DAVIS EXPEDITION FUND
REPORT ON EXPEDITION / PROJECT

Expedition/Project Title: Macrofungal Biodiversity Surveys in the Coastal Temperate Rainforests of Vodudahue, Los Lagos Region, Chile

Travel Dates: May to June 2022

Location: Vodudahue, Chile

Group Members: Alice Peace, Kai Cartwright

Aims: To characterise macrofungal diversity and ecology in the coastal temperate rainforest of the isolated Vodudahe estuary in the Los Lagos region of Chile, to collect RBGE herbarium specimens of macrofungi, and to collect soil and root tip samples adjacent to specimens of the the endangered conifer *Fitzroya cupressoides* for metagenome analysis by partners at the Royal Botanic Gardens, Kew to characterise mycorrhizal symbionts.

Photography consent form attached: (please refer to your award letter) ☒ Yes ☐ No

Macrofungal Biodiversity Surveys in the Coastal Temperate Rainforests of Vodudahue, Los Lagos Region, Chile

Alice Peace & Kai Cartwright
May - June 2022

Summary

In May 2022, we travelled to the Los Lagos Region of Chile in northern Patagonia to conduct ecological and biodiversity surveys of macrofungi and collect specimens for the Royal Botanic Gardens Edinburgh herbarium. Over ten days, we collected fungi and recorded field notes within different forest types and between altitudes of 5-400m. We also collected soil and root tip samples adjacent to seven specimens of the endangered conifer Alerce (*Fitzroya cupressoides*) for metagenome analysis with partners at the Royal Botanic Gardens, Kew in London. These analyses will help to characterise mycorrhizal fungi that associate with Alerce in the wild, which could be of significant importance in the *in-* and *ex-situ* conservation of the species. Our fieldwork was based at the Fundacion Alerce 3000 site in the Vodudahue estuary in the Hualaihué commune, at the generous invitation of the Chilean conservation NGO Fundacion Chilco and the landowner Nicolás Ibáñez. Fundacion Chilco is an important international partner of the RBGE in initiatives such as the International Conifer Conservation Programme, and our fieldwork contributed to ongoing strong collaborations between the two institutions. In addition to collecting important ecological data and herbarium specimens, this experience was of great educational value to us, for which we are immensely grateful to the Davis Expedition Fund.

Figure 1: En route to the field to survey macrofungal diversity. This photograph shows Alice Peace (left) and Kai Cartwright (right) equipped for the very wet conditions of the temperate Nothofagus forest where surveys were conducted and specimens collected.

