How long and how frequent is sufficient? Long time study (2003-2022) of variation in occurrence, species richness and fructification of grassland-fungi in a semi-natural grassland in South-Western Norway

Per Fadnes
Faculty of Education, Science and Sport, Institute for Sport, Nutrition andNatural Sciences, Western Norway University of Applied Sciences, campus Stord

Corresponding author: per.fadnes@hvl.no

Norsk tittel: Hvor ofte og hvor lenge er nok? Langtidsstudium (2003-2022) av variasjon i forekomst, artsmangfold og fruktifisering av beitemarksopp i en naturbeitemark på Stord.

How long and how frequent is sufficient? Long time study (2003-2022) of variation in occurrence, species richness and fructification of grassland-fungi in a semi-natural grassland in South-Western Norway. Agarica 2023 vol. 43: 55-83.

KEYWORDS
Grassland-fungi, biodiversity, frequency of survey, monitoring, seasonality, longtime study, fructification

NØKKELORD
Beitemarksopp, biomangfold, frekvensen av besøk, overvåkning, sesongmessige forhold, langtidsstudium, fruktifisering

SAMMENDRAG
Hovaneset på Stord har vært inventert for beitemarksopp i 20 år, fra 2003 og frem til i dag (2022). Dette har resultert i funn av 92 forskjellige arter etter totalt 180 besøk. Endring i frekvensen av inventering til ca. ukentlige besøk i soppsesongen i årene mellom 2010 og 2022 resulterte i stor økning av artsmangfoldet også av rødlistearter. I dette tidsrommet mer enn doblet det totale artsantallet seg, og tallet på rødlistearter mer enn tredoblet seg, noe som tydelig viser den store effekten i endring av inventeringsfrekvens. Inventeringen viser også klart at antall funn ved en inventering varierer stort fra år til å og fra uke til uke. I et optimalt år for beitemarksopp kan en totalt finne i overkant av 70% av artene som vokser der ved en ukentlig inventering hele sesongen, mens et dårligere år gir maksimalt funn av 40% av artene som vokser der. Inventeringen viser også at tidspunkt for besøk i et bestemt år er avgjørende for hvor mange arter som blir funnet. I et optimalt år kan en ved best mulig treff med tidspunkt, finne oppunder 50% av de artene som vokser der på ett besøk, men mest sannsynlig vil det ligge på rundt 35%. Dette viser ganske tydelig at besøk gjennom hele sesongen er nødvendig for å få oversikt over alle soppene som vokser her.

Langtidsserien viser også at mange beitemarksopp har en svært uregelmessig fruktifisering fra år til år, noe som særlig gjelder arter innenfor slekten *Clavaria* og *Entoloma*. Bare ti arter ble funnet hvert år i løpet av 20 år med inventering, der de fleste var vokssopper (*Hygrocybe* s.l.), mens 14 arter bare ble funnet i ett av årene i inventeringen.

Tidspunkt for fruktifisering for mange arter synes også å ha endret seg i løpet av de 20 årene undersøkelsen har pågått. Uken med flest funn i 2021/22 er ca. 3-4 uker seinere enn den var i 2013/14. Dette er særlig tydelig for slekten *Entoloma* som normalt har en tidlig og begrenset fruktifiseringsperiode.

ABSTRACT
The semi natural grassland described in this paper, Hovaneset in Stord municipality, South-Western Norway, has been examined for grassland fungi for 20 years, from 2003 until today (2022). This has resulted in the discovery of 92 different species after a total of 180 visits to the locality. Change in the frequency of survey to approximately a weekly visit during the seasons from 2010 resulted in a large increase in species diversity, also of red-listed species. During these last 13 years of survey, the total number of species more than doubled, and the number of red-listed species more than tripled, which clearly shows the huge effect in changing the frequency of visits to a visit once a week during the season. The survey also clearly shows that the number of finds during a visit varies greatly from year to year and from week to week. In an optimal year for grassland fungi, around 70% of the species that grow there can be found in a weekly survey throughout the season, while a less good year gives a maximum of 40% finds. The survey also shows that the time of visit in a particular year is decisive for how many species are found in one visit. In an optimal year for grassland fungi, at the best possible match with time of survey, you can find up to 50% of the species growing there in one visit, but more likely it will be around 35%. It therefore shows very clearly that visits during the whole season is necessary to get hold on all fungi growing here.

The survey also shows that many grassland fungi have very irregular fructification from one year to the other, which particularly applies to species within the genera *Clavaria* and *Entoloma*. Only ten species were found each year during the survey, most of which were waxcaps (*Hygrocybe* s.l.), while 14 species were only found in one of the years of survey. The time of fruiting also seems to have changed during the 20 years the survey has been ongoing. The week with the highest number of species recorded in 2021/22 is approximately 3-4 weeks later than it was in 2013/14. This is particularly clear for the genus *Entoloma*, which normally has an early and limited fruiting period.

INTRODUCTION
Grassland fungi is a concept normally restricted to a few genera and families of fungi that primarily grow in unfertilized pastures, often described as semi-natural grasslands. This is in accordance with several authors (Nitare 1988, Griffith et. al. 2013). Concepts like “grassland fungi” and “waxcap-grasslands” (seminatural grasslands rich in *Hygrocybe* species) were given attention as far as back in the 18th century (Griffith et. al. 2013).

Grassland fungi, used as described above, consist of a taxonomical diverse group of fungi with similar ecological preferences. These are *Hygrocybe* (s. l.) (waxcaps), *Entoloma* (redspores), *Camarophyllopsis* (s. l.), *Clavaria*, *Clavulinopsis*, *Ramariopsis*, *Pseudotricholoma* (*Porpoloma*), *Dermoloma* and Geoglossaceae (*Geoglossum* (s. l.) and *Trichoglossum*) (Boertmann 2010, Nitare 1988, Noordeloos 1992, Jordal 1997, McHugh et. al. 2001, Griffith et al. 2013), and the former member of Geoglossaceae, *Microglossum,* which is now placed in the Leotimycetes (Ohenoja et al. 2010, Hustad et al. 2013). In Norway and Sweden around 150-160 different species of grasslandfungi have so far been registered (Nitare 1988, Jordal 2011, 2013).

In the United Kingdom, these fungi are also often referred to as CHEGD-fungi, which is an acronym of most of the names listed above (Griffith et. al. 20013). In accordance with Evans (2003) and Griffith et. al. (2013), the group consists of 180-200 species in the UK.

Many grassland fungi are rare and grow only in unfertilized pastures and meadows, which are the main habitats for these fungi. Termination of grazing leads to invading of

trees, and fertilization are favoring fungi and plants which prefer more nutrition.

Overgrowing can, to some extent, be reversed by reintroducing grazing animals, since the grassland fungi might have survived and start fructification again, while fertilization seems to be an irreversible process wiping out most of the species (Arnolds 1989, Vesterholt and Knudsen 1990). Some species prefer calcareous soil, but we can also find many rare and red-listed species growing on more poor and acidic soil, especially if the locality has long continuity. Many of these species are therefore good indicators of valuable grasslands, probably better indicators than vascular plants (Rald 1985, Öster 2008). Different species of grassland fungi often occur in clusters on the same locality.

The ecology of grassland fungi is to date not clearly understood. Are they saprotrophes, decaying dead materials, or do they have biotrophic relationships with herbs or grasses? New results indicate that hyphae from several waxcaps do colonize plant roots, indicating a biotrophic lifestyle (Seitzman et al. 2011, Halbwachs et al. 2013).

Very few longtime studies of fungal fructification have been performed, and those that exist are relatively old. The existing surveys of fungi have been criticized for being too short and random not hitting the right time for the best results (Halme & Kotiaho 2012).

Generally, there is a clear correlation between the number of species found at one location and the intensity of visits (Parker-Rhodes 1955, Watling 1995). According to Parker-Rhodes, you will seldom find more than 25% of the species that grow at a specific site in one visit. Orton (1986) concluded that the data from at least 10 years of investigation is needed to get an accurate picture of the species growing in a locality. Newton et al. (2003) visited five localities with old pastures between 10 and 16 times over a period of three years (1999-2001). Species diversity increased with each visit, and they concluded that at least 16 visits to a certain site is necessary to get a good picture of fungal diversity at the locality. Straatsma et al. (2001) surveyed a forest area for 21 years from May to September. New species appeared every year, but only eight species occurred every year.

The most species rich localities in Norway until now had 71 different species recorded, including 32 waxcaps (Fadnes 2014), in Sweden one locality had 76 species of which 33 were waxcaps (Phil 1992), and in UK the most species rich locality had 78 species, of which 34 were waxcaps (Griffith et. al. 2013).

Increased survey of grasslands in SW-Norway the last decade has led to a great increase in the number of grassland fungi known, and today between 60 and 130 species, including many rare and red-listed species are found in the municipalities situated here (Brandrud et al. 2021). It has previously been shown that SW-Norway has a particularly rich and varied occurrence of earthtongues (Fadnes 2011), and for most of the other genera the numbers are high (Artsdatabanken 2022). SW-Norway therefore appears to be a “hot spot” for localities with rare and red-listed grassland fungi. After around 20 years of survey of old grasslands in SW-Norway, it is apparent that there are many factors in addition to fertilizing, continuity, geology and soil, that have an influence on which species we actually find on a particular locality at a certain time (Fadnes 2014).

Results from the first 11 years of survey have previously been published (Fadnes 2014). The number of species presented in this study differs to a degree from those presented here. This is due to several new finds the last nine years, some changes in red listed species (Brandrud et.al. 2021), and that some species, especially species of *Entoloma*, have been subjected to molecular studies.

In 2003, when this study started, no grasslandfungi were known from the location

studied. However, every year of survey gradually increased species diversity. Some new fungi appeared every year, and some found the last year did not reappear. Such a long-term study of a semi-natural grassland has probably not been carried out before.

The aim of this study will focus on the variations in diversity and fruiting of grassland fungi through a 20-year survey with almost weekly visits from July to November of this particular grassland in SW-Norway, described in detail below.

MATERIALS AND METHODS

Sampling procedure

The locality that is the main basis for this article, Hovaneset (a more detailed description follows under), was investigated from 2003 to 2022. From 2003 to 2009 the frequency of visits was random during the season.

The intensity ranged from two to five visits every year. In 2010, the number of visits increased to ten, and the last 12 years (2011-22) the locality was visited almost every week from late July to November, totaling 10 to 15 times a season. The total number of visits to the locality accounts to around 180 performed during the last 20 years. A path of about 2 km has been followed, and in this way, the whole area has been systematically surveyed on each visit. Every find was recorded at each visit, and for rare and red-listed species coordinates were noted. Dried specimens of red-listed species were sent to the fungaria of Natural History Museum (O), (University of Oslo 2023), all other finds were registered at the Norwegian Species Observation Service (Artsdatabanken 2022). Both datasets are available for downloading at GBIF-org (2023).

