</i> (Hedge Woundwort)	V L	
		<i>Stellaria graminea</i> (Lesser Stitchwort)	Y	
		<i>Symphytum officinale</i> (Common Comfrey)	R	
		<i>Trifolium pratense</i> (Red Clover)	O/ F	
		<i>Trifolium repens</i> (White Clover)	O	
		<i>Urtica dioica</i> (Stinging Nettle)	L A	
		<i>Viola riviniana</i> (Common Dog-violet)	O	-

Table 28.3 - eDNA SURVEY					
SITE NAME: Whitehouse Farm, Llanvihangel Ystern Llewern		FIELD NUMBER: 1		DATE:	10/05/20 22
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis_cap_gig</i>	1.52 %	<i>Achillea_millefolium</i>	5.33%		
<i>Alopecurus_pratensis</i>	0.82 %	<b>Cardamine_prat_flex</b>	<b>0.18%</b>		
<i>Anthoxanthum_odoratum</i>	1.66 %	<b>Centaurea_nigra</b>	<b>0.13%</b>		
<i>Dactylis_glomerata</i>	0.11 %	<i>Cirsium_palustre</i>	1.45%		
<i>Festuca_rubra</i>	1.40 %	<b>Lathyrus_pratensis</b>	<b>0.90%</b>		
<i>Holcus_lanatus</i>	0.24 %	<b>Lotus_corniculatus</b>	<b>34.62 %</b>		
<i>Poa_trivialis</i>	0.10 %	<i>Lotus_pedunculatus</i>	0.64%		
		<i>Plantago_lanceolata</i>	9.78%		
-		<b>Potentilla_erecta</b>	<b>4.91%</b>		
		<i>Potentilla_reptans</i>	2.33%		
		<i>Ranunculus_acris_occid</i>	4.42%		
		<b>Ranunculus_bulb_rep</b>	<b>0.57%</b>		
		<b>Rhinanthus_minor</b>	<b>9.17%</b>		
		<i>Rumex_acetosa</i>	2.41%		
		<b>Stellaria_alsine-graminea</b>	<b>0.10%</b>		
		<i>Taraxacum_officinale_agg.</i>	2.90%		
		<b>Trifolium_pratense</b>	<b>3.49%</b>		
		<i>Trifolium_rep_occini</i>	5.54%		

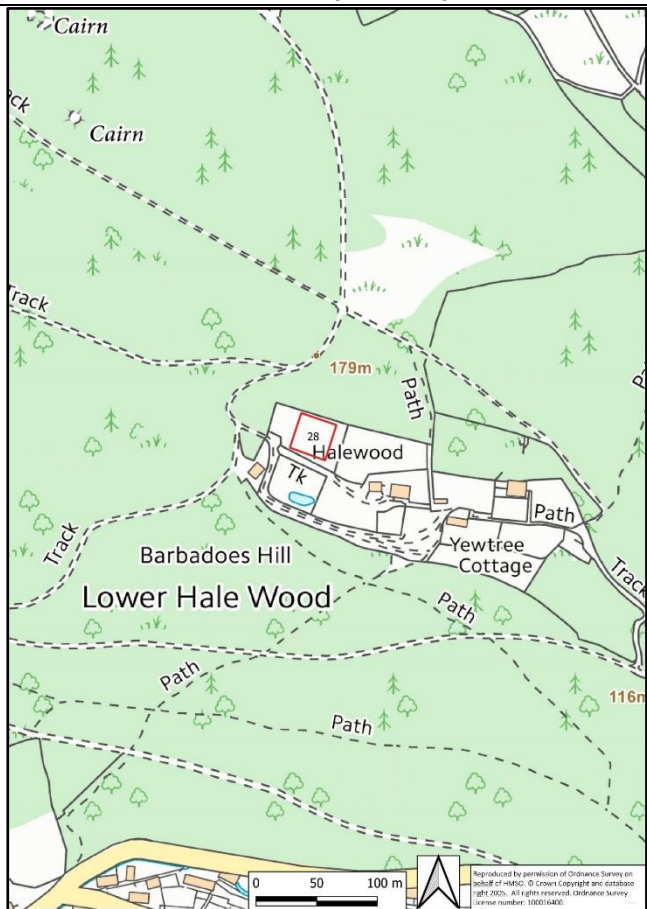
It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The original survey recorded 16 Indicator Species, the eDNA method recorded 8/9 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be be enough to recognise the site as a LWS.

