

FINAL REPORT

eDNA analysis of fungal populations in waxcap fungi from soil samples collected at Severalls Hospital site before and after sward translocation.

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Introduction

The former Severalls Hospital site (Fig. 1A) has been redeveloped for housing and in addition to demolition of the former hospital buildings, and house construction; this also requires landscaping of the site. During ecological surveys, several of the grassland areas were found to contain diverse populations of waxcap fungi. Surveys by Ian Rose in 2001 and more recently by Tony Boniface (three visits in autumn 2015) identified 12 grassland areas with waxcaps present (Table 1). Based on these earlier surveys, several of these grassland areas were home to moderately diverse waxcap populations (6-8 spp.; areas 1,3,5,8,11), whereas less diverse populations were recorded in the other areas (1-5 spp.). These areas are shown in Fig. 1B.

In an attempt to mitigate the destruction of the waxcap populations in the grasslands at the Severalls site, it was decided to explore the possibility that large turves (ca. 100W x 100L x 30D cm) can be removed from the five more promising areas, stored off-site in an adjacent field until the landscaping is complete and then repositioned in the reprofiled grassland areas. This the first rigorous attempt to determine whether the translocation of waxcap fungi is possible and as such is potentially of wider significance for mitigation of loss of important waxcap population and re-establishment of waxcap populations in areas of restored grassland.

Translocation of soil fungi has not previously been tested but the use of DNA-based methodologies allows much more sensitive quantification of changes in the relative biomass of different species that was previously possible by fruitbody surveys. One informal attempt in 2002 (Griffith et al., 2004), using 30x30x30cm turves, was not successful, as judged by the absence of any subsequent fruiting by the transplanted species (*Porpolomopsis calyptriformis*). Griffith et al. (2014) translocated 2x2 m turves (3 cm deep) from waxcap fairy rings but no subsequent fruiting was observed on either the recipient or donor areas. Similar approaches have also been tested for orchids on lawns (e.g. at the National Trust property Tyntesfield House in Somerset, where turves covering a ca. 40 m² area (5-10 cm depth) were temporarily translocated in an attempt to preserve a population of *Spiranthes spiralis* (Autumn ladies tresses). However, these orchids did not survive the translocation process (John Bailey, pers. comm., 2017).

More recently Wright (2015) suggested that waxcap population could be translocated by placing excised basidiocarps over the recipient site; however, there is no evidence that this would work and theoretical considerations (mainly relating to the very low amount of inoculum that would be transferred) would suggest that it is highly unlikely to be effective. However, eDNA analysis of the donor and recipient sites at Heysham (as described by Wright) found no evidence of successful translocation of species by this method (Griffith, unpublished report, 2018).

The aim of this study is to determine whether the translocation of larger turves may allow survival of waxcap mycelia in the soil and permit their recolonisation of the re-profiled soil following replacement at the redeveloped Severalls site. The first stage of this process, was the identification of the fungi initially present in the turves prior to their translocation to provide a baseline study of fungal populations in the areas of soil earmarked for

translocation, as reported by Griffith and Detheridge (Griffith and Detheridge, 2017). This was done via DNA metabarcoding of soil fungal populations, using a methodology we have devised at Aberystwyth University. Use of DNA metabarcoding to monitor fungal populations provides a rigorous method for determining mycelial survival (and to monitor changes in wider fungal populations) at the various stages of this process. **This report provides an update to the original report with an analysis of the fungi detected in the translocated swards to determine whether the waxcaps have survived the *ex-situ* storage and subsequent relocation on the Severalls site.**

Timeline and methods summary

Stage 1

The original site sampling, prior to turf removal, was undertaken on Monday 10th October 2016 by Dr. Andrew Detheridge, with assistance from Ms. Ella Barnett (now Gibbs) from SES Ltd.. Within each of the five grassland areas, sub areas had been fenced off (Fig. 2) to protect them from construction work in surrounding areas. Six 1x1 m quadrat were marked out (with bamboo stakes, each numbered to identify the quadrat) across the fenced off grassland patches (Fig. 2). These are the turves to be translocated. No waxcaps were present at the time. After marking, quadrat areas were logged with GPS (Fig. 3).

Soil sampling, Oct 2016: For each 1 m² quadrat, nine soil cores (15mm diameter x 10 cm deep) were collected on a 3x3 grid pattern and pooled in a sample bag. Thus 30 soil samples in all were taken, each comprising 9 pooled cores from a single 1 m² quadrat). Cores were stored cold (4°C) during transport back to Aberystwyth and frozen at -80°C. The soil was freeze-dried, homogenised by grinding through a 0.5 mm mesh sieve and then thoroughly mixed before storage at -80°C. DNA was extracted from a 250 mg sub-sample and the PCR amplification conducted using bespoke PCR primers specific to the large (28S) subunit of the rRNA operon (one of the DNA barcoding loci for Fungi). Next Generation sequencing was conducted using an Ion Torrent PGM sequencer, as described by Detheridge et al. (2016; 2018; 2020).

Stage 2

In spring 2017 turves were removed to an adjacent cricket pitch to the south of the main site (Figs. 3, 7) where they were stored until December 2019. Total of 25x 1m² turves were moved and turves from same original area were pushed together (Fig. 7a). It was necessary to move the turves to a second area (Fig. 7B) within the storage site (in January 2018) to make way for other activities. During this process the labelling (red flags) on the turves was lost and there was some loss of integrity of the original monoliths. During the storage period, turves were strimmed annually but otherwise not managed. Occasional waxcaps seen during autumn visits in 2017/2018.

Stage 3

In December 2019, the turves were moved a third time to their final location in the western edge of the site (Fig. 8). The receptor site was an area of undisturbed grassland, partly wooded, where no waxcaps had been observed during earlier surveys (Fig. 9). The turves (ca. 1m² area, 15-20cm deep) were placed into pre-excavated holes, in 8 areas, all 15-20m apart. However, during storage the labelling of the original sampling quadrats could not be identified and some of the turves had broken into smaller fragments, so it is not possible to say which of the original quadrats were translocated.

A total of 30 quadrats (each 1m²) were sampled in October 2016. However, only 8 (composite) turves (each 1m²) were translocated to the recipient sites (Fig. 10). Therefore,

only about a quarter of the originally surveyed soil was relocated so it would be expected that not all of the CHEGD fungi originally identified would be present in the translocated turves.

Soil sampling Oct 2020: On Wed 4th November 2020, approximately 11 months after translocation, 9 cores were taken in a grid at a distance of 40cm apart, across the inserted turves and pooled (Fig. 11). Nine cores were taken in a circle centred on the inserted turf but 2m away from its edge and again pooled. This gave a total of 16 samples in pairs. The soil was stored cold until Fri 6th November when it was transported via courier to Aberystwyth (arrived Mon 9th), frozen and was processed as per the original sampling.

Results

1) eDNA analysis of original turves (Oct16-Apr17)

A total of ca. 160 million basepairs (bp) of sequence data were obtained during the sequence run, corresponding to 650,772 separate DNA barcodes (each barcode is ca. 215 bp long). The raw data from the sequencing run of the sample were first subjected to quality control, removal of incomplete/chimeric reads and removal of unique sequences (potentially the result of sequencing errors). Sequences were then clustered at 97% identity into operational taxonomic units (OTUs), with OTUs containing ≤ 2 sequences removed. Following clustering there remained a total of 576,127 sequences from the 30 quadrat samples analysed (mean 19204 [range 8915-34283] per quadrat). Of these 96.4% were fungal sequences, the remainder comprising Rhizarians (eg. *Cercozoa/Bodonomorpha* spp.; flagellate/amoeboid protozoa) or Oomycetes (eg. *Peronospora/Pythium*; pseudofungi). Some non-fungal sequences (42-46%) were not identifiable at any taxonomic level, since our reference sample database is focused on Fungi so other eukaryotes are not as well represented.

The fungal sequences from the 30 samples were classified into 1177 OTUs with the number of OTUs in each sample ranging from 85 to 397 (Table. 2). An OTU, based on DNA sequence data (>97% identical to other members of the same OTU), is broadly equivalent to a genus or subgenus. For different fungal groups the genetic similarity (ie % sequence identity) between sister species or genera is variable, so the method used here can provide species level discrimination for some groups (eg. waxcaps/Hygrophoraceae) but only genus level discrimination for others (eg. bracket fungi/Polyporales). However, relative to other loci used for similar metabarcoding (eg ITS locus), the approach used here (D1 region of the LSU locus) is better at providing genus/family/order level identification for less characterised taxa, where reference DNA barcodes are absent.

Only 100,000 of the estimated 1.5 million fungal species have hitherto been named (and many named species do not yet have defined DNA barcode). Therefore, one would not expect to identify all fungal sequences. The bioinformatics 'pipeline' which we have designed in order to conduct quality control, clustering and identification of sequences, will designate such unidentified sequence clusters as "OTU[number]". For example, the 20th most abundant taxon overall across all quadrats (mean abundance 1.2%; present in 9/30 quadrats) was identified only to class level and is thus designated Agaricomycetes OTU30 (Table 2). We undertook additional analyses of this sequence and found it to only distantly related to any known (barcoded) fungi, and probably a member of the poorly studied order Trechisporales which are common in temperate soils; the most similar sequences to this (also not linked to any named species) were obtained from forest soils in the USA, so this probably represents a hitherto undiscovered species. Despite the occurrence of unidentified OTUs, >83% of sequences were identified to genus level and >90% to family level. The most abundant 100 taxa accounted for 98.7% of all the sequences.

The more reads per OTU, the more abundant is that particular fungal taxon in the sample. We usually express this as a percentage of the total fungal reads for each sample - the data in [Table 2](#) are ranked in order of decreasing abundance. A total of 22 fungal taxa were present at a mean abundance of >1% per quadrat and 14 of these taxa were CHEG fungi. CHEG fungi together accounted for 51.3% (mostly Hygrophoraceae [waxcaps] and Clavariaceae [fairy clubs]) of all fungal sequences across all samples and were thus the most abundant in all samples. However, there was a high level of variability between quadrats, with waxcaps accounting for 90.7% of all fungal sequences in one quadrat (11-6) but absent in two others (5-5, 5-6); and mean abundance of 24.2% overall. It is important to note that the data presented here are relative abundances.

The data presented here allows identification of which fungi are present and their rank abundance in each quadrat (but not absolute biomass). However, the total amount of fungal DNA present and also total fungal biomass, is likely to vary across quadrats. In order to obtain data for absolute amounts of fungal biomass per quadrat, it would be necessary to quantify total fungal biomass (e.g. using a fungal –specific biomarker such as ergosterol). Then the relative abundance data could be converted to absolute biomass data.

Our initial analyses across all fungi, clustered the sequences into generic or subgeneric groupings. More detailed analysis of the CHEG fungi allows species level identification. In [Table 3](#), the data are presented as numbers of sequences rather than relative percentages. The number of reads (=sequences) per OTU for CHEG species in each samples ranged from 3 (the minimal number, since we discarded smaller clusters during quality control) to 26,610 (*Gliophorus laetus* in quadrat 11-6; [Table 3](#)).

The most abundant OTU was *G. laetus* (77% of all reads in sample Q11-6) this species was highly localised in its distribution, mostly in one quadrat. The most common CHEG species across all 30 quadrats was *Calvulinopsis helvola* (mean abundance of 9% of all reads; present in 27/30 quadrats). The second most abundant waxcap (after *Gliophorus laetus*) was *Hygrocybe glutinipes* (mean abundance of 4.6% of all reads; present in 13/30 quadrats) ([Table 2](#)).

