Chapter 1

Introduction

1.1 Soil free-living and root-associated fungi in the temperate forests: an overview

Temperate forests represent one of the major terrestrial biomes (Reich and Frelich, 2002). They have been the most common forests in the densely populated and industrialized regions in the world, such as Europe, North America, and China. Hence, the temperate forests have been prone to anthropogenic pollution and intensive land-use for a long time, suffering massive changes in composition, structure and diversity with only a small part of the original vegetation remaining (Leuschner and Ellenberg, 2017; Reich and Frelich, 2002). Despite that, the contribution of temperate forests to ecosystem services, primarily their role in biogeochemical cycles and carbon (C) sequestration, remains of global interest. Based on a carbon to nitrogen (C:N) ratio, which is well above average due to the high (>300) wood C:N ratio (Monson, 2014), the temperate forest ecosystems represent a large terrestrial C sink (14% of the global C stock, Pan et al., 2011).

About 44% of the C storage in forest ecosystems are localized in the soil to 1-m depth (Pan et al., 2011) being together with other nutrients under the continuous recycling action of the soil fungi (Baldrian, 2017; Crowther et al., 2019; Zanne et al., 2020). In the temperate forests, the soil mycobiome is dominated in terms of biodiversity, biomass and functional importance by two fungal groups, which contrast in their lifestyle and trophic mode: **(1)** the free-living saprotrophs, which obtain C for their metabolism by degrading the organic matter, and **(2)** the root-associated mycorrhizal fungi, which primarily rely on C supplied by their plant-hosts.

Saprotrophic fungi appear in all major fungal lineages (Zanne et al., 2020), exposing a wide functional variety (Boddy, 1999). Although the enzymatic apparatus complexity enables decaying substrates of various qualities, a high substrate specialization commonly occurs (Elsas et al., 2019; Sahu et al., 2021). Thus, fungal saprotrophic communities change with increasing soil depth following the progress of organic matter decomposition (Štursová et al., 2020; Voříšková and Baldrian, 2013). Under the incipient decomposition stage in the forest floor, saprotrophic communities consist of species that use the soluble compounds released from the organic substrate. In deeper organic layers, communities contain species that decompose structural polymers or species that produce enzymes for degrading the more recalcitrant substrates, such as lignocellulose (Algora Gallardo et al., 2021; Bhatnagar et al., 2018; Lindahl et al., 2010).

Mycorrhizal fungi are symbiotically associated with temperate forest trees colonizing 90% to 100% of their root tips (Brundrett, 2009; Brundrett and Tedersoo, 2018; Frank, 2005; Read, 1991). They receive carbohydrates from the host tree. In return, mycorrhizal fungi may improve the tree access to the growth limiting nutrients (Smith and Read, 2010). Mycorrhizal symbiosis is categorized into four major types based on structure and function: arbuscular mycorrhiza, ectomycorrhiza, orchid mycorrhiza, and ericoid mycorrhiza (Brundrett, 2009; Brundrett and Tedersoo, 2018; Read, 1991). Mycorrhizal fungi reside around or inside the cortex cells and around the epidermal cells of the roots, on the root surface, and outside the root as extramatrical mycelium (Fig. 1) (Smith and Read, 2010). In temperate forests, the trees associate either with ectomycorrhizal fungi (80% to 100% from all tree individuals) or arbuscular fungi (Read, 1991; Soudzilovskaia et al., 2020; Steidinger et al., 2019). Arbuscular mycorrhizas are formed with taxa from the Glomeromycota clade. They are distinguished from other mycorrhizas by the ingrowth of hyphae inside the cortical root cells, where the fungus develops storage vesicles or tree-like structures called arbuscules (Fig. 1 C, D) (Brundrett, 2009). Ectomycorrhizas contain taxa from Basidiomycota and Ascomycota and show three specific structural components as follows: (1) at the root surface, the fungal sheath (mantle) that encapsulates the root tips (Fig. 1 D, E); (2) in the cortical intercellular space, a continuous densely netted hyphal layer build around each cell and connected in a so-called Hartig net (Fig. 1 E); and (3) the extramatrical hyphal networks which extend into the surrounding soil (hundreds of meters per cubic centimetre of soil, Awad et al., 2019; Johnson et al., 2003). The hyphal mantle represents a protective barrier for the root tip (Taylor and Peterson, 2005) and a storage place for carbohydrates and lipids (Jordy et al., 1998; Laczko et al., 2004). The Hartig net serves as an interface of nutrient exchange between the fungus and the host plant (Peterson et al., 2004). The extramatrical mycelium is the structure involved in scavenging for nutrients, enhancing the access of host plants to less reachable nutrient pools (Hodge et al., 2010; Kohler et al., 2015; Pena, 2016; Read and Perez-Moreno, 2003). The Hartig net structure is similar in all ectomycorrhizas, but the anatomy and morphology of the fungal mantle and extramatrical mycelium vary with the fungal species identity (Agerer, 1987). Based on the extramatrical mycelium architecture and morphology, mycorrhizal fungi have been classified into four exploration types: contact, short-distance, medium-distance, and long-distance (Agerer, 2001). The medium-distance exploration type is further divided based on mantle surface, hyphal frequency, and rhizomorph morphology into three subtypes, the medium-distance mat, medium-distance fringe, and medium-distance smooth (Table 1).