**Site Number/Name:** Site 28 – Barbadoes Hill (Halewood Cottage)

**Date Surveyed:** 10<sup>th</sup> May 2022

**British National Grid (centre):** SO52291 00634



The site is located on a south facing slope. The fields are not managed as such but the sward is kept very low by rabbits. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field is unknown, however the owner did report grassland fungi present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown, however there are no previous results to compare these to.

Figure 2.28 – Barbadoes Hill Site Location

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 29.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Camarophyllopsis_schulzeri</i>		[VU]	17	0.02%
<i>Clavaria_falcata</i>			30	0.10%
<i>Clavaria_flavipes</i>	Straw Club		29	0.33%
<i>Clavulinopsis_helvola</i>	Yellow Club		25	0.98%
<i>Clavulinopsis_laeticolor</i>	Handsome Club		21	0.64%
<i>Clavulinopsis_luteoalba</i>	Apricot Club		18	0.04%
<i>Ramariopsis_avellaneo-inversa</i>	a coral fungus sp.		29	0.03%
<i>Ramariopsis_crocea</i>	a coral fungus sp.		23	0.01%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	3.14%



Cuphophyllus_flavipes	Yellow Foot Waxcap	[VU]	10	3.84%
Cuphophyllus_flavipesoides	a Waxcap sp.	[VU]	1	0.87%
Cuphophyllus_pratensis	Meadow Waxcap		22	1.08%
Hygrocybe_conica	Blackening Waxcap		25	1.33%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.07%
Hygrocybe_inspida	Spangle Waxcap		19	1.26%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.04%
Entoloma_conferendum	a pinkgill sp.		25	0.06%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.04%
Geoglossum_fallax	Deceptive Earthtongue		16	0.02%
Glutinoglossum_heptaseptatum	an Earthtongue sp.		4	0.16%
Glutinoglossum_pseudoglutinsum	an Earthtongue sp.		21	0.19%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	2.47%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.86%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			6
Entolomataceae			2
Geoglossomycetes			4
Dermoloma			0
CHEGD Score			20
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			4
Hygrophoraceae			6
Entolomataceae			1
Geoglossomycetes			3
Dermoloma			0
CHEGD Score			14

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

This is a quite valuable Grassland Fungi site with a fairly high CHEGD score, and an impressive number of Hygrocybe (Waxcap) species recorded.

The site is already a Local Wildlife Site based on floristic composition. The results of the eDNA Survey would be almost sufficient for the site to also be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 4 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (29.2 and 29.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

SITE NAME: Barbadoes Hill (Halewood Cottage)		FIELD NUMBER: 1		DATE: 14/07/2015	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
Agrostis capillaris (common bent)	F	Achillea millefolium (yarrow)	LF		
Anthoxanthum odoratum (Sweet Vernal grass)	LF	<b>Centaurea nigra (common knapweed)</b>	<b>O</b>		
Arrhenatherum elatius (false oat grass)	LF	Chamerion angustifolium (Rosebay Willowherb)	R		
Festuca rubra (red fescue)	O	Cirsium arvense (creeping thistle)	O/LF		
Holcus lanatus (yorkshire fog)	F	Crepis capillaris (smooth hawks-beard)	LF		
Lolium perenne (perennial rye grass)	O/VL F	Cytisus scoparius (Broom)	R		
<b>Luzula campestris (field wood rush)</b>	<b>O</b>	Galium aparine (Cleavers)	R		
Pteridium aquilinum (bracken)	VLA	<b>Hypochaeris radicata (common cats ear)</b>	<b>F</b>		
		Leontodon autumnalis (autumn hawkbit)	O		
		<b>Leucanthemum vulgare (oxeye daisy)</b>	<b>O/VL F</b>		
		<b>Lotus corniculatus (birds-foot trefoil)</b>	<b>LF</b>		
		Lotus uliginosus (greater birds-foot trefoil)	LF		
		Plantago lanceolata (ribwort plantain)	F		
		Prunella vulgaris (self-heal)	O		
		Ranunculus acris (meadow buttercup)	O		
		Ranunculus repens (creeping buttercup)	LF		
		<b>Rhinanthus minor (yellow rattle)</b>	<b>VL</b>		
		Rubus fruticosus (bramble)	O		
		Rumex acetosa (common sorrel)	O		
		Rumex acetosella (Sheeps Sorrel)	LF		
		Rumex obtusifolius (Broad-leaved Dock)	LF		
		Senecio jacobea (ragwort)	R		
		Silene dioica (Red Campion)	R		
		<b>Stellaria graminea (lesser stichwort)</b>	<b>O/VL F</b>		
		<b>Trifolium pratense (red clover)</b>	<b>F</b>		
		Trifolium repens (white clover)	F		
		Urtica dioica (Stinging Nettle)	O/VL A		
		Veronica chamaedrys (Germander speedwell)	O		
		Vicia sativa (common vetch)	O		
		Vicia sepium (bush vetch)	VLF		

