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Conservation of Edible Ectomycorrhizal Mushrooms: Understanding of the ECM Fungi Mediated Carbon and Nitrogen Movement within Forest Ecosystems

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Abstract

Most edible ectomycorrhizal (ECM) mushrooms are currently harvested from nature and many of them are high-priced. Demand for the wild mushrooms as a culinary delicacy has stimulated research that aims to understand (1) the puzzled role that the ECM fungi play in the forest ecosystem, and (2) nutritional and other requirements for fruiting, which is highly variable. In this review, we focus on understanding of the ECM fungi mediated carbon and nitrogen movement between the symbiotic partners and on the interactions with other fungi in forest ecosystems. Thereby, we better understand the diverse nitrogen requirements for edible ECM fungal growth and mushroom fruiting. We attempt to provide a theoretical basis for the future research of edible ECM mushrooms in wild and controlled conditions.

Keywords: culture, cultivation, ectomycorrhizal fungi, edible mushrooms, nitrogen uptake

1. Introduction

Forests play a crucial role in the global environment and economy. Forest-based wood products as well as non-wood forest products have offered remarkable resources and benefits for the well-being of people [1, 2]. A healthy and well-growing forest system is largely dependent on available soil nutrients and efficient nutrient cycling [3, 4], especially nitrogen (N). As we know, nitrogen is a limiting resource for plant growth in many temperate forests.

Nitrogen is necessary for plants. Most crops require N relatively high amounts, but only a small amount of available N is present in soil at a time. A large source of soil N is the atmospheric dinitrogen (N₂), the major gas of air (79%) [5]. Only certain microorganisms can bind molecular nitrogen from air. All other organisms need to take up nitrogen from soil. Soil organic matter (especially humus) acts as a storage and supplier of nitrogen for plant roots and microorganisms;

almost 90–95% of soil total nitrogen originates from soil organic matter [6, 7]. Plants acquire N mostly from the inorganic forms such as ammonium and nitrate. However, plants that associate with mycorrhizal fungi are considered to have greater access to organic nitrogen pools when compared to non-mycorrhizal plants [5].

ECM fungi play an important role in the nutrient cycle of terrestrial ecosystems. Especially in a forest poor in nutrients, the growth of trees depends on the existence of mycorrhizal fungi. The value of ECM fungi is evaluated from the global framework. ECM fungi provide hidden biological fertilizers for increasing plant biomass, conventional afforestation, and ecosystem restoration practices; they also control soil pathogens [8–10].

In addition to benefits for forests, many ECM fungi produce edible mushrooms that are widely appreciated for their nutritional, medicinal, and gastronomic properties [11]. One of the major challenges of the twenty-first century is to produce sufficient food. From that perspective, wild mushrooms as non-wood forest products are getting more and more attention globally [12]. It would be convenient if these mushrooms could be cultivated. However, most edible ECM mushrooms can only be collected from nature and not cultivated artificially [11]. The main obstacle to the cultivation of edible ECM mushroom is their need to be associated with a host plant in plantations. The association is obligatory for the successful growth and fruiting of the mushrooms. The unanimous discussion of the nutritional growth requirements of ECM edible fungi is a topic of interest for scientists.

An in-depth understanding of the nutritional requirements of ECM fungi and the role of ECM fungi in nutrient cycling, particularly in ECM fungi mediating carbon and nitrogen movement within forest ecosystems will be summarized in this chapter. The nutritional requirements to successfully culture and cultivate ECM fungi will be discussed.

2. Ectomycorrhizal fungi

2.1 Ectomycorrhizal fungi

Ectomycorrhizal fungi are found in association with the roots of most forest trees throughout the world. ECM fungi form obligate symbioses with many of the dominant trees in temperate and boreal forest, as well as in some tropical forests. ECM fungi do not penetrate their host's cell walls. Instead, they form an entirely intercellular interface, known as Hartig net, consisting of highly branched hyphae that forms a latticework between epidermal and cortical cells [13]. Hartig net provides a large surface area for the two symbiotic partners and it is the site of nutrient exchange. Carbon (C) is transported to the fungus from a tree that receives limiting nutrients in exchange. The fungus can transport nutrients beyond the nutrient depletion zone surrounding the host's root system and release from immobilized sources inaccessible to the plant [13, 14]. ECM fungi are thus regarded as key elements of forest nutrient cycles and as strong drivers of forest ecosystem processes [15].

Most (86%) terrestrial plant species obtain mineral nutrients through mycorrhizal symbionts as estimated using taxonomic and ecological extrapolation methods [16]. An estimate of ECM fungal species richness is likely between 20,000 and 25,000 [16, 17]. These ECM fungi belong to more than 80 independently evolved lineages and to more than 250 genera, mainly in Basidiomycetes and Ascomycetes [18].

2.2 General roles of ectomycorrhizal fungi in forest ecosystems

Ectomycorrhizal fungi are essential contributors in forest ecosystems by forming beneficial symbiosis plants. These fungi drive forest soil processes such as soil organic matter decomposition, nutrient cycling, and carbon sequestration [19–21].

ECM fungi have the ability to provide hosts not only nitrogen but a variety of other major nutrients, including phosphorus, potassium, calcium, magnesium, sulfur, as well as micronutrients such as iron, zinc, copper, and manganese. However, they are often ignored because N is the main growth-limiting element in many forest ecosystems, particularly in the Northern Hemisphere [19, 22, 23]. In addition to nutrients, trees receive several other benefits. First, the resistance of trees against pathogens is improved due to the mycelial network [24]. Second, the ECM mycelial networks are involved in water transport [25]. Third, ECM fungi can relieve salt and heavy metal stress of the host plants [9]. The benefits that the ECM fungi offer are complicatedly regulated by the host type, ECM species, as well as climatic and environmental conditions. Recently, a study based on a climate change model predicted that the global abundance of ECM-associated trees will decline by 10% by the end of 2070, and the majority of this will take place in boreal and temperate ecotones [26]. Therefore, the conservation of ECM fungi should be taken as an important issue.

2.3 Structure of ectomycorrhizas is diverse

Fungal mycelium has been estimated as one of the largest living organisms on Earth [27]. Hyphae is composed of fungal mycelium and other structures including rhizomorphs. Rhizomorphs are structures through which fungi can spread in their environment and search for new substrates to colonize. The structure of ectomycorrhiza is diverse. Agerer [28] proposed that ECM mycelia systems influence on their patterns of differentiation and putative ecological importance. Mycorrhizal fungi have been classified into four exploitation types depending on the extent of hyphal development: contact, short-distance, medium-distance, and long-distance.

ECM fungi are characterized according to the water repellence of the mycelium. Fungi vary from extremely hydrophobic to extremely hydrophilic types [29]. All fungal growth parameters such as hyphal hydrophilicity, presence of rhizomorphs, and mat formation correspond together to how fungi interact with and exploit the environment [28, 30]. The function of extraradical mycelia of ECM fungi is the transportation of nutrients between plant and soil environment [13, 31].

Ectomycorrhizas differ in their ability to take up and transport nutrients, and thus, promote tree growth [32, 33]. The differences in ECM effectiveness are often species specific or even strain specific [34]. It is evident that the amount and differentiation of extraradical mycelium is an important ecological factor for tree performance [35–37] and soil nutrition [38].

3. Contribution of ectomycorrhizal fungi to nitrogen cycling in forest ecosystems

3.1 Forms of nitrogen in forest soil

The major N sources in the forest floor can be divided into external and internal sources. Atmospheric nitrogen deposition is an external source, and the living organisms and their decomposition products are an internal source [39, 40].

Ammonium and nitrate are the two major pools of inorganic N. Ammonium is most often the dominant inorganic N pool available to trees in coniferous ecosystems. Nitrate concentrations are usually relatively low in mature forests [41].

Most of the nitrogen in forest soils is bound to organic compounds [42]. It is well known that over 90% of N occurs in organic forms in most surface soils [7, 43]. The forms of organic N can be roughly divided into two categories. (I) Organic residues consisting of undecomposed animal and plant residues and partial decomposition products, and (ii) soil organic matter or humus. The humus is composed of non-humic, easily identifiable compounds (e.g. amino acids, carbohydrates, nucleic acids, etc.) and complex humic substances, such as high-molecular-weight amorphous and aromatic compounds, formed during the decomposition process. The importance of humus is widely recognized in maintaining and improving soil fertility [7].