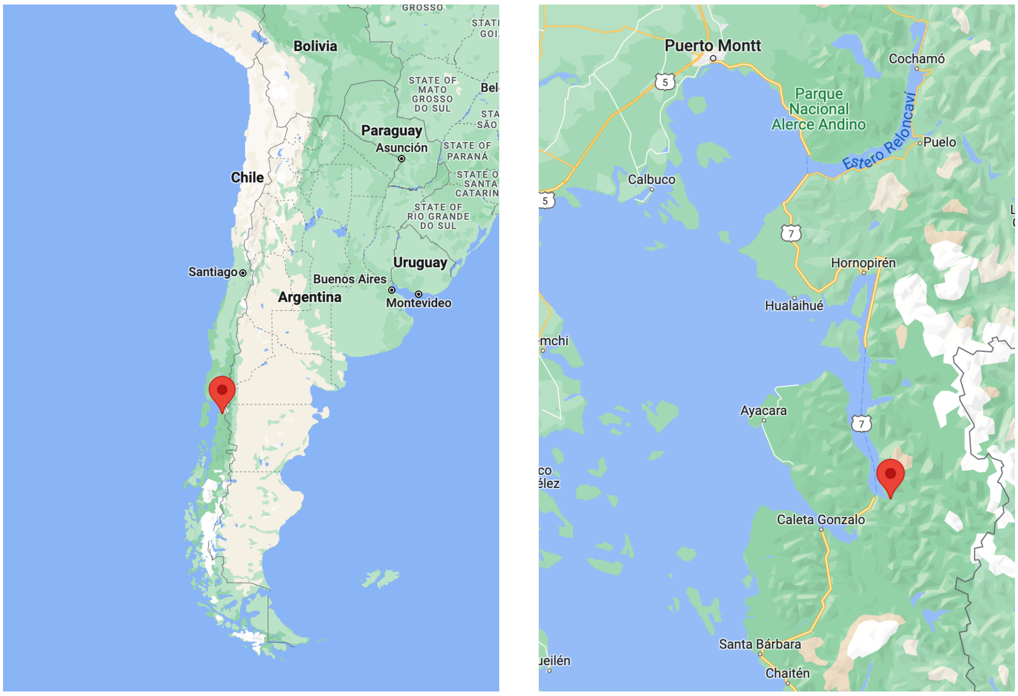

Figure 2: Location of the Fundacion Alerce 3000 site within Chile (left) and relative to the largest regional city, Puerto Montt (right). Source: Google Maps.

Context

Fungi are amongst the most diverse eukaryotic lineages of life, and play critical and diverse roles in terrestrial ecosystems. Almost all land plants form associations with mycorrhizal fungi important for their growth and survival (Taylor *et al*., 2014), whilst diverse arrays of enzymes and metabolic plasticity enable white rot fungi in the Basidiomycota to degrade lignin, making them the principal decomposers in terrestrial ecosystems (Akhtar *et a*l., 1997). Fungi are highly metabolically diverse, and are the sources of many essential medicines and a vast potential reservoir of novel ones (Rai *et al*., 2018). Estimates place the number of extant fungal taxa around several million, of which less than 10% are thought to have been described (Bass and Richards, 2011). Whilst this discrepancy is partially due to the fact that most fungi do not produce conspicuous fruiting bodies, the macrofungi of many terrestrial regions, particularly rural areas with low populations that are difficult to access, are very understudied (He *et al*., 2022).

The old-growth temperate and coastal rainforests of Chilean Patagonia are unique ecosystems in South America, characterised by high precipitation, very low atmospheric pollution and flora derived from Gondwanan elements. Whilst work by Butin and Peredo (1986) and Furci (2008) indicates that these ecosystems are relatively rich in fungal taxa, in comparison to other regions of South America they have been subject to relatively little mycological exploration (Truong *et al*., 2017). Several hitherto unknown, unique fungal lineages have recently been characterised from the Patagonian Andes (Nouhra *et a*l., 2013), indicating that the region may harbour even more diversity than previously estimated. Additionally, general ecological traits of fungal communities in Patagonian forests differ widely to fungal ecologies in Northern Hemisphere forests - for example, angiosperm trees in the genus Nothofagus widely associate with ectomycorrhizal (EM) fungi, whilst native conifers generally form associations with arbuscular mycorrhizal (AM) fungi, in contrast to Northern Hemisphere forests, where conifers generally associate with EM fungi and angiosperms with AM fungi (Marin *et al*., 2017). The temperate and coastal rainforests of Chilean Patagonia therefore represent important regions for mycological exploration to develop knowledge of global fungal diversity and ecology and were selected for our macrofungal surveys and collections.

Alerce (*Fitzroya cupressoides*) is a native conifer that was once a common species in old growth temperate coastal Patagonian rainforests, but that was extensively logged for centuries due to its high quality, durable wood and is now classified as Endangered on the IUCN Red List (Premoli *et al*., 2013). Within the extant population are some of the oldest non-clonal individual trees on the planet, providing dendrochronology records of climate reaching thousands of years into the past, living windows into deep time (Lara & Villalba, 1993). Mycorrhizal fungi are extremely important for the growth, health and survival of most land plants, but the mycorrhizal partners of Alerce have never been characterised. The conservation status of these fungal partners therefore also remains unknown, constituting another major threat to the survival of the species. Characterising the fungal symbiotic partners of Alerce is therefore a high research priority to enable appropriate and rapid action if the fungi are threatened, so that the Alerce is not lost forever.