It is possible to analyse the fungal communities present in each quadrat in order to determine whether different fungal communities occur in different areas of the Severalls sites. Various methods are used in plant or microbial ecology (for instance DCA: detrended correspondence analysis), and we have found NMDS (Non-metric multidimensional scaling) to be the most suitable for comparison on fungal populations. Comparison of the fungal populations present in the 30 quadrats did not indicate any very strong differentiation of quadrats from any particular area. However, fungal populations in area 11 were somewhat divergent from this in other areas of the site, mostly likely due to the abundance of *G. laetus* and *Cuphophyllus pratensis* in this area (both rare/absent elsewhere). Also slightly divergent from other samples was a cluster of samples which contained large amounts of DNA from ectomycorrhizal fungi ([Fig. 4](#); blue polygon). It is likely that these quadrats were located close to trees with whose roots these fungi are associated.

[Table 3](#) shows which CHEG species are present in which quadrats, with summary data for numbers of CHEG spp. and also the numbers of waxcap spp. for each quadrat and also for each area. Care must be taken in interpreting these data, since some species are only represented by small numbers of sequences and this likely to represent only very small mycelial systems. It is unlikely that small mycelial systems would contain enough biomass to form fruitbodies. However, since a conservative approach has already been taken in determining whether a particular species is present (ie the occurrence of >4 corresponding identical DNA barcode sequences per sample), we are confident that the species are present

albeit in potentially small amounts. It is also important to note that the distribution of fungal mycelia is spatially very heterogeneous and that the nine cores taken per quadrat is only a small proportion of the total soil present <0.2%; ie. <20 cm² of each 1m² quadrat).

2) eDNA analysis of translocated turves and surrounding receptor area (Oct20-Jun21)

Sequencing of environmental DNA from these 16 samples resulted in a total of 67989 fungal sequences with an average of 4249 sequences per sample (minimum: 3174 maximum 6225). Sequence data were processed as summarised above and the data from the original sampling and the current data were combined so that they could be compared.

Substantial changes in the mean abundance of the main phyla in the sequence data were observed (Fig. 5; Table 5). In the original samples, Basidiomycota, the phylum to which most CHEGD fungi apart from earth tongues belong, was dominant, with mean relative abundance of 77%. The other major group of fungi typically found in soils is phylum Ascomycota which was present at a mean relative abundance of 16%. Such a pattern is typical of 'waxcap' grasslands, with two dominant groups within Basidiomycota being Hygrophoraceae (waxcaps) and Clavariaceae (fairy clubs). However, in samples from 2020, a much lower relative abundance of Basidiomycota was observed. In the both translocated turves (37% B vs. 51% A) and surrounding receptor soils (44% B vs. 44% A) Ascomycota showed higher relative abundance. Mean relative abundance of CHEGD fungi was correspondingly reduced from 54% to 10% (7.6% in inserted turves and 12.6% in receiving turves).

Principal coordinate ordination of both sets of data (Fig. 6) in which most of the original samples ordinated separately from the translocated (IN) samples, suggests that populations of fungi present had changed considerably from the original (pre-translocation) condition. The inserted turves and the receiving turves do not cluster separately suggesting that some colonisation of the inserted turves by fungi in the surrounding receiver area has occurred.

The relative abundance of CHEGD species in the translocated turves was low, ranging from identified 0.9% to 19.6% (mean 6.4%) relative abundance compared to 3.7-94.4% (mean 51.5%) for the original turves pre-translocation (Table 6). For four of the translocated turf locations, CHEGD fungi were more abundant in the surrounding receiver soil (mean 11.3%; range 0.04%-39.3%) than in the translocated turves.

Of the 12 original Hygrophoraceae (Waxcap) species, nine were detected in the inserted turves (*H. chlorophana*, *H. coccinea*, *H. pseudoconica* were absent), and one species not previously detected in the 2016 sampling (*Gliophorus irrigatus*) but reported by Rose from a fruitbody survey in 2001 (Table 1) was found (turf 216I). However, in all cases the numbers of sequences were low (Table 6), with relative abundance of the CHEGD species across all 8 translocated turves being at least 10-fold lower than the average for the original turves prior to translocation. Three waxcap species detected in the receiving soil, in moderate abundance (>250 sequences) across the translocated turves (*Cuphophyllus pratensis*, *Hygrocybe acutoconica*, *Hygrocybe conica*) but for other species, the number of sequences for the waxcap species was very low (<10sequences =ca. 0.05% relative abundance), and it is possible that this represents residual DNA from dead fungal hyphae rather than actual living mycelium. The exception was *H. conica*, found in all the translocated turves. However, *H. conica* is widely recognised as a pioneer species amongst waxcaps, so may potentially have recently colonised the turves.

In a third of cases (10/29), waxcap species present both in either the translocated or adjacent receiving soils were present in both (Table 5), so it is possible that colonisation one way or the other had occurred in the 11 months since turf insertion. Six species were present in translocated turves and the original soils but not detected in receiver soils

(*Cuphophyllus pratensis*, *Gliophorus irrigatus*, *Gliophorus laetus*, *Gliophorus psittacinus*, *Hygrocybe ceracea*, *Hygrocybe glutinipes*), though often at low sequence abundance and it is more likely that these illustrate survival of the translocation process (as opposed to in-growth from the receiving soils).

Of the other CHEGD fungi (except Entolomataceae [pink gills], where there is great taxonomic uncertainty and poor coverage by reliable DNA barcodes), most of the Clavariaceae (16/21 spp.) and all the Geoglossaceae detected in the original soils were found in translocated turves but as with waxcaps at much lower relative abundance (only *Hodophilus foetens* found with >100 sequences). Two species (*Trichoglossum hirsutum*, *Dermoloma cuneifolium*) were detected in translocated turves but not in the original soils. Since *D. cuneifolium* was found in two receiving soil samples, it is likely that the translocated turves were colonised during 2020 after final relocation of the turves.

The general decrease in abundance of CHEGD fungi in the translocated turves is clear, as is the increase in relative abundance of Ascomycota. However, without quantification of fungal biomass (eg. via ergosterol analysis; (Detheridge et al., 2018)), it is not possible to determine whether other species have expanded to fill the 'gap' left by the declining CHEGD fungi or whether there has been a general decline in fungal biomass. Perusal of the most abundant species (Table 6) does not reveal any consistent pattern across the translocated turves but it was noteworthy that a *Mycena* sp., and *Typhula* sp., often associated with decaying roots and litter, was present at high abundance in turves 216I and 218I respectively.

With regard to the receiving soils, some samples contained high levels of wood decay fungi, for example *Gymnopus*, *Marasmius*, *Parasola*, *Coprinellus* in 221O and *Endoperplexa*, *Calyptella* in 220O. This suggests the presence of buried woody debris or roots, consistent with the presence nearby of trees. Similarly, ectomycorrhizal fungi (*Inocybe*, *Cortinarius*, *Scleroderma*, Thelephoraceae) were abundant in 218O suggesting that living tree roots were present in the soil. The presence of *Microdochium* and *Limonomyces*, found on senescent grass tissues was observed in 214O and 220O.

Conclusion

Waxcaps and other fungi characteristically found in undisturbed grasslands dominated the fungal populations in all five donor sites. The numbers of waxcap species present in each area was similar to the numbers found in earlier fruitbody surveys and there was good (but not exact) overlap in the species detected and where they were found. This is to be expected since the quadrats themselves comprised only a small part of each area so it would be expected that some species would be missed (since their mycelia would not be present in the quadrat). This likely explains the instances where waxcap species were found in surveys but not in the DNA (red cells in Table 4).

Of the three species found in DNA but not by fruitbody surveys, two of these (*Hygrocybe acutoconica* [formerly *H. persistens*] and *H. pseudoconica*) were likely missed because they are morphologically very similar to *H. conica*, and often fruit rather early in the season (July/August). From the perspective of the translocation experiment, the original sampling provided baseline data as to which CHEG (and other) fungi are present in the turves. From this set of baseline data, we were able to determine whether the abundance of waxcap mycelia was altered by the translocation.

The difference in the community structure between the original samples of the turves in situ and after translocation (Fig. 5) indicates that the fungal population in the turves has changed considerably, most likely during the 30 months of storage. There was evidence of invasion of

translocated cores by species absent from the donor site but only limited evidence of possibly spread of the CHEGD fungi into the recipient soils.

Many of the original CHEG species have disappeared or exist at very low sequence abundance. It is not possible to exclude the possibility that the eDNA detected was from relict (i.e. dead) mycelia. Overall, the sequence data indicate that the translocation has not been successful in re-establishing the original populations of CHEGD fungi in the receiving location. Longer term sampling (e.g. resampling in late 2023/24) would determine whether the CHEGD fungi still present in late 2020 will recover or are in terminal decline.

Recommendations for future translocation trials

This translocation experiment met with limited success, in part due to its complexity. It is likely that the survival of CHEGD fungi would have been greater had the donor turves been moved directly to the final receiving site. The storage for 30 months at two storage sites exposed the turves to drought and physical disruption which would have negatively impacted on the CHEGD fungi which are biotrophic and rely on living host plants for nutrition. Ideally translocation should be undertaken with a single handling to minimise physical deterioration of the turves and the risk of excessive droughting/waterlogging in storage.

With regard to timing, translocation in winter (as was done) when plants and soil organisms are less active would reduce disturbance, although the higher water content of soils in winter and problems of heavy machinery moving over sensitive soils, risks logistical problems. Late spring, before the growing season is in full swing, would be preferable to autumn when the grassland fungi are fruiting.

The ultimate destination of the turves was undisturbed ('retained') grassland with nearby trees. It is possible that fungi already present in receiver soils might outcompete the fungi in the turves at such locations. In hindsight, translocation into newly created (reprofiled) grassland might have been preferable, if possible, but in this case it is unlikely that this would have made a difference.

The most widely deployed wildlife translocations in Europe are for amphibians and reptiles and in many cases these fail or the translocated population shows continued decline (Edgar et al., 2005). However, translocations of larger populations are found to be more successful (Germano and Bishop, 2009). Analogous to this in the case of grassland fungi would be the translocation of larger soil areas, attempting where possible to avoid break-up of the monoliths in transit. Specialised earthmoving equipment capable of moving 2x2x0.25 m monoliths intact likely do exist but are not regularly to hand on building sites. However, diggers equipped with a wide scoop should permit the moving on 1.5x1m turves would be more readily available but the operator would likely need some training /practice (e.g. when creating the holes at the receiver site) in order to undertake the delicate turf transfer operation effectively.

From a monitoring perspective, eDNA analysis should be ideally conducted immediately before the translocation from the donor site and include sampling of the removed monoliths at the receiver area, as well as the areas surrounding the receiver site. Translocation inevitably causes disturbance to the organisms involved and whilst changes in fungal populations can be sensitively monitored using eDNA metabarcoding, it would also be informative to monitor changes in total fungal biomass and also in vegetation over the months/years following translocation.