The distribution of major N compounds was investigated in different climatic and geological conditions including arctic, cool, temperate, subtropical, and tropical climates early [44]. The results indicated that about 33–42% of soil N occurs as free and protein amino acids. The amino acid composition of all soils, however, was remarkably similar. The composition and concentration of amino acids has shown generally constant throughout the growing season [45], which suggests that amino acids originate from a common source or through similar biochemical processes. However, the distribution of N compounds at different regions seems to be related to decomposition process and as well as forest types [46]. Soil proteins are often not free, they are bound to humic compounds and are not soluble. These N forms cannot directly be used by plants, they need to be depolymerized by microorganisms and converted into plant available monomeric organic or mineral N forms.

3.2 Diversity in nitrogen uptake in Ectomycorrhizal Fungi

Ectomycorrhizas occur widely in forest ecosystems. Most of the terrestrial plant species are in symbiosis with mycorrhizal fungi, about 3% of them are ectomycorrhizal. The most common tree species belong to Pinaceae, Salicaceae, Betulaceae, Fagaceae and Myrtaceae [13, 47]. The general mechanism of ECM fungi to improve plant nutrition is the so called Hartig net structure that increase the surface area of roots to absorb nutrients.

Ectomycorrhizal fungi are able to take up both inorganic and organic forms of N. Ammonium is generally recognized as the most readily utilizable form for most ECM fungi when studied in mycelial cultures [48, 49] or with ECM roots in vitro and in the field [50]. Nygren and colleagues [51] demonstrated that 68 species of ECM fungi used nitrate as the sole N source in a pure culture. However, the pure culture conditions do not reflect the N preference of ECM fungi in nature [52]. *Laccaria laccata* was shown to uptake nitrate and transfer it to the host plant when in nitrate-rich conditions [53].

In other studies, ECM seedlings demonstrate a strong preference for amino acids over ammonium [54]. Already in 1953, Melin and Nilsson [55] demonstrated that ¹⁵N labelled glutamate was absorbed by the mycelium of *Boletus variegatus*, and that the nitrogen was transferred to the shoots of pine seedlings that had been infected with the fungi in an aseptic culture. Many ECM fungi are able to grow with amino acids as the N source in pure culture and also in association with host trees [56–59].

The capacities of ECM fungi to mineralize organic N differ. Abuzinadah and Read [60] found that ECM fungi such as *Suillus bovinus*, *Amanita muscaria*, *Paxillus involutus*, *Cenococcum geophilum*, and *Rhizopogon roseolus* were able to use peptides and proteins as their sole N sources. In contrast, *Laccaria laccata* and *Lactarius rufus* had little ability to grow with peptides and proteins but they grew well with

ammonium. It was further demonstrated that different fungal species, even different strains had different abilities to utilize organic N and/or transfer the assimilated N to their host plants [60]. Some ECM fungi might take up the nitrogen compound completely and some break the molecules into smaller organic or inorganic forms. The difference in the ability of ECM fungi to transfer N from chitin, protein, and other organic substances in litter and humus was explained by differences in their enzyme secretion profiles [61].

ECM fungi have several functionally distinct metabolic pathways to transfer N. ECM fungal hyphal morphology, species niche (original living conditions), genetic characteristics and carbon costs to host plants may influence on their capacity to utilize and mineralize organic N.

3.2.1 Mycelium structure determines the efficiency of ECM transport nitrogen

ECM fungal hyphae morphology is diverse. Morphology seems to have a great influence on the hyphal enzymatic ability of ECM fungi. ECM species with hydrophilic ectomycorrhizal hyphae have proteolytic activities and they are adapted to N-limited conditions [62]. In contrast, other ECM fungi with hydrophobic ectomycorrhizal hyphae, similar to many saprotrophic fungi, form aggregated hyphae (rhizomorphs) for long-distance transport of elements. This is presumably an adaptation for patchily distributed resources [63].

In addition to hydrophobicity, another aspect is to consider the size of mycelia. The species that form extensive extraradical mycelia (e.g. *Cortinarius*, *Suillus*, *Tricholoma* species) have different capacity to utilize organic N than those species that form diffuse, spatially limited extraradical mycelia (e.g. *Amanita*, *Lactarius* species). These differences in mycelia are thought to be associated with different reproductive and colonization strategies [58, 62]. It is believed that extensive mycelia are established infrequently, but it is long-living. In contrary, the diffuse mycelia become more stable, usually by spores for the generation, but the mycelia do not persist. The long-living extraradical mycelia is believed to be more efficient to process N than short-living mycelia.

Studies based on the stable N isotope ratios in ECM fungal fruitbodies have provided new insights and evidence for the N sources of ECM fungi. As we know, the relative abundance of stable isotopes in food webs follows from discrimination against heavier isotopes in several biochemical processes [64]. The ratio is useful particularly in studying nitrogen cycling mediated by mycorrhizal fungi [65]. Stable N isotope ratios in ECM fungal fruitbodies showed that those having long-living mycelia exhibited higher $\delta^{15}\text{N}$ than those having short-living mycelia [58, 66, 67].

Thus, the signature of ^{15}N in ECM fruitbodies was determined by the morphological characteristics of the mycelia. Another observation revealed by the isotope studies is that ECM fungal species that can utilize organic N exhibited higher $\delta^{15}\text{N}$ in their fruitbodies than those that are restricted to mineral N sources [67, 68].

3.2.2 Nitrogen utilization of ECM fungi is related to the nitrogen status of the habitat

The form of nitrogen in the environment influences N mobilization by ECM fungi. The species common in low inorganic N soils grew well with protein, glutamine, and serine whereas species in high inorganic N soils grew well with glutamine, but poorly with protein and serine [67]. Differences among ECM fungal species in their ability to access and take up different N forms indicate that the form and abundance of N in the environment may be a defining factor for ECM fungal species niche [69]. ECM species are selected by the N form that is predominant in

their environment. Recently, an increasing number of studies showed that inorganic N enrichment in forest soils caused by pollution, fertilization or natural causes are leading to a reduction in the level of plant root colonization by ECM fungi, also shift fungal community in soils away from ECM fungi specialized in organic N acquisition to more generalist nitrophilic species and saprotrophs [70–72].

Other studies have concluded that differences in proteolytic activity between the species of ECM fungi could be explained by soil-derived selection pressures. For example, *Hebeloma crustuliniforme* expressed proteolytic activity in the presence of a readily available N source such as ammonium [73]. Ammonium has also been shown to repress the expression of amino-acid transporters and enzymes in N assimilation pathways in ECM fungi [74, 75]. The presence of inorganic N tightly down regulated soil organic matter degradation by *Paxillus involutus* as proved [76]. Such facts suggest that ECM fungal degradation activity would be controlled by environmental factors.

Different ECM species occupy different successional stages in forest development. This seems to be related to the proteolytic activity of fungi. When resource quality declines and organic matter accumulation declines during forest development, fungi with limited proteolytic activity is favored. For the cultivation of edible mushrooms, this means that we should pay attention to the natural preferences of the species for nitrogen uptake. This may concern especially the ECM species that are difficult to cultivate artificially.

3.2.3 Fungal genetic characteristics determines the efficiency of N transition

Recently, advances in genetics and molecular biological techniques have provided better understanding about nitrogen metabolism. The acquisition of inorganic N and the mineralization of organic N by ECM fungi have been proved by many molecular investigations. Ectomycorrhizal fungi encode a number of transporters to acquire nitrate and ammonium from soil, as well as a suite of enzymes and transporters necessary for utilizing organic N sources [77–79]. Ammonium importers such as AMT1, AMT2 and AMT3 have been functionally characterized in several ECM fungal species, such as, *Hebeloma cylindrosporum* [75, 80], *Tuber borchii* [81] and *Amanita muscaria* [82]. Nitrate transporters, such as LbNRT2 in *Laccaria bicolor* [83] and HcNRT2 in *H. cylindrosporum* [84], are also present in ECM genomes allowing N transport.

Ectomycorrhizal fungi have all evolved from their saprotrophic ancestors, and hence, ECM have the ability to decompose organic matter [85, 86]. The utilization of proteins by fungi requires the enzymatic degradation of proteins to peptides and amino acids before cellular uptake. Lindahl and Taylor [87] studied the genetic potential of ECM fungi to produce N-acetylhexosaminidases that hydrolyze chitin to N-acetylglucosamine. Thus, N-acetylglucosamine and amino acids replace ammonium and nitrate as the N sources [19].

