UNEARTHING THE DIVERSITY OF ALPINE FUNGI IN THE CAIRNGORMS

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Soil is one of the last great frontiers for biodiversity exploration on planet earth. In 2021, scientists from the James Hutton Institute in Aberdeen worked with the plant and fungi conservation charity Plantlife and volunteers from the mountaineering community in and around north-east Scotland to explore the belowground biodiversity of the Cairngorms National Park.

Recent estimates suggest that around 60% of all terrestrial biodiversity resides in the soil, encompassing all types of life forms, from microbes to mammals, making the soil the singular, most biodiverse habitat on Earth. Despite this, and despite our growing understanding of the importance of soil biodiversity in the functioning of ecosystems and the services they provide to human societies, we know far more about aboveground biodiversity than we do about what inhabits the ground beneath our feet.

This huge knowledge gap is particularly acute for our most remote and inaccessible habitats, such as those found in the alpine zone, with much of our present knowledge derived from lowland and agricultural habitats. Given the rapid pace of climate change and the impacts of other drivers such as nitrogen pollution, our mountain habitats are under greater pressure than ever before. Now is a critical time to fully explore the unique biodiversity they support, both above and below ground, before that biodiversity is potentially lost.

High alpine plateau on Ben Avon. The mosaic of different plant species and habitats is clearly visible, but what about the biodiversity below ground? Photo credit Andrea Britton

Soil fungi

Fungi are an essential component of soil biodiversity and play critical roles in ecosystem functioning. Many species are decomposers of organic materials, some form mutualistic associations with plants, while others are parasites or pathogens of other organisms. Decomposer fungi play a particularly important role in carbon and nutrient cycling, breaking down complex organic matter into simpler molecules which are then consumed or re-cycled within the ecosystem. Their activity is an important control on the amount of carbon which is stored in the soil or released to the atmosphere. Mutualistic fungi include many species that colonise the roots of plants forming arbuscular, ericoid, or ectomycorrhizal associations. Arbuscular and ericoid mycorrhizal fungi make structures inside the root cells which look like little trees (arbuscules) or coils respectively, while ectomycorrhizal fungi make dense sheath of fungal threads around the root tips. These mutualistic associations are essential for plant growth, with the fungi supplying nutrients and water to plants in exchange for sugars from photosynthesis. Neither the plants nor the fungi can survive in the absence of the other. While some mycorrhizal fungi are able to associate with a wide range of plant partners, other species are specialists on particular plant hosts. In many cases, establishment and growth of a plant is only possible if a suitable fungal partner is present in the soil.

Despite the clear functional importance of fungi, our knowledge of their diversity and distribution is still very limited. There are currently thought to be around 2.5 million species of fungi globally (although some estimates are as high as 165 million) with around 90% yet to be described and named. With the vast majority of fungi living in soil, this means that there could be literally millions of unknown species in our soils, waiting to be discovered.

Foxy bolete (*Leccinum vulpinum*) in alpine dwarf-shrub heath in the Cairngorms. This is a mycorrhizal species that commonly grows with Scots pine, but which also grows with bearberry and has been recorded at several locations around the Cairngorms. Photo credit Andrea Britton

Soil fungi can usually only be detected visually when they produce aboveground macroscopic structures – the fruit bodies (i.e., 'mushrooms'). But the great majority of species either fruit infrequently, or not at all, or actually produce fruit bodies underground (e.g., truffles). Even if the fungi do produce above ground fruit bodies, their detection and recording require that a person is present to see the fruit body *and* that they have the necessary skills to identify the species or can pass information on to someone who does. Despite the UK being a country with a strong tradition of amateur naturalists and biological recording, skilled field-mycologists are few and far between and the likelihood of the right person being in the right place at the right time to detect a species is usually very low. This is particularly true for our alpine habitats where large areas may be rarely visited.