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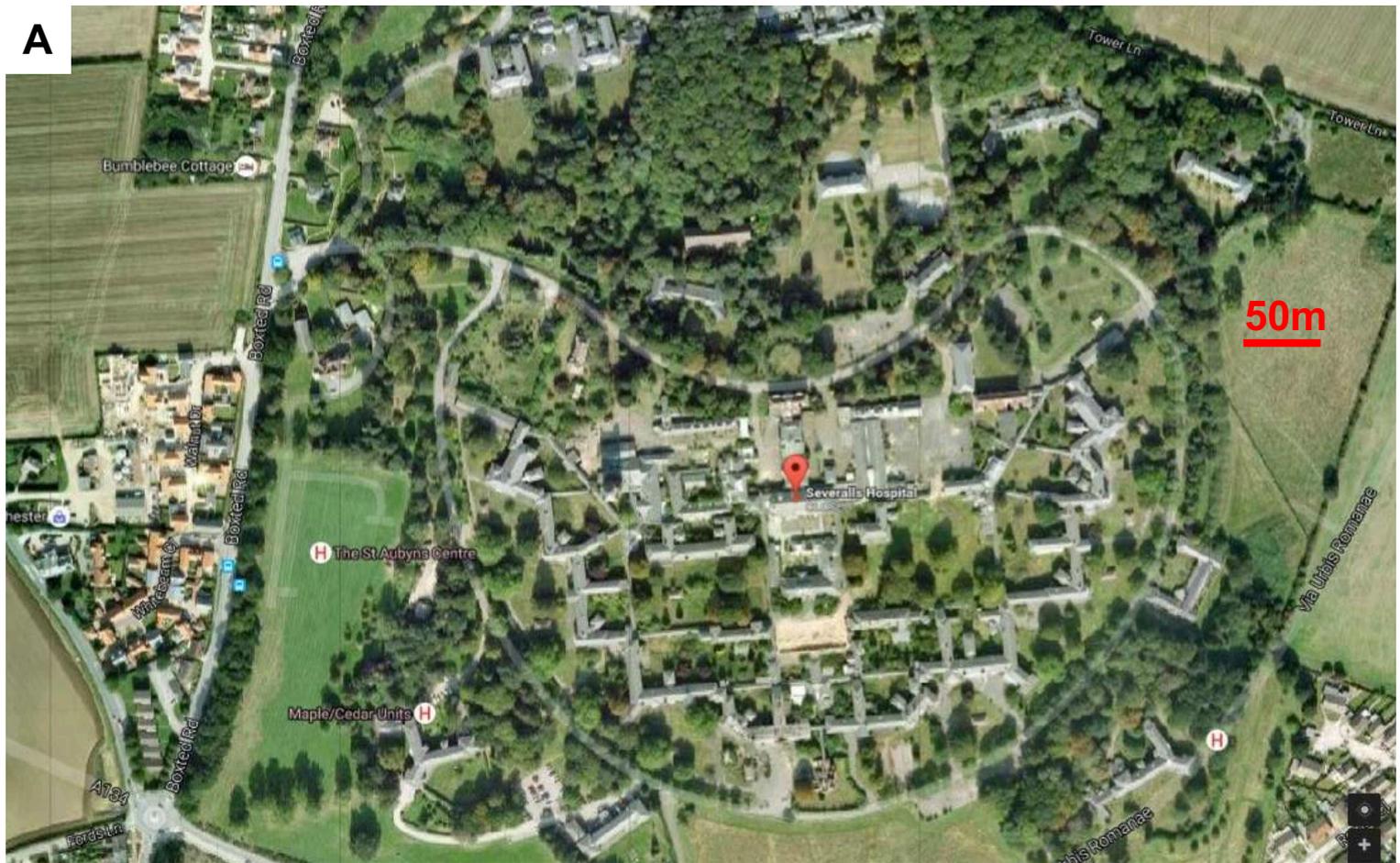


Fig. 1. **A.** Aerial photograph (Googlemaps) of the Severalls site. **B.** Map of the Severalls site indicating the grassland areas with no waxcaps (**blue**), 1-5 waxcap species (**light pink**) or 6-10 waxcap species (**bright pink**) based on survey data from Tony Boniface in 2015.

Table 1. Summary of the waxcap survey undertaken by Tony Boniface and Lucy Addison (29th Oct/4th Nov/12th Nov 2015) indicating which species were found in which areas. Three species are highlighted in pink, indicating those found during an earlier survey by Ian Rose (Andrew Martin Associates) in 2001 but not by Boniface & Addison; the exact location of these was not reported. Those highlighted in blue indicate three species found by Boniface but not by Rose. Of the 12 sub-areas defined by Boniface & Addison, the five most species-rich are highlighted in green and these were the areas studied during the current investigation. Note that current Latin names are used here (specific names are unchanged, but we now know that 'waxcaps' fall into 6 different genera within the family Hygrophoraceae. Note also that *C. berkeleyi* is now considered a separate species (no longer a subspecies of *C. pratensis*). Only Latin names are used here since use of English names is prone to cause confusion.

SPECIES	Three Gardens 1	Driveway 2	Behind Birchwood 3	Garden 4	Garden 5	Garden 6	Garden 7	Myland Court 8	Courtyard 9	Garden bh. Larch House 10	Behind Medway 11	Near the Church 12	TOTAL
2 <i>Cuphophyllus berkeleyi</i>										1			1
3 <i>Cuphophyllus fornicatus</i>							1				1		2
4 <i>Cuphophyllus pratensis</i>	1	1	1		1	1	1	1		1	1		9
1 <i>Cuphophyllus russocoriaceus</i>	Recorded by Rose (2001) but no location given												1
5 <i>Cuphophyllus virgineus</i>	1	1	1		1	1	1	1	1		1		9
6 <i>Gliophorus irrigatus</i>	1		1					1					3
7 <i>Gliophorus psittacinus</i>	1		1		1	1	1				1		6
8 <i>Hygrocybe ceracea</i>	1		1	1	1			1	1		1	1	8
9 <i>Hygrocybe chlorophana</i>			1		1			1	1		1	1	6
10 <i>Hygrocybe coccinea</i>	Recorded by Rose (2001) but no location given												1
11 <i>Hygrocybe conica</i>	1		1			1		1					4
12 <i>Hygrocybe glutinipes</i>												1	1
13 <i>Hygrocybe insipida</i>					1								2
14 <i>Hygrocybe miniata</i>	Recorded by Rose (2001) but no location given												1
	6	2	8	1	6	4	4	6	3	2	6	3	

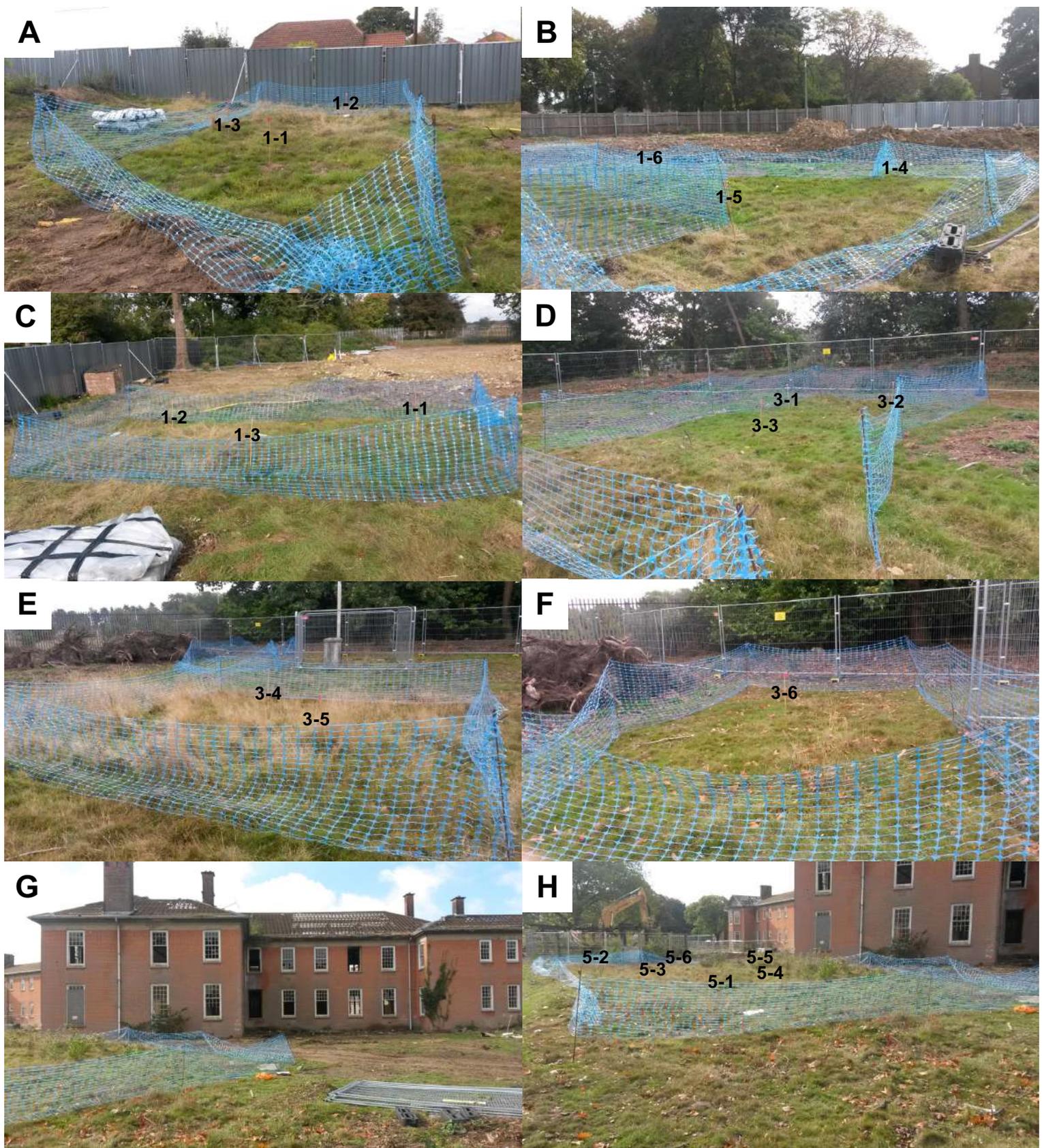


Fig. 2. Photographs of the five sampling areas. A,B,C: Area1(Three Gardens), D,E,F: Area 3 (Behind Birchwood) and G,H: Area 5 (Garden). Bamboo stakes with red flags indicate the quadrat locations

