Recent advances in the biology, genetics and ecology of the ectomycorrhizal ascomycete *Cenococcum geophilum*

Huayong Wang[1], **Annegret Kohler**[1] **& Francis M. Martin**[1*]

[1] Université de Lorraine, INRAE, UMR Interactions Arbre/Micro-organismes, Centre INRAE Grand-Est Nancy, 54280 Champenoux, France

*** Correspondence:**
Huayong WANG
huayong.wang@inrae.fr
Francis M. Martin
francis.martin@inrae.fr

Keywords: Drought1, environmental stresses2, forest3, genomics4, mycorrhizal symbiosis5, population genetics6

Abstract

The ascomycete *Cenococcum geophilum* is a cosmopolitan and ecologically significant ectomycorrhizal (ECM) fungus that forms symbiotic associations with diverse host plants globally across various ecosystems. As the only known ECM member of Dothideomycetes, *C. geophilum* exhibits several distinctive characteristics that distinguish it from other ECM fungi. Its high genetic diversity is particularly noteworthy considering its asexual reproduction mode. This genetic variability may contribute to its adaptability and extensive distribution across habitats. As an early colonizer of primary forest succession, *C. geophilum* significantly contributes to ecosystem stability and development. Its ability to establish quickly in disturbed or newly formed habitats helps create conditions favorable for the establishment of other plant species, thereby playing a crucial role in ecological succession and ecosystem recovery. *C. geophilum* plays several key roles in forest and other terrestrial ecosystems. It is particularly important in nutrient cycling as it facilitates the transfer of nutrients, especially nitrogen and phosphorus, from the soil to its host plants. Additionally, *C. geophilum* has been demonstrated to enhance the drought resistance of its host plants, a trait that is becoming increasingly important in the context of climate change and frequent drought events. Recent advances in molecular biology and genomics have revealed that *C. geophilum* is not a single species but rather a species complex comprising multiple cryptic lineages. These lineages exhibit potential host preferences, suggesting a degree of specialization within the complex. The genome of *C. geophilum* has been sequenced and analyzed, providing valuable insights into its molecular biology and symbiotic capabilities. Notably, this genome encodes a reduced repertoire of carbohydrate-active enzymes and a large set of effector-like, small secreted proteins. These molecular traits likely facilitate host colonization across diverse plant taxa by enabling the fungus to interact with and modify plant cell walls, as well as to communicate with its host plants through various signaling pathways. Therefore, *C. geophilum* is a compelling model system for research on fungal ecology, evolution, and mycorrhizal symbiosis.

1 Introduction

Most land plants establish symbiotic relationships with mycorrhizal fungi, which play a critical role in terrestrial ecosystems by regulating nutrient and carbon cycles, influencing soil structure, and contributing to ecosystem multifunctionality (Martin & van der Heijden, 2024). Approximately 80% of plant N and P is provided by these mutualistic fungi, and the majority of plant species depend on them for growth and survival. An estimated 20,000 fungal species, primarily belonging to the phyla Basidiomycota and Ascomycota, establish ectomycorrhizal (ECM) associations with approximately 6,000 plant species, mostly trees and shrubs (van der Heijden et al., 2015). ECM fungi are present in a diverse range of terrestrial ecosystems and are responsible for colonizing 60% of trees in temperate and boreal forest ecosystems. These tree species, belonging to families including the Pinaceae, Fagaceae, Betulaceae, Nothofagaceae, Myrtaceae and Dipterocarpaceae, play crucial ecological and economic roles in both the northern and southern hemispheres.

During symbiosis development, ECM fungi differentiate the hyphal mantle, ensheating the rootlets, and an intraradical Hartig net penetrating the host roots. In numerous ECM associations, an extraradical mycelium permeating the soil environment extends from ECM roots. Mycelial networks facilitate the acquisition of water and nutrients for plants and enhance their resistance to environmental stress. ECM symbionts can secrete extracellular enzymes that degrade soil organic matter (SOM) to facilitate nitrogen acquisition in their hosts (Ward et al., 2022). ECM from different independently evolved lineages exhibits varying capacities to degrade SOM and transfer nitrogen to their host (Nicolás et al., 2019). In boreal and temperate forests, ECM fungi provide 70% of N flux to their hosts (Smith and Read, 2010). Consequently, ECM plays a crucial role in C and N cycles in forest soils. In exchange for soil minerals, 10–20% of photoassimilates are allocated to fungal partners by the host plant. Plant communities allocate 9.07 Gt of CO2e per year to their mycorrhizal symbionts (Hawkins et al., 2023).

The ascomycetous fungus *Cenococcum geophilum*, previously known as *C. graniforme* (Ferdinandsen and Winge, 1925), is a cosmopolitan ECM fungus and is one of the most prevalent mutualistic species found in soil fungal communities worldwide (LoBuglio et al., 1999) (Figure 1). It forms mycorrhizal associations with over 200 trees, shrubs, and herbaceous species in boreal, temperate, and subtropical forests as well as in savannas and alpine meadows. As the only known ECM member of Dothideomycetes, *C. geophilum* exhibits several distinctive characteristics that distinguish it from other ECM fungi. Its high genetic diversity is particularly noteworthy considering its asexual reproduction mode. This genetic variability may contribute to its adaptability and extensive distribution across habitats. As an early colonizer of primary forest succession, *C. geophilum* significantly contributes to ecosystem stability and development. It is particularly important in nutrient cycling as it facilitates the transfer of nutrients, especially nitrogen and phosphorus, from the soil to its host plants. Additionally, *C. geophilum* has been demonstrated to enhance the drought resistance of its host plants, a trait that is becoming increasingly important in the context of climate change and frequent drought events. *C. geophilum* is therefore a compelling model system for research on fungal ecology, evolution, and mycorrhizal symbiosis.

The biological and ecological attributes of *C. geophilum* have been extensively documented in multiple reviews (LoBuglio, 1999; Obase et al., 2017). In recent years, genomics has emerged as a crucial tool for investigating the biology, evolution, and ecology of mutualistic symbionts. This approach not only provides essential mechanistic insights, but also identifies key traits, such as drought resistance, which can be prioritized for the application of this mycorrhizal symbiont in forestry. This review offers a brief overview of the current understanding of *C. geophilum* biology and ecology, followed by an examination of recent studies that demonstrate the impact of genomics and related techniques (e.g., DNA metabarcoding and transcriptomics) on our understanding of this enigmatic mycorrhizal fungus. Additionally, we briefly explore the potential applications of *C. geophilum* in sustainable forestry and ecosystem restoration, highlighting the significance of understanding the functional traits and ecological roles of this ECM fungi in adapting to environmental changes. By consolidating the latest research findings, this review aims to identify knowledge gaps and suggest future research directions for this ubiquitous symbiont to address the global challenges in forestry and environmental sustainability.

2 Morphological features and Life Cycle

The black fungus *C. geophilum* is distinguished by its septate dematiaceous hyphae, which contain high concentrations of melanin in their cell walls (Fernandez and Koide, 2013). This pigmentation enables mycelia to endure various environmental challenges, including UV exposure, dehydration, temperature extremes, enzymatic breakdown, antimicrobial agents, and heavy metal exposure (Pal et al., 2014). This hardiness allows *C. geophilum* to flourish under harsh conditions where other mycorrhizal species might struggle. Furthermore, the fungus produces melanized sclerotia that resist decomposition by soil microorganisms (Fernandez and Koide, 2014). The ECM of *C. geophilum* can persist in the soil for 2.7 years (McCormack et al., 2017), whereas its sclerotia can remain viable for up to 40 years (Nyamsanjaa et al., 2021). *C. geophilum* utilizes the 3,4-dihydroxyphenylalanine (DOPA) and 1,8-dihydroxynaphthalene (DHN) pathways to produce melanin (Peter et al., 2016). In ECM roots, the DOPA pathway shows higher upregulation than the DHN pathway (Peter et al., 2016).

Dark septate root endophyte (DSE) fungi (e.g., *Piceirhiza bicolorata* and *Cadophora finlandia*) share the same soil habitats and many morphological features as *C. geophilum* (Rosling et al., 2003; Vrålstad et al., 2002). Thus, identification of *C. geophilum* relies on a combination of morphological and molecular methods (Rosling et al., 2003; Vrålstad et al., 2002). *C. geophilum* isolates can be cultured on modified MMN medium containing biotin and hydrolyzed casein (Trappe, 1962). Its hyphae show various shapes according to the growth medium and the age of the mycelial colony (Trappe, 1962; Flores et al., 1997; LoBuglio, 1999). Chlamydospores-like structures have been observed in both solid and liquid media (Massicotte et al. 1992). These chlamydospores-like structures are always intercalary and rarely terminal in the mycelia (Mikola, 1948). This structure also exists in the taxonomically related species *Glonium* spp. (Amano, 1983), and *Pseudocenococcum floridanum* (Obase et al., 2016).

C. geophilum can differentiate sclerotium, which is a compact mass of hardened fungal mycelium containing food reserves. One role of sclerotia is to survive extreme environmental conditions. They constitute an underestimated source of polysaccharides in forest soils, accounting for 3.6% of the total carbohydrates in subalpine forest soils (Murayama & Sugiura, 2021). The carbohydrate composition of these storage structures is dominated by glucose (80%), followed by a small proportion of mannose and galactose. Sclerotia hosts a specific fungal and bacterial community (Obase et al. 2014; Narisawa et al. 2021, Massicotte et al. 1992).

Although molecular evidence, such as recombination and diploidy (see below), suggests the presence of unknown sexual stages in the life cycle of *C. geophilum*, no sexual structures have been observed under laboratory or field conditions (Bourne et al., 2014; Dauphin et al., 2021). The number of nuclei per cell remains unknown because of the difficulty in nuclear staining in the presence of melanin. During the co-culture of mycelia, different isolates failed to fuse, indicating the presence of vegetative incompatibility genes.

The only ECM fossil related to *C. geophilum* is *Eomelanomyces cenococcoides* gen. spec. nov., discovered in a 52-million-year-old amber specimen from a lignite mine in Gujarat State, India. This amber was produced by representatives of Dipterocarpaceae trees in the early tropical broadleaf forests. The fossil is similar to the extant *Cenococcum;* however, it is distinguished by the high variability in the branching of ECM systems and by the regular formation of microsclerotia and chlamydospore-like structures (Beimforde et al., 2011).