Environmental DNA

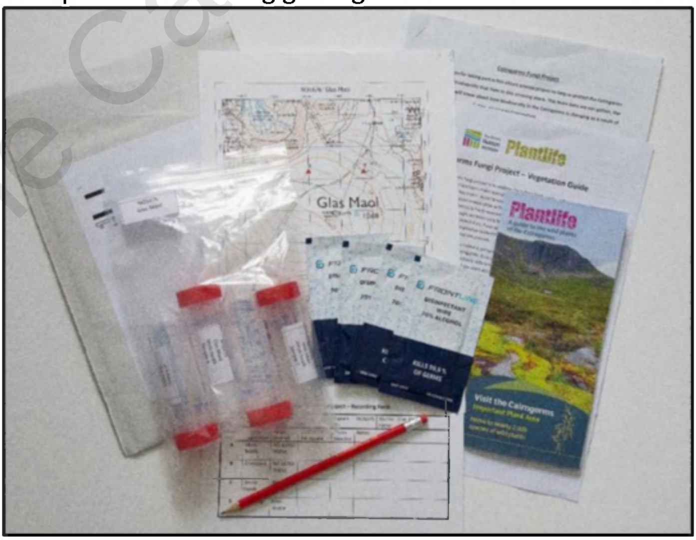
In the past 10 years or so, the analysis of DNA from environmental samples such as soil and water (so-called eDNA) has started to transform our ability to detect organisms which may leave few visible clues of their presence. DNA of organisms living in the soil is present both in the living organisms and in dead cells shed into the soil and can be extracted and used for identification purposes. Identification relies upon extracting DNA and then selectively generating short DNA sequences of the organisms of interest. These short sequences are called barcodes, and function in much the same way as the unique barcodes on produce in supermarkets. The number of different barcode sequences detected in a sample indicates the number of species which are present. Individual barcodes generated from environmental samples are compared and identified using reference barcodes in online databases. The reference barcodes are derived from previously identified fungi. The major drawback of this is that only a tiny fraction of the vast diversity of fungal species are actually represented in databases, which means that only a small number of barcodes can usually be identified to species. The majority of the barcodes remain unidentified – sometimes all we can say is simply that they are fungi.

While DNA analysis of environmental samples was initially very expensive, rapid advances in sequencing technology have greatly reduced the costs involved, to the point where it is now possible to extract and analyse DNA from large numbers of environmental samples, providing a cost-effective method of characterising soil biodiversity. The greatest barrier to fully exploring soil biodiversity in alpine habitats is now the logistical challenge of obtaining soil samples from remote areas.

The Cairngorms Fungi project

In Scotland, completing a round of the Munros is a popular objective for an ever-increasing number of people. Popular summits and those in the more accessible mountain regions may receive many thousands of visits per year. If these mountain visits could be used to gather biodiversity data, we could vastly increase our understanding of the species present in our alpine habitats. In 2021, the Cairngorms Fungi project was conceived to test whether scientists and the hill-going public could work together utilising eDNA methods to explore alpine soil biodiversity.