Recently, the genomes of ECM fungi were found to contain the same or smaller number of copies of genes coding for secreted N and P targeting hydrolases than saprotrophs, pathogens, or ericoid mycorrhizal fungi [88]. This observation is surprising because the well-documented ability of ECM fungi to hydrolyze organic phosphate compounds and scavenge nitrogen through the degradation of proteins accumulated in litter. Miyauchi and colleagues [88] also showed that the ECM fungus *Paxillus involutus* was able, while assimilating organic N, to significantly modify organic matter with a free-radical-based mechanism similar to that of saprophytic brown-rot fungi [76]. Unlike the saprophytic fungi, *P. involutus* did not show any expression of genes encoding extracellular enzymes needed to metabolize the released C. This suggests that the degradation mechanism of this ECM fungus has evolved to assimilate organic N rather than C.

3.2.4 ECM utilizing organic N in relation to receiving C from trees

ECM fungi are able to breakdown soil organic N with differing efficiencies. It has been found that the uptake of amino acids by mycorrhizal fungi is related to the N content and carbon structure of the amino acid [89]. One hypothesis was proposed that the rate at which mycorrhizal fungi degrade large organic N polymers in soils is also controlled by the plant C resources available to the fungi to construct extracellular enzymes, as well as the bond strength and structural diversity of the target organic N compound although the direct tests of the hypothetical mechanism is still needed. Another study by Näsholm et al. [90] tested a model for C–N exchange between trees and mycorrhizal fungi. They found that ECM fungi transport smaller amounts of absorbed N to trees in N-limited than in N-rich conditions. The study found further that the greater allocation of C from trees to ECM fungi increases N retention into soil mycelium. The growth of these fungi is stimulated, and thus, N is immobilized and sequestered in soil. This mechanism was suggested to drive boreal forests towards a more severe N limitation at low N supply.

ECM fungi have diverse evolutionary origins and they use diverse decomposition mechanisms to access organic nitrogen entrapped in soil organic matter [91]. The timing and magnitude of decomposition activity seem to be controlled by the below-ground nitrogen quality and the above-ground carbon supply. Some ECM fungi might act as decomposers, not primarily to obtain C to their metabolism, but to search for organic N in the absence of readily available inorganic N [76, 92–94].

4. Challenges in establishing edible ectomycorrhizal fungal culture with fruitbody formation

More than thousand species of ECM fungi produce edible mushrooms [95]. Some of them, such as *Amanita caesarea* (Scop.) Pers. *Boletus edulis* Bull., *Cantharellus cibarius* Fr. and *Tricholoma matsutake* (S. Ito and S. Imai) Singer, have economical value on international markets. The problem is that edible ECM fungi are usually more difficult to cultivate than saprophytic fungi because of the symbiotic relationship with a host tree is needed. In the past few decades, significant progress has been made in the cultivation of some fungi, such as *Lactarius deliciosus* (L.) Gray [96–98], *Lactarius hatsudake* Nobuj. Tanaka [99], *Suillus granulatus* (L.) Roussel [96], *Rhizopogon roseolus* (Corda) Th. Fr. [100], and *Lyophyllum shimeji* (Kawam.) Hongo [101]. In controlled conditions, however, the successful fruitbody or primordium formations are limited. Most of edible ECM fungi still cannot be cultivated. The major issues that need to be understood are the trophic relationships, biotic, edaphic, and climatic requirements for each mushroom. In this review, we focus on the nitrogen acquisition of edible ECM fungi for their mycelial culture and its effect on fruitbody formation. Secondly, we take *T. matsutake* as an example and discuss in detail about its ability to acquire nitrogen, its preferences, and possible strategies. Finally, we discuss about the further challenges – to conserve proper ecological conditions for edible ECM fungi to grow.

4.1 Nitrogen sources in edible ECM fungal cultures

We summarize the nitrogen sources used in mycelium culture and the cultivation experiments of edible ECM fungi in combination with ECM fungal morphological characteristics reported from the published studies (**Table 1**). As known, most edible ECM fungi are difficult for cultivation so far. We could get some hints for the ECM cultivation from experimentally observed nitrogen preferences

ECM fungi	Mycelium growth	Mycorrhization	Fruitbody formation	Ref	Hydrophobicity	Exploration type	$\delta^{15}\text{N}$ (‰) (Mean $\pm$ SD) (n)	Ref.
Amanita	NH ₄ ⁺ (poor on orgN)			[103]	Hi	Medium-smooth	3.1 $\pm$ 0.5 (35)	[102]
<i>A. caesarea</i>								
Boletus					Ho	Long	5.8 $\pm$ 1.0 (17)	[102]
							8.66 (1)	[104]
<i>B. edulis</i>		orgN		[105]				
<i>B. reticulatus</i>			NH ₄ ⁺ and orgN	[106]				
<i>Boletus</i> sp.			NH ₄ ⁺ and orgN	[107]				
Cantharellus					Hi		4.3 $\pm$ 1.4 (8)	[102]
<i>C. cibarius</i>	NH ₄ ⁺ (poor on orgN)			[48]				
Cortinarius					Ho	Medium-fringe	6.8 $\pm$ 0.3 (100)	[102]
<i>C. variecolor</i>			orgN	[67]				
Hebeloma					Ho	Short/ medium-fringe	2.7 $\pm$ 1.1 (7)	[102]
<i>H. cylindrosporum</i>			orgN (but a variable among strains)	[59]				
			ON	[57]				
<i>H. radicosum</i>			NH ₄ ⁺	[108]				
<i>Hebeloma</i> sp.			orgN	[108]				
Hydnum					Ho	Medium-fringe	12 (1) cap	[102]
<i>H. repandum</i>	NO ₃ ⁻ or ON (poor on NH ₄ ⁺)			[109]				
Laccaria					Hi	Short	0.5 $\pm$ 0.6 (15)	[102]

ECM fungi	Mycelium growth	Mycorrhization	Fruitbody formation	Ref	Hydrophobicity	Exploration type	$\delta^{15}\text{N}$ (‰) (Mean $\pm$ SD) (n)	Ref.
<i>L. lacata</i>	orgN			[110]			3.0 $\pm$ 0.4 (3) cap	[111]
	NH ₄ ⁺ (poor on orgN)			[112]				
<i>L. bicolor</i> NH ₄ ⁺ (poor on NO ₃ ⁻ , or orgN)				[113]			1.8 (1) cap	[111]
	NH ₄ ⁺ , NO ₃ ⁻ (poor on amino acid, good on urea)			[114]				
Lactarius	NH ₄ ⁺ (poor on orgN)			[67]				
					Hi	Contact/ Medium-smooth	4.2 $\pm$ 0.3 (54)	[102]
							4.3 $\pm$ 0.5 (3)	[111]
<i>L. deliciosus</i>	NH ₄ ⁺ plus orgN			[98]				
<i>L. rufus</i>	orgN (a variable among strains)			[67]				
Lyophyllus								
<i>L. shimeji</i>			NH ₄ ⁺ and orgN	[115]				
Paxillus					Ho	Long	7.1 $\pm$ 0.7 (7)	[102]
<i>P. involutus</i>	orgN			[113]				
Scleroderma								
<i>S. citrinum</i>	NH ₄ ⁺ or orgN			[112]	Ho	Long		
Suillus					Ho	Long	8.2 $\pm$ 0.7 (17)	[102]
<i>S. bovinus</i>			Forest soil	[116]				

ECM fungi	Mycelium growth	Mycorrhization	Fruitbody formation	Ref	Hydrophobicity	Exploration type	$\delta^{15}\text{N}$ (‰) (Mean $\pm$ SD) (n)	Ref.
<i>S. luteus</i>	NH_4^+ (poor on orgN)			[112]				
<i>S. variegatus</i>	orgN			[113]			5.7 $\pm$ 1.1 (4) cap	[111]
Tricholoma					Ho	Medium-fringe	9.3 $\pm$ 0.6 (35)	[102]
<i>T. imbricatum</i>	NH_4^+ or NO_3^- or orgN (gained better growth in iorgN)			[117]				
<i>T. bakamatsutake</i>	NH_4^+ or orgN (poor on NO_3^-)			[118]				
<i>T. matsutake</i>	NH_4^+ plus orgN			[119]			16.8 $\pm$ 2.3 (15)	[120]
	orgN			[121]				
		orgN (sustaining symbiotic relationship)		[122]				
<i>T. terreum</i>	orgN (gained better growth)			[123]				
Tuber					Hi	Short	15.1 $\pm$ 0.6 (9)	[102]
<i>T. sinense</i>	orgN (gained better growth)			[124]				

* NH_4^+ , ammonium nitrogen; NO_3^- , nitrate nitrogen; orgN, organic nitrogen. Ho, hydrophobic; Hi, hydrophilic.