3 An ecologically important ectomycorrhizal symbiont

C. geophilum plays a key role in nutrient cycling in forests and other terrestrial ecosystems, such as alpine meadows. Additionally, *C. geophilum* has been demonstrated to enhance the drought resistance of its host plants, a trait that is becoming increasingly important in the context of climate change and frequent drought events. As an early colonizer in primary succession, *C. geophilum* significantly contributes to ecosystem stability and development. Its ability to establish quickly in disturbed or newly formed habitats helps create conditions favorable for the establishment of other plant species, thereby playing a crucial role in ecological succession and ecosystem recovery.

3.1 Global and Local Distributions

C. geophilum is a cosmopolitan ECM fungus and one of the most prevalent ECM fungi found in soil fungal communities worldwide (LoBuglio et al., 1999) (Figure1). It forms ECM or ectendomycorrhizal associations with over 200 trees, shrubs, and herbaceous species in boreal, temperate, and subtropical forests as well as in savannas. In Alpine and Arctic biomes, *C. geophilum* establishes ectendomycorrhizas or ECM with herbaceous plants such as sedges or shrubs (Obase et al., 2017). Its abundance in tropical ecosystems is relatively low (Tedersoo et al., 2010; Bakray et al., 2024), although high colonization and genetic diversity have been reported in the dry deciduous forests of Thailand (Phosri et al., 2012). However, ECM of *C. geophilum* ECM are rare in African and South American tropical forests (Bâ et al., 2012; Becerra & Zak, 2011). *C. geophilum* has also been recorded at the edge of deserts (Massicotte et al., 1992) and sandy forests of *Picea mongolica* (Qiuli, 2005).

C. geophilum ECM is abundant in the 0–5 cm soil layer (Rosling et al., 2003; Genney et al., 2006; Scattolin et al., 2008), but can be found in much deeper soil layers, such as the mineral layer at a depth of 20 cm (Genney et al., 2006). *C. geophilum* is known as a pioneer species because of its propensity to partner with pioneer host trees, such as those colonizing newly exposed glacier moraines (Trappe, 1988). Root samples collected from trees located above 2700 m exhibited a high rate of colonization by *C. geophilum* (90%), whereas in the lower areas, the colonization rate decreased to less than half (Trappe, 1988). Moreover, the symbiont is recognized as a "multi-stage" fungus in secondary forest succession, meaning that it forms ECM associations in both seedlings and adult host plants (Visser, 1995; Danielson, 1991). In the volcanic desert of Fuji Mountain, *C. geophilum* is present in both the early and later stages of vegetation development, colonizing young and old pioneer shrubs such as *Salix reinii,* as well as herbaceous species such as *Polygonum cuspidatum* (Nara, 2006). Interestingly, *C. geophilum* has been found in old growth forests (Peter, 2003), although it is known to primarily colonize young trees in alpine regions near tree lines (Hasselquist et al., 2005). In particular, *C. geophilum* is associated with seedlings and juvenile trees of *Picea engelmannii* and *Abies lasiocarpa*, with colonization rates 20 times greater for juveniles than for seedlings. These findings suggest that *C. geophilum* plays an important role in the early stages of forest succession, and that this symbiont may be necessary for survival in certain areas.

However, *C. geophilum* is not replaced by late-stage ECM species in older forest stands. The high prevalence of *C. geophilum* in mature alpine forest ecosystems, which are known for their cold climate, slow litter breakdown, and organic matter buildup in the soil, is believed to be a consequence of the substantial presence of sclerotia (approximately 3600 kg ha^{-1}) and synchronization of rootlet growth surge with sclerotia germination in autumn (Vogt et al. 1981). Furthermore, *C. geophilum* is among the most frequent ECM symbionts following a short fire return interval (Buscardo et al., 2010).

3.2 Drought tolerance

C. geophilum exhibits drought tolerance and is prevalent in water-stressed environments (Pigott, 1982; McCormack et al., 2017). Several surveys of soil fungal communities have shown that the proportions of *C. geophilum* ECM and extramatrical mycelia increase under water stress conditions and are often higher during summer in natural settings (Pigott, 1982; Querejeta et al., 2009). This tolerance has been further verified through *in vitro* culture experiments using osmotically adjusted media (Mexal and Reid, 1973; Coleman et al., 1989), cell damage tests following desiccation (Di Pietro et al., 2007), and respiration measurements under water stress (Jany et al., 2003). The level of tolerance varies among geographical isolates (Coleman et al., 1989; Jany et al., 2003). However, the physiological mechanisms responsible for the success of this species under water stress remain largely unknown. Multiple factors likely contribute to this trait, such as the accumulation of compatible osmolytes (e.g., polyols), heat shock proteins, hydrophobic proteins (e.g., hydrophobins), and melanin in cell walls. It has also been suggested that drought resistance in *C. geophilum* may be associated with the increased expression of aquaporin water channels (see below, Peter et al., 2016). Although *C. geophilum* is widely recognized as a drought-tolerant symbiont, this assertion has recently been debated. A study utilizing *Pinus seedlings* colonized by *C. geophilum* and subjected to drought stress demonstrated that the drought resistance of mycorrhizal plantlets was not directly correlated with that of *C. geophilum* isolates cultivated in liquid medium (Zhang et al., 2024). Xie et al. (2024) used inoculated *Quercus mongolica* and *Tilia amurensis* to investigate the response of ECM fungal communities and their exploration types under drought conditions in a pot system. The relative abundance of *C. geophilum* in both hosts decreased. Nickel et al. (2017) examined ECM fungal community diversity changes of European beech and Norway spruce forests under drought conditions by utilizing retractable roofs to exclude rain for three years. The results indicated that the abundance of *C. geophilum* decreased irrespective of the depth, year, or host. Pellitier et al. (2024) investigated ECM-fungal communities inhabiting *Populus trichocarpa* roots distributed across a precipitation gradient in the Pacific Northwest USA. These communities were profiled using taxonomic (metabarcoding) and functional (metagenomic) approaches. Their findings revealed that genes involved in fungal drought-stress tolerance and fungal-mediated plant water uptake (including genes of melanin synthesis, hydrophobins, aquaporins, trehalose synthases, and other gene families) were not the most abundant in drier soils, and fungal communities in dry soils were composed of distinct aquaporin and hydrophobin gene sequences.

3.3 Heat stress

Although laboratory experiments have shown that temperatures of 26°C can inhibit the growth of several *C. geophilum* isolates (Yan et al., 2022), this species is still capable of forming mycorrhizal associations after exposure to heat stress at approximately 70°C for a short time or 5°C higher than the environment. However, double stress, consisting of drought and heat stress, with a temperature 5°C higher than the environment and 50% precipitation, can be fatal to *C. geophilum* (Gehring et al., 2020; Kipfer et al., 2010). Herzog et al. (2013) showed that increases in temperature and drought can differentially affect the relative ECM abundance and exoenzyme activities of *C. geophilum* associated with various oak species, that is *Q. robur*, *Q. petraea* and *Q. pubescens*.

3.4 Cold stress

Furthermore, because of its abundance as a symbiont in Arctic and Alpine ecosystems, *C. geophilum* mycelia and ECM are likely to possess high tolerance to cold stress. Corbery and Le Tacon (1997) indicated that the mycelium of *C. geophilum* remains viable even when subjected to freezing temperatures of -80°C for a short duration, displaying a greater resistance to cold than other selected ECM fungi. Moreover, reports have suggested that *C. geophilum* can thrive at temperatures below 1°C (Vogt et al., 1982). This cold stress resistance in *C. geophilum* may be related to its high mannitol synthesis rate (Martin et al., 1985; Table 5). Mannitol is known to play a protective role under drought conditions and can be used to safeguard fungi from harsh cryoenvironments (Weinstein et al., 1997).

3.5 Heavy metal tolerance

C. geophilum isolates have shown patterns of local adaptation to serpentine soils, with a significant effect of nickel concentrations on fitness-related traits (Gonçalves et al., 2009; Bazzicalupo et al., 2020).

3.6 Phosphorus acquisition

Lussenhop and Fogel (1999) investigated phosphorus (P) dynamics in the ECM sheath of *C. geophilum* associated with *Pinus strobus*. Seasonal shifts in sheath P content were observed: a decline in spring during tree growth, stabilization in summer and fall when only fungal growth occurred, and an increase in early spring. Their findings suggested three phases of P dynamics: early spring uptake, translocation during tree growth, and depletion during fungal growth. These cycles indicate that P in ECM sheaths is shared between the fungus and the host tree.

4 Host Preferences

C. geophilum is recognized as a mycorrhizal generalist species. This symbiont can form ecto- or ectendomycorrhizal associations with a broad range of host plants. It does not form specialized structures such as cellular arbuscules or coils; however, it can penetrate cortical cells in certain plants to form ectendomycorrhizae. Based on the morphology and anatomy of the mycorrhizal roots sampled in natural settings, three groups of host plants were identified (Trappe 1962; LoBuglio 1999) (Figure 2. Photos of *C. geophilum*):

Group 1 hosts include members of the Salicaceae and Betulaceae families (excluding *Corylus* spp.) as well as ectotrophic genera within the Rosaceae family. The ECM root tips of these hosts are typically monopodial or occasionally branched, with the mantle covering only the root tip and measuring approximately 15 µm thick. The Hartig net in these hosts never extends deeper than the third layer of cortical cells, and intracellular penetration is sparse and limited to occasional cells.

The group 2 hosts were *Pinus* species. ECM root tips are monopodial, dichotomous, or occasionally irregularly branched. Mantles typically cover all short roots and have a thickness ranging between 8-60µm, with a median thickness of 20-30µm. The diameter of the shortest roots ranges from 0.35-1.0 mm, with an average of approximately 0.5 mm. The Hartig net extends inward to the innermost layer of cortical cells, and the cortex experiences strong intracellular infection. It is rare for these trees to form endotrophic mycorrhizal fungi.

The host species that formed ECM associations in Group 3 were predominantly found in Fagaceae, such as *the Corylus* and Pinaceae families, with the exception of *Pinus* spp. The root tips of these associations display a range of morphologies, including monopodial, racemose, irregularly branched, long, or short structures. The mantle typically covers a significant portion or all of the short roots, and its thickness ranges from to 8-60 µm, typically falling within the range of to 20-30 µm. The diameter of the short roots varies depending on the host species, with values ranging from 0.2 to l.0 mm, and generally falling within the range of 0.30.6 mm. The Hartig net extends to the innermost layer of the cortical cells, and intracellular infection is prevalent throughout the cortex. Although some host species form endotrophic mycorrhizae, ectotrophic associations are more common.