<b>Table 29.3 - eDNA SURVEY</b>					
<b>SITE NAME: Barbadoes Hill (Halewood Cottage)</b>		<b>FIELD NUMBER: 1</b>		<b>DATE: 10/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Woody Species</b>	<b>%</b>
<i>Agrostis_cap_gig</i>	2.93 %	<i>Achillea_millefolium</i>	0.66%	<i>Fagus_sylvatica</i>	0.31 %
<i>Anthoxanthum_odoratum</i>	11.69 %	<i>Centaurea_nigra</i>	0.07%	<i>Quercus_petraea_robur</i>	0.49 %
<i>Festuca_rubra</i>	0.95 %	<i>Cerastium_fontanum</i>	0.23%		
<i>Holcus_lanatus</i>	0.22 %	<i>Crepis_capillaris</i>	0.04%		
<i>Luzula_campestris</i>	0.07 %	<i>Cucumis_sativus</i>	0.03%		
<i>Poa_trivialis</i>	1.38 %	<i>Euphrasia_agg</i>	0.09%		
		<i>Hypochaeris_radicata</i>	4.37%		
		<i>Jacobaea_vulgaris</i>	0.08%		
		<i>Leucanthemum_vulgare</i>	0.51%		
		<i>Lotus_corniculatus</i>	0.06%		
		<i>Lotus_pedunculatus</i>	15.66 %		
		<i>Plantago_lanceolata</i>	16.84 %		
		<i>Prunella_vulgaris</i>	0.10%		
		<i>Ranunculus_bulb_repe</i>	0.55%		
		<i>Rhinanthus_minor</i>	8.18%		
		<i>Rumex_acetosa</i>	1.19%		
		<i>Rumex_acetosella</i>	2.83%		
		<i>Stellaria_alsine-graminea</i>	0.70%		
		<i>Taraxacum_officinale_agg.</i>	2.11%		
		<i>Trifolium_pratense</i>	0.72%		
		<i>Trifolium_rep_occi_nigr</i>	6.76%		
		<i>Veronica_chamaedrys</i>	1.44%		

It can be seen that the eDNA survey returned fairly similar numbers of species, although there were quite a few only recorded in one or the other survey. The eDNA doesn't give much indication of abundance. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges where certain other species may be located so a full species list is not realistic.

The original survey recorded 8 Indicator Species, the eDNA method recorded 9/10 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a LWS.

Interestingly this was the only site where the eDNA actually recorded a higher number of Indicator species, perhaps because the turf was grazed very low by rabbits thus making plants difficult to see and more challenging to identify conventionally.