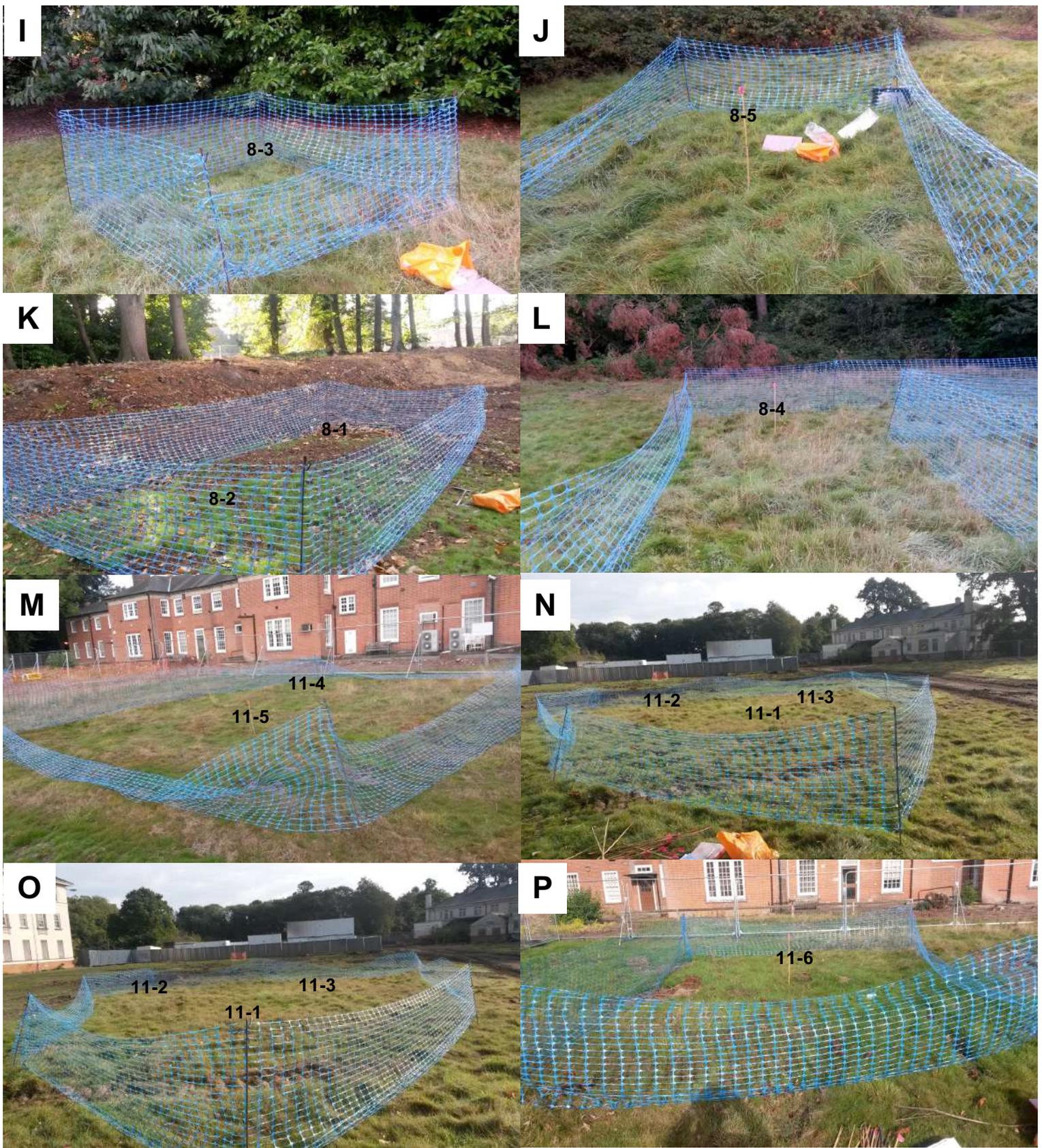


Fig. 2 (Continued). Photographs of the five sampling areas. I,J,K,L: Area 8 (Myland Court); M,N,O,P: Area 11 (Behind Medway). Bamboo stakes with red flags indicate the quadrat locations.

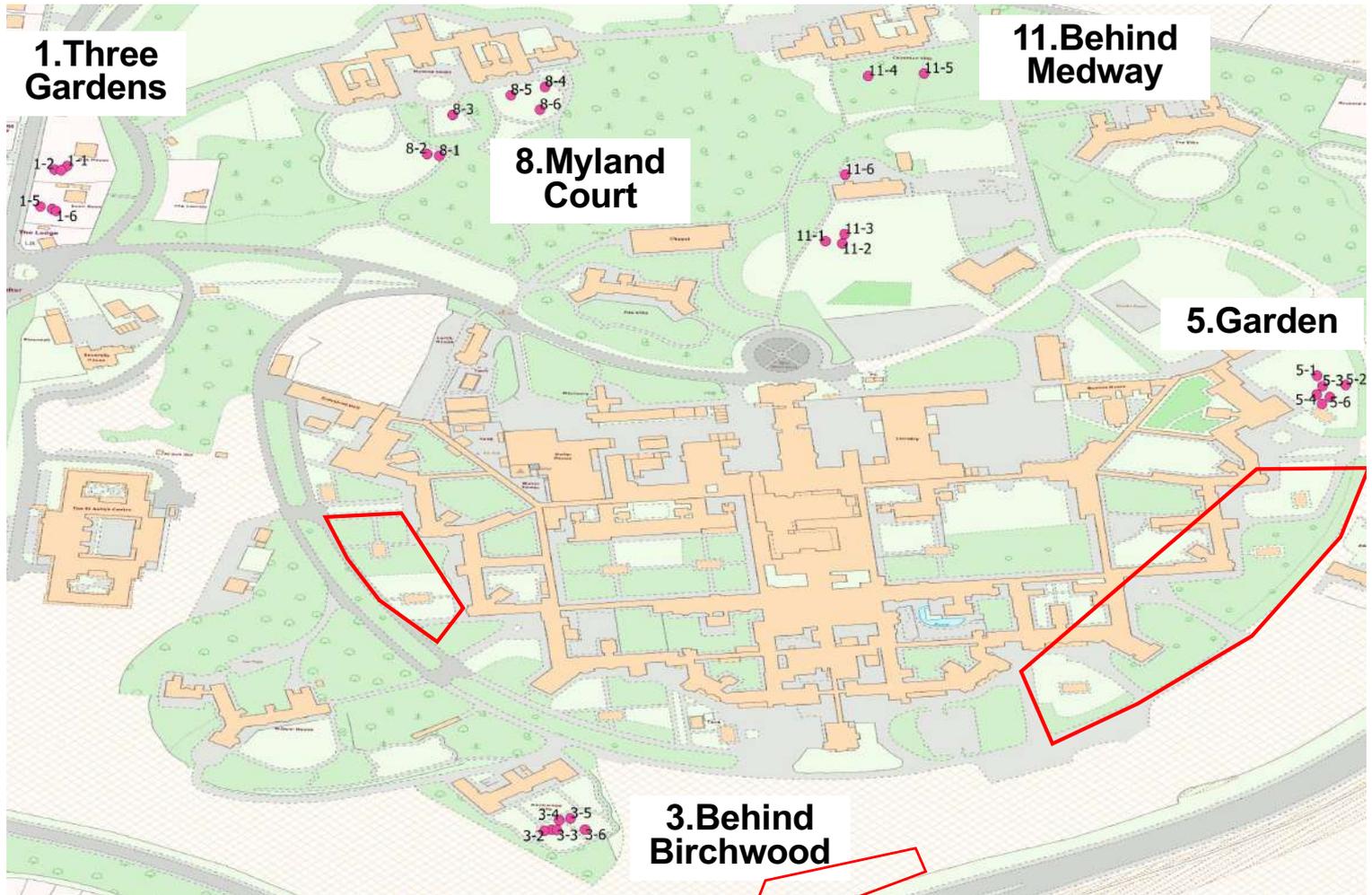


Fig. 3. GIS layer showing the locations of the original quadrats across the Severalls site. At the southern end is the approximate location of the storage sites and to the west the two final destination areas.

Table 3. Summary of the number of sequences of each CHEG species detected in DNA from each quadrat sampled at the Severalls site. Cells highlighted in pink show area totals (sum of sequences from each quadrat). Cells highlighted in green show the more abundant sequences (>100 sequences). Note that for Clavariaceae (fairy clubs), the molecular taxonomy of the family is less well studied than for Hygrophoraceae (waxcaps); as a result, several putative species shown here have not been formally named. At the bottom of the table the total number of CHEG and also waxcap species are shown for each quadrat/area.

Code	Species	Summary data -per area												No. Quads where present	Total No. Sequences																								
		Area1	Area3	Area5	Area8	Area11	Q1-1	Q1-2	Q1-3	Q1-4	Q1-5	Q1-6	Q3-1			Q3-2	Q3-3	Q3-4	Q3-5	Q3-6	Q5-1	Q5-2	Q5-3	Q5-4	Q5-5	Q5-6	Q8-1	Q8-2	Q8-3	Q8-4	Q8-5	Q8-6	Q11-1	Q11-2	Q11-3	Q11-4	Q11-5	Q11-6	
C1	Camarophyllopsis schulzeri	13546	8	140	15671	1664	12788	758	16	8	8	16	8	27	222	8	117	23	25	598	314	456	1296	276	174	935	400	623	7716	5997	12	5	1647	13	31029				
C2	Clavaria acuta	335	257	2965	476	1934	121	286	16	8	21	16	8	27	222	8	117	23	25	598	314	456	1296	276	174	935	400	623	7716	5997	12	5	1647	13	31029				
C3	Clavaria roseaFF	2487	304	2401	531	292	40	1079	48	1268	54	8	77	86	88	525	1351	274	60	66	11	160	226	8	56	131	13	41	51	27	8015								
C4	Clavaria straminea	40	19	206	213	47	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30		
C5	Clavaria sp. JMB10061001	47	7262	229	162	47	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30		
C6	Clavaria fragilis	47	7262	229	162	47	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30	5	7	10	30		
C7	Clavaria incarnata	27	8	116	30	75	16	5	6	6	17	5	10	79	564	17	5	10	79	564	17	5	10	79	564	17	5	10	79	564	17	5	10	79	564	17	5	10	
C8	Clavulimopsis sulcata	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	268	
C9	Clavaria guillermii	3369	11683	11496	24790	2924	337	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	
C10	Clavaria sp. BRACR16032	3286	1485	4291	55	25	337	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	
C11	Clavulimopsis corticulata	3369	11683	11496	24790	2924	337	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	
C12	Clavulimopsis luteoalba	266	81	372	574	1148	147	38	31	50	38	7	50	38	7	50	38	7	50	38	7	50	38	7	50	38	7	50	38	7	50	38	7	50	38	7	50	38	
C13	Clavulimopsis helvola	75	6	474	449	608	12	58	5	5	12	33	3942	1559	18	2493	5	762	446	15433	4899	3225	8	10	2025	2913	11	110	10	18	2441	15	1612	15	1612	15	1612		
C14	Clavulimopsis laeticolor	564	1206	1171	557	537	371	111	82	82	53	5	53	5	42	42	12	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
C15	Ramanopopsis sp. JMB-2011	22	100	29	233	366	6	7	9	53	53	5	53	5	42	42	12	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
C16	Ramanopopsis crocea	22	100	29	233	366	6	7	9	53	53	5	53	5	42	42	12	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
C17	Ramanopopsis kunzei	22	100	29	233	366	6	7	9	53	53	5	53	5	42	42	12	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
C18	Ramanopopsis pulchella	22	100	29	233	366	6	7	9	53	53	5	53	5	42	42	12	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6		
C19	Clavulimopsis corallinorosea	5	60	13204	60	13204	5	60	13204	5	60	13204	60	13204	60	13204	5	60	13204	60	13204	60	13204	60	13204	60	13204	60	13204	60	13204	60	13204	60	13204	60	13204	60	
H1	Cuphophyllus pratensis	149	96	169	2804	13	107	42	5	96	96	6	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421
H2	Cuphophyllus russocorticaceus	13	6	1421	7	37778	9	13	42	5	9	6	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421	169	1421
H3	Cuphophyllus virgineus	1135	11	536	8	12474	1135	2894	413	6	11	51	8116	3238	4891	95	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	
H4	Gliophorus laetus	3307	11	536	8	12474	1135	2894	413	6	11	51	8116	3238	4891	95	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538
H5	Gliophorus peltacinus	6	11405	4986	4761	13	2894	413	6	11	51	8116	3238	4891	95	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	538	
H6	Hygrocybe acitocoinca	3206	6455	36	55	5368	12	3074	58	62	6	12	1597	3315	1525	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	
H7	Hygrocybe ceratocina	17666	7	9557	4820	15	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
H8	Hygrocybe chlorophana	3672	1036	6459	17	1238	267	103	3196	8	267	103	3196	8	267	103	3196	8	267	103	3196	8	267	103	3196	8	267	103	3196	8	267	103	3196	8	267	103	3196	8	267
H9	Hygrocybe coccinea	300	260	309	83	49	37	56	207	30	37	56	207	30	37	56	207	30	37	56	207	30	37	56	207	30	37	56	207	30	37	56	207	30	37	56	207	30	
H10	Hygrocybe conica	421	24	154	20	8	128	19	53	221	43	59	117	41	219	82	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
H11	Hygrocybe glutipes	28023	128059	117173	142502	135015	23477	13330	11395	26709	24205	28907	19875	20932	25377	24995	16649	20131	20587	20018	17198	14870	26463	18037	15051	31475	9586	34430	31868	20092	14657	31021	21358	12267	18783	36929			
H12	Hygrocybe pseudocoinca	55704	54118	35122	67453	91176	17322	681	2374	20970	6199	8158	6406	2975	12177	15276	7658	9626	6306	8810	7522	4431	2798	5255	1224	992	6249	23987	25242	9759	7826	11965	16587	10933	12073	31792			
G1	Geoglossum fallax	44%	42%	30%	47%	68%	74%	5%	21%	79%	25%	28%	32%	14%	48%	61%	48%	31%	44%	44%	44%	30%	11%	29%	8%	3%	65%	70%	79%	49%	53%	39%	78%	89%	64%	86%			
G2	Geoglossum simile	26	22	23	25	28	7	9	12	14	11	9	5	10	10	11	16	4	12	8	8	7	12	18	9	17	16	18	9	11	6	4	2	4	2	4			
G3	Geoglossum glutinosum	46	42	42	42	42	46	42	42	42	46	42	42	42	46	42	42	42	46	42	42	42	46	42	42	42	46	42	42	42	46	42	42	42	46	42	42	46	
*G*4	Microglossum spp.	9	7	5	8	8	1	1	1	3	4	7	2	1	3	5	2	2	0	4	1	1	0	0	1	2	1	2	5	2	2	2	4	2	4	2	4		