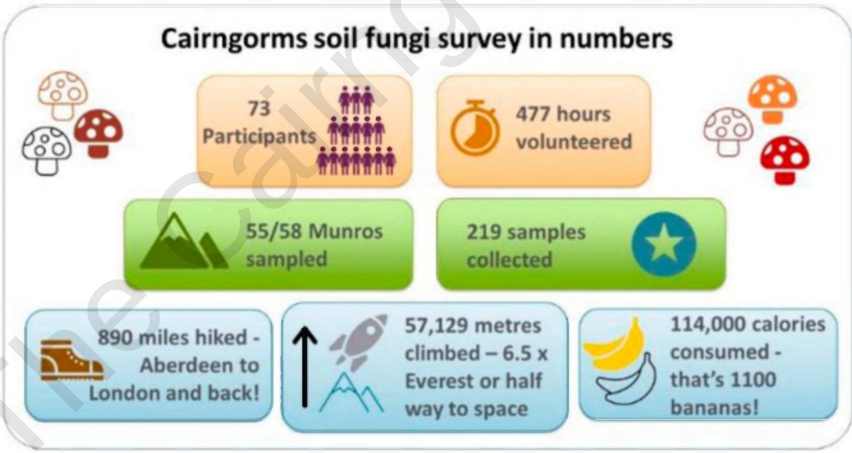
The project aimed to collect soil samples from the 58 Munros located within the Cairngorms National Park boundary and to identify the fungi present in the soil using DNA analysis. Volunteers could 'adopt' a Munro of their choice and were sent a sampling pack with instructions on how to collect a soil sample for DNA analysis and a map with three pre-defined locations for sampling. DNA analysis uses a very small amount of soil and so volunteers only needed to collect a 50ml sample which was sufficient for both DNA extraction and chemical analysis of soil pH and carbon and nitrogen contents. Their small size also meant that samples could be collected with negligible damage to the environment. The DNA analysis technique is very sensitive, so it is important to avoid any contamination of the samples. Volunteers were provided with alcohol wipes to clean sampling implements between samples and instructions on how to sample while avoiding getting their own DNA into the tube.



Example of a sampling pack sent out to volunteer citizen scientists. Photo credit Andrea Britton

The sampling was targeted at three of the most widespread alpine habitats in the Cairngorm National Park, namely Woolly fringe moss (*Racomitrium lanuginosum*) moss heath, alpine dwarf-shrub heath, and Mat grass (*Nardus stricta*) grassland. High resolution aerial photography and pre-existing habitat maps were used to locate areas of these three habitats on each Munro and grid references for suitable sampling points were supplied to the volunteers. In addition, the volunteers were supplied with a fourth sampling tube for them to collect a 'lucky dip' soil sample from a location and habitat of their choice (wherever they thought would be interesting).

The project was launched in July 2021. This was a nervous moment for the project team while we waited to find out if the project would appeal to the hill-going public – would people be prepared to take time out of their walk to collect a sample? Would finding out about soil fungi be interesting to them? Fortunately, the answer to both questions was a resounding yes, and, over the following 3 months, a total of 73 volunteers hiked, biked and ran up 55 of the 58 Munros in the park and gathered a total of 219 samples.



Collection of the samples represented a huge input of time and effort from our dedicated group of volunteers. Photo credit Andrea Britton

Volunteer sampling soil from Racomitrium moss heath, one of the target habitats for the survey, on Glas Maol. Photo credit Andrea Britton

The results

So, what did the survey reveal? In total, we obtained more than 9.3 million DNA barcode sequences from the 219 samples, ranging from 18- to 100-thousand sequences per sample. These sequences were grouped into 2748 unique barcodes or taxa, of which 268 (10%) could be given a species name, 304 (11%) could only be identified as a fungus, and the remaining 2176 could be identified to varying taxonomic levels of detail (genus, family etc).

We found between 17 and 160 taxa in each soil sample, with the highest diversity being recorded in a moss heath sample from Beinn a' Bhuird. Only 48 taxa could be classed as common, being present in 25% or more of the samples. Most taxa (2700) were present in less than 25% of samples and an astonishing 45% (1161) were actually only found in a single sample. This type of distribution, with a few common species but many rare species making up the bulk of the biodiversity in a habitat is typical for many communities.

In terms of the total number of fungal taxa found on individual mountain summits, Beinn a' Bhuird came out on top with 359 taxa detected, closely followed by Beinn Mheadhoin (358), Cairn Gorm (352), Broad Cairn (309) and Glas Maol (304). Species found only in a single sample are also an important component of biodiversity, because they are likely to represent scarce species and those with more specialised habitat requirements. Beinn a' Bhuird also had the greatest number of unique fungi detected (56), followed by Beinn Mheadhoin, Ben Macdui and The Cairnwell (all 49 unique taxa) and Cairn Gorm (45).