Fungi mainly growing in nutritious soil and manure were noted, but not included in this survey. Some species had to be examined microscopically. Fresh material of the fungus was studied after soaking in water using a LEICA DM750 microscope.

Based on the number of visits to the location, the survey can be divided into two periods:

Period 1. The first seven years (2003-2009) with a more random number of visits, ranging from two to five.

Period 2. The last 13 years (2010-2022) with a more systematic survey with a visit almost every week during the season (10-15 visits/year).

Description of the investigated locality

Hovaneset, the location studied here, is a peninsula in the Hardangerfjord just north of the village Leirvik on the east side of the island Stord and about 70 km south of Bergen defined by the coordinates 32VLM0534 (Figure 1). It is a large natural grassland of approximately 5 ha with some small marshy areas and is grazed by sheep (Figure 2). Probably some fertilizer has been provided earlier on the upper flattest part of the area. Therefore, the prevalence of grassland fungi is mainly highest in the rugged peripheral zones of the peninsula (Figure 2).

Calcareous bedrocks surround the pasture where you can find plants like *Lysimachia minima* (EN), *Sagina nodosa* and *Erophila verna* in addition to typical mountain plants like *Saxifraga oppositifolia* (NT) and *S. aizoides*. In the pasture we find a variety of demanding grass and herbs like *Cynosurus cristatus*, *Briza media* (NT), *Linum catharticum* and *Orchis mascula*. The soil quality varies from thin soil with *Pimpinella saxifraga* and *Aira praecox* to more humid areas with *Carex hostiana* and *C. flacca* (NT). Here we also find the rare and red-listed species *Isolepis setacea* (EN).

Seen from archaeological traces, the area has a continuous use by man from the Bronze Age up to present (Figure 2c) At the end of the last ice age (10000 years ago) the area was mainly under the sea level because the land had been pressed down by the large icecap. Later, in the Bronze Age, Hovaneset

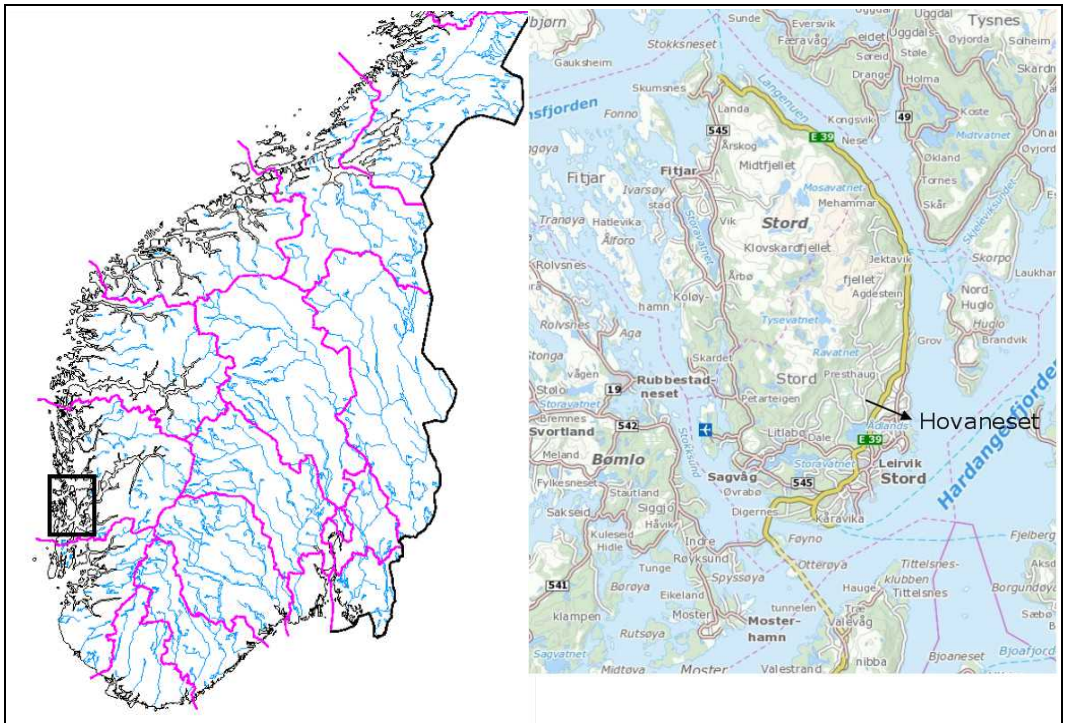


Figure 1. Map showing the location of the locality Hovaneset in Western Norway

Figure 2. a. Arial photo of the locality Hovaneset (Norgeskart), b. The peninsula Hovaneset seen from the south, c. Archaeological traces (burial mound) going back to the Bronze age, d. Part of the grassland from Hovaneset. Photo P. Fadnes.

was an island, and as the mainland gradually raised from the pressure of the ice, it became a peninsula, which is how it looks today.

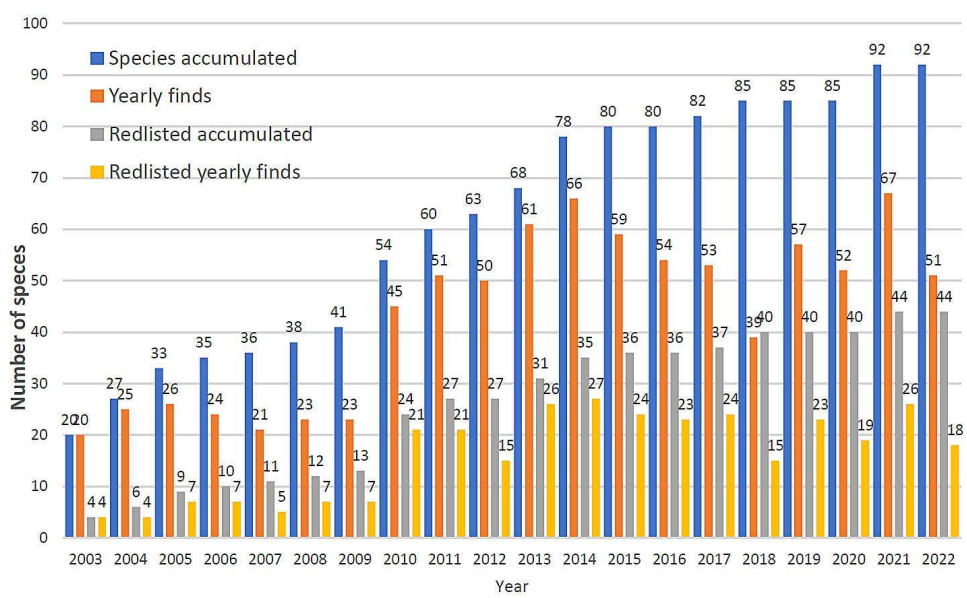
After 20 years of investigation, a total of 92 different species of grassland fungi have been discovered on Hovaneset, where 44 are on the Norwegian red list and 21 of them are vulnerable (VU) or endangered (EN) while five are defined as data deficient (DD) (Table 1). In addition, more than 15 species not belonging to the grasslandfungi are found in the area (*Arrhenia* spp., *Agaricus* sp., *Mycena* spp, *Rickenella* sp., *Panaeolus* spp., *Cystoderma* sp., *Coprinopsis* sp., *and Stropharia* sp.), making the total number of fungal species more than 100. Some of them are also relatively rare in Norway like *Arrhenia rickenii,* and *Agaricus phorphyrocephalus*).

With a totality of 180 visits during the last 20 years, the locality is probably the best investigated semi natural grassland in Norway and the most species rich known today (Fadnes 2014, 2021). Most of the species from Hovneset are well documented on the authors webpage "Grasslandfungi.no" (Fadnes 2023).

Molecular studies

Molecular studies were performed with standard procedures. DNA extraction, PCR amplification and sequencing were done in accordance with the Norwegian Barcode of Life Network (NorBOL) as part of BOLD by the University of Oslo or following Alvalab (Alvarado et al. 2012) or Dima et al. (2016). For BOLD methods see Ratnasingham & Hebert (2007, 2013).

For the DNA extraction and PCR reactions the Phire® Plant Direct PCR Kit (Thermo Scientific, USA) was used following the recommendations of the manufacturer, but in some cases the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) was also used. The ITS region of the nrDNA was amplified with the primer pairs ITS1F/ITS4 (White et al. 1990, Gardes & Bruns 1993). PCR protocols were followed Papp & Dima (2018). The amplicons were sequenced at LGC Genomics



Fgure 3. Changing in diversity of grassland fungi during 20 years of survey.

(Berlin, Germany) with the same primers used in PCR reactions. The electropherograms were checked, assembled, and edited with the CodonCodeAligner package (CodonCode Corporation, Centerville, MA, U.S.A).

RESULTS

Change in number, diversity and fructifying of grassland fungi during 20 years of survey.

The number of visits to the locality was sparse from 2003 to 2009 (period 1), during the season from July until November, varying between two and five random visits per year. The yearly records of different species varied from 20 to 26, even if the accumulated number of different species found during the first seven years increased from 20 to 41 (Figure 3). The same effect was seen among the fungi listed in the Norwegian red list (Brandrud et. al. 2021). The number of red-listed species found in Period 1 varied between four and seven each year, and the accumulated number found during Period 1 increased to 13 in 2009 (Figure 3).

In period 2, from 2010 until 2022, the investigation of the locality increased to a visit almost once a week during the season from end of July to medio November. This change in method had a profound impact on the number of fungi recorded. It resulted in a doubling in number of species found in one year from 2009 to 2010, from 23 to 45 species. This also increased the total (accumulated) number of species found at the locality to 54 in 2010. A total of 21 of 24 red-listed species were found that year, showing the profound effect of the increased survey activity from one year to the next, especially on red-listed species which normally are rarer than the others.

