Master Thesis: Effects of logging and girdling on ectomycorrhizal communities in a beech forest in the calcareous Alps

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Abbreviations:

AM	Arbuscular mycorrhiza
C	Contact exploration type
CA	Correspondence Analysis
CC	Clear-cut
CCW	Clear-cut with woody debris
EM	Ectomycorrhizae, ectomycorrhizal
ERM	Ericoid mycorrhizae
FM	Facultatively mycorrhizal
G	Girdling
LD	Long distance exploration type
MD	Medium distance exploration type
NM	Non-mycorrhizal
OM	Obligatory mycorrhizal
SD	Short distance exploration type

Abstract:

Symbiotic relationships between plant roots and mycorrhizal fungi are the basis for the existence of plants in many ecosystems. In temperate forests, a majority of trees rely on ectomycorrhizal fungi (EM) for their supply with vital nutrients like phosphorous and nitrogen, which makes EM a major player with regard to net primary production. While forest ecosystems are increasingly confronted with natural and human-induced disturbance events like bark-beetle attacks, windthrows, clear-cutting or fire outbreaks, the effects on ectomycorrhizal fungi are still not fully understood. The present work addressed changes in EM community composition induced by the establishment of clear-cut and girdling disturbance treatments in a semi-natural beech forest of the Austrian calcareous Alps. Additionally, differences in undisturbed plots between two sampling dates in May and September were monitored and mycorrhization patterns of young beech trees were analyzed. The examination of the communities was done by morphotyping, which refers to the visual assessment of EM structures under a stereomicroscope, and by subsequent DNA analysis of the mycorrhizal root tips. Analysis in disturbance treatment plots showed that roots were dead three years after treatment establishment. Results from control plots and young beech trees confirmed a high variation of species presence and abundance between samples, blocks, seasons and whether they came from young or mature trees. The work gives evidence to EM status of species of the families Sebacinaceae, Hygrophoraceae, Pyronemataceae and Clavulinaceae and suggests a possible mycorrhizal status of three species of the Hyaloscyphaceae.

Symbiotische Beziehungen zwischen Pflanzenwurzeln und Pilzen sind Voraussetzung für das Wachstum und Überleben von Pflanzen in vielen Ökosystemen. In Wäldern der gemäßigten Breiten spielen Ectomycorrhiza (EM) für den Großteil der Bäume eine wichtige Rolle in der Versorgung mit Nährstoffen wie Phosphor und Stickstoff, was EM zu einem wichtigen Einflussfaktor der Nettoprimärproduktion macht. Während Waldökosysteme zunehmend mit natürlichen und durch Menschen induzierte Störungen, wie Borkenkäferbefall, Windwürfe, Kahlschläge oder Feuer, konfrontiert werden, ist der Einfluss den diese auf EM Gemeinschaften haben, zu einem großen Teil unklar. Die vorliegende Arbeit beschäftigt sich mit den Auswirkungen, die Störungen durch Kahlschlag und Ringelung auf EM Gemeinschaften in einem semi-natürlichen Buchenwald in den österreichischen Kalkalpen haben. Auch Änderungen in den Kontrollflächen zwischen zwei Probenahmen im Mai und September sowie der Mykorrhizierungsgrad von Jungbäumen wurden untersucht. Die Analyse erfolgte mittels Morphotypisierung, d.i. die visuelle Einschätzung von EM Strukturen mit Hilfe eines Stereomikroskops, sowie nachfolgender DNA Anlayse von mykorrhizierten Wurzelspitzen. Die visuelle Analyse zeigte, dass die Wurzeln in den Störflächen bereits abgestorben waren. Die Ergebnisse der Kontrollflächen und Jungbäume zeigten eine hohe Variabilität in der Zusammensetzung der Gemeinschaften, mit unterschiedlichen Arten und deren Häufigkeiten sowohl in einzelnen Proben, Blöcken, Jahreszeiten, Jung- oder adulten Bäumen. Die Arbeit bestätigt den EM Status einiger Arten der Familien der Sebacinaceae, Hygrophoraceae, Pyronemataceae und Clavulinaceae und gibt Hinweise auf einen möglichen EM Status von drei Arten aus der Familie der Hyaloscyphaceae.

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1. Introduction

Natural and anthropogenic disturbances like fire, bark beetle attacks or clear-cut harvesting have a significant impact on forest ecosystems due to their extensive destruction of tree cover. In temperate climates, the frequency of natural disturbances like bark beetle attacks or windthrows has increased over the last decades and it is expected that climate change will increase disturbance events further (Silva Pedro et al., 2015). While more than 65,000 hectares of forests were destroyed by windthrows and snow in the years 2007 and 2008 in Austria, damage by bark beetles resulted in the loss of 132,000 hectares of forests between 2003 and 2011 (FAO, 2015) with an all-time high in 2017 (BFW, 2018). A deeper understanding of the impact of disturbances on soil biogeochemical processes and microbial communities can help to minimize negative trade-offs for a more sustainable resource management. The objective of this master thesis is to determine the impact of clear-cut logging and girdling on the diversity and structure of ectomycorrhizal communities in a beech (*Fagus sylvatica*) dominated forest stand located in the Northern Calcareous Alps. Fungi are a diverse group of organisms that play a vital part in the functioning of ecosystems. They promote net primary production as plant symbionts, they play an important role in the degradation of organic material as decomposers, and they are a main player in the global carbon cycle. The total number of fungal species is still under debate with estimations ranging from 600,000 up to 6 Mio. species worldwide, with the vast majority still undescribed (Taylor et al., 2014). A focus will be put on ectomycorrhizal fungi (EM) which are essential for the uptake of vital nutrients like nitrogen and phosphorus for trees in temperate and boreal forests and are an important factor for C cycling and soil formation (Dickie et al., 2013).

In this work the effects of clear-cut disturbances on abundance and composition of EM communities in a semi-natural mountain forest are analyzed by morphotyping and subsequent DNA analysis of ectomycorrhizal root tips. Additionally, changes in EM community composition are compared between two sampling dates in May and September. In the course of the May sampling, root tips of cut and girdled trees in clear-cut and girdling plots were found to be dead after the three-year period from treatment establishment. Therefore, during September sampling a small number of young trees from treatment and control plots were collected and their root tips analyzed, to get an indication whether mycorrhization patterns were different between them and compared to soil samples with root tips supposedly coming from mature trees. As soil fungal communities at the project site had already been studied before and right after the establishment of the clear-cut treatments three years before, in the discussion also a reference to these results is given.

2. Literature review

2.1. Roles of fungi in forest ecosystems

2.1.1. Mutualists

Mutualistic fungi live in symbiotic relationships with another organism. They are characterized by an intensive transfer of nutrients between the partners (Brundrett, 2004). The most prominent examples are lichens, where fungi make associations with algae, and mycorrhizae, where fungi make associations with plant roots. This mutualistic relationship is regarded as having a significant influence on the composition, diversity and productivity of plants (van der Heijden et al., 2015). Mycorrhizae play an important role in the uptake of the nutrients phosphorous and nitrogen from the soil, which they pass on to plant roots in exchange for assimilated carbon (van der Heijden et al., 2015).

There are four main types of mycorrhizae: arbuscular mycorrhizae (AM), ectomycorrhizae (EM), ericoid mycorrhizae (ERM) and orchid mycorrhizae. While EM species form a net-like tissue for nutrient transfer, the so called Hartig net, and a dense mantle around the plants' tips of fine roots, AM fungi penetrate the host plants' root cortex cell walls and form arbuscles. Moreover, the mycelia of mycorrhizal fungi form a dense network in soils which, in addition to the uptake of nutrients and water, connects plants with each other and seems to be the reason for nutrient flows among them (van der Heijden et al., 2015).

AM fungi come from the division of Glomeromycota and tend to form symbiotic relations with herbs, grasses and many trees (van der Heijden et al., 2015). In temperate climates they are much more abundant in grasslands than in forests (Gerz et al., 2016). EM fungi, in their majority from the division of Basidiomycota and to a smaller extent from the division of Ascomycota, form symbiotic relationships with 6,000 plant species which are mainly trees and shrubs (van der Heijden et al., 2015). In temperate climates they show a high species richness in forests (Gerz et al., 2016). Ericoid mycorrhizae (ERM) form relationships with plants of the Ericaceae family, whereas orchid mycorrhizae are associated with the 20,000 to 35,000 orchid species worldwide (van der Heijden et al., 2015). Even though the big majority of land plants form mycorrhizal relationships, some plant families do not. Among them are the Brassicaceae, Caryophyllaceae, Juncaceae, Polygonaceae or Saxifragaceae (Brundrett, 2009). Compiled data from Western, Central and Northern Europe, have listed the status of plant species to be by 66 % arbuscular mycorrhizal, 4 % ectomycorrhizal, 4 % both arbuscular and ectomycorrhizal, 1 % ericoid mycorrhizal and 25 % non-mycorrhizal (NM). All mycorrhizal types were present in European zones, with AM species forming most symbiotic relationships. Nevertheless, higher proportions of EM, ERM and NM plant species could be observed in Northern Europe being coupled with reduced medium annual temperatures, soil pH and net primary production, along with an increased rate of AM and obligatory mycorrhizal (OM) plant species in Southern Europe. Against the trend there was an increased share of OM species in Central European mountains (Bueno et al., 2017).

Interactions between plants and their mutualistic fungi are complex, nevertheless there seems to be a pattern for plant fungal associations. According to the review of Hempel et al. (2013), where the mycorrhizal status of 1.758 Central European plant species were compared taking into account their habitat specifics, obligatory mycorrhizal plants were observed to live in drier, less nutrient containing, higher pH and higher soil temperature habitats while they tended to be underrepresented in fertile, moister or acidic soils. Facultative mycorrhizal species showed the highest variability regarding environmental conditions (Hempel et al., 2013). This higher variability might be due to molecular regulatives that allow them to form mycorrhizal associations in poor soils (Brundrett, 2004) where increased nutrient uptake outweighs the drawback of carbon loss, whereas in nutrient rich soil they stay non-mycorrhizal. Even though there is a range of interdependence between fungi and host plants with differing rates of benefits among the partners, it seems probable that most mycorrhizal associations are balanced and beneficial for both partners (Brundrett, 2004).

When looking at forest ecosystems, the most important mutualistic partners for trees are ectomycorrhizal fungi (EM). In exchange for the uptake of nutrients like phosphorus and nitrogen which is facilitated by the fungal species, trees provide carbon to the fungi which constitutes a main factor of carbon flow into the soil (Rosinger et al., 2018). It is estimated that there are around 5,000 to 6,000 ectomycorrhizal fungi worldwide (Agerer, 2006).

The composition of EM communities has been shown to differ widely among sites (Pena et al., 2017) and may be influenced by various factors like plant species composition, tree stand age and chemical and physical soil parameters (Twieg et al., 2009). EM species display different association patterns:

there are generalist EM species which are able to colonize several host tree species, while others can only be found on one host tree species (Lang et al., 2011). EM communities tend to consist of a few dominant and a high number of rare species, where rare species constitute the main factor of EM diversity at each site (Rosinger et al., 2018). In their study covering the mycorrhizal status of three broad-leafed tree species, Lang et al. (2011) observed a colonization rate of 75 to 85 % of root tips by only 35 % of EM species. These species tended to be generalists with little or intermediate host preference, while only 15 to 25 % of colonized root tips were made up by 65 % rare species with narrow host range (2011). A similar pattern was observed by Pena et al. (2017) covering forests in three major forest ecosystems across Germany where 50 % of all root tips were inhabited by host generalists. Nevertheless, Rosinger et al. (2018) showed in their work summarizing studies of EM communities in Western Europe, that some rare species occurring only at one studied site can also have a dominant position there.

In addition to the composition of EM communities, in recent research there has been a trend to focus on the functions of these communities down to the levels of single EM species. Starting with the categorization into exploration types that reflect different foraging capabilities by the abundance and extension of hyphal networks and rhizomorphs that enable EM to interact with the surrounding soil particles (Agerer, 2001), enzymatic analyses covering these interactions have become important. An interesting finding was that even though mycorrhizal community composition is different, these communities seem to be able to fulfill the same functions in different ecosystems (Wang et al, 2017) and are even able to adapt their functional responses to major ecosystem changes (Nicholson and Jones, 2017).

Through recent advances in molecular biology regarding sequencing, transcriptomics or bioinformatic analyses, new insights have been gained in the lifestyles of EM fungi and their relationships with their host plants. The sequencing of several fungal genomes, beginning with *Laccaria bicolor* (Martin et al., 2008) and *Tuber melanosporum* (Martin et al., 2010), the black truffel species, has brought about interesting details, like the presence of genes for enzymes for the degradation of organic substances like glycosyl hydralases, proteases, chitinases and glucanases, which might allow EM species to change for some time to a saprophytic lifestyle. Conversely, plant tissue degrading enzymes like pectinases or enzymes for the break-down of lignocellulose are either completely absent or down-regulated to avoid the host's defense reactions (Bonfante and Genre, 2010). The loss of lignocellulose degrading enzymes makes the big majority of EM dependent on the supply of assimilated carbon from their host plants (van der Heijden et al., 2015).

From the plant's perspective, costs for the establishment and functioning of the symbiosis are substantial: it has been shown that up to one third of assimilated carbon is transferred belowground, with up to 20 % of net primary production flowing into EM fungi (Hobbie and Hobbie, 2008). This figure, together with the evidence that most ecosystems are inhabited by a large majority of mycorrhizal plants, makes it likely that mycorrhizal fungi are a main player in the global C cycle (van der Heijden et al., 2015). Even though not all molecular processes in the interfaces between soil, fungal mycelia and host plants have been uncovered, several genes in EM genomes for specialized transporters for phosphorous, organic and inorganic nitrogen forms and hexose transporters give an indication for the importance of nutrient transfer between the symbiotic partners (Bonfante and Genre, 2010).

The discovery of carbon and nutrient transfer among plants via mycorrhizal networks has received a lot of attention in the past two decades. As most trees form mycorrhizal relations with various EM species and these in turn colonize several trees of the same or different species, networks are formed which connect plant roots with each other (van der Heijden et al., 2015). By using ^{14}C isotopes, net

carbon flows of 3 – 10 % between different trees could be observed (Simard et al., 1997), while other studies have shown the transfer of nutrients like phosphorus or nitrogen between different plants (van der Heijden et al., 2015). The access to carbon and nutrients via mycorrhizal networks seems to be especially important for tree seedlings under harsh environmental conditions, as lower mortality rates have been observed for seedlings having access to mycorrhizal networks compared to seedlings that have not (Teste et al., 2009). In addition to the transfer of nutrients, in the last years mycorrhizal networks have been analyzed with regard to their capability to pass on defense signals from plants confronted with attacks of herbivores or pathogens to neighboring plants (Dickie et al., 2015). As an example, Song et al. (2010) observed in their experiments that healthy plants connected by mycorrhizal networks activated their own defense mechanisms after a neighbouring plant had been infected with a fungal pathogen.

