Charles University in Prague

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Significance of mycorrhizal symbiosis in invasiveness of Pinus strobus

Význam mykorhizní symbiózy v invazivnosti borovice vejmutovky

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Diploma thesis

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Poděkování

Děkuji všem, co mě podporovali při mojí magisterské práci. Zejména pak své rodině, blízkým přátelům a všem kolegům z Botanického ústavu za jejich ochotu kdykoliv pomoci a za jejich neutuchající trpělivost se mnou. Děkuji svému školiteli Petrovi Kohoutovi, který mou práci odborně vedl a také mě motivoval k lepším výkonům. Děkuji konzultantům a kolegům za nejen odbornou, ale hlavně konstruktivní kritiku mé práce a vědeckého projektu. Bez všech výše jmenovaných by tato práce nedosahovala kvalit, jaké má.

Abbreviations

AM - Abruscular mycorrhiza / Arbuscular mycorrhizal

AMf - Arbuscular mycorrhizal fungi

CMN/s - Common mycelial network / networks

EeM - Ectendomycorrhiza / Ectendomycorrhizal

EcM - Ectomycorrhiza / Ectomycorrhizal

EcMf - Ectomycorrhizal fungi / fungal

EMM - Extramatrical mycelia

ErM - Ericoid mycorrhiza / Ericoid mycorrhizal

ErMf - Ericoid mycorrhizal fungi

OTU - Operational taxonomic unit

OrM - Orchid mycorrhiza / Orchid mycorrhizal

OrMf - Orchid mycorrhizal fungi

NM - Non mycorrhizal

SAP - Saprotrophy

SAPf - Saprotrophic fungi / fungal

SIP - Stable isotope probing

WWW - Wood Wide Web

Abstrakt

Předkládaná práce si klade za cíl porovnat komunity ektomykorhizních (EcM) hub, které kolonizují kořenový systém invazní borovice vejmutovky a domácí borovice lesní. Cílem práce bylo posoudit, jaký má mykorhizní symbióza vliv na invazivitu borovice vejmutovky na území Národního Parku České Švýcarsko a určit jak borovice vejmutovka působí na druhové složení a četnost EcM hub. Porovnáváno bylo také množství alokovaného uhlíku do nadzemních a podzemních ektomykorhizních struktur, jehličí ve formě opadu a do jemných kořínků obou druhů borovic. Měřena byla i produkce extramatrikálního (EMM) mycelia v experimentálních nylonových sáčcích, pomocí stanovení obsahu ergosterolu - specifické složky houbových membrán. Jednotlivé polní experimenty probíhaly během dvou let na lokalitách, kde se monodominantně vyskytovaly jednotlivé druhy borovic. Oproti očekávání, byla druhová bohatost EcM hub na kořenech borovice vejmutovky poměrně vysoká, jelikož byla srovnatelná s druhovou bohatostí původní borovice lesní. Výsledky prokázaly významný rozdíl v produkci ectomykorhizních plodnic. Oproti plochám, kde se vyskytuje nepůvodní borovice vejmutovka, byl zaznamenán o 100% nižší výskyt EcM plodnic, ve srovnání s plochami obývanými borovicí lesní. Průkazně nižší byl i obsah ergosterolu ve vzorcích pocházejících ze stanovišť invazní borovice vejmutovky. Borovice lesní oproti tomu produkuje vetší množství kořínků, ale naopak méně opadu jehlic. Z výsledků též vyplynulo, že borovice vejmutovka si zřejmě vybírá EcM druhy hub jiných exploračních typů a je možné, že uspořené karbohydráty využije pro svůj vlastní růst a invazní potenciál.

Klíčová slova

Mykorhiza, borovice vejmutovka, druhové složení a bohatost ektomykorhizních hub, invazní druh, koloběh uhlíku

Abstract

This study aimed to compare the mycorrhizal fungal communities inhabiting the roots of invasive *Pinus strobus* L. and native *Pinus sylvestris* L. We also compared carbon allocation into ectomycorrhizal fungal (EcMf) and other structures of the two pine species. The aim was to assess the influence of mycorrhiza on the invasive potential of *P. strobus* in the protected areas of National Park Bohemian Switzerland. The two field experiments were conducted on three locations of each species. We estimated the EcM extramatrical mycelium (EMM) production by measuring the ergosterol content in sterile sand filled mesh-bags. Next measured variables were: biomass of ectomycorrhizal and saprotrophic sporocarps, fine roots biomass and leaf litter biomass to compare the one season production of each measured variables. The results revealed a major difference in EcM sporocarps production, whereas on the *P. sylvestris* sites was the production 100% higher. Same results came from the fine roots measurements: *P. sylvestris* had a higher fine roots production, which may be also related with the production of EMM, which was about 60% higher as well. The EcMf species richness on the *P. strobus* root-tips was as high as the native pine, but the species composition was different. The *P. strobus* prefers EcMf species with different exploration types, when compared to the native pine. And this might be the clue to the mechanism of the *P. strobus* invasion - we hypothesise, that it allocates less photosynthates into EcMf structures and uses them for its own intensive apex growth.

Key words

Mycorrhiza, *Pinus strobus*, species richness and assemblage of EcMf, invasive species, carbon cycle

1. Introduction

Mycorrhizal symbiosis is a ubiquitous phenomenon in the plant world and if studying plant ecology, it needs to be taken into account due to its importance and influence on the plant individuals and communities. Mycorrhizal fungi colonize the plant root systems and help to gain nutrients, water, increase the host plants immunity against pathogens etc. The amount of fungi in the forest soil was estimated up to 900 kg/ha (Wallander *et al.*, 2001). Such amount of fungal mycelia in the soil is not only a major global carbon sink, but also a nutrition and maybe informational highway connecting many individual plants together (Simard *et al.*, 2012). Next variable in this study are invasions of alien species into novel ecosystems, which cause ecological damage when outcompeting the native species and thus decreasing the diversity. The damage caused by the alien plants is not just biological, but also financial. Eradication of alien species costs substantial portion of national budgets. If the alien plant is mycorrhizal, it is necessary to assess if there is any influence of mycorrhiza on the process of invasion.

The presented study aimed to reveal the significance of mycorrhizal symbiosis in the case of *Pinus strobus* L. invasion into native forests in National Park Bohemian Switzerland, inhabited by indigenous *Pinus sylvestris* L. It continues with the study (Kohout *et al.*, 2011b) which aimed to determine the interaction between ericaceous understorey shrubs and the diversity and abundance of ectomycorrhizal fungi (EcMf) associated with the invasive *P. strobus* and native *P. sylvestris*. The experiment was conducted in mesocosmic systems and revealed the possible influence of *P. strobus* on the EcMf community. This study was a field experiment, which aimed to compare the EcMf species richness and assemblage of both Pine species. In addition, we compared the fine roots production, carbon allocation into the soilborne EcMf mycelium, abundance of fungal sporocarps in the tree undergrowth and biomass of leaf litter.

2. Literature review

2.1 Mycorrhizal symbiosis

Mycorrhizal symbiosis is a key factor not only in fungal but plant physiology, ecology and evolution (Read *et al.*, 2004; Smith & Read, 2008). This intimate relation is at least 460 million years old (Redecker *et al.*, 2000) and according to some authors is the cooperation of plants and fungi the one essential evolutionary step for plant terrestrialization (Selosse & Le Tacon, 1998). Recent findings show (Fig. 2) that more than 80% of angiosperm plant species and over 300 gymnosperm species associate with some kind of mycorrhizal fungi (Brundrett, 2009). Mycorrhiza was common feature to all higher plants, but some of them during the evolution lost the ability to associate with the mycorrhizal fungi (Wang & Qiu, 2006). The fact, that in the whole plant kingdom are only few plant families which do not create mycorrhizae shows, how much is the mycorrhiza linked together with plants. The most common or important families of non-mycorrhizal representatives are Brassicaceae, Caryophyllaceae, Cyperaceae, Proteaceae, etc. (Tester *et al.*, 1987).

The basic principle of mycorrhizal symbiosis is nutrient exchange between photosyntheticaly active plant and its symbiotic fungi. Mycorrhizal fungi are able to reach with their extramatrical mycelium (EMM) into distant areas of the soil and obtain the inaccessible mineral nutrients or water and provide it for its host plants (Marschner & Dell, 1994). The main nutrients that mycorrhizal fungi transport into the plant roots are P and N. The P in the soil is usually hardly accessible for the plants, because it is bound into insoluble or very hardly soluble substances. Mycorrhizal fungi have the ability to harvest and provide it to the plants. The nutrient support is even possible in cases when the P concentration is below the reachable level for the non-colonized plant (Smith & Read, 2008). In return the plant rewards its symbiotic fungi with carbohydrates originating from the photosynthesis process. The mycorrhizal fungal EMM production directly correlates with the host plant C allocation into its roots and gradually transferred into mycorrhizal structures as root-tips, mycelia and rhizomorphs. Carbon donation from the plant is dependent on various factors as mineral nutrients availability (Bahr *et al.*, 2013), precipitation, biotic factors, etc.

Mycorrhizas are traditionally divided into three basic anatomo-morphological groups - (Fig. 1) endomycorrhizae, ectomycorrhizae (EcM) and ect-endomycorrhizae (EeM).

Ericoid mycorrhiza (ErM), Orchid mycorrhiza (OrM) and the most frequent Arbuscular mycorrhiza (AM) belong in the endomycorrhizal group. Typical feature of endomycorrhizas is the fungal hyphae penetration through the primary cortex or rhizodermal cell walls (Genre *et al.*, 2005) and creation of typical intracellular structures as arbuscules in case of AM, hyphal coils in case of ErM and pelotons in case of OrM. EeM structures, both intercellular and intracellular can be found in Arbutoid and Monotropoid mycorrhizal plants. EcM fungi do not penetrate the plant cell walls and create an intercellular structures and hyphal sheet on the outer surface of the plant roots (Peterson *et al.*, 2004). The involved symbiotic fungi are in most cases from the Basidiomycota, Acsomycota and Glomeromycota phyla (Smith & Read, 2008).

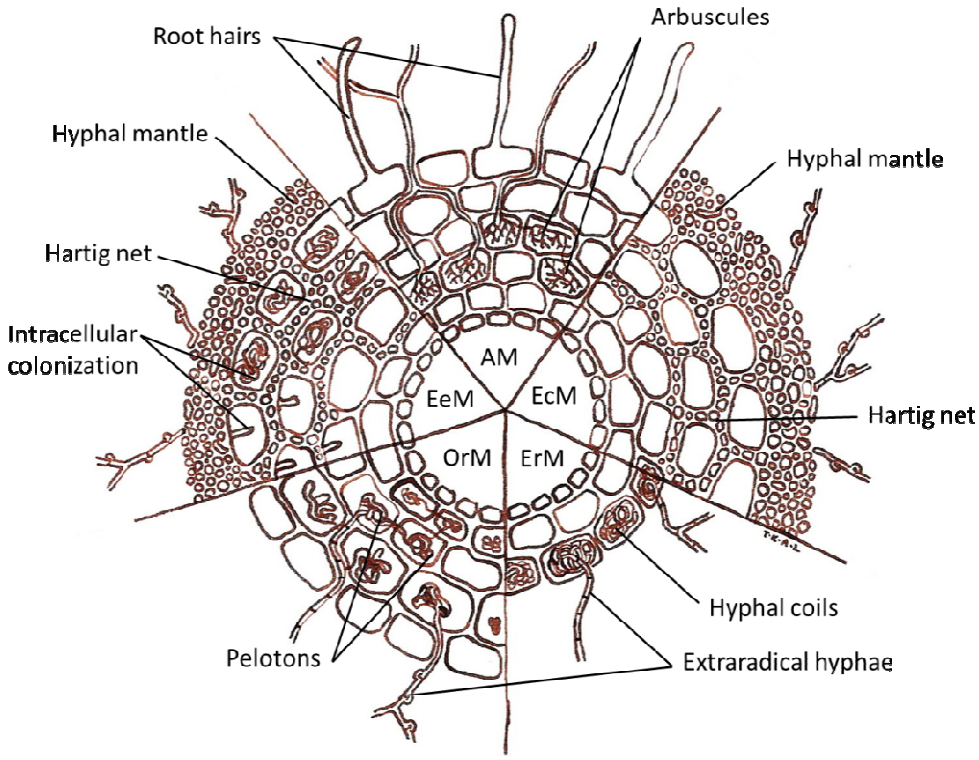


Figure 1 (Types of mycorrhiza, endomycorrhizas: AM, ErM, OrM, ectomycorrhizas: EcM, ectendomycorrhiza: EeM, author: T. Lukešová)

2.2 Plant - fungus nutrient exchange

The way how the EcMf obtain nutritious substances varies between the saprotrophic feeding on organic matter and obtaining the photosynthetized C from the mycorrhizal host plants (Zeller *et al.,* 2007). High diversity of fungal species with different degrees of trophic level can be found especially in temperate forest ecosystems (Tedersoo *et al.,* 2010a). The EcMf are able to decompose some organic matter from the soil, but the amount of saprotrophicaly gained carbon is almost negligible (Talbot *et al.,* 2008). Due to an inability to process sacharosis are EcMf almost fully dependent on their host plants which provide them glucose and fructose exchanging it for glucose-6-P and other nutrients (Nehls *et al.*, 2010). In the study (Wallander *et al.,* 2001) the stable isotope probing (SIP) experiment was performed and the results showed, that carbon incorporated in the EcMf cell bodies originated mainly from the host trees. The EcMf ^{13}C isotopic trace was compared to the saprotrophic fungal trace, host plants trace and was significantly closer to the green plants autotrophic nutrition level.

The physiology of plants and their mycorrhizal fungi is linked together. As an example of such connection can be taken the results of two following studies. Carbon donated by a plant into its fungal symbionts can reach up to 20% of the total amount of net primary production (Hobbie, 2006) and in laboratory experiment was shown, that mycorrhiza increases the net assimilation rate of the host plant (Loewe *et al.*, 2000). When performing tree girdling experiments, which is a rather cruel method that practically kills the tree by cutting the phloem and xylem around the tree trunk and stops the C flow into the underground. Those experiments showed evidence, that EcMf are a substantial part of the woody plants soil C sinks. Without the host plant carbon donation are the EcMf not able to continue growing and fruiting. After one month, there was a 41% decrease of microbial carbon content in the soil and 45% decrease of dissolved organic carbon abundance in the soil on the plots where the girdling was executed (Högberg & Högberg, 2002). The fluxes of mineral nutrients and carbohydrates between the plant and fungal partners have a great influence on pedosphere dynamics as soil respiration, fungal growth (Högberg *et al.,* 2001) and plant communities in the aboveground as well (van der Heijden & Horton, 2009).

2.3 Types of mycorrhiza

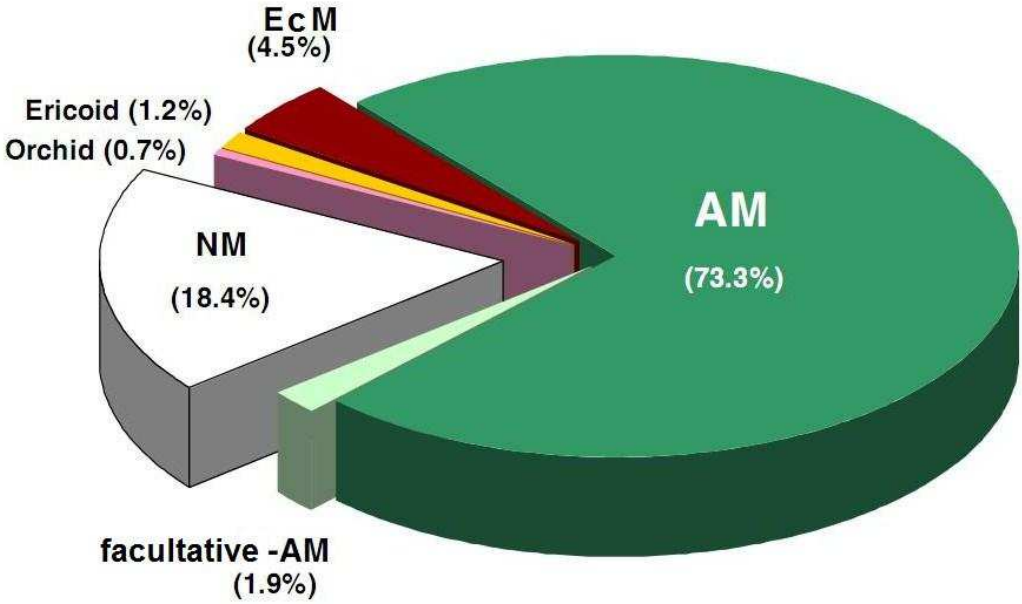


Figure 2 - Abundance of various types of mycorrhizas among plant species, picture taken from the study (Brundrett, 2009) - literature review from 128 publications includes ca 8000 plant species, AM – arbuscular mycorrhiza, EcM – ectomycorrhiza, NM – nonmycorrhizal, Ericoid – ErM, Orchid – OrM.