The next 12 years with intensive survey increased the total number of species almost every year and reached the substantial number of 92 species accumulated in 2021 (Figure 3). The number of red-listed species also increased gradually during these years, with the numbers starting to level out in 2015, ending up at 44 species in 2021, which constitute almost half of the total number of

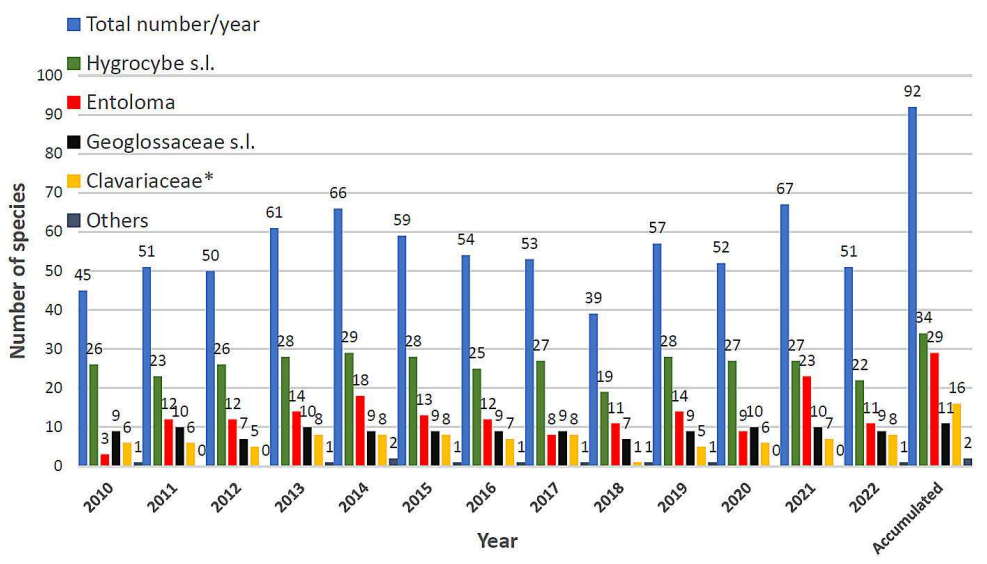


Figure 4. Occurrence of different groups of grassland fungi the 13 last years of survey (Period 2).

species. The rise in number of species in 2021 is partly due to the molecular study of several *Entoloma* species which could have occurred earlier but have not been registered before (Table 3). Moreover, some species of waxcaps and Clavariaceae were identified by molecular studies in 2021 and contributed to the rise in number of species (Table 3). Since the change to a more intensive survey in 2010, the number of red-listed species more than tripled, and the total number of species has more than doubled at the end of the survey (Figure 3).

Figure 4 shows the changing in number of the different groups of grassland fungi in the years from 2010 until 2022. The waxcaps show a stable number of species from one year to the other. Species of *Entoloma* varies a lot from one year to the other and had two years with relatively high numbers, 2014 and 2021. The number of earthtongues and members of the Clavariaceae show like the waxcaps a relatively stable number from year to year. The exception was 2018 which had an extraordinary dry summer (NMI 2022), resulting in a dramatic drop in the number of finds of all groups compared with all years back to 2010, especially for Clavariaceae which had only one find in 2018. This shows the clear effect of weather on the number of records in a given year. However, much unexpected was the occurrence of tree new species that year, all on the Norwegian red list. The most surprising species was *Hygrocybe spadicea*, occurring for the first time, together with *Entoloma neglectum* and *E. ameides*. *H. spadicea* is a rare, southerly species which probably prefers warm summers. *E. ameides* is a species also occurring on many other locations this year. The year 2014 also had a relatively warm summer in Western Norway but not as dry as in 2018 (NMI 2022), and was as shown, one of the most species rich years in the survey (Figure 3) together with 2021.

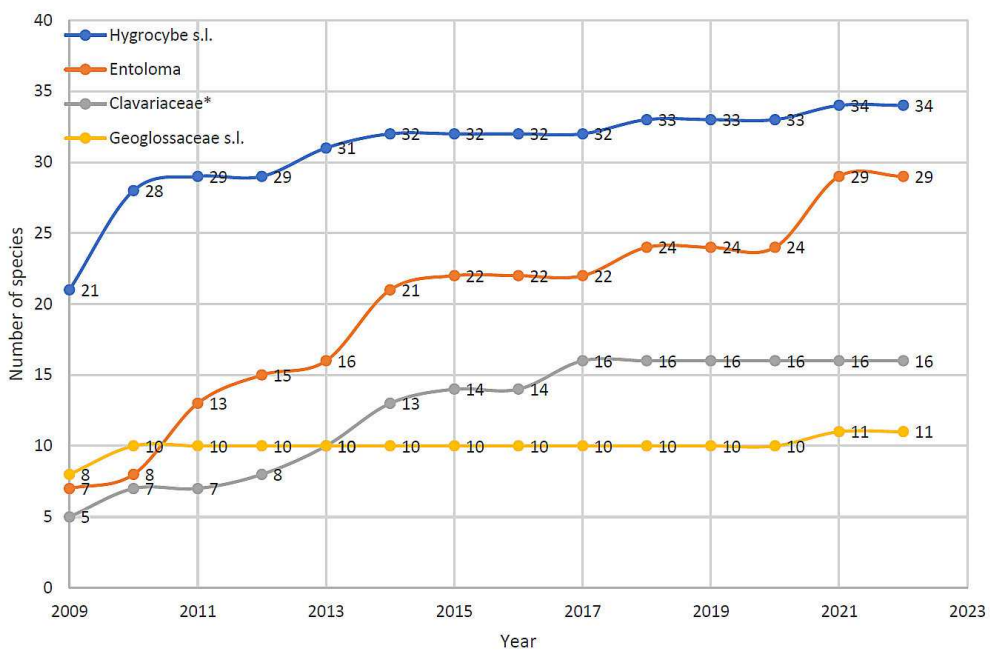


Figure 5. Accumulated finds of different groups of grassland fungi from Period 1 to Period 2.

Figure 5 shows the accumulated number of the different groups of grassland fungi from 2009 to 2022. The year 2009 represents the accumulated number for the first seven years of survey (Period 1). This shows that waxcaps constituted the most abundant group in Period 1 with as many as 21 species where six are redlisted. This number raised significantly already the first year of Period 2, and later to 34 species in 2021, which is the final number of waxcaps. Among these are 13 on the Norwegian red list (Brandrud et al. 2021) (Table 1).

The genus *Entoloma* has a slower development in the number of species found, starting with only seven species from Period 1, but starting to increase already the first years of Period 2 and ending at 29 species in 2021 (Figure 5), with as many as 13 species on the red list (Brandrud et al. 2021).

Most of the earthtongues (s.l.) were already found in Period 1, with eight different species, and increasing to 11 as late as 2021. The earthtongues from the South-Western Norway are presented in an earlier article (Fadnes 2011).

Few species (5) from the family Clavariaceae were found during Period 1 but showed a significant rise in number in 2013/14 adding seldom species like *Clavaria tenuipes* and *C. flavipes* to the genus. Especially many of the club fungi in the genus *Clacvaria* have a very irregular fructification from year to year which can explain this development.

Figure 6 shows the appearance of different species during 20 years of survey. As many as 14 species have only appeared once during the survey period: *Pseudotricholoma metapodium, Clavaria tenuipes, Clavulinopsis umbrinella, Entoloma ameides, E. neglectum, E. indutoides, Hygrocybe spadicea, H. aurantiosplendens, H. coccineocrenata, Geoglossum cookeanum.* This also includes some

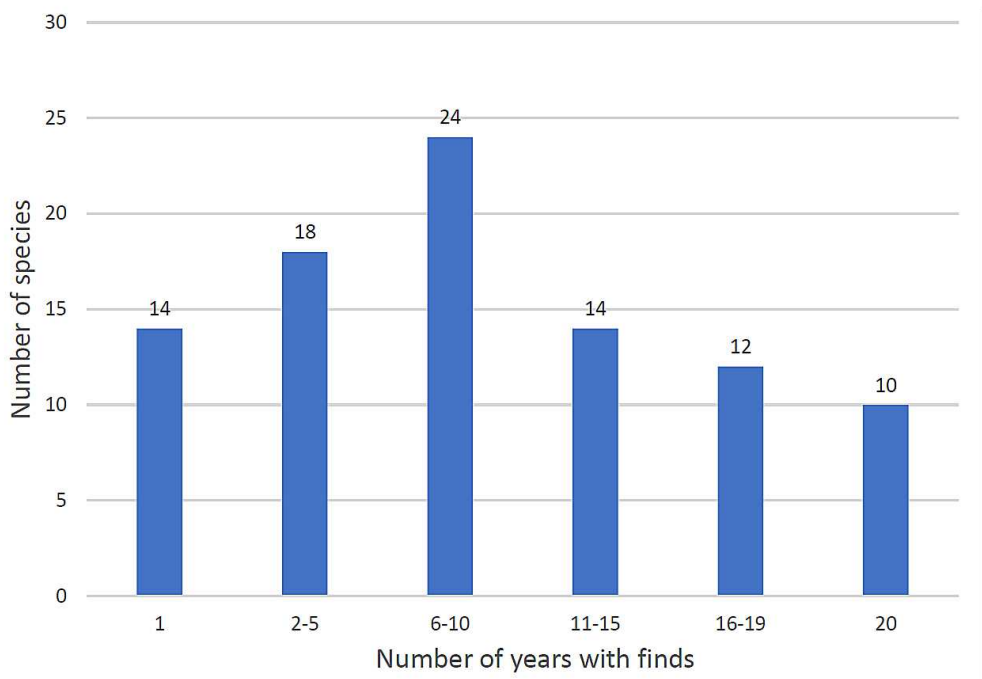


Figure 6. Number of years with number of different species.

new species identified by molecular study in 2021/22: *Entoloma violaceoviride, E. allospermum, E. carneogriseum, Hygrocybe flavipesoides* and *Lamelloclavaria petersenii.* Some *Entoloma* species could of course have occurred earlier without being discovered, since some of them have just been described. Only 10 species were registered every single year of the survey. They were mostly relatively common species and mainly waxcaps: *Geoglossum fallax, Hygrocybe chlorophana, H. coccinea, H. conica, Cuphophyllus pratensis, C. virgineus, C. colemannianus, C. russiocoriaceus, Gliophorus psittacinus* and *G. laetus.*

Figure 6 also shows that 56 different species are only found between one and ten years, while 36 species were found between 11 and 20 years of the survey (se also Table 1).

All species in the location but one (91) were found during Period 2, while only 41 species were found in Period 1. This shows that a more intensive survey with almost a visit every week in the season results in more finds, especially of more rare species.

If we only look at period 2, a total of 22 different species were found every year, and 13 species were found only once during this period, again indicating that these fungi are rare and/or have a very irregular fructification.

Table 2 shows the number of different species found each year the last ten years. It varies from 39 to 67 species and constitute from 42 to 73% of the total number (92). These high numbers presuppose an intense survey of the location during the whole season.