Apart from their role in plant nutrition and signaling, mycorrhizal fungi, together with their saprotrophic relatives, also fulfill an important function in pedogenesis: with their extensive hyphal networks and the exudation of acids they bind together soil particles to form aggregates and they contribute to the weathering of minerals (Dickie et al., 2013). As mentioned above, mycorrhizal fungi are expected to be of main relevance to C cycling. Apart from their role in carbon transfer through their networks, they seem to be important for the formation of soil organic matter as their cell components tend to be difficult to degrade for many organisms. Additionally, they themselves play a role in the decomposition and uptake of organic matter (Ekblad et al., 2013).

As a last aspect, mycorrhizae are suspected to play a key role in shaping the communities of microorganisms in the rhizosphere (Frey-Klett et al., 2005). Bacterial communities colonizing EM root tips have been observed to be highly diverse on different EM species and changing rapidly in abundance with time (Marupakula et al., 2016). Relations between EM fungi and bacteria, and their possible effects on plant life, are still unclear, with evidence that mycorrhizae could also have an antagonistic effect on bacterial species (Moore et al., 2015). Nevertheless, research in this field is still at the beginning, with new molecular methods possibly being able to gain new insights into the plant-soil-EM-bacterial interface in the coming years.

New molecular methods might also help to find answers to open questions regarding plant host and mycorrhizal fungi interactions, like whether hosts are able to select among possible symbionts according their specific needs, if they can apply regulations regarding carbon transfer, or if fungi interact antagonistically with other possible symbionts to strengthen their own position (van der Heijden, 2015).

2.1.2. Saprotrophs

The second big functional group of fungi in forest ecosystems are saprotrophs. Saprotrophic fungi are vital for the break-down of complex organic matter (Fernandez and Kennedy, 2016), especially their ability to produce lignocellulose-degrading enzymes like Laccase or Mn-peroxidase is a prerequisite for further break-down of organic matter through other microorganisms. By removing or changing lignin in the litter or wood, other organisms gain access to plant components like hemi-cellulose or cellulose, the C/N ratio is reduced, and further decomposition, e.g. by bacteria, is possible. Nevertheless, there are differences in substrate preference and degrading abilities of species. While saprotrophs of the Basidiomycetes tend to be able to degrade lignin, saprotrophs of the ascomycetes seem to prefer carbohydrates and have lower ligninolytic capabilities (Valasková et al, 2007).

Like EM fungi, saprotrophic fungi play an important role in the improvement of soil structure (Lehmann and Rillig, 2015). Hyphae of saprophytic fungi have been shown to create aggregates with fungal exudates (Tisdall et al., 2012). It is assumed that aggregate formation is achieved by the growth of hyphae which has influences on the movement of soil particles, along with compression and adhesion processes. Stabilization is achieved through the exudation of fungal biopolymers, whereas also the disintegration of soil aggregates could be influenced by fungal growth along with degrading enzymes (Lehmann and Rillig, 2015).

The diversity of saprotrophs has been shown to be correlated to the variability of substrates, namely the availability of dead wood at various stages of decay (Heilmann-Clausen et al., 2005) and of different tree species, with the highest species numbers in forests with many different stages of decaying wood of diverse tree species (both angiosperms and gymnosperms) (Baber et al., 2016; van der Wal et al., 2017)). Moreover, saprotrophic fungal genera's abundances were observed to change with availability of litter. There are genera that thrive on senescent or freshly fallen leaves, others that are specialized on fresh litter, while typical saprotrophs are abundant on older litter at the end of the winter (Vorisková et al., 2014).

It is still under debate how the presence of EM influences the composition of saprotrophic fungal communities and how decomposition rates are altered. Studies in the 20th century suggested the inhibition of saprotrophic species and a reduction of litter decomposition rates in the presence of EM fungi, which is referred to as the 'Gadgil effect'. Nevertheless, from then on other studies have obtained differing results which suggest that interactions between saprotrophic and mycorrhizal communities are complex and the degree of antagonism is also dependent on biotic and abiotic environmental variables (Fernandez and Kennedy, 2016).

2.1.3. Pathogens

Pathogenic fungi in the soil are another fungal group characterized by strong interaction with plants. In contrast to mycorrhizal fungi, they do not form mutualist relations but adhere to a parasitic lifestyle (Raaijmakers et al, 2009). Soil pathogens can survive in the soil matter or on decaying wood (van der Wal et al., 2017), nevertheless it is the rhizosphere where they interact with plant roots to start a parasitic relationship with their host. As the rhizosphere is a diverse biome made up of countless species of bacteria, fungal mutualists and other micro and macro organisms, strategies of soil pathogens are counteracted by organisms beneficial to plants which makes the analysis of rhizosphere processes a complex and interesting field of research (Raaijmakers et al., 2009). Moreover, the outbreak of diseases is often related to biotic and abiotic environmental conditions. It was shown by Desprez-Loustau et al. that water stress through drought correlated with disease incidences (2006), while Gómez-Aparicio et al. observed that pathogen distribution varied within sites according to soil texture and tree species (2012).

While many fungal plant pathogens that occur on upper plant parts are biotrophic, i.e. they are reliant on living plant tissue for survival, the largest part of soil-borne fungal pathogens is necrotrophic and kills plant cells after infection. In contrast to biotrophic fungi, which are usually highly host specific, necrotrophic soil pathogens tend to have a wide host range. Compared to bacteria and viruses which can only enter plant cells through wounds or natural openings like stomata, fungal pathogens can infect intact plant cells by specialized organs for attachment and penetration along with enzymes for degradation. After having killed the infected plant cells, the fungus can spread internally and externally

by spores and fungal hyphae and continues to attack new tissues which is finally causing root rot (Raaijmakers et al., 2009).

2.1.4. Community composition

Forest soils in temperate climates are variable in their physical and chemical properties, resulting from factors like topography, precipitation, temperatures, insolation, the mineral soil or different plant cover (broadleaved versus coniferous species). Soil fungal community composition is a result of differing success in the competition for space and nutrients, mainly root exudates, in the rhizosphere (Raaijmakers et al., 2009).

Voriskova et al. (2014) have shown in their study of a deciduous oak forest in the Czech Republic that organic matter contents can vary significantly between soil horizons, ranging from more than 80 % in the L horizon to 42 % in the H horizon and 16 % in the Ah horizon. This, along with correlated N contents, had implications on the distribution of fungal communities and their activities. While fungal biomass and enzymatic activities were highest in the L horizon, this layer was also dominated by saprophytic fungi. With soil depth, variables like pH, fungal biomass and enzymatic activities decreased while the proportion of ectomycorrhizal fungi increased. Additionally, there were substantial seasonal changes in community composition: especially in the litter horizon, abundance of dominant saprotrophic genera changed completely over the year with the highest quantity of species in autumn at the time of litterfall and the highest enzymatic activity in winter (2014). This is in line with observations in Finnish and Alaskan boreal forests, where saprotrophs also dominated in horizons with high soil organic matter (McGuire et al., 2013; Santalahti et al., 2016). Saprotrophs tended to dominate during seasons with low photosynthetic production (winter), while ectomycorrhizal fungi dominated in layers with lower dead organic matter content (organic and mineral soil horizons) and during growing season (spring to autumn). Community composition also showed a high variability between sites (Santalahti et al., 2016). As for EM species, their abundance and diversity highly increased from spring to summer, with H and Ah horizons showing the same pattern. Abundance of specific EM species varied significantly with the season (Voriskova, 2014), which was also observed by Buée et al. in a beech forest in France (2005). This was attributed to different drought resistance in summer (Buée et al., 2005) or the ability of some species to change to a saprotrophic lifestyle in winter (Voriskova, 2014). It was also shown that different EM species have a different time pattern for their enzymatic activities (Buée et al, 2005) which lead to the assumption that EM fungi have different preferences for physical and chemical soil properties and show a variety of physiological responses (Koide et al., 2007).

The main influencing factors for the composition of fungal communities in forests seem to be tree species, pH values and C:N values. While fungal species in coniferous forests have been shown to differ significantly from the composition in deciduous forests, higher pH values seem to favor EM diversity and abundance, whereas increasing C:N ratios seem to have the opposite effect (Goldmann et al., 2015). Species composition has also been observed to differ with stand-age and the availability of organic nitrogen (LeDuc et al., 2013). Likewise, in their review covering EM communities across Western Europe, Rosinger et al. recognized the main factors for EM diversity to be host tree species, pH values, N deposition, mean annual temperatures and precipitation (Rosinger et al., 2018).

An interesting question still under debate is whether fungal diversity is necessary for the functioning of forest ecosystems (Leake, 2001) or if some species are sufficient for fulfilling all functional roles. Nicholson and Jones (2017) have shown in their study of 1-year-old tree seedlings from a natural environment, hosting EM communities of later successional species, compared to seedlings in a disturbed environment with early successional species, that after transplant into the other

environment their communities stayed the same but adjusted their functional responses and were able to adapt to the new situation successfully.

2.2. General effects of disturbances on forest ecosystems

Disturbances are part of natural forest ecosystem dynamics. Nevertheless, disturbance events have increased worldwide significantly over the last 50 years and are expected to rise further in the coming decades due to climate change. In Europe, disturbances in boreal and temperate forests are mainly due to windthrows and bark beetle attacks, while in Southern and Mediterranean countries forest fires are the dominant source of disturbances (Seidl et al., 2014).

Sites affected by disturbances are confronted with a rapid change in environmental factors, like higher forest soil temperatures and wider daily amplitudes (Likens et al., 2004), higher air and surface temperatures especially in disturbed zones with dead trunks and branches left, fewer days with snow-cover (Hesslerová et al., 2018), higher insolation and wind speed (Stern et al., 2018), and higher soil temperatures (Buée et al., 2005). Disturbed sites, especially with remaining woody debris, may be subject to nitrate leaching due to missing uptake by plant roots and higher mineralization (Scharenbroch and Bockheim, 2008) and the leaching of dissolved organic carbon (Schelker et al., 2013).

Forests are a major player in the global carbon cycle, where disturbances have been observed to result in significant changes of CO_2 fluxes from the soil (Mayer et al., 2017; Zehetgruber et al., 2017)). The main sources of soil respiration in undisturbed forests were identified to be by 60 % of heterotrophic, 15 % of plant root and 25 % of mycorrhizal hyphal origin (Heinemeyer, 2007). In their study covering changes after major windthrow events, Mayer et al. (2017b) observed a reduction in autotrophic respiration by roots and fungal symbionts due to the reduction in plant cover, whereas heterotrophic respiration increased by 60 %. This increase was attributed to higher soil temperatures and it was accompanied by a respective decline in soil organic carbon stocks. In another study, they provided evidence that this decline in carbon stocks could be reduced or totally prevented by advance tree generation (Mayer et al., 2017a). Similar results were shown by Zehetgruber et al. (2017), where disturbances by clear-cutting resulted in C effluxes in the first years but slowed down after dense ground vegetation, mainly consisting of grasses, had been established.

Effects of disturbances depend on the size of the affected area and seem to vary with time passed since the disturbance event. While the effect of higher soil temperatures and soil moisture could already be observed with anthropogenic small-scale disturbances like reduced tree cover by thinning practices (Buée et al., 2005), it was shown that for small scale disturbances with the removal of tree cover of less than 60 %, higher access to photoactive radiation enhanced photosynthetic production in the remaining vegetation. This resulted in similar above-ground net primary production levels as before the disturbance. Nevertheless, after exceeding this threshold, production also decreased non-linearly (Stuart-Haentjens et al., 2015). Conversely, in the Central Alps increasing numbers of disturbed areas have been confronted with difficult or failing tree regeneration which makes a permanent loss of tree cover likely unless costly measures of replanting are taken (Zehetgruber et al., 2017). In another study, Kishchuk et al. did not encounter significant differences in the soil parameters pH, carbon content and extractable ammonium 10 years after clear-cut logging, even though there had been differences immediately after establishment of the treatments, which suggests no long-term effect on these values (2015).

2.3. Effects of disturbances on fungal communities in forest ecosystems

Disturbances like clear-cut logging affect forest ecosystems in various ways. When looking at EM fungi, in addition to the changed physical and chemical conditions, they are confronted with the reduction and eventual stop of carbon flow from their host trees as soon as their tree roots have died. The eventual death of EM on root tips results in a loss of EM diversity over time (Jones et al., 2003), which makes the availability of spore banks vital for recolonization after disturbances (Glassman et al., 2015). In their comparison of EM communities in mature soils to their spore banks across North American ecosystems, Glassman et al. (2015) observed a significant difference between species in soils to those present in spore banks. While spore banks only consisted of a small number of species which might play a role in early colonization after disturbances, even abundant species in mature soils did not find their representation there. This goes in line with observations that colonization of tree fine roots after clear-cutting decreased substantially with increasing distance from the borders of un-cut forest, which suggests that living mycorrhizal hyphae play an important role for inoculation (Hagerman et al., 1999). Their propagation methods, along with their ability to cope with higher temperatures and insolation, changed soil moisture, and a different plant structure like grasses or herbal plants that could lead to the competition by AM fungi, seem to be the main factors why EM communities after regeneration were observed to differ significantly in their composition to the time before clear-cuts (Jones et al., 2003). The time that EM species can survive on the roots of logged trees seems to depend on their ability to change to a saprophytic lifestyle. When analyzing the genome of two EM species, namely *Laccaria bicolor* and *Tuber melanosporum*, enzymes aimed at the degradation of organic matter, namely glycosyl hydrolases, proteases, chitinases and glucanases were present, whereas the lack of ligninolytic enzymes signifies a major drawback for a saprotrophic lifestyle (Bonfante and Genre, 2010). Studies have shown a reduction of EM species in the growing seasons after the clear-cut harvesting with a total loss of all species 3 years after logging (Hagerman et al., 1999). In addition to the loss of species, also a significant reduction or total loss of mycorrhizal networks in disturbed ecosystems is likely (van der Heijden et al., 2015).

Even though it was shown that mycorrhizal relationships are beneficial for the re-establishment of healthy forest ecosystems after disturbances, there is still debate on how many EM species are necessary for successful reforestation. Even though a large amount of species seems to be favorable in the long run, in shorter terms a small selection of species seems to be sufficient for successful re-establishment of tree cover (Hawkins et al., 2015).

As for fungal community composition, the increased quantity of dead wood in disturbed plots may lead to a change of saprotrophic fungi communities in disturbed plots. Nevertheless, contrary to the expectation that with increased dead wood also the species number of saprotrophs should increase, Bässler et al. (2016) could only confirm an increase of the species number of lichens, probably due to higher insolation levels, after bark beetle dieback, but no increase in species numbers of saprophytic fungi. Nevertheless, a change of community composition of both guilds was confirmed.