Data collection and analysis

Fungal Diversity and Ecology Surveys

Over ten days of fieldwork, we followed established trails through coastal temperate rainforest within the Fundacion Alerce 3000 estate, accompanied by guides and at times other visiting Chilean mycologists. The predominant tree species in the forest through which the trail passed were coigüe (*Nothofagus dombeyi*), coigüe de Chiloé (*N.nitida*), canelo (*Drimys winteri*), and *Podocarpus* trees, with stands of tepú (*Tepualia stipularis*) and arrayán (*Luma apiculata*) forming distinct habitats. Using the trails as transects, we collected specimens of all macrofungi encountered within two metres of either side of the trail where it was safe to do so. Detailed field notes and photographs were taken for each specimen, recording characteristics including location, substrate, morphology and smell. Coordinates and altitude were established where possible using a GPS. Samples were then extracted by gently lifting substrate around the base of the stipe, or carefully removing part of the substrate, whilst causing minimal damage to the environment in which the specimen was growing. Any new details, such as colour changes when bruised, stain and exuding liquid were recorded and samples were carefully packaged alongside specimen cards for transport back to our accommodation facility.

Generally, mornings (8am - 2pm) were spent in the field, whilst afternoons (2pm - 7pm) were spent preparing spore prints and identifying samples to genus and species level where possible with principal reference to the most comprehensive Chilean field guide *Hongos de Chile* (*Furci, 2018*). Browsing regional research grade observations on the citizen science app iNaturalist also helped narrow down identifications of genera with which we were unfamiliar. Subsequently, specimens were partially air dried alongside their respective specimen cards before being placed into brown paper bags and into ziploc bags with silica gel for complete dehydration and storage for transportation back to the RBGE herbarium.

Figure 3: Kai Cartwright combing through leaf litter underneath a Nothofagus tree in search of elusive mycorrhizal fungi.

Soil and Root Tip Sampling

For the collection of soil and root tip samples, Fundacion Chilco members and Fundacion Alerce 3000 guides took us to visit three wild and four nursery *Fitzroya cupressoides* specimens. The wild specimens included a very young (<5y) individual at sea level and an ancient (>1500y) individual at an elevation of approximately 400m (figure 4). At each tree, a soil corer was used to take 20cm soil cores 15cm from the trunk base, away from any visible roots. GPS and altitude data was recorded for each sample. Upon returning to the accommodation facility, samples were thoroughly air dried following Claysen et al. (2020) and packaged in airtight, opaque soil sample bags for transport to Kew Gardens for metagenome analysis.

Figure 4: Range in the size and age of Alerce specimens. On the left is a veteran tree at least 1,500 years of age, and on the right a young individual of natural regeneration.

Results

Diversity and Ecology Surveys

Our collections included at least 30 distinct species from an estimated 26 genera, and comprised mostly saprotrophic, white-rot fungi, and some mycorrhizal taxa (table 1, figure 5).

Table 1: Macrofungi collected in temperate coastal rainforest around the Fundacion Alerce 3000 estate.

Taxon	Ecology	Habitat	Date Collected
Hypholoma spp.	Saprotrophic	Grass adjacent to mixed stands of young *N. nitida* and *L. apiculata*	21/05/22
Stereum hirsuta	Saprotrophic	Blanketing a thin, long, dead *N. nitida* branch trapped in the canopy	22/05/22
Austropaxillus boletinoides	Mycorrhizal	Growing amongst leaf litter adjacent to *N. dombeyi* trees	22/05/22