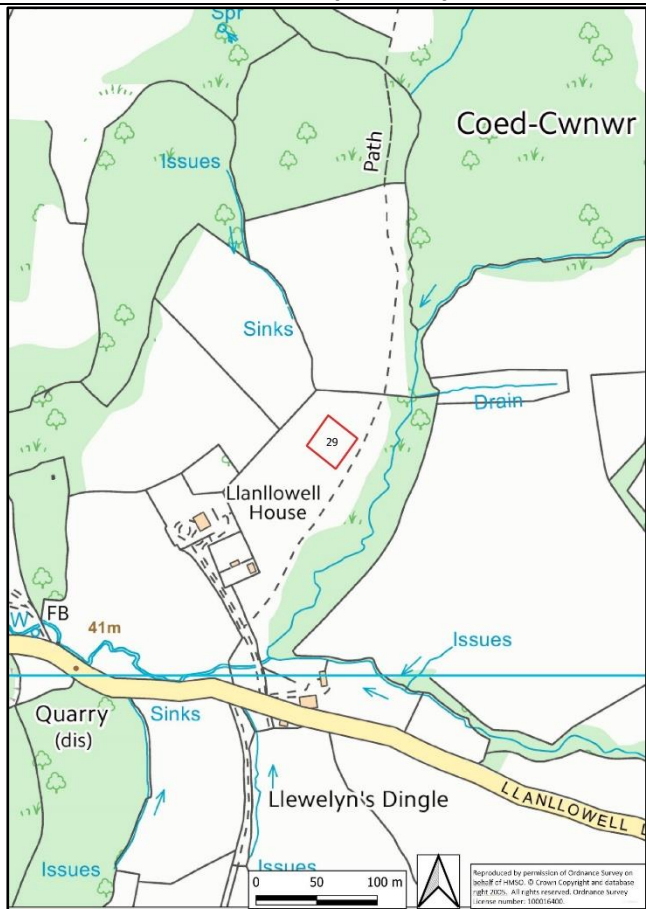
<b>Site Number/Name:</b>	<b>Site 29 – Llanllowell Meadows</b>
<b>Date Surveyed:</b>	<b>10<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>ST40446 99193</b>
	<p>The site is located on a gentle south facing slope. The fields are managed as Hay Meadows with aftermath grazing. It is known to be floristically species-rich and as such forms part of a Local Wildlife Site because of this. The fungal diversity of the field was investigated on a single visit on 26/11/21, the owner also reported grassland fungi present. The full floral list and comparison with the floral eDNA is shown below. The eDNA Fungal results are also shown and some comparisons made, however these are limited.</p>

Figure 2.29 – Llanllowell Meadows Site Location

## Results and comparison of these between conventional and eDNA.

### Fungi

The results of the eDNA Survey are shown within Table 30.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
<i>Clavaria californica</i>			6	0.01%
<i>Clavaria falcata</i>			30	0.18%
<i>Clavaria flavipes</i>	Straw Club		29	0.02%
<i>Clavaria redoleoalii</i>			2	0.01%
<i>Clavulinopsis corniculata</i>	Meadow Coral		22	1.22%
<i>Clavulinopsis helvola</i>	Yellow Club		25	0.09%
<i>Clavulinopsis luteonana</i>	Dwarf Spindles		2	0.53%
<i>Ramariopsis avellaneo-inversa</i>	a coral fungus sp.		29	0.37%
<i>Ramariopsis flavescens</i>	a coral fungus sp.		16	0.07%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	1.82%
<i>Gliophorus psittacinus</i>	Parrot Waxcap		12	3.48%

Hygrocybe_conica	Blackening Waxcap		25	0.79%
Hygrocybe_glutinipes	Glutinous Waxcap		22	0.07%
Cuphophyllus_russocoriaceus	Cedarwood Waxcap		4	1.48%
<b>UNIDENTIFIED WAXCAPS</b>			13	0.01%
Entoloma_ameides	a pinkgill sp.		15	0.02%
Entoloma_asprellum	a pinkgill sp.		18	0.12%
Entoloma_clandestinum	a pinkgill sp.		13	0.02%
Entoloma_dysthales	a pinkgill sp.		10	0.18%
Entoloma_henricii	a pinkgill sp.	[VU]	12	0.08%
Entoloma_infula	a pinkgill sp.		12	0.01%
Entoloma_longistriatum	a pinkgill sp.		6	0.02%
Entoloma_poliopus	a pinkgill sp.		14	0.01%
Entoloma_pseudocoelestinum	a pinkgill sp.		16	0.01%
Entoloma_sericeum	Silky Pinkgill		24	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.20%
Geoglossum_aff_simile	an Earthtongue sp.		19	0.22%
Geoglossum_fallax	Deceptive Earthtongue		16	0.01%
Geoglossum_nigritum	an Earthtongue sp.		15	0.16%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.02%
Hemileucoglossum_aff_alveolatum	an Earthtongue sp.		25	0.18%
Trichoglossum aff. variabile	an Earthtongue sp.		19	0.25%
Trichoglossum_walteri	Short-spored Earthtongue	VU	21	0.14%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.36%
Dermoloma_magicum	Black Magic	[VU]	16	1.65%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	1.03%

<b>SPECIES COUNT</b>			
Clavariaceae			9
Hygrophoraceae			4
Entolomataceae			10
Geoglossomycetes			7
Dermoloma			2
CHEGD Score			32
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			6
Hygrophoraceae			4
Entolomataceae			3
Geoglossomycetes			5
Dermoloma			2
CHEGD Score			20

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.