3. Objectives, questions and hypotheses

This work is part of a 3-years-project studying the effects of clear-cut disturbances on a mountain forest ecosystem in the calcareous Alps (Godbold et al., 2018). Within this project, changes in environmental parameters like soil moisture, pH values, soil temperatures and soil organic carbon

contents were monitored. As heterotrophic soil respiration by microbial and fungal communities was suspected to be the main driver for carbon effluxes after severe windthrow disturbance events in a similar project (Mayer et al., 2017b), it was decided to take a closer look at the changes in soil fungal community composition along the three years period within this project. Due to their vital importance for plant nutrition (van der Heijden et al., 2015), this work puts a focus on ectomycorrhizal species where the diversity, abundance and temporal variation are analyzed. EM species form a mantle around the host tree's root tips (van der Heijden et al., 2015), therefore a method for measuring their diversity and abundance is morphotyping (Rosinger et al., 2018), which consists mainly of the visual analysis of tree root tips under a stereomicroscope, the assignment of encountered ectomycorrhizal fungi to a probable genus or species level, and the counting of their abundance. As morphotyping is a time consuming and subjective method which requires a lot of experience of the researcher, in the last decades the preferred methods for identification have become molecular techniques (Rosinger et al., 2018). Nevertheless, if identification is done based on molecular data from soil samples, the question is whether this data reflects the abundance of species present on tree root tips. Therefore, based on the results of the current work, another objective of the 3-years-project is to get an indication whether high-throughput molecular methods based on soil samples reflect the structure of fungal communities on tree root tips.

The assumptions for the current work can be summed up in the following hypotheses:

H1: EM communities on tree root tips vary between root tips in plots with disturbance treatments (girdling, clear-cut) and control plots

H2: EM communities on tree root tips vary between spring and autumn

H3: EM communities on tree root tips vary between tree root tips of mature versus young trees

4. Materials and methods

4.1. Design

The study was conducted in a south-facing mixed forest dominated almost exclusively by beech (*Fagus sylvatica*) at a height of around 1,100 m in the calcareous Alps close to the town of Molln in Upper Austria. The design was established as part of a project for the research of effects of disturbance events on the carbon cycle (Godbold et al., 2018). In 2015, 4 blocks were designed in the research area at the same elevation. In each block 4 plots were established, each comprising an area of 30x30 m, and subjected to the following treatments: the disturbance treatments clear-cut (CC), clear-cut with woody debris (CCW) and girdling (G) and the control treatment (C). For sampling, each plot was again divided into 4 subplots, which covered the area within a triangle of three beech trees. The treatments were established in summer 2015. In clear-cut plots all trees were harvested at around breast-height and debris was removed. In clear-cut with woody debris plots thin branches and leaves were left on the ground and open spaces were covered with woody debris in the same density. For girdling plots a 15 cm wide strip of bark was taken off from all trees at breast height. Figures 1 and 2 show the research site and an overview of the research design.

Figure 1. Research site (provided by Mathias Mayer)

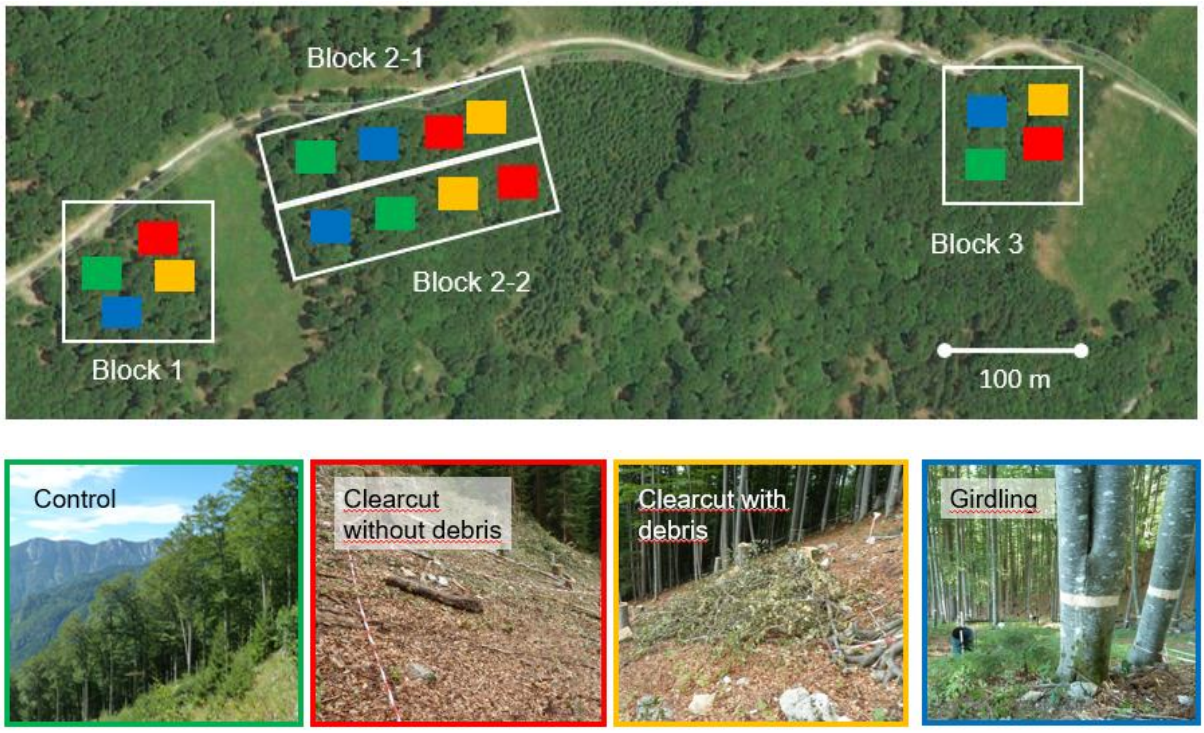

Figure 2. Overview Block design and treatments (provided by Mathias Mayer)

4.2. Morphotyping and DNA analysis of mycorrhizal communities on fine root tips

For morphotyping of the mycorrhizal communities on fine tree root tips the first sampling took place on 28th and 29th of May 2018. In all treatment plots of the four blocks around 1 liter of soil was taken from the A horizon from 3 subplots, with the exception of plot 2C2, where only samples of 2 subplots were taken as the organic layer of the third subplot was so deep that no A horizon was encountered within a depth of 50 cm. Samples were stored in a box with cool-packs and analyzed within 3 weeks from the sampling date. In the laboratory fine roots were carefully removed from the soil and washed in tap water, afterwards roots were cut into pieces of 2-3 cm length. From each sample around 300 root tips were chosen randomly, which summed up to a number of 700 to 1000 root tips per subplot and 2800 to 4000 root tips per treatment. Root tips were analyzed under a stereomicroscope, counted and assigned to the following categories: Mycorrhizal, Non-mycorrhizal, Semi-Vital/Dead/Broken. The assignment of the mycorrhizal root tips to morphotypes was done based on the comparison with pictures and specifications from Agerer and Rambold (2004–2018). For DNA analysis, between 1 and 10 root tips of each identified morphotype was cut from the analyzed piece of fine roots, placed into a 1.5 ml Eppendorf tube, and frozen. The September sampling was done from 17 to 21 of September 2018. Sampling was done according to procedures in May, with the exception that samples were taken from 4 subplots of each plot, which summed up to around 1.200 root tips at each plot. In addition to the soil samples, in Block 1 four young beech trees of around 50 to 80 cm height from the clear-cut plots (CC and CCW) and 4 young beech trees of the control plot were collected and stored at around 4 °C. Roots of soil samples were washed immediately. Analysis under the stereomicroscope was done within 2 weeks from the sampling date. For a better respresentation, the brightness and contrasts of the pictures were increased by 40 % in MS Word.

For DNA analysis, at least one representative sample per morphotype and a number of samples with unclear morphotype were selected and put into a 96-piece plate with granules and buffer solution (Qiagen Solution C1). DNA extraction was done with a Qiagen DNeasy PowerSoil HTP 96 Kit according to the Qiagen centrifuge protocol, dated June 2016, with the change that each centrifuging step was performed for 15 min at 3900 × g instead of the 6 min at 4500 × g in the standard protocol. The extracted samples showed a low DNA content of mostly below 30 ng/µl which is why a nested PCR was chosen for amplification. As a trial nested PCR for 8 samples showed the best results with undiluted DNA, undiluted DNA was chosen for all samples. The first round of amplification was performed with the fungi specific primers FQ-F (GGRAAACTCACCAGGTCCAG; Liu et al., 2012)) and TW13 (GGTCCGTGTTTCAAGACG; O'Donnell, 1993). 1 µl of the DNA template was added to a mastermix consisting of 7.5 µl of GoTaq solution, 0.75 µl of forward and reverse primers (10 µM each) and 5 µl of distilled deionized water. For the PCR reaction a program (54013035) with initial denaturation at 95 °C for 2:30 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec, and extension at 72 °C for 1:30 min, and a final extension step at 72 °C for 5 min was chosen The PCR products were then diluted 1:10 with distilled deionized water and subjected to a second PCR with the primer pair ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC). 3 µl of the template was added to the mastermix of 22.5 µl GoTaq solution, 2.25 µl of forward and reverse primers and 15 µl of distilled deionized water. The program (54004535) chosen for this PCR had an initial denaturation at 95 °C for 2:30 min followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 54 °C for 30 sec, and extension at 72 °C for 1:30 min, and a final extension step at 72 °C for 5 min. All PCR were run on a Biometra TRIO cycler. The quality of the obtained products was analyzed by loading 5 µl of each sample on a 2 % agarose gel with the stain Midori Green and watching the result under fluorescent light. The remaining PCR products were sent to LGC Genomics in Berlin/Germany for sequencing. The analysis of the sequences was conducted with the software program CLC

Genomics Workbench 6.9.2. The sequences were trimmed, forward and reverse reads were assembled and edited. Where necessary, reference sequences were obtained from a BLAST search on the homepage of the National Center for Biotechnology Information of the U.S. National Library of Medicine (NCBI) to assist the assembly for improvement of the quality. Taxonomic affiliation was done by BLAST searches at NCBI and, if possible, assigned to genus or species level based on similarity with a reference sequence from the BLAST search. In the BLAST search, fungal sequences of the UNITE database were included. If sequences showed a similarity of more than 97 %, it was assumed to be from the same species. As more than one third of all samples showed sequences of poor quality or seemed to result from contamination by other fungal species, it was decided to run a second nested PCR with basidiomycete specific primers for these samples. In the first round the DNA templates were amplified with the basidiomycetes specific primer pair Basid 2R+ (ACCGTTGTAGTCTTAACAG; Lynch and Thorn, 2006) and LB-WT (CTTTTCATCTTTCCCTCACGGT; modified from Tedersoo et al., 2008), whereas the second PCR round was conducted with the primers ITS1-F and ITS4. The procedure and programs for thermocycling stayed the same as in the first nested PCR process. The obtained products were again sent for sequencing and assigned to taxonomic levels as mentioned above. With this approach, the majority of the samples could be assigned to species, genus or family level. Based on these reference species, the rest of the identified EM were assigned to one of these reference species by visual comparison of the microscope pictures.

Support for morphotyping of ectomycorrhizal root tips was provided by Prof. Douglas Godbold and coworkers at the Institute of Forest Ecology, BOKU. Support for DNA analysis of EM root tips was given by Dr. Markus Gorfer and coworkers at the AIT, Tulln.

4.3. Statistics

Statistical calculations were done with the help of the statistical software PAST version 3 (Hammer et al., 2001). For comparing the degree of mycorrhization between blocks, seasons and young beech trees, a two-sample t-test with confidence interval of 95 % and a p-value of 0.05 was used. Correspondence Analysis was chosen for the visual analysis of patterns between samples. Correspondence Analysis is a method widely used in various fields of natural and social sciences for the visualization of proximities among variables in a dataset (Greenacre, 2010).

5. Results

5.1. Mycorrhizal communities on tree root tips in disturbance treatment plots

In May and September sampling, a large majority of roots from soil samples in disturbance plots were encountered to be dead, with the rare exception of single root tips seemingly coming from young beech trees. Interestingly, in May some of the trees in the girdling treatment plots still had green leaves which suggested that these trees were still alive. In contrast, in September they had already shed all leaves, while trees in control plots had not shed them yet. Examples of dead root tips from treatment plots are shown in Figures 3 and 4. For a better representation, the brightness and contrasts were increased in MS Word. The original pictures can be found in the Appendix.

On some of the dead roots EM are still recognizable, as can be observed in Figures 5 and 6.

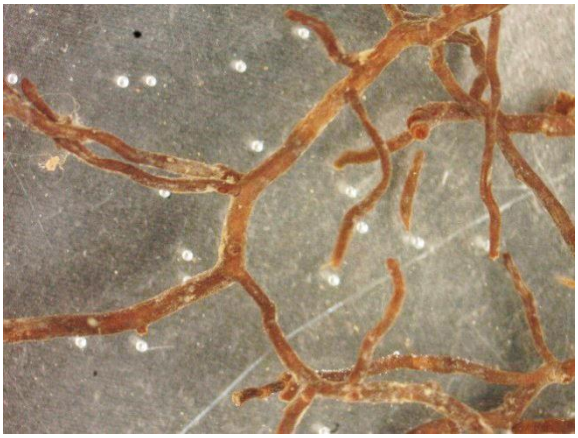

Figure 3. Dead root tips from May Block 3, treatment clear-cut with debris, edited

Figure 4. Dead root tips from May Block 1, treatment girdling, edited

Figure 5. Dead root tips with recognizable EM from May Block 2-2, treatment girdling, edited

Figure 6. Dead root tips with recognizable EM from May Block 2-2, treatment clear-cut, edited

Consequently, only root tips in control plots were analyzed. In May, 3,679 root tips, with 988 root tips coming from Block 1, 992 root tips from Block 2C1, 766 root tips from Block 2C2 and 933 root tips coming from Block 3C, were analyzed. For September sampling, the total number of root tips was 5,020, with 1,253 root tips coming from Block 1, 1,289 root tips from Block 2C1, 1,251 root tips from Block 2C2, and 1,227 root tips from Block 3. Additionally, in September 1,348 root tips of young beech trees from the control plot and 1,362 root tips from treatment plots of Block 1 were analyzed.