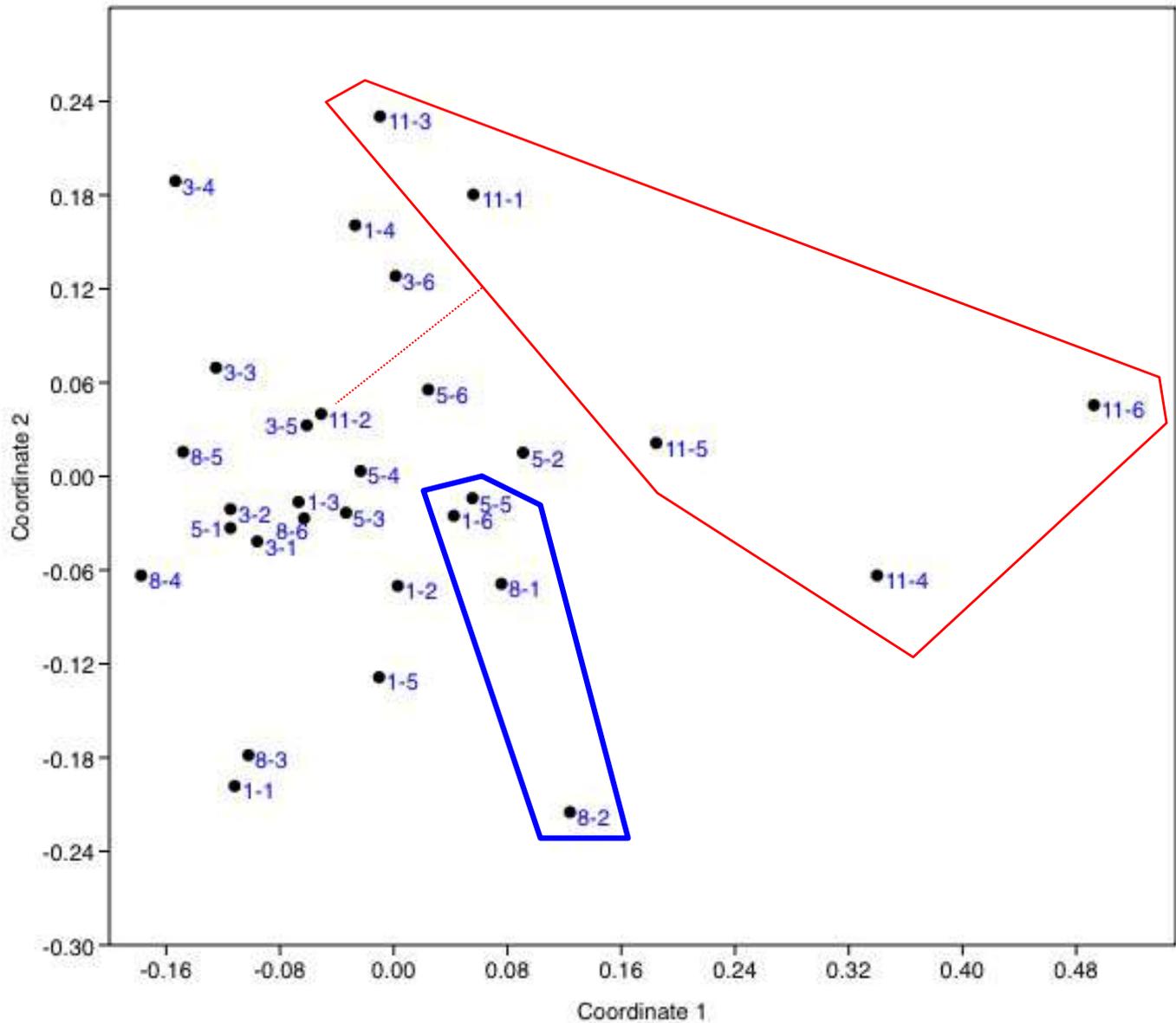


Fig. 4. Non-metric multidimensional scaling ordination of the fungal populations in the Severalls quadrats. The samples from area 11 (red polygon) form a broadly distinct cluster suggesting the presence of a distinctive fungal population (in large part dominated by the waxcap *G. laetus*). Four quadrats (blue polygon) contained large amounts (27-70%) of DNA from ectomycorrhizal basidiomycetes (*Laccaria*, *Lactarius*, *Russula*, *Thelephora*; Table 2). This suggests the nearby presence of suitable host trees (oak, beech, birch, conifers).

Table 4. Summary of the quadrats where waxcap species were detected, correlated with the results of earlier fruitbody surveys (Table 1). Columns highlighted in green indicate those areas sampled in the present investigation and the numbers in these cells show the number of sequences of each species detected.

Cells highlighted pink/red indicate where species were found in previous surveys, with red boxes surrounded by black line showing those areas where a particular species had been found during FB surveys but not detected in the DNA from any of the quadrats in that area.

DNA metabarcoding detected the presence of twelve waxcap species, including three species not previously recorded from Severalls (*Gliophorus laetus*, *Hygrocybe acutoconica*, *Hygrocybe pseudoconica*) whereas four species previously detected in the areas sampled here were not detected (*Cuphophyllus fornicatus*, *Gliophorus irrigatus*, *Hygrocybe insipida*, *Hygrocybe miniata*).

Waxcap Species	Three Gardens	Driveway	Behind Birchwood	Garden	Garden	Garden	Garden	Myland Court	Courtyard	10	Behind Medway	Near the Church	TOTAL
	1	2	3	4	5	6	7	8	9	10	11	12	
1 <i>Cuphophyllus berkleyi</i>	0	*	0	*	0	*	*	0	*	*	0	*	0
2 <i>Cuphophyllus fornicatus</i>	0	*	0	*	0	*	*	0	*	*	0	*	0
3 <i>Cuphophyllus pratensis</i>	5	*	0	*	0	*	*	60	*	*	13204	*	13269
4 <i>Cuphophyllus russocoriaceus</i>	149	*	96	*	169	*	*	2804	*	*	0	*	3218
5 <i>Cuphophyllus virgineus</i>	13	*	6	*	1421	*	*	0	*	*	0	*	1440
6 <i>Gliophorus irrigatus</i>	0	*	0	*	0	*	*	0	*	*	0	*	0
7 <i>Gliophorus laetus</i>	0	*	9	*	0	*	*	7	*	*	37778	*	37794
8 <i>Gliophorus psittacinus</i>	1135	*	0	*	0	*	*	0	*	*	135	*	1270
9 <i>Hygrocybe acutoconica</i>	3307	*	11	*	536	*	*	8	*	*	12474	*	16336
10 <i>Hygrocybe ceracea</i>	6	*	11405	*	4989	*	*	4761	*	*	13	*	21174
11 <i>Hygrocybe chlorophana</i>	0	*	0	*	0	*	*	4120	*	*	0	*	4120
12 <i>Hygrocybe coccinea</i>	0	*	0	*	36	*	*	0	*	*	0	*	36
13 <i>Hygrocybe conica</i>	3206	*	6455	*	3	*	*	55	*	*	5368	*	15087
14 <i>Hygrocybe glutinipes</i>	17666	*	14	*	0	*	*	4920	*	*	15	*	22615
15 <i>Hygrocybe insipida</i>	0	*	0	*	0	*	*	0	*	*	0	*	0
16 <i>Hygrocybe miniata</i>	0	*	0	*	0	*	*	0	*	*	0	*	0
17 <i>Hygrocybe pseudoconica</i>	7	*	9557	*	0	*	*	0	*	*	8	*	9572
	25494	*	27553	*	7154	*	*	16735	*	*	68995	*	145931

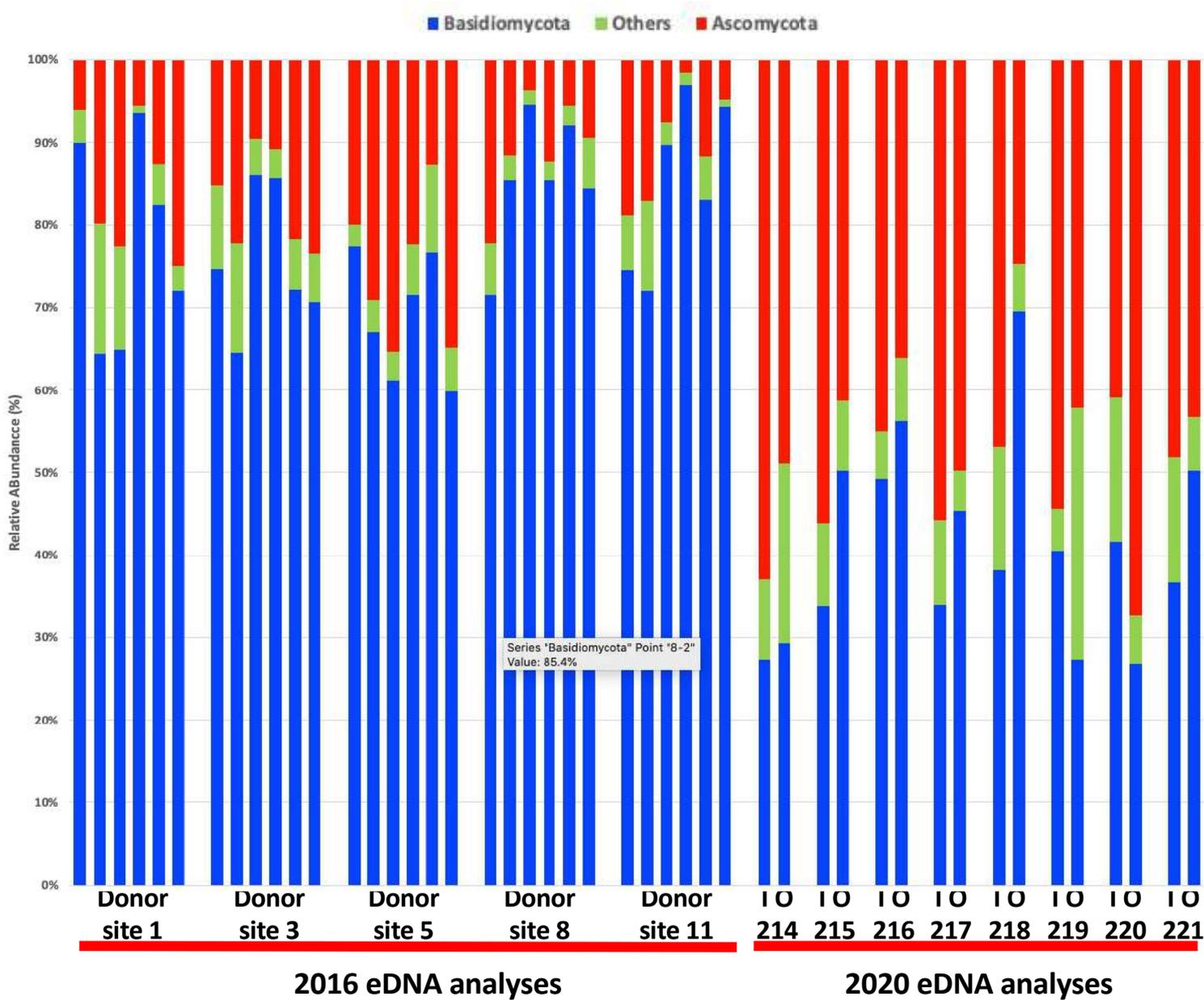


Fig. 5. Stacked barchart showing the relative abundance of Basidiomycota, Ascomycota and other fungal phyla in the soils samples from 2016 and 2020.