#### 2021 Results

- *Hygrocybe chloropana* Golden Waxcap
- *Entoloma sericeum* Silky Pinkgill
- *Cuphophylls pratensis* Meadow Waxcap
- *Entoloma rhodopolium* Wood Pinkgill

Neither of the two Waxcaps recorded in 2021 (Golden and Meadow) were picked up by the eDNA, although it must be remembered that the eDNA only surveyed a 30mx30m quadrat of the field. The Silky Pinkgill was picked up in both surveys.

This is a valuable Grassland Fungi site with a high CHEGD score, the number of Hygrocybe (Waxcap) species recorded is decent, although perhaps lower than might be expected with the impressive number of Clavarioids, Entoloma and Geoglossum present.

The fact that 3 Vulnerable species were identified further reinforces its value and the importance of preserving this.

## Flora

The following two tables (30.2 and 30.3) show the results the original flora survey and the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at.

KEY
<b>Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)</b>
Recorded in both surveys
Just recorded in that survey
Dubious ID from eDNA

Table 30.2 - ORIGINAL SURVEY					
SITE NAME: Llanllowell Meadow		FIELD NUMBER: 1		DATE: 22/05/2015	
Grasses, Sedges, Rushes & Ferns	D	Herbs	D	Woody Species	D
<i>Agrostis tenuis</i> (common bent)	F	<b><i>Ajuga reptans</i> (bugle)</b>	O/VLF	<i>Acer pseudoplatanus</i> (Sycamore)	R
<i>Alopecurus pratensis</i> (meadow foxtail)	O	<i>Anthriscus sylvestris</i> (Cow Parsley)	VL	<i>Prunus spinosa</i> (Blackthorn)	V L
<i>Anthoxanthum odoratum</i> (sweet vnl grass)	F	<i>Cardamine pratensis</i> (cuckoo flower)	O		
<b><i>Briza media</i> (quaking grass)</b>	LO	<b><i>Centaurea nigra</i> (common knapweed)</b>	F		
<i>Bromus hordeaceus</i> (Soft Brome)	VL	<i>Cerastium holosteoides</i> (cmn. mouse-ear)	VO		
<b><i>Carex caryophylla</i> (spring sedge)</b>	VL	<i>Chamerion angustifolium</i> (Rosebay Willowherb)	VLF		
<b><i>Carex flacca</i> (glaucous sedge)</b>	LO	<i>Chrysosplenium oppositifolium</i> (Opposite Leaved Golden Saxifrage)	VL		
<i>Carex hirta</i> (Hairy Sedge)	LO	<i>Cirsium arvense</i> (creeping thistle)	R		
<b><i>Carex panicea</i> (carnation sedge)</b>	VL A	<b><i>Conopodium majus</i> (pignut)</b>	VL		
<i>Cynosurus cristatus</i> (crested dog's tail)	F	<b><i>Dactylorhiza fuchsii</i> (c. spotted orchid)</b>	F		
<i>Dactylis glomerata</i> (cock's foot)	VO	<i>Filipendula ulmaria</i> (meadowsweet)	O/VLF		
<i>Festuca rubra</i> (red fescue)	LF	<i>Glechoma hederacea</i> (ground ivy)	VL		
<i>Holcus lanatus</i> (yorkshire fog)	O	<i>Heracleum sphondylium</i> (hogweed)	O/VLF		
<i>Juncus acutiflorus</i> (sharp-flowered rush)	LF	<i>Hyacinthoides non-scripta</i> (bluebell)	VO		
<i>Juncus conglomeratus</i> (compact rush)	R	<b><i>Hypericum maculatum</i> (imp St Johns-Wort)</b>	R		
<i>Lolium perenne</i> (perennial rye grass)	VO	<b><i>Hypericum perforatum</i> (pf St Johns-Wort)</b>	R		
<b><i>Luzula campestris</i> (field wood rush)</b>	F	<b><i>Hypochoeris radicata</i> (common cats ear)</b>	O		
<i>Poa trivialis</i> (rough meadow grass)	VL	<b><i>Lathyrus pratensis</i> (meadow vetchling)</b>	VL		
<i>Pteridium aquilinum</i> (bracken)	VL F	<i>Leontodon autumnalis</i> (autumn hawkbit)	R		
		<b><i>Leontodon hispidus</i> (rough hawkbit)</b>	O/LF/V LA		
		<b><i>Lotus corniculatus</i> (birds-foot trefoil)</b>	F		
		<i>Medicago lupulina</i> (Black Medick)	VO		
		<i>Oenanthe crocata</i> (Hemlock water dropwort)	O/VLF		