5.2. Degree of Mycorrhization

The degree of mycorrhization between different blocks within May and September samplings showed no significant difference. Figure 7 shows the comparison of the degree of mycorrhization between May and September samples. While the degree of non-mycorrhizal root tips was similar in May and September plots with 15 % and 11 % respectively, in September a significantly higher proportion of dead/semi-vital/broken root tips with a mean of 56 % compared to 37 % in May, and a lower proportion of mycorrhizal root tips with a mean of 33 % in September and 48 % in May was observed. On root tips of young beech trees from the control plot of Block 1, the mycorrhization rate of 68 % was significantly higher than in September soil samples of presumably mature tree roots, and the ratio of 1 % of non-mycorrhizal root tips and 31 % of dead/semi-vital/broken root tips was significantly lower.

On root tips of young beech trees from disturbance plots, the ratio of 29 % of dead/semi-vital/broken root tips was significantly lower compared to roots from soil samples while, in contrast to trees from the control plot, the degree of non-mycorrhizal root tips was much higher with 23% (p=0.07).

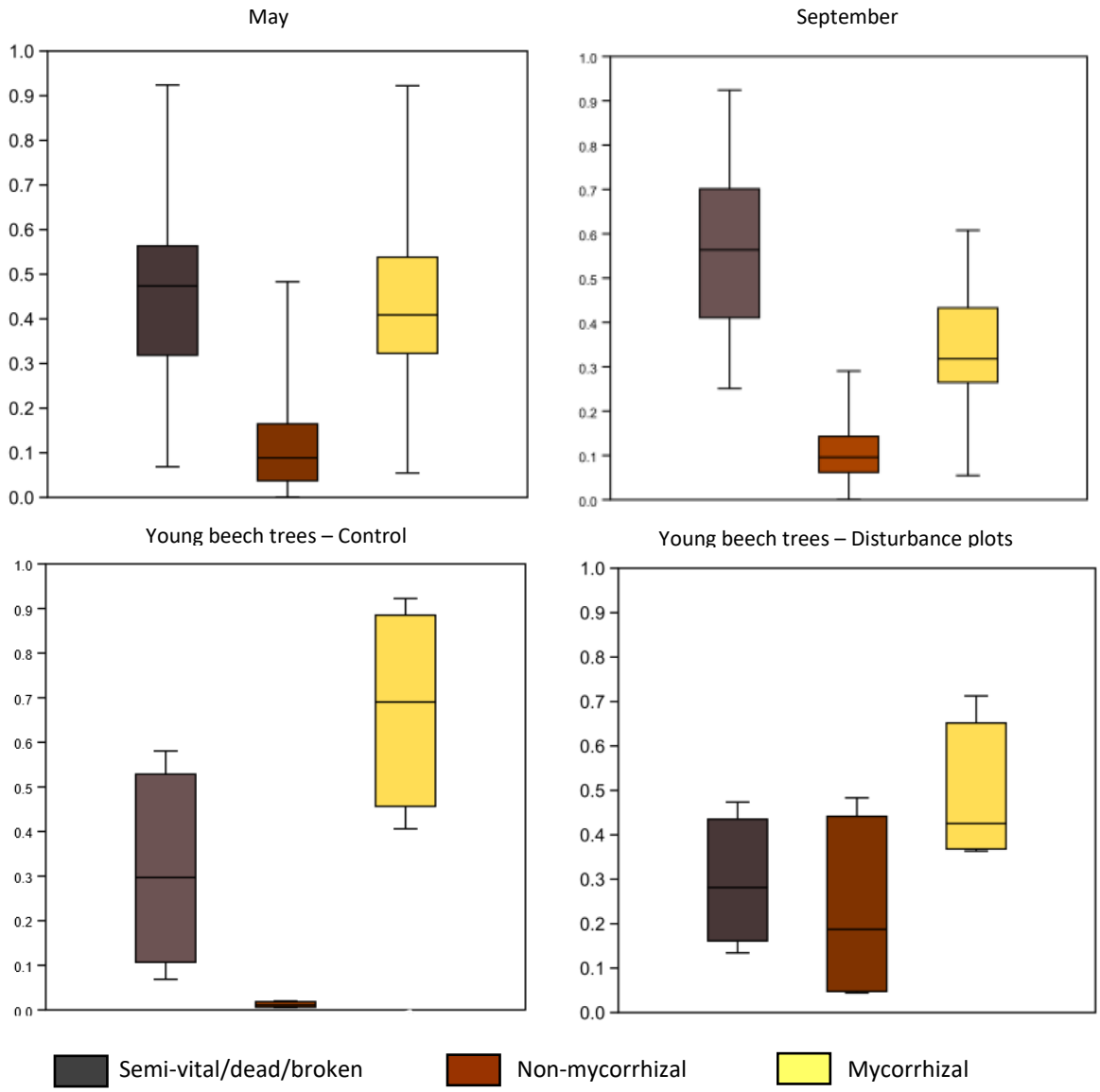

Figure 7. Degree of mycorrhization on root tips of May and September soil samples and from young beech trees

5.3. Morphotyping

Identified EM on tree root tips were photographed and categorized into morphotypes based on visual assessment and comparison with reference pictures and descriptions from Agerer and Rambold (2004 to 2018). The identified morphotypes from May samples amounted to 34 morphotypes, for September sampling to 30 morphotypes, and for young beech trees to 14 morphotypes.

Interestingly, DNA extraction of 96 samples had the result that species showed a wide range of morphotypes which required many amendments of the first classification that relied solely on the analysis of morphotypes. While some species showed a consistent morphotype (for an example see Figure 8 for *Lactarius helvus/pallidus*), other samples that were identified by DNA analysis to come from the same or closely related species showed a wide range of morphotypes (Figures 9 to 12), or samples showing a similar morphotype were identified as being from – often unrelated - species by DNA analysis (Examples Figures 13 to 16). Details regarding the assignment of samples to a specific species can be found in the accompanying information.