Nidula sp.	Saprotrophic	Growing on a decaying, wet fence post in grass close to the river	22/05/22
Ganoderma australe	Saprotrophic-rots hearts of trees	Growing on dead *Nothofagus*	22/05/2022
Russula nothofaginea	Mycorrhizal	Growing underneath a *N. nitida* tree in leaf litter	22/05/22
Mycena subulifera	Saprotrophic	Growing in mossy soil on the base of a fallen, dead tree	23/05/22
Hypholoma frowardii	Saprotrophic	Growing on dead *Nothofagus* tree stump	23/05/22
Scytinotus longiquinus	Saprotrophic	Growing on wet, dead stick suspended in undergrowth	23/05/22
Calycina citrina	Saprotrophic	Widespread on fallen logs throughout forest	23/05/22
Nidula niveotomentosa	Saprotrophic	On dead *Podocarpus log*- on sheltered side in moss	23/05/22
Mycena chusqueophila	Saprotrophic	Growing from wood fragment and leaf in leaf litter beneath *N. dombeyi*	24/05/22
Mycena cyanocephala	Saprotrophic	Growing in a cluster on a dead branch	24/05/2022
Xylaria sp.	Saprotrophic	Growing on mossy, dead log, possibly *N. nitida*, within an *L. apiculata* stand	24/05/2022
Lachnum sp.	Saprotrophic	Growing on stick on the forest floor	24/05/2022
Ascocoryne sarcoides	Saprotrophic	Growing on dead *N. dombey*i log	24/05/2022
Aleuria aurantia	Saprotrophic	Growing on gravel path where a road	24/05/2022

		was being constructed	
Antrodia sp.	Saprotrophic	Growing on dead branch of possibly *T. stipularis*	25/05/2022
Calocera cornea	Saprotrophic	Growing on dead *N. dombeyi* log	25/05/22
Inocybe sp.	Saprotrophic	Growing in leaf litter amongst *N. dombeyi* and *N. nitida*	25/05/22
Gymnopilus sp.	Saprotrophic	Growing in cluster on fallen *N. dombeyi* tree	25/05/22
Mycena sp.	Saprotrophic	Growing on Nothofagus leaves in leaf litter	25/05/22
Mycena sp.	Saprotrophic	Growing on bamboo canes in undergrowth	25/05/2022
Marasmius alliaceus	Saprotrophic	Growing on Nothofagus leaves in leaf litter	25/05/22
Lycoperdon sp.	Saprotrophic	Amongst ferns on newly exposed soil formed by a minor landslide	26/05/22
Trametes versicolor	Saprotrophic	On logs and gates	26/05/22
Cortinarius magellanicus	Mycorrhizal	In leaf litter next to *N. dombeyi* trees	26/05/22
Cortinarius subs. myxacium	Mycorrhizal	In leaf litter next to N. dombeyi trees	26/05/22
Bjerkandera adusta	Saprotrophic	Growing from dead Nothofagus log	26/05/22
Galiella coffeata	Saprotrophic	Growing on mossy dead log	27/05/2022