-	Plantago lanceolata (ribwort plantain)	O	
-	Potentilla anserina (silverweed)	R	
-	<b>Potentilla erecta (tormentil)</b>	<b>VLF</b>	
	<b>Primula veris (cowslip)</b>	<b>VLF</b>	
	Prunella vulgaris (self-heal)	O	
	Ranunculus acris (meadow buttercup)	O	
	<b>Ranunculus bulbosus (bulbous buttercup)</b>	<b>VLF</b>	
	Ranunculus repens (creeping buttercup)	VL	
	<b>Rhinanthus minor (yellow rattle)</b>	<b>A</b>	
	Rubus fruticosus (bramble)	VL	
	Rumex acetosa (common sorrel)	O	
	Rumex obtusifolius (Broad-leaved Dock)	R/VLF	
	<b>Stachys officinalis (betony)</b>	<b>LO</b>	
	Stellaria holostea (greater stichwort)	VL	
	<b>Succisa pratensis (devils bit-scabious)</b>	<b>R</b>	
	Taraxacum officinale (dandelion)	VO	
	Trifolium dubium (Lesser Trefoil)	O	
	<b>Trifolium pratense (red clover)</b>	<b>O/VLF</b>	
	Trifolium repens (white clover)	O	
	Veronica chamaedrys (gemdr. speedwell)	R	
	Vicia sativa (common vetch)	R	
	Vicia sepium (bush vetch)	VL	

<b>Table 30.3 - eDNA SURVEY</b>					
<b>SITE NAME: Llanlloell Meadow</b>		<b>FIELD NUMBER: 1</b>		<b>DATE: 10/05/2022</b>	
<b>Grasses, Sedges, Rushes &amp; Ferns</b>	<b>%</b>	<b>Herbs</b>	<b>%</b>	<b>Woody Species</b>	<b>%</b>
Agrostis_cap_gig	0.75%	Ajuga_reptans	0.28%		
Anthoxanthum_odoratum	2.63%	Centaurea_nigra	25.39%		
Briza_media	0.04%	Dactylorhiza_maculata	0.04%		
Carex_caryophyllea	0.23%	Ficaria_verna	0.21%		
Carex_flacca	0.03%	Heracleum_sphondylium	0.07%		
Cynosurus_cristatus	0.16%	Hypochaeris_radicata	3.65%		
Dactylis_glomerata	0.11%	Leontodon_hispidus	16.76%		
Festuca_rubra	0.45%	Leontodon_saxatilis	0.60%		
Holcus_lanatus	0.04%	Linum_catharticum	0.13%		
		Lotus_corniculatus	21.66%		
		Lotus_pedunculatus	1.45%		
		Plantago_lanceolata	7.19%		
		Potentilla_erecta	0.86%		
		Potentilla_reptans	0.03%		
		Primula_vulgaris	0.04%		
		Prunella_vulgaris	0.88%		
		Ranunculus_acris_occid	2.23%		
		Ranunculus_bulb_repe	0.57%		
		Rhinanthus_minor	1.33%		

		Rumex acetosa	0.04%		
		Taraxacum officinale agg.	0.23%		
		Trifolium dubium	0.02%		
		<b>Trifolium pratense</b>	<b>6.72%</b>		
-		Trifolium rep. occi. nigr	2.54%		

It can be seen that the eDNA survey didn't return anywhere near as many species as the original "Traditional Survey", it also doesn't give much indication of abundance. However, it should be noted that a number of additional species were detected. It also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges where certain other species may be located so a full species list is not realistic.

The original survey recorded 23 Indicator Species, the eDNA method recorded 14/15 (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This would be enough to recognise the site as a LWS.