Seasonal variation of grasslandfungi

Based on own experiences grasslandfungi fructify at different times during the season depending on latitude. In the end of July and beginning of August the fructification in the north of Norway is optimal especially among *Entoloma* species. At that time there is almost nothing in the south-western part of Norway. In the UK the best time to look for grassland-

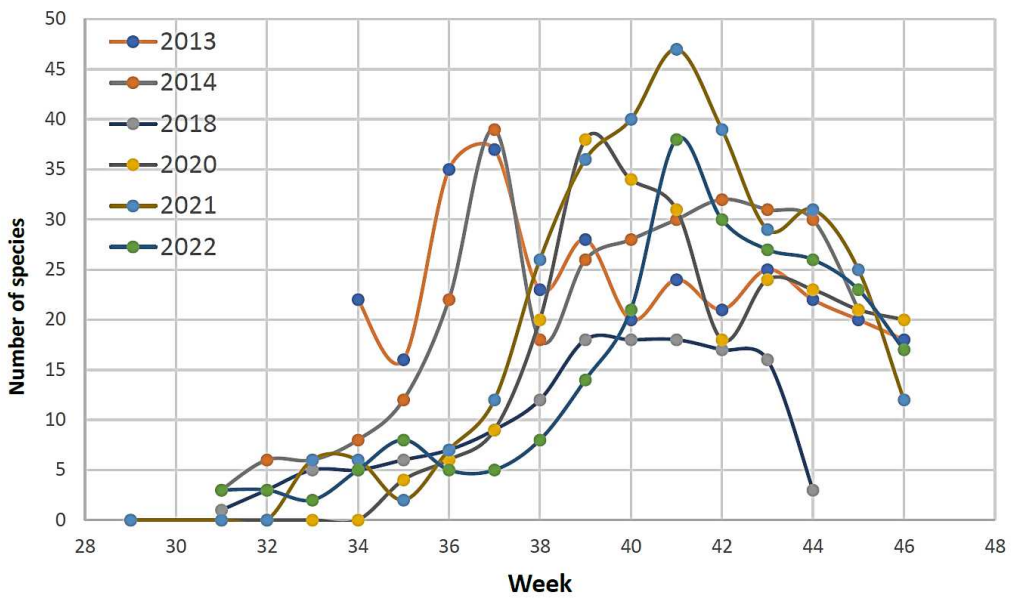


Figure 7. Weekly finds of grassland fungi in some selected years in Period 2. (July: 29-30, August: 31-34, September: 35-39, October: 40-43, November: 44-46.)

fungi is in October and November, showing the effect of latitude. The seasonal variation among the different genera and species of grasslandfungi is also great. Different species are fructifying to different time of the season resulting in different numbers from one week to the other.

Figure 7 shows the variation of finds of different species from week to week for six different years. The season in SW-Norway normally starts in the beginning of September with relative few numbers of finds, but the number increases as September passes and October begins. However, it seems that the highest peak of different fungi has changed from 2013 to 2022. The last years the main peak has been in the beginning to middle of October, which is a change in several weeks from 2013 (Figure 7). This is particular evident for the genus *Entoloma* which is normally an early fructifying genus, but the last years have fructified three to four weeks later compared to 2013-2014 (Figure 8). This figure also shows the great variation of finds of different *Entoloma* species between years.

The total number is kept relatively high over a longer period mostly due to the waxcaps which fructify over a longer period of time and where there also are some early and late fructifying species (Figure 10). Many waxcaps were found during the whole season like the more common species *Hygrocybe chlorophana, H. conica* and the more seldom *H. quieta.* The early fructifying species are species like *Hygrocybe helobia, H. acutoconica, H. intermedia* and *Neohygrocybe ingrata*. None of those were found in the late visits to the locality. The late fructifying species like *Hygrocybe splendidissima, H. punicea and Cuphophyllus russocoriaceus* were not found in the start of the season but started to show up in the middle of September or later. The

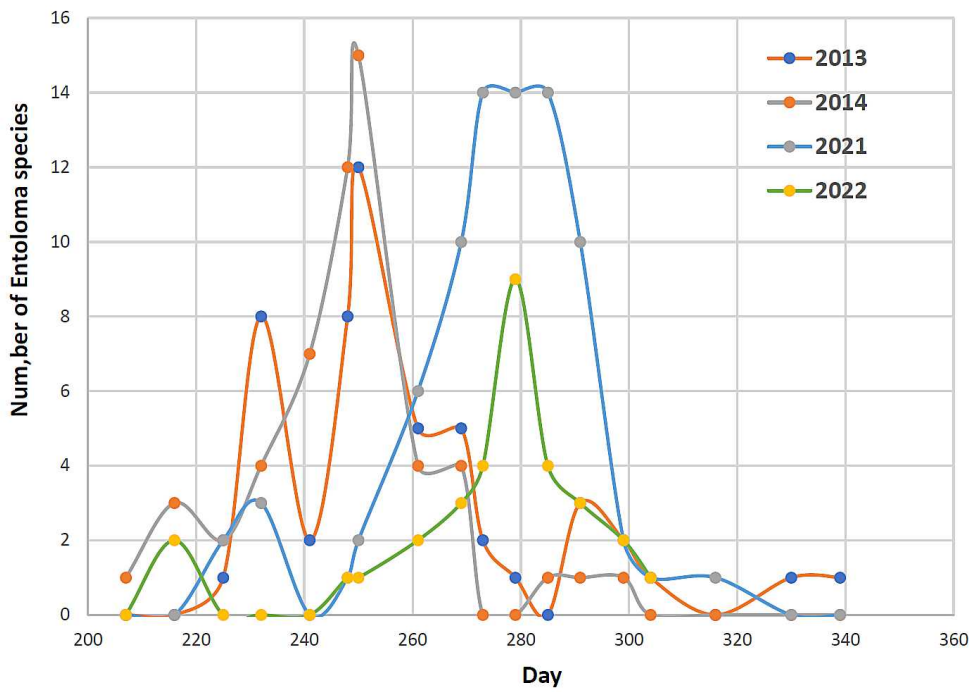


Figure 8. Weekly finds of Entoloma species for four selected years in Period 2.

latest fungi to show up in the location was always *Cuphophyllus fornicatus*. As shown in figure 7 the start of the season has shifted the last years, which will also be the case for many of the species shown in Figure 10.

The high number of fungi found in a particular year is dependent on several visits which is the case in this study. However, figure 7 shows a great variation in finds depending on the time of visit. Normally you only visit a locality a few times during the season, and the figure shows that you should be lucky to hit the most perfect week concerning the number of different species you can expect to find.

Table 2 gives you a picture of this. The maximal number of different species you will find, provided you choose the optimal week, varies between 18 and 47 in the years 2013 to 2022. The most probable number is between 30 and 40 of a total number of 92 species. This makes the maximal percentage of different species found in one visit in this survey around 50% (2021), but the most probable number is between 34% and 38%.

Figure 7 also shows the dramatic effect of high and stabile summer temperatures and little precipitation on the number of collected fungi, which was the case in 2018.

Survey statistic

Figure 9 shows the connection between the accumulating number of fungi and the number of visits during 20 years of survey. It seems like the accumulated number of species found correlate very well with the number of visits until year 2009 (Figure 9). Then with an increased number of visits the number of species still increases until 2014 and around 90 visits and 78 different species, when the number seems to even out. A further increase of visits from 2014 still adds some new species and ended in 2021 on 92 different species. So even if the number of new species doesn't follow the number of visits from 2015, the intense survey still added 12 new species to the total number during these last eight years, which constitute 13% of the total number of species, and the last 90 visits were therefore not in vain even if it can seem overwhelming.

Some comments on the survey of different groups of grassland fungi

Waxcaps (*Hygrocybe s.l.)*

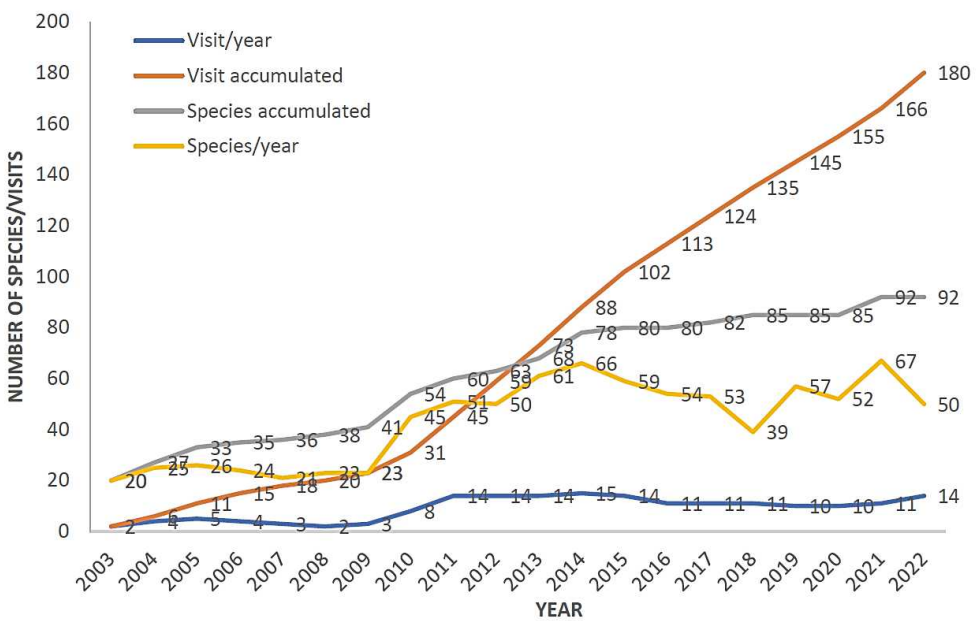
Waxcaps growing in semi-natural grasslands were until recently placed in the genus *Hygrocybe*, distinguished from the ectomycorrhizal species associated with trees belonging to the genus *Hygrophorus*. During the last years, there has been a revision of both genera resulting in splitting the genus *Hygrocybe* into several genera based on molecular studies (Lodge et al. 2014). In the survey during 20 years on this locality, 34 different waxcaps were found. It raised from 21 species to 28 from 2009 to 2010 after a more intensive survey started (Period 2) (Figure 5). As late as 2018 the very rare waxcap *Hygrocybe spadicea* was found for the first time (Figure 11a). The summer of 2018 was unusual warm and dry (NMI 2022), and this seems to be advantageous to this fungus. The same year it was found on several other localities in Norway (Artsdatabanken 2022) indicating the same. In addition, the species *H. coccineocrenata* and *H. aurantiosplendens* were also only found one year. Other waxcaps with relatively few yearly finds are *Neohygrocybe nitrata* (4) and *H. subpappilata* (3). *Cuphophyllus flavipes* has with molecular studies been shown to be two species (Jordal & Larsson 2021). In 2021/22 material of *C. flavipes* coll. were subjected to molecular studies to see which of the two species were present. The study showed that both species were growing in the location. Most molecular studies of this complex in the region have showed that the most common species of the two is *C. flavipesoides*, and that *C. flavipes*

probably is a southernly species reaching as far north as SW-Norway (Jordal & Larsson 2021). At the moment it is not possible to separate the two species by morphological differences, only by sequencing, making it difficult determining the right species in the field. Among the other waxcaps, nine species were found every year, and these were relatively abundant species mentioned earlier. In addition to the 34 different waxcaps species, two subspecies have also been found in the locality. These are *Cuphophyllus pratensis* subsp. *pallidus* and *C. virgineus* subsp. *ochraceopallidus,* the last one is a very common species occurring almost every year.