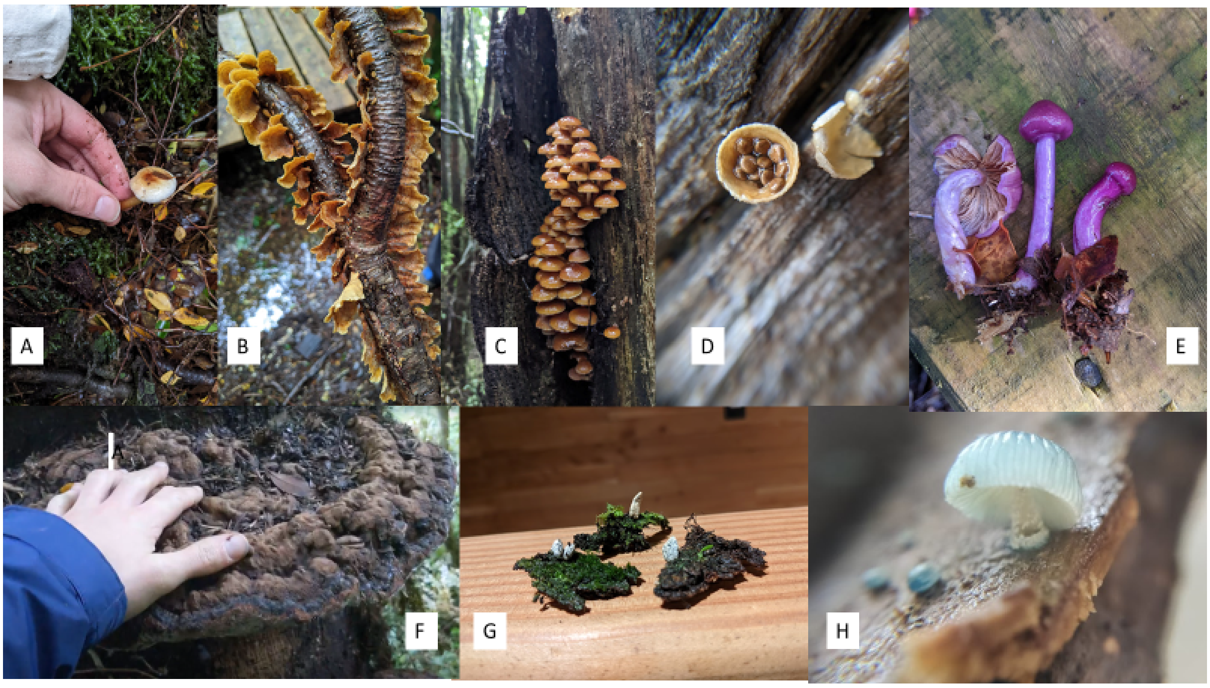

Figure 5: Examples of some of the macrofungi that were collected: A) Austropaxillus boletinoides, B) Stereum hirsutum, C) Hypholoma spp., D) Nidula sp., E) Cortinarius magellanicus, F) Ganoderma australe, G) Xylaria sp., H) Mycena cyanocephala. Photography by Kai Cartwright and Alice Peace.

Metabarcoding of Soil and Root Tip Samples

Eight soil and root tip cores were successfully sampled, dried, labelled, packaged and transported, and are awaiting metabarcoding analysis and sequence comparisons at Kew Gardens.

Discussion

We visited Vodudahue at the end of the autumn/beginning of winter season, when the main autumn macrofungal fruiting period draws to a close (Furci, 2018). Our findings are therefore unlikely to be representative of macrofungal diversity at Vodudahue at the peak of the fruiting season, but reflect diversity at the end of the autumn. Notably, we saw relatively fewer ectomycorrhizal fungi than would be expected at the peak of the autumn season in the wider region (Furci, personal communication). Our observations are therefore consistent with trends in macrofungal fruiting phenology previously observed in the wider region. Diminished fruiting of ectomycorrhizal fungi towards the end of the autumn compared to ongoing fruiting of saprotrophs could be associated with diminishing flow of carbohydrates from trees to mycosymbionts with reduced photosynthesis at lower temperatures, as has been suggested to be a potential driver of similar patterns in forests in the Northern Hemisphere (Kauserud *et al*., 2012).

Historically, macrofungi were classified using morphology alone. However, with the advancement of sequencing technologies, sequence-based phylogenetic analyses are demonstrating that more and more taxa hitherto grouped as species by morphology are in fact species complexes separated by significant evolutionary history and genetic divergence. To increase knowledge of the biodiversity of our planet, increasing numbers of herbarium specimens is very important, particularly for fungi. Even if particular taxa are thought to be well-represented in collections, sequencing of morphologically similar novel specimens can sometimes lead to major changes and developments in our understanding of the evolutionary history and diversity of complexes. Recently, a collection of specimens all morphologically recognised as *Cortinarius magellanicus* were subjected to sequence-based phylogenetic comparisons and found to constitute a complex of species, with strong regionalism and distinct host associations (Salomon *et al*., 2018). Our herbarium collections include *C. magellanicus* and other taxa in the genus *Cortinarius*, and could prove important in the ongoing characterisation and revision of the genus. Accurately characterising fungal biodiversity is extremely important not only for the protection of the diversity itself, but for the effective conservation of the wider ecosystems in which they play essential and diverse functions.