The forest soil mycobiome also includes the pathogenic and endophytic groups (Baldrian, 2017). The fungal pathogens obtain the nutrients from living plants by producing diseases that may kill (necrotrophs) or not (biotrophs) the hosts (Elsas et al., 2019). The fungal endophytes occur in the living roots as asymptomatic residents receiving their nutrients from host plants (Elsas et al., 2019). In temperate forests, the tree roots are colonized by a phenotypically distinct group of dark septate endophytes that have a complex, largely ambiguous ecology. They are living like mycorrhizal fungi in mutualistic or parasitic relationships with host roots (Baldrian, 2017; Jumpponen, 2001; Jumpponen and Trappe, 1998).

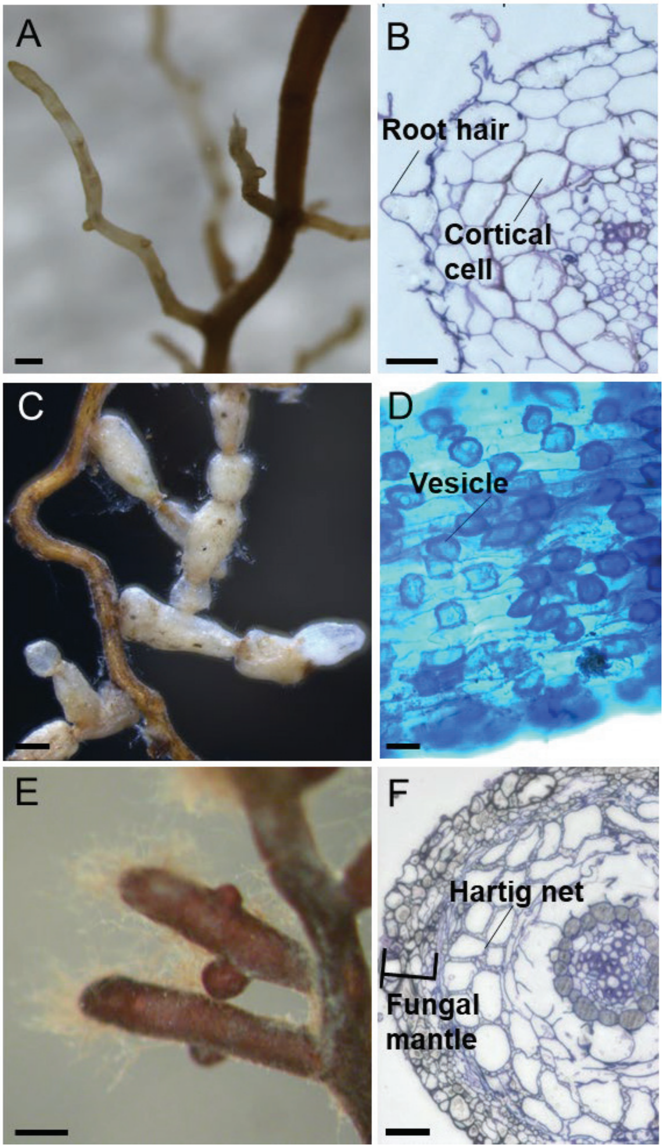
A
B
Root hair
Cortical cell
C
D
Vesicle
E
F
Hartig net
Fungal mantle