Additionally, many shrubs and herbaceous plants can form mycorrhizae with *C. geophilum*. The shrubs and herbaceous plants involved are *Pedicularis capitata* (Kohn & Stasovski, 1990), *Cistus* spp. (Massicotte et al., 2010), *Helianthemum* spp. (Read et al., 1977), *Dryas octopetala* (Bjorbækmo et al., 2010), *Bistorta vivipara* (Massicotte et al., 1998), *Carex myosuroides* (Massicotte et al., 1998), *Pulsatilla patens* (Hoeksema et al., 2018), *Pyrola* spp. (Jia et al., 2017), *Potentilla hyparctica* (Gardes & Dahlberg, 1996), *Vaccinium* spp. (Largent et al., 1980; Vohník et al., 2007) and *Rhododendron* spp. (Largent et al., 1980; Vohník et al., 2007). *Helianthemum* and *Cistus* species in the Cistaceae family exhibit distinct patterns of mycorrhizal associations and Hartig net development. *Helianthemum* forms ectendomycorrhiza and establishes the inner-most Hartig net (Group 3), whereas *Cistus* forms ECM associations with hyphae confined to the radially elongated epidermal cells (Group 1). These differences make Cistaceae an ideal model for investigating the molecular mechanisms underlying the establishment of either ECM or ectendomycorrhiza (Giovannetti and Fontana 1982; Massicotte et al. 2010). Unusual for an ECM symbiont, *C. geophilum* can also establish ectendomycorrhizal associations with shrubs and herbaceous plants, sharing mycelial networks with woody plants such as oak and *Helianthemum bicknellii* (Dickie et al., 2005), or the *Dryas octopetala-Bistorta vivipara-Salix herbacea* association (Mühlmann et al., 2008). Symbiosis with herbaceous plants appears to enhance the colonization of woody plants (Dickie et al., 2005; Hoeksema et al., 2018).

C. geophilum forms ECM and ectendomycorrhizae with shrubs and herbaceous plants in the Ericaceae family, including Monotropoideae, Arbutoideae, Pyroloideae, and Vaccinioideae, as well as herbaceous plant species (as detailed in section 4). A single *C. geophilum* hyphae can extend up to 2 m from a mycorrhizal root tip and differentiate up to 43 hyphal branches that connect with other mycorrhizal root tips (Trappe & Fogel, 1982). Although there is no evidence of nutrient transfer between herbaceous and woody plants sharing a *C. geophilum* common mycorrhizal networks (CMN), this structure could possibly act as a physical link between roots of herbaceous and woody plants, thereby enhancing *C. geophilum* colonization in sharing plants. CMN may also alter the bacterial communities of the hyphosphere (Vik et al., 2013). This CMN can also translocate nutrients to different regions of the soil (Frąc et al., 2018).

Strains from diverse geographical origins can exhibit widely different mycorrhizal rates for the same host species. In a comprehensive mycorrhizal inoculation assay, over 200 *C. geophilum* isolates from North America, Asia, and Europe were assessed for their colonization rates on *Pinus densiflora* and *Betula platyphylla* (Wang et al., 2021). These results indicated that *C. geophilum* had a relatively higher colonization rate with *Pinus densiflora*. In addition, some isolates formed ectomycorrhizae with both host trees, while other isolates colonized only a single host. A similar experiment has been conducted using an *in vitro* system in a controlled laboratory environment (Lian et al. 2022). Although some isolates displayed varying colonization rates with *Betula platyphylla* and *Pinus densiflora*, a small subset of *C. geophilum* strains was unable to colonize one or both hosts. Comparable findings have been reported in a *Salix rotundifola* inoculation experiment involving three *C. geophilum* isolates, one of which was unable to form ECM (Antibus et al. 1981). It is noteworthy that field observations may differ from laboratory experimental results (Trappe 1962). The inability of certain isolates to colonize host plants suggests that several *C. geophilum* strains possess host preferences or host specificity. Variations in colonization rates and/or host preferences can be attributed to genetic factors in both partners, as well as environmental factors, such as soil organic matter content, total N, and available P (Wurentaoges et al., 2012). For example, initial root ingress and subsequent Hartig net formation may be affected by the polysaccharide composition of the host plant cell walls and their depolymerization by secreted fungal enzymes. Mannans are the main component of gymnosperm wood, whereas xylans are the major polysaccharides in angiosperm wood (Godin et al., 2013); the types, proportions, and distributions of hemicellulose, especially β-1,4 mannan and β-1,4 xylan, and polygalacturonans may affect the loosening of the apoplastic space during penetration of ECM fungi, and thus, determine the Hartig net depth. Colonization of the root middle lamella by ECM hyphae relies on both the mechanical force generated by hydrostatic pressure at the tip of the growing hyphae (Massicotte et al., 1986; Cairney & Burke, 1994) and the activity of fungal plant cell-wall-degrading enzymes (PCWDEs) (Veneault-Fourrey et al., 2014; Zhang et al., 2018). Using comprehensive microarray polymer profiling technology, Sillo et al. (2016) showed that a localized degradation of pectin occurs during root colonization in *Tuber melanosporum*–*Coryllus avellana* ECM. Zhang *et al.* (2018, 2021) reported that the symbiosis-induced b-1,4-endoglucanase LbGH5-CBM1 and polygalacturonase LbGH28A act on poplar cell walls and that these enzymes are key factors for successful symbiotic fungal colonization. Symbiosis-induced GH5 and GH28 have been found in the *C. geophilum* genome (Peter et al., 2016), and their expression is upregulated in ECM roots.

The melanin content in *C. geophilum* cell walls may play a role in host recognition and root penetration (Paris et al., 1993). As the mycelium of *C. geophilum* shifts from a saprophytic free-living to a symbiotic state, its cell wall structure becomes less complex. The outer hyphal wall layer, which is highly reactive to the Gomori-Swift test that detects cysteine-rich proteins during the saprophytic phase, becomes less reactive and indistinguishable from the inner wall layer in Hartig net hyphae. This intensely stained outer wall layer was also not observed in pure *C. geophilum* cultures grown in low-sugar media. This alteration in cell wall composition may result from a decrease in melanin or a combination of melanin and cystine-rich proteins. Such changes could be essential for host colonization and/or improved nutrient exchange between symbiotic partners through the hyphal walls.

5 Molecular Biology, Genomics and Population Genetics

5.1 Genomics

As mentioned above, *C. geophilum* is a widely distributed ECM fungus, known for its resilience and broad ecological amplitude. It is one of the most ubiquitous ECM fungi, forming symbiotic associations with a wide variety of host plants, particularly with trees and shrubs. Its unique ability to thrive under diverse and often harsh environmental conditions such as drought and poor soils is likely related to its genomic blueprint. Recent studies have shown that *C. geophilum* is characterized by several distinctive molecular features that contribute to its survival and adaptability. As reported by Peter et al. (2016), ECM ecology is imprinted in the *C. geophilum* genome. The genome of the sequenced strain, 1.58, is notably large, approximately 178 Mb, and is one of the largest ECM-fungal genomes sequenced to date. This large genome size is primarily due to the high content of repetitive sequences, particularly transposable elements, which make up 81% of the genome. This is in contrast to its close saprotrophic relatives, *Glonium stellatum* and *Lepidopterella palustris*, which have significantly smaller genomes of 41 Mb and 46 Mb, respectively. Despite its close taxonomic relationship with these saprotrophs, *C. geophilum* exhibits unique genomic features that are consistent with its ECM lifestyle. *C. geophilum* contains 14,748 gene models, with 2,176 of these genes being unique to the species. Many of these unique genes are involved in protein-protein interactions and defense mechanisms, which are likely crucial for their symbiotic relationships with plants. Additionally, the fungus showed a reduction in plant cell wall-degrading enzymes compared to saprotrophic fungi, aligning with its symbiotic rather than decomposer role.

5.2 Gene Expression and Regulation

Peter et al. (2016) revealed that 3% of *C. geophilum* genes were upregulated during symbiosis, as determined by comparing RNA sequences from mycorrhizal roots and free-living mycelia. The most highly expressed and upregulated genes in symbiosis include transporters and small secreted proteins (SSPs), which are proteins of less than 300 amino acids with a predicted signal peptide. Notably, 18–23% of the upregulated genes were specific to *C. geophilum*, with SSPs being overrepresented in these taxon-specific orphan genes compared to their proportion in the overall gene repertoire. These SSPs may function as novel symbiosis-related effectors, similar to the mycorrhiza-induced protein MiSSPs in *Laccaria bicolor*, which regulate the defense-related pathway in host roots (Martin et al., 2016).

Pereira *et al.* (2018) compared the secretome of *C. geophilum* interacting with pine and poplar trees, and found that the levels of transcripts encoding carbohydrate-active enzymes (CAZymes) and mycorrhiza-induced small secreted proteins (MiSSPs) were strikingly different. This may be related to the different cell wall compositions of the pine and poplar roots (Sarkar et al. 2009). Colonizing *C. geophilum* hyphae may require different cell wall-loosening enzymes to penetrate the roots and effectors to dampen the host immune system.

Gene expression analysis also revealed changes in aquaporin (AQP) gene expression, which codes for water channels during symbiosis. The high expression of two highly water-permeable AQPs in the ECM may be triggered by plant water and/or nutrient requirements during interaction. Expression studies have indicated the precise regulation of AQP genes under drought conditions. However, Peter et al. (2016) were unable to demonstrate a significant effect of *C. geophilum* mycorrhization on the plant physiological parameters during drought. In contrast, Zhang et al. (2024) investigated the effect of *C. geophilum* on the drought resistance of *Pinus massoniana* seedlings in arid regions. They found that inoculation with various strains of *C. geophilum* improved the drought resistance of seedlings by affecting the water content, photosynthesis, osmotic adjustment substances, and antioxidant enzyme activities. Transcriptome analysis revealed that seedlings primarily regulated energy metabolism and redox reactions to cope with early drought stress. The effectiveness of the inoculation did not depend on the drought tolerance level of the *C. geophilum* strains; that is, the drought resistance of mycorrhizal seedlings did not correlate with the inherent drought resistance of the *C. geophilum* strain itself.