<b>Site Number/Name:</b>	<b>Site 30 – St.Woolos Cemetery</b>
<b>Date Surveyed:</b>	<b>11<sup>th</sup> May 2022</b>
<b>British National Grid (centre):</b>	<b>SO29524 87592</b>

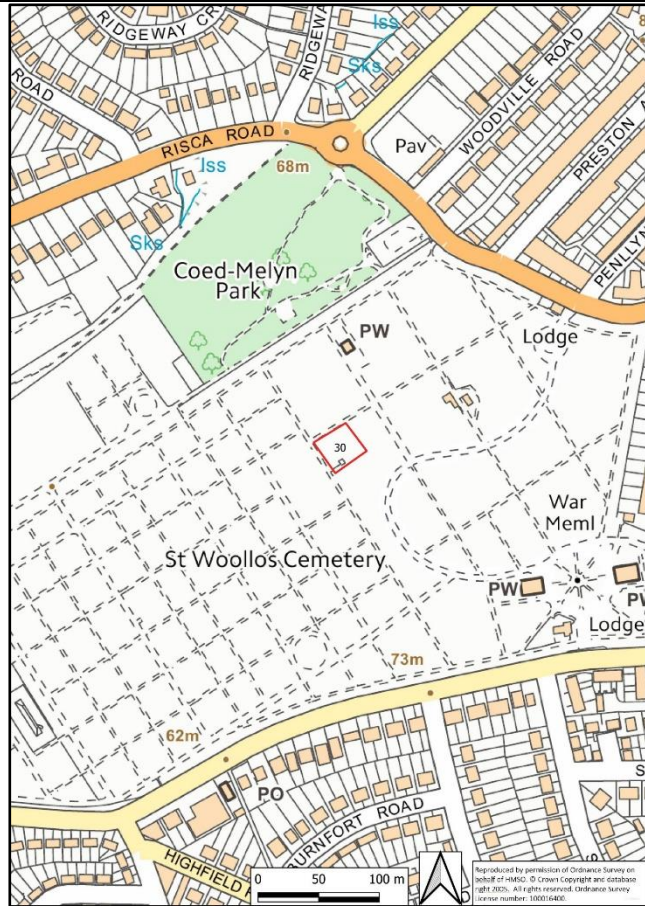


Figure 2.30 – St. Woolos Cemetery Site Location

The site is located on a gentle south-west facing slope. The site is a cemetery and grass is cut at various times of the year. The area surveyed was flanked by graves but the actual survey area was currently grave free. The fungal diversity of St. Woolos Cemetery is well know however no species lists could be tracked down and it was unclear where the best location in the cemetery was to undertake the survey. Both the floral & fungi eDNA are shown below, however no comparisons could be made.

**Results and comparison of these between conventional and eDNA.**

**Fungi**

The results of the eDNA Survey are shown within Table 31.1

Scientific Name	English Name	IUCN Status	No. of the 30 sites it was recorded at	% of DNA
Clavaria_californica			6	0.01%
Clavaria_falcata			30	0.20%
Clavaria_flavipes	Straw Club		29	0.18%
Clavulinopsis_corniculata	Meadow Coral		22	0.30%
Clavulinopsis_hisingeri			2	0.01%
Clavulinopsis_laeticolor	Handsome Club		21	0.05%
Hodophilus_micaceus			4	0.03%
Ramariopsis_avellaneo-inversa	a coral fungus sp.		29	0.11%
<b>UNIDENTIFIED FAIRY CLUBS</b>			30	0.97%
Hygrocybe_citrinovirens	Citrine Waxcap	<b>VU</b>	11	0.51%
Hygrocybe_conica	Blackening Waxcap		25	0.58%

Clitopilus_baronii			4	0.01%
Entoloma_conferendum	a pinkgill sp.		25	0.01%
Entoloma_neglectum	a pinkgill sp.		5	0.01%
<b>UNIDENTIFIED PINK GILLS</b>			29	0.28%
Glutinoglossum_pseudoglutinosum	an Earthtongue sp.		21	0.03%
Microglossum_nudipes_aff	an Earthtongue sp.	Sect7	1	0.18%
<b>UNIDENTIFIED EARTHTONGUES</b>			27	0.12%
Dermoloma_cuneifolium	Crazed Cap Mushroom		22	2.25%

<b>SPECIES COUNT</b>			
Clavariaceae			8
Hygrophoraceae			2
Entolomataceae			3
Geoglossomycetes			2
Dermoloma			1
CHEGD Score			16
<b>CONSERVATIVE' SPECIES COUNT (ONLY SPP. PRESENT AT &gt;0.05% ABUNDANCE)</b>			
Clavariaceae			5
Hygrophoraceae			2
Entolomataceae			0
Geoglossomycetes			1
Dermoloma			1
CHEGD Score			9

**IUCN** (International Union for Conservation of Nature) – **VU = Vulnerable** – *IUCN Criteria suggest that best available evidence indicates that it is facing a high risk of extinction in the wild.*

St. Woolos Cemetery is a known high value Grassland Fungi site so the results are slightly disappointing. It however couldn't be ascertained which were the best locations within this large site so perhaps a poorer quality area was surveyed. This perhaps demonstrates the value of pin-pointing the best area when only 30mx30m is being surveyed.