Fig. 1 Morphological and anatomical structures of non-mycorrhizal root tips (A, B), arbuscular mycorrhizas (C, D), and ectomycorrhizas (E, F). Non-mycorrhizal root tips of beech (Fagus sylvatica) showing root hairs. Arbuscular mycorrhizal maple (Acer sp) root tips are forming distinctive vesicles in the root cortex. Ectomycorrhizal the root tips are completely sheathed by the fungal mantle and external hyphae forming the soil mycelium. Cross-section of ectomycorrhizal root tip showing the fungal mantle around root cortex and Hartig net hyphae, which envelope each cortex cells. Shown is an ectomycorrhiza formed between the fungus Tomentella sp. and beech root tips. Bar, 200 µm (A, C, E) and 50 µm (B, D, F).

Table 1. Morphological characteristics of ectomycorrhizal fungal mycelium exploration types classified after (Agerer, 2001; Hobbie and Agerer, 2009).

Exploration type	Description
Contact	Smooth mantle with very few if any hyphae growing into the soil. The mantle is hydrophilic. The rhizomorphs are lacking.
Short-distance	Dense emanating hyphae grow to a short extent into the soil. The mantle and hyphae are hydrophilic. No rhizomorphs present.
Medium-distance	The hyphae spread into the soil to a considerable distance from the mantle. The rhizomorphs are present.
Medium-distance fringe	The most frequent medium-distance sub-type. Fungi form many coarse rhizomorphs that are netted with thinner rhizomorphs and dense emanating hyphae in fan-like structures. The rhizomorph surface is hairy. The entire ectomycorrhiza is hydrophobic.
Medium-distance mat	It is similar to the fringe type, but the rhizomorphs and hyphae are aggregated in very dense mats that no other ectomycorrhizas can colonise the roots within the mats.
Medium-distance smooth	The surface of the mantle and rhizomorphs are smooth. There are only a few emanating hyphae present. The rhizomorphs and the hyphal mantle are mostly hydrophilic.
Long-distance	The rhizomorphs are hydrophobic and extend into the soils for many decimetres from the mantle. The rhizomorphs possess vessel-like hyphae capable of transporting water and nutrients on long distances.

1.2 The role of saprotrophic and mycorrhizal fungi in the ecosystem

Saprotrophic and mycorrhizal fungi are critical players in forest ecosystem functioning (*sensu* Glatthorn et al., 2021; Baldrian, 2017; Frąc et al., 2018; Phillips et al., 2013). They contribute both to **regulating** (soil C sequestration and decomposition) and **supporting** (nutrient cycling and primary production) **ecosystem services** (Table 2, Fig. 2).

In the temperate forest ecosystems, saprotrophic and mycorrhizal fungi are likely the most prominent functional groups in driving C fluxes between trees, soil, and atmosphere (Fig. 2) (Bödeker et al., 2016; Clemmensen et al., 2015, 2013; Lindahl and Clemmensen, 2016; Soudzilovskaia et al., 2019). However, the two fungal guilds play contrasting roles in the C cycle: saprotrophic fungi degrade the organic matter to retrieve C, involving a massive CO_2 release to the atmosphere (E. Boddy et al., 2007); while mycorrhizal fungi channel the (photosynthetic) C to the soil through mycelium biomass and fungal exudates (Valtanen et al., 2014), promote the accumulation of slow-cycling soil C, and also contribute to the formation of stable, decomposition-resistant, organic matter aggregates (Frey, 2019 and references therein; Keller et al., 2021). Although there is consensus on the positive influence of mycorrhizal fungi on the soil C sequestration (Frey, 2019), some ectomycorrhizal fungi have retained a set of genes that potentially enable them to produce enzymes to degrade the organic substrates (Kohler et al., 2015; Müller et al., 2020; Treseder and Allen, 2000). Thus, ectomycorrhizal fungi may contribute to organic matter loss by mining for nutrients, particularly nitrogen (N) and phosphorus (P), and less by acquiring C (Baldrian, 2009; Lindahl and Tunlid, 2015; Zak et al., 2019).