The survival of *C. geophilum* in various environments depends on its capacity to regulate the expression of stress-related genes. Transcriptome profiling has shown that *C. geophilum* can enhance the expression of numerous genes associated with stress resistance, including those associated with osmotic/drought stress (Li et al., 2022), salt stress (Li et al., 2022b), oxidative stress, heat shock responses (Yan et al., 2022), and heavy metal tolerance (Shi et al., 2022). These genes, which are involved in processes such as organic acid secretion, antioxidant activity (e.g., peroxidase and superoxide dismutase), membrane transport, and sphingolipid metabolism, are regulated in a synchronized manner. This suggests that their expression is controlled by transcription factors that react to environmental changes, such as heat shock factors (HSFs) and elements responsive to osmotic stress.

5.3 Population Genomics

For additional requirements for specific article types and further information please refer to "Article types" on every Frontiers journal page. Dauphin et al. (2021) conducted a study of 16 European isolates of *C. geophilum* using whole-genome re-sequencing. Their findings revealed divergent lineages in geographically confined sampling locations, without strong geographic structuring. Genome-wide polymorphism analyses indicated species subdivisions and suggested two primary genetic groups: clonal and recombinant. The lineage phylogeny and groupings were largely corroborated by the numerous copy number variations (CNVs) discovered among the genomes. Although the clonal cluster contained nearly twice as many strains, gene diversity analyses showed higher genetic diversity in the recombinant group. The top candidate genes potentially under positive selection, based on Tajima's D statistics, differed between the two groups. The recombinant cluster exhibited more genes from lineage-specific expanded gene families involved in self/non-self-recognition, while the more clonal cluster showed genes related to secondary metabolism. Additionally, this study confirmed *C. geophilum* heterothallism through chromosomal synteny analysis of MAT1-1 and MAT1-2 idiomorphs. It also revealed significant genetic rearrangements in the surrounding coding and non-coding regions for strains carrying both the same and opposite MAT1 idiomorphs. These results highlight the complex genome architecture of *C. geophilum*, possibly due to cryptic sex-and/or transposon-related mechanisms.

Li C *et al.* (2022) assembled five *C. geophilum* genomes representing different geographical regions and generated a pan-genome comprising 7,556 core gene families and 12,686 dispensable gene families. Genome re-sequencing of 304 isolates was performed to estimate the genetic diversity, structure, and demographic history of C. geophilum isolates. Millions of SNPs and 0.04%–0.2% structural variations were identified, suggesting the occurrence of several ecotypes (Zhang et al., 2024), which are isolates with different drought resilience levels.

5.4 Population Structure

Population genetic studies have revealed a complex population structure in *C. geophilum*, even at the soil core sample level, with evidence of both local adaptation and limited gene flow between populations (Jany et al. 2002; Douhan and Rizzo, 2005; Matsuda et al. 2015; Obase et al. 2016). Population structure is influenced by several factors, including geographic distance, environmental gradients, and host-plant associations. *C. geophilum* populations are often structured according to environmental conditions, such as soil type, moisture levels, and temperature. For example, populations from dry nutrient-poor soils tend to be genetically distinct from those in more fertile environments, suggesting a local adaptation to specific ecological niches (Douhan and Rizzo 2005, Lian et al. 2006, Bahram et al., 2011). A subtle geographic structure with long-distance disjunction suggests (Obase et al., 2016) a complex alternation of sexual and asexual reproduction over space and time (Obase et al., 2017). However, gene flow between populations can occur through sclerotia dispersal, leading to a combination of local adaptation and genetic exchange. The presence of cryptic species within *C. geophilum* has also been suggested, with distinct genetic lineages corresponding to different ecological and geographic regions (Obase et al., 2017; Vélez et al., 2021). These cryptic species may represent locally adapted forms of *C. geophilum*, which have evolved in response to specific environmental conditions.

The genetic diversity of angiosperm-associated *C. geophilum* populations is higher than that of gymnosperm-associated populations, suggesting that angiosperm and gymnosperm hosts exert different selective pressures on their symbionts (Field et al. 2018). Furthermore, the population genetic diversity and structure of Cg were analyzed based on the ITS2 sequences of 219 root samples collected from three plant families (Betulaceae, Fagaceae, and Pinaceae) from 10 Chinese forest sites using high-throughput sequencing techniques (Guo et al., 2021). Analysis of molecular variance (AMOVA) showed that genetic differentiation was evident within each geographical population and population in each host plant family. The Fagaceae population was distant from the Betulaceae and Pinaceae populations, and the haplotype composition was conspicuously different among the three plant families.

6 Applications in forestry and conservation

Through the utilization of genomics and other -omics techniques to examine the mycorrhizal symbiosis formed by *C. geophilum*, we have acquired a more comprehensive understanding of the molecular, physiological, and ecological mechanisms underlying this mutually beneficial relationship. DNA metabarcoding surveys can foster applications and predict the conditions under which *C. geophilum* inoculation is beneficial to foresters and land managers. These genotyping approaches can also be used to identify whether specific isolates are characteristic of well-performing systems. This enhanced knowledge should be leveraged to develop practical applications that enhance ecosystem function and preservation, aid in the overall reduction of climate change impacts, and maintain the long-term viability of arable lands. *C. geophilum* is a highly adaptable ECM fungus that demonstrates significant potential for ecological restoration and environmental remediation. Its extensive distribution, broad host range, and high stress tolerance make it particularly valuable for addressing desertification and adapting to climate change (Zhai et al. 2023). This fungus is found in diverse ecosystems worldwide, from boreal forests to tropical regions, and can thrive in a wide range of soil conditions, including extreme pH levels and nutrient deficiencies. *C. geophilum* establishes symbiotic relationships with diverse tree species, including poplars and pines, thereby enhancing drought resistance and growth in arid regions (Wang et al., 2020). The fungus forms a dense network of melanized hyphae around the roots of host plants, creating a protective sheath that improves water and nutrient uptake. This symbiotic association is particularly beneficial in water-limited environments, where Cg helps trees to maintain hydraulic conductivity and photosynthetic activity under drought stress. Additionally, the fungus has been shown to enhance salt tolerance of host plants, making it valuable for reclaiming saline soils.

Furthermore, the fungus colonizes herbaceous plants, potentially fostering a balanced ecosystem that supports forest stability and mitigates desertification. By forming associations with both woody and non-woody plants, Cg can contribute to the development of diverse plant communities in challenging environments. The ability to support multiple plant species can lead to increased soil stability, reduced erosion, and improved nutrient cycling in degraded ecosystems. In arid regions, *C. geophilum* colonizes both ECM and Cistaceae plants. The physical connection of *C. geophilum* mycelial networks with both tree roots and herbaceous plants could redistribute water from the deeper roots of the tree, retain a portion of the water in the upper soil layers, and facilitate enhanced nutrient acquisition by the host plant. This Cistaceae- *C. geophilum* -ECM tree symbiotic community is likely to contribute to forest stability, mitigate soil desertification, and promote afforestation. Furthermore, *Carex myosuroides* can be incorporated into the community when implemented in northern regions (e.g., northern China, Huebner, and Al-Quraishi). Environmental changes are a problem for plants in which the area is located between two climate regions, the bounder and the savanna. For example, vulnerable areas in China in the forest–grassland ecotone suffer from an increasing frequency or severity of drought because of global climate change (Piao et al., 2010). Because *C. geophilum* has a wide distribution, host range, and high resistance to stresses (such as drought stress), it could help these plants adapt to climate change.

In contaminated environments, *C. geophilum* has shown promising results in the remediation of soils affected by heavy metals and petroleum (Danielson and Visser, 2019; Danielson and Visser, 1989). The fungus has demonstrated the ability to accumulate and sequester various heavy metals, including Pb, Cd, and Zn, in melanized cell walls (Huang et al. 2014, Azaiez et al. 2018, Shi et al. 2022, Zhang et al. 2023,). This characteristic makes Cg a potential candidate for mycoremediation in polluted soils. Moreover, its association with host plants can enhance phytoremediation efforts by improving plant survival and growth at contaminated sites.

C. geophilum thrives in urban ecosystems and enhances the vitality of urban trees, such as Tilia tomentosa, further underscoring its adaptability and ecological significance (Garbaye et al., 1996; Van Geel et al., 2018). Urban environments often present challenging conditions for plant growth, such as soil compaction, elevated temperatures, and air pollution. *C. geophilum* is often the most abundant ECM symbiont in urban trees (Hui et al. 2017, Van Geel et al. 2018, Olchowik et al. 2021), and the ability to form symbiotic relationships can improve resilience to these stressors, potentially leading to increased tree longevity and enhanced ecosystem services in cities.

The genetic and physiological adaptations that allow *C. geophilum* to flourish in diverse and challenging environments are the subject of ongoing research. Understanding these mechanisms could provide insight into fungal stress tolerance and adaptation strategies, potentially leading to the development of novel biotechnological applications. For instance, genes or compounds derived from *C. geophilum* can be used to enhance the stress tolerance of agricultural crops or improve the efficiency of bioremediation processes. In the context of climate change, the role of *C. geophilum* in ecosystem resilience has become increasingly important. As extreme weather events and environmental stressors become more frequent, the ability of fungi to support plant growth and survival under adverse conditions could be crucial for maintaining ecosystem stability and biodiversity. Furthermore, its potential to enhance carbon sequestration through increased plant growth and soil organic matter accumulation may contribute to climate change mitigation.

7 Future directions

Several enduring challenges persist in utilizing genomics and other -omics approaches, such as metabarcoding and metatranscriptomics, to enhance our understanding of mycorrhizal symbioses in *C. geophilum*, encompassing their evolutionary history, developmental processes, functional aspects, and ecological roles. We identified ten critical areas that warrant further investigation.

- In what ways does the genetic diversity of *C. geophilum* facilitates its wide distribution.
- Which gene families are crucial for *C. geophilum* to tolerate stress?

- How does the mutualistic association between *C. geophilum* and its plant partners fluctuate across various environmental settings?
- What is the significance of horizontal gene transfer (if any) in *the evolutionary* trajectory of *C. geophilum*?
- How does *C. geophilum* modulate gene expression in response to drought and other abiotic stressors?
- What patterns have emerged in the population genomics of *C. geophilum* across different geographical areas?
- How do the secondary metabolites produced by *C. geophilum*, such as melanin, influence its interactions with soil microbial communities such as soil and litter decomposers?
- What role do epigenetic modifications play in the ability of *C. geophilum* to adapt to different environments?
- How do the genomic functions of *C. geophilum* affect nutrient cycling processes within forest ecosystems?
- What molecular mechanisms underlie the resilience of *C. geophilum* to extreme environmental conditions, including heavy metal contamination or high salinity?