A Brittlegill fungus (*Russula sp.*) fruiting in amongst mosses and lichens on Cairngorm. This species forms a mycorrhizal association with dwarf willow (*Salix herbacea*). Four species of Russula were detected in the survey. Photo credit Andrea Britton

The most common fungi detected in the survey were *Solicoccozyma terricola* - which is a common soil yeast, *Mortierella humilis* and *Mortierella macrocystis* - which are part of a group of widespread soil saprotrophs (decomposers of dead organic material), and a species of *Tolypocladium* - a group of species which are saprotrophic, or which live on other fungi. Despite these species being extremely common, found in more than half of the samples in the survey, we know very little about their ecology and some have not been recorded in the UK before.

Of those taxa which could be identified to species level, a number of interesting finds were made which illustrate the variety of life histories found among our alpine fungi and give some potential insights into the environmental conditions experienced by alpine communities in Scotland and their relationships to extreme habitats in other parts of the world.

The Antarctic connection

Perhaps one of the biggest surprises and possibly the rarest fungus we found in the survey was the species *Acrodontium antarcticum*. As far as we know this species was previously only known from one locality in the world - the Danco Coast, in the northern part of the Antarctic continent. The fungus was first described in 1989 by a mycologist called Cabello after being isolated and grown from the Danco Coast soil collected from around the roots of one of the only two native flowering plants which grow on Antarctica, the Antarctic pearlwort (*Colobanthus quitensis*). It is likely that the fungus was surviving partly on decomposing dead plant matter from the Pearlwort while also getting sugars from the plant roots. The appearance of this Antarctic fungus in the survey gives a good indication of the harsh conditions experienced by soil organisms on Scottish mountain summits. The fungus also demonstrates very nicely how little we know about the biodiversity in our alpine soils. However, this fungus may not be as rare as it first appears, as it was found in soil samples from 18 out of the 55 Munros sampled. This would strongly suggest that the fungus may actually be much more widespread than previously thought. This contradiction can partly be explained by the fact that it produces no visible structures above ground which could be seen and recorded, and its detection requires either isolation and identification by an expert fungal taxonomist (who are as rare as many of the fungi they study) or detection by DNA methods.

An ancient group of unknown fungi

Most of the fungi we recorded in the samples could not be identified down to the level of species but only to higher levels of classification, such as genus or family. The reason for this is partly due to the fact that most of the amazing diversity of fungi do not yet have a species name: only about 3% of the estimated fungal diversity has been formally described. Coupled with this is the problem that even when a fungus has a species name, only a small proportion of species are actually included in the international reference datasets we use to identify the DNA sequences we retrieve from the soil samples. A very good example which exemplifies the massive challenge we face in the identification of fungi are the group known as the Archaeorhizomycetes (which translates as 'ancient root fungi'). This was a very common group of fungi in our study with 53 taxa found, occurring in 80% of the samples and making up 12% of all the sequences. However, not a single one could be assigned to a species name. It is thought that globally there may be hundreds if not thousands of undescribed species within this group of fungi – but there is only a single example of a reference barcode and associated species name available in the databases. We also know nothing about what this group of fungi do – for all we know they could be critical for the growth and survival of alpine plants, but currently they are something of a mystery.

Arctic-alpine

Mountain habitats in the Cairngorms are often referred to as arctic-alpine, because they support species more commonly found in arctic regions which are present in the UK at the southern edge of their global range, together with species typical of mountain regions on the continent and in Scandinavia which may be at the northern or western edge of their distribution. This combination of species gives rise to the unique character of alpine habitats in the UK and adds to their biodiversity value. We currently know little about how our soil biodiversity compares with that in other alpine or arctic regions of the world, but one species found in the survey which demonstrates this potential arctic connection is *Amanita groenlandica*. As the name suggests, this species was first described from Greenland where it is a common species associated with willow and birch. Although it produces a large fruit body, up to 15 cm tall, this species has not previously been recorded in the UK. It was found on only one mountain in the survey (Mullach Clach a' Bhlair) but given that mountain willows are widespread across Scotland, there is the potential for it to be found in additional locations.