Table 1.
Fungal growth, symbiosis and fruitbody formation observed using different nitrogen sources in edible ectomycorrhizal fungi in combination with the information of hydrophobicity, exploration type and $\delta^{15}\text{N}$ of the fruitbodies.

and mycorrhizal formation. In pure culture conditions, most of the studied fungi appeared to favor ammonium N. Some species, namely *Amanita caesarea*, *Cantharellus cibarius*, *Lactarius bicolor*, *Suillus variegatus* were not able to grow nitrate as the sole N source [48, 103, 113].

However, many of the edible ECM fungi, namely *Amanita caesarea* [103, 105], *Cantharellus cibarius* [48], *Cortinarius variegatus* [67], *Paxillus involutus*, *Suillus variegatus* [113], *Tricholoma terreum* [123], and *Tuber sinense* [124] were able to grow on the media containing organic N (protein) as the sole nitrogen. Moreover, some fungi belonging to *Lactarius* genus had limited capacity to utilize protein N [113, 114]. *Hebeloma cylindrosporum* was able to experimentally utilize a wide range of amino acids and other simple (e.g. urea) or complex (e.g. proteins) compounds [6, 59].

The studied forms of N often predominate soil solution and the culturing results might be assumed to hold true in nature. However, it is worth mentioning is that the optimal nitrogen in the mycelium culture does not necessarily reflect the nitrogen preference of the ECM fungus under natural conditions because environmental factors affect. This was shown with *H. cylindrosporum* growing in nature. Wild dikaryotic strains of *H. cylindrosporum* isolated from two different habitat types had different N preferences [6].

Cultivation of edible ECM mushrooms has been successful in cases of two truffles *Tuber melanosporum* Vittad. and *Tuber aestivum* Vittad. They are cultivated commercially around the world [125]. In addition, some success has been achieved with *Lactarius deliciosus* [126, 127] and *Boletus edulis* [128]. Regarding truffle production, it has been suggested that most soils contain enough N to maintain both fungal and tree growth [125]. Similarly, *Lactarius deliciosus* was cultivated experimentally in forest soil, which was observed to meet the demands for fruitbody formation [126]. It has also been demonstrated that the nutritional properties of soil and the forestry history the natural development of ECM mushrooms in forest ecosystems [129]. A productive and diverse ECM mushroom community resembling natural communities developed when abandoned farmland in Mediterranean dry area was forested with *Pinus* sp.

In summary, productive ECM community can grow in natural soils. However, the challenges faced in artificial cultivation has not been solved.

4.2 Nitrogen source requirements for *Tricholoma matsutake* mycelial culture and mycorrhizal synthesis

Tricholoma matsutake is among the most economically valuable mushrooms in the world. Its taxonomy, distribution, ecology, physiology, and cultivation has been studied widely [130]. Here, we summarize the key results linking matsutake ecological characteristics and nutrient requirements focusing on nitrogen.

Matsutake colonizes the roots of its host trees via an ECM association (**Figure 1a** and **b**). It develops an extraradical mycelium in the rhizosphere and in the surrounding soil area. This can be seen as a white rhizosphere area and it corresponds to the mycelium-soil aggregated zone, called a shiro [131] (**Figure 1c** and **d**). Matsutake shiro grows in the form of a concentric or horseshoe-like circle, depending on the rhizosphere conditions, around the host plant at the rate of approximately 10–15 cm per year [131, 132]. The production of matsutake mushrooms changes periodically. Based on our field observations, the part of mycorrhizal root tips is degraded prior to matsutake fruiting. The extraradical mycelium might grow towards new roots and colonizing. Such a hyphal growth strategy indicates that matsutake symbiosis may often need to be renewed and form new mycorrhizas to acquire nutrients (data not published). Among the mycorrhizal associations, such

Figure 1.

The ectomycorrhizal edible mushroom—*Tricholoma matsutake* (a) the root of *Pinus sylvestris* seedling is colonized by *T. matsutake* fungal mycelium, forms mycorrhizas; (b) the transverse section of ECM root showing the Hartig net (hn) development within the cortex; (c) matsutake mushrooms form in a conifer mixed forest in southern of Finland; (d) the matsutake shiro (arrow) after the mushrooms be harvested (photos were taken by Lu Min Vaario).

a phenomenon does not seem to be rare. Hortal and colleagues [133] found that the plant had the ability to limit the root tip colonization of the least cooperative symbiont, and therefore, influence the outcome of ECM fungi competition. Such reduction in colonization did not result in a reduction in carbon allocation to the fungus providing the lowest amount of nitrogen.

It is worth noting that decayed mycorrhizal roots together with mycelium-soil aggregated zone might be important organic nutrient sources for matsutake. Recently, the natural abundance of isotopes data showed a very high $\delta^{15}\text{N}$ value in *T. matsutake* fruitbodies, which were sampled from Finland and Japan [120]. Matsutake usually grow at B layer of mineral soil [131], such taxa obtain their N could explain for high $\delta^{15}\text{N}$ values (see review [102]). More importantly, the high $\delta^{15}\text{N}$ value in matsutake is an indicator of organic N uptake from soil because the great variation of ^{15}N content observed among ECM taxa has been reported to be related to the differences in organic N utilization [111]. In addition, a literature study shows that mycorrhizal taxa with proteolytic activities generally show high $\delta^{15}\text{N}$ values [67]. Therefore, we conclude that matsutake has a greater proteolytic activity to digest chemically complex ^{15}N -enriched organic matter in soil during matsutake fruitbody development.

In addition to proteases, matsutake produces organic matter degradation enzymes such as acid proteinase [134, 135] and β -glucosidase [136]. Relatively high enzyme activities, β -glucosidase and xylosidase, were detected from matsutake cultures in vitro and in shiro soil [137, 138]. The genome of *T. matsutake* encodes

two GH7 cellobiohydrolases [88], which is in agreement with its known facultative saprotrophic activity [136, 138]. However, no further evidence of any strong saprotrophic characteristics of matsutake was found. It could be speculated that these ECM fungi produce certain levels of carbohydrase, not to fully degrade organic matter to access C but N. Kawai and Abe [121] reported that dried beer yeast, corn steep liquor, casein hydrolysate, and polypeptone were good N sources for matsutake mycelium culture whereas nitrate was not. Dry beer yeast (Ebios, Asahi Beer Inc., Tokyo, Japan), as the sole N source, showed promising matsutake mycelium growth and as well mycorrhizal formation [139] (personal communication with Dr. A. Yamada).

Several agar media such as MMN, MNC, Hamada containing both inorganic and organic N are widely used to culture the mycelium of *T. matsutake* [119, 140]. However, the question whether matsutake prefers organic nitrogen is worth of considering. Usually, more inorganic N than organic N is present in the soil top layer. Some studies suggested that increased N deposition could reduce fruitbody production [141]. Nohrstedt [142] reported a 30% decrease in sporocarp production by *Cantharellus cibarius* in a central Swedish pine forest after the application of 150 kg N ha⁻¹ ammonium nitrate. The presence of nitrate ions has been shown to have negative effects on the development of some ECM fungi both in vitro and in soil [143, 144]. Removal of the litter layer has been considered an important method to improve the productivity of matsutake in many Asian countries [145]. It has also been shown that the removal of the upper organic soil layers of the forest floor can improve the sporocarp production of some other ECM fungi [146, 147]. The explanation might be that competition with other microbes diminishes. Litter and organic soil provide carbon and nutrients for microbes, especially for saprotrophic fungi that would compete with *T. matsutake* in the shiro [148].