8 References

Amano, N. (1983). Saprobic loculoascomycetous fungi from Japan 1. Hysteriaceous fungi. Transactions of the Mycological Society of Japan, 24, 283-297.

Antibus, R. K., Croxdale, J. G., Miller, O. K., & Linkins, A. E. (1981). Ectomycorrhizal fungi of *Salix rotundifolia* III. Resynthesized mycorrhizal complexes and their surface phosphatase activities. Canadian Journal of Botany, 59, 2458-2465.

Azaiez, A., Beaudoin Nadeau, M., Bertrand, A. & Khasa, D. P. (2018). In vitro selection of ecologically adapted ectomycorrhizal fungi through production of fungal biomass and metabolites for use in reclamation of biotite mine tailings. Mycologia, 110**,** 1017-1032.

Bâ, A. M., Duponnois, R., Moyersoen, B., & Diédhiou, A. G. (2012). Ectomycorrhizal symbiosis of tropical African trees. Mycorrhiza, 22, 1-29.

Bakray, N. A. M., Azman, A. S., Kin, T. B., Mansor, P., Mohti, A., Jamar, N. H., ... & Said, M. N. M. (2024). Effects of soil nutrient availability on ectomycorrhizal communities's distribution in two *Dipterocarps* species under normal and elevated atmospheric carbon dioxide. Research Square, https://doi.org/10.21203/rs.3.rs-3780828/v1

Bazzicalupo, A. L., Ruytinx, J., Ke, Y. H., Coninx, L., Colpaert, J. V., Nguyen, N. H., Vilgalys, R. & Branco, S. (2020). Fungal heavy metal adaptation through single nucleotide polymorphisms and copy-number variation. Molecular Ecology, 29**,** 4157-4169.

Becerra, A.G., Zak, M.R. (2011). The Ectomycorrhizal Symbiosis in South America: Morphology, Colonization, and Diversity. In: Rai, M., Varma, A. (eds) Diversity and Biotechnology of Ectomycorrhizae. Soil Biology, Springer, Berlin, Heidelberg, pp.19-41.

Beimforde, C., Schafer, N., Dorfelt, H., Nascimbene, P. C., Singh, H., Heinrichs, J., Reitner, J., Rana, R. S. & Schmidt, A. R. (2011). Ectomycorrhizas from a Lower Eocene angiosperm forest. New Phytol, 192**,** 988-996.

Bjorbækmo, M. F. M., Carlsen, T., Brysting, A., Vrålstad, T., Høiland, K., Ugland, K. I., Geml, J., Schumacher, T. & Kauserud, H. (2010). High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. BMC Plant Biology, 10**,** 244.

Bourne, E. C., Mina, D., Goncalves, S. C., Loureiro, J., Freitas, H. & Muller, L. A. (2014). Large and variable genome size unrelated to serpentine adaptation but supportive of cryptic sexuality in Cenococcum geophilum. Mycorrhiza, 24**,** 13-20.

Buscardo, E., Rodríguez-Echeverría, S., Martín, M. P., De Angelis, P., Pereira, J. S., & Freitas, H. (2010). Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. Fungal Biology, 114, 628-636.

Cairney JWG, Burke RM. (1994). Fungal enzymes degrading plant cell walls: their possible significance in the ectomycorrhizal symbiosis. Mycological Research, 98, 1345–1356.

Coleman, M. D., Bledsoe, C. S. & Lopushinsky, W. (1989). Pure culture response of ectomycorrhizal fungi to imposed water stress. Canadian Journal of Botany, 67**,** 29-39.

Corbery, Y., & Le Tacon, F. (1997). Storage of ectomycorrhizal fungi by freezing. In Annales des sciences forestières. EDP Sciences. 54, 211-217

Danielson R M, Visser S. 1989. Host response to inoculation and behaviour of introduced and indigenous ectomycorrhizal fungi of jack pine grown on oil-sands tailings. Canadian Journal of Forest Research, 19, 1412-1421.

Danielson, R. M. (1991). Temporal changes and effects of amendments on the occurrence of sheating (ecto-) mycorrhizas of conifers growing in oil sands tailings and coal spoil. Agriculture, ecosystems & environment, 35, 261-281.

Dauphin, B., De Freitas Pereira, M., Kohler, A., Grigoriev, I. V., Barry, K., Na, H., Amirebrahimi, M., Lipzen, A., Martin, F., Peter, M. & Croll, D. (2021). Cryptic genetic structure and copy-number variation in the ubiquitous forest symbiotic fungus *Cenococcum geophilum*. Environ Microbiol, 23**,** 6536-6556.

De Freitas Pereira, M., Veneault-Fourrey, C., Vion, P., Guinet, F., Morin, E., Barry, K. W., Lipzen, A., Singan, V., Pfister, S., Na, H., Kennedy, M., Egli, S., Grigoriev, I., Martin, F., Kohler, A. & Peter, M. (2018). Secretome Analysis from the Ectomycorrhizal Ascomycete *Cenococcum geophilum.* Front Microbiol, 9**,** 141.

Di Pietro, M., Churin, J. L. & Garbaye, J. (2007). Differential ability of ectomycorrhizas to survive drying. Mycorrhiza, 17**,** 547-550.

DICKIE, IAN. A., & Reich, P. B. (2005). Ectomycorrhizal fungal communities at forest edges. Journal of Ecology, 93, 244-255.

Douhan, G. W. & Rizzo, D. M. (2005). Phylogenetic divergence in a local population of the ectomycorrhizal fungus Cenococcum geophilum. New Phytol, 166**,** 263-71.

Ferdinandsen, C., & Winge, Ö. (1925). *Cenococcum* Fr. A monographic study.

Fernandez, C. W. & Koide, R. T. (2013). The function of melanin in the ectomycorrhizal fungus *Cenococcum geophilum* under water stress. Fungal Ecology, 6**,** 479-486.

Fernandez, C. W. & Koide, R. T. (2014). Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. Soil Biology and Biochemistry, 77**,** 150-157.

Field, K. J. & Pressel, S. (2018). Unity in diversity: structural and functional insights into the ancient partnerships between plants and fungi. New Phytol, 220**,** 996-1011.

Flores, R., Godoy, B., & Palfner, G. (1997). Morfo-anatomía de la ectomicorriza *Cenococcum geophilum* Fr. en *Nothofagus alessandrii* Esp.

Frąc, M., Hannula, S. E., Bełka, M. & Jędryczka, M. (2018). Fungal Biodiversity and Their Role in Soil Health. Frontiers in Microbiology, 9.

Garbaye, J. & Churin, J.-L. (1996). Effect of ectomycorrhizal inoculation at planting on growth and foliage quality of *Tilia tomentosa*. Arboriculture & Urban Forestry (AUF), 22**,** 29-34.

Gardes, M. & Dahlberg, A. (1996). Mycorrhizal diversity in arctic and alpine tundra: an open question. New Phytologist, 133**,** 147-157.

Gehring, C., Sevanto, S., Patterson, A., Ulrich, D. E. M. & Kuske, C. R. (2020). Ectomycorrhizal and Dark Septate Fungal Associations of Pinyon Pine Are Differentially Affected by Experimental Drought and Warming. Front Plant Sci, 11**,** 582-574.

Genney, D. R., Anderson, I. C. & Alexander, I. J. (2006). Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. New Phytol, 170**,** 381-90.

Giovannetti, G. & Fontana, A. (2006). Mycorrhizal Synthesis between *Cistaceae* and *Tuberaceae*. New Phytologist, 92**,** 533-537.

Godin, B., Lamaudière, S., Agneessens, R., Schmit, T., Goffart, J.-P., Stilmant, D., Gerin, P. A. & Delcarte, J. (2013). Chemical Composition and Biofuel Potentials of a Wide Diversity of Plant Biomasses. Energy & Fuels, 27**,** 2588-2598.

Gonçalves, S. C., Martins-Loução, M. A., & Freitas, H. (2009). Evidence of adaptive tolerance to nickel in isolates of *Cenococcum geophilum* from serpentine soils. Mycorrhiza, 19, 221-230.

GUO Zi-Xuan, WANG Yong-Long, WU Bin-Wei, XU Ying, YAO Hui, LI Zhong-Feng, YU Qing-Yi, LI Xing-Chun, GUO Liang-Dong. (2021). Population genetic diversity and structure of ectomycorrhizal fungus *Cenococcum geophilum*. Mycosystema, 40**,** 920-935.

Han, S., & Yan, W. (2012). The relationship between natural infection rate of *Cenococcum geophilum* and vegetation, rhizosphere soil factors. Chin. Agric. Sci. Bull., 28, 47-51.

Hasselquist, N., Germino, M. J., McGonigle, T., & Smith, W. K. (2005). Variability of Cenococcum colonization and its ecophysiological significance for young conifers at alpine–treeline. New Phytologist, 165, 867-873.

Hawkins, H.-J., Cargill, R. I. M., Van Nuland, M. E., Hagen, S. C., Field, K. J., Sheldrake, M., Soudzilovskaia, N. A. & Kiers, E. T. (2023). Mycorrhizal mycelium as a global carbon pool. Current Biology, 33**,** 560-573.

Herzog, C., Peter, M., Pritsch, K., Günthardt ‐ Goerg, M. S., & Egli, S. (2013). Drought and air warming affects abundance and exoenzyme profiles of *Cenococcum geophilum* associated with *Quercus robur, Q. petraea and Q. pubescens*. Plant Biology, 15, 230-237.

Hobbie, E. A. & Agerer, R. (2009). Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. Plant and Soil, 327**,** 71-83.

Hoeksema, J., Roy, M., Łaska, G., Sienkiewicz, A., Horning, A., Abbott, M. J., Tran, C. & Mattox, J. (2018). *Pulsatilla patens* (*Ranunculaceae*), a perennial herb, is ectomycorrhizal in northeastern Poland and likely shares ectomycorrhizal fungi with Pinus sylvestris. Acta Societatis Botanicorum Poloniae, 87, 3578.