Ectomycorrhizal and saprotrophic fungi are phylogenetically related (Miyauchi et al., 2020). Ectomycorrhizal fungi evolved independently in different saprotrophic fungal clades (Kohler et al., 2015; Martin et al., 2016; Martin and Nehls, 2009). Thus, the two fungal groups share a range of similar functional features that may result in competition for the non-C soil resources (Lindahl et al., 2001; Sterkenburg et al., 2018). As ectomycorrhizal fungal C requirements are alleviated by plant C supply, the competitive interactions with saprotrophic fungi may inhibit saprotrophic fungal activity and, subsequently, cause deceleration of organic matter decomposition (Averill et al., 2014; Averill and Hawkes, 2016; Fernandez and Kennedy, 2016; Gadgil and Gadgil, 1971).

Contributions of saprotrophic and mycorrhizal fungi to nutrient cycling and tree productivity have been long-time documented (Cooke and Rayner, 1984; Frank, 2005;

Smith and Read, 2010). Saprotrophic fungi control plant nutrient availability by releasing nutrients from organic matter and distribute them into the soil through their mycelia (Boddy and Watkinson, 1995). Mycorrhizal fungi forage for and transfer growth-limiting nutrients from mineral (Heijden et al., 2015) or organic sources to the host plant (Näsholm and Persson, 2001; Read and Perez-Moreno, 2003). Mycorrhizal fungi facilitate the movement of resources among colonized plants through the extramatrical mycelium network (He et al., 2003; Teste et al., 2010, 2009). This process is particularly important for the nutrition and establishment of tree seedlings (O'Donnell et al., 2020; Simard and Austin, 2010; Simard et al., 2012). The extensive mycorrhizal nutrient uptake leads to reduced nutrient loss through leaching (Bahr et al., 2015).

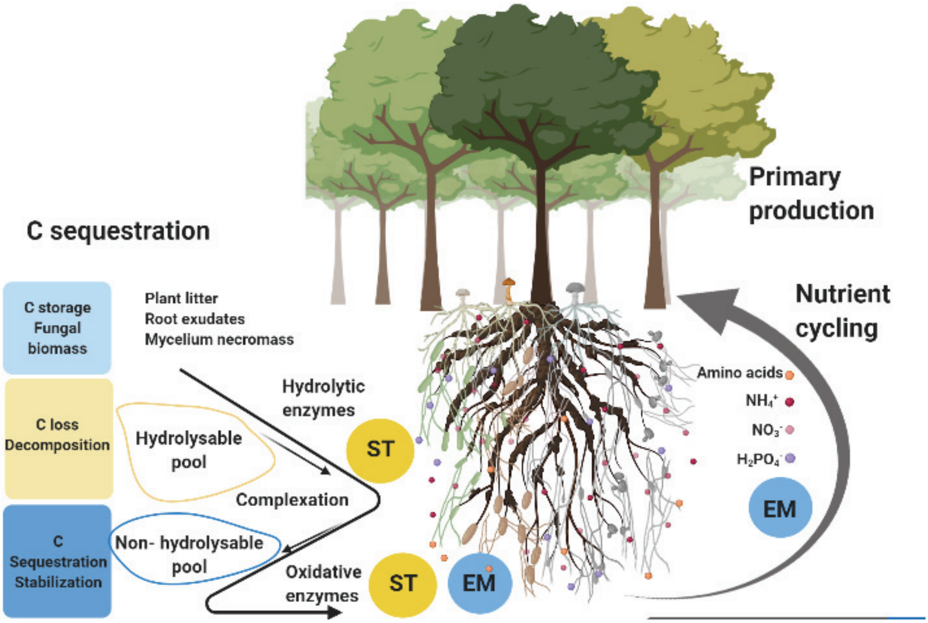

Fig. 2 Schematic overview of the role of ectomycorrhizal (EM) and saprotrophic (ST) fungi in the ecosystem services: soil carbon (C) sequestration, nutrient cycling, and primary production. Fungi can degrade the plant litter using their enzymes. During decomposition,