2.3.1 Arbuscular mycorrhiza

The most common and abundant mycorrhiza is AM which belongs into the endomycorrhizas and inter alia associates with approximately 74% of all Angiosperm plant species, number of Gymnosperm species and some Cryptogamic species (Brundrett, 2009). AM is formed only by one obligatory mycorrhizal fungal phylum called Glomeromycota. AMf are generalists and they form mycorrhizal symbiosis with various plants species. Glomeromycota are vitally dependent on their autotrophic hosts since the earliest time of plant evolution. Some authors enforce a theory, that the AM was the symbiosis, which allowed the plants to leave the water environment and gradually colonize the dry land (Selosse & Le Tacon, 1998).

The colonization of plant root cells by AM fungi starts with an attraction by strigolactone molecules released by the plant roots (Besserer *et al.*, 2006) and afterwards continues with a cascade of signals which are induced by the fungal hyphae and gradually leads to penetration of the cortical plant cell walls (Oldroyd *et al.*, 2005). The plasmatic membrane of penetrated root cells remains untouched and covers the whole surface of fungal

hyphae which gradually forms a typical tree like structure called arbuscule. The branched structure of arbuscule builds a highly multiplied surface between the fungal and plant phospolipid membranes, which gives formation of space called interfacial matrix where the nutrients exchanging processes are held (Peterson & Massicotte, 2004).

2.3.2 Orchid mycorrhiza

OrM is a unique symbiosis between fungi from Basidiomycota and Ascomycota (Zelmer *et al.*, 1996) and plants originating from the family Orchideaceae. OrMf same as AMf colonize the intercellular space of the plant root cells. Unlike AMf form the OrMf in the cortical cells a specialized coiled structures called pelotons which represent and build the interfacial matrix (Peterson & Massicotte, 2004). The relation between orchids and OrMf is an example of highly evolved symbiosis where both members of the association are highly dependent on each other. Most of the orchids are fully dependent on their symbiotic fungi in the seed germination stadium. The dust seeds of plants from Orchideaceae family are utterly lacking of an endosperm and are fully reliant on the nutrients provided by its symbiotic fungus in the earliest stages of germination (Arditti & Ghani, 2000). This stage of orchid life cycle is called protocorm and the plant is practicaly saprotrophic until it is able to create its own chloroplasts and photosynthetise the vital cabrohydrates. A number of Orchid species remain in mixotrophic or mycoheterotrophic nutrition state (Roy *et al.*, 2009), when partially acquiring carbon from its OrMf and partially photosynthetizing its own carbohydrates (Selosse & Roy, 2009). It happens commonly, that the pelotons are eventually digested by the host plant. The orchid nutrition acquirement is highly dependent on its OrMf. Some orchid species root systems are highly reduced and its function is to provide a refuge for OrM fungi and the fungal hyphae emanating from the root system explores the surrounding soil and provides collected nutrients and practically replaces the function of the plant root system (Smith & Read, 2008).

2.3.3 Ericoid mycorrhiza

ErM is a symbiotic relation between Ericaceous plants and Ascomycota or Basidiomycota. ErM can be found from the arctic and boreal to the Mediterranean, subtropical and tropical ecosystems. ErM plants can thrive with the same success in wet and acidic soils of marshes and wetlands and as well in the dry and nutrient poor Mediterranean

soils which contain high amount of hardly accessible minerals. The main effect of ErM is that the ErMf are able to provide the mineral nutrients to their plant hosts in even very unfavourable soil conditions such as low pH, drought, heavy metal contamination etc. (Cairney & Meharg, 2003). The ErM fungi produce a spectrum of hydrolytic and oxidative enzymes, which helps to mobilize and acquire the minerals from the soil complexes (Burke & Cairney, 2002). This process helps to close the carbon cycle very efficiently by digesting the dead plant material by the ErMf and transferring the nutrients back to the host plant.

2.3.4 Mycoheterotrophy

Photosynthesis is a crucial process of fixing atmospheric CO2 and incorporating the derived carbon into the plant tissues and gradually into the soil organic matter. The organic matter from autotrophic plants becomes a source of carbon for decomposers and then the rest of food chain members. Except autotrophs and heterotrophs exists another group of organisms. For example, plants that evolved from Orchideaceae and Ericaceae family are organisms with marginal portion or without any chloroplasts and their carbon requirements are fully supported by the mycorrhizal fungus and are called mycoheterotrophs and plants with partial dependence on the mycorrhiza are called mixotrophs (Leake, 1994; Taylor & Bruns, 1999; Selosse & Roy, 2009). Mycoheterotrophs are on the way between autotrophy and heterotrophy. The name resembles ability of these non-photosynthetic plants to gain carbon not only from mycorrhizal fungi, which acquire carbon from other host plants, but also from catabolic processes, which are performed by the saprotrophic fungi. It natural conditions can easily happen, that symbiotic fungi of the mycoheterotrophic plant associates with other plant species that are photosyntheticaly active. If those fungi provide the derived carbon to the mycoheterotroph it turns the plant into a epiparasite. Epiparasite is thus a non-green plant, which thrives on carbohydrates photosynthetized by other green plants (Bidartondo, 2005).

An odd case of plant nutrition are mycoheterotroph plants associating with saprotrophic fungi. The principle is similar to the mycoheterotrophic and epiparasitic plants, because the fungi are donor of carbon, but the source of carbon are only dead plant tissues and other organic matter. Orchids as for example *Gastrodia confusa* presented in the study (Ogura-Tsujita *et al.*, 2009), are terrestrial non-photosynthetic plants, which form a symbiosis with saprotrophic fungi form genus *Mycena* which is usually characterized as a

non-mycorrhizal fungal species. Thus the *Gastrodia* derives the carbon, which is acquired from fungal decaying processes of wood or organic materials.

2.3.5 Ectomycorrhiza

The typical anatomical characteristic of EcM is the fungal intercellular structure called Hartig net (Fig. 3) which creates a large surface area for the efficient contact between fungal cells and the host cells, allowing to effectively transfer the exchanged substances. Another typical anatomical feature of EcM is hyphal sheet or mantle, which covers the outer surface of the host plant roots (Peterson *et al.*, 2004) and creates the typical EcMf structure: the root-tip. According to (Agerer, 1997) there are two main types of hyphal development within EcM mantles: pseudoparenchymatous which is formed compactly with highly differentiated hyphal elements, and plectenchymatous which has loosely tangled hyphae and their stringy form is still visible. Mycelial hyphae emanating from the mantle are able to create more complex structures called rhizomorphs, which explore the soil and are able to reach to relatively far distances. Various EcMf lineages (Tedersoo *et al.*, 2010a) create their own typical hyphal features and are divided into exploration types. The mycelial hyphae and rhizomorphs greatly increase the surface and radius of the whole root and mycorrhizal system and enables to reach further and acquire more nutrients from the soil (Agerer, 2001). The nutrients provided from the EcM fungi for its host plants are as mentioned P and mainly N, water and other nutritious substances.

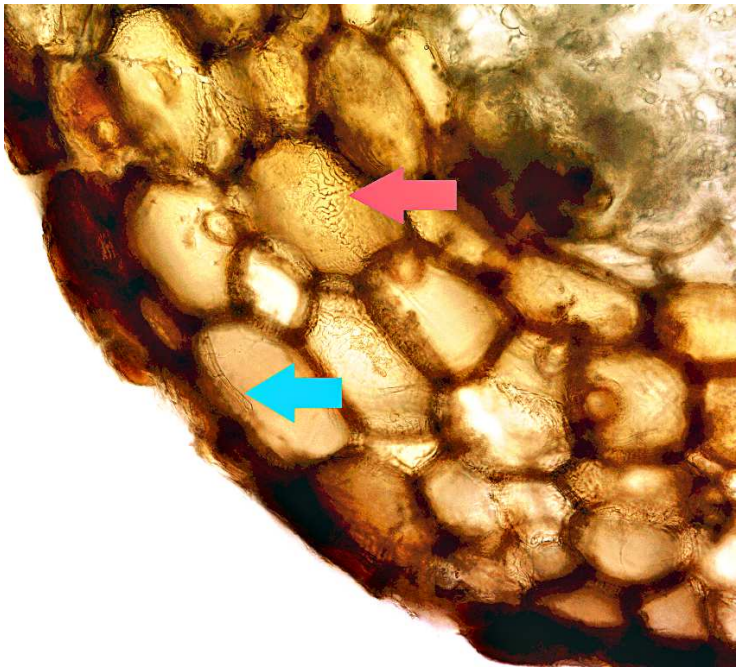


Figure 3 (Cross-section of EcM *Pinus* sp. root, blue arrow - Fungal hyphae, red arrow - Hartig net, author: T. Antl)

Due to the hyphal sheet characteristics, such as hydrophobic surface and the ability to control the flow of exchanged substances to the root (Ashford *et al.*, 1988), is the fungus practically the main or even only way, trough the substance exchange is held. Considering the possibility, that the plant roots colonization can reach up to 100%, it means that the host plant is almost isolated from the pedosphere. This means, that any nutrients and water entering the root must first pass through the fungal structures and same in case of leaving the root and EcMf therefore occupy and probably control the interface between the soil environment and the host plant (Taylor & Alexander, 2005). This theory needs to be tested in future research, due to its only indirect evidence. EcM fungi may have therefore an irreplaceable role in boreal and temperate forests ecology and soil dynamics (Anderson & Cairney, 2007). They colonize a major part of the global pedosphere (Wallander *et al.*, 2001) and form mycorrhizal symbiosis with most of the woody plants. It is known that approximately 6000 plant species which are mainly trees or shrubs (Brundrett, 2009) form ectomycorrhiza with more than 20 000 species of the Basidiomycota, Ascomycota and Zygomycota (Tedersoo *et al.*, 2010b) in the geographical range from the arctic to tropical ecosystems.

The plant preference of its symbiotic EcMf (and vice versa) varies from species to species and the general distinction is: if the species is a generalist or specialist. An example of generalist EcM plant is *Pseudotsuga menziesii* which naturally associates with approximately 2000 EcM fungal species (Molina *et al.*, 1992), that is probably the widest range of mutualists preference among the EcM plant species. EcM plant species can either form only one type of mycorrhiza, or like some plants, besides having a EcM fungal symbionts are able to simultaneously associate with more types of mycorrhizal fungi or other mutualistic organisms. *Pseudotsuga menziesii* has a broad range of symbiotic and mutualistic fungi, except EcM it associates with, AMf (Salgado Salomón *et al.*, 2013) and various others endophytes (Hoff *et al.*, 2004) such as the special ecological group - the Dark Septate Endophytes (DSE fungi). Another example of multisymbiotic partnership is the genus Alnus, which representatives form a mutualistic symbiosis with EcMf, AMf and Actinobacteria (Benson & Clawson, 2000; Tedersoo *et al.*, 2009). *Alnus* is with its narrow range of EcM mutualists, compared to *Pseudotsuga*, located on the other side of EcM fungal preference spectra and is an example of a specialist. Recent findings show that the 22 species of genus *Alnus* associate with circa 150 EcM fungal species and it is estimated that the number can reach up to 200 EcM species (Põlme *et al.*, 2013).

2.4 Common mycelial networks

The simplest CMN structure consists of one or more mycorrhizal fungi that connect at least two plant root systems. Another possibility is when the fungal hyphae fuse together and connect the root systems of their host plants. The two main types of CMNs are AM networks, typical for grassland ecosystems and agroecosystems (Helgason *et al.*, 1998), which can originate from the mycorrhizal hyphal fuses and EcMf - woody plants networks which are typical for forest ecosystems (Selosse *et al.*, 2006). An example, which supports the fascinating theory of wood-wide-web (WWW), is the study (Beiler *et al.*, 2010) where was shown, that a single tree root system might be linked with 37 other trees by the EcM mycelial connection (Fig. 5). From this point of view might be the whole forest a single living system of multiple plant species nodes interconnected by fungal links and sharing the mineral nutrients, carbohydrates, water and maybe even information (Song *et al.*, 2010; Simard *et al.*, 2012). Although the demonstrated studies show, that the wood-wide-web phenomenon has a theoretic chance to exist, it should be approached critically and need a

future rigorous research. For example the sustainability of the untouched mycelial web, which is able to connect plants and distribute various substances, is questionable and needs to be proven by more future studies. The mycelia of all kinds of fungi is in the soil environment severely attacked by spectrum of pathogens, grazers (Crowther *et al.*, 2012) and suffers multiple other disturbances. It has been discovered that adult plants create CMN by providing mycorrhizal inoculum to their seedlings. A field and glass-house experiments with early succesional EcM *Salix reinii,* which grows in volcanic rock substrates on slopes of mount Fuji, showed that seedlings grown in close distance and with access to adult plants mycorhizosphere had a significantly higher survival rate than the non-mycorrhizal controls. And yet T-RFLP analysis proved, that the shared EcM fungi between the seedlings and adults were the same individual mycobionts (Nara, 2006). From all these mentioned results can be said, that plant communities interconnected by CMNs might exhibit a higher seedlings survival rate and thus incensement of the whole ecosystem stability.

2.4.1 AM mycelial networks

The Glomeromycota have a coenocytic mycelium, which allows free mobility of numerous nuclei in the cytoplasmatic content, within one fungal individual. In the same time, they are able to create a conjoined cell growth called anastomosis. The anastomosis development is possible even between two different fungal individuals (Giovannetti *et al.*, 2001). This system of anastomosis between number of individual fungi can indirectly link together number of plant root systems and create a complex net called wood-wide-web (Helgason *et al.*, 1998) or common mycelial network (CMN), which is a terminus used for EcM networks as well. The plants are able to share water, mineral nutrients and possiliby even carbohydrates via the CMNs. As an example can be taken a SIP experiment which proved a two-way transfer of P and N from *Pisum sativum* L. to *Hordeum vulgare* L via the CMN (Johansen & Jensen, 1996). This system of various plants linked by mycorrhizal mycelial and root systems takes up other various neutral, pathogenic and or mutualist symbiotic organisms and increases the local and even global ecosystem fitness (Bonfante & Anca, 2009).

2.4.2 EcM mycelial networks and exploration types

The CMNs can develop within the EcM plant and fungal communities as well. The process of interconnecting the plant root systems is not exactely the same as in the AM networks. The principle is that one EcMf can associate with two plant hosts. The amount of EcM fungal biomass in the boreal forest soil is estimated to around 900 kg ha−1 (Wallander *et al.*, 2001). Considering this quantity of fungal mycelia in the soil, it is easily possible that the whole forest ecosystem might be interconnected by a fungal web called common mycorrhizal network (CMN) (Peter, 2006; Lekberg *et al.*, 2010).

An indirect evidence of WWW might be co called Fairy rings (Fig. 4). When the EcM mycobiont remains untouched during several seasons, than the fruiting sporocarps grow in the same time in a circle pattern (Peter, 2006). EcM plants support this way its seedlings by providing them already established mycorrhiza in the soil and facilitate the development of mycorrhiza in the seedling root systems. This phenomenon is called Nurse effect and increases the number of successfully developed young seedlings with a higher chance to survive (Nara, 2006). The supportive transport of nutrients from the fully grown trees to their seedlings can cause an competition advantage what can eventually lead to domination in the local ecosystem (McGuire, 2007).

The hyphae emanating from the EcMf root-tip mantel have a unique morphology and ecophysiological importance. According to (Agerer, 2001) there are several exploration types of the emanating hyphae. Some types are very smooth and their contact surface is distinctively small and maybe even isolated from the soil environment due to its hydrophobic surface. Other types are very rich in hyphae emanating either few millimetres or centimetres from the root-tip and some create a thicker threads called rhizomorphs, which can reach even decimeters and meters from the root-tip. The /suillus-rhizopogon lineage cerates so called tuberculate structures, that look like a bulk or tuber like structures. Every EcMf community on the host tree has its unique exploration types assemblage and thus have a different carbon demands, enzimatic activity, soil exploration ability and many other important ecological and physiological implications (Kjøller, 2006; Hobbie & Agerer, 2010; Peay *et al.*, 2011; Tedersoo *et al.*, 2012).