Huang, J., Nara, K., Zong, K., Wang, J., Xue, S., Peng, K., Shen, Z. & Lian, C. (2014). Ectomycorrhizal fungal communities associated with Masson pine (*Pinus massoniana*) and white oak (*Quercus fabri*) in a manganese mining region in Hunan Province, China. Fungal Ecology, 9**,** 1-10.

Hui, N., Liu, X., Kotze, D. J., Jumpponen, A., Francini, G. & Setälä, H. (2017). Ectomycorrhizal fungal communities in urban parks are similar to those in natural forests but shaped by vegetation and park age. Applied and Environmental Microbiology, 83**,** e01797-17.

Jany, J. L., Martin, F., & Garbaye, J. (2003). Respiration activity of ectomycorrhizas from *Cenococcum geophilum* and *Lactarius* sp. in relation to soil water potential in five beech forests. Plant and Soil, 255, 487-494.

Jia, S., Nakano, T., Hattori, M. & Nara, K. (2017). Root-associated fungal communities in three *Pyroleae* species and their mycobiont sharing with surrounding trees in subalpine coniferous forests on Mount Fuji, Japan. Mycorrhiza, 27**,** 733-745.

Kipfer, T., Egli, S., Ghazoul, J., Moser, B., & Wohlgemuth, T. (2010). Susceptibility of ectomycorrhizal fungi to soil heating. Fungal biology, 114, 467-472.

Kohn, L. M., & Stasovski, E. (1990). The Mycorrhizal Status of Plants at Alexandra Fiord, Ellesmere Island, Canada, a High Arctic Site. Mycologia, 82, 23–35.

Largent, D., Sugihara N., and Wishner, C. (1980). Occurrence of mycorrhizae on ericaceous and pyrolaceous plants in northern California. Canadian Journal of Botany. 58, 2274-2279

Li, J., Li, C., Tsuruta, M., Matsushita, N., Goto, S., Shen, Z., Tsugama, D., Zhang, S. & Lian, C. (2022). Physiological and transcriptional responses of the ectomycorrhizal fungus *Cenococcum geophilum* to salt stress. Mycorrhiza, 32**,** 327-340.

Li, M., Yuan, C., Zhang, X., Pang, W., Zhang, P., Xie, R., Lian, C. & Zhang, T. (2022). The Transcriptional Responses of Ectomycorrhizal Fungus, *Cenococcum geophilum*, to Drought Stress. J Fungi (Basel), 9.

Lian, C., Narimatsu, M., Nara, K. & Hogetsu, T. (2006). *Tricholoma matsutake* in a natural *Pinus densiflora* forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities. New Phytol, 171**,** 825-36.

Lian, C., Zhang, J., Li, C., Matsushita, N., Tsuruta, M., Wang, H. & Zhang, T. (2022). Exploiting natural variation in *Cenococcum geophilum* to identify genes for ectomycorrhizal formation. The Japanese Forest Society Congress, 133**,** 208.

LoBuglio, K.F. (1999). *Cenococcum*. In: Cairney, J.W.G., Chambers, S.M. (eds) Ectomycorrhizal Fungi Key Genera in Profile. Springer, Berlin, Heidelberg, pp. 287-309.

Lussenhop, J., & Fogel, R. (1999). Seasonal change in phosphorus content of *Pinus strobus – Cenococcum geophilum* ectomycorrhizae. Mycologia, 91, 742–746.

Martin, F. M. & Van Der Heijden, M. G. A. (2024). The mycorrhizal symbiosis: research frontiers in genomics, ecology, and agricultural application. New Phytol, 242**,** 1486-1506.

Martin, F., Aerts, A., Ahrén, D., Brun, A., Danchin, E. G. J., Duchaussoy, F., Gibon, J., Kohler, A., Lindquist, E., Pereda, V., Salamov, A., Shapiro, H. J., Wuyts, J., Blaudez, D., Buée, M., Brokstein, P., Canbäck, B., Cohen, D., Courty, P. E., Coutinho, P. M., Delaruelle, C., Detter, J. C., Deveau, A., Difazio, S., Duplessis, S., Fraissinet-Tachet, L., Lucic, E., Frey-Klett, P., Fourrey, C., Feussner, I., Gay, G., Grimwood, J., Hoegger, P. J., Jain, P., Kilaru, S., Labbé, J., Lin, Y. C., Legué, V., Le Tacon, F., Marmeisse, R., Melayah, D., Montanini, B., Muratet, M., Nehls, U., Niculita-Hirzel, H., Secq, M. P. O.-L., Peter, M., Quesneville, H., Rajashekar, B., Reich, M., Rouhier, N., Schmutz, J., Yin, T., Chalot, M., Henrissat, B., Kües, U., Lucas, S., Van De Peer, Y., Podila, G. K., Polle, A., Pukkila, P. J., Richardson, P. M., Rouzé, P., Sanders, I. R., Stajich, J. E., Tunlid, A., Tuskan, G. & Grigoriev, I. V. (2008). The genome of Laccaria bicolor provides insights into mycorrhizal symbiosis. Nature, 452**,** 88-92.

Martin, F., Canet, D. & Marchal, J. P. (1985). 13C nuclear magnetic resonance study of mannitol cycle and trehalose synthesis during glucose utilization by the ectomycorrhizal ascomycete Cenococcum graniforme. Plant Physiology, 77**,** 499-502.

Martin, F., Kohler, A., Murat, C., Veneault-Fourrey, C., & Hibbett, D. S. (2016). Unearthing the roots of ectomycorrhizal symbioses. Nature Reviews Microbiology, 14, 760-773.

Massicotte, D., Peronnet, F., Allah, C., Hillaire-Marcel, C., Ledoux, M., & Brisson, G. (1986). Metabolic response to [13C] glucose and [13C] fructose ingestion during exercise. Journal of Applied Physiology, 61, 1180-1184.

Massicotte, H. B., Melville, L. H., Peterson, R. L. & Luoma, D. L. (1998). Anatomical aspects of field ectomycorrhizas on *Polygonum viviparum* (Polygonaceae) and *Kobresia bellardii* (Cyperaceae). Mycorrhiza, 7**,** 287-292.

Massicotte, H. B., Peterson, R. L., Melville, L. H. & Tackaberry, L. E. (2010). *Hudsonia ericoides* and *Hudsonia tomentosa*: Anatomy of mycorrhizas of two members in the *Cistaceae* from Eastern Canada. Botany, 88**,** 607-616.

Massicotte, H. B., Trappe, J. M., Peterson, R. L. & Melville, L. H. (1992). Studies on *Cenococcum geophilum*. II. Sclerotium morphology, germination, and formation in pure culture and growth pouches. Canadian Journal of Botany, 70**,** 125-132.

Matsuda, Y., Takeuchi, K., Obase, K. & Ito, S. (2015). Spatial distribution and genetic structure of *Cenococcum geophilum* in coastal pine forests in Japan. FEMS Microbiol Ecol, 91.

Mccormack, M. L., Fernandez, C. W., Brooks, H. & Pritchard, S. G. (2017). Production dynamics of *Cenococcum geophilum* ectomycorrhizas in response to long-term elevated CO2 and N fertilization. Fungal Ecology, 26**,** 11-19.

Mexal, J., & Reid, C. P. P. (1973). The growth of selected mycorrhizal fungi in response to induced water stress. Canadian Journal of Botany, 51, 1579-1588.

Mikola P. (1948). On the physiology and ecology of *Cenococcum graniforme* especially as a mycorrhizal fungus of birch. Inst Forest Fenn Commun, 36, 1–104.

Murayama, S., Sugiura, Y. (2021). Origin of Soil Polysaccharides, and Ectomycorrhizal Fungal Sclerotia as Sources of Forest Soil Polysaccharides. In: Watanabe, M. (eds) Sclerotia Grains in Soils. Progress in Soil Science. Springer, Singapore, pp. 91-117.

Nara, K. (2006). Ectomycorrhizal networks and seedling establishment during early primary succession. New Phytologist, 169, 169-178.

Narisawa, K., Amasya, A., Nonoyama, Y.S., Obase, K. (2021). Fungal Communities of Sclerotia Grains from Forest Soils. In: Watanabe, M. (eds) Sclerotia Grains in Soils. Progress in Soil Science. Springer, Singapore, pp. 17-34.

Nicolas, C., Martin-Bertelsen, T., Floudas, D., Bentzer, J., Smits, M., Johansson, T., Troein, C., Persson, P. & Tunlid, A. (2019). The soil organic matter decomposition mechanisms in ectomycorrhizal fungi are tuned for liberating soil organic nitrogen. ISME J, 13**,** 977-988.

Nickel, U. T., Weikl, F., Kerner, R., Schäfer, C., Kallenbach, C., Munch, J. C. & Pritsch, K. (2018). Quantitative losses vs. qualitative stability of ectomycorrhizal community responses to 3 years of experimental summer drought in a beech–spruce forest. Global change biology, 24**,** e560-e576.

Nyamsanjaa, K., Oyuntsetseg, B., Takashima, Y., Sakagami, N. & Watanabe, M. (2021). Characteristics of *Cenococcum geophilum* sclerotia found in steppe forest soil in Mongolia. Journal of Forest Research, 27**,** 76-82.

Obase, K., Douhan, G. W., Matsuda, Y. & Smith, M. E. (2014). Culturable fungal assemblages growing within *Cenococcum* sclerotia in forest soils. FEMS Microbiol Ecol, 90**,** 708-17.

Obase, K., Douhan, G. W., Matsuda, Y. & Smith, M. E. (2016). Revisiting phylogenetic diversity and cryptic species of *Cenococcum geophilum* sensu lato. Mycorrhiza, 26**,** 529-40.

Obase, K., Douhan, G. W., Matsuda, Y. & Smith, M. E. (2017). Progress and Challenges in Understanding the Biology, Diversity, and Biogeography of of *Cenococcum geophilum*. In: Tedersoo, L. (eds) Biogeography of Mycorrhizal Symbiosis. Springer, Cham, pp. 299-317.

Olchowik, J., Suchocka, M., Jankowski, P., Malewski, T. & Hilszczańska, D. (2021). The ectomycorrhizal community of urban linden trees in Gdańsk, Poland. Plos one, 16**,** e0237551.Pal, A. K., Gajjar, D. U. & Vasavada, A. R. (2014). DOPA and DHN pathway orchestrate melanin synthesis in Aspergillus species. Med Mycol, 52**,** 10-8.