Figure 8. Morphotypes of Lactarius helvus/pallidus, *edited*

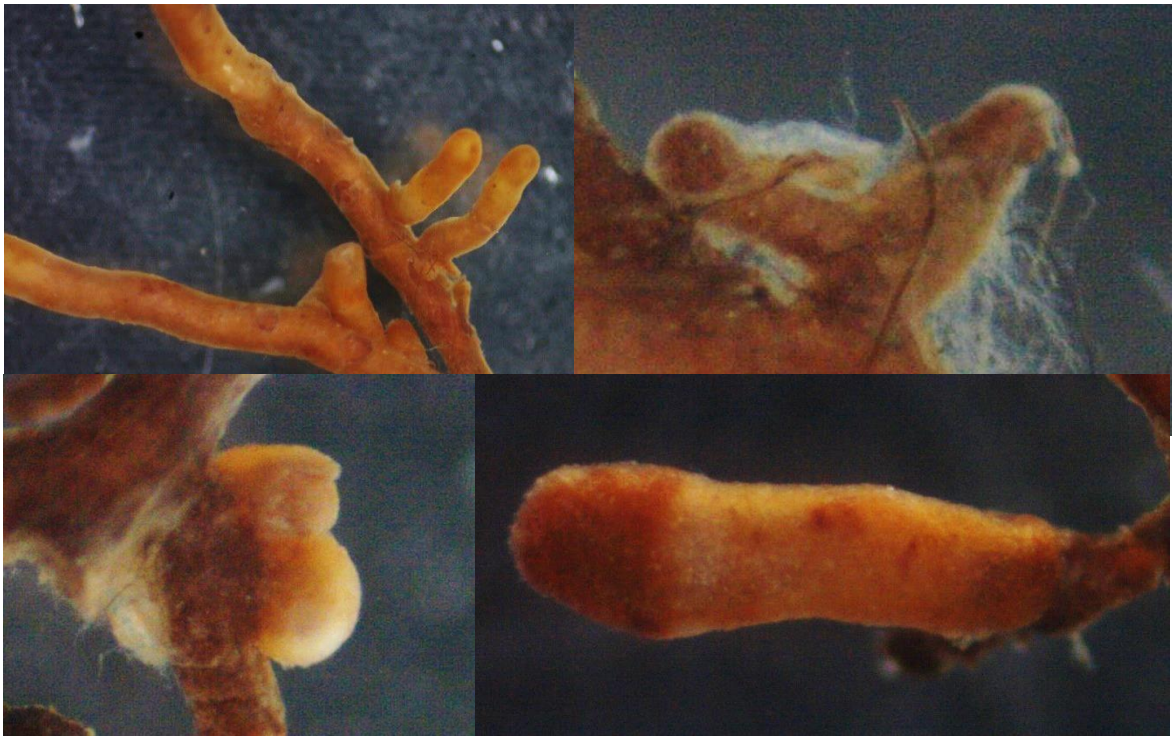

Figure 9. Morphotypes of Lactarius rubrocinctus, *edited*

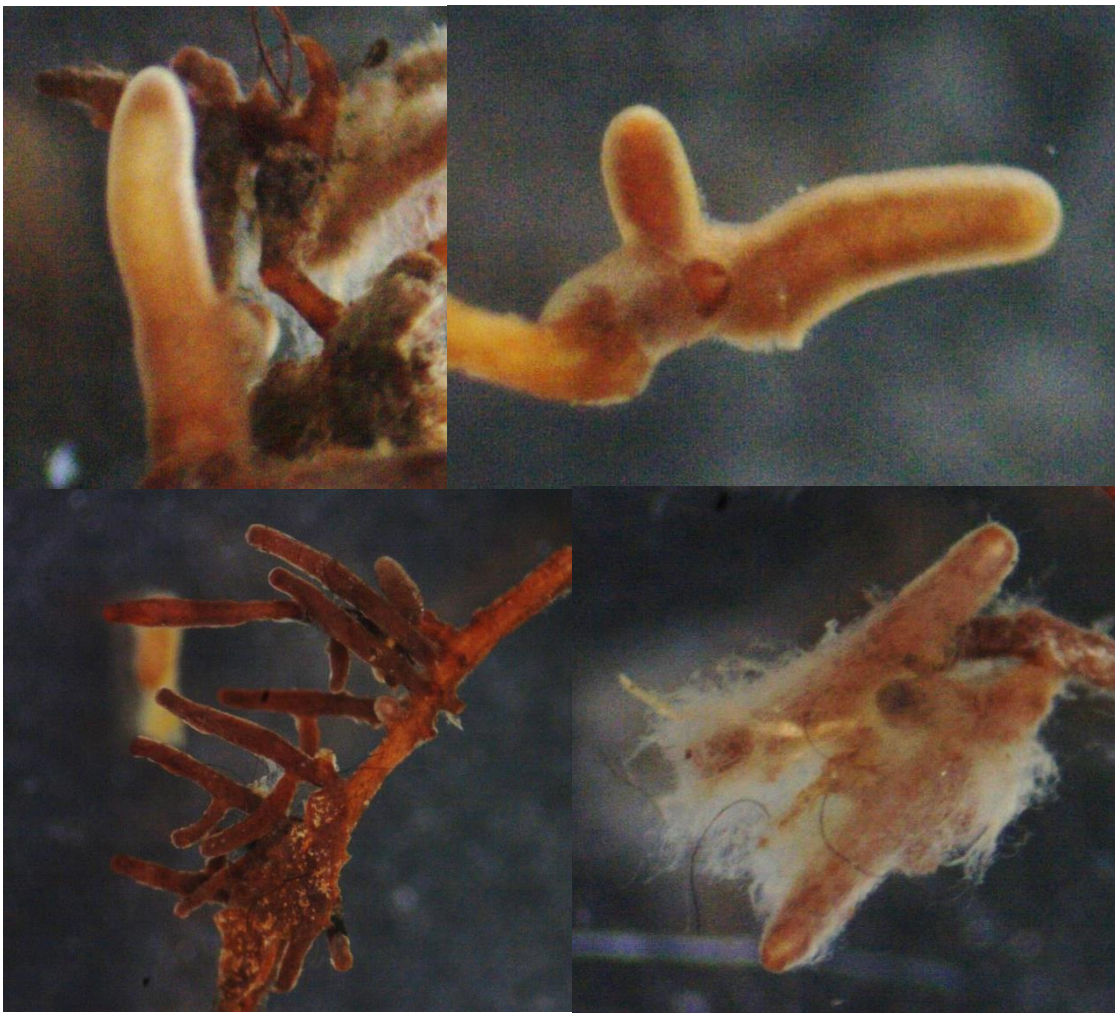

Figure 10. Morphotypes of Lactarius blennius, *edited*

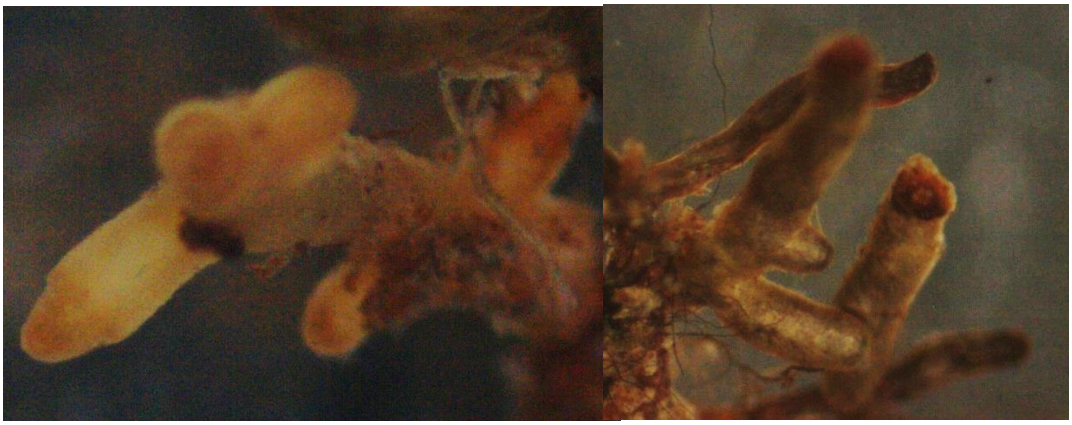

Figure 11. Morphotypes of Sebacina aff. incrustans, *edited*

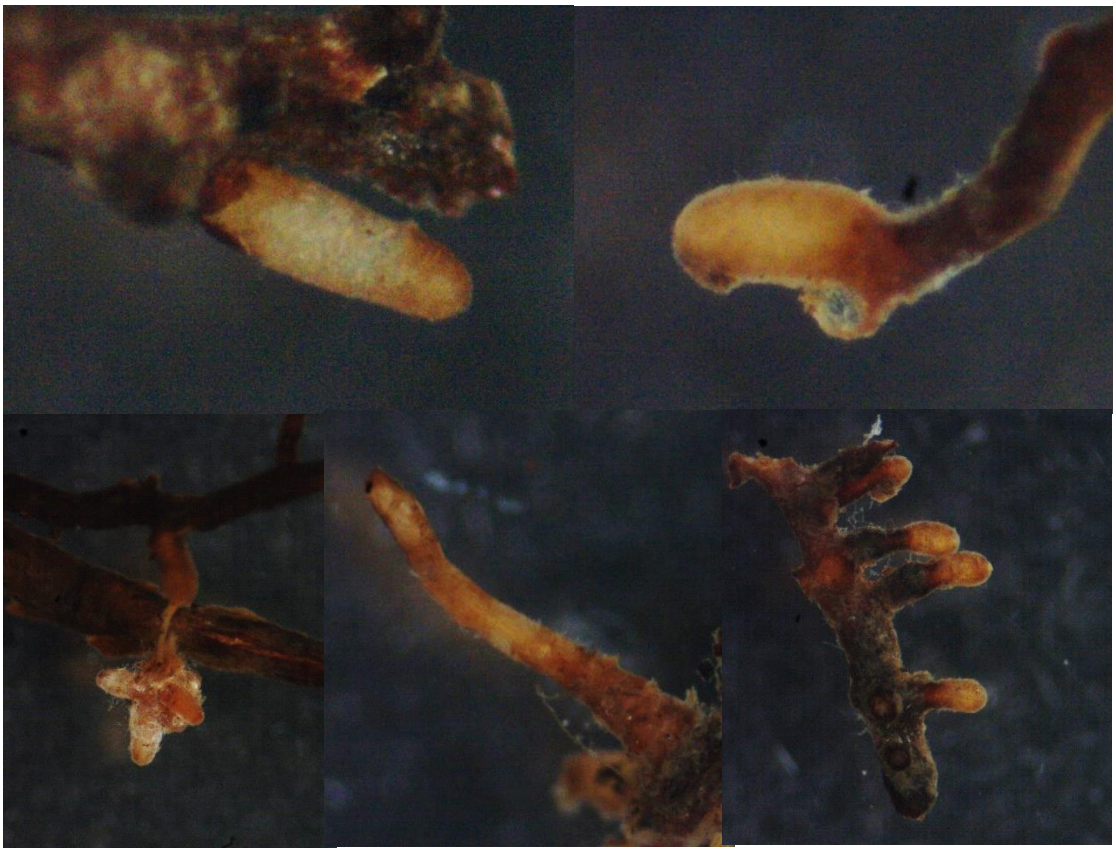

Figure 12. Morphotypes of Hygrophorus discoxanthus, *edited*

Figure 13. Morphotype of Hydnobolites sp., *edited*

Figure 14. Morphotype of Lactarius pallidus/helvus, *edited*

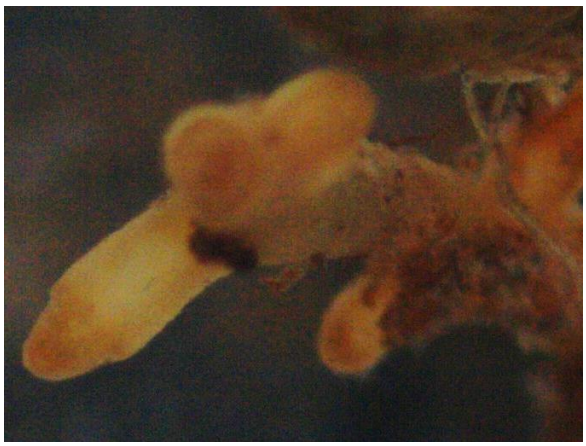

Figure 15. Morphotype of Sebacina aff. incrustans, *edited*

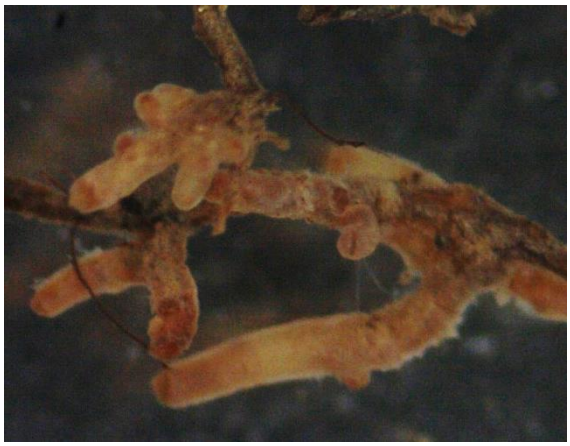

Figure 16. Morphotype of Inocybe hirtella *var.* bispora*, edited*

5.4. DNA analysis

Based on results from DNA extraction, the presence and abundance of EM on tree root tips is summarized in Figure 17. In total, 44 species were identified by DNA analysis. Two additional species were assigned based on the morphotypes, as DNA sequencing did not give a valid result but the morphotypes suggested two *Tomentella* species (*Tomentella* sp. 7 and 8). 6 mycorrhizal root tips from May sampling, amounting to a percentage of 0.3 %, could not be assigned visually as the quality of the microscope pictures was insufficient.

29 species were encountered in soil samples from May, 31 species in soil samples from September, 15 species on young beech trees in the control plot and 11 species on young beech trees in the treatment plots of Block 1 in September. 8 species were exclusively found in soil samples from May, 9 species solely in soil samples from September, and 5 species were only encountered on roots of young beech trees. Species richness and abundance were highly variable between blocks, between seasons and between roots from soil samples and young beech root tips. As regards abundance patterns, in May, 9 species made up 82 % of total abundance, whereas in September abundance was more evenly distributed with 15 species making up 81 % of total abundance.

Looking at the distribution of EM on root tips in the four blocks in May and September, it can be deduced that even though many species can be found in more than one block, different species tend to be dominant in each block. Figure 17 shows the changes in species community composition and abundance between May and September in the four blocks. The figures suggest a major change in community composition with many of the abundant species from May becoming less frequent in September or being completely absent. Their role was taken over mainly by species that had not been encountered in that block in May.

Species	May 1C	May 2C1	May 2C2	May 3C	Sep 1C	Sep 2C1	Sep 2C2	Sep 3C	1CB	1B
Cortinarius aff. *ferrugineovelatus*										
Hebeloma sinapizans										
Lactarius helvus/pallidus										
Lactarius rubrocinctus										
Lactarius blennius										
Peziza depressa										
Hydnobolites sp.										
Hygrophorus discoxanthus										
Hygrophorus unicolor										
Sebacina sp. 1										
Sebacina sp. 2										
Sebacina sp. 3										
Sebacina sp. 4										
Sebacina sp. 5										
Sebacina sp. 6										
Sebacina incrustans sp. 1										
Sebacina incrustans sp. 2										
Sebacina aff. *incrustans*										
Sebacina flagelliformis										
Sebacina epigaea										
Tomentella sp. 1										
Tomentella sp. 2										
Tomentella sp. 3										
Tomentella sp. 4										
Tomentella sp. 5										
Tomentella sp. 6										
Tomentella pilosa										
Pseudotomentella sp.										
Thelephora sp.										
Tomentella sp. 7										
Tomentella sp. 8										
Elaphomyces barrioi										
Tuber puberulum										
Inocybe curcumina										
Inocybe cf. *fuscidula/splendens*										
Inocybe glabripes										
Inocybe erinaceomorpha										
Inocybe hirtella var. *bispora*										
Hyaloscyphaceae sp. 1										
Hyaloscyphaceae sp. 2										
Hyaloscyphaceae sp. 3										
Tarzetta catinus										
Clavulinaceae sp.										
Cenococcum geophilum										
Hypholoma sublateritium										
Hysterangium sp.										
not assigned										

Figure 17. Presence of EM on tree root tips and their relative abundances in this block/on young beech trees

Species distribution among the samples was highly uneven (Figure 18). More than 55 % of all species (i.e. 26 out of 47) were found only in 1, 2 or 3 samples from a total of 35 samples. The maximum number was a single species that was encountered in 13 samples. This uneven distribution highlights the high diversity of EM at the Molln experimental site but makes statistically significant comparisons between blocks, seasons and sample types (soil cores vs. young trees) difficult.

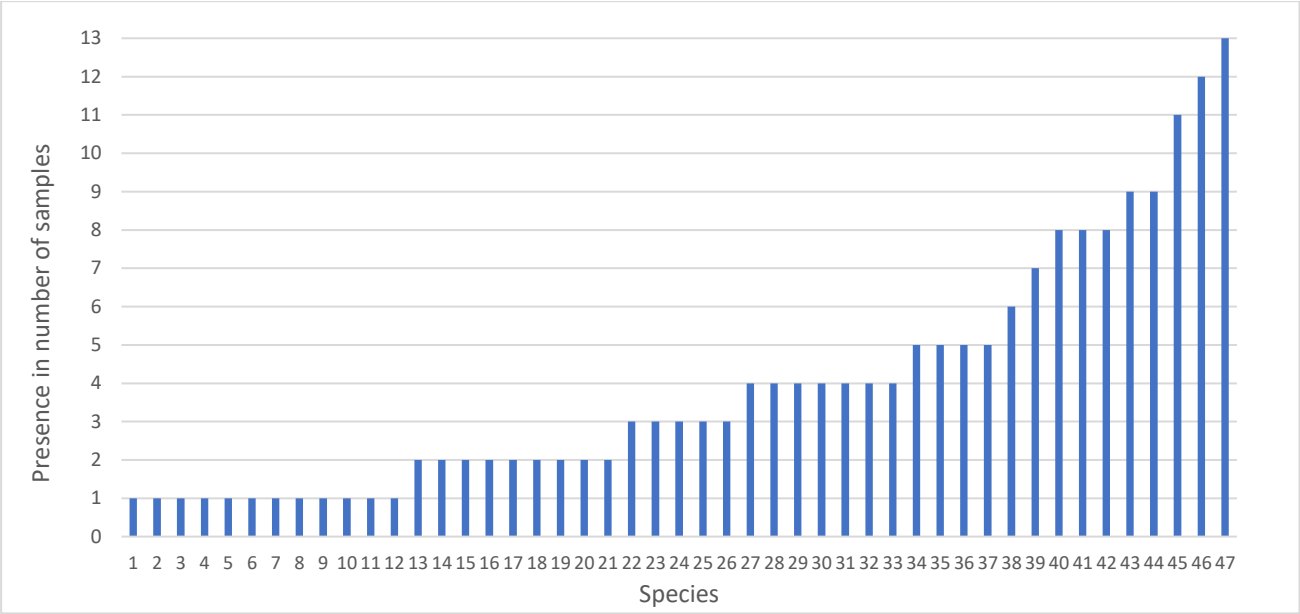

Figure 18. Species presence in number of samples

Figures 19 to 21 show an overview of the abundance of encountered taxa in May, September and on young beech trees, on family, genus and species level. The highest proportion of species in May, September and on young beech trees came from the family of Sebacinaceae (Figure 19). The families Thelephoraceae, Hyaloscyphaceae and Cortinariaceae were also encountered in all samplings, whereas Inocybaceae could be found in May samples only in small quantities, whereas they were abundant in September soil and young tree samples. Hygrophoraceae and Russulaceae from the genus *Lactarius* were only encountered in soil samples of presumably mature trees but not on roots of young trees (Figure 20).

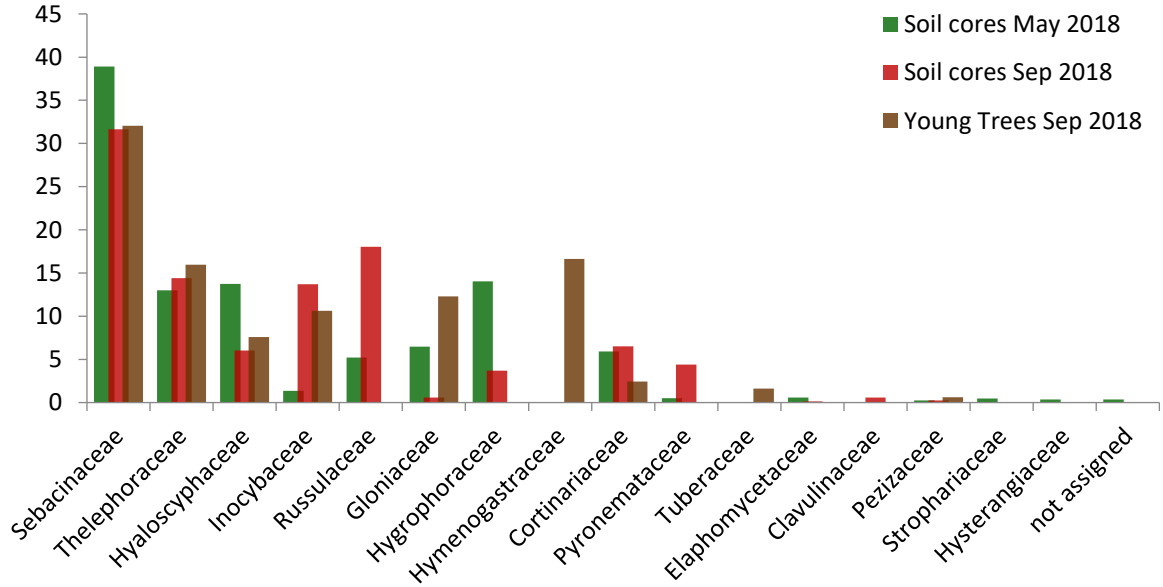

Figure 19. Abundance of EM on tree root tips (family level)

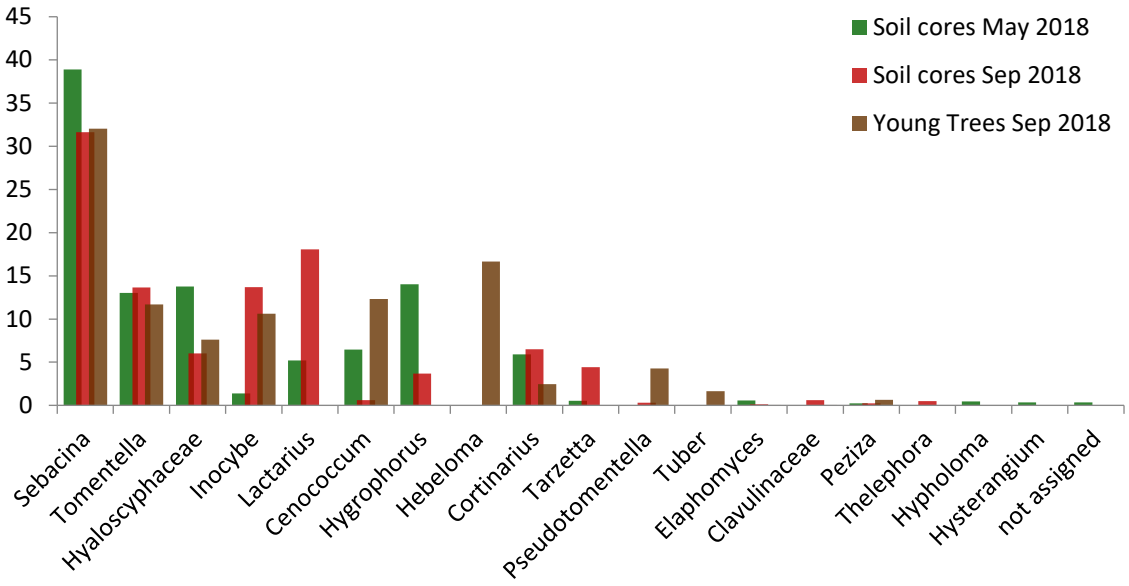

Figure 20. Abundance of EM on tree root tips (genus level)

While the two *Hygrophorus* species changed roles between seasons, with *Hygrophorus unicolor* being one of the most abundant species in May and *Hygrophorus discoxanthus* present only in September, Russulaceae of the genus *Lactarius* had a higher abundance and species variety in September (Figure 21). The species *Hebeloma sinapizans* from the family of Hymenogastraceae could only be found on root tips of young beech trees in the control plot of Block 1, where it nevertheless was present on almost 30 % of all root tips. *Cenococcum geophilum* was present in all samplings, but it was only abundant in May samples and on young beech trees in September and did not play an important role in September soil samples.