Figure 4 (photography of EcM *Cantharellus* sp. fiary ring, source: http://novicemushroomer.blogspot.cz/2013/07/chanterelle-fairy-ring.html)

There is some evidence, that plants are able to share nutrients or water via the CMNs with plants from different species. For example the nodulating and EcM Australian *Casuarina cunninghamiana* transferred its labeled N into the mycorrhizal structures and thus indirectly into the *Eucalyptus* roots connected to the hyphal network. The amount of N from *Casuarina* transferred via the CMN made whole 30% of total N in the tested *Eucalyptus* seedlings (He *et al.*, 2005). Not only mineral nutrients can be shared by the plants connected to the CMNs. There is some evidence, that in the controlled conditions, the plants are able to share photosytheticaly produced carbohydrates. Laboratory experiments using SIP method showed, that a significant amount of labeled C was transferred via EcM mycelial network between *Pseudotsuga menziesii* and *Betula papyrifera* seedlings. The transfer was bidirectional but in total the *Pseudotsuga menziesii* received higher amount of C specifically 2-3% of the net C gain (Philip *et al.*, 2010). The future research need to prove, weather there is a possibility of carbohydrates sharing among plants in the nature, which might have a great

ecological impact. Another laboratory experiment discovered a transfer of very small amount deuterium-labelled water via the mycelia hyphae from the EcM *Arctostaphylos viscida* to *Pseudotsuga menziesii* seedlings (Plamboeck *et al.*, 2007). The proportion of thus transferred water was very small (0.01–0.04% of the total volume of water in the tested seedlings), which implies that the ecological importance of that phenomenon might be probably ecologically negligible.

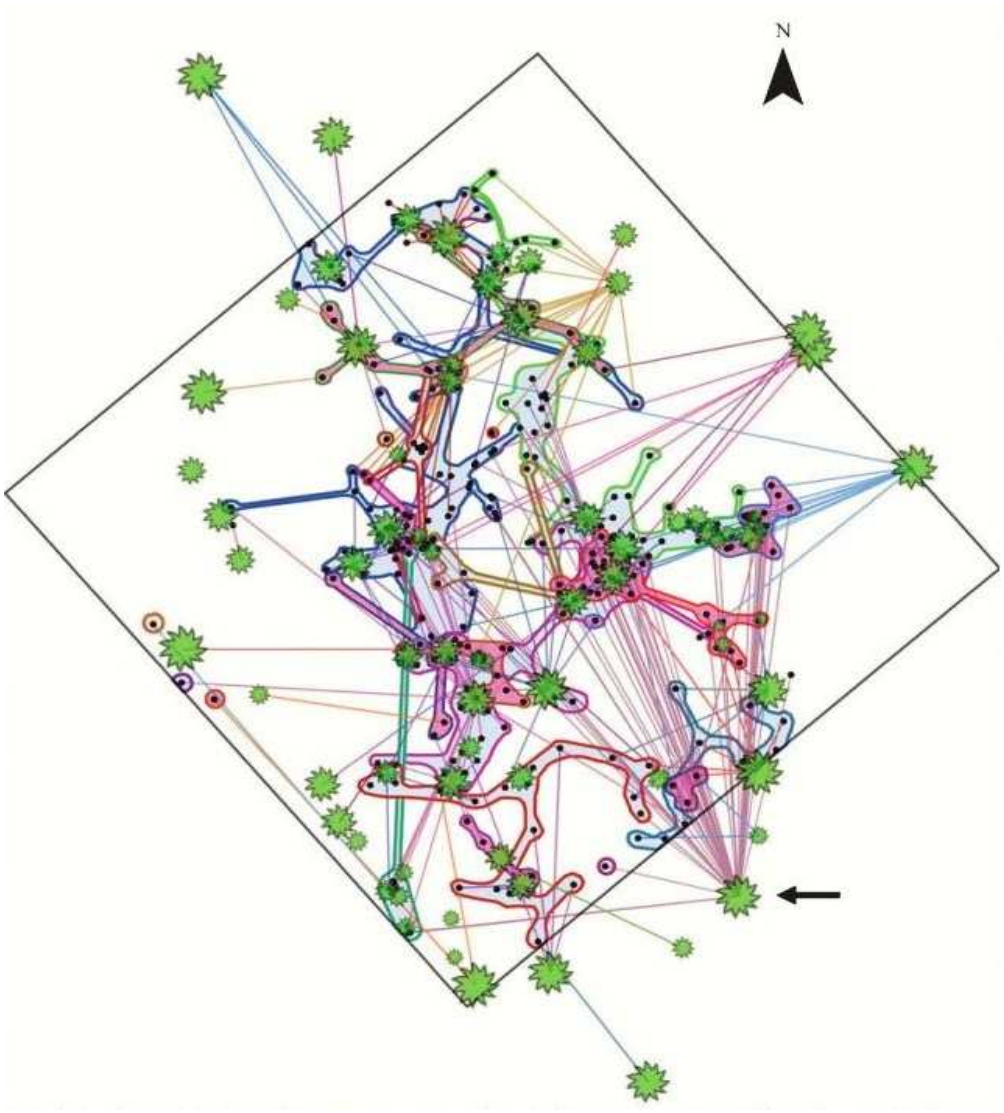


Figure. 5 from the study (Beiler *et al.*, 2010), the genets of EcMf from the genus Rhizopogon and Douglas firs (*Pseudotsuga menziesii* Mirb.) the study site view from above, the black dots represent the places of EcM roots extraction, *Rhizopogon vesiculosus* A.H. Sm. is colored blue and *Rhizopogon vinicolor* A.H. Sm. is pink, the lines represent the connections of trees by the EcM mycelia, the arrow shows the most linked tree called The Hub tree, this tree is possibly connected with 37 other trees by 8 genets of *Rhizopogon vesiculosus*.

2.5 Terminology of invasion issues

Before focusing on the invasion issues it is unnecessary to elucidate the terminology. The scientific literature dealing with the biological invasions is often not uniform in its terminology. In many cases there are used duplicate, analogic, multiple-meaning words, which can cause ambiguity and misunderstanding. First of all, it is needed to explain the terminus native, to explain all the other names.

Using the terminology according to (Pysek *et al.*, 2004), the **native** (syn. indigenous) plants are those plants that have originated in a given area without human involvement or that have arrived there without intentional or unintentional intervention of humans from an area in which they are native.

Alien plants (syn. exotic, introduced, non-native, non-indigenous) are those plants species in a given area whose presence there is due to intentional or unintentional human involvement, or which have arrived there without the help of people from an area in which they are alien.

Naturalized plants (syn. established) are plants that sustain self-replacing populations for at least 10 years without direct intervention by people (or in spite of human intervention) by recruitment from seed or ramets (tillers, tubers, bulbs, fragments, etc.) capable of independent growth.

Invasive plants are those plants, that are a subset of naturalized plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plants, and thus have the potential to spread over a large area.

2.6 Mycorrhizal invasive species

Ecosystems can be in various ways disrupted by alien plants or fungi, which can cause a serious damage of the ecosystems and even extinction of some species (Gilbert & Levine, 2013). The most cases of alien fungal species are Basidiomycota and especialy EcMf associating with introduced plants used for timber production, which usually do not expand into the local native ecosystem. The alien and invasive species of EcMf have a potential to alter the local fungal community or allow to facilitate an introduction or invasion of EcM plant species (Vellinga *et al.*, 2009) and indirectly alter the native plant community as well. Despite the fully developed invasions of EcMf are rare, they can cause a significant changes in native fungal species community and alter diversity and overall fungal biomass production as in the case of invasive *Amanita phaloides* in the North America (Wolfe *et al.*, 2010).

The invasions of mycorrhizal plant species have an extra factor, which is primary the mycorrhizal status and secondary its mycorrhizal growth response (Pringle *et al.*, 2009). There are several observed scenarios what can happen when an alien mycorrhizal plant species is transported into a novel ecosystem. One possibility is, that the alien species are not able to associate with the local spectrum of mycorrhizal fungi and the native ecosystem endures the invasion (Nuñez et al., 2009, Tedersoo et al., 2007).

If the alien plant species go through all the four stages of invasion (transport, establishment, spread and impact) as described in (Lockwood *et al.*, 2007) it becomes invasive and starts to alter the local habitats. Some invasive plant species inhibit the local mycorrhizal fungal growth and this way indirectly suppress the local plant species. This gives the invaders a competitive advantage and allows them to thrive. Such process occurs in case of non-mycorrhizal *Alliaria petiolata* which exudes a allelopathic substance benzyl isothio-cyanate (BITS), which is toxic for the EcM fungi associated with local EcM plants and AM fungi (Roberts & Anderson, 2001) as well. The anti-fungal effect of BITS was tested and observed in field, glasshouse and laboratory experiment. The native pine seedlings biomass, mycorrhizal colonization and EcM root-tips abundance decreased in the presence of *Alliaria petiolata* (Wolfe & Rodgers, 2008). Similarly, the alien *Berberis thunbergii*, alters the native local soil by exuding various enzymes and thereby decreases the local EcMf abundance (Kourtev *et al.*, 2003). Another way for the invader to overwhelm the new

environment is to disrupt the carbon fluxes of the local mycorrhizal plants as it happens in case of *Centaurea maculosa* invasion in north American AM grasslands (Carey *et al.*, 2004).

2.7 Invasions of Pinaceae

An example of very successful EcM invasive plants is the family Pinaceae, where 28 genuses with some invasive representatives can be found. Therefore is the Pinaceae family compared with other plant families considered as the most invasive and especially the genus *Pinus*, which contains 21 invasive species (Richardson & Rejmánek, 2004). South from the equator, *Pinus* invasions are among other invasions one of the most important, widespread and have the biggest influence on the local native ecosytems. Many *Pinus* species with economical and ecological importance were there intentionally or accidentally imported and established. Gradually some species spread into the local habitats and became invasive (Richardson *et al.*, 1994). Some of the invasive Pines have a significant impact on local EcM fungal and plant communities. For example *Pinus contorta* invading the New Zealand forests and adjoining grasslands inhabited by the native *Nothofagus solandri var. cliffortioides*. The invasion of *Pinus contorta* is with a high probability enhanced by the presence of its coinvaded symbiotic EcM fungi. Despite having significantly lower count of EcM mutualists (Fig. 6) it is able to dominate the novel habitat and suppress the growth of the native *Nothofagus solandri var. cliffortioides* and its EcMf (Dickie *et al.*, 2010). *Pinus contorta* in this case indirectly disturbes and alters the local EcMf community.

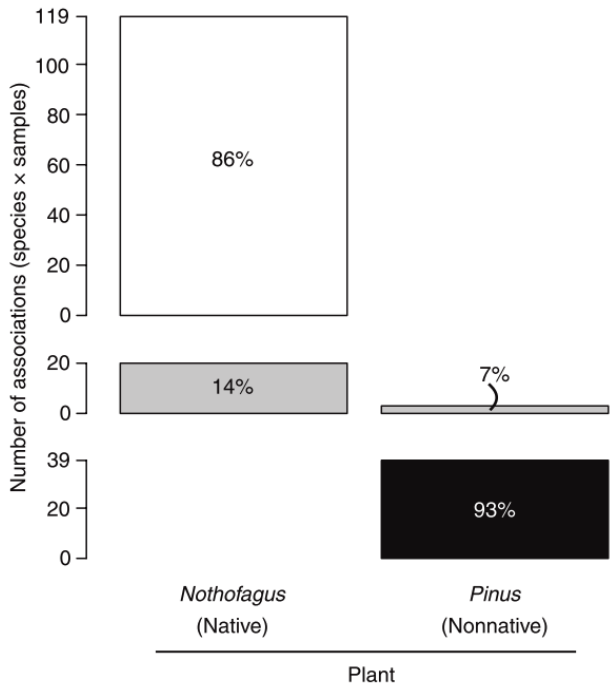


Figure 6: Summary of the number of total occurrences (species within the soil core) of native (white), cosmopolitan (gray), and non- native (black) fungi on native *Nothofagus solandri var. cliffortioides* and nonnative *Pinus contorta* based on the 89% of ectomycorrhizal associations that could be attributed on the basis of fungal origin. Percentages give a proportion of associations within each plant species. Graph and comment taken from (Dickie *et al.*, 2010)

2.6.1 Pinaceae in Europe

On the other hand, on the Northern hemisphere and specifically in Europe are the cases of Pinaceae invasions much fewer. One of the reasons is that there is lesser scientific attention on this topic. In the year 2009 the European database DAISIE (www.europe-aliens.org) recorded 30 alien species from the Pinaceae family, while more than half originates from North America and a third originates from Asia. Studies about invasion subjects exclusively to *Pinus strobus* in the Czech republic. In general, in the scientific literature dedicated to alien plants, there are about 190 studies about Pinaceae (Carrillo-Gavilán & Vilà, 2010), which might show its significant invasive potential or an artefact caused by the scientific teams focused attention on the Pinaceae. In every way, the cases of conifer introductions and invasions on the North hemisphere are happening in a lesser extent than on the South hemishpere. The explanation might be socio-economical or ecological. The first explanation is related to different historical date of the introductions. Most of the species from Pinaceae family were introduced to the Europe at the 18th century. The oldest record of alien species presence in Europe is around the year 1800, which refers to the previously mentioned *Pinus strobus* in the Czech republic.

2.6.2 Pinus strobus in the Czech Republic

Pinus strobus is native to North America and in the Czech republic is classified as an invasive species. It was introduced to Europe in the end of the 18th century to enrich the species diversity in the monoculture timber production plantations and enhance this way the plantation immunity against the pests. It was also appreciated because of its rapid growth and creation of distinctly straight stems used in carpentry. The first record of *P. strobus* occurrence in the Czech republic territory was 1784 and the first timber producing plantations were established in 1789 in the Elbe sandstones area (Nožička, 1965), where the National Park Bohemian Switzerland is located in recent time. *P. strobus* is no more component of the timber plantations nowadays and spreads without control into the native habitats and gradually displaces the native *P. sylvestris*. It has ability to spread by intensive seed dispersal into great distances (Münzbergová *et al.*, 2010) and to grow on steep sandmountain cliffs and mountains where is unreachable for any kind of intervention by the National park management. *P. strobus* is thus able to spread quickly and suppress not only

native tree seedlings but also the growth of Cryptogams and smaller plants in the undergrowth (Härtel & Gardens, 2007).

3. Aims of the study

Aim of this study is to reveal the influence of invasive *P. strobus* on the native EcM fungal community and carbon flows into the underground and aboveground structures of EcM symbionts.

We tested:

(1) Possible unequality of carbon fluxes into its underground EcM mycelia and aboveground EcM sporocarps from *P. strobus* in comparison to the native P. sylvestris.

(2) Different P. strobus preference of its symbiotic fungal species in comparison to the native *P. sylvestris*.

(3) Possible inequality of energy which *P. strobus* allocates into its roots and litterfall in comparison to the native *P. sylvestris*.

4. Materials and Methods

4.1 Study sites

The studied sites are located in north-west of Czech republic in the Bohemian Switzerland National Park. This area is known for its rock cities and steep sandstone mountains on both shores of the Elbe River. The average annual temperature is around 8°C and average annual precipitation is around 850 mm. Among other Czech sandstone regions is this the place with the widest altitudinal range from 110 - 726 m a.s.l. (Härtel *et al.*, 2007). The dominant and native ecosystems are a dry *P. sylvestris* forests at higher altitudes with Ericaceous undergrowth, wet *P. sylvestris* forests at lower altitudes with Cryptogamic undergrowth and its combinations. In all of these types of forests is *P. strobus* able to outcompete the native *P. sylvestris.*

In the year 2011 we selected three sites with a monodominant overgrowth of *P. sylvestris* and three sites with a monodominant *P. strobus* overgrowth. One of our *P. strobus* sites was in the year 2011 unexpectedly cut down, which resulted in loss of data front that site. In the year 2012 we have chosen three new *P. strobus* sites to avoid any similar problems. The *P. sylvestris* sites (see supplementary) named Babylon, Hrby, Icko are located in the protected areas of the National park and represent the higher altitudes ether in dry (B,H) or partially wet conditions (I). The *P. strobus* sites (see supplementary) chosen in the year 2011 are Rynartice, Falknstein and Tap (R,T1,F). The *P. strobus* sites chosen in the year 2012 are Kaja, Tom and Zvire, all represent the dry type of habitat and are located inside (K,T2) and outside (Z) the National park territory.

4.2 Root tips sampling

The root tips were examined to determine the EcM fungal diversity within each site. The sampling of all sites was performed within one day on the 11th of June 2011. First the root tips were excavated by using a soil corer (12 cm in diameter) and put into separate plastic bags. Fifteen samples were taken randomly on each site (at least 3 meters apart) which makes in total 90 soilcore samples and stored at 5°C the same day. The next step was

cleaning the roots from the surrounding soil by tap water and examining them under a binocular microscope. The EcM root tips from each soil core were determined into number of moprhotypes by their morphological characters such as shape, colour, surface structure and type of emanating hyphae, following (Agerer, 2001). One to five root tips representing one morphotype were put into 0,5 ml eppendorf tubes and preserved by 90% ethanol to prevent any degradation of DNA before sequenation. In total we isolated 1052 root tips which gave us 590 reliable sequences.