4.3 Research prospects

Cultivation of ectomycorrhizal mushrooms is still facing many challenges. Although some species of ECM fungi can form the primordium of fruiting bodies on several media, they usually do not develop further into mature fruiting bodies. So far, the most successful efforts have been carried out with the mycorrhizal plants growing in soil. Soil nutrients and soil microbial communities together with climatic factors have shown to affect significantly the persistence of ectomycorrhizas in outplanted inoculated plants, and further, the successful fruiting. The observed suppression of many mycorrhizal mushrooms has been linked to indirect effects of air pollution, in particular to increases in nitrogen deposition accumulating into litter and humus [149, 150]. A thorough understanding of the ecological and environmental factors regulating the ECM fungal species is a prerequisite for their cultivation.

Ectomycorrhizal fungi colonize the roots of their host plants and improve plants' access to nutrients, especially nitrogen. In exchange, host plants deliver a significant portion of their photosynthesized carbon to the ECM fungi. However, we need more accurate understanding of the ECM fungi mediated C and N movement within forest ecosystems. ECM fungi may follow a similar pattern with the amount of C delivered being related to the amount of N sourced by the fungus [77, 151], although this is still controversial [133]. Production of ECM mushrooms do need a balanced nutrient either assimilating by ECM fungi or by other soil microbial.

It has been suggested that the growth of ECM fungi and the formation of mycorrhizas are promoted by certain mycorrhizosphere bacteria, termed 'mycorrhizal helper bacteria' [152]. Some mycorrhizal fungi-associated bacteria are also known to fix nitrogen [153, 154]. However, there is still no evidence that the fungus would

directly benefit from its associated bacteria. Sporocarps of *Cantharellus cibarius* contain large amount of bacteria, in particular fluorescent *Pseudomonas* [155]. Some species of bacteria such as *Streptomyces* spp., *Paenibacillus* spp. and *Bacillales* spp. were isolated from the mycorrhizal root tips and fruitbodies of *T. matsutake* as well [156–158]. Otherwise, the information about mycorrhizas-associated bacteria and their effect on the nutrient uptake of ECM fungi is limited. These studies, however, hint that the production of ectomycorrhizal mushrooms may require teamwork to obtain enough nutrients from the environment.

In conclusion, ECM fungi play an important role in the nutrient cycle of forest ecosystems, especially on mediating C and N movement. A better understanding of the nitrogen status of the habitat of ECM fungi, nutrients movements within the ecosystems, as well as the ECM fungal hyphal structures should be the first step for cultivation of ECM edible mushrooms. The methodological advances in these areas in combination with forest management may allow the successful establishment of commercial plantations and production of edible ECM mushrooms in forests.

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References

- [1] Pilz D, Molina R. *Managing forest ecosystems to conserve fungus diversity and sustain wild mushroom harvests*. US Department of Agriculture, Forest Service, Pacific Northwest Research Station, 1996.
- [2] Führer E. Forest functions, ecosystem stability and management. *For Ecol Manage* 2000; 132: 29-38.
- [3] Mandzak JM, Moore JA. The role of nutrition in the health of inland western forests. *J Sustain For* 1994; 2: 191-210.
- [4] O'Neill KP. *Soils as an indicator of forest health: a guide to the collection, analysis, and interpretation of soil indicator data in the Forest Inventory and Analysis program*. USDA Forest Service, North Central Research Station, 2005.
- [5] Schimel JP, Bennett J. Nitrogen mineralization: challenges of a changing paradigm. *Ecology* 2004; 85: 591-602.
- [6] Abuarhub SM, Read DJ. The biology of mycorrhiza in the Ericaceae XI. The distribution of nitrogen in soil of a typical upland Callunetum with special reference to the 'free' amino acids. *New Phytol* 1988; 108: 425-431.
- [7] Kelley KR, Stevenson FJ. Forms and nature of organic N in soil. In: *Nitrogen Economy in Tropical Soils*. Springer, 1995, pp. 1-11.
- [8] Hartley J, Cairney JWG, Meharg AA. Do ectomycorrhizal fungi exhibit adaptive tolerance to potentially toxic metals in the environment? *Plant Soil* 1997; 189: 303-319.
- [9] Bandou E, Lebailly F, Muller F, et al. The ectomycorrhizal fungus *Scleroderma bermudense* alleviates salt stress in seagrape (*Coccoloba uvifera* L.) seedlings. *Mycorrhiza* 2006; 16: 559-565.
- [10] Nadeem SM, Ahmad M, Zahir ZA, et al. The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnol Adv* 2014; 32: 429-448.
- [11] Hall IR, Yun W, Amicucci A. Cultivation of edible ectomycorrhizal mushrooms. *TRENDS Biotechnol* 2003; 21: 433-438.
- [12] Boa E. Non-wood forest products. Wild edible fungi a glob overview of their use and importance to people. 2004 FAO.
- [13] Smith SE, Read DJ 1997. *Mycorrhizal Symbiosis*. Academic Press London.
- [14] Landeweert R, Hoffland E, Finlay RD, et al. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. *Trends Ecol Evol* 2001; 16: 248-254.
- [15] Phillips RP, Brzostek E, Midgley MG. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon–nutrient couplings in temperate forests. *New Phytol* 2013; 199: 41-51.
- [16] Brundrett MC. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 2009; 320: 37-77.
- [17] Rinaldi AC, Comandini O, Kuyper TW. Ectomycorrhizal fungal diversity: separating the wheat from the chaff. *Fungal Divers* 2008; 33: 1-45.
- [18] Tedersoo L, Mett M, Ishida TA, et al. Phylogenetic relationships among host plants explain differences in

fungus species richness and community composition in ectomycorrhizal symbiosis. *New Phytol* 2013; 199: 822-831.

[19] Read DJ, Perez-Moreno J. Mycorrhizas and nutrient cycling in ecosystems—a journey towards relevance? *New Phytol* 2003; 157: 475-492.

[20] Orwin KH, Kirschbaum MUF, St John MG, et al. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecol Lett* 2011; 14: 493-502.

[21] Zak DR, Pellitier PT, Argiroff W, et al. Exploring the role of ectomycorrhizal fungi in soil carbon dynamics. *New Phytol* 2019; 223: 33-39.

[22] Toljander JF, Eberhardt U, Toljander YK, et al. Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytol* 2006; 170: 873-884.

[23] Lin G, McCormack ML, Ma C, et al. Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. *New Phytol* 2017; 213: 1440-1451.

[24] Laliberté E, Lambers H, Burgess TI, et al. Phosphorus limitation, soil-borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. *New Phytol* 2015; 206: 507-521.

[25] Egerton-Warburton LM, Johnson NC, Allen EB. Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. *Ecol Monogr* 2007; 77: 527-544.

[26] Steidinger BS, Crowther TW, Liang J, et al. Climatic controls of

decomposition drive the global biogeography of forest-tree symbioses. *Nature* 2019; 569: 404-408.

[27] Smith ML, Bruhn JN, Anderson JB. The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. *Nature* 1992; 356: 428-431.

[28] Agerer R. Exploration types of ectomycorrhizae. *Mycorrhiza* 2001; 11: 107-114.

[29] Unestam T, Sun Y-P. Extramatrical structures of hydrophobic and hydrophilic ectomycorrhizal fungi. *Mycorrhiza* 1995; 5: 301-311.

[30] Hobbie EA, Agerer R. Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant Soil* 2010; 327: 71-83.

[31] Finlay RD, Read DJ. The structure and function of the vegetative mycelium of ectomycorrhizal plants: II. The uptake and distribution of phosphorus by mycelial strands interconnecting host plants. *New Phytol* 1986; 103: 157-165.

[32] Burgess T, Dell B, Malajczuk N. Variation in mycorrhizal development and growth stimulation by 20 *Pisolithus* isolates inoculated on to *Eucalyptus grandis* W. Hill ex Maiden. *New Phytol* 1994; 127: 731-739.

[33] Burgess TI, Malajczuk N, Grove TS. The ability of 16 ectomycorrhizal fungi to increase growth and phosphorus uptake of *Eucalyptus globulus* Labill. and *E. diversicolor* F. Muell. *Plant Soil* 1993; 153: 155-164.

[34] Dell B, Malajczuk N, Bougher NL, et al. Development and function of *Pisolithus* and *Scleroderma* ectomycorrhizas formed in vivo with *Allocasuarina*, *Casuarina* and *Eucalyptus*. *Mycorrhiza* 1994; 5: 129-138.

[35] Colpaert J V, Van Assche JA, Luijters K. The growth of the

extramatrical mycelium of ectomycorrhizal fungi and the growth response of *Pinus sylvestris* L. *New Phytol* 1992; 120: 127-135.