All together a number of 41 different species of waxcaps have until now been recorded in SW-Norway, which means that a substantial number (34) of these fungi grow in this particular grassland studied here (Artsdatabanken 2022).

Earth tongues (Geoglossaceae s. l.)

The taxonomy of Geoglossaceae has historically been difficult due to lack of good morphological characters to distinguish the different species. Therefore, they have until lately been placed in the same family Geoglossaceae. However, after several revisions of the family there have been great changes. The genus *Geoglossum* has recently been revised by many authors. Schoch et. al. (2009) reduced the family only to include the genera *Sarcoleotia*, *Geoglossum* and *Trichoglossum*. *Microglossum,* which has earlier been included in Geoglossaceae, is now excluded, and shown to be very distant related based on molecular methods (Schoch et al. 2009, Sandnes 2006). This genus is now placed in the Leotiomycetes (Schoch et al. 2009). Hustad et al. (2011 and 2013) also included the genus *Nothomitra* in Geoglossaceae and created a new genus *Sabuloglossum* (including the former *Geoglossum arenarium*) and *Glutinoglossum*



Fgure 9. Survey statistic: Number of visits vs. number of accumulated species and yearly finds of grassland fungi during 20 years of survey.

(including the former *Geoglossum glutinosum*). Studies of Fedosova et al. (2018) have later proposed 13 species in the genus *Glutinoglossum* by molecular studies, where seven species are known from Europe.

The studies of Arauzo & Iglesias (2014) confirmed the genus *Leucoglossum* proposed by Imai (1942). They also proposed a new genus *Hemileucoglossum,* which includes species with hyaline spores and setae on the stipe showing resemblance with those of the genus *Trichoglossum*.

Eleven different earthtongues are found in the locality, and they are often appearing in great numbers. This is true for *Geoglossum fallax* which is also a very macroscopically variable species and could very well be a complex of different species. In the wet part of the locality, we find *Geoglossum uliginosum* (Figure 11f), *G. simile* and *Trichoglossum hirsutum*. The latter also grows in completely dry areas where we also find *Geoglossum umbratile*, *G. starbaeckii*, *Glutinoglossum glutinosum*, *Trichoglossum walteri*, *Microglossum atropurpureum* and *Hemileucoglossum pusillum*. The last one was found for the first time in 2010 but was confirmed by molecular studies in 2020 (Fadnes et al. 2021). This is the only known record of this species in Norway and is otherwise only known from a few localities in Slovakia and Spain. Surprisingly a record of a new earthtongue was made in 2021, *Geoglossum cookeanum*. It could probably have been recorded earlier, but since the occurrence of earthtongues are high, it can occasionally have been overlooked.

Redspores (*Entoloma*)

The genus *Entoloma* consist of many different species where many are restricted to semi-natural grasslands, especially in the subgenus *Cyanula* with their characteristic scaly cap and blue colors on stem and/or hat. However, also species from the subgenus *Nolanea* and *Trichopilus* are common in this habitat.

Around 30 different species of *Entoloma* are found at Hovaneset until now, based on the keys developed by Noordeloos (1992 and 2004) and Vesterholt (2002). However, in the Norwegian Entoloma project (Brandrud et al. 2020, Noordeloos et al. 2020) where many collections have been subjected to molecular study, the present keys show substantial failures in determination for a great number of species. This is especially the case for many of the *Cyanula* species with blue colors. In Noordeloos et al. (2022b) they are also confirming that species of the genus *Entoloma* very frequently have been misidentified in literature resulting in high level of incorrect names in gene databases as Gen Bank and UNITE. This study has also shown that a species like *Entoloma corvinum*, which was known as a common species in Norway, now seems not to grow here. In many cases in this survey, it has been shown by molecular studies to be *E. atrocoeruleum* (Table 3). The number of *Entoloma* species in the locality is probably higher than the number reported here. This is due to the rejection of several species because of unsecure determination. In 2021, which was an optimal year with many *Entoloma* collections, many species were subjected to molecular studies (Table 3). This resulted in seven new species of *Entoloma* to the locality: *Entoloma allospermum, E. glaucobasis, E. carneogriseum, E. cyaneoliliacinum, E. soropratulense, E. violaceoviride* and *E. majusculum*, explaining the rise in numbers of *Entoloma* recorded in 2021 (Figure 4). Some of these species have just recently been described (Noordeloos et al. 2021, Dima et al. 2023). The same is for *E. pentagonale* which is a member of the Rhombisporum clade (Noordeloos et al. 2022a). All the species are also described in Fungi Europaei: *Entoloma s.l.* (Noordeloos et al. 2022b). Some of the *Entoloma* species described here are shown in Figure 11.

Most *Entoloma* species normally appears early in the season, and the season for most of them is relatively short. The slope of the locality facing north has the richest occurrence of *Entoloma* species, and the ground here probably also contains calcareous sediments that many *Entoloma* species seems to prefer (Figure 2).

In the years before 2010 when the survey was conducted in a smaller scale (Period 1), only seven different species of *Entoloma* were found (Figure 5). This could partly be due to visits not conducted on the optimal time for *Entoloma* species, or to difficulties in determining the right species. However, the number generally differs much from one year to the other. The years 2014 and 2021, which had warm summers in the western part of Norway and enough precipitation later in the autumn (NMI 2022), provided a high number of species of *Entoloma*, with 20 and 25 species found, respectively. However, in the years in between and especially after the dry summer in 2018, the occurrences have been sparser (Figure 4). This change in occurrence from year to year is difficult to explain, but difference in weather from one year to the other and irregular fructification of many species is worth mentioning.

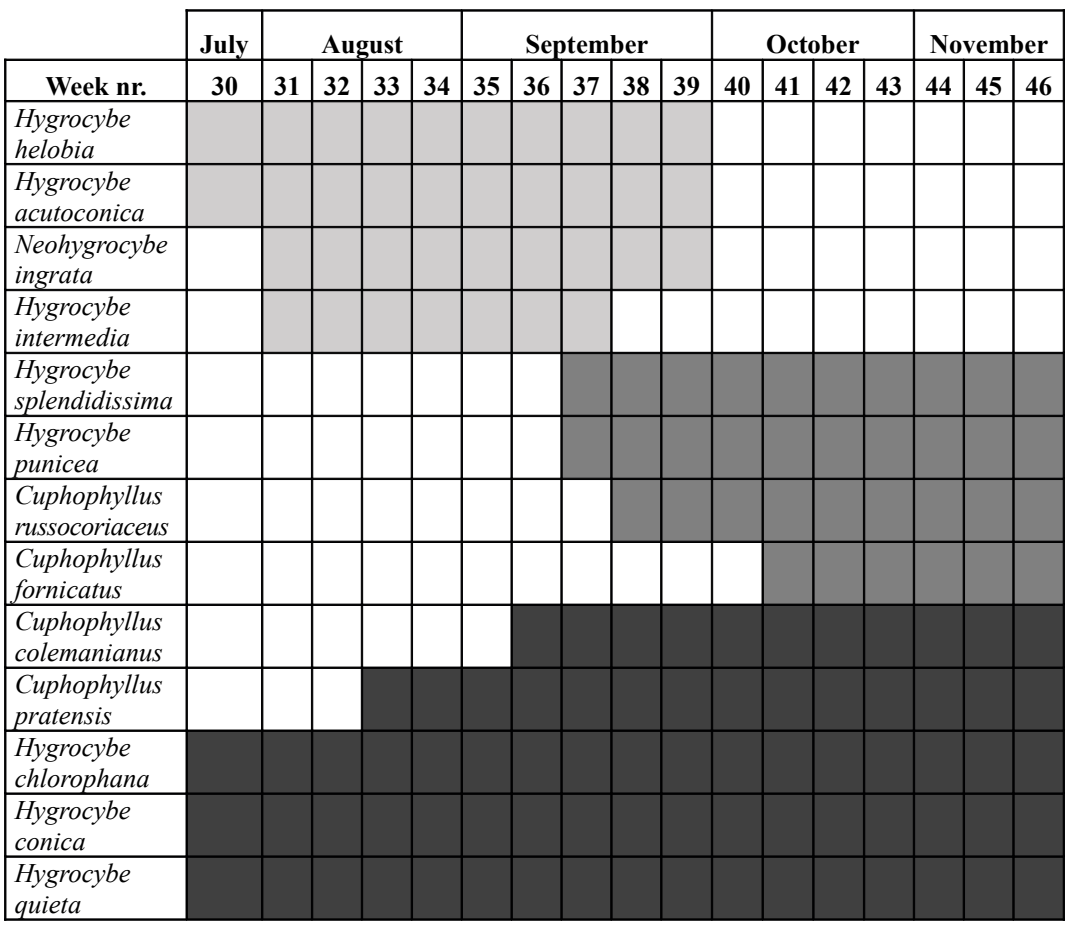


Figure 10. Time of fructifying for some selected waxcaps based on 20 years of survey.

One species of *Entoloma,* which has been subjected to molecular studies, came out as an unknown species by two occasions (Table 3) (Figure 11f). This species will be described and published in a coming paper.

Clavariaceae

A total of 16 different species in the family Clavariaceae are registered in the locality, representing the genera *Clavaria* (7), *Clavulinopsis* (5), *Ramariopsis* (1), *Camarophyllopsis* (1), *Hodophilus* (1) and *Lamelloclavaria* (1) (Figure 4). Most of these can be characterized as club-fungi, while the genera *Hodophilus* and *Camarophyllopsis* (which were earlier in the same genera) have many morphological similarities with waxcaps. The same applies to *Lamelloclavaria petersonii* which was found once in 2017 and confirmed with molecular studies in 2022 (Table 3) (Figure 12d). This fungus was first described in 2016 (Birkebak et. al. 2016) and this is the second find in Norway and the first in Western Norway (Brandrud et al. 2021). Two additional finds are made in Finland (type sp.) and Germany (Brandrud et al. 2021), which indicate that this must be a very rare species. Because of lack of data, it was listed as *data deficient* (DD) in the Norwegian red-list (Brandrud et al. 2021).