Tables 1 and 2 show the 10 species with highest abundance in May and September soil samples and their corresponding abundance in the second sampling. Four of the most abundant species in May were not encountered in September samples (Table 1), whereas four species, namely an uncultured *Sebacina* species (*Sebacina sp. 3*), an uncultured *Tomentella* species (*Tomentella sp. 1*), *Cortinarius* aff. *ferrugineovelatus* and *Lactarius blennius*, were abundant in both samplings. Three of the abundant species in September were not encountered in May (Table 2).

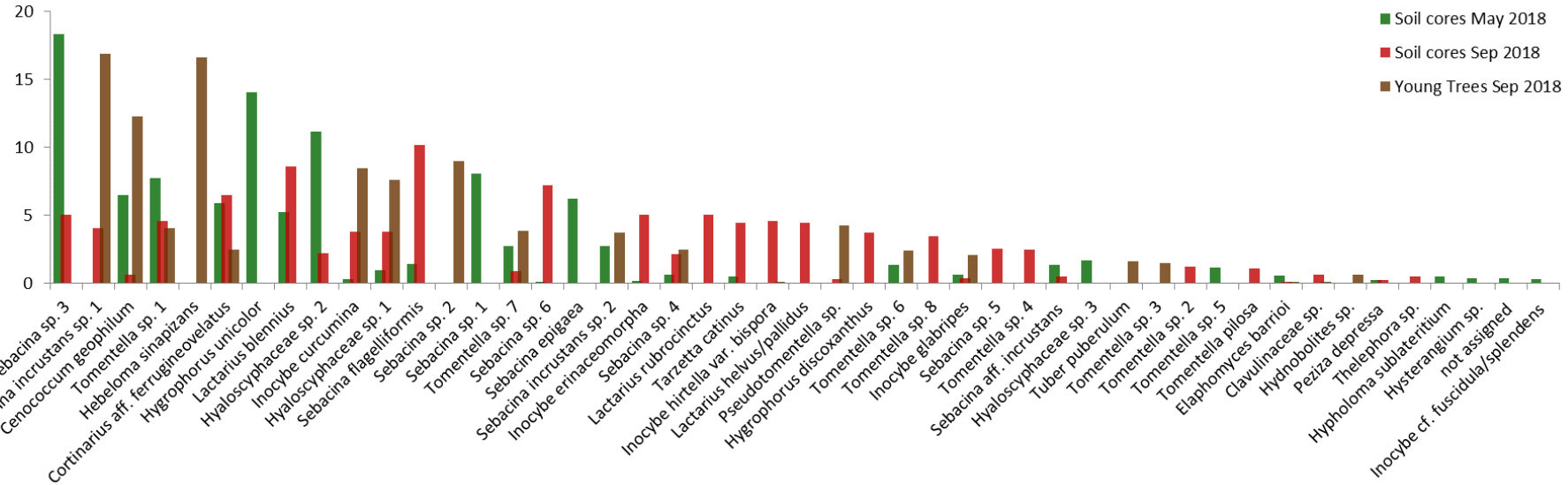

Figure 21. Abundance of EM on tree root tips (species level)

Species	Family	Sum May	Sum Sep
Sebacina sp. 3	Sebacinaceae	**18,13%**	5,01%
Hygrophorus unicolor	Hygrophoraceae	**13,88%**	
Hyaloscyphaceae sp. 2	Hyaloscyphaceae	**11,05%**	2,21%
Sebacina sp. 1	Sebacinaceae	**7,99%**	
Tomentella sp. 1	Thelephoraceae	**7,65%**	4,59%
Cenococcum geophilum	Gloniaceae	**6,40%**	0,60%
Sebacina epigaea	Sebacinaceae	**6,12%**	
Cortinarius aff. *ferrugineovelatus*	Cortinariaceae	**5,84%**	6,50%
Lactarius blennius	Russulaceae	**5,16%**	8,58%
Sebacina incrustans sp. 2	Sebacinaceae	**2,72%**	

Table 1. Ten most abundant species in May soil samples

Species	Family	Sum May	Sum Sep
Sebacina flagelliformis	Sebacinaceae	1,42%	**10,19%**
Lactarius blennius	Russulaceae	5,16%	**8,58%**
Sebacina sp. 6	Sebacinaceae	0,11%	**7,21%**
Cortinarius aff. ferrugineovelatus	Cortinariaceae	5,84%	**6,50%**
Lactarius rubrocinctus	Russulaceae		**5,01%**
Sebacina sp. 3	Sebacinaceae	18,13%	**5,01%**
Inocybe erinaceomorpha	Inocybaceae	0,17%	**5,01%**
Tomentella sp. 1	Thelephoraceae	7,65%	**4,59%**
Inocybe hirtella var. bispora	Inocybaceae		**4,59%**
Lactarius helvus/pallidus	Russulaceae		**4,47%**

Table 2. Ten most abundant species in September soil samples

For the visual presentation of distribution patterns between samples, Figures 22 and 23 show a Correspondence Analysis based on species abundance data. In Figure 22 there is an overview of all blocks with respective species, whereas Figure 23 gives a close-up of proximities for all samplings without showing species details.

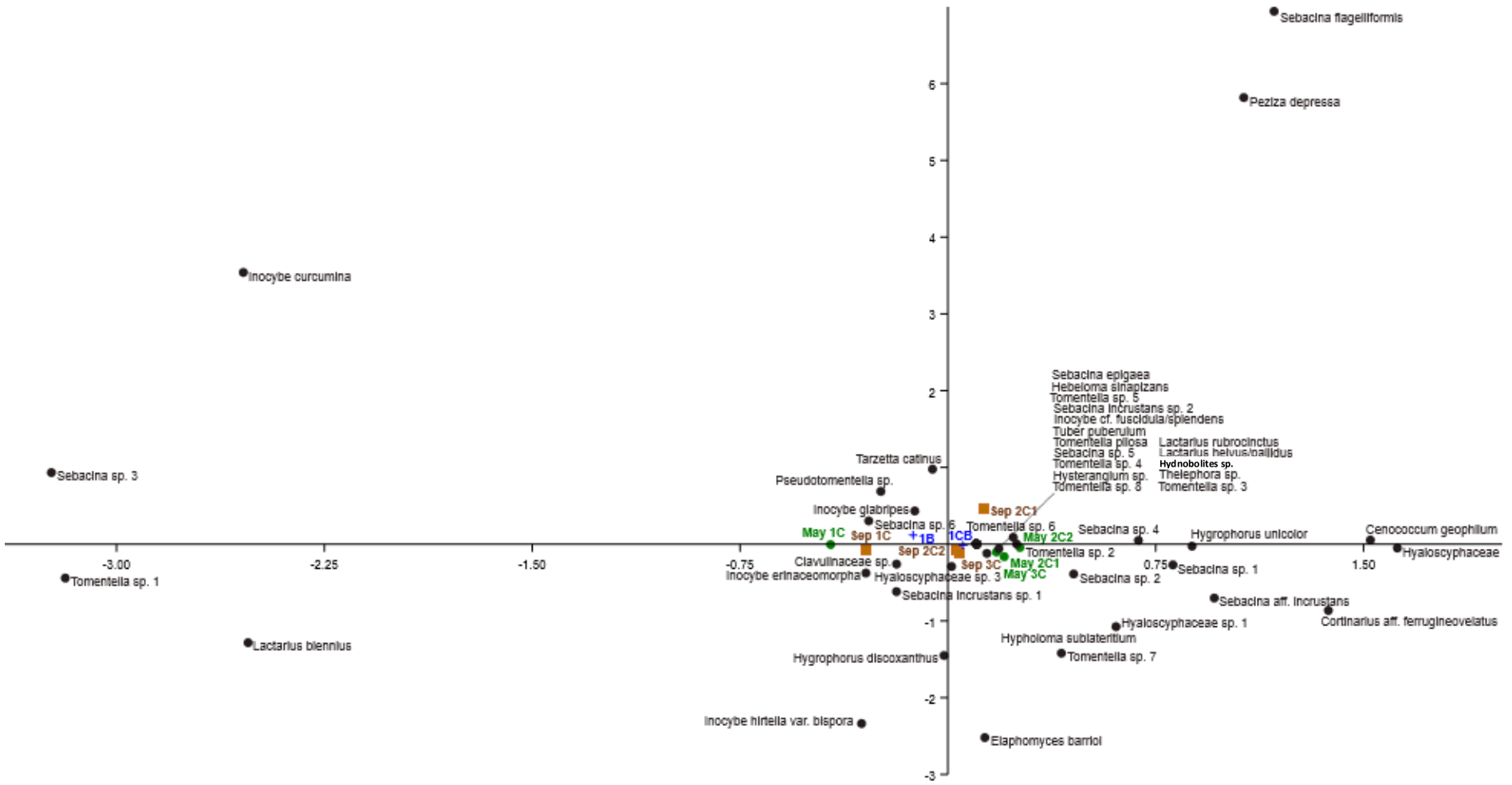

Figure 22. Correspondence Analysis of based on abundance of species

The Correspondence Analysis shows a proximity among May samples and, to a lesser extent, also among September samples of Blocks 2C1, 2C2 and 3C, whereas Block 1C stands out because of three dominant species, an uncultured *Sebacina* species (*Sebacina* sp. 3) in May, *Lactarius blennius* in September and, on both sampling dates, an uncultured *Tomentella* species (*Tomentella* sp. 1). In September, Plot 2C1 stands apart as it has a large number of species only encountered in this plot (7 out of 17) and a high abundance of *Sebacina flagelliformis*, which was encountered in all blocks in September and in three of the four blocks in May, although in the blocks 1C, 2C2 and 3C at lower abundance rates.

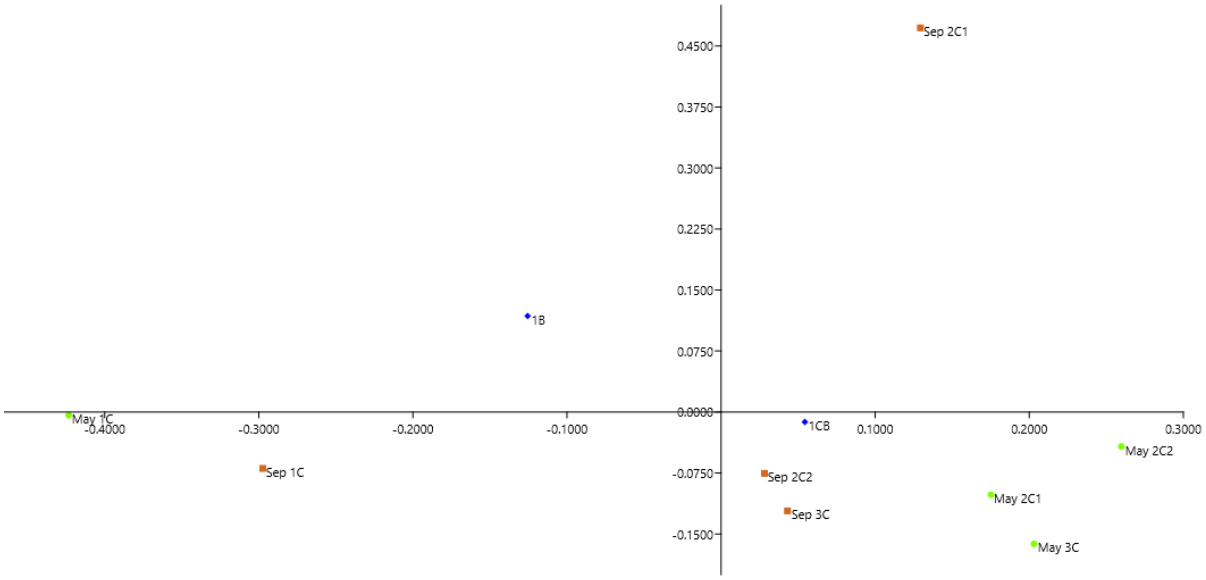

Figure 23. Correspondence Analysis based on abundance of species (close-up of blocks and young beech trees)

When looking at the family level, the proximity of families with different blocks and samplings is depicted in Fig. 24. While May samples of plots 2C1 and 2C2 have a higher share of species of the Gloniaceae, Hyaloscyphaceae and Hygrophoraceae families, in September there seems to be a shift to families like the Thelephoraceae or Sebacinaceae, which had also been important in May samples of plots 1C and 3C. The family of Russulaceae is especially important in September samples of plots 1C and 3C, the family of Inocybaceae only played a major role in September samples.