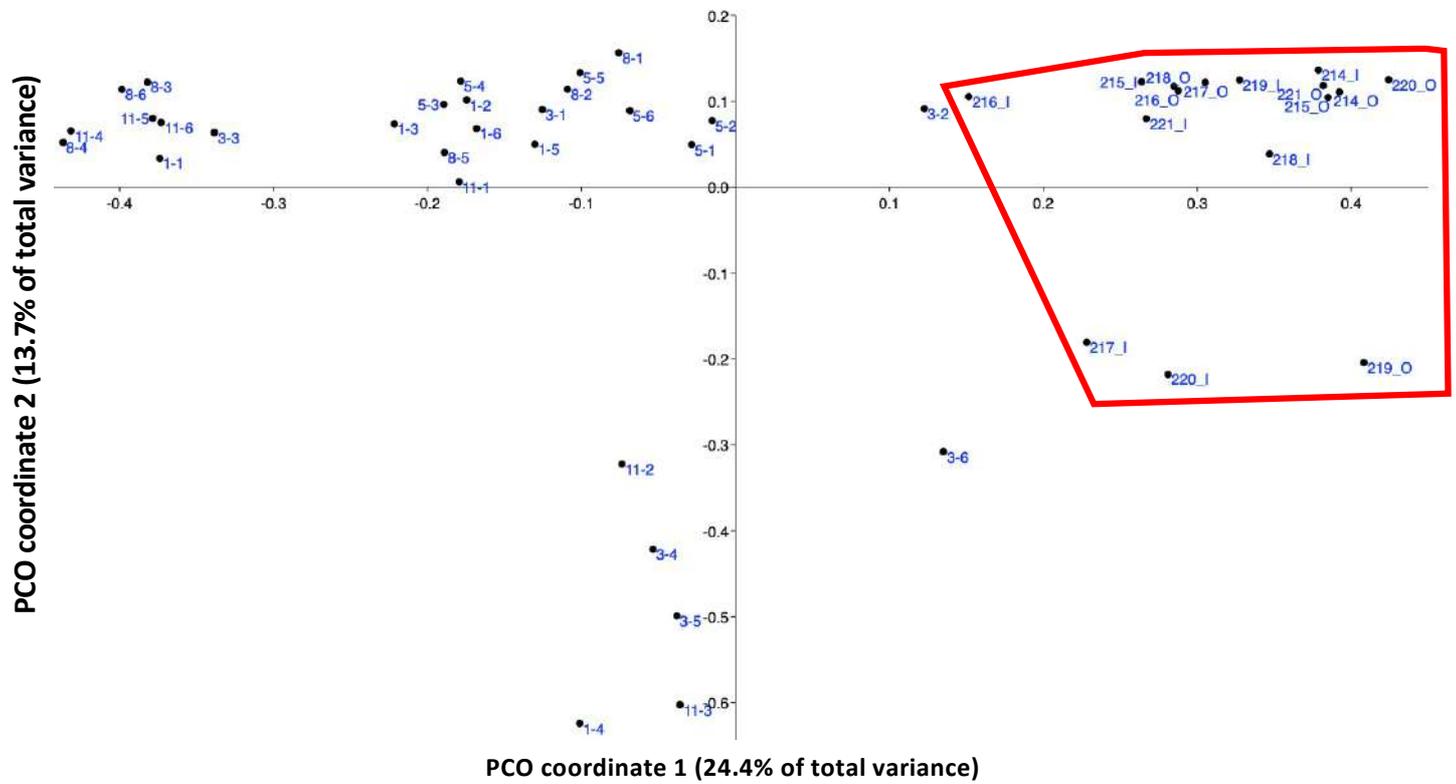


Fig. 6. Principal coordinate ordination of the new samples taken in 2020 (red polygon) after translocation and the original samples before translocation taken in 2016. The clear separation between the two sampling points indicates distinct fungal populations