Experience with the family Clavariaceae from different locations is a very irregular fructification from year to year for some of the members. Two species have only appeared once at Hovaneset, namely *Clavaria tenuipes* and *Clavulinopsis umbrinella. Clavaria flavipes* appeared twice, *Clavaria fumosa* appeared three times while the rare species *Clavaria pullei* was found in six years since 2013 (Figure 12c). In the genus *Clavulinopsis* most of the species were found almost every year, *Ramariopsis subtilis* was found six times from 2010 and *Hodophilus foetens* was found eight times since 2004 (Figure 12b). However, in the dry year 2018, only one species in the family was found, namely *Clavaria spagnophila,* which grows in the wet part of the locality. Several of the species have been confirmed by molecular studies (Table 3).

Other grasslandfungi

Only two different species of typical grasslandfungi belonging to other genera are found in the locality. These are *Dermoloma cuneifolium* and *Pseudotricholoma metapodium* (*Porpoloma*) (Figure 12e). The last one has only been found once, in 2014, an optimal year for grasslandfungi, and has been confirmed by molecular studies (Table 3). *D. cuneifolium* is found almost every year since 2010 at least on three different spots.

DISCUSSION

The most interesting aspect with the survey is the 20-year long-time study of fungal sporocarps in a selected semi natural grassland in SW-Norway with a total of 180 visits during the years, which makes it probably the most intensive survey ever performed in a semi natural grassland.

No records of grassland fungi were made in the locality prior to the start of this survey back in 2003. The first seven years (2003-2009) differs from the rest of the survey with more random visits throughout the season compared with visits almost every week of the season in the last 13 years.

The results from the first seven years are therefore difficult to compare with the last 13 years, but it is interesting to see how the change in sampling method affects the number of fungi recorded during the first and second period (Period 1 and 2).

No year give anywhere near the full information about the fungi on the location even with a visit once a week in the season. Comparing Period 1 and 2 it's obvious that the low score in period 1 is due to random and low number of visits these years. The 180

visits to the locality for 20 years resulted in 92 different species of grasslandfungi (Figure 9). Since nearly all species (91) were found in period 2, this result (92 species) probably could have been obtained with fewer visits during fewer years given a systematic survey as in Period 2. With this high number of different grassland fungi, Hovaneset is today the most species rich grassland in Norway, and it probably could compete with the richest grassland in Sweden and UK (Phil 1992, Griffith et. al. 2013). The survey indicates how many fungi you can find in one visit. If you are choosing the optimal year and the optimal week of that year, you will only find maximum 50% of the fungi growing there (Table 2). This is more than stated by Parker-Rhodes (1955), who tells that not more than 25% of the fungi in a location will be found in one visit even with many visits. Orton (1986) confirms that several visits are necessary to get the right picture of the species growing on a location, but the timespan he refers to is much less than what is the case in this study both in number of years and number of visits.

Newton et al. (2003) concluded that at least 16 visits to a certain pasture were necessary to get a good understanding of the diversity of grassland fungi on the spot. This is not consistent with my survey. If you picked 16 random visits from the survey described in this paper during three years, you would probably find only a fraction of the fungi growing there. This is at least true if the pasture has a great potential for grasslandfungi with many rare species with an irregular fructification from one year to the other. The survey of Newton et al. (2003) is almost consistent with the sampling method of Halme & Kotiaho (2012), who are criticizing the more random sampling of fungi shown in literature. In the article they are reporting an intensive repeated study of wood-inhabiting fungi with the aim to determine the optimal timing and number of the surveys for reliable estimation of the diversity of this group of fungi. In this study they surveyed a forest once a month in the snowless season throughout four years, resulting in a total of 24 visits. They conclude that this method will give the optimal number of fungi in the location.

Even if the survey presented in this paper is about grasslandfungi, it could be interesting to challenge the statement made by Halme & Kotiaho (2012) using data from this survey. Based on data from Period 2 in this survey, results from one visit in the middle of each month (August -November) during the season in four following years has been tested. This results in ten different studies (2010-13, 2011-14 etc.), which can be compared with the total survey described above with 180 visits for 20 years. In none of these ten studies the number of fungi registered exceeded 67% of the number of fungi found in the total survey. The results ranged from 60 to 67% with an average of 63% for all the ten studies. Even if we included all years in Period 2 with one visit every month, it ended with a find percentage of 82, and many of the most seldom species from the survey would not have been found.

As a conclusion, the survey of Hovaneset shows that between 30 and 40% of the fungi from the location would have been excluded from the results using this method.

It not realistic that every survey of fungi should last for 20 years and almost 200 visits, but it shows clearly that it is not easy to get the full overview of the biodiversity of fungi in a location. One visit is normally only “a blink of the eye” of the total number of fungi on the spot, and the intense survey of this grassland has shown that several visits during the whole season for several years are necessary to get a hold on the diversity of fungi in the area. This is clearly shown in figure 9, indicating that even if the last 100 visits did not add many new species, it still count for

Figure 11. a. *Entoloma violaceoviride**, b. *E. cyaneolilliacinum**, c. *E. soropratulense**, d. *E. indutoides**, e. *E. neglectum*, f. *Entoloma sp.* (new species). Photo P. Fadnes.
*Sequenced

13% of the species found in the location. This indicates with all clarity that fructification for many rare and redlisted grassland fungi is a seldom and often irregular event.

The number of species found every single year of the survey was only ten, which coincide well with observation done by Staatsma et al. (2001), who found only eight species each

Figure 12. a. *Hygrocybe spadicea*, b. *Hodophilus foetens**, c. *Clavaria pullei**, d. *Lamelloclavaria petersenii**, e. *Pseudotricholoma metapodium**, f. *Geoglossum uliginosum*. Photo P. Fadnes.
*Sequenced

single year during a survey lasting for 21 years. As also shown in that study, new species appeared every year, which is almost the same results described in this paper.

The yearly surveys with more than ten visits to the locality gives you an impression of how many different species you can find in one visit, depending on the time of the visit and the year of visit (Table 2). If you hit the

right week for survey you will find between 18 (2018) and 47 (2021) different species in one visit, ranging from 20 to 51% of the total number growing there. That means that by "choosing" the right year and the right week you will find not more than 50% of the species in the location. More likely you are going to find far less on an occasional visit. The number varies, but shows that 2013, 2014 and 2021 were the most optimal years for grassland fungi in SW Norway.

The most common grassland fungi on the locality are found on many spots and could probably represent different mycelia. Others have only been found once at one spot and are probably seldom registered because there probably is only one mycelium. This is most likely also the case for many red-listed species found several times. As an example, *Clavaria pullei* is found six times since 2013, always on the same spot and therefore probably consist of only one mycelium. The same is for *Hodophilus foetens* which is found eight times always on the same three spots and could therefore probably consist of three different mycelia.

One interesting observation made was that in 2013 and 2014 there was many different *Entoloma* species found, and they also appeared in huge amounts (Figure 8). However, the following years the occurrence of *Entoloma* species was sparse, and for some species very often only a single basidiocarp was found. In 2021, a new year with many *Entoloma* finds occurred, resulting in many new species verified by molecular studies (Table 3). This shows, at least in SW-Norway, that the genus *Entoloma* has great variation in fructifying and in number of basidiocarps from one year to the other. In this study there was six to seven years between years with high numbers of *Entoloma* species (Figure 4 and 8).

The same is true for the family Clavariaceae, where the irregular fructifying is particularly observed among species in the genus *Clavaria*. Seven *Clavaria* species are found in the location, and especially the red-listed species C. *fumosa, C. flavipes, C. pullei and C. tenuipes* have few years of finds, varying from one to six. This observation coincides with observations made in other semi natural grasslands in SW-Norway.

The species *Lamelloclavaria petersenii* was found on the location as the second find in Norway and appeared only once in 2017 and has never been found in the later years. It is obviously a rare species with irregular fructification.

The earthtongues (s.l.) seems to be more stable in fructifying from one year to the other, varying from finds of seven to ten different species each year. Even the exceptionally dry year 2018 resulted in finds of seven different species although the total number of species was low (Figure 4). Some earthtongues like *Geoglossum simile*, *Trichoglossum hirsutum* and *T. walterii* seem to have wide ecological preferences when it comes to soil consistency. On the other hand, *Geoglossum uliginosum* was always found on swampy areas. The variation in habitat in the location probably explains the relatively high number of earthtongues growing here.

Based on the results from this study it is legitimate to ask if the season for the bloom of grasslandfungi have changed during this study. The maximum number of fungi found in one visit has been delayed by several weeks from 2014 to 2021/22 (Figure 7). In 2013/14 the maximum peak was in week 37 (medio September) and in 2021/22 it was in week 41 (medio October). This is even more evident if we only look at the genus *Entoloma* which has a shorter fruiting period (Figure 8). This shows a delay in fruiting of around 30 days in 2021/22 compared with 2013/14. This is also shown to a degree in Table 2. The reason for this delay can only be speculation. It could be due to great variation in fructifying of some of the species or local vari-

ation in weather from one year to the other. However, the actual weather in SW-Norway does not show that great variation between years and can hardly count for these differences (NMI 2022). A nearby speculation is of course climate change, which makes the season for grassland fungi in SW-Norway gradually more like the season in UK. The impact of climate change on fruiting season of fungi has been pointed out by several authors (Kauserud et al. 2007, Andrew et al. 2018), and could therefore also be the reason for the observation made in this study.

One interesting follow-up study of my survey should be comparing the carpophores found with fungi DNA from soil samples on the location.

REFERENCES

Alvarado P, Moreno G, Manjón JL, 2012. Comparison between *Tuber gennadii* and *T. oligospermum* lineages reveals the existence of the new species *T. cistophilum* (Tuberaceae, Pezizales). Mycologia 104:894–910.

Andrew C, Heegaard E, Gange AC, Senn-Irlet B, Egli S, Kirk PM, Büntgen U, Kauserud H, Boddy L, 2018 Congruency in fungal phenology patterns across dataset sources and scales. Fungal Ecology.32: 9-17.

Arauzo S, Iglesias P, 2014. La familia Geoglossaceae en la península Ibérica y Macaronesia. Errotari. 11: 166-259.

Arnolds E, 1989. The influence of increased fertilization on the macrofungi of av sheep meadow in Drenthe, the Netherlands. Opera Botanica 100: 7-21.