many polymers (e.g., cellulose, hemicellulose, proteins) are depolymerised by hydrolytic enzymes by a large contribution of ST fungi with a high CO_2 loss. The non-hydrolysable polymers (e.g., lignin, melanin, chitin) are degraded by oxidation. Phenols and intermediate oxidation products react chemically and form even more recalcitrant complexes than before, thus contributing to soil organic matter stabilisation. Both ST and EM fungi may contribute to organic matter oxidation. However, commonly, EM fungi are associated with C sequestration, while ST fungi with C loss through decomposition. The interactions between ST and EM fungi can be competitive, facilitative, or neutral (not displayed). The type of ST-EM interactions plays a crucial role in the balance between organic matter decomposition and stabilisation. Ectomycorrhizal fungi provide nitrogen (N) and phosphorus (P) to their host plants, particularly under low nutrient availability. Thus, in nutrient-limited ecosystems, EM fungi contribute to the reduction of nutrient losses and increase plant productivity. Figure made in BioRender.com.

Worldwide, forest ecosystem services such as biogeochemical cycles (Bonan, 2008; Canadell et al., 2007; Schulze et al., 2019), tree nutrition (Dirks et al., 2019; Jonard et al., 2015; Talkner et al., 2015; Veresoglou et al., 2014) and tree productivity (Ciais et al., 2005; Jonard et al., 2015) are under the threat of anthropogenic disturbance and climate change. Although saprotrophic and ectomycorrhizal fungi are essential contributors to multiple ecosystem services, their role in forest ecosystem resistance and resilience is poorly understood.

Table 2. Contribution of free-living and root-associated soil fungi to temperate forest ecosystem services.

Ecosystem process	**Fungal type**	**Contribution**
Carbon Sequestration (regulating service)		
Decomposition of soil organic matter	Saprotrophic	The most efficient leaf litter and wood decayers [1,2]
	Ectomycorrhizal	Some taxa possess abilities to decay lignocellulose complexes [3,4]

		Inhibit plant litter decomposition due to the competition with saprotrophs [5–7]
Litter decomposability	Saprotrophic	They are key regulators of nutrient cycling in forest ecosystems, affecting plant nutrient availability, and subsequently plant litter chemical composition [8,9]
	Ectomycorrhizal	Senescent colonised roots decompose slower than arbuscular or non-mycorrhizal roots [10]
		Decrease the host plant leaf litter decomposability[11,12]
Contribution to soil organic C pool	Saprotrophic	Fungal necromass forms a relatively stable C pool due to the potentially recalcitrant chitin and melanin compounds [13,14,15]
	Ectomycorrhizal	Mycelium has a slower turnover rate than saprotrophic mycelium [16]
		Channel the plant photosynthetic C to the soil through mycelial biomass or exudates [17–21]
Soil aggregation	Saprotrophic and Ectomycorrhizal	Increase soil aggregation by their filamentous growth and by producing hydrophobins and other biopolymers involved in mycelium attachment to mineral surfaces [22,23]
Nutrient cycling (supporting service)		
Biological rock weathering	Saprotrophic	Increase the rates of mineral weathering [24]
	Ectomycorrhizal	Likely the most critical fungi in weathering as they can support the high costs of producing organic anions [25–27]
Plant nitrogen acquisition	Saprotrophic	Increase N mineralisation, which may result in higher plant N availability. However, under no C limitation, they may decrease plant N acquisition through competition for mineral N [24]