4.3 Molecular analysis

The DNA was extracted from the root tips using the DNeasy Plant Mini extraction kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. DNA was eluted in 50 µl of sterile ddH_2O and kept at -20°C. Polymerase chain reaction (PCR) amplification of the ITS region was performed as in the study (Kohout *et al.*, 2011a) using the primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990). The PCR mix included 2.5 µl of 10× PCR buffer without MgCl2, 2 µl dNTPs mixture (200 nM), 2.5 µlMgCl2 (2 mM), 0.5 µlof each primer (10 mM), 1 µl of Taq DNA polymerase (Fermentas International Inc, Burlington, ON, Canada), 15.8 µl of sterile dd H_2O and 8 µl of the template (DNA extract diluted 1:10 in sterile water) in a final volume of 25 µl. Thermal cycling parameters were as follows: initial denaturation step of 4 min at 94°C, 35 cycles consisting of a denaturation step at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 70 s and a final extension at 72°C for 10 min. The length and quality/quantity of the PCR products were checked using gel electrophoresis (1% agarose). Samples that yielded double-banded PCR products were excluded from further analyses. In the case of barely visible PCR products, a semi-nested or nested PCR was performed using primers ITS1 and ITS4

Each sample was separately sequenced with the primer ITS1 or ITS1F in Macrogen Inc. (Seoul, South Korea). The DNA sequences were checked for possible machine errors and edited in Sequence Scanner 1.0 (Applied Biosystems, Forest City, CA, USA). Preliminary identification of EcMF was achieved by conducting a nucleotide Basic Local Alignment Search Tool (BLASTn) search of the GenBank and UNITE (Abarenkov *et al.*, 2010) public sequence databases.

4.4 Leaf litter and fine roots biomass measurement

We compared the difference of carbon allocation between the two Pine species by measuring the amount of needles and fine roots produced during one vegetative season (June - November).

Litter fall: We placed randomly 30 plastic boxes (at least 2 meters apart) with open top (13 × 23 cm) on each site and collected the needles which naturally fell into the boxes. The placement was done on the 11th of June 2011 and then the boxes were exposed to the natural litterfall during the vegetative season. The content of each box was in the 28th of October 2011 collected and replaced into paper bag, dried at 25°C for 24 hours and the dry biomass was measured with scientific scales.

Fine roots: To measure the season production of fine roots, we buried 10 soil cores within each plot in the 11th of June 2011. The soil cores were made from plastic tubes (5 cm in diameter, 25 cm length) with three cut out windows in it (2 x 10 cm) so the fine roots could grow into the soil core. When collecting the soil cores, the roots were trenched by cutting the roots along the windows. The collection was done in the 28th of October 2011. Content of every soil core was placed into separate plastic bag and stored in 5°C the same day. Next step was removing the surrounding soil from the roots using tap water and drying the clean roots in 50°C for 8 hours. The dry roots biomass was measured with scientific scales.

4.5 Mesh bags

Ingrowth triangle shape bags made of nylon mesh (6 × 6 × 6 cm, 30 µm) were used for the estimation of EcM fungal underground biomass. The mesh size allows ingrowth of fungal hyphae, but not the plant roots (Wallander *et al.,* 2001). The mesh bags were filled with 20 grams of burned quartz sand originating from study sites and sealed by plastic-glue gun. Before filling the bags, the sand was heated up to 600°C to burn out the entire content of organic matter. This step prevents the mesh-bags from the colonization of saprotrophic organisms.

In total 60 mesh bags (10 bags × 6 locations) were buried on the *P. sylvestris* sites (B,H,I) and *P. strobus* sites (K,T,Z). The bags were placed on each site between the organic

and mineral layer of the soil. Than randomly distributed (at least 5 meters apart) and incubated for four months during the vegetative season from the 23th of June till the 15th November 2012. After excavation were the bags stored in portable cooler to prevent any decomposition in the samples and stored at the same day in -20°C until subsequent processing.

4.6 Ergosterol analysis

Each mesh bag was cut open and half of its sand volume (10 g) was transferred separately into a plastic bag. The other half was stored in -20°C for later analysis. The half prepared for ergosterol analysis was divided into two subsets (5 and 5 g). The content of each mesh bag was mixed thoroughly by a sterile stirrer and then separately transferred into a glass test tubes. The ergosterol content of each subset was measured and the results were compared. The differences between the values of those two repetitions from each mesh bag were negligible, which proved the accuracy of the method. The means of both values were used as the original data for statistical analysis.

Ergosterol was measured according to the protocol (Nylund & Wallander, 1992), extracted with 5 ml 10% KOH in methanol and sonicated for 15 minutes. After this step the test tubes were placed into 70°C water bath for 90 minutes. After cooling down 1 ml H_2O and 2 ml cyclohexane were added and the samples were mixed in a vortex apparatus for 20 s. After 5 minute centrifugation at 900g was the separated cyclohexane phase extracted and replaced into a new test tube and the residual methanol was extracted with a another 1.5 ml cyclohexane. The cyclohexane was evaporated under N_2 and the samples were dissolved in methanol.

Before the HPLC quantification of ergosterol, the samples were filtered through a 0.5 µm Teflon syringe filter (Millex LCR- 4; Millipore). The chromatographic system is composed by a high-performance liquid chromatograph (Hitachi model L2130, Japan), UV detector (Hitachi model L2400, Japan) and a C18 reversed-phase column (Chromolith, Merck) preceded by a C18 reversed-phase guard column (Elite LaChrome; Hitachi). The extracts were eluted with methanol at a flow rate of 1 ml/min and absorbance measured at 282 nm.

4.7 Fungal sporocarps biomass measurement

The EcM and saprotrophic fungal aboveground production was compared by measuring the dry weight of sporocarps on *P. sylvestris* sites (B,H,I) and *P. strobus* sites (K,T,Z). Epigeous sporocarps of EcM and saprotrophic fungi were harvested nine times within each plot during the peak fruiting period (Jun 24th, Jul 25th, Aug 8th, 30th, Sep 28th, Oct 5th, 20th, Nov 4th, 19th, 2012). Sporocarps were determined into genuses and species by morphological features or by molecular determination of ITS region of rDNA. The DNA from the sporocarps was extracted using the SIGMA Extract-N-Amp TM Plant Kit (Sigma-Aldrich) following the manufacturer's instructions and the Polymerase chain reaction (PCR) amplification of the ITS region and sequenation was conducted the same way as described above. The dry biomass (50°C, 8 hours) of the sporocarps was measured. The weight of saprotrophic and EcM fungi was separated. The weight of dry sporocarps from each of the 9 collections was divided by the sum of the total weight of collected sporocarps. This gave us 54 numbers (6 sites × 9 collections) ranging between 0 and 1 which represents a percentage of one day collection of the total fungal weight.

4.8 Phylogenetic analysis

All high quality fungal ITS sequences recieved from Macrogen company were edited using Finch TV 1.4.0. (Geospiza Inc.) and were used for taxonomic identification and delimitation of operational taxonomic units (OTUs) based on 97% similarity. The first identification of all OTUs was achieved by conducting a BLASTn search in the GenBank and PlutoF (Abarenkov *et al.*, 2010) sequence databases. Representative The sequences were aligned with database sequences originating from fungal sporocarps (retrieved from NCBI and UNITE) by using MAFFT 6.6 (http://mafft.cbrc.jp/alignment/server/index.html). Phylogenetic trees were primarily obtained by neighbour joining analyses in MEGA 6 (Tamura *et al.*, 2013).

4.8 Statistical analysis

All statistical analyses and graphics were computed using R 3.0.2 software (R Foundation, Vienna, Austria).

4.8.1 Species richness and exploration types analysis: To calculate species accumulation (rarefaction) curves within each site we used the EstimateS computer program, version 9. (Colwell *et al*., 2012). The calculations were based on the number of isolates of each OTU obtained from the soil-cores.

The data from sequenation results were put into presence/absence table and processed in R program using vegan and vegdist package. Next step was the Hellinger transformation, Bray Curtis calculation following by Adonis analysis what represents a multivariate analysis of variance, which allows simultaneous testing of multiple factors and covariates based on permutation tests. As a visualization tool was used the Non Metric Dimensional Scaling (NMDS) in the R program.

4.8.2 Ergosterol: From the 60 HPLC analysis results 6 outlayer values were discarded (one within each site). The data had not changed significantly after removing the outlayers, especialy the median values. The data were tested for normal distribution (Shapiro-Wilk test) after the logarithmic transformation. To test the effect of site and species we conducted a GLM (generalised linear model) with a Gauss error distribution and its analysis of variance (ANOVA).

4.8.3 Leaf litter and fine roots: Data from the leaf litter and fine roots collection were tested for normal distribution (Shapiro-Wilk test) and to test the effect of site and species we conducted a GLM with a Gauss error distribution and its ANOVA. The only difference is that in the leaf litter dataset was the data transformation not performed.

4.8.4 Sporocarp biomass: This data were checked for a normal distribution and then we performed a arcus-sinus transformation. Finally we conducted a GLM (generalised linear model) with a Gauss error distribution and its analysis of variance (ANOVA) to test the effect of site and species.

5. Results

5.1 List of EcMf OTU and their exploration types

The following figure shows overview of all EcMf found in the root-tips.

Pinus strobus root-tips	/lineage	exploration type	*Pinus sylvestris* root-tips	/lineage	exploration type
Amanita fulva	/amanita	medium	*Amanita citrina*	/amanita	medium
Amanita spissa	/amanita	medium	*Amanita rubescens*	/amanita	medium
Amphinema sp.	/amphinema-tylospora	medium	*Amanita sp.*	/amanita	medium
Cenococcum geophilum	/cenococcum	short	*Amanita spissa*	/amanita	medium
Lactarius camphoratus	/russula-lactarius	contact	*Amphinema sp.*	/amphinema-tylospora	medium
Lactarius helvus	/russula-lactarius	contact	*Cenococcum geophilum*	/cenococcum	short
Lactarius necator	/russula-lactarius	contact	*Clavulina cristata*	/clavulina	short
Lactarius rufus	/russula-lactarius	contact	*Entoloma*	/entoloma	medium
Meliniomyces bicolor	/meliniomyces	short	*Lactarius helvus*	/russula-lactarius	contact
Piloderma sp.	/piloderma	short	*Lactarius rufus*	/russula-lactarius	contact
Piloderma sphaerosporum	/piloderma	short	*Meliniomyces bicolor*	/meliniomyces	short
Pseudotomentella	/pseudotomentella	short	*Piloderma sp.*	/piloderma	short
Rhizopogon	/suillus-rhizopogon	long	*Piloderma sphaerosporum*	/piloderma	short
Russula decolorans	/russula-lactarius	contact	*Pseudotomentella*	/pseudotomentella	short
Russula densifolia	/russula-lactarius	contact	*Pseudotomentella vepallidospora*	/pseudotomentella	short
Russula ochroleuca	/russula-lactarius	contact	*Russula densifolia*	/russula-lactarius	contact
Russula paludosa	/russula-lactarius	contact	*Russula paludosa*	/russula-lactarius	contact
Scleroderma citrinum	/pisolithus-scleroderma	long	*Russula silvicola*	/russula-lactarius	contact
Thelephora terrestris	/tomentella-thelephora	medium	*Sistotrema*	/cantharellus	medium
Tomentellopsis submollis	/tomentellopsis	medium	*Sistotrema muscicola*	/cantharellus	medium
Tylopilus felleus	/boletus	long	*Sphaerosporella sp*	/sphaerosporella	short
Tylospora asterophora	/amphinema-tylospora	short	*Theleophoraceae*	/tomentella-thelephora	medium
Tylospora fibrillosa	/amphinema-tylospora	short	*Tylopilus felleus*	/boletus	long
Xerocomus badius	/boletus	long	*Tylospora fibrillosa*	/amphinema-tylospora	short
Xerocomus pruinatus	/boletus	long	*Xerocomus badius*	/boletus	long

Fig. 7 (List of EcM OTU, lineages and their exploration types.)

5.1.1 EcM root-tips fungal diversity and species richness

In total we isolated 1052 root tips which gave us 590 reliable sequences. The results from diversity statistics show that the locations were undersampled and more density of samples would be needed for the full insight. We have found a significant difference in EcM species assemblage between the two pine species (df = 1, p = 0.016, significance level < 0.05) and between locations as well (df = 4, p = 0.002, significance level < 0.01). We also tested the difference of EcMf exploration types. *P. strobus* prefers the contact exploration type and *P. sylvestris* prefers the medium type (df = 1, p = 0.007, significance level < 0.01).

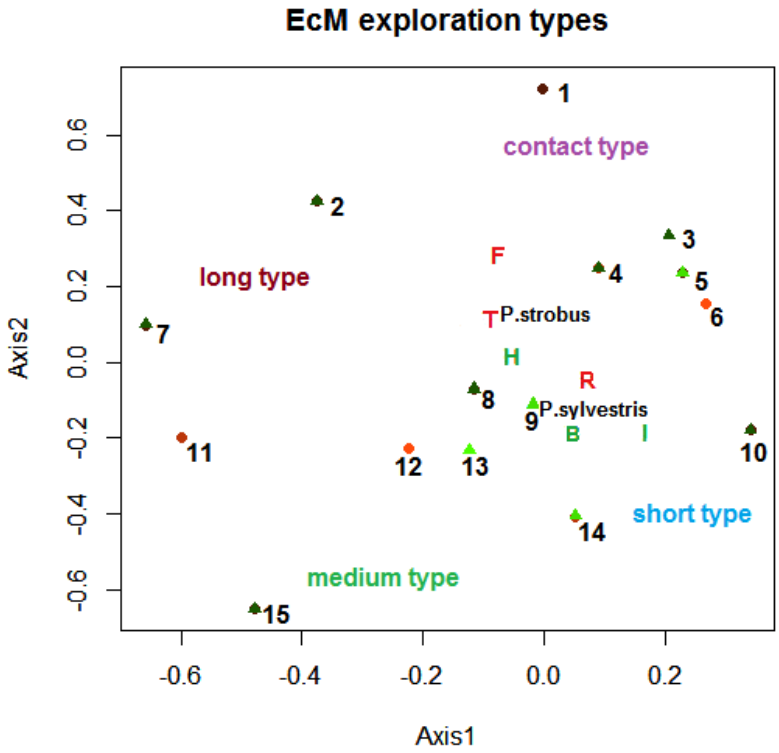


Fig. 8 (NMDS visualisation of the EcMf exploration types, *P. sylvestris* - triangle, *P. strobus* - circle. Each point represent more soil-core samples and present exploration types. The labels of exploration types are illustrative and have not s statistical meaning. Points legend: **point number**: (exploration type) - *species* (appearances on location).