Artsdatabanken, 2022. Artskart. https://artskart.artsdatabanken.no (Downloaded December 2022.).

Birkebak JM, Adamčík S, Looney BP, & Matheny PB. 2016. Multilocus phylogenetic reconstruction of the Clavariaceae (Agaricales) reveals polyphyly of agaricoid members. Mycologia, 108(5), 860–868. doi:10.3852/15-370.

Boertmann D, 2010. The genus Hygrocybe. 2nd revised edition. Fungi of Northern Europe 1. Danish Mycological Society. 200 pp.

Brandrud TE, Bendiksen E, Blaalid R, Hofton TH, Jordal JB, Nordén J, Nordén B and Wollan AK. 2021. Sopper. Rødlista for arter 2021. Artsdatabanken. https://artsdatabanken.no/rodlisteforarter2021/Artsgruppene/Sopp

Brandrud TE, Bendiksen E, Jordal JB, Weholt Ø, Dima B, Morozova O, Noordeloos ME, 2020. On some new or little known *Entoloma* species (Tricholomatinae, Basidiomycota) from Norway. Agarica 39: 31-52.

Dima B, Lindström H, Liimatainen K, Olson A, Soop K, Kytövuori I, Dahlberg A, Niskanen T, 2016. Typification of Friesian names in Cortinarius sections Anomali, Spilomei, and Bolares, and description of two new species from northern Europe. Mycol. Prog. 15:903–919.

Dima B et al. 2023. Phylogenetic relationships of the European species of *Entoloma* subgenus *Cyanula* in a global perspective inferred from ribosomal DNA sequences. Persoonia (in press).

Evans S, 2003. Waxcap-grasslands - an assessment of English sites. Peterborough, UK. English Nature Research Reports No. 555. 49 pp.

Fadnes P, 2011. Jordtunger (Geoglossaceae) i Sunnhordland, Vest Norge - økologiske obsevasjoner og oversikt over nyfunn og utbredelse. Agarica 30: 47-62. [Earthtongues (Geoglossaceae) in Sunnhordland West-Norway – ecological observations, new records and distribution].

Fadnes P, 2014. Variasjoner i mangfold og fruktifisering av beitemarksopp basert på 11 års inventering av kulturlandskap i Sunnhordland, Vest-Norge. Agarica 35:93-105. [Variations in diversity and fructification of grassland fungi based on 11years investigation of cultural landscapes in South-Western Norway].

Fadnes P, 2021. Ny og supplerende kartlegging av biologisk mangfold i Sunnhordland og Nord-Rogaland 2015-2020. 2021. HVL-rapport nr. 10. 2021 61s. [New and supplementary survey of biological diversity in South-Western Norway 2015-2020.] Fadnes P, 2023. Grasslandfungi.no: https://grasslandfungi.no/

Fadnes P, Fedosova AG, Kučera V, 2021. *Hemileucoglossum pusillum*, an earthtongues new to Norway. Agarica 42:65-73.

Fedosova AG, Popov ES, Lizoň P, Kučera V, 2018. Towards an understanding of the genus *Glutinoglossum* with emphasis on the *Glutinoglossum glutinosum* species complex (*Geoglossaceae*, *Ascomycota*). Persoonia 41: 18-38.

Gardes M, Bruns TD, 1993. ITS primers with enhanced specifity for basidiomycetes, application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113–118.

GBIF.org (9 March 2023) GBIF Occurrence Download https://doi.org/10.15468/dl.mfx8ej.

Griffith GW, Gamarra JGP, Holden EM, Mitchel D, Graham A, Evans DA, Evans SE, Aron C, Noordeloos ME, Kirk PM, Smith SLN, Woods RG, Hale AD, Easton GL, Ratkowsky DA, Stevens DP, Halbwachs H, 2013. The international conservation importance of Welsh 'waxcap' grasslands. Mycosphere 4:969-984.

Halbwachs H, Dentinger BTM, Detheridge AP, Karasch P, Griffith GW, 2013. Hyphae of waxcap fungi colonise plant roots. Fungal ecology 6(6):487-492.

Halme P, Kotiaho JS, 2012. The importance of time and number of surveys in fungal biodiversity research. Biodivers. Conserv. 21:205-219.

Hustad VP, Miller AN, Moingeon JM, Priou JP, 2011. Inclusion of *Nothomitra* in *Geoglossomycetes*. *Mycosphere* 2(6) :646-654.

Hustad VP, Miller A, Dentinger BTM, Cannon PF, 2013. Generic circumscriptions in Geoglossomycetes. Persoonia 31:101–111.

Imai S, 1942. Contributiones ad studia monographica Geoglossacarum. Bot. Mag., Tokyo 56:523-527.

Jordal JB, 1997. Sopp i naturbeitemarker i Norge. En kunnskapsstatus over utbredelse, økologi, indikatorverdi og trusler i et europeisk perspektiv. Direktoratet for Naturforvaltning, Utredning for DN nr. 6 - 1997. 112 pp. [Fungi of seminatural grasslands in Norway. Status of knowledge about distribution, ecology, indicator value and threats in a European perspective].

Jordal JB, 2011. Åtgärdsprogram för svampar i ängs- och betesmarker 2011-2015. Naturvårdsverket rapport 6423. Stockholm. 78 pp. [Actionplan for the conservation of grassland fungi 2011-2015].

Jordal JB, 2013. Naturfagleg utgreiing om truga beitemarkssoppar, med forslag til utval av prioriterte artar. Rapport J.B. Jordal nr. 2-2013. 47 pp. [Analysis of endangered species of grasslandfungi, with suggestions for a selection of priority species.]

Jordal JB, Larsson E, 2021. *Cuphophyllus flavipesoides*. Fungal Planet 1237. In Crous PW, Cowan DE, Maggs-Kölling G, et al. 2021. Persoonia 46: 313-528.

Kauserud H, Stige LC, Vik JO, Økland RH, Stenseth NC, 2008. Mushroom fruiting and climate change. PNAS.105: 3811-3814.

Lodge DJ, Padamsee M, Matheny PB, Aime MC, Cantrell SA, Boertmann D, Kovalenko A, Vizzini A, Dentinger BTM, Kirk PM, Ainsworth AM, Moncalvo J-M, Vilgalys R, Larsson E, Lücking R, Griffith GW, Smith ME, Norvell LL, Desjardin DE, Redhead SA, Ovrebo CL, Lickey EB, Ercole E, Hughes KW, Courtecuisse R, Young A, Binder M, Minnis AM, Lindner DL, Ortiz-Santana B, Haight J, Læssøe T, Baroni TJ, Geml J, Hattori T, 2014. Molecular phylogeny, morphology, pigment chemistry and ecology in Hygrophoraceae (Agaricales). Fungal Diversity. 64:1-99.

McHugh R, Mitchel D, Wright M, Anderson R, 2001. The fungi of Irish grasslands and their value for nature conservation. Biol. Environ. 101B: 225-242.

Newton AC, Davy LM, Holden E, Silverside A, Watling R, Ward SD, 2003. Status, distribution and definition of mycologically important grasslands in Scotland. Biological conservation 111: 11-23.

Nitare J, 1988. Jordtungor, en svampgrupp på tillbakagång i naturliga fodermarker. Svensk-Bot. Tidskr. 82: 341-368. [Earth tongues, a declining group of fungi in seminatural grasslands].

NMI. Norwegian Meteorological institute. Stord airport. https://seklima.met.no/ (Downloaded December 2022).

Noordeloos ME, 1992. Entoloma s.l. Fungi Europaei 5. Saronno, Italia, 1-760.

Noordeloos ME, 2004. Entoloma s.l. Supplemento. Fungi Europaei 5b, Italia 1-1378.

Noordeloos ME, Lorås J, Eidissen SE, Brandrud TE, Bendiksen E, Morozova O, Jordal JB, Weholt Ø, Jansen GM, Larsson E, Dima B, 2020. Three new *Entoloma* species of the Cyanula clade (Entolomataceae, Agaricales) from (sub)alpine habitats in Northern Norway and Sweden. *Sydowia* 73: 185–196.

Noordeloos ME, Jordal JB, Brandrud TE, Dima B, 2021. *Entoloma cyaneoliliacinum*. Fungal planet 1243. In Crous PW, Cowan DE, Maggs-Kölling G. et al. 2021. Persoonia 46: 313-528.

Noordeloos ME, Jansen GM, Jordal JB, Morozova O, Weholt Ø, Brandrud TE, Vila J, Karich A, Rechke K, Lorås J, Krisai-Greilhuber I, Dima B, 2022a. Molecular and morphological diversity in the Rhombisporum clade of the genus Entoloma with a note on E. cocles. Mycologica progress. https://doi.org/10.1007/s11557-022-01779-z

Noordeloos ME, Morozova, Dima B, Reschke K, Jansen G, Brandrud TE, Jordal JB, Bendiksen E, Vila J, 2022b. ENTOLOMA s.l. Flora agaricina neerlandica, vol 1. supplement. Subgenera *Cyanula, Leptonia, Nolanea, Trichophilus*, and the Rhombisporum clade. Fungi Europaei. Candusso Editrice Italia.

Ohenoja E, Wang Z, Townsend JP, Mitchel D, Voitk A, 2010. Northern species of earth tongue genus Thuemenidium revisited, considering morphology, ecology and molecular phylogeny. Mycologia 102(5):1089-1095.

Orton PD, 1986. Fungi of Northern pine and Boreal woods. Bulletin of the British Mycological Sociely 20: 130-144.

Parker-Rhodes AF, 1955. Statistical aspects of fungus forays. Transactions of the British Mycological Sociery 38: 283-290.

Papp V, Dima B, 2018. New systematic position of Aurantiporus alborubescens (Meruliaceae, Basidiomycota), a threatened old-growth forest polypore. Mycol Progress 17: 319–332.

Pihl R, 1992. Presentation av två biotoptyper i Södra Älvsborg. Jordstjärnan 13: 23-28. [Presentation of two habitat types in Södra Älvsborg].

Rald E, 1985. Vokshatte som indikatorarter for mykologisk verdifulle. overdrevslokaliteter. Svampe 11:1-9. [Waxcaps as indicator for mycological valuable grasslands].

Ratnasingham S, Hebert PDN, 2007. BOLD: The Barcode of Life Data System. (www.barcodinglife.org). Molecular Ecology Notes 7, 355-364. DOI:10.1111/j.1471-8286.2006.01678.x

Ratnasingham S, Hebert PDN. 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. PLoS ONE 8(8): e66213. DOI:10.1371/journal.pone.0066213

Sandnes ACS, 2006. Phylogenetic relationships among species and genera of Geoglossaceae (Helotiales) based on ITS and LSU nrDNA sequences. Cand. Scient. Thesis., University of Oslo, Dep. of biology.