	phylum	class	order	family	genus	Count	relative t	Mean	Max	Min	214	214.0	215	215.0	216	216.0	217	217.0	218	218.0	219	219.0	220	220.0	221	221.0	Ave2016	AveIN20	AveOUT20					
1	Fun	Basidiomycota	Agaricomycetes	Agaricales	Tricholomatales	Mycena	35	56.4%	1.23%	32.7%	0.01%	2.62%	0.00%	0.95%	0.00%	34.71%	0.17%	0.06%	0.47%	0.05%	0.00%	0.00%	0.00%	0.00%	0.18%	0.00%	0.65%	4.86%	0.08%					
2	Fun	Ascomycota	Dothideomycetes	Pleosporales	Pleosporales	Pyrenochaeta	43	40.1%	0.87%	7.0%	0.04%	2.25%	1.87%	1.39%	2.22%	0.31%	0.28%	1.15%	1.37%	0.84%	0.42%	0.27%	0.00%	0.00%	4.09%	2.16%	0.00%	0.18%	0.00%	0.28%	2.77%	1.74%		
3	Fun	Basidiomycota	Agaricomycetes	Trechsporales	Trechsporales	Trechspora	44	148.6%	3.23%	20.2%	0.04%	0.07%	0.00%	2.27%	1.06%	0.61%	0.58%	3.37%	1.67%	0.24%	0.50%	0.64%	0.68%	0.25%	0.00%	12.68%	1.24%	4.30%	2.52%	0.57%				
4	Fun	Ascomycota	Dothideomycetes	Capnodiales	Cladosporidales	Cladosporida	44	44.9%	0.98%	4.0%	0.01%	3.84%	3.87%	1.29%	2.09%	1.88%	2.79%	0.87%	2.52%	3.58%	2.37%	1.81%	1.46%	4.20%	2.39%	1.78%	0.30%	2.32%	2.68%	0.30%				
5	Fun	Basidiomycota	Dothideomycetes	Agaricales	Hydrophorales	Hydrophora	48	101.6%	2.21%	24.0%	0.01%	0.07%	0.00%	0.38%	0.54%	0.88%	0.00%	0.57%	1.76%	0.17%	0.52%	0.53%	0.67%	0.00%	0.18%	0.06%	2.80%	1.98%	0.84%	0.16%	1.78%	0.60%		
6	Fun	Ascomycota	Dothideomycetes	Dothiorales	Collemasphaerales	Collemasphaera	35	22.2%	0.48%	3.2%	0.01%	3.35%	3.59%	1.20%	0.98%	3.22%	1.32%	1.57%	0.61%	2.24%	0.34%	1.95%	0.24%	0.11%	0.33%	0.57%	0.37%	0.00%	1.78%	0.60%	0.00%	1.67%	0.52%	
7	Fun	Ascomycota	Leotiomycetes	Helotiales	Helotiales	OTU 151	12	16.1%	0.35%	6.6%	0.04%	1.94%	0.37%	0.19%	2.60%	0.98%	2.83%	0.27%	1.15%	0.85%	0.27%	0.00%	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	1.60%	1.67%	0.60%			
8	Fun	Ascomycota	Eurotiomycetes	Chaetothiales	Heptrichiales	OTU 30	46	33.2%	0.72%	3.8%	0.01%	1.15%	0.26%	1.64%	1.60%	2.78%	0.46%	3.56%	2.31%	0.72%	0.57%	1.07%	1.24%	1.01%	0.68%	0.48%	0.42%	1.00%	1.29%	0.61%	1.29%	0.61%		
9	Fun	Pseudofungi	Oomycetes	Saprolegniales	Saprolegniales	OTU 96	43	17.0%	0.37%	4.1%	0.01%	0.21%	0.13%	0.91%	0.34%	0.63%	0.50%	0.42%	0.10%	0.26%	0.38%	0.11%	0.34%	2.62%	0.06%	0.68%	0.00%	0.08%	1.53%	0.61%	0.72%	0.61%		
10	Fun	Basidiomycota	Tremellomycetes	Tremellales	Timporales	Saitozyma	45	17.4%	0.40%	2.9%	0.01%	2.86%	0.70%	1.76%	1.16%	1.15%	0.24%	0.53%	1.46%	1.44%	0.97%	1.75%	1.04%	0.70%	3.01%	0.62%	1.21%	0.00%	1.28%	1.22%	1.00%	1.51%	0.72%	
11	Fun	Ascomycota	Sordariomycetes	Sordariales	Lasiopeltiales	Schizothecium	21	18.4%	0.40%	2.9%	0.01%	2.86%	0.70%	1.76%	1.16%	1.15%	0.24%	0.53%	1.46%	1.44%	0.97%	1.75%	1.04%	0.70%	3.01%	0.62%	1.21%	0.00%	1.28%	1.22%	1.00%	1.51%	0.72%	
12	Fun	Basidiomycota	Tremellomycetes	Fibrosariales	Piskurozoriales	Solkocozyma	46	38.8%	0.84%	4.9%	0.04%	4.47%	2.67%	1.48%	1.68%	1.49%	4.34%	1.91%	2.13%	2.36%	0.91%	0.04%	1.59%	0.24%	1.75%	0.90%	0.42%	0.28%	0.69%	1.25%	1.26%	0.69%	1.25%	1.26%
13	Fun	Ascomycota	Leotiomycetes	Thelebolales	Thelebolales	Thelebolus	46	23.6%	0.51%	3.9%	0.01%	0.54%	0.37%	0.79%	0.49%	4.16%	1.71%	1.53%	0.35%	0.15%	0.43%	0.89%	0.49%	1.01%	0.65%	0.30%	1.24%	0.78%	0.30%	1.24%	0.78%	0.30%	1.24%	0.78%
14	Fun	Ascomycota	Eurotiomycetes	Eurotiales	Trechcospora	Penicillium	45	27.3%	0.59%	3.1%	0.02%	0.84%	0.39%	2.21%	0.21%	0.70%	0.61%	3.42%	0.92%	0.24%	0.11%	0.23%	0.74%	0.68%	0.47%	0.47%	0.54%	0.54%	1.10%	0.50%	0.54%	1.10%	0.50%	
15	Fun	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriales	Sarcosotoma	9	9.0%	0.20%	3.7%	0.11%	0.12%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.10%	0.16%	0.16%	1.10%	0.16%	
16	Fun	Basidiomycota	Agaricomycetes	Agaricales	Clavariales	Hedophium	3	7.4%	0.16%	7.2%	0.08%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	1.06%	0.03%	0.03%	1.06%	0.03%	
17	Fun	Ascomycota	Leotiomycetes	Helotiales	Helotiales	Cudoniella	46	28.6%	0.62%	2.2%	0.03%	2.30%	0.59%	0.69%	0.18%	0.20%	0.74%	1.04%	0.08%	0.76%	0.46%	1.86%	0.46%	0.76%	0.51%	0.91%	1.38%	0.56%	1.05%	0.62%	0.56%	1.05%	0.62%	
18	Fun	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasiales	Thamnetophonus	13	14.4%	0.31%	6.9%	0.01%	0.80%	0.05%	0.13%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	1.03%	1.04%	0.01%	1.03%	1.04%	
19	Fun	Ascomycota	X	X	OTU 154	21	20.7%	0.45%	4.1%	0.01%	0.59%	0.74%	1.45%	2.55%	0.00%	0.48%	0.84%	1.64%	1.36%	0.78%	0.55%	2.55%	3.21%	4.30%	0.21%	1.55%	0.01%	1.02%	1.82%	0.01%	1.02%	1.82%		
20	Fun	Fungi	incaei	Mortierellomycetes	Mortierellales	Mortierella	28	14.1%	0.31%	2.1%	0.01%	0.70%	0.54%	0.79%	0.95%	1.32%	1.15%	1.05%	0.55%	0.55%	0.55%	0.55%	0.55%	0.55%	0.55%	0.55%	0.55%	0.01%	1.01%	0.96%	0.01%	1.01%	0.96%	
21	Fun	Basidiomycota	Agaricomycetes	Thelephorales	Typhulales	Typhula	5	7.0%	0.15%	6.5%	0.05%	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.99%	0.05%	0.05%	0.99%	0.05%	
22	Fun	Basidiomycota	Pucciniales	Pucciniales	Pucciniales	Puccinia	29	15.0%	0.33%	6.9%	0.01%	0.00%	0.26%	7.40%	1.19%	0.00%	0.00%	0.22%	0.79%	0.21%	0.23%	0.90%	0.12%	0.00%	0.06%	0.00%	0.17%	0.18%	0.98%	0.35%	0.18%	0.98%	0.35%	
23	Fun	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasiales	Waitea	4	7.8%	0.17%	7.2%	0.04%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.98%	0.08%	0.00%	0.98%	0.08%	
24	Fun	Basidiomycota	Agaricomycetes	Trechsporales	Trechsporales	OTU 10	39	73.2%	1.59%	10.0%	0.01%	0.19%	0.00%	2.39%	0.00%	0.09%	0.41%	0.87%	0.85%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.22%	0.93%	0.16%	2.22%	0.93%		
25	Fun	Basidiomycota	Agaricomycetes	Agaricales	Hydrophorales	Hydrophora	17	109.0%	2.37%	4.4%	0.01%	0.00%	0.00%	0.22%	0.00%	0.11%	0.00%	0.00%	0.36%	0.43%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	3.39%	0.92%	0.46%	3.39%	0.92%		
26	Fun	Basidiomycota	Agaricomycetes	Agaricales	Strophariales	Tubaria	9	6.9%	0.15%	4.4%	0.01%	1.52%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.88%	0.11%	0.88%	0.11%	0.88%		
27	Fun	Basidiomycota	Agaricomycetes	Agaricales	Hydrophorales	Cuophylyus	15	94.8%	2.06%	75.4%	0.01%	0.00%	0.00%	0.00%	0.00%	0.07%	0.00%	0.05%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	2.96%	0.86%	0.00%	2.96%	0.86%	0.00%	2.96%	0.86%
28	Fun	Fungi	incaei	Mortierellomycetes	Mortierellales	OTU 777	44	15.2%	0.33%	2.0%	0.02%	0.30%	1.15%	0.44%	0.59%	0.63%	0.54%	0.73%	0.48%	1.44%	0.88%	0.59%	2.58%	1.41%	0.39%	1.25%	0.59%	0.10%	0.85%	0.90%	0.10%	0.85%	0.90%	
29	Fun	Basidiomycota	Agaricomycetes	X	X	OTU 295	3	6.3%	0.14%	6.1%	0.06%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.83%	0.00%	0.00%	0.83%	0.00%		
30	Fun	Ascomycota	Dothideomycetes	Pleosporales	Phaeosporales	OTU 118	41	18.3%	0.40%	2.4%	0.01%	1.97%	2.74%	0.60%	1.37%	0.31%	0.24%	2.22%	0.64%	1.33%	0.65%	1.02%	0.15%	0.39%	1.80%	0.78%	0.20%	0.18%	0.83%	0.97%	0.18%	0.83%	0.97%	
31	Fun	Ascomycota	X	X	OTU 217	18	8.5%	0.18%	5.1%	0.01%	0.00%	0.33%	0.06%	0.00%	0.00%	0.50%	0.15%	0.66%	0.13%	0.00%	0.06%	0.06%	0.06%	0.06%	0.06%	0.06%	0.01%	0.82%	0.33%	0.01%	0.82%	0.33%		
32	Fun	Ascomycota	Dothideomycetes	Pleosporales	Didymellales	OTU 60	42	15.5%	0.34%	1.5%	0.01%	0.54%	0.15%	1.58%	0.82%	0.26%	0.93%	1.00%	1.07%	0.50%	0.50%	0.74%	0.59%	0.45%	0.57%	0.28%	0.18%	0.80%	0.61%	0.18%	0.80%	0.61%		
33	Fun	Ascomycota	Sordariomycetes	Hypocreales	Clavicipitales	Paecilomyces	32	11.3%	0.24%	2.1%	0.01%	0.30%	0.15%	0.60%	0.90%	0.94%	1.27%	2.36%	1.98%	0.43%	0.23%	0.27%	0.80%	0.42%	0.08%	1.01%	0.25%	0.02%	0.79%	0.71%	0.02%	0.79%	0.71%	
34	Fun	Basidiomycota	Agaricomycetes	Trechsporales	X	OTU 346	12	7.4%	0.16%	2.5%	0.04%	2.44%	0.26%	0.16%	0.49%	0.04%	0.00%	0.35%	0.04%	0.73%	0.74%	0.20%	0.00%	0.05%	0.65%	0.00%	0.74%	0.27%	0.00%	0.74%	0.27%			
35	Fun	Ascomycota	Dothideomycetes	Pleosporales	Cochliobolales	OTU 17	45	17.2%	0.37%	1.8%	0.03%	0.94%	0.24%	0.47%	0.28%	0.39%	0.54%	1.60%	1.93%	1.15%	0.42%	0.61%	0.50%	0.59%	0.14%	0.16%	0.28%	0.27%	0.74%	0.54%	0.27%	0.74%	0.54%	
36	Fun	Ascomycota	Leotiomycetes	Helotiales	Spirosphaerales	OTU 9	21	9.8%	0.21%	2.1%	0.01%	0.19%	0.15%	2.27%	1.57%	0.39%	0.26%	0.53%	0.14%	0.53%	0.08%	0.25%	2.64%	1.24%	0.12%	0.75%	0.20%	0.01%	0.73%	0.65%	0.01%	0.73%	0.65%	
37	Fun	Ascomycota	Leotiomycetes	Helotiales	Helotiales	Trichadium	43	12.4%	0.27%	1.6%	0.01%	0.28%	0.04%	1.70%	0.31%	0.48%	0.47%	0.53%	0.56%	0.29%	0.75%	0.36%	0.59%	0.00%	0.00%	0.47%	0.17%	0.19%	0.69%	0.28%	0.19%	0.69%	0.28%	
38	Fun	Ascomycota	Leotiomycetes	Helotiales	X	OTU 32	41	30.7%	0.67%	2.9%	0.05%	0.30%	0.00%	1.01%	0.41%	0.39%	2.50%	2.14%	0.35%	0.38%	0.25%	0.00%	0.59%	0.00%	0.08%	0.00%	0.77%	0.68%	0.41%	0.77%	0.68%	0.41%		
39	Fun	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphales	Laetium	45	24.2%	0.53%	4.1%	0.02%	2.25%	2.26%	0.44%	0.23%	0.24%	0.06%	0.17%	1.09%	0.24%	0.19%	0.14%	0.00%	1.63%	4.32%	0.23%	0.11%	0.40%	0.67%	1.03%	0.40%	0.67%	1.03%	
40	Fun	Pseudofungi	Oomycetes	Pythiales	Pythiaceae	Pythium_B	12	6.9%	0.15%	3.3%	0.01%	3.42%	1.39%	0.00%	0.00%	0.00%	0.00%	0.30%	1.54%	0.03%	0.00%	0.06%	0.00%	0.14%	0.18%	0.08%	0.00%	0.65%	0.29%	0.00%	0.65%	0.29%		
41	Fun	Ascomycota	Sordariomycetes	Xylariales	Amphisphaeriales	Microdothium	13	31.9%	0.69%	11.8%	0.05%	2.11%	12.92%	1.04%	0.80%	0.00%	0.00%	0.62%	0.06%															

Table 6. List of CHEGD species detected via eDNA at the Severalls site, with species only detected in 2020 surveys listed in red font. Data for the original 2016 samples are shown in light blue and translocated soils from 2020 in light green. Summary data for the original soils, the translocated turves and receiving soils are shown in pink highlight. For species present in either the translocated or receiving soils, boxes shaded dark green indicate turves where a given species was found in both translocated and receiving soils and yellow boxes indicate where a given species was found only in the translocated soil.

Species	2020 soil sampling													2016 soil sampling (grouped by area)										
	Tot2016	TotIN20	TotOUT20	214	215	216	217	218	219	220	221	222	Area1	Area3	Area5	Area8	Area11							
C1 <i>Camarophyllopsis schulzen</i>	29802												13158	9	143	14862	1630							
C2 <i>Clavaria acuta</i>	5637	41	110			2	3	7	9	65	18	36		8		3								
C3 <i>Clavaria amoenoides</i>	418	16															2740							
C4 <i>Clavaria fragilis</i>	7247	31				11			9								264							
C5 <i>Clavaria guileminii</i>	178	11															6999							
C6 <i>Clavaria incarnata</i>	157																205							
C7 <i>Clavaria rosea</i> AFF	2622	14															77							
C8 <i>Clavaria</i> sp. BRACR 16032	3438	3															0							
C9 <i>Clavaria</i> sp. JMB10061001	455	11															2							
C10 <i>Clavaria straminea</i>	5527	52				10	2		38	7	2	12					187							
C11 <i>Clavulinopsis corallinosacea</i>	589																214							
C12 <i>Clavulinopsis comiculata</i>	9657	130	2			2	2	18	39								187							
C13 <i>Clavulinopsis helvola</i>	50574	27	29			15	5	9	20								214							
C14 <i>Clavulinopsis laeticolor</i>	2882	31				2			29								2							
C15 <i>Clavulinopsis luteoalba</i> AFF	6038	5	23						3	23							187							
C16 <i>Clavulinopsis sulcata</i>	566																214							
C17 <i>Hodophilus foetens</i>	12	301	10														187							
C18 <i>Ramaniopsis crocea</i>	1906	4															214							
C19 <i>Ramaniopsis kunzei</i> AFF	4153	36	114														214							
C20 <i>Ramaniopsis pulchella</i>	3261	25	179			68	78	23	31								214							
C21 <i>Ramaniopsis</i> sp. JMB-2011	784																214							
G1 <i>Geoglossum fallax</i>	12194	8	128														214							
G2 <i>Geoglossum simile</i>	75	17															214							
G3 <i>Glutinoglossum glutinosum</i>	941	104	24														214							
G4 <i>Trichoglossum hirsutum</i>		8				19	4	3									214							
G5 <i>Trichoglossum octopartitum</i>																	214							
D1 <i>Dermotoma cubae</i> affilium	26	686				15	508	11		178							214							
H1 <i>Cuphophyllus pratensis</i>	12804	257															214							
H2 <i>Cuphophyllus russocefacea</i>	77	2	253														214							
H3 <i>Cuphophyllus virgineus</i>	6577	16	2999			2	35	2	1974	3	990						214							
H4 <i>Gliophorus irrigatus</i>		7															214							
H5 <i>Gliophorus laetus</i>	36911	55															214							
H6 <i>Gliophorus psittacinus</i>	1250	8															214							
H7 <i>Hygrocybe ceracea</i>	20567	2															214							
H8 <i>Hygrocybe acutoconica</i>	16267	267	228			7											214							
H9 <i>Hygrocybe chlorophana</i>	4040																214							
H10 <i>Hygrocybe coccinea</i>	32																214							
H11 <i>Hygrocybe conica</i>	16684	594	236			3	12	21	33	213	12	66	8	23	193	237	7							
H12 <i>Hygrocybe glutinipes</i>	22064	71															2							
H13 <i>Hygrocybe pseudoconica</i>	6565																2							
Total No. Fungal Sequences	544408	34583	40796	4443	5348	3421	4321	4854	5772	3971	6694	4545	5242	4748	4250	4209	5331	4392	3838	108888	102252	94388	122459	116421
Total No. CHEGD Sequences	293151	2180	5103	38	2	68	693	101	2270	585	1731	137	77	251	307	827	4	173	19	55221	50252	33297	66039	88342
%CHEGD	53.8%	6.3%	12.5%	0.9%	0.0%	2.0%	16.0%	2.1%	39.3%	14.7%	25.9%	3.0%	1.5%	5.3%	7.2%	19.6%	0.1%	3.9%	0.5%	50.7%	49.1%	35.3%	53.9%	75.9%
No. CHEGD species	36	31	17	5	1	8	10	15	6	14	11	6	4	15	2	14	1	7	5	26	24	25	29	29
No. waxcap species	12	10	4	1	0	3	2	6	2	4	4	3	1	4	1	5	0	4	2	11	9	8	9	9