1:(contact) - *ST* (T,F), **2**:(contact, long) - *ST* (R,T,F) / *SYL* (H), **3**:(contact, short) - *SY* (H), **4**:(contact, short, medium) - *ST* (R) / *SY* (H), **5**:(contact, short) - *ST* (R,T,F / *SY* (I), **6**:(contact, short) - ST (T), **7**:(long) - STR (T, F) / SY (B, H), **8**:(long, short) - SY (R, T, F) / SY (B, H, I), **9**: (long, short) - SY (B,I), **10**:(short) -ST (R, T, F) / SY (B, H, I), **11**:(medium, long) - ST (R), **12**:(short, medium, long) - ST (R. T), **13**:(short, medium, long) - SY (B), **14**:(short, medium) - ST (R) / SY (B, I), **15**:(medium) - ST (F) / SY (B, H, I)

5.1.2 EcMf species richness on the *P. sylvestris* and *P. strobus* root-tips

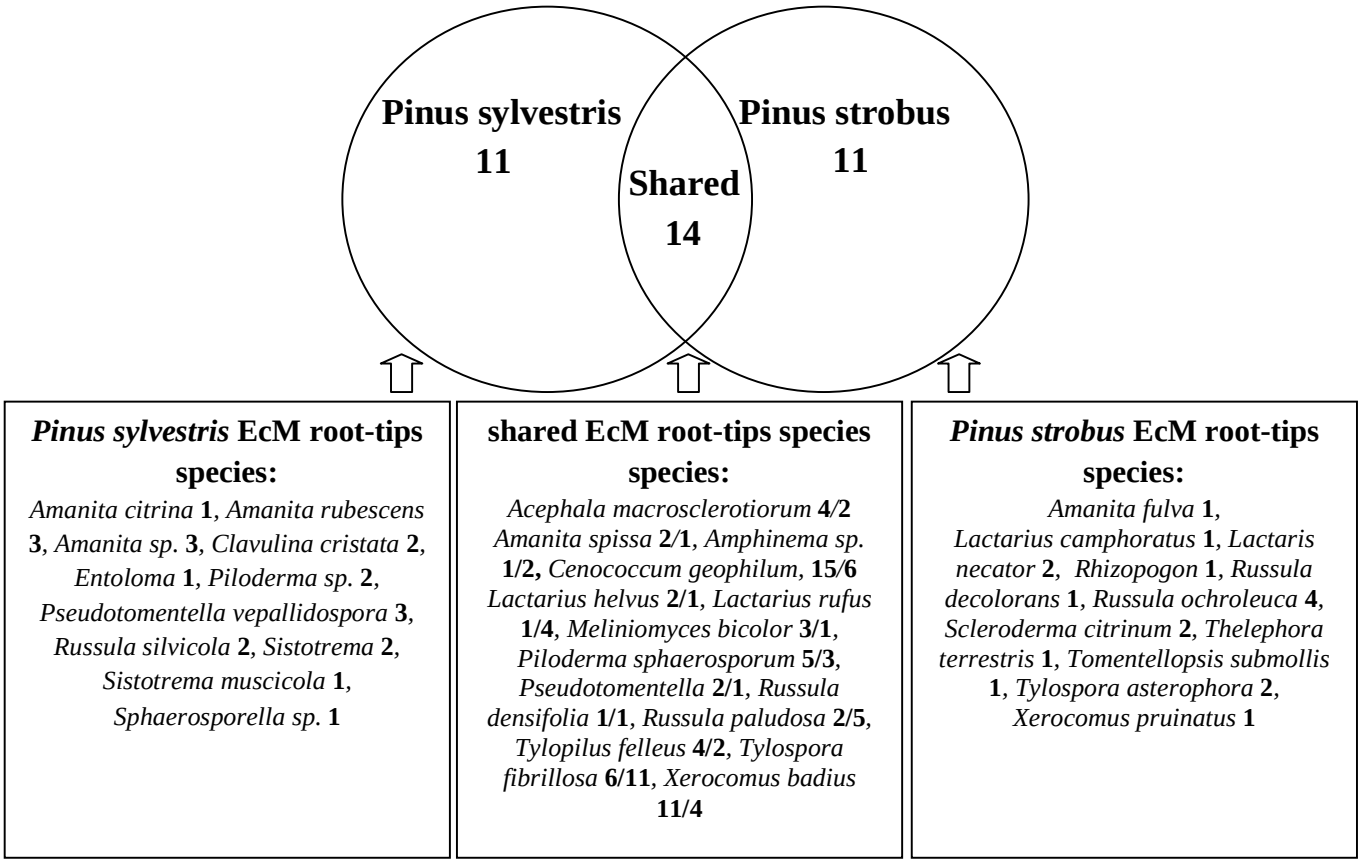
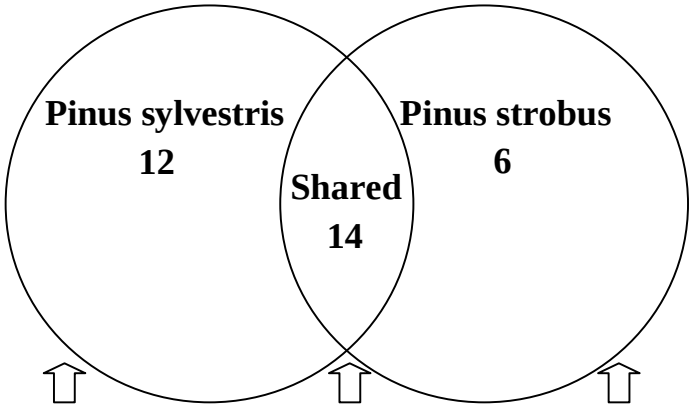


Fig. 9 (Numbers of EcMf species on root-tips and numbers of occurrence in the soil core samples, shared species - 1st number: occurrence in P. sylvestris soil cores / 2nd number: occurrence in P. strobus soil cores)

5.1.3 EcMf sporocarps species richness on the *P. sylvestris* and *P. strobus* sites



EcM sporocarps on the *Pinus sylvestris* sites:	**shared species of EcM sporocarps:**	**EcM sporocarps on the *Pinus strobus* sites:**
Amanita muscaria **4**, *Amanita rubescens* **3**, *Boletus edulis* **3**, *Boletus chrysenteron* **6**, *Cantharellus cibarius* **1**, *Leccinum scabrum* **3**, *Russula decolorans* **1**, *Russula paludosa* **4**, *Russula silvicola* **1**, *Russula sp.* **1**, *Russula xerampelina* **1**, *Suillus variegatus* **1**	*Amanita alba* **4/1**, *Amanita citrina* **5/2**, *Cantharellus tubaeformis* **2/5,** *Lactarius helvus* **4/5**, *Lactarius rufus* **2/1**, *Ramaria pallidosaponaria* **1/2**, *Russula amethystina* **2/5**, *Russula emetica* **3/2**, *Russula ochroleuca* **1/1**, *Russula vesca* **1/1**, *Scleroderma citrinum* **8/1**, Suillus bovinus **4/1**, *Tylopilus felleus* **1/1**, *Xerocomus badius* **7/1**	*Amanita rubra* **1**, *Lactarius camphoratus* **1**, *Russula adusta* **1**, *Russula densifolia* **2** *Russula puellaris* **1,** *Russula sardonia* **1**,

Figure 10 (EcMf sporocarps and numbers of occurrence in the 9 samplings, shared species - 1st number: occurrence on P. sylvestris sites / 2nd number: occurence on P. strobus sites)

5.1.4 Comparison of aboveground EcMf sporocarps and underground EcM root-tips on the *P. sylvestris* sites

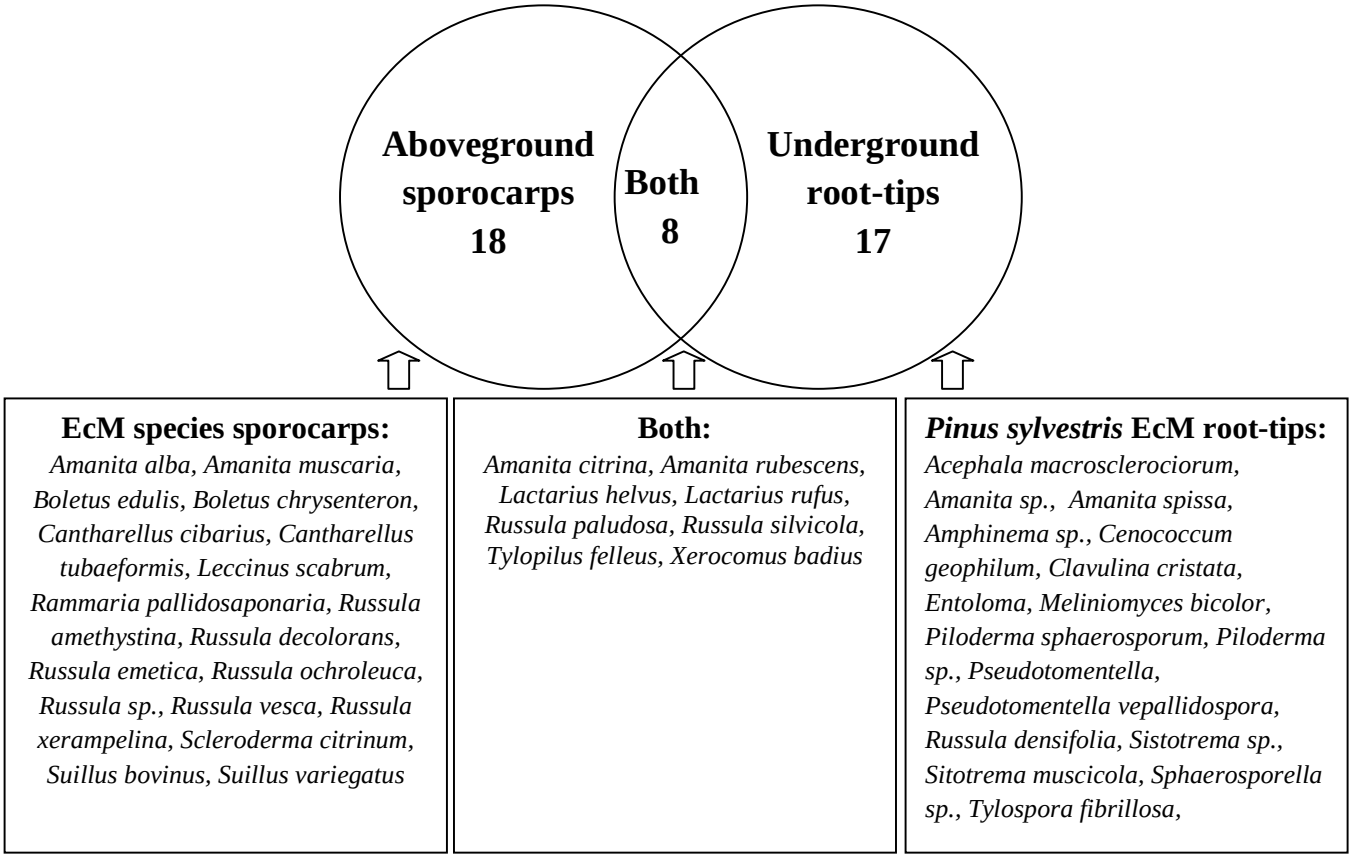


Figure 11 (Illustration of above and below ground EcMf appearances on *P. sylvestris* sites)

5.1.5 Comparison of aboveground EcMf sporocarps and underground EcM root-tips on the *P. strobus* sites

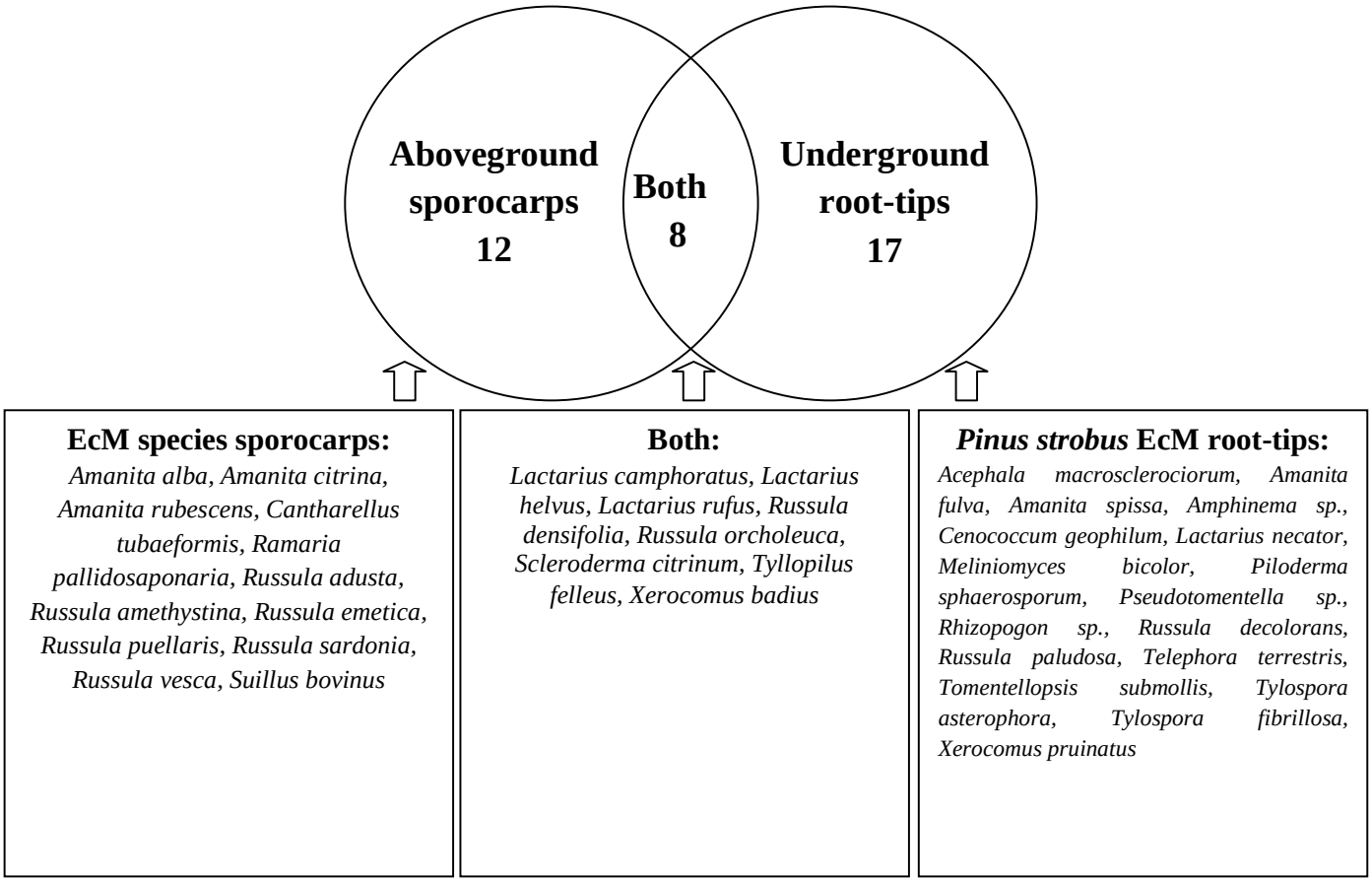
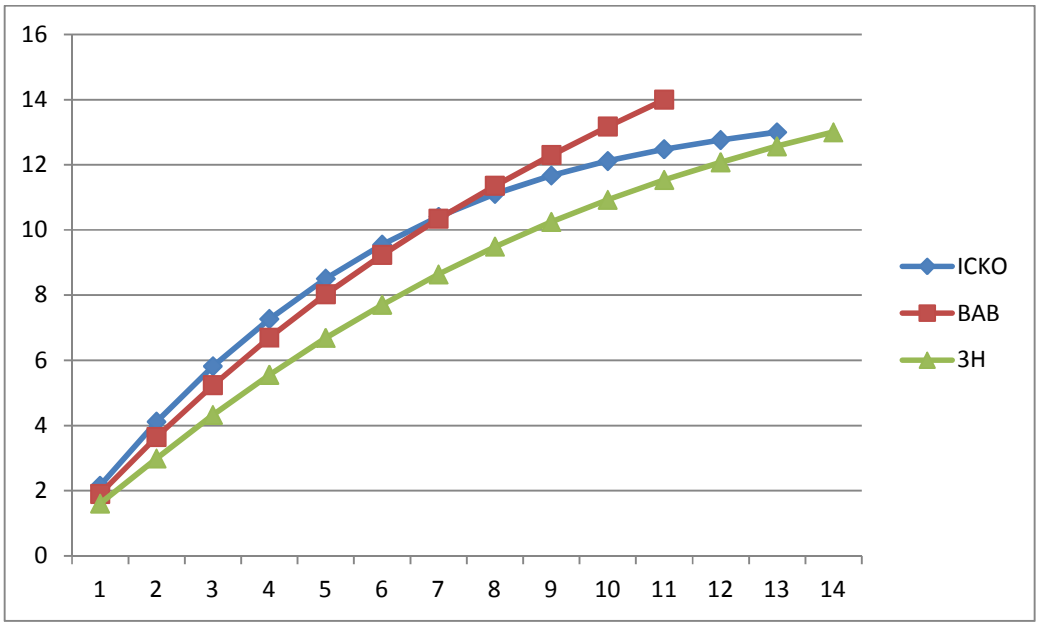


Figure 12 (Illustration of above and below ground EcMf appearances on *P. strobus* sites)

5.1.6 Accumulation curves

The line represents the S (est) vaule computed by EstimateS program (Colwell *et al.*, 2012), which is a identical to *MaoTau* in earlier versions of the program. The tables represent species accumulation curves of ectomycorrhizal (ECM) fungi in study sites with increasing sample size.