	Ectomycorrhizal	Enhance plant N acquisition and mediate the access to organic N sources [28–30]
Reduction of nitrate leaching, denitrification, and N_2O losses	Saprotrophic	By immobilising high amounts of N in their mycelia, soil fungi prevent nitrification, N leaching, denitrification, and N_2O emissions [31]
	Ectomycorrhizal	Reduces soil N losses: immobilise N in their mycelium and enhance plant N immobilisation [32,33]
		Alter soil structure, soil aeration, and C availability for bacterial communities decreasing denitrification rates [34,35]
Plant phosphorus uptake	Ectomycorrhizal	Improving plant P acquisition under P deficiency by enhancing inorganic P solubilisation, and enzymatic hydrolysation of organic P [36,37], P absorption through high-affinity transporters [38], efficiency in competition with soil microbes [39]
Primary production (supporting service)		
Seedling Survival	Saprotrophic	Improve soil nutrient availability that benefits plant productivity [40].
Plant productivity	Ectomycorrhizal	Increase plant growth and photosynthesis rate [41–43]

1. Boddy et al., 2007 **2.** Boddy and Watkinson, 1995 **3.** Miyauchi et al., 2020 **4.** Kohler et al., 2015 **5.** Smith and Wan, 2019 **6.** Fernandez et al., 2020 **7.** Zak et al., 2019 **8.** Prescott, 2010 **9.** Keeler et al., 2009 **10.** Langley et al., 2006 **11.** Cornelissen et al., 2001 **12.** Dickie et al., 2014 **13.** Martin and Haider, 1979. **14.** Adamczyk et al., 2020. **15.** Awad et al., 2019 **16.** Ekblad et al., 2013 **17.** Calderón et al., 2012 **18.** Frey, 2019 **19**. Hobbie, 2006 **20.** Johansson et al., 2009 **21.** Godbold et al., 2006 **22.** Lehmann and Rillig, 2015 **23.** Lehmann et al., 2020 **24.** Dighton, 2007 **25.** Hoffland et al., 2004 **26.** Landeweert et al., 2001 **27.** Thorley et al., 2015 **28.** Hobbie and Hobbie, 2006 **29.** Näsholm et al., 2009 **30.** Read and Perez-Moreno, 2003 **31.** Vries and Bardgett, 2012 **32.** Almeida et al., 2018 **33.** Bahr et al., 2015 **34.** Bender et al., 2014 **35.** Bender and Heijden, 2015 **36.** Meeds et al., 2021 **37.** Finlay et al., 2020 **38.** Nehls and Plassard, 2018 **39.** Clausing and Polle, 2020 **40.** Clocchiatti et al., 2020 **41.** Hoeksema et al., 2010 **42.** Shi et al., 2017 **43.** Heinonsalo et al., 2015.

1.3 The importance of ectomycorrhizal functional traits in the biodiversity-ecosystem functioning relationships

Ecosystem services are often positively related to biodiversity (Cardinale et al., 2013, 2012; Hooper et al., 2005). Because of the unprecedented high rate of changes in Earth's ecosystems (Vitousek et al., 1997) accompanied by a severe global biodiversity loss (Mace, 2005; Mace et al., 2012), research on the relationships between biodiversity and ecosystem functioning (BEF) has become a major facet of ecology (Balvanera et al., 2006; Cardinale et al., 2006; Hillebrand and Matthiessen, 2009).

The positive relationship between species richness and ecosystem function (Cardinale et al., 2013, 2012; Hooper et al., 2005) invokes two mechanisms: selection effect and niche differentiation. The selection effect is based on a higher probability that a species with a particular trait is present in the species-rich than species-poor communities (Hooper et al., 2005; Lefcheck et al., 2015). The niche differentiation employs the fact that a rich community offers the chance of complementary use of resources, such as niche partitioning or beneficial interactions among species (Isbell et al., 2011). However, the positive relationships between species richness and functional richness are not always apparent as species could also perform similar functions that lead to functional redundancy (P mobilization, Müller et al., 2020; Spohn et al., 2018). The direct BEF positive relationships may occur only when the species have unique functions that contribute to the ecosystem functioning, and the presence of other species cannot compensate for their loss. Therefore, although species richness is essential as a reservoir of species with potentially important traits, addressing BEF relationships requires a functional framework, which includes the species functional traits and functional richness (Cardinale et al., 2012; Weiss and Ray, 2019; Crowther et al., 2014; Dawson et al., 2019).

Functional traits represent the base in defining organismal fitness (Darwin, 1859; Grime, 1973). Diversity of functional traits within a community, namely functional diversity, reflects **(i)** the processes of local species coexistence and their interaction with the