[36] Islam MR, Tudryn G, Bucinell R, et al. Morphology and mechanics of fungal mycelium. *Sci Rep* 2017; 7: 1-12.

[37] Thomson BD, Grove TS, Malajczuk N, et al. The effectiveness of ectomycorrhizal fungi in increasing the growth of *Eucalyptus globulus* Labill. in relation to root colonization and hyphal development in soil. *New Phytol* 1994; 126: 517-524.

[38] Cairney JWG. Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biol Biochem* 2012; 47: 198-208.

[39] Vestgarden LS. Carbon and nitrogen turnover in the early stage of Scots pine (*Pinus sylvestris* L.) needle litter decomposition: effects of internal and external nitrogen. *Soil Biol Biochem* 2001; 33: 465-474.

[40] Wu T. Can ectomycorrhizal fungi circumvent the nitrogen mineralization for plant nutrition in temperate forest ecosystems? *Soil Biol Biochem* 2011; 43: 1109-1117.

[41] Alexander IJ. The significance of ectomycorrhizas in the nitrogen cycle. In 'Nitrogen as an Ecological Factor'. (Eds J. A. Lee, S. McNeill and IH Rorison.). 1983; pp. 69-94.

[42] Schnitzer M, Schulten H-R. New ideas on the chemical make-up of soil humic and fulvic acids. *Futur Prospect soil Chem* 1998; 55: 153-177.

[43] Jones DL, Kielland K. Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biol Biochem* 2002; 34: 209-219.

[44] Sowden FJ, Chen Y, Schnitzer M. The nitrogen distribution in soils

formed under widely differing climatic conditions. *Geochim Cosmochim Acta* 1977; 41: 1524-1526.

[45] Werdin-Pfisterer NR, Kielland K, Boone RD. Soil amino acid composition across a boreal forest successional sequence. *Soil Biol Biochem* 2009; 41: 1210-1220.

[46] Vancampenhout K, Wouters K, De Vos B, et al. Differences in chemical composition of soil organic matter in natural ecosystems from different climatic regions—A pyrolysis–GC/MS study. *Soil Biol Biochem* 2009; 41: 568-579.

[47] Meyer FH. Distribution of ectomycorrhizae in native and man-made forests. *Ectomycorrhizae their Ecol Physiol* 1973; 79-105.

[48] Rangel-Castro IJ, Danell E, Taylor AF. Use of different nitrogen sources by the edible ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycorrhiza* 2002; 12: 131-137.

[49] Chalot M, Plassard C. Ectomycorrhiza and nitrogen provision to the host tree. *Ecol Asp nitrogen Metab plants* 2011; 69-94.

[50] Clemmensen KE, Sorensen PL, Michelsen A, et al. Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes and ectomycorrhizal fungi. *Oecologia* 2008; 155: 771-783.

[51] Nygren CMR, Eberhardt U, Karlsson M, et al. Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. *New Phytol* 2008; 180: 875-889.

[52] Turnbull MH, Goodall R, Stewart GR. The impact of mycorrhizal colonization upon nitrogen source utilization and metabolism in seedlings of *Eucalyptus grandis* Hill ex Maiden

- and *Eucalyptus maculata* Hook. *Plant Cell Environ* 1995; 18: 1386-1394.
- [53] Hobbie EA, Colpaert J V, White MW, et al. Nitrogen form, availability, and mycorrhizal colonization affect biomass and nitrogen isotope patterns in *Pinus sylvestris*. *Plant Soil* 2008; 310: 121.
- [54] Read DJ, Leake JR, Perez-Moreno J. Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Can J Bot* 2004; 82: 1243-1263.
- [55] Melin E, Nilsson H. Transfer of labelled nitrogen from glutamic acid to pine seedlings through the mycelium of *Boletus variegatus* (Sw.) Fr. *Nature* 1953; 171: 134.
- [56] Chalot M, Brun A. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiol Rev* 1998; 22: 21-44.
- [57] Plassard C, Bonafos B, Touraine B. Differential effects of mineral and organic N sources, and of ectomycorrhizal infection by *Hebeloma cylindrosporum*, on growth and N utilization in *Pinus pinaster*. *Plant Cell Environ* 2000; 23: 1195-1205.
- [58] Taylor AFS, Fransson PM, Högberg P, et al. Species level patterns in ^{13}C and ^{15}N abundance of ectomycorrhizal and saprotrophic fungal sporocarps. *New Phytol* 2003; 159: 757-774.
- [59] Guidot A, Verner M-C, Debaud J-C, et al. Intraspecific variation in use of different organic nitrogen sources by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Mycorrhiza* 2005; 15: 167-177.
- [60] Abuzinadah RA, Read DJ. The role of proteins in the nitrogen nutrition of ectomycorrhizal plants: I. Utilization of peptides and proteins by ectomycorrhizal fungi. *New Phytol* 1986; 103: 481-493.
- [61] Courty P, Pritsch K, Schlöter M, et al. Activity profiling of ectomycorrhiza communities in two forest soils using multiple enzymatic tests. *New Phytol* 2005; 167: 309-319.
- [62] Lilleskov EA, Hobbie EA, Horton TR. Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *fungus Ecol* 2011; 4: 174-183.
- [63] Peay KG, Kennedy PG, Bruns TD. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *fungus Ecol* 2011; 4: 233-240.
- [64] Dawson TE, Mambelli S, Plamboeck AH, et al. Stable isotopes in plant ecology. *Annu Rev Ecol Syst* 2002; 33: 507-559.
- [65] Hobbie EA, Hobbie JE. Natural abundance of ^{15}N in nitrogen-limited forests and tundra can estimate nitrogen cycling through mycorrhizal fungi: a review. *Ecosystems* 2008; 11: 815-830.
- [66] Trudell SA, Rygielwicz PT, Edmonds RL. Patterns of nitrogen and carbon stable isotope ratios in macrofungi, plants and soils in two old-growth conifer forests. *New Phytol* 2004; 164: 317-335.
- [67] Lilleskov EA, Hobbie EA, Fahey TJ. Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes. *New Phytol* 2002; 154: 219-231.
- [68] Hobbie EA, Sánchez FS, Rygielwicz PT. Controls of isotopic patterns in saprotrophic and ectomycorrhizal fungi. *Soil Biol Biochem* 2012; 48: 60-68.

- [69] Kranabetter JM. Ectomycorrhizal fungi and the nitrogen economy of conifers—implications for genecology and climate change mitigation. *Botany* 2014; 92: 417-423.
- [70] Pardo LH, Fenn ME, Goodale CL, et al. Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecol Appl* 2011; 21: 3049-3082.
- [71] Morrison EW, Frey SD, Sadowsky JJ, et al. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. *fungal Ecol* 2016; 23: 48-57.
- [72] Corrales A, Turner BL, Tedersoo L, et al. Nitrogen addition alters ectomycorrhizal fungal communities and soil enzyme activities in a tropical montane forest. *fungal Ecol* 2017; 27: 14-23.
- [73] Zhu H, Dancik BP, Higginbotham KO. Regulation of extracellular proteinase production in an ectomycorrhizal fungus *Hebeloma crustuliniforme*. *Mycologia* 1994; 86: 227-234.
- [74] Nehls UWE, Kleber R, Wiese J, et al. Isolation and characterization of a general amino acid permease from the ectomycorrhizal fungus *Amanita muscaria*. *New Phytol* 1999; 144: 343-349.
- [75] Javelle A, Morel M, Rodríguez-Pastrana B, et al. Molecular characterization, function and regulation of ammonium transporters (Amt) and ammonium-metabolizing enzymes (GS, NADP-GDH) in the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *Mol Microbiol* 2003; 47: 411-430.
- [76] Rineau F, Shah F, Smits MM, et al. Carbon availability triggers the decomposition of plant litter and assimilation of nitrogen by an ectomycorrhizal fungus. *ISME J* 2013; 7: 2010-2022.
- [77] Casieri L, Lahmidi NA, Doidy J, et al. Biotrophic transportome in mutualistic plant–fungal interactions. *Mycorrhiza* 2013; 23: 597-625.
- [78] Nehls U, Plassard C. Nitrogen and phosphate metabolism in ectomycorrhizas. *New Phytol* 2018; 220: 1047-1058.
- [79] Becquer A, Guerrero-Galán C, Eibensteiner JL, et al. The ectomycorrhizal contribution to tree nutrition. In: *Advances in Botanical Research*. Elsevier, 2019, pp. 77-126.
- [80] Javelle A, Rodríguez-Pastrana B-R, Jacob C, et al. Molecular characterization of two ammonium transporters from the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *FEBS Lett* 2001; 505: 393-398.
- [81] Montanini B, Moretto N, Soragni E, et al. A high-affinity ammonium transporter from the mycorrhizal ascomycete *Tuber borchii*. *Fungal Genet Biol* 2002; 36: 22-34.
- [82] Willmann A, Weiß M, Nehls U. Ectomycorrhiza-mediated repression of the high-affinity ammonium importer gene *AmAMT2* in *Amanita muscaria*. *Curr Genet* 2007; 51: 71.
- [83] Kemppainen MJ, Pardo AG. pHg/pSILBA γ vector system for efficient gene silencing in homobasidiomycetes: optimization of ihpRNA-triggering in the mycorrhizal fungus *Laccaria bicolor*. *Microb Biotechnol* 2010; 3: 178-200.
- [84] Jargeat P, Rekangalt D, Verner M-C, et al. Characterisation and expression analysis of a nitrate transporter and nitrite reductase genes, two members of a gene cluster for nitrate assimilation from the symbiotic basidiomycete *Hebeloma cylindrosporum*. *Curr Genet* 2003; 43: 199-205.