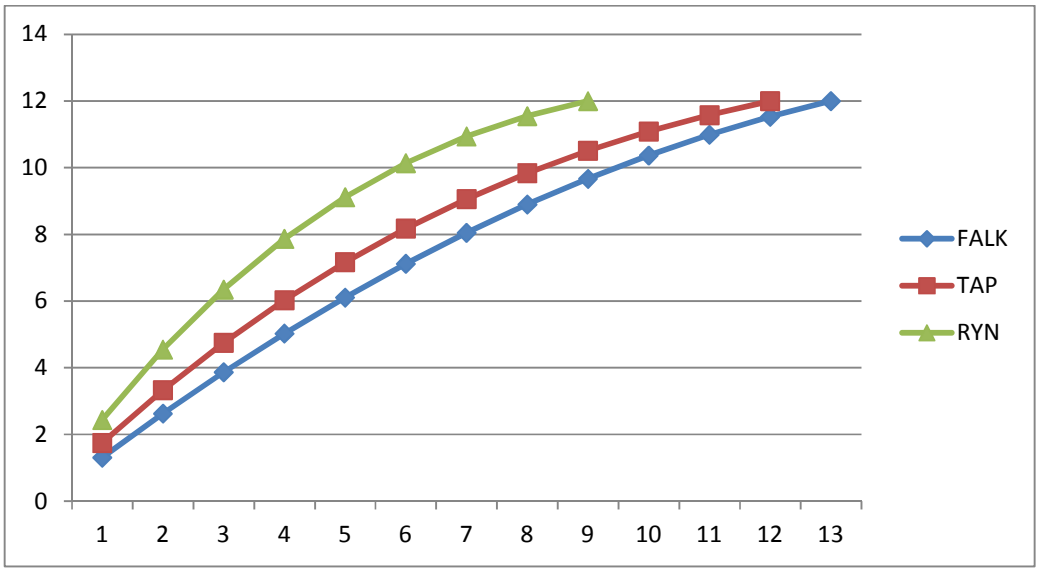


Figure 13 (EcMf species accumulation curves) *P. sylvestris* sites - (ICKO,BAB,3H) *P. strobus* sites - (FALK, TAP, RYN)

5.2 Biomass measurements

Biomass type	P. sylvestris	P. strobus	Significnace level
EcM sporocarps	+	-	*
Sapro. sporocarps	-	+	x
Leaf litter	-	+	***
Fine roots	+	-	**
Ergosterol	+	-	**

Figure 14 (Comparison of all measured biomass: the symbols + and - represent trends of prevailing values. Levels of statistical ignificance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'x' 1)

5.2.1 Ergosterol

The amount of ergosterol in the mesh bag samples varied between 0,0258 µg/g and 0,45 µg/g (mean 0,103 ; median 0,091 µg/g). The highest values have been found on the location Babylon which is located in the most protected area of the NP. We found a significant difference between the amount of ergosterol in *P. strobus* and *P. sylvestris* samples. There was in average nearly 60% higher values of ergosterol (µg/gram sample) in the *P. sylvestris* underground (df = 1, p = 0.002012) compared to *P. strobus* (see fig. 15). This indicates the lower production of EcM fungal mycelia in the *P. strobus* underground samples. There was also a marginally significant difference between sites (df = 5, p = 0.076340)

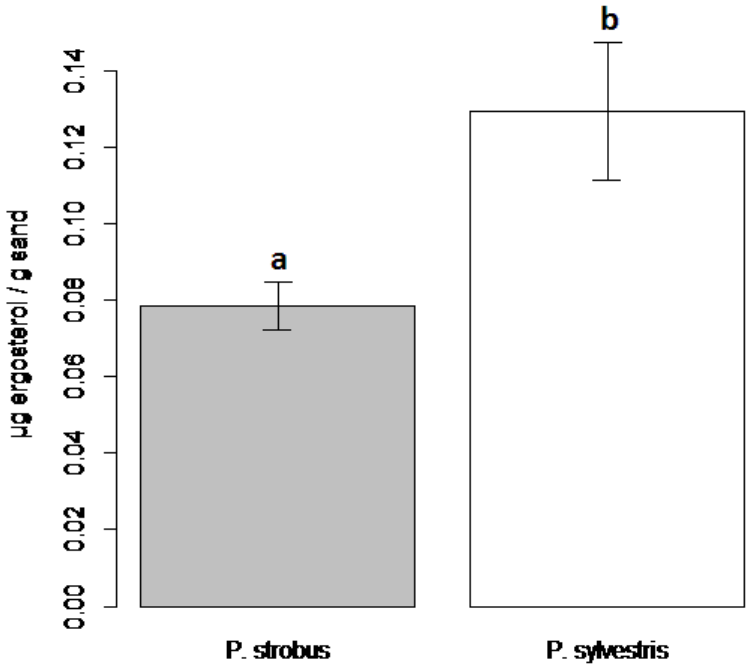


Figure 15 (mean values per species, y axis = µg erosterol / gram sand, Gray column = *P.strobus*, White column = *P.sylvestris*, the letters a, b represent statistical difference between each data sets)

5.2.2 EcM fungal sporocarps biomass

The greatest difference was observed while comparing the biomass of EcM fungal sporocarps. There was almost 100% more (df = 1, p = 0.03411, significance level < 0.05) EcM sporocarp biomass in the undergrowth of *P. sylvestris* (see fig. 16, left). This result was almost obvious while seeing the plots on first sight, especially during the peak of fungal growth season. There was also a significant difference between each sites (df = 4, p = 0.02115, significance level < 0.05).

5.2.3 Saprotrophic fungal sporocarps biomass

We observed no difficulties of saprotrophic fungal growth on in the undergrowth of *P. strobus*. Our measurements showed that the saprotrophic sporocarp production on the *P. strobus* sites is on the same level as on *P. sylvestris* sites. The amount of saprotroph sporocarp biomass seemed subjectively higher, but this result has not reached any level of statistical significance (df = 1, p = 0.15).

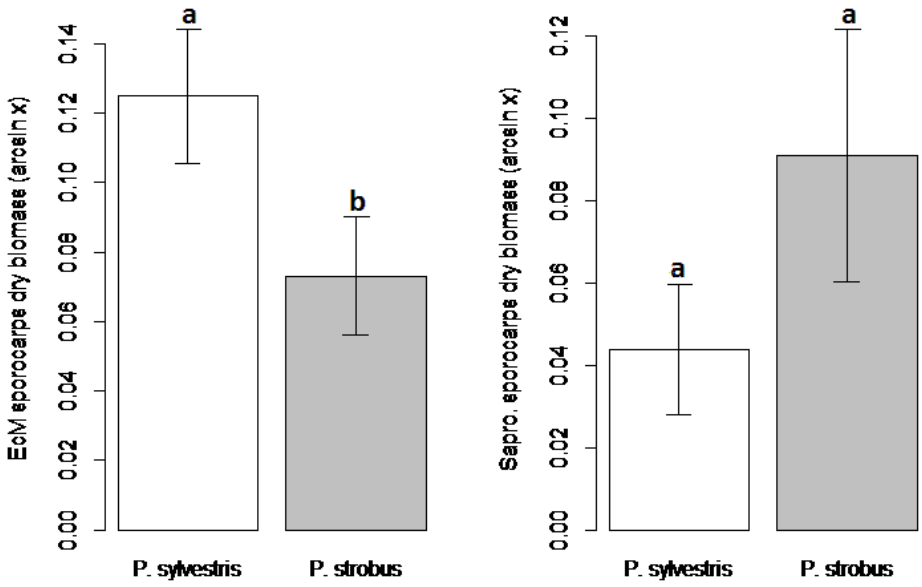


Figure 16 (Gray column = *P. strobus*, White column = *P. sylvestris*, left table - grams of dry EcM fungal biomass, right table - grams of saprotrophic sporocarps biomass ,y axis = data after arc-sin transformation, the letters a, b represent statistical difference between each data sets)

5.2.4 Leaf litter and fine roots biomass

The results from leaf litter and fine roots measuring are limited by the loss of one location (Falknstejn). As expected the leaf litter production is exceedingly higher in case of *P. strobus* (df = 1, p = 2.2e-16). The leaf litter production of *P. strobus* is over 60% higher (fig. 17, left), than the *P.sylvestris* production. There was a significant difference between sites as well (df = 3, p = 2.961e-14).

Contrary, the rate of *P.sylvestris* fine roots production is over 200% higher (pic. 1, right) than the production of *P. strobus* (df = 1, p = 0.004533). There was a significant difference between sites also (df = 4, p = 1.525e-06).

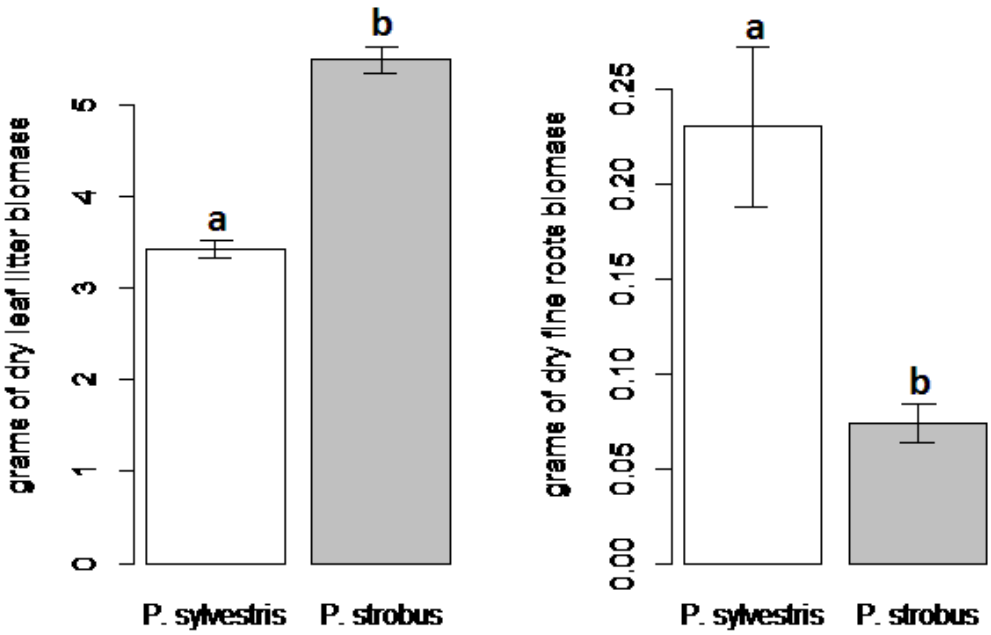


Figure 17 (Gray column = *P.strobus*, White column = *P.sylvestris*, left - grams of dry leaf litter biomass, right - grams of fine roots biomass, the letters a, b represent statistical difference between each data sets)

6. Discussion

Even with the recent scientific methods, it is very difficult to measure the exact amounts of carbon allocated by the trees to their EcMf and other structures in the field. In this study, the comparison of the two pine species carbon allocation into EcMf and plant structures was based on a few parameters, which are easy to measure and give an approximate estimation of the overall carbon balance of each species. Therefore we used comparative methods and our effort was not to quantify the exact values, but to relatively compare the measurable variables of each species (see fig. 14). We monitored the abundance of EcM sporocarps, which might be related with the amount of EMM in the underground, respectively in the mesh-bags. The production of EcM mycelia is linked with fine roots production (Neumann & Matzner, 2013), which was estimated as well. We compared the litter fall production to verify, weather there is any relation between the fungal sporocarps abundance and the amount of littered needles in the undergrowth. The EcMf diversity on the root tips was estimated to discover the possible preference of the alien *P. strobus* of certain EcM fungal species or possible lower diversity compared to the native *P. sylvestris.*

Our results showed a significantly higher *P. strobus* litter fall compared to *P. sylvestris,* which confirmed the first observations on the sites and results from the literature (Scott & Binkley, 1997). The thick layer of littered needles shades the soil surface and probably prevents the growth of other plant species by restricting them the light source and lack of understory plant species on the *P. strobus* sites might be related to a excessive *P. strobus* litter fall (Ferrari, 1999). It is known, that the *P. strobus* seedlings are able to germinate and grow in restricted light conditions and the shade actually promotes their germination and emergence (Herr *et al.*, 1999), which explains why the seedlings are able to evade this problem.

Preliminary observation of the studied sites indicated possible inequality in EcM sporocarps production in the undergrowth of each species and the following measurements proved the highly significant difference. The production of EcM sporocarps was distinctly higher on the *P. sylvestris* sites. There was also a weakly significant difference among each sites. Effect of the sites probably originates from unequal microclimate, altitude, different

plant species in the trees undergrowht and possibly also different fungal species assemblage, whereas each fungal species has an unique mycelial and sporocarp productivity (Gardes & Bruns, 1996; Baldrian *et al.*, 2013). When compared to the precipitation data from Czech Hydrometeorological Institute (see Suplementary), the abundance of sporocarps showed no correlation. The timing of sporocarp growth (De la Varga *et al.*, 2013) is much more complex phenomenon, which would need a whole study, to reveal. Assuming that the abundance of EcM fungal sporocarps relies almost completely on the host plant donation (Högberg *et al.*, 2001), we take those results as an evidence of lowered investment of *P. strobus* into the EcM fungal structures. It is necessary to mention, that the results on the Fig. 12, which describe the aboveground and underground fungal structures, are not from the same locations. So the comparison is just approximate and for the relevant results it needs to be done on the same sites.

We took into account the fact that there is a range of variables which influence the EcM and saprotroph fungal sporocarps abundance, for example allelopathic substances (Javaid & Samad, 2012), litterfall production (Ferrari, 1999) and number of other biotic and abiotic factors. For this reason, we monitored the biomass of saprotrophic fungal species sporocarps on each site as well. Despite the thick layer of leaf litter, which might provide decomposable organic matter and almost no native higher green plants in the *P. strobus* undergrowth, were the saprotrophic fungal sporocarps relatively abundant when compared to the *P.sylvestris* stands (see Fig. 16, right). The measured amount of saprotroph sporocarp biomass on *P. strobus* sites was compared to the *P. sylvestris* sites slightly higher, but this result had not reached any level of statistical significance. However it is a clear evidence of non-limited growth of saprotrophic fungal species on the *P. strobus* sites.

The influence of *P. strobus* on the fungal sporocarps abundance should be investigated in the future. A suitable method is to measure the isotopic trace of various elements, especially C, N and P, which might be a way to reveal some further information about the carbon flows from the host trees into their EcMf structures (Högberg *et al.*, 1999). Principle of such experiment would be the ability of some EcMf species to partially gain carbon by saprotrophic processes. The ^{13}C isotopic trace indicates to the trophic level of the individual fungus (Högberg *et al.*, 1999). By comparing it to the isotopic trace of its host tree, it might be possible to estimate the degree of saprotrophy or EcM nutrition of that

individual mycobiont. If the fungal ^{13}C isotopic trace would be closer to the host tree, it would mean that the fungus is mainly supported by the plant carbohydrates. On the other hand, if the fungal isotopic trace would be distant from the host plant, it would mean that the fungus is more dependent on its saprotrophic ability. The main idea is, that if *P. strobus* allocates less carbohydrates to its EcMf, than the fungi start to gain the carbon more intensively by saprotrophic processes and it would affect its ^{13}C isotopic trace. Another question to test, might be for example weather *P. strobus* chooses its symbiotic fungi with higher saprotrophic potential (Zeller *et al.*, 2007) and safe this way the C for itself.

We measured the EMM production on each site using ingrowth mesh-bags to compare the EcM fungal biomass in the underground. The method using mesh-bags is considered as a reliable way to estimate the underground production of EcMf (Wallander *et al.*, 2013) and was chosen specifically for purpose in this study, because the aim was to relatively compare the EMM production of EcMf in the underground of each Pine species (Wallander *et al.*, 2001). Assurance, that the fungal hyphae in mesh-bags origins from the EcMf, is based on the fact that other fungi than EcMf have no effort of growing in to the bags. The fungal mycelia contained in the mesh-bags is from 80 - 90% EcM origin due to the lack of any organic compounds in the sterile sand content (Wallander *et al.*, 2010). The abundance of EcM mycelia in the ingrowth mesh bags, respectively with the amount of measured ergosterol, follows the same pattern as the EcM sporocarp biomass on the sites. The results showed lower production of EcM fungal mycelia on the sites dominated with *P. strobus*. The lower amount of EcM mycelia in the *P. strobus* underground indicates a lower amount of donated carbon in comparison to *P. sylvestris*. Providing that the same difference would occur in the whole underground on the observed sites, we might extrapolate that the total amount of EcM mycelia would be in case of *P. strobus* 60% smaller. This points to the possibility, that *P. strobus* has a direct influence on the EcM fungi abundance in the forest.