Fitzroya cupressoides trees are known to form associations with arbuscular mycorrhizal (AM) fungi (Diehl *et al*., 2003), but these associations are very little characterised in the wild-we currently do not know with which fungi they associate, or have any knowledge of the conservation status of these important symbionts. Whilst protection of the trees in and ex-situ are essential, so is conservation of the mycorrhizal symbionts. The first and crucial step to understanding their conservation status and being able to respond appropriately is to characterise them. In tree nurseries, previous successes inoculating saplings of other tree species with their wild mycorrhizal partners suggest that it may be possible to inoculate Alerce saplings with cultures of these symbionts to improve health and growth (*e.g.* Urgiles *et al*., 2004). It is our hope that metabarcoding of our soil samples will provide important data that helps to characterise these unknown mycorrhizal symbionts so that their conservation status can be assessed and for potential introductions to Alerce nurseries. In this way, I hope that we can contribute towards the huge efforts to conserve and restore this most majestic of tree species that was once such a significant part of Patagonian temperate rainforest ecosystems.

Concluding Remarks

This fieldwork expedition to Chilean Patagonia was an invaluable experience for us both educationally and spiritually, as the COVID-19 pandemic had severely limited our opportunities to gain fieldwork experience (either as a part of our respective degrees, or as an extracurricular self-organised activity). Planning the expedition, from the location and objectives to the budget and logistics, was excellent preparation for the reality of what will be expected as we graduate this year and begin careers as plant and fungal ecologists. The opportunity to work and learn alongside experienced mycologists in the field was extremely complementary to our independent and university studies, and nourished and nurtured our passion and enthusiasm for fungi ever higher.

Our fieldwork expedition involved and would not have been possible without researchers and conservationists at the Royal Botanic Gardens Edinburgh, Fundacion Chilco, The Royal Botanic Gardens, Kew, the Fundacion Fungi, the Fundacion Alerce 3000 and independent Chilean mycologists. We hope to continue to build and develop these relationships and

collaborations in our careers to come, particularly Kai, who volunteers with the Fundacion Fungi and will begin an MSc in Plant and Fungal Taxonomy, Diversity and Conservation at Kew in September, and hopes to work with the conservation of Alerce and its mycorrhizal symbionts in the future. We would also like to express personal thanks to Martin Gardner, Josefina Hepp, Paulina Hechtenleiter and Colin Pendry, for guiding us to Vodudahue and advising and supporting us throughout the entire process; to Antonio, Helmut, Nicolas, Joaquin, Oscar and all the guides and workers at Vodudahue for the kindest welcome, sharing of their knowledge and the most fun time, to Daniela Torres and Giuliana Furci at the Fundacion Fungi for being the initial inspiration and teachers, to Pablo and Adriana for all the mycology we learnt from them and the fantastic days in the field, and very importantly to Nicolas Ibanez for receiving us so generously at his property. We are profoundly grateful to the trustees of the Davis Expedition Fund for supporting our fieldwork and helping us to launch our careers as plant and fungal ecologists.

References

Bass, D. and Richards, T.A., 2011. Three reasons to re-evaluate fungal diversity ‘on Earth and in the ocean’. Fungal biology reviews, 25(4), pp.159-164.

Butin H, Peredo H (1986) Hongos parásitos en coníferas de América del Sur con especial referencia a Chile. Stuttgart, Berlin

Clasen, L.A., Detheridge, A.P., Scullion, J. and Griffith, G.W., 2020. Soil stabilisatizion for DNA metabarcoding of plants and fungi. Implications for sampling at remote locations or via third-parties. bioRxiv.

Diehl, P., Mazzarino, M.J., Funes, F., Fontenla, S., Gobbi, M. and Ferrari, J., 2003. Nutrient conservation strategies in native Andean-Patagonian forests. Journal of Vegetation Science, 14(1), pp.63-70.

Furci G (2008) Diversidad de especies Hongos. In: Ocho libros (ed) Biodiversidad de Chile: Patrimonio y Desafíos, Chile, pp 366-375

Furci, G., 2018. Guía de campo Hongos de Chile Vol. I y II. Ed. Fundación Fungi, Santiago, Chile.

He, M.Q., Zhao, R.L., Liu, D.M., Denchev, T.T., Begerow, D., Yurkov, A., Kemler, M., Millanes, A.M., Wedin, M., McTaggart, A.R. and Shivas, R.G., 2022. Species diversity of basidiomycota. Fungal Diversity, pp.1-45.