A new parasitic fungus for the UK

Many fungi have interesting and sometimes slightly macabre lifestyles. Several groups of parasitic fungi infect the larvae of insects, which they then consume from the inside. Eventually they produce a structure projecting out from the larva which sheds spores into the air with the aim of finding a new victim. The survey found one such parasitic fungal species which is new to the UK, with the wonderful name of *Ophiocordyceps macroacicularis*. This species was only described in 2015 from Japan and is a parasite of moth larvae which inhabit plant tissue. This may be the first record of this fungus in Europe. This species again highlights how little we know about soil biodiversity in alpine systems, and the multitude of complex interactions that occur between different groups of organisms.

A new strangler in the hills?

Fungi do not only parasitise other types of organisms – many fungi are parasites of other fungi (mycoparasites). One very weird example of this is the group of fungi known as Stranglers. These fungi have a unique approach to fruiting because they develop their own fruit bodies on top of the partly formed fruit bodies of other fungal species. Hence the strangler nickname. The end result is a chimera with the bottom part of the stipe (stalk) belonging to the host fungus, while the upper part of the stipe, the cap and gills beneath are formed by the strangler. This type of mycoparasitism is very unusual and the fruit bodies of stranglers are very rarely observed. There are three strangler species reported from Scotland with only one or two records of each. We found a barcode in the Munro study which strongly supports the idea that there is a fourth species, but one which currently has no name. This is a very exciting find from this bizarre group of globally rare fungi. The unknown strangler was detected on four different Munros and, since the sampling locations were all geopositioned, we could go back to the exact sites where the unknown strangler was detected and if we were unbelievably lucky, we might find the fruit bodies which would allow us to describe a new strangler species.

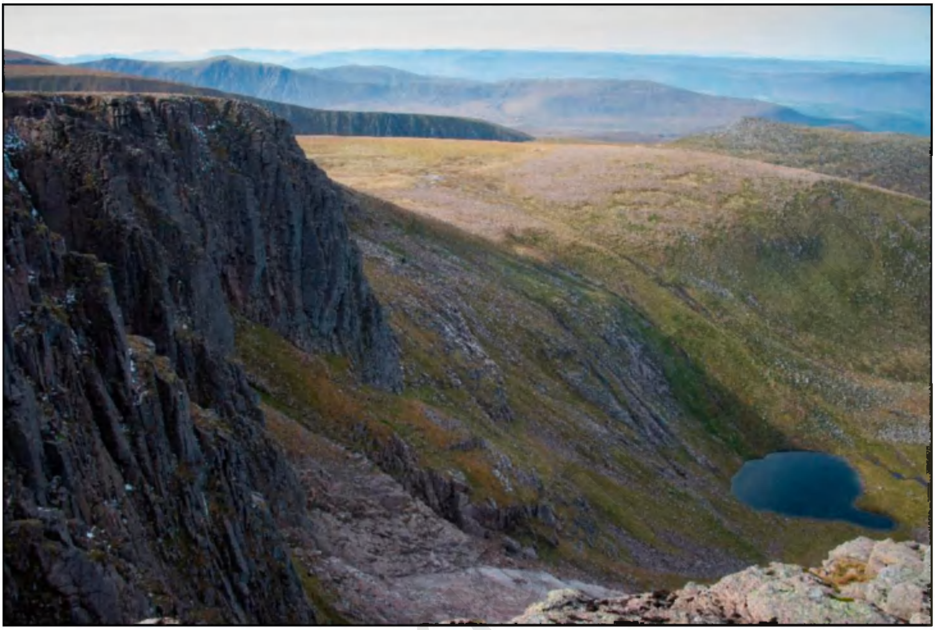
Scarlet waxcap (*Hygrocybe coccinea*) in grassland at Gleann an t-Slugain. Waxcap fungi are commonly found in nutrient-poor grasslands. Seven species were detected in the survey, including the rare mountain waxcap.
Photo credit Andrea Britton