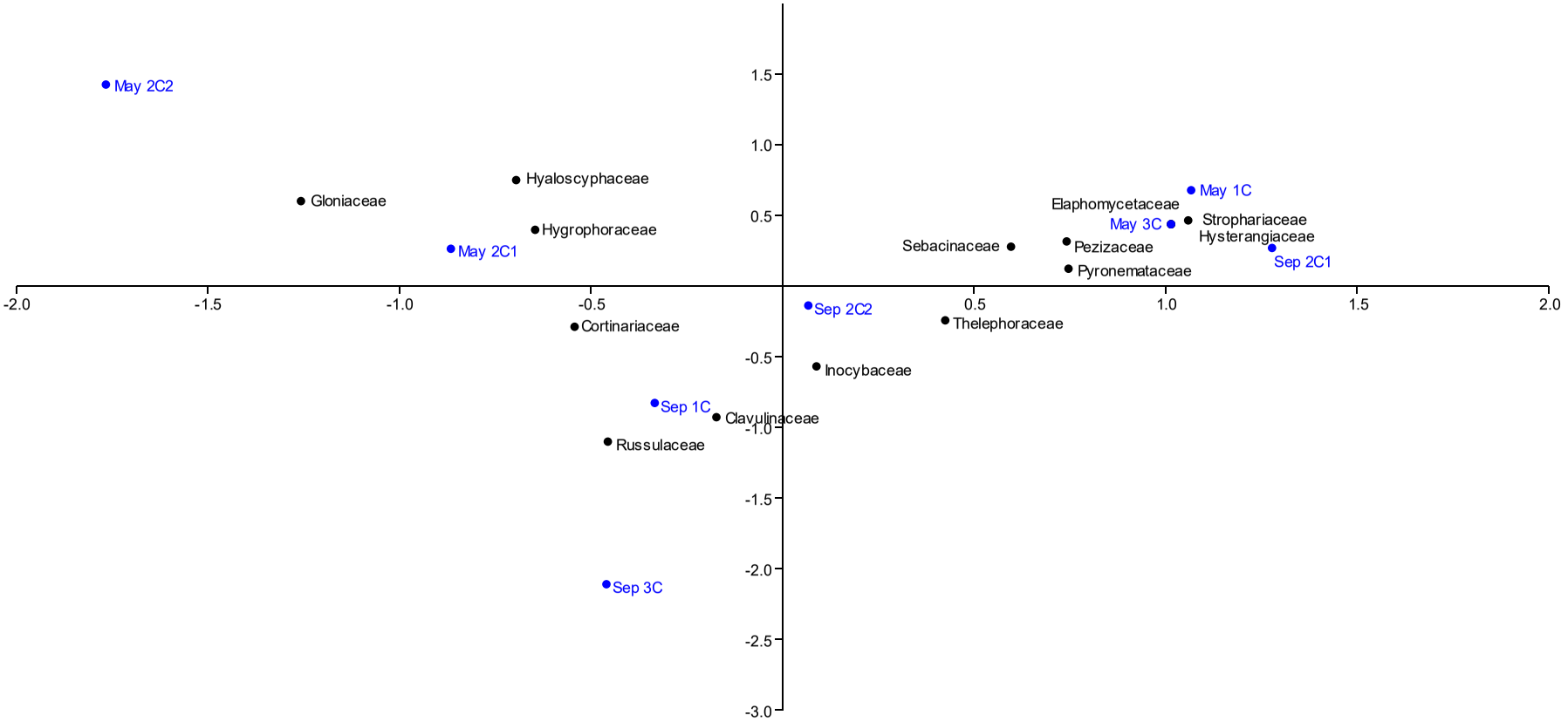

Figure 24. Correspondence Analysis of May and September soil samples based on abundance of families

Looking at the abundance values of EM species on root tips of young beech trees from Block 1 in September, similar patterns as in soil samplings can be observed. Species abundance levels vary highly between young beech trees of control and treatment plots, as well as between samples of young trees and soil samples. In each sampling there are different dominant species, a majority of all encountered species (16 of 25) were only encountered either on young trees of control or treatment plots or in September soil samples and there were only 4 species that were encountered in all three samplings.

Tables 3 and 4 show the ten most abundant species present on young beech trees of control and treatment plots of Block 1 and their respective levels in the other samplings. The most abundant species on young beech trees from the control plot was not encountered on young beech trees from treatment plots and vice versa. On young beech trees from the control plot a higher number of species (15) was found compared to beech trees from treatment plots (11), whereas in both cases the five most abundant species colonized around 80 % of all mycorrhizal root tips (80,86 % in treatment plots versus 81,73 % in control plot).

Species	**Family**	**1CB**	**1B**	**1C**
Hebeloma sinapizans	Hymenogastraceae	**28,65%**		
Cenococcum geophilum	Gloniaceae	**18,16%**	4,20%	0,60%
Sebacina sp. 2	Sebacinaceae	**14,92%**	0,75%	
Hyaloscyphaceae sp. 1	Hyaloscyphaceae	**13,08%**		3,81%
Tomentella sp. 1	Thelephoraceae	**6,92%**		4,59%
Tomentella sp. 7	Thelephoraceae	**6,59%**		0,89%
Cortinarius aff. ferrugineovelatus	Cortinariaceae	**3,78%**	0,60%	6,50%
Pseudotomentella sp.	Thelephoraceae	**2,59%**	6,60%	0,30%
Tomentella sp. 3	Thelephoraceae	**2,49%**		
Hydnobolites sp.	Pezizaceae	**1,08%**		

Table 3. Ten most abundant species in September samples of young beech trees in the control plot (1CB) compared to young beech trees of treatment plots (1B) and soil samples of Block 1 (1C)

Species	**Family**	**1CB**	**1B**	**1C**
Sebacina incrustans sp. 1	Sebacinaceae		**40,33%**	4,05%
Inocybe curcumina	Inocybaceae	0,65%	**19,34%**	3,75%
Sebacina incrustans sp. 2	Sebacinaceae		**8,85%**	
Pseudotomentella sp.	Thelephoraceae	2,59%	**6,60%**	0,30%
Tomentella sp. 6	Thelephoraceae		**5,70%**	
Inocybe glabripes	Inocybaceae		**4,95%**	0,36%
Sebacina sp. 4	Sebacinaceae	0,76%	**4,80%**	2,15%
Cenococcum geophilum	Gloniaceae	18,16%	**4,20%**	0,60%
Tuber puberulum	Tuberaceae		**3,90%**	
Sebacina sp. 2	Sebacinaceae	14,92%	**0,75%**	

Table 4. Ten most abundant species in September samples of young beech trees in treatment plots (1B) compared to young beech trees of control plot (1CB) and soil samples of Block 1 (1C)

When doing a Correspondence Analysis of the species distribution of EM on root tips of young beech trees compared to those of September and May soil samples in Block 1, we see a diverse picture (Fig.

25): not only do species and their abundances differ to a high degree between soil samplings in May and September, also species on young beech trees are different based on whether the samples come from beech trees in control plots or from treatment plots. Interestingly, of all the 30 species that were encountered in Block 1 from all samplings, only 1 species, namely an uncultered *Sebacina* species (*Sebacina* sp. 4) was encountered in May and September soil samples, as well as on young beech trees of control and treatment plots. In contrast, 16 out of the 30 species were only found in one of the four samplings.

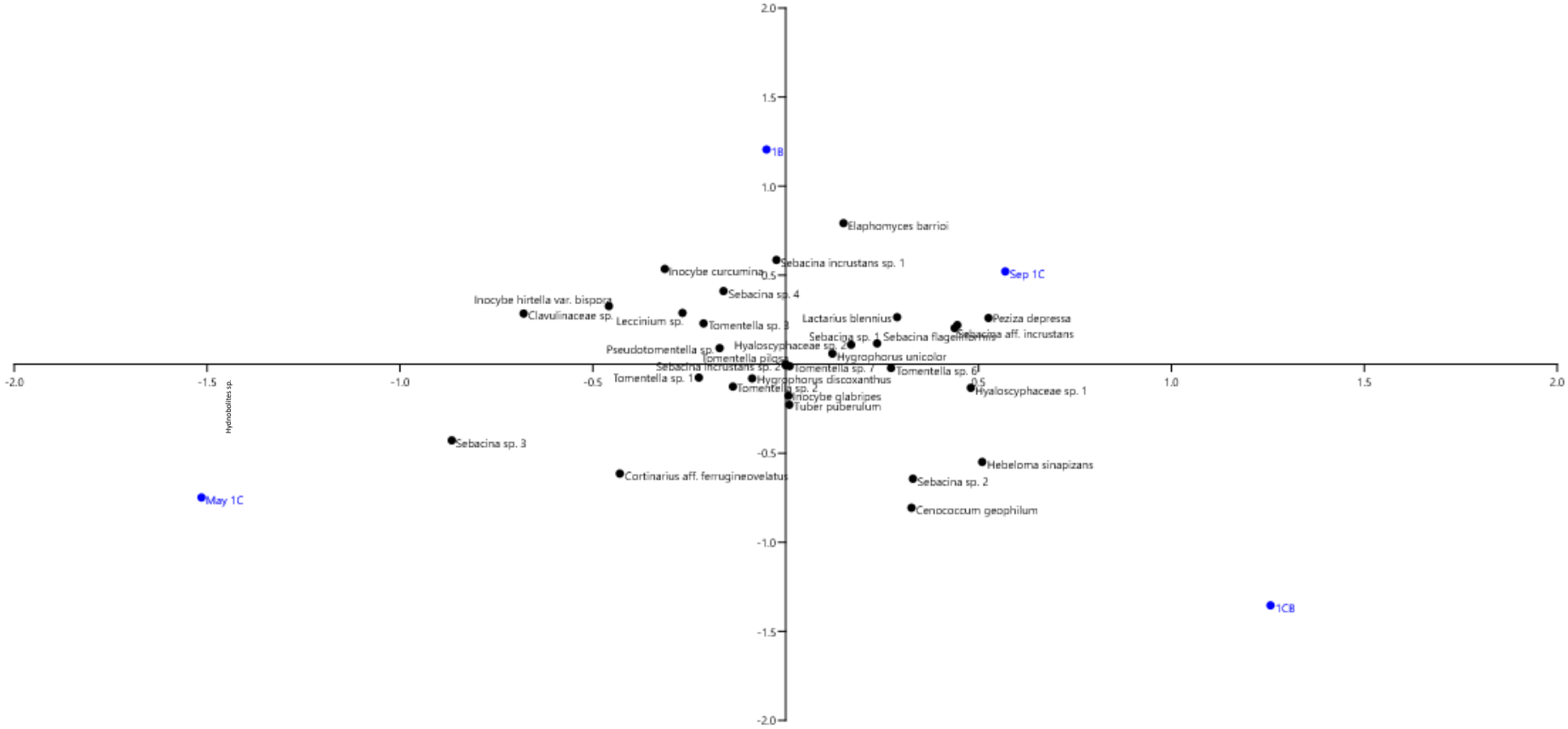

Figure 25. Correspondence Analysis of Analysis of Block 1 May (May 1C) and September (Sep 1C) soil samples and samples of young beech trees of control (1CB) and treatment plots (1B)

Looking at the family level, Fig. 26 shows a correspondence analysis of Block 1 soil and young beech tree samples. While in September soil samples, species of the families of the Russulaceae are prominent, species of the Hymenogastraceae and Gloniaceae have a higher share on young beech trees of the control plot whereas species from May soil samples of young beech trees from treatment plots are closer to the Sebacinaceae. *Tuber puberulum*, as the only encountered species of the Tuberaceae, is only present on young beech trees of treatment plots.

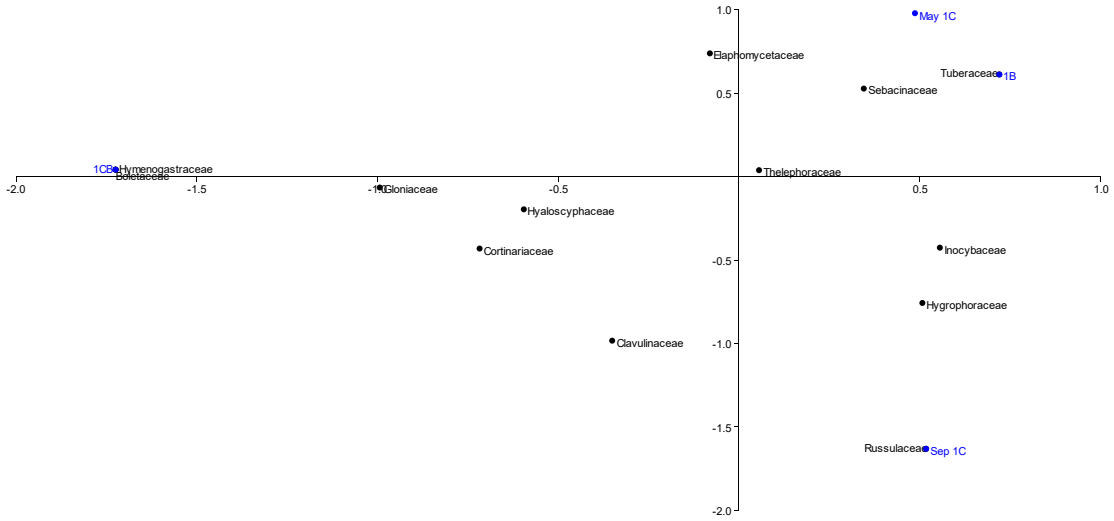

Figure 26. Correspondence Analysis on family level of Block 1 May (May 1C) and September (Sep 1C) soil samples and samples of young beech trees of control (1CB) and treatment plots (1B)

5.5. Exploration Types

In order to analyze whether the temporal change of EM communities is reflected in a change in Exploration Types, all encountered species were assigned to an Exploration Type (Table 5) based on the assignment of Agerer (2006). For species where no Exploration Type could be found in literature, they were assigned to an Exploration Type based on visual characteristics in the microscope pictures. These Exploration Types are marked with an * in Table 5.

Species	**Family**	**Exploration Type**
Lactarius helvus/pallidus	Russulaceae	C
Lactarius rubrocinctus	Russulaceae	C
Lactarius blennius	Russulaceae	C
Peziza depressa	Pezizaceae	SD°
Hygrophorus discoxanthus	Hygrophoraceae	SD*
Hygrophorus unicolor	Hygrophoraceae	SD*
Sebacina sp. 1	Sebacinaceae	SD
Sebacina sp. 2	Sebacinaceae	SD

Sebacina sp. 3	Sebacinaceae	SD
Sebacina sp. 4	Sebacinaceae	SD
Sebacina sp. 5	Sebacinaceae	SD
Sebacina sp. 6	Sebacinaceae	SD
Sebacina incrustans sp. 1	Sebacinaceae	SD
Sebacina incrustans sp. 2	Sebacinaceae	SD
Sebacina aff. incrustans	Sebacinaceae	SD
Sebacina flagelliformis	Sebacinaceae	SD
Sebacina epigaea	Sebacinaceae	SD
Tomentella sp. 1	Thelephoraceae	SD*
Tomentella sp. 2	Thelephoraceae	SD*
Tomentella sp. 3	Thelephoraceae	SD*
Tomentella sp. 5	Thelephoraceae	SD*
Tomentella sp. 6	Thelephoraceae	SD*
Tomentella pilosa	Thelephoraceae	SD*
Pseudotomentella sp.	Thelephoraceae	SD
Tomentella sp. 7	Thelephoraceae	SD*
Tomentella sp. 8	Thelephoraceae	SD*
Tomentella sp. 4	Thelephoraceae	MD*
Thelephora sp.	Thelephoraceae	MD
Elaphomyces barrioi	Elaphomycetaceae	SD
Tuber puberulum	Tuberaceae	SD
Inocybe curcumina	Inocybaceae	SD
Inocybe cf. *fuscidula/splendens*	Inocybaceae	SD
Inocybe glabripes	Inocybaceae	SD
Inocybe erinaceomorpha	Inocybaceae	SD
Inocybe hirtella var. *bispora*	Inocybaceae	SD
Hyaloscyphaceae sp. 1	Hyaloscyphaceae	SD*
Hyaloscyphaceae sp. 2	Hyaloscyphaceae	SD*
Hyaloscyphaceae sp. 3	Hyaloscyphaceae	SD*
Tarzetta catinus	Pyronemataceae	SD*
Clavulinaceae sp.	Clavulinaceae	SD*
Cenococcum geophilum	Gloniaceae	SD
Hypholoma sublateritium	Strophariaceae	SD*
Cortinarius aff. *ferrugineovelatus*	Cortinariaceae	MD
Hebeloma sinapizans	Hymenogastraceae	MD*
Hysterangium sp.	Hysterangiaceae	MD
Hydnobolites sp.	Pezizaceae	C/SD°
not assigned		

Table 5. Exploration Types of encountered species

*Exploration Types marked with * were assigned based on microscope pictures, exploration types marked with ° based on Tedersoo and Smith (2013), exploration types of all other species are based on Agerer (2006)*

Figure 27 shows the distribution of Exploration Types of species from May and September soil samples and of young beech trees in control (1CB) and treatment plots (1B). In all samplings a large majority of around 95 % consisted of species with a Short Distance (SD) Exploration Type. While on root tips from soil samples species with a Contact (C) Exploration Type were encountered, in some plots even at high abundance, they were absent on root tips of young beech trees. In contrast to the other samplings, young beech trees of control plots had a higher proportion of species with a Medium Distance (MD) Exploration Type and the only species with a Long Distance (LD) Exploration Type.