- [85] Kohler A, Kuo A, Nagy LG, et al. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nat Genet* 2015; 47: 410-415.
- [86] Shah F, Nicolás C, Bentzer J, et al. Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytol* 2016; 209: 1705-1719.
- [87] Lindahl BD, Taylor AFS. Occurrence of N-acetylhexosaminidase-encoding genes in ectomycorrhizal basidiomycetes. *New Phytol* 2004; 164: 193-199.
- [88] Miyauchi S, Kiss E, Kuo A, et al. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits. *Nat Commun* 2020; 11: 1-17.
- [89] Talbot JM, Treseder KK. Controls over mycorrhizal uptake of organic nitrogen. *Pedobiologia (Jena)* 2010; 53: 169-179.
- [90] Näsholm T, Högborg P, Franklin O, et al. Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytol* 2013; 198: 214-221.
- [91] Nicolás C, Martin-Bertelsen T, Floudas D, et al. The soil organic matter decomposition mechanisms in ectomycorrhizal fungi are tuned for liberating soil organic nitrogen. *ISME J* 2019; 13: 977-988.
- [92] Lindahl BD, Ihrmark K, Boberg J, et al. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol* 2007; 173: 611-620.
- [93] Talbot JM, Allison SD, Treseder KK. Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change. *Funct Ecol* 2008; 22: 955-963.
- [94] Sinsabaugh RL. Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biol Biochem* 2010; 42: 391-404.
- [95] Hall IR, Zambonelli A. Laying the foundations. In: *Edible ectomycorrhizal mushrooms*. Springer, 2012, pp. 3-16.
- [96] Poitou N, Mamoun M, Ducamp M, et al. After *Boletus granulatus*, *Lactarius deliciosus* fructification is obtained in the field from inoculated plants. *PHM Rev Hortic* 1984; 244: 65-68.
- [97] Guerin-Laguette A, Cummings N, Butler RC, et al. *Lactarius deliciosus* and *Pinus radiata* in New Zealand: towards the development of innovative gourmet mushroom orchards. *Mycorrhiza* 2014; 24: 511-523.
- [98] Wang R, Guerin-Laguette A, Yu F-Q. Optimum media for hyphal growth and mycorrhizal synthesis of two *Lactarius* species. *Mycosystema* 2020; 39: 1346-1355.
- [99] Tan ZM, Danell E, Shen AR, et al. Successful cultivation of *Lactarius hatsutake*—an evaluation with molecular methods. *Acta Edulis Fungi* 2008; 15: 85-88.
- [100] Visnovsky SB, Guerin-Laguette A, Wang Y, et al. Traceability of marketable Japanese shoro in New Zealand: using multiplex PCR to exploit phylogeographic variation among taxa in the *Rhizopogon* subgenus *Roseoli*. *Appl Environ Microbiol* 2010; 76: 294-302.
- [101] Yamanaka K. Commercial cultivation of *Lyophyllum shimeji*. GAMU GmbH, Institut für Pilzforschung, 2008; pp. 197-202.
- [102] Hobbie EA, Högborg P. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytol* 2012; 196: 367-382.

- [103] Daza A, Manjón JL, Camacho M, et al. Effect of carbon and nitrogen sources, pH and temperature on in vitro culture of several isolates of *Amanita caesarea* (Scop.: Fr.) Pers. *Mycorrhiza* 2006; 16: 133-136.
- [104] Tedersoo L, Naadel T, Bahram M, et al. Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytol* 2012; 195: 832-843.
- [105] Endo N, Gisusi S, Fukuda M, et al. In vitro mycorrhization and acclimatization of *Amanita caesareoides* and its relatives on *Pinus densiflora*. *Mycorrhiza* 2013; 23: 303-315.
- [106] Yamanaka K, Namba K, Tajiri A. Fruit body formation of *Boletus reticulatus* in pure culture. *Mycoscience* 2000; 41: 189.
- [107] Ohta A, Fujiwara N. Fruit-body production of an ectomycorrhizal fungus in genus *Boletus* in pure culture. *Mycoscience* 2003; 44: 295-300.
- [108] Ohta A. Fruit-body production of two ectomycorrhizal fungi in the genus *Hebeloma* in pure culture. *Mycoscience* 1998; 39: 15-19.
- [109] Peksen A, Kibar B, Yakupoglu G. Favourable culture conditions for mycelial growth of *Hydnum repandum*, a medicinal mushroom. *African J Tradit Complement Altern Med* 2013; 10: 431-434.
- [110] Davis EE, Jong SC. Basidiocarp formation by *Laccaria laccata* in agar culture. *Mycologia* 1976; 68: 211-214.
- [111] Taylor AFS, Högbom L, Högbom M, et al. Natural ^{15}N abundance in fruit bodies of ectomycorrhizal fungi from boreal forests. *New Phytol* 1997; 136: 713-720.
- [112] Itoo ZA, Reshi ZA. Effect of different nitrogen and carbon sources and concentrations on the mycelial growth of ectomycorrhizal fungi under in-vitro conditions. *Scand J For Res* 2014; 29: 619-628.
- [113] Finlay RD, Frostegård Å, Sonnerfeldt A. Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. *New Phytol* 1992; 120: 105-115.
- [114] Yamanaka T. Utilization of inorganic and organic nitrogen in pure cultures by saprotrophic and ectomycorrhizal fungi producing sporophores on urea-treated forest floor. *Mycol Res* 1999; 103: 811-816.
- [115] Ohta A. Production of fruit-bodies of a mycorrhizal fungus, *Lyophyllum shimeji*, in pure culture. *Mycoscience* 1994; 35: 147-151.
- [116] Sun Q, Li J, Finlay RD, et al. Oxalotrophic bacterial assemblages in the ectomycorrhizosphere of forest trees and their effects on oxalate degradation and carbon fixation potential Oxalotrophic bacterial assemblages in the ectomycorrhizosphere of forest trees and their effects on oxalate. *Chem Geol* 2019; 514: 54-64.
- [117] Lazarević J, Stojičić D, Keča N. Effects of temperature, pH and carbon and nitrogen sources on growth of in vitro cultures of ectomycorrhizal isolates from *Pinus heldreichii* forest. *For Syst* 2016; 25: 3.
- [118] Terashima Y. Carbon and nitrogen utilization and acid production by mycelia of the ectomycorrhizal fungus *Tricholoma bakamatsutake* in vitro. *Mycoscience* 1999; 40: 51.
- [119] Hamada M. Physiology and ecology of *Armillaria matsutake*. *Bot Mag* 1950; 63: 40-41.