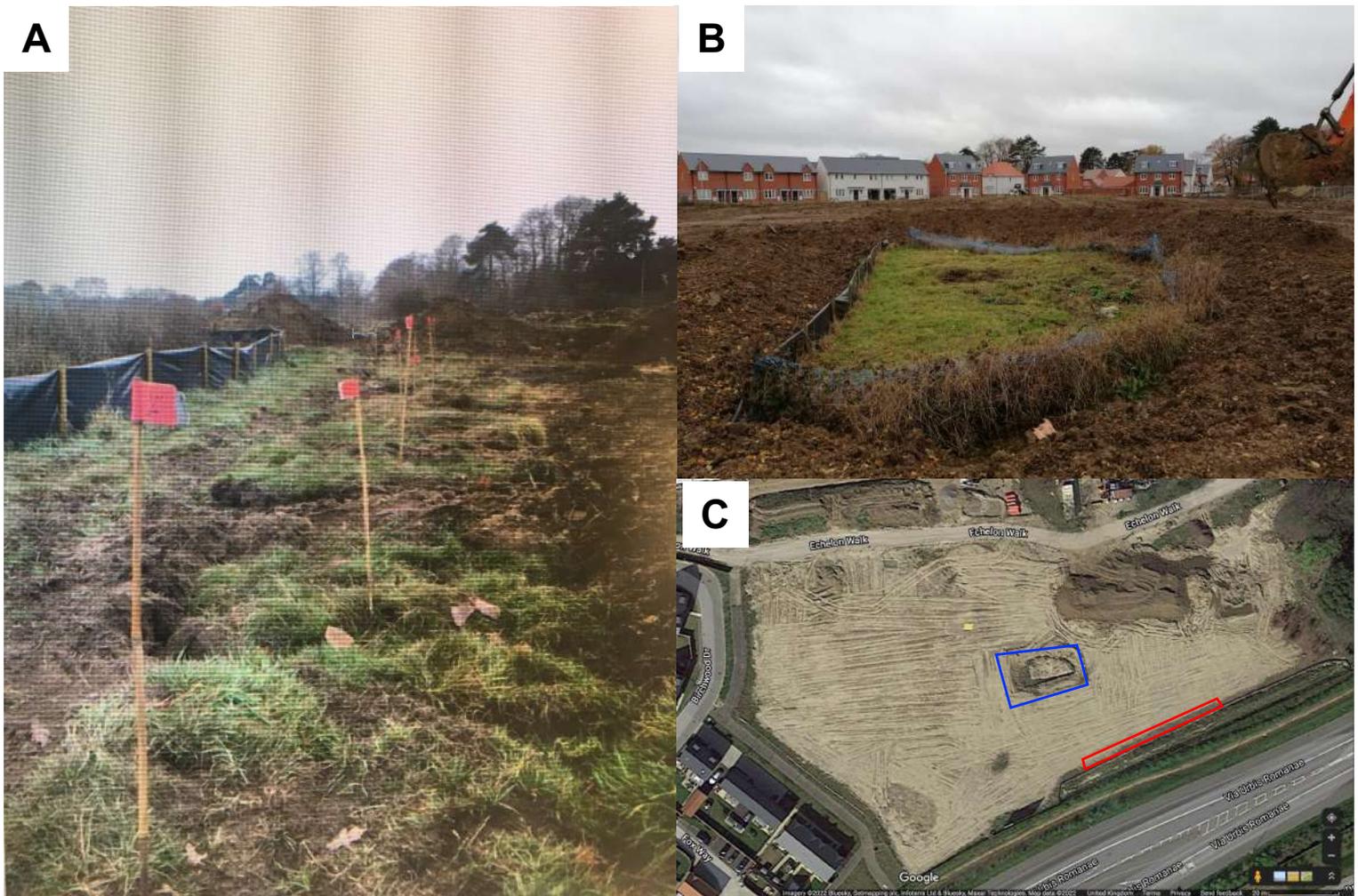


Fig. 7. Red flags (A) indicate the initial storage site area from XX 2017 (black fence is reptile receptor site boundary). The cores were later moved to storage site 2 in XX 2018. The red flags which initially identified each monolith were lost in transit. The map (C) shows the relative locations of the two storage sites on the former cricket pitch at the south of the site (now levelled as a recreational area).

Severalls translocation timeline:

- 1) 10th Oct 2016: APD/EB initial sampling of marked 1m² turves in five areas (1,3,5,8,11; 5 turves per area)
- 2) Spring 2017: Turves translocated to storage site 1 (south end of cricket pitch) at south of site (51.9163,0.8975).
- 3) Turves had to be relocated in January 2018 to 2nd storage area.
- 4) Turves moved to final locations in December 2019 - into retained (undisturbed) grassland areas around the three Airing shelters.
- 5) Soil cores taken on 4th November 2020 for 2nd eDNA analysis of waxcaps



Fig. 8. Stage (3) of turf monolith translocation process: transfer from the storage area. Turves were excavated from the storage site using a large digger bucket (ca. 100 cm wide; A) used to translocate the intact turf monoliths. Initial attempts involved placing the turf directly onto wooden pallet (B,C) with the pallets located on adjacent hardstanding. A smaller tipper truck was used for final placement of some turves (E) and a digger bucket for others (F).



Fig. 9. Stage 3 of turf monolith translocation process: final placement on the turves in the destination area. The receptor site is at the SE end of the site (A,B,C). A hole (1m x 1m 1m deep) was dug(D) and the monolith fragments transported to the receptor site in a digger bucket and dropped into the hole. As can be seen the structure of the monoliths had degraded during storage.

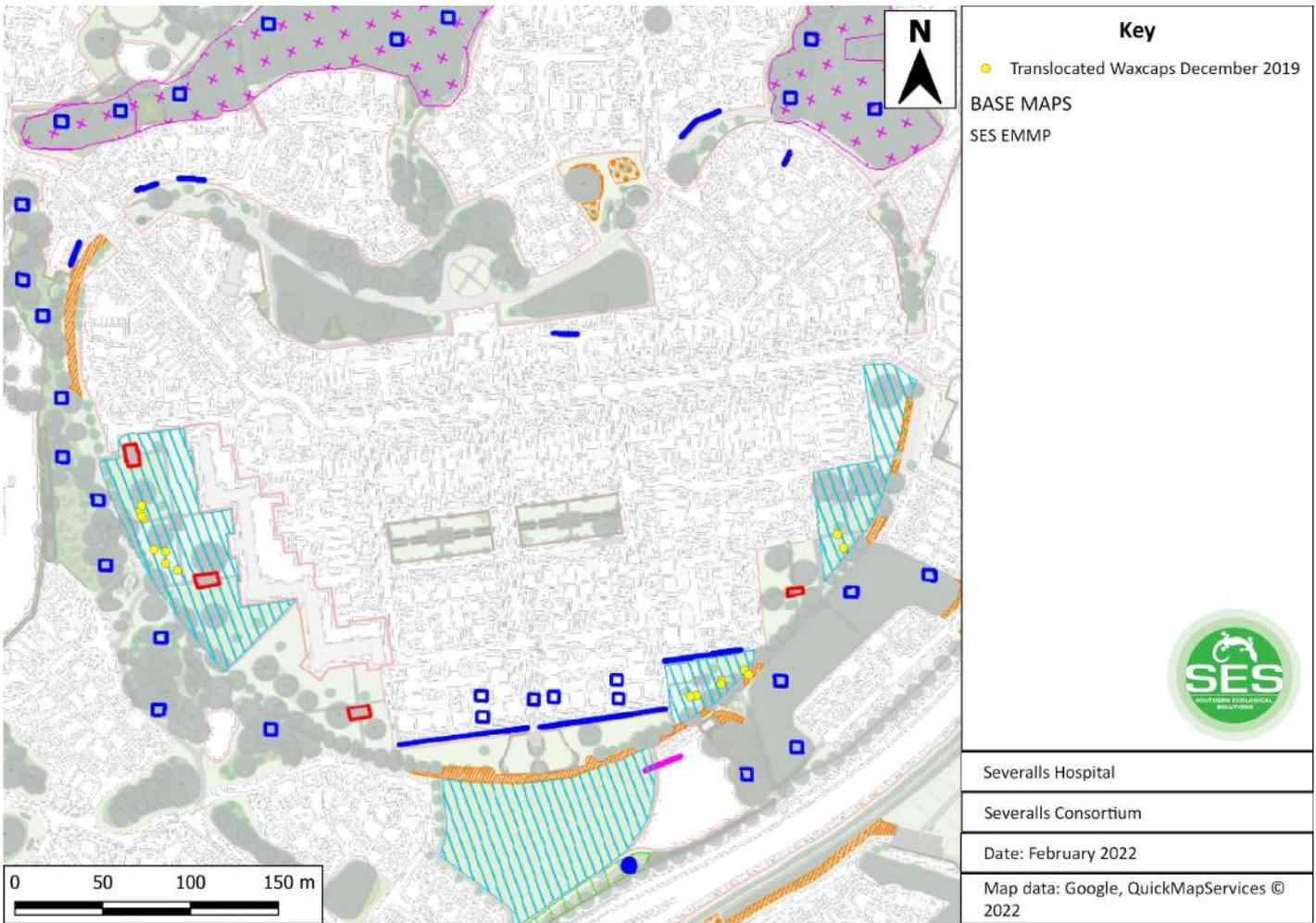


Fig. 10. Final location of the translocated turves in the peripheral retained grassland areas.



Fig. 11. Sampling of the receptor site on 4th November 2020, 11 mths after the placement of the monolith. Cores were taken as outlined above (A-D). Some mushrooms (nn-waxcap) were observed at the receptor site during sampling but not directly on the translocated turves (E).



2000



10Sept 2006