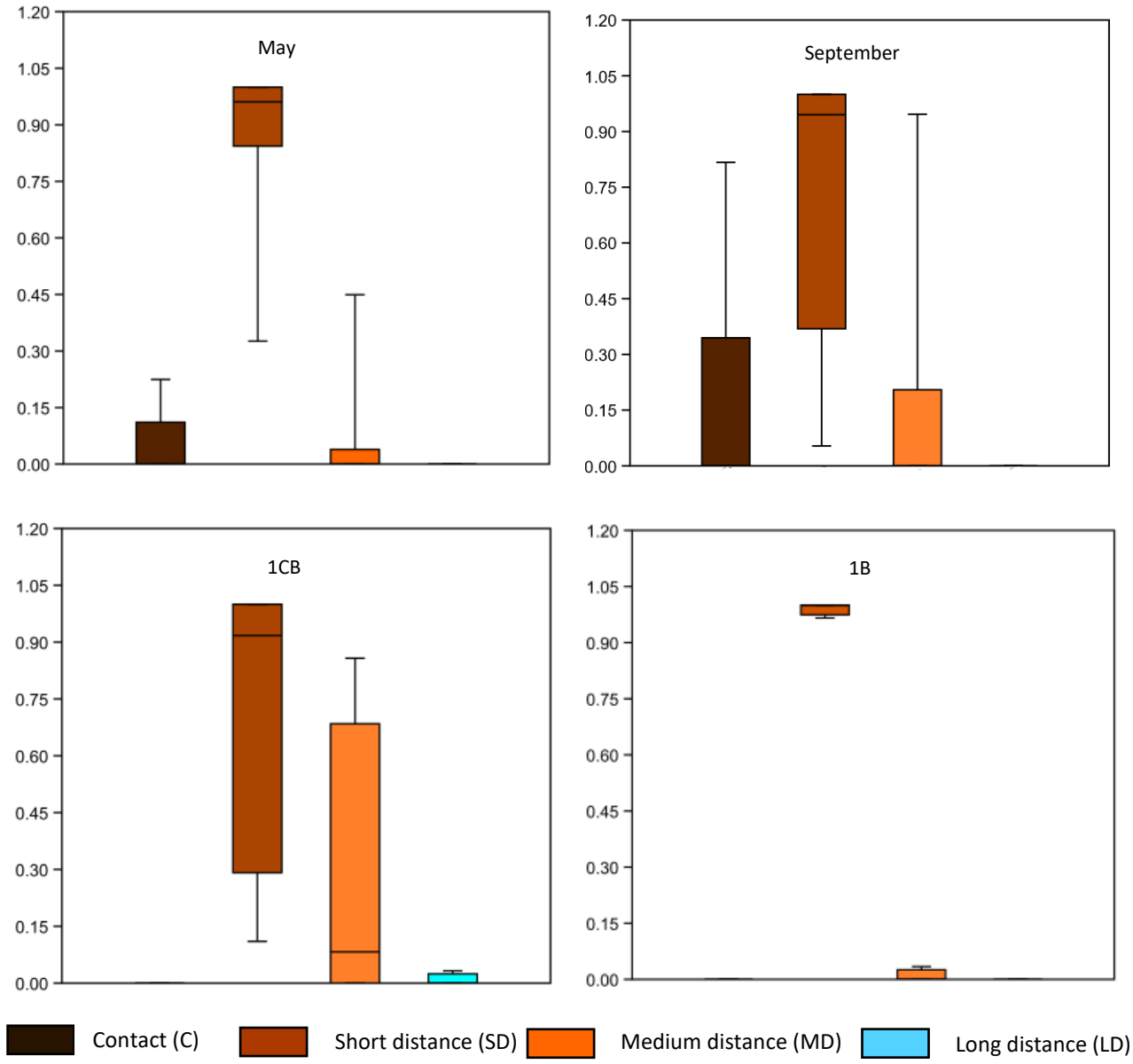

Figure 27. Distribution of Exploration Types of species in May and September soil samples and on young beech trees from control (1CB) and treatment plots (1B)

6. Discussion

The finding that tree roots in treatment plots were dead three years after treatment establishment, made comparisons between EM on fine root tips in disturbance treatment plots and control plots impossible. Even though in some samples single roots seemed to be alive, their visual appearance gave the impression that they were coming from young beech trees growing in or close to the sampling

spots. The observation that on some dead root tips EM mantles were still recognizable, could be taken as indication for active EM. Nevertheless, as all of these EM were black, it is more likely that these structures were recalcitrant to degradation. A study of Fernandez et al. (2013) showed that black EM species like *Cenococcum geophilum* had 4 to 10 times longer degradation times compared to other EM which they attributed to the high melanin contents of their mantles. Studies regarding the persistence of metabolically active EM on root tips after clear-cut treatment establishment show differing results. Even though some researchers could not find active EM on tree root tips two years after clear-cut treatment establishment (Harvey et al., 1980, Parsons et al., 1994, cited in Hagerman et al., 1999), Hagerman et al. (1999) found seemingly active EM even three years after treatment establishment which they attributed to saprophytic capabilities of some EM species.

Looking at the EM community composition in control plots and on young beech trees, it can be concluded that there seems to be hardly any pattern behind the distribution and abundance of observed ectomycorrhizal fungi. Less than half of the species could be found in more than three of the 35 samples and also abundant species often were only abundant in some samples or plots while they were rare or could not be found in others. This suggests a high diversity of species in a relatively small area. Nevertheless, the fact that 27 out of the encountered 46 species were found in more than one block without a clear correlation between them, could indicate that all blocks share a common species pool where environmental conditions and/or microbial or plant interactions of a specific microsite are the decisive factors for the successful formation of a symbiotic relationship of an EM species with a tree.

The mechanisms behind the overrepresentation of one EM species compared to another are still largely not understood. Nevertheless, the encountered distribution seems to be typical for EM communities in temperate regions. When summing up studies of 98 sites from EM communities on beech, spruce and pine trees across Europe, Rosinger et al. (2018) found that research sites usually were characterized by communities with a unique profile. While there was a low number of abundant species present across sites, on beech trees more than 80 % of all species were only encountered in one or two sites. Even though many of these species were found at low abundances, others represented the dominant taxa at these sites. From their results they drew the conclusion that abundant species across various sites had a low host-specificity and were able to thrive within a larger band of environmental parameters, whereas rare species were dependent on their ecological niche. Nevertheless, mechanisms are largely unclear, as when looking at their functional roles, EM communities seem to be highly dynamic: on the one hand, EM communities consisting of different species and living in different ecosystems were shown to fulfill the same functional roles (Wang et al., 2017), whereas on the other hand, communities were also able to adapt to new environments and adjust their functional responses when they were transferred between sites (Jones and Nicholson, 2017).

A drawback for the study of EM communities is that there still is a high number of species which have not been clearly described yet, e.g. in our study, of the 44 species identified by DNA analysis, 23 could not be assigned to a described species so far. Even though for most of them DNA alignments with a similarity of more than 97 % could be found in the UNITE database, these sequences usually came from uncultured species, which makes statistical comparisons across studies difficult. For this purpose, Tedersoo et al. (2017), who, in a comprehensive study of soil inhabiting fungi from 38 countries in temperate and tropical regions, found that only a minority of around 15 % could be assigned to family level or below, with the rest of taxa still undescribed, suggested a common provisional naming based on the fungal ITS region and rRNA genes for these often difficult to cultivate fungi. In this respect, an interesting question could be whether species, especially rare species, can only be found in

geographically restricted areas, or if they are distributed over large areas with higher abundances only in their ecological niche.

The difference in community composition between May and September gives an example of how quick communities can change within a rather short time. Abundant species in May did not necessarily correlate with abundant species in September. Some abundant species in May could not be encountered in September and vice versa. Even though studies have observed changes in community composition over time (Koide et al., 2007, Santalahti et al., 2016), this complete change within a period of 3.5 months is striking. A possible explanation might lie in an extraordinarily warm and dry summer in the region. When looking at climate data from a time series of the climate station in Bad Ischl, which is at a distance of around 50 kilometers from the research site at a height of 512 m, the period between June and August 2018 was ranked the 5th highest for mean temperatures and the 8th lowest in precipitation since the beginning of measurements in the 1850s (ZAMG, 2019). This suggests that many of the prevalent species in May were not able to thrive in the harsh summer months with low soil moisture and high upper soil temperatures. It seems that their places were occupied by species that were more adapted to these conditions. Moreover, as many of the abundant September species were not encountered there in May or only at low abundances, the results suggest that they nevertheless were present in the soil either as living hyphae, on root tips at low abundances, or in spore banks. The results of the present work confirm findings of a study conducted by Courty et al. (2008), who found high spatial and temporal variations in EM community composition along a time series of 15 months in a French oak forest. Even though the sites were dominated by four species, their abundance differed highly between seasons and communities were observed to change significantly within even one month.

As regards exploration types, more than 90 % of encountered EM on tree root tips showed a Short Distance exploration type. In comparison to compiled results of Rosinger et al. (2018) of European EM communities, this value is untypical, as in their results EM with SD exploration type accounted for only around 30 % across sites. The reason for this high value is unclear. A possible explanation could be that precipitation values in the region, with values between 1,250 mm and 2,000 mm per year (ZAMG, 2019), are high, which is why there had not been much evolutionary pressure for the selection of species that are able to transport water over large distances. One limitation to the obtained result is that some of the encountered species had to be assigned to an exploration type by the author based on the microscope pictures as no exploration type could be found in literature or the mentioned exploration types did not allow for assignment to only one exploration type. This may have led to wrong assignments. An interesting detail is that species with Medium and Long Distance exploration types reached a higher proportion of mycorrhization only on young beech trees in the control plot of Block 1. This might be an indication for their role in forming hyphal networks between young and adult trees for a better support of young trees with nutrients and water (Teste et al., 2009). On roots of young trees in disturbance treatment plots no species with LD and only a very low proportion of species with MD exploration types were encountered. Moreover, the mycorrhization rate was much lower than on young beech trees in control plots. Judging from their height, these young trees seem to have started growing shortly before disturbance treatment establishment which must have given them the opportunity to profit from inoculation via living hyphae or spore banks. Nevertheless, over the three years of disturbance treatment establishment, compared to young beech trees in control plots, their EM communities seem to have evolved differently.

An interesting question is whether the species of the families Hygrophoraceae, Hyaloscyphaceae, Clavulinaceae, Pyronemataceae and Strophariaceae can be classified as EM. It has been observed that several saprotrophic species interact with roots mycorrhized by EM fungi. Sometimes fungi also live as endophytes within tree roots (Baldrian and Kohout, 2017) which makes classification difficult.

Additionally, Brundrett and Tedersoo (2019) pointed out, that mycorrhizal status of plants is frequently interpreted incorrectly which may have led to wrong conclusions in many studies. In order not to misinterpret EM status, they suggested to scrutinize samples based on the root age and number of mycorrhizal root tips and judge them according to whether the mantle is uniformly present and the Hartig net can easily be distinguished. Moreover, it is important that the formation and branching of EM is synchronized with root tip growth, so that the presence of saprotrophic fungi on older root tips is not misjudged as mycorrhizal association. In their review of mycorrhizal status, Tedersoo et al. (2010) list the genera *Hygrophorus*, *Clavulina*, *Tarzetta* as well as the taxa *Peziza depressa* as ectomycorrhizal based on results of recent studies. As regards *Hypholoma sublateritium*, no hints regarding ectomycorrhizal activities could be found in literature, which probably indicates that the wood-decaying fungi was a contaminant. Likewise, also for the family of Hyaloscyphaceae no studies supporting a mycorrhizal status could be found in literature. There are species of the Hyaloscyphaceae family that act as plant endophytes and there are indications for a strong interaction between them and EM species (Nakamura et al., 2018), which might be the reason for their presence in the samples. Nevertheless, the pictures shown below highly suggest a mycorrhizal status of the samples, and there is also indications in literature suggesting mycorrhizal status of unnamed species belonging to the order of Helotiales (Tedersoo and Smith, 2013). This is why the three species identified as belonging to the family of Hyaloscyphaceae by DNA analysis were included in the results as EM.

In Figures 28 to 37 the samples assigned by DNA analysis to the families of Hygrophoraceae, Hyaloscyphaceae, Pyronemataceae, Clavulinaceae and Strophariaceae are depicted. Even though the branching is not always fulfilled, root tips seem vital and the Hartig net is visible. Nevertheless, it is possible that the chosen primers were not suitable for detection of a specific EM and the DNA result came from contaminant hyphae or spores present on the sample. As this is seems to be a common problem in studies, for the confirmation whether a fungal species really adheres to a mycorrhizal lifestyle, Heijden et al. (2015) suggested the analysis of the fungi's genome with focus on EM specific genes, e.g. for transporters needed for nutrient exchange.

Figure 28. Sample of Hygrophorus unicolor, edited

Figure 29. Sample of Hygrophorus unicolor*, edited*

Figure 30. Sample of Hygrophorus unicolor, *edited*