- [120] Vaario LM, Sah SP, Norisada M, et al. *Tricholoma matsutake* may take more nitrogen in the organic form than other ectomycorrhizal fungi for its sporocarp development: the isotopic evidence. *Mycorrhiza* 2019; 29: 51-59.
- [121] Kawai M, Abe S. Studies on the artificial reproduction of *Tricholoma matsutake* (S. Ito et Imai) Sing. i. effects of carbon and nitrogen sources in media on the vegetative growth of *Tricholoma matsutake*. *Transactions*. 1976.
- [122] Yamada A, Maeda K, Kobayashi H, et al. Ectomycorrhizal symbiosis in vitro between *Tricholoma matsutake* and *Pinus densiflora* seedlings that resembles naturally occurring 'shiro'. *Mycorrhiza* 2006; 16: 111-116.
- [123] Kibar B, Peksen A. Nutritional and environmental requirements for vegetative growth of edible ectomycorrhizal mushroom *Tricholoma terreum*. *Agriculture* 2011; 98: 409-414.
- [124] Liu R-S, Li D-S, Li H-M, et al. Response surface modeling the significance of nitrogen source on the cell growth and Tuber polysaccharides production by submerged cultivation of Chinese truffle *Tuber sinense*. *Process Biochem* 2008; 43: 868-876.
- [125] Bonet JA, Oliach D, Fischer C, et al. Cultivation methods of the black truffle, the most profitable mediterranean non-wood forest product; a state of the art review. In *EFI proceedings* 2009; 57: 57-71.
- [126] Guerin-Laguette A, Plassard C, Mousain D. Effects of experimental conditions on mycorrhizal relationships between *Pinus sylvestris* and *Lactarius deliciosus* and unprecedented fruit-body formation of the Saffron milk cap under controlled soilless conditions. *Can J Microbiol* 2000; 46: 790-799.
- [127] Parladé J, Pera J, Luque J. Evaluation of mycelial inocula of edible *Lactarius* species for the production of *Pinus pinaster* and *P. sylvestris* mycorrhizal seedlings under greenhouse conditions. *Mycorrhiza* 2004; 14: 171-175.
- [128] Águeda B, Parladé J, Fernández-Toirán LM, et al. Mycorrhizal synthesis between *Boletus edulis* species complex and rockroses (*Cistus* sp.). *Mycorrhiza* 2008; 18: 443-449.
- [129] Oria-de-Rueda JA, Hernández-Rodríguez M, Martín-Pinto P, et al. Could artificial reforestations provide as much production and diversity of fungal species as natural forest stands in marginal Mediterranean areas? *For Ecol Manage* 2010; 260: 171-180.
- [130] Yamanaka T, Yamada A, Furukawa H. Advances in the cultivation of the highly-prized ectomycorrhizal mushroom *Tricholoma matsutake*. *Mycoscience* 2020; 61: 49-57.
- [131] Ogawa M. Microbial ecology of mycorrhizal fungus, *Tricholoma matsutake* Sing. in pine forest 1. Fungal colony ('Shiro') of *T. matsutake*. *Bull Prod Res Inst* 1975; 272: 79-121.
- [132] Narimatsu M, Koiwa T, Sakamoto Y, et al. Estimation of novel colony establishment and persistence of the ectomycorrhizal basidiomycete *Tricholoma matsutake* in a *Pinus densiflora* forest. *fungus Ecol* 2016; 24: 35-43.
- [133] Hortal S, Plett KL, Plett JM, et al. Role of plant-fungal nutrient trading and host control in determining the competitive success of ectomycorrhizal fungi. *ISME J* 2017; 11: 2666-2676.
- [134] Terashita T, Kono M. Carboxyl proteinases from *Tricholoma matsutake* and its related species. *Mem Fac Agric Kinki Univ* 1989; 22:5-12.
- [135] Terashita T, Kono M, Yoshikawa K, et al. Productivity of

hydrolytic enzymes by mycorrhizal mushrooms. *Mycoscience* 1995; 36: 221-225.

[136] Kusuda M, Ueda M, Konishi Y, et al. Detection of β -glucosidase as saprotrophic ability from an ectomycorrhizal mushroom, *Tricholoma matsutake*. *Mycoscience* 2006; 47: 184-189.

[137] Vaario L-M, Guerin-Laguette A, Matsushita N, et al. Saprobic potential of *Tricholoma matsutake*: growth over pine bark treated with surfactants. *Mycorrhiza* 2002; 12: 1-5.

[138] Vaario L-M, Heinonsalo J, Spetz P, et al. The ectomycorrhizal fungus *Tricholoma matsutake* is a facultative saprotroph in vitro. *Mycorrhiza* 2012; 22: 409-418.

[139] Endo N, Dokmai P, Suwannasai N, et al. Ectomycorrhization of *Tricholoma matsutake* with *Abies veitchii* and *Tsuga diversifolia* in the subalpine forests of Japan. *Mycoscience* 2015; 56: 402-412.

[140] Yamada A, Ogura T, Degawa Y, et al. Isolation of *Tricholoma matsutake* and *T. bakamatsutake* cultures from field-collected ectomycorrhizas. *Mycoscience* 2001; 42: 43-50.

[141] Van Der Eerden L, De Vries W, Van Dobben H. Effects of ammonia deposition on forests in the Netherlands. *Atmos Environ* 1998; 32: 525-532.

[142] Nohrstedt HO. Fruit-body production and ^{137}Cs -activity of *Cantharellus cibarius* after nitrogen and potassium fertilization. Forestry Research Inst. of Sweden, 1994.

[143] Harley JL, Smith SE. *Mycorrhizal Symbiosis* Academic Press. London/ New York. 1983.

[144] Richards BN. *Mycorrhiza* development of loblolly pine seedlings in relation to soil reaction and the

supply of nitrate. *Plant Soil* 1965; 22: 187-199.

[145] Takeshi I, Ogawa M. Cultivating method of the mycorrhizal fungus, *Tricholoma matsutake* (Ito et Imai) Sing. (II) increasing number of Shiro (fungal colony) of *T. matsutake* by thinning the understory vegetation. *J Jpn For Soc* 1979; 61: 163-173.

[146] Baar J, Ter Braak CJF. Ectomycorrhizal sporocarp occurrence as affected by manipulation of litter and humus layers in Scots pine stands of different age. *Appl Soil Ecol* 1996; 4: 61-73.

[147] Gundersen P, Callesen I, De Vries W. Nitrate leaching in forest ecosystems is related to forest floor CN ratios. *Environ Pollut* 1998; 102: 403-407.

[148] Vaario LM, Kiikkilä O, Hamberg L. The influences of litter cover and understorey vegetation on fruitbody formation of *Tricholoma matsutake* in southern Finland. *Appl Soil Ecol* 2013; 66: 56-60.

[149] Yun W, Hall IR. Edible ectomycorrhizal mushrooms: challenges and achievements. *Can J Bot* 2004; 82: 1063-1073.

[150] Smit E, Veenman C, Baar J. Molecular analysis of ectomycorrhizal basidiomycete communities in a *Pinus sylvestris* L. stand reveals long-term increased diversity after removal of litter and humus layers. *FEMS Microbiol Ecol* 2003; 45: 49-57.

[151] Bogar L, Peay K, Kornfeld A, et al. Plant-mediated partner discrimination in ectomycorrhizal mutualisms. *Mycorrhiza* 2019; 29: 97-111.

[152] Garbaye J. Tansley review no. 76 helper bacteria: a new dimension to the mycorrhizal symbiosis. *New Phytol* 1994; 128: 197-210.

[153] Paul LR, Chapman BK, Chanway CP. Nitrogen fixation associated with *Suillus tomentosus* tuberculate ectomycorrhizae on *Pinus contorta* var. *latifolia*. *Ann Bot* 2007; 99: 1101-1109.

[154] Barbieri E, Ceccaroli P, Saltarelli R, et al. New evidence for nitrogen fixation within the Italian white truffle *Tuber magnatum*. *Fungal Biol* 2010; 114: 936-942.

[155] Danell E, Alström S, Ternström A. *Pseudomonas fluorescens* in association with fruit bodies of the ectomycorrhizal mushroom *Cantharellus cibarius*. *Mycol Res* 1993; 97: 1148-1152.

[156] Oh S-Y, Lim YW. Root-associated bacteria influencing mycelial growth of *Tricholoma matsutake* (pine mushroom). *J Microbiol* 2018; 56: 399-407.

[157] Vaario L-M, Asamizu S, Sarjala T, et al. Bioactive properties of streptomyces may affect the dominance of *Tricholoma matsutake* in shiro. *Symbiosis* 2020; 81: 1-13.

[158] Oh S-Y, Kim M, Eimes JA, et al. Effect of fruiting body bacteria on the growth of *Tricholoma matsutake* and its related molds. *PLoS One* 2018; 13: e0190948.