Out of the 1059 sequenced root-tips 462 remained undetected probably due to the degraded DNA in necrotic root-tips and *in situ* contamination by parasitic or saprotrophic fungi resulting in multiple bands or eventual inability to detect some fungal species by ITS1, ITS1F and ITS4 primers. Our data revealed relatively narrow insight into the EcMf and other

symbiotic fungi on the root-tips. The major handicap of our root-tips sampling was the fact that the sampling on all sites was made in one day, which gives us a limited view on the fungal community inhabiting the pine root-tips. EcMf show a large variation in their belowground and aboveground abundance. Most of the species are rare or low in abundance. In the study (Gardes & Bruns, 1996) where the common EcM species divided into a few groups: with (a) balanced presence of root-tips and sporocarps, for example some members of genus *Russula*. Other groups of species are those, which are (b) frequent on the root-tips and rare when producing sporocarps. Some species are (c) common fruitbodies and rare as a root-tips below ground. And finally there are (d) EcMf species which create the ectomycorrhizae on the root-tips and do not have any sporocarps in its known lifecycle, such as *Cenococcum geophilum* (Pigott, 1982) or they create subterranean sporocarps, for example *Tuber* (Smith & Read, 2008) or resupinate fruitbodies as does for example *Sistotrema* (Münzenberger & Schneider, 2012). In this study, we made the similar observations (see fig. 11 and 12), because the individuals of *Russula* genus were found in most cases when collecting the sporocarps and on the root-tips were frequent as well (a). On the other hand *Suillus* sp. was rare on the root-tips and rich in sporocarps (c) in the mentioned study and our results as well. It is important to mention, that those partitions are just an estimation and the real distinction is a spectrum of all those groups combined. The sporocarp production is dependent not only on the fungal species, but also on the succession stage of the ecosystem (Wu *et al.*, 2005). The community of EcM and other symbiotic fungi is a highly dynamic system which changes under various abiotic and biotic influences and many other factors as for example season period (Anderson & Cairney, 2007). According to our results, the future study that would reveal the actual whole fungal community on the sites, would need a extensively larger sampling design and a multiple replications within one year and several years as well (Lindahl *et al.*, 2013). Such a large scale experiment might uncover the whole extent of EcMf community and also the most influential factors.

In our study, the diversity of EcMf was in the case of *P. strobus* higher than expected. This expectation originated from the observation of lower EcM sporocarps occurrence. We found 25 OTUs with affinities to EcMF species on the root tips of *P. strobus* compared to 25 OTUs on the *P. sylvestris* roots. Thirteen EcMF species were shared by both pine species (fig 9). This indicates that *P. strobus* has an ability to form mycorrhizal

symbiosis naturally with the local fungal species and its competitive advantage is not depending on the exceptional cooperation with a narrow group of EcM symbionts. Our results showed, that the EcMf community of each investigated *Pine* species differs in some features (fig. 7,8,9,10). Compared to the native *P. sylvestris* was the invasive *P. strobus* symbiotic fungal species richness on the same level. It needs to be taken in consideration, that the locations were undersampled (see fig. 13) and for the relevant results would need a thorough sampling design. Besides the EcMf species we found in the root-tips a amount of root-endophytes from the order Helotiales and DSE (Dark septate endophytes) fungi. DSE fungi are root-endophytes, which live associated with mycorrhizal fungi. Effect of DSE fungi on host plant is mostly neutral, but there are also few cases of positive or negative effects (Jumpponen, 2001). One species from the DSE ecological group known for its ability to create EcM structures and positive effect on the hosts is *Acephala macrosclerotiorum* from the Helotiales (Lukešová, 2013). The importance and effect of DSE and other endophytes associations with EcMf and plants are still unresolved.

It seems that *P. strobus* is easily able to associate with the whole range of local fungi and shows no signs of narrowed EcM fungal richness as some other invasive Pines (fig. 6). A possible explanation might be the effect of the floristic region from which the invasive plant originates. Some studies imply that if the invasive species originates from the same plant kingdom as is the novel invaded ecosystem, than it has less problem to associate with the local fungi (Kohout *et al.*, 2011a). For example *P. sylvestris*, which invades the Northern Iranian forests dominated by native broadleaf woody trees form Fagaceae family, associates with more than 80% of the local EcMf (Bahram *et al.*, 2013). On the other hand, if the alien plant species invades a area of the different floristic region, than is mostly dependent on own co-invaded EcMf symbionts (Díez *et al.*, 2001; Dickie *et al.*, 2010; Jairus *et al.*, 2011). If the alien species fail to associate with the local EcMf, than is not able to establish in the novel ecosystem and has no chance to become invasive (Parker, 2001; Nuñez *et al.*, 2009). This theory deserves attention and might be investigated in the future research. The idea originates from the fact, that plants within each floristic region are relatively closely related as well as the EcMf. Thus the alien plant species have less barriers when trying to associate with the local fungal species, while invading the same region of their own origin and vice versa. Our data showed, that the EcMf species assemblage of *P. strobus* root-tips is

significantly different from the local *P. sylvestris.* We discovered a significant difference between the preference of each Pines on their EcMf species considering the mycelial exploration types. *P. strobus* prefers EcMf species with contact exploration type. This might indicate the *P. strobus* preference of less carbon demanding fungal species and possible connection with its invasive potential. This idea need to be investigated in future research.

Abundance of the root-tips, EcM fungal mycelia and sporocarps is dependent on the host tree fine roots production (Neumann & Matzner, 2013). This statement is based on fact, that the more fine roots the host tree produces, the more niches to colonize for the EcMf it offers. Our results agree with this statement, while the *P. strobus* fine roots production is compared to the *P. sylvestris* significantly lower. Is the lower production of fine roots really linked to the lower production of EcM mycelia in mesh-bags? Recent studies show a relation of fine roots growth and EMM production (Ekblad *et al.*, 2013). In this study was the unequal fine roots production of each Pine species measured only in the first 30 cm of soil. Weather this phenomenon occurs in the other levels of root system might be another question for future research. Our results show only indirect evidence and a future rigorous field and laboratory experiments need to prove that idea.

The major question is, weather *P. strobus* allocates the photosynthates into the fine roots to a lesser extent compared to *P. sylvestris* (Vanninen & Mäkelä, 1999; Makkonen & Helmisaari, 2001; Peichl & Arain, 2007)? If yes it could mean, that the saved carbohydrates might be used for intense apex growth during the early stages of the trees development, leading to competitional advantage in ecosystems inhabited by *P. sylvestris*. This question should be tested in future research by comparing the root production of each species in the laboratory or field experiments.

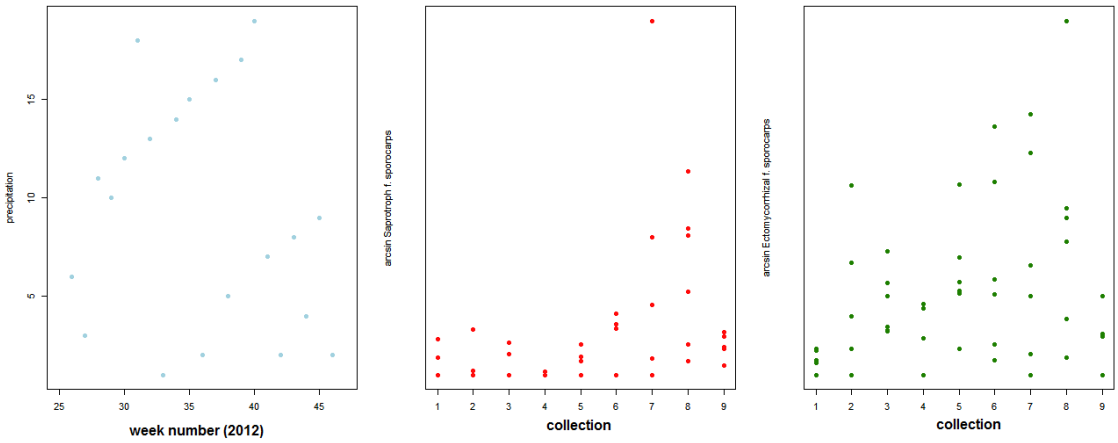
The exact mechanism of *P. strobus* invasion into the *P. sylvestris* forests is not known and it raises several questions. Is it possible, that the *P. strobus* gains an inadequate portion of energy by receiving the full support form EcM fungal symbionts and saving the carbon substances by allocating them less into the mycorrhizal structures? Is the invasive potential given just by natural growing patterns and physiologic properties of each Pine species? Results from the study (Booth, 2004) showed clearly a positive influence of CMNs on the *P. strobus* seedling survival and growth and (Herr *et al.*, 1999) proved the positive

effect of shading on the *P. strobus* seedlings emergence and germination. These hints might explain, why is *P. strobus* in the National Park Bohemian Switzerland and generally in the Czech republic more efficient and is able to outcompete the native *P. sylvestris*.

But how to distinguish the causality - is the observed invasive and growth potential linked with mycorrhizal symbiosis or is it just a naturally higher growing properties of *P. strobus?* It seems that *P. sylvestris* has surprisingly higher growing properties than *P. strobus* when cultivated in the same controlled conditions (Hanzélyová, 1998; Grotkopp *et al.*, 2002). But when growing in natural conditions as presented in this study, it is completely opposite and *P. strobus* outgrows the local *P. sylvestris*. This might the clue, which points to the possible *P. strobus* ability to cheat the mycorrhizal network. It seems that *P. strobus* gains the full mineral nutrient and water support from its EcMf living in the root system and allocates less amount of carbon into the roots and EcMf, which results in low sporocarp and EMM abundance. Could there be a similarity with the case of invasive *Centaurea maculosa* in the North American AM grassland community (Carey *et al.*, 2004)? This theory should be tested in the future experiments. Either in laboratory when trying to distinguish the effect of mycorrhiza on both species in separated and mixed growing experiments. Or even in the field by measuring the net photosynthesis of the host trees and allocation of carbon into its mycorrhizal symbionts at the same time.

7. Suplementary material:

Precipitation and measured growth of EcMf and SAPf sporocarps



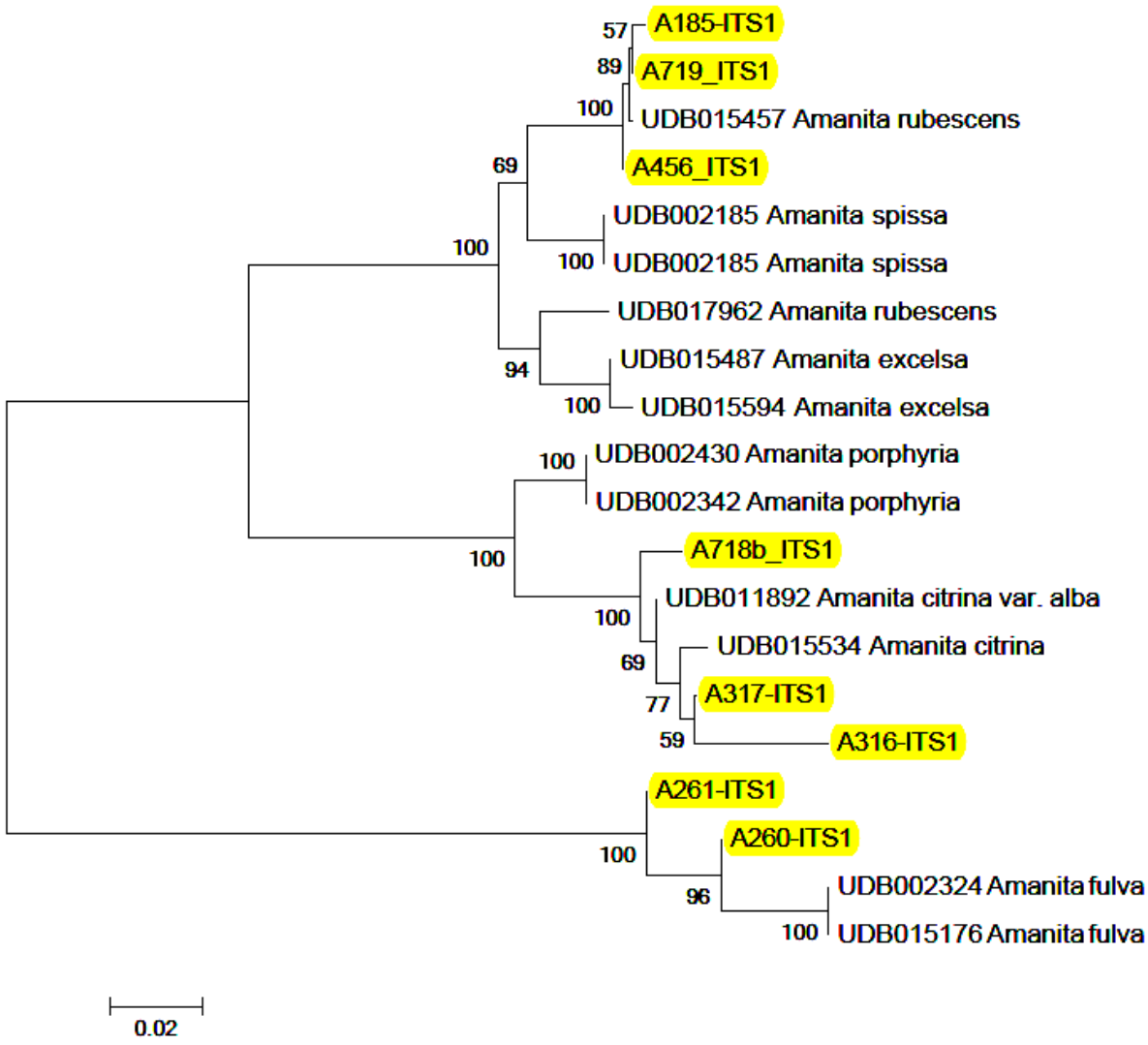
(Precipitation and measured biomass of EcM and SAP sporocarps within each collection, blue points - precipitation in mm/week, red points - SAPf, green points EcMf,)