Since the DNA approach is not reliant on finding fungal fruit bodies, it provides a much more comprehensive picture of fungal diversity in soil than was previously possible. As well as identifying which species are present in the soil and finding species new to the UK, this type of data allows us to start investigating how fungal diversity varies with factors such as climate, elevation, habitat type and soil conditions. This type of information could provide a much more secure basis for conservation decisions than ad hoc records of fruit bodies.

The survey data showed that there were significant differences in the numbers of fungi found between the three target habitats of moss heath, dwarf shrub heath and grassland. Each habitat type also supported a unique group of species. Diversity was highest in the grassland samples (average of 87 taxa per sample) and lower in the shrub heath and moss heath (average of 76 and 75 species per sample respectively). The 'lucky dip' samples chosen by the volunteers, which were located in a wide variety of habitats, also showed that there could be high diversity in wet 'flushed' habitats and in the fell-field habitats of the highest summit areas where bare gravel is interspersed with patches of woolly fringe moss and three-leaved rush. When looking at above ground plant diversity, the number of species present tends to decline with increasing elevation, but we didn't find this pattern for the soil fungi, if anything there was a weak trend for increased number of species per sample in the highest elevation plots.

We also carried out some basic chemical analysis on the soil samples, measuring acidity (pH) and the amounts of carbon and nitrogen contained in the soil. This showed that the total number of fungi present in a sample, and also the number of unique fungi, was higher in less acidic soils. Much of the Cairngorms National Park is underlain by granitic rocks which produce acidic, nutrient-poor soils. This result points to the potential importance of areas such as the Cairnwell and Glas Maol region where limestone and other more base-rich rocks produce less acidic soils. Some of these locations are already well known for the special plants that they support, and it seems likely that they might be important for fungi too. The soil chemical data also showed that the richness of fungi tended to be lower in soils with a very high carbon content, suggesting that the deep peat soils found on lower slopes and in high altitude blanket-bog may be less important for fungal biodiversity than they are for carbon storage.

The discoveries discussed here are only the start for the exploration of soil biodiversity in the Cairngorms. Future analyses will dig deeper into the factors controlling fungal diversity and distributions with the aim that the biodiversity beneath our feet be as well-understood as that we can easily see around us when we are walking in the hills (continued).



The Cairngorm plateau at Cairn Lochan, looking towards Creag an Leth-choin. We now know that our alpine soils support a rich diversity of fungi. Photo credit Andrea Britton

What next?

The data collected by the Cairngorms Soil Fungi project provides a baseline assessment of alpine fungal communities in the Cairngorms, against which the effects of future climate and environmental change could be assessed. As the climate changes, distributions of alpine species are also likely to change, and understanding which species are moving and how fast will be vital to predict how ecosystem function might change in future.

The success of the survey has inspired us to think big and to expand the project to explore soil biodiversity across all of Scotland's Munros. In 2023 we launched 'Mountain Heights, Hidden Depths' – a 4-year citizen science project which aims to work with the hill going public to collect soil samples for DNA analysis from the 270 Munros readily accessible to hillwalkers, building on the 55 Munros already sampled. In this project the DNA analysis will be expanded to include all forms of life in the soil, not just the fungi. We hope that this project will allow us to build a more complete picture of the biodiversity in Scotland's alpine soils and to gain a better understanding of the factors influencing soil biodiversity and the most important areas for its conservation.

Acknowledgements

This work would not have been possible without the enthusiastic participation of our many volunteer citizen scientists who climbed Munros and gathered soil samples in all weathers – we are immensely grateful to each and every one of them.

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