Paris, F., Dexheimer, J., & Lapeyrie, F. (1993). Cytochemical evidence of a fungal cell wall alteration during infection of Eucalyptus roots by the ectomycorrhizal fungus *Cenococcum geophilum*. Archives of microbiology, 159, 526-529.

Pellitier, P. T., Van Nuland, M., Salamov, A., Grigoriev, I. V. & Peay, K. G. (2024). Potential for functional divergence in ectomycorrhizal fungal communities across a precipitation gradient. ISME communications, 4**,** ycae031.

Peter, M. (2003). Volcanic deserts and primary succession: when and how do mycorrhizal fungi participate? New Phytologist, 159, 534-536.

Peter, M., Kohler, A., Ohm, R. A., Kuo, A., Krutzmann, J., Morin, E., Arend, M., Barry, K. W., Binder, M., Choi, C., Clum, A., Copeland, A., Grisel, N., Haridas, S., Kipfer, T., Labutti, K., Lindquist, E., Lipzen, A., Maire, R., Meier, B., Mihaltcheva, S., Molinier, V., Murat, C., Poggeler, S., Quandt, C. A., Sperisen, C., Tritt, A., Tisserant, E., Crous, P. W., Henrissat, B., Nehls, U., Egli, S., Spatafora, J. W., Grigoriev, I. V. & Martin, F. M. (2016). Ectomycorrhizal ecology is imprinted in the genome of the dominant symbiotic fungus *Cenococcum geophilum*. Nat Commun, 7**,** 12662.

Phosri, C., Polme, S., Taylor, A. F., Koljalg, U., Suwannasai, N., & Tedersoo, L. (2012). Diversity and community composition of ectomycorrhizal fungi in a dry deciduous dipterocarp forest in Thailand. Biodiversity and Conservation, 21, 2287-2298.

Piao, S., Ciais, P., Huang, Y., Shen, Z., Peng, S., Li, J., Zhou, L., Liu, H., Ma, Y., Ding, Y., Friedlingstein, P., Liu, C., Tan, K., Yu, Y., Zhang, T. & Fang, J. (2010). The impacts of climate change on water resources and agriculture in China. Nature, 467**,** 43-51.

Pigott, C. D. (1982). Survival of mycorrhiza formed by *Cenococcum geophilum* Fr. in dry soils. New Phytologist, 92, 513-517.

Põlme, S., Bahram, M., Kõljalg, U. & Tedersoo, L. (2017). Biogeography and Specificity of Ectomycorrhizal Fungi of Coccoloba uvifera. Biogeography of Mycorrhizal Symbiosis.

Qiuli Bao. (2005). Survey of Ectomycorrhizal Fungus Flora Association with Picea Mongolica and Study on the Mycelial Culture Condition of *Cenococcum geophilum* Fr.in Baiyinaobao (Doctoral dissertation). Inner Mongolia Agricultural University.

Querejeta, J., Egerton-Warburton, L. M., & Allen, M. F. (2009). Topographic position modulates the mycorrhizal response of oak trees to interannual rainfall variability. Ecology, 90, 649-662.

Read, D. J., Kianmehr, H., & Malibari, A. (1977). The Biology of Mycorrhiza in *Helianthemum* Mill. The New Phytologist, *78*, 305–312.

Rosling, A., Landeweert, R., Lindahl, B. D., Larsson, K. H., Kuyper, T. W., Taylor, A. F. S. & Finlay, R. D. (2003). Vertical distribution of ectomycorrhizal fungal taxa in a podzol soil profile. New Phytol, 159**,** 775-783.

Sarkar, P., Bosneaga, E. & Auer, M. (2009). Plant cell walls throughout evolution: towards a molecular understanding of their design principles. Journal of Experimental Botany, 60**,** 3615-3635.

Scattolin, L., Montecchio, L., Mosca, E. & Agerer, R. (2008). Vertical distribution of the ectomycorrhizal community in the top soil of Norway spruce stands. European Journal of Forest Research, 127**,** 347-357.

Shi, Y., Yan, T., Yuan, C., Li, C., Rensing, C., Chen, Y., Xie, R., Zhang, T. & Lian, C. (2022). Comparative Physiological and Transcriptome Analysis Provide Insights into the Response of *Cenococcum geophilum,* an Ectomycorrhizal Fungus to Cadmium Stress. J Fungi (Basel), 8.

Sillo, F., Fangel, J. U., Henrissat, B., Faccio, A., Bonfante, P., Martin, F., Willats, W. G. & Balestrini, R. (2016). Understanding plant cell-wall remodelling during the symbiotic interaction between *Tuber melanosporum* and *Corylus avellana* using a carbohydrate microarray. Planta, 244**,** 347-59.

Smith, S. E. & Read, D. J. (2010). Mycorrhizal Symbiosis, Third Edition.

Tang, Y., Shi, L., Zhong, K., Shen, Z. & Chen, Y. (2019). Ectomycorrhizal fungi may not act as a barrier inhibiting host plant absorption of heavy metals. Chemosphere, 215**,** 115-123.

Tedersoo, L., May, T. W. & Smith, M. E. (2010). Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza, 20**,** 217-63.

Trappe, J. M. (1988). Lessons from alpine fungi. Mycologia, 80, 1-10.

Trappe, J. M., & Fogel, R. D. (1977). Ecosystematic functions of mycorrhizae.

Trappe, J., (1962). Cenococcum graniforme e Its Distribution, Ecology, Mycorrhiza Formation and Inherent Variation (Doctoral dissertation). University of Washington.

Van Der Heijden, M. G., Martin, F. M., Selosse, M. A. & Sanders, I. R. (2015). Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytol, 205**,** 1406-23.

Van Geel, M., Yu, K., Ceulemans, T., Peeters, G., Van Acker, K., Geerts, W., Ramos, M. A., Serafim, C., Kastendeuch, P. & Najjar, G. (2018). Variation in ectomycorrhizal fungal communities associated with Silver linden (*Tilia tomentosa*) within and across urban areas. FEMS Microbiology Ecology, 94**,** fiy207.

Vélez, J. M., Morris, R. M., Vilgalys, R., Labbé, J., & Schadt, C. W. (2021). Phylogenetic diversity of 200+ isolates of the ectomycorrhizal fungus *Cenococcum geophilum* associated with *Populus trichocarpa* soils in the Pacific Northwest, USA and comparison to globally distributed representatives. PLoS One, 16, e0231367.

Veneault-Fourrey, C., Commun, C., Kohler, A., Morin, E., Balestrini, R., Plett, J., Danchin, E., Coutinho P., Wiebenga A., De Vries R.P., Henrissat B., Martin, F. (2014). Genomic and transcriptomic analysis of *Laccaria bicolor* CAZome reveals insights into polysaccharides remodelling during symbiosis establishment. Fungal Genetics and Biology, 72, 168-181.

Větrovský, T., Morais, D., Kohout, P., Lepinay, C., Algora, C., Awokunle Hollá, S., Bahnmann, B. D., Bílohnědá, K., Brabcová, V. & D'alò, F. (2020). GlobalFungi, a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. Scientific Data, 7**,** 228.

Vik, U., Logares, R., Blaalid, R., Halvorsen, R., Carlsen, T., Bakke, I., Kolstø, A.-B., Økstad, O. A. & Kauserud, H. (2013). Different bacterial communities in ectomycorrhizae and surrounding soil. Scientific Reports, 3**,** 3471.

Visser, S. (1995). Ectomycorrhizal fungal succession in jack pine stands following wildfire. New Phytologist, 129, 389-401.

Vogt, K. A., Grier, C. C., Meier, C. E., & Edmonds, R. L. (1982). Mycorrhizal role in net primary priduction and nutrient cytcling in *Abies amabilis* ecosystems in Western Washington. Ecology, 63, 370-380.

Vohník, M., Fendrych, M., Albrechtová, J. & Vosátka, M. (2007). Intracellular colonization of *Rhododendron* and *Vaccinium* roots by *Cenococcum geophilum*, Geomyces pannorum and *Meliniomyces variabilis*. Folia Microbiologica, 52**,** 407-414.

Wang, F., Pan, X., Gerlein-Safdi, C., Cao, X., Wang, S., Gu, L., Wang, D. & Lu, Q. (2020). Vegetation restoration in Northern China: A contrasted picture. Land Degradation & Development, 31**,** 669-676.

Wang, H, (2021). Study on host selectivity of *Cenococcum geophilum* from different regions (master's dissertation). The University of Tokyo.

Ward, E. B., Duguid, M. C., Kuebbing, S. E., Lendemer, J. C. & Bradford, M. A. (2022). The functional role of ericoid mycorrhizal plants and fungi on carbon and nitrogen dynamics in forests. New Phytol, 235**,** 1701-1718.

Weinstein, R. N., Palm, M. E., Johnstone, K., & Wynn-Williams, D. D. (1997). Ecological and physiological characterization of *Humicola marvinii*, a new psychrophilic fungus from fellfield soils in the maritime Antarctic. Mycologia, 89, 706-711.

Xie, L., Yang, Y., Ma, J., Lin, G., Deng, J., Robson, T. M., Peng, H., Zhou, L., Yu, D. & Wang, Q. W. (2024). Variations in ectomycorrhizal exploration types parallel seedling fine root traits of two temperate tree species under extreme drought and contrasting solar radiation treatments. Plant, Cell & Environment.

Yan, T., Zhang, P., Pang, W., Zhang, X., Lian, C. & Zhang, T. (2022). Effects of High Temperature-Triggered Transcriptomics on the Physiological Adaptability of *Cenococcum geophilum*, an Ectomycorrhizal Fungus. Microorganisms, 10, 2039.

Zhai, J., Wang, L., Liu, Y., Wang, C., & Mao, X. (2023). Assessing the effects of China's three-north shelter forest program over 40 years. Science of the Total Environment, 857, 159354.

Zhang, F., Anasontzis, G. E., Labourel, A., Champion, C., Haon, M., Kemppainen, M., Commun, C., Deveau, A., Pardo, A., Veneault-Fourrey, C., Kohler, A., Rosso, M. N., Henrissat, B., Berrin, J. G. & Martin, F. (2018). The ectomycorrhizal basidiomycete *Laccaria bicolor* releases a secreted beta-1,4 endoglucanase that plays a key role in symbiosis development. New Phytol, 220**,** 1309-1321.