Seitzman BH, Ouimette A, Mixon RL, Hobbie EA, Hibbett DS, 2011. Conservation of biotrophy in Hygrophoraceae inferred from combined stable isotope and phylogenetic analyses. Mycologia 103(2): 280-290. DOI:10.3852/10-195

Straatsma G, Ayer F, Egli S, 2001. Species richness, abundance and phenology of fungal fruit bodies over 21 years in a Swiss forest plot. Myco. Res. 105 (5): 515-523.

Schoch CL,Wang Z, Townsend JP, Spatafora JW, 2009. *Geoglossomycetes* cl. nov., *Geoglossales* ord. nov. and taxa above class rank in the *Ascomycota* Tree of Life. Persoonia 22: 129-138.

University of Oslo (2023). Fungarium, Oslo (O) UiO. Version 1.2091. Occurrence dataset https://doi.org/10.15468/6xjrdn accessed via GB.

Vesterholt J, Knudsen H, 1990. Truede storsvampe i Danmark – en rødliste. Foreningen til svampekundskapens fremme, København. [Threatened macrofungi in Denmark- a redlist].

Watling R, 1995. Assessment of fungal diversity-Macromycetes, the problems. Canadian Journal of Botany 73; Suppl. I: 1-24.

Öster M, 2008. Low congruence between the diversity of Waxcap (Hygrocybe spp.) fungi and vascular plants in semi-natural grasslands. Basic and Applied Ecology 9: 514-522.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and amplifications. Academic, New York, pp 315–322.

ATTACHEMENTS

Tables 1, 2 and 3 on the following pages.

Table 1. Different species of grassland fungi found at Hovaneset 2003-2022, redlist-category (RL) and number of yearly finds.
* One find molecular determined: *Cuphophyllus flavipes*
** One find molecular determined: *Entoloma pentagonale*
*** *Entoloma* sp. subjected to molecular studies: no match

Species	RL	Yearly finds	Species	RL	Yearly finds
Camarophyllopsis schulzeri	NT	10	*Entoloma soropratulense*		2
Clavaria falcata		2	*Entoloma turci*	NT	2
Clavaria flavipes	VU	2	*Entoloma violaceoviride*	DD	1
Clavaria fragilis		2	*Entoloma sp.***		6
Clavaria fumosa	NT	2	*Geoglossum cookeanum*	NT	1
Clavaria pullei	VU	6	*Geoglossum fallax*		20
Clavaria sphagnicola		4	*Glutinoglossum glutinosum*		16
Clavaria tenuipes	VU	1	*Geoglossum simile*	NT	10
Clavulinopsis corniculata		16	*Geoglossum starbaeckii*		7
Clavulinopsis helvola		19	*Geoglossum uliginosum*	VU	14
Clavulinopsis laeticolor		5	*Geoglossum umbratile*		14
Clavulinopsis luteoalba		12	*Gliophorus irrigatus*		19
Clavulinopsis umbrinella	NT	1	*Gliophorus laetus*		20
Cuphophyllus colemannianus	VU	20	*Gliophorus psittacinus*		20
*Cuphophyllus flavipes (coll)**	VU	10	*Hemileucoglossum pusillum*	DD	12
Cuphophyllus flavipesoides		1	*Hodophilus foetens*	VU	8
Cuphophyllus fornicatus	VU	7	*Hygrocybe acutoconica*		12
Cuphophyllus pratensis		20	*Hygrocybe aurantiosplendens*	NT	1
Cuphophyllus russocoriaceus	NT	20	*Hygrocybe cantharellus*		13
Cuphophyllus virgineus		20	*Hygrocybe ceracea*		18
Dermoloma cuneifolium	NT	9	*Hygrocybe chlorophana*		20
Entoloma indutoides	DD	1	*Hygrocybe coccinea*		20
Entoloma allospermum		1	*Hygrocybe coccineocrenata*		1
Entoloma ameides	NT	1	*Hygrocybe conica*		20
Entoloma atrocoeruleum	NT	16	*Hygrocybe glutinipes*		8

Table 1. Different species of grassland fungi found at Hovaneset 2003-2022, redlist-category (RL) and number of yearly finds.
* One find molecular determined: *Cuphophyllus flavipes*
** One find molecular determined: *Entoloma pentagonale*
*** *Entoloma* sp. subjected to molecular studies: no match

Entoloma caesiocinctum		8	*Hygrocybe helobia*		10
Entoloma carneogriseum	DD	1	*Hygrocybe insipida*		10
Entoloma chalybaeum	NT	9	*Hygrocybe intermedia*	VU	10
Entoloma clandestinum		8	*Hygrocybe miniata*		16
Entoloma conferendum		13	*Hygrocybe mucronella*	NT	9
Entoloma cyaneolilacinum		2	*Hygrocybe phaeococcinea*		9
Entoloma exile		12	*Hygrocybe punicea*		19
Entoloma formosum		4	*Hygrocybe quieta*	NT	15
Entoloma glaucobasis	VU	1	*Hygrocybe reidii*		19
Entoloma griseocyaneum	NT	7	*Hygrocybe spadicea*	VU	1
Entoloma infula		8	*Hygrocybe splendidissima*	VU	18
Entoloma juncinum		2	*Hygrocybe subpapillata*	VU	3
Entoloma majusculum		5	*Lamelloclavaria petersenii*	DD	1
Entoloma mougeotii	NT	9	*Mikroglossum atropurpureum*	VU	18
Entoloma neglectum	VU	1	*Neohygrocybe ingrata*	VU	10
*Entoloma rhombisporum (coll)***	VU	5	*Neohygrocybe nitrata*	NT	4
Entoloma poliopus		2	*Neohygrocybe ovina*	VU	15
Entoloma prunuloides	VU	10	*Pseudotricholoma metapodium*	EN	1
Entoloma sericellum		13	*Ramariopsis subtilis*	NT	6
Entoloma sericeum		12	*Trichoglossum hirsutum*		16
Entoloma serrulatum		12	*Trichoglossum walterii*	VU	15

Table 2. Annual and weekly finds as well as percent of finds of grassland fungi the last ten years of survey.

	Weekly Finds			Yearly Finds	
Year	Maximal different finds/visit	% of total (92)	Week nr.	Number of different finds	% of total (92)
2013	35	38 %	37	61	66 %
2014	35	38 %	37	66	72 %
2015	32	35 %	39	59	64 %
2016	33	36 %	38	54	59 %
2017	34	37 %	37	53	58 %
2018	18	20 %	39	39	42 %
2019	31	34 %	42	57	63 %
2020	38	41 %	39	52	57 %
2021	47	51 %	41	67	73 %
2022	38	41 %	41	51	55 %

Table 3. List of grassland fungi subjected to molecular studies. For a description of methods used and procedures followed, with references, see “Materials and methods”.
*Noordeloos et al. 2022a
**Fadnes et al. 2021

Sequenced specimens	**Voucher**	**Dato**	**Sequencing**
Agaricus phorphyrocephalus	missing	20.08.2018	Alvalab
Clavaria flavipes	O-F-303977	12.10.2014	NorBOL
Clavaria pullei	O-F-245863	21.08.2013	NorBOL
Clavaria tenuipes	O-F-303976	02.10.2014	NorBOL
Cuphophyllus flavipes	O-F-204303	16.08.2021	Dima
Cuphophyllus flavipesoides	O-F-259048	11.10.2021	Dima
Entoloma allospermum	O-F-204173	28.09.2021	Dima
Entoloma allospermum	O-F-204178	11.10.2021	Dima
Entoloma allospermum	O-F-204182	11.10.2021	Dima
Entoloma atrocoeruleum (OTU 33)	O-F-242498	22.08.2011	NorBOL
Entoloma atrocoeruleum (OTU 33)	O-F-204175	28.09.2021	Dima
Entoloma atrocoeruleum (OTU 33)	O-F-204176	22.09.2021	Dima
Entoloma atrocoeruleum (OTU 33)	O-F-204183	11.10.2021	Dima
Entoloma atrocoeruleum (OTU 33)	O-F-204304	22.09.2021	Dima
Entoloma carneogriseum	O-F-204185	11.10.2021	Dima
Entoloma chalybeum	O-F-256344	04.09.2019	NorBOL
Entoloma clandestinum	O-F-204170	14.09.2020	Dima
Entoloma clandestinum	O-F-204177	22.09.2121	Dima
Entoloma cyaneoliliacinum	O-F-259049	28.09.2021	Dima
Entoloma cyaneoliliacinum	O-F-204184	11.10.2021	Dima
Entoloma exile	O-F-204174	28.09.2021	Dima
Entoloma glaucobasis	missing	16.08.2021	Dima
Entoloma indutoides	missing	28.08.2010	Alvalab
Entoloma majusculum	O-F-204291	23.08.2021	Dima
Entoloma majusculum	O-F-204306	22.09.2021	Dima
Entoloma mougeotii (OTU 31)	O-F-245808	05.08.2012	NorBOL
*Entoloma pentagonale**	PF 1-16*	14.09.2016	Alvarado

Table 3. List of grassland fungi subjected to molecular studies. For a description of methods used and procedures followed, with references, see “Materials and methods”.
*Noordeloos et al. 2022a
**Fadnes et al. 2021

Entoloma poliopus	O-F-304593	19.08.2016	NorBOL
Entoloma poliopus	O-F-204172	28.09.2021	Dima
Entoloma poliopus	O-F-204181	11.10.2021	Dima
Entoloma sorapratulense	O-F-259050	10.08.2014	Dima
Entoloma unknown	O-F-245862	21.08.2013	NorBOL
Entoloma unknown	O-F-75970	08.09.2014	NorBOL
Entoloma violaceoviride	O-F-204171	28.09.2021	Dima
Entoloma violaceoviride	O-F-204179	11.10.2021	Dima
Gliophorus irrigatus	O-F-256325	04.09.2021	NorBOL
Gliophorus psittacinus	O-F259051	28.09.2021	Dima
*Hemileucoglossum pusillum***	O-F-757329	10.09.2017	Alvalab
Hodophilus foetens	O-F-242513	11.09.2011	NorBOL
Hygrocybe glutinipes	O-F-293270	25.09.2010	NorBOL
Hygrocybe mucronella	O-F-245865	13.10.2013	NorBOL
Lamelloclavaria petersenii	O-F-204305	10.09.2017	Dima
Pseudotricholoma metapodium	O-F-303974	12.10.2014	NorBOL
Trichoglossum hirsutum	O-F-204289	16.12.2020	Dima