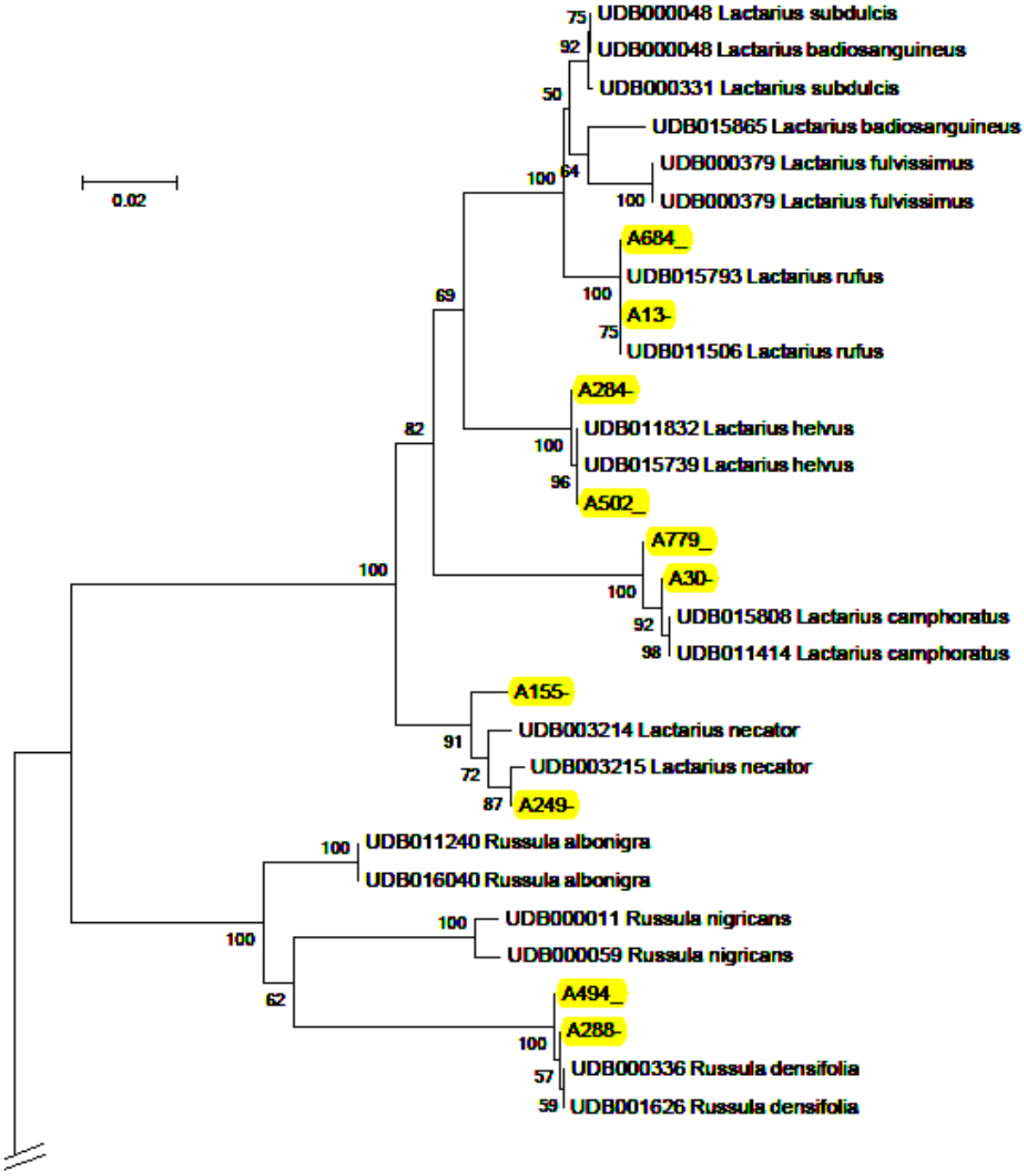
Study sites

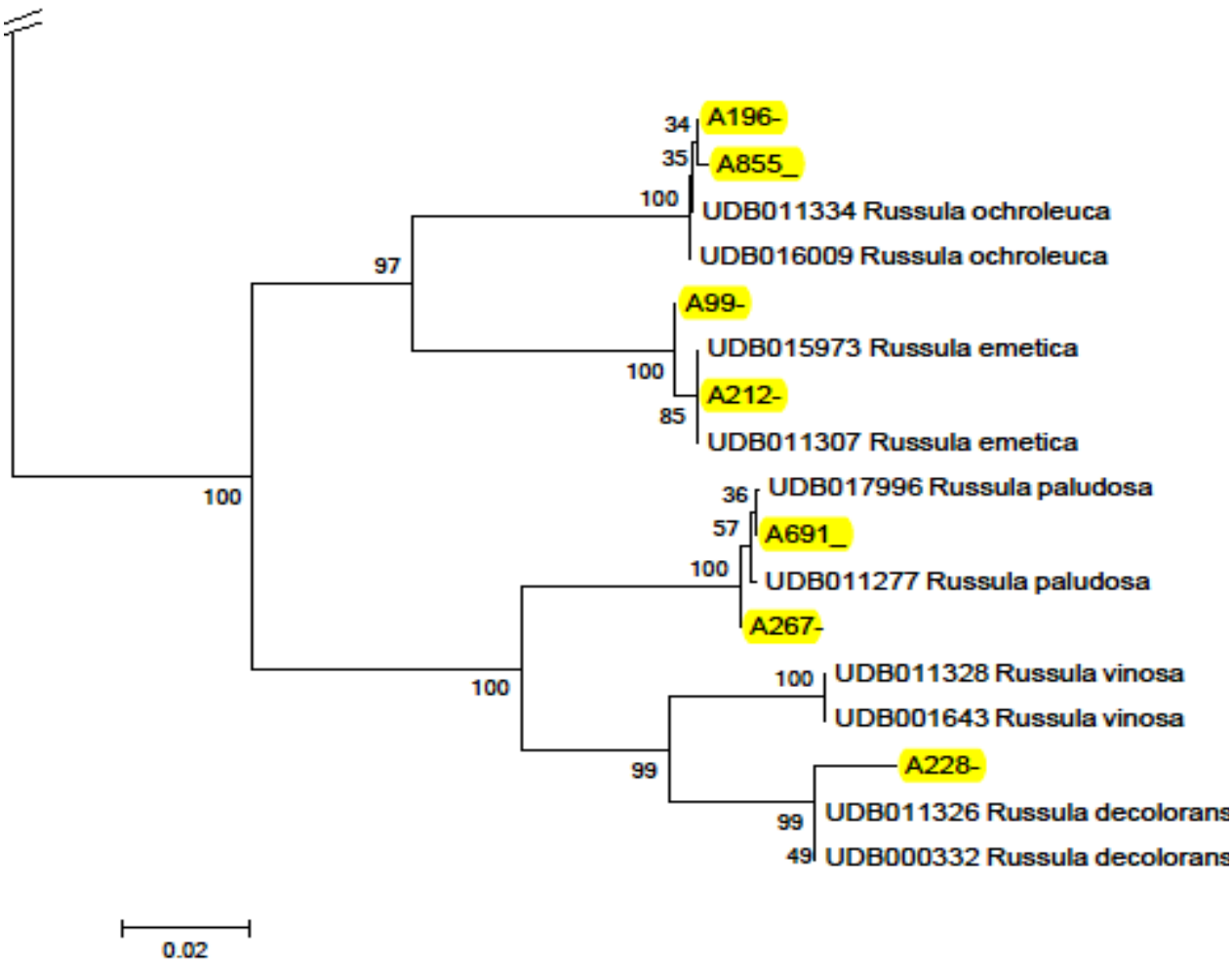
Name (**code**)	GPS	Sampling	Year of sampling
Babylon (**B**)	N50°52,190´ E14°22,903´	root-tips, sporocarps, leaf litter, fine roots, mesh-bags	2011, 2012
Hrby (**H**)	N50°52,719´ E14°22,806´	root-tips, sporocarps, leaf litter, fine roots, mesh-bags	2011, 2012
Icko (**I**)	N50°52,387´ E14°26,725´	root-tips, sporocarps, leaf litter, fine roots, mesh-bags	2011, 2012
Rynartice (**R**)	N50°50,481´ E14°24,145´	root-tips, leaf litter, fine roots	2011
Tap (**T**)	N50°52,431´ E14°26,158´	root-tips, leaf litter, fine roots	2011
Falknstejn (**F**)	N50°51,139´ E14°24,262´	root-tips	2011
Kaja (**K**)	N50°52,283´ E14°14,988´	sporocarps, mesh-bags	2012
Tom (**T2**)	N50°52,098´ E14°15,607´	sporocarps, mesh-bags	2012
Zvireci stezka (**Z**)	N50°49,656´ E14°22,544´	sporocarps, mesh-bags	2012

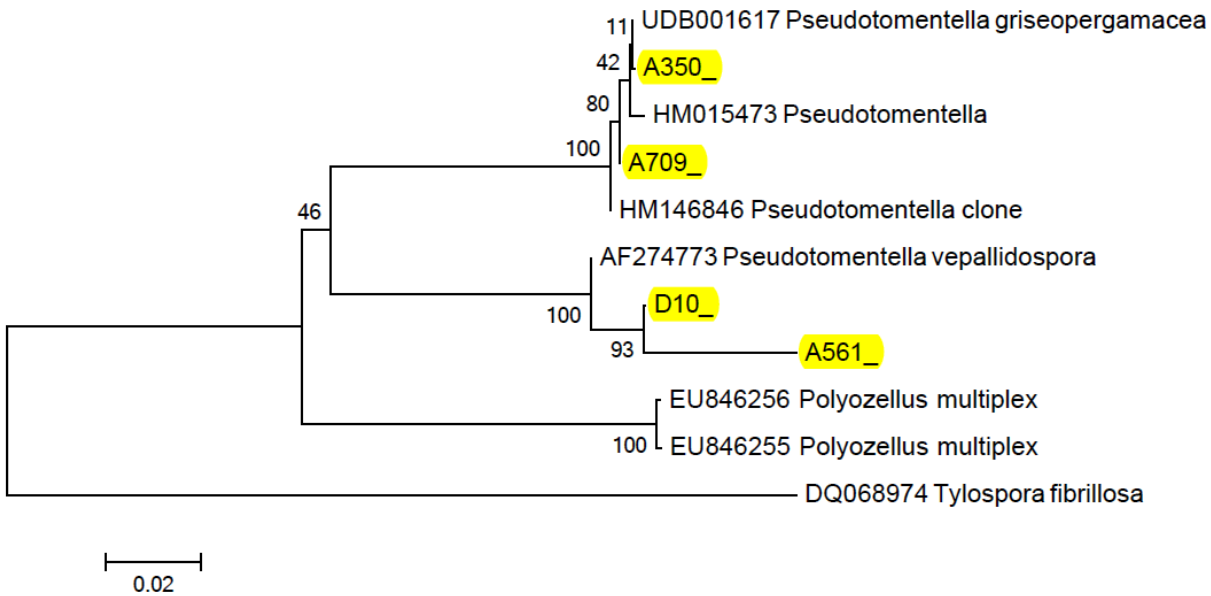
7.1 Phylogenetic analysis of EcM fungal lineages on the root-tips

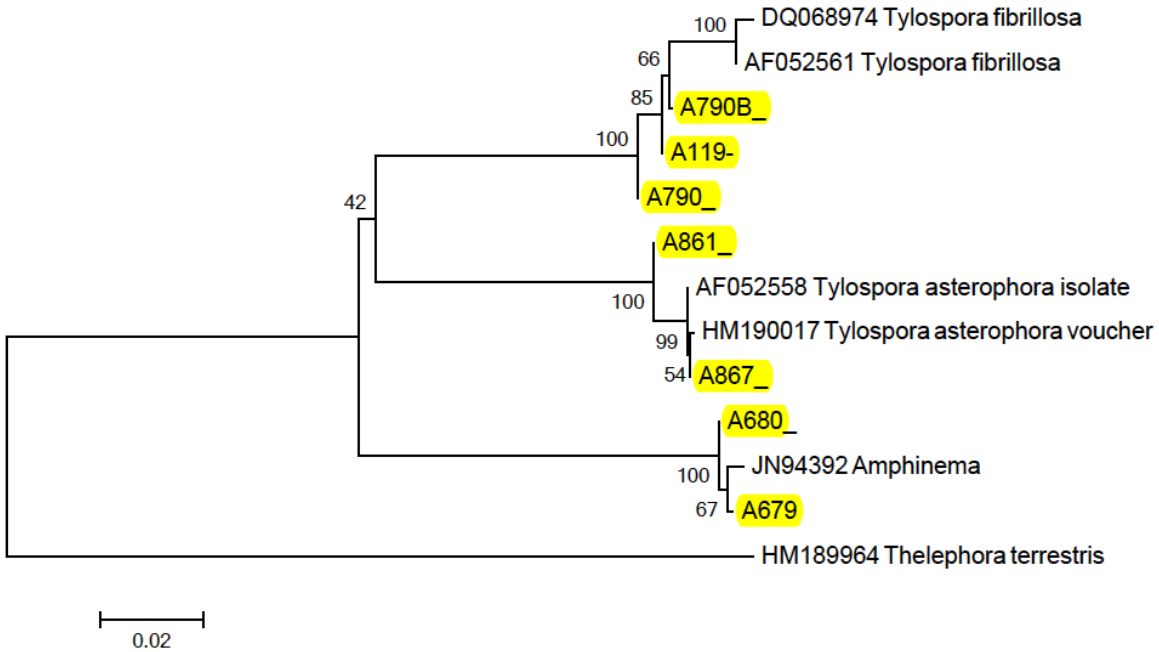
Figure 19. The fylogenetic trees represent each EcM lineage according to (Tedersoo *et al.*, 2010b) found on the root-tips of *P. strobus* and *P. sylvestris*. The highlighted samples are from the EcM root-tips and the samples with names are sequences isolated from sporocarps. Sequences are from UNITE (UDB... codes) and NCBI (other codes) database.




0.02
UDB000048 Lactarius subdulcis
UDB000048 Lactarius badiosanguineus
UDB000331 Lactarius subdulcis
UDB015865 Lactarius badiosanguineus
UDB000379 Lactarius fulvissimus
UDB000379 Lactarius fulvissimus
A684_
UDB015793 Lactarius rufus
A13-
UDB011506 Lactarius rufus
A284-
UDB011832 Lactarius helvus
UDB015739 Lactarius helvus
A502_
A779_
A30-
UDB015808 Lactarius camphoratus
UDB011414 Lactarius camphoratus
A155-
UDB003214 Lactarius necator
UDB003215 Lactarius necator
A249-
UDB011240 Russula albonigra
UDB016040 Russula albonigra
UDB000011 Russula nigricans
UDB000059 Russula nigricans
A494_
A288-
UDB000336 Russula densifolia
UDB001626 Russula densifolia


A196-
A855_
UDB011334 Russula ochroleuca
UDB016009 Russula ochroleuca
A99-
UDB015973 Russula emetica
A212-
UDB011307 Russula emetica
UDB017996 Russula paludosa
A691_
UDB011277 Russula paludosa
A267-
UDB011328 Russula vinosa
UDB001643 Russula vinosa
A228-
UDB011326 Russula decolorans
UDB000332 Russula decolorans
34
35
100
97
100
36
57
100
100
100
99
99
49
0.02


UDB001617 Pseudotomentella griseopergamacea
A350_
HM015473 Pseudotomentella
A709_
HM146846 Pseudotomentella clone
AF274773 Pseudotomentella vepallidospora
D10_
A561_
EU846256 Polyozellus multiplex
EU846255 Polyozellus multiplex
DQ068974 Tylospora fibrillosa
11
42
80
100
46
100
93
100
0.02


100
DQ068974 Tylospora fibrillosa
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AF052561 Tylospora fibrillosa
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A790B_
100
A119-
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A790_
A861_
100
AF052558 Tylospora asterophora isolate
99
HM190017 Tylospora asterophora voucher
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A867_
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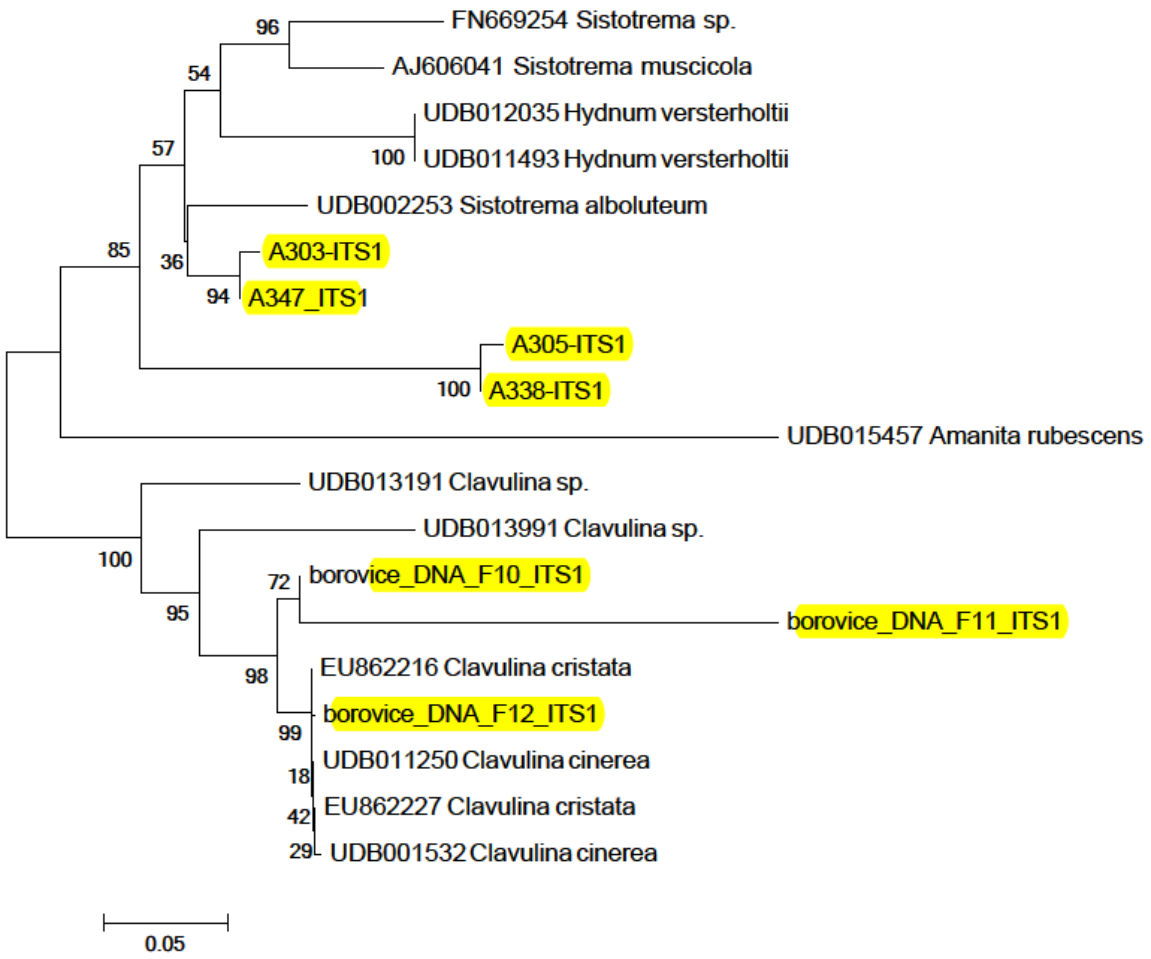
A679
HM189964 Thelephora terrestris
0.02


96
FN669254 Sistotrema sp.
54
AJ606041 Sistotrema muscicola
UDB012035 Hydnum versterholtii
57
100
UDB011493 Hydnum versterholtii
UDB002253 Sistotrema alboluteum
85
36
A303-ITS1
94
A347_ITS1
A305-ITS1
100
A338-ITS1
UDB015457 Amanita rubescens
UDB013191 Clavulina sp.
UDB013991 Clavulina sp.
100
72
borovice_DNA_F10_ITS1
95
borovice_DNA_F11_ITS1
98
EU862216 Clavulina cristata
borovice_DNA_F12_ITS1
99
UDB011250 Clavulina cinerea
18
EU862227 Clavulina cristata
42
29
UDB001532 Clavulina cinerea
0.05

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