Zhang, F., Labourel, A., Haon, M., Kemppainen, M., Da Silva Machado, E., Brouilly, N., Veneault-Fourrey, C., Kohler, A., Rosso, M.-N., Pardo, A., Henrissat, B., Berrin, J.-G. & Martin, F. (2022). The ectomycorrhizal basidiomycete *Laccaria bicolor* releases a GH28 polygalacturonase that plays a key role in symbiosis establishment. New Phytologist, 233**,** 2534-2547.

Zhang, T., Pang, W., Yan, T., Zhang, P., He, J., Rensing, C., Yang, W. & Lian, C. (2023). Metal-non-tolerant ecotypes of ectomycorrhizal fungi can protect plants from cadmium pollution. Frontiers in Plant Science, 14**,** 1301791.

Zhang, X., Zhang, J., He, J., Li, M., Matsushita, N., Geng, Q., Lian, C. & Zhang, S. (2024). Physiological and Transcriptome Responses of *Pinus massoniana* Seedlings Inoculated by Various Ecotypes of the Ectomycorrhizal Fungus *Cenococcum geophilum* during the Early Stage of Drought Stress. J Fungi (Basel), 10, 71.

9 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

10 Author Contributions

HW, FM, and AK: wrote, drafted, read, corrected, improved, revised, and accepted the last version of manuscript.

11 Funding

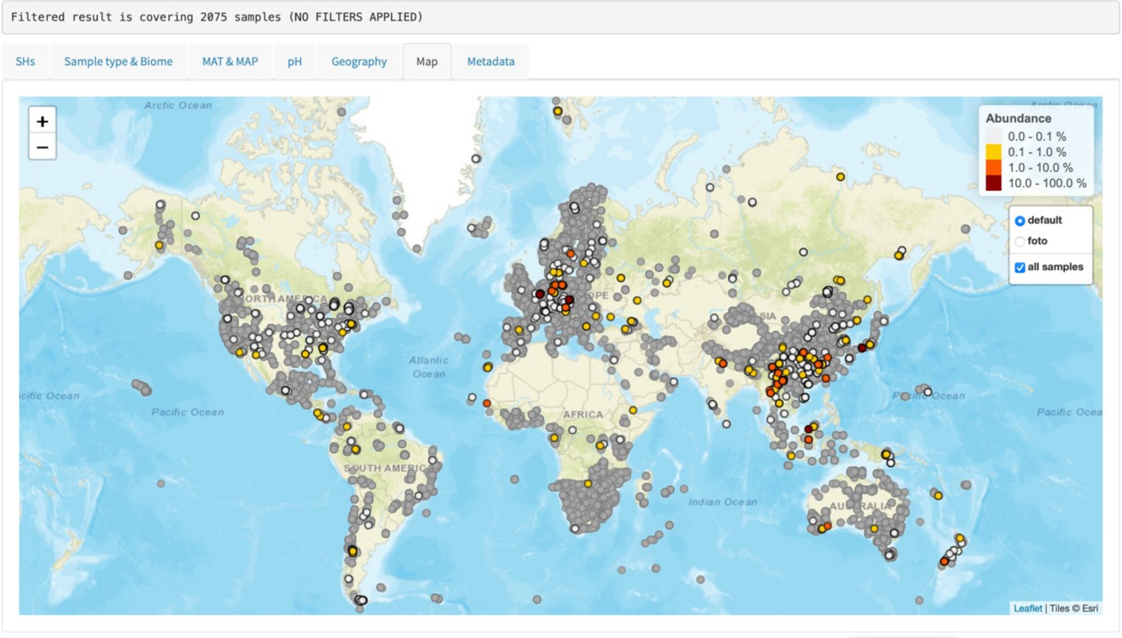
HW was supported by a scholarship from the China Scholarship Council and Agreenium. This study was supported by the Laboratory of Excellence ARBRE (ANR-11-LABX-0002-01).

12 Acknowledgments

The authors greatly thank Dr. Obase Kesuke, Woodland Bioecoloy Group, Hokkaido Research Center, Forestry and Forest Products Research Institute, Japan for the graph of *Cenococcum geophilum* phylogenetic tree.

Figure 1. The worldwide abundance and distribution of *Cenococcum geophilum* (**A**) and distribution (in % per site) among biomes and continents (**B**) were assessed using the GlobalFungi database (https://globalfungi.com) (Větrovský et al., 2020) *Scientific Data* 7, 228).

A



Filtered result is covering 2075 samples (NO FILTERS APPLIED)

B

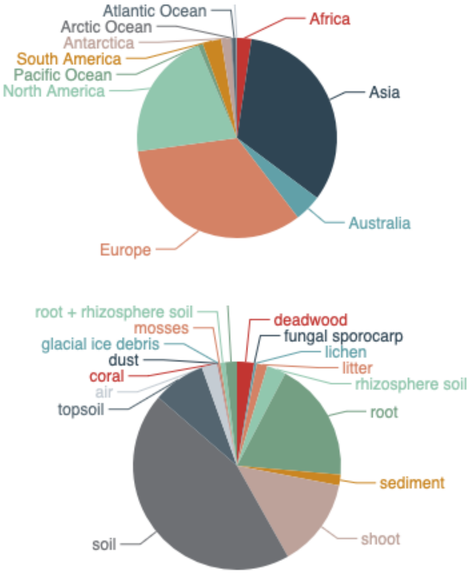


Figure 2. The (A) Melanized mycelium, (B) colony, (C) ectomycorrhizas, and (D) sclerotia of *Cenococcum geophilum*.

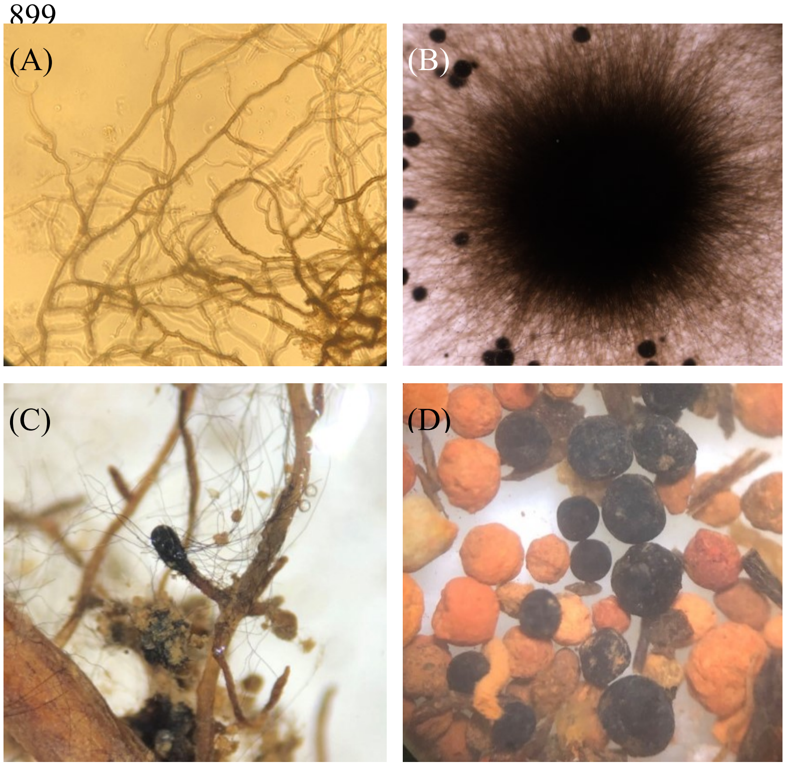

(A)
(B)
(C)
(D)

Figure 3. The phylogenetic tree of *Cenococcum geophilum* and the distribution (CA: California, USA, FL: Florida, USA, FR; France, NL: Netherland, OR: Oregon, USA, SP: Spain, SW: Switzerland) of each clade. A copy from Obase (2017). The phylogenetic tree of *C. geophilum* based on maximum likelihood analysis of seven concatenated loci (ITS, SSU, LSU, TEF, RPB1, RPB2 and GAPDH). Isolates of *Pseudocenococcum floridanum* are outgroups.

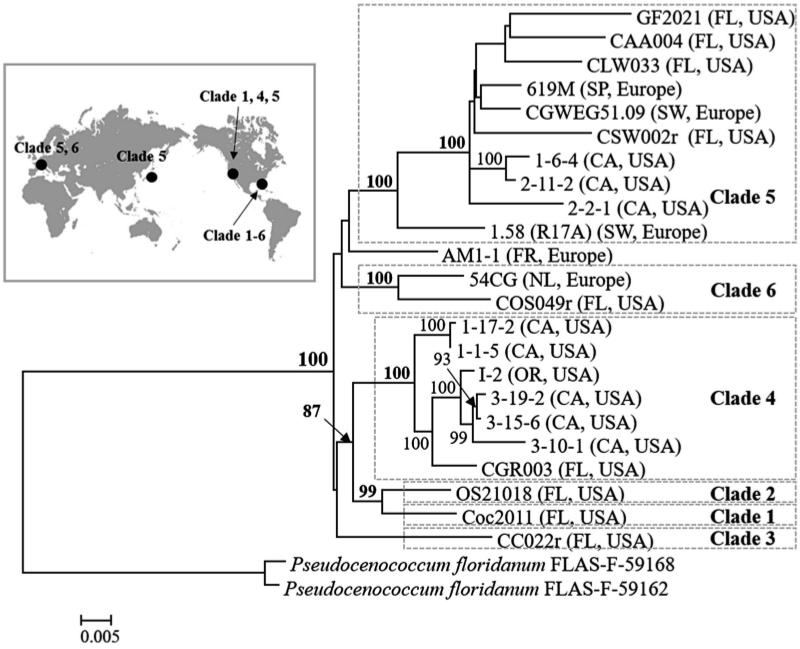


Fig. 14.2 The optimum phylogenetic tree of *C. geophilum* based on maximum likelihood analysis of seven concatenated loci (ITS, SSU, LSU, TEF, RPB1, RPB2 and GAPDH). The placement of each lineage is highlighted and clades 1–6 are named according to Obase et al. (2016a). Inset in the *upper left corner* shows the known global distribution of each lineage based on multi-gene data (although vast areas of the globe have not been sampled). Isolates of *P. floridanum* are included as outgroups. CA: California, USA, FL: Florida, USA, FR; France, NL: Netherland, OR: Oregon, USA, SP: Spain, SW: Switzerland