Kauserud, H., Heegaard, E., Büntgen, U., Halvorsen, R., Egli, S., Senn-Irlet, B., Krisai-Greilhuber, I., Dämon, W., Sparks, T., Nordén, J. and Høiland, K., 2012. Warming-induced shift in European mushroom fruiting phenology. Proceedings of the National Academy of Sciences, 109(36), pp.14488-14493.

Marín, C., Aguilera, P., Oehl, F. and Godoy, R., 2017. Factors affecting arbuscular mycorrhizal fungi of Chilean temperate rainforests. Journal of soil science and plant nutrition, 17(4), pp.966-984.

Nouhra, E., Urcelay, C., Longo, S. and Tedersoo, L., 2013. Ectomycorrhizal fungal communities associated to Nothofagus species in Northern Patagonia. Mycorrhiza, 23(6), pp.487-496.
Rai, M., Ingle, A., Gaikwad, S., Gupta, I., Yadav, A., Gade, A. and Duran, N., 2018. Fungi: myconanofactory, mycoremediation and medicine. In Fungi (pp. 201-219). CRC Press.

Premoli, A., Quiroga, P., Souto, C. & Gardner, M. (2013) Fitzroya cupressoides. In IUCN Red List of Threatened Species v. 2013.2.

Salomón, M.E.S., Dresch, P., Horak, E., Galleguillos, F., Barroetaveña, C. and Peintner, U., 2018. The enigmatic Cortinarius magellanicus complex occurring in Nothofagaceae forests of the Southern Hemisphere. Fungal Biology, 122(11), pp.1077-1097.

Taylor DL, Hollingsworth TN, McFarland JW, Lennon NJ, Nusbaum C, Ruess RW. 2014. A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs 84: 3–20.

Truong, C., Mujic, A.B., Healy, R., Kuhar, F., Furci, G., Torres, D., Niskanen, T., Sandoval‐Leiva, P.A., Fernández, N., Escobar, J.M. and Moretto, A., 2017. How to know the fungi: combining field inventories and DNA‐barcoding to document fungal diversity. New Phytologist, 214(3), pp.913-919.

Urgiles, N., Loján, P., Aguirre, N., Blaschke, H., Günter, S., Stimm, B. and Kottke, I., 2009. Application of mycorrhizal roots improves growth of tropical tree seedlings in the nursery: a step towards reforestation with native species in the Andes of Ecuador. New Forests, 38(3), pp.229-239.

Appendix: Table of expenditure

Alice

Item	**Cost (£)**	**Justification**
Train Edinburgh to London	£30.05	Train to London for departure from LHR
Return flight London to Santiago	£905.17	Return flight from LHR to SCL
Hostel Santiago	£59.99	Necessary to quarantine 24h at destination until PCR results received
Return flight Santiago-Puerto Montt	£166.34	Return flight from SCL to PMC
Train London to Edinburgh	£31.19	Return train from London to Edinburgh
Food during fieldwork	£66.34	Food
Travel Insurance	£194.40	Required for entry to Chile. Included full cover for Covid-19 (mandatory). Additionally, the area we were working in was very remote, so also included cover for helicopter evacuation in case of emergency.
Total	**£1453.48**	We are very grateful for the generous £1375 per person that the Davis Expedition Fund contributed to our trip. The remainder of the trip expenses, including food, overland transportation and necessary personal equipment was paid for using personal

		contributions (not listed here)

Kai

Item	**Cost**	**Justification**
Return flight to Santiago	865.62	
Return transport to London	84.48	
Return travel to Puerto Montt	135.32	
Hostel in Hornopiren	35.80	
PCR Test	55.00	
Travel insurance	52.93	
Food during fieldwork	£66.34	
Equipment: ziploc and paper bags, soil sampler	£34.90	
Total	**1330.39**	We are very grateful for the generous £1375 per person that the Davis Expedition Fund contributed to our trip. The remainder of the trip expenses, including food, overland transportation and necessary personal equipment was paid for using personal contributions (not listed here)