The results of the eDNA Survey would be sufficient for the site to be recognised as a Local Wildlife Site on Criteria S8) – Fungi:

**S8) FUNGI** The following should be considered for selection:

- all grassland sites supporting 8 or more species of waxcap (Hygrocybe spp.)
- any site which supports a species, which is listed in the UK Red Data Book (NCC, 1987) or in the "Section 74 List"\* (WAG 2003). \*Now Section 7 species

The fact that 1 Vulnerable species and 1 Section 7 species were identified further reinforces its value and the importance of preserving this.

## Flora

The following table (31.1) shows the flora results returned by the eDNA. It should be borne in mind that the flora was not the primary focus of the eDNA survey as this was focussed on Fungi, however as these results could also be obtained at no extra cost they are worth looking at. For many sites a comparison of the eDNA with Traditional surveys has been undertaken, this was not possible for this site as no previous survey had been undertaken.

### KEY

**Bold = Indicator Species of good quality Neutral Grassland used to assess the ecological value of the site against Local Wildlife Site Criteria (8 or more species is LWS quality)**

Dubious ID from eDNA

**Table 31.2 - eDNA SURVEY**

SITE NAME: St. Woolos		FIELD NUMBER:		DATE: 11/05/2022	
Grasses, Sedges, Rushes & Ferns	%	Herbs	%	Woody Species	%
Agrostis_cap_gig	5.79 %	Ajuga_reptans	0.03 %		
Alopecurus_pratensis	0.05 %	Cardamine_prat_flex	13.90 %		
Anthoxanthum_odoratum	0.57 %	Cerastium_fontanum	0.04 %		
Arrhenatherum_elatius	5.85 %	Cucumis_sativus	0.02 %		
Dactylis_glomerata	0.58 %	Ficaria_verna	0.32 %		
Elymus_repens	0.87 %	Geranium_dissectum	0.09 %		
Festuca_rubra	0.60 %	Hypochaeris_radicata	5.26 %		
Holcus_lanatus	4.57 %	Jacobaea_vulgaris	0.03 %		
Luzula_campestris	0.04 %	Leucanthemum_vulgare	0.53 %		
Poa_prat_calc_parv	2.13 %	Lysimachia_nummularia	1.36 %		
Poa_trivialis	8.63 %	Potentilla_sterilis	0.07 %		
		Ranunculus_acris_occid	1.07 %		
		Ranunculus_bulb_repe	2.32 %		
		Rumex_acetosa	0.33 %		
		Taraxacum_officinale_agg.	15.65 %		
		Trifolium_rep_occi_nigr	10.16 %		
		Veronica_praecox	0.04 %		
		Veronica_chamaedrys	10.98 %		

The eDNA survey returned a reasonable number of species but likely short of the overall diversity in the field and doesn't give much idea of abundance. However, it also should be remembered that the survey just focussed on one 30mx30m area of the field and was generally away from field edges were certain other species may be located so a full species list is not realistic.

The survey recorded 5/6 Indicator Species, (It can't separate Creeping and Bulbous Buttercup, the later being an Indicator). This wouldn't be enough to recognise the site as a Local Wildlife Site, however it would give a good idea of potential value and warrant further survey work.

## 4.0 CONCLUSION

Both the Fungi and Flora eDNA has been very useful.

The Fungi eDNA has been demonstrated to be a very powerful tool, producing high quality data, and in excess of that which could be achieved by more Traditional Surveys, despite only focussing on 30mx30m areas of a site. It has the added bonus of being able to be undertaken throughout the year rather than a very narrow survey window. The results returned were sufficient to identify the fungal value of all sites and in many cases to a sufficient level that assessments could be made of whether they were considered Local Wildlife Site quality.

The Flora eDNA was not as strong in comparison with Traditionnal Survey methods, however it was still in many cases to a sufficient level that assessments could be made of whether they were considered Local Wildlife Site quality, or at least flag up potential value.

The sending of individual site data to all the Landowners has been of great benefit in enthusing the landowners, making them aware of the sites ecological value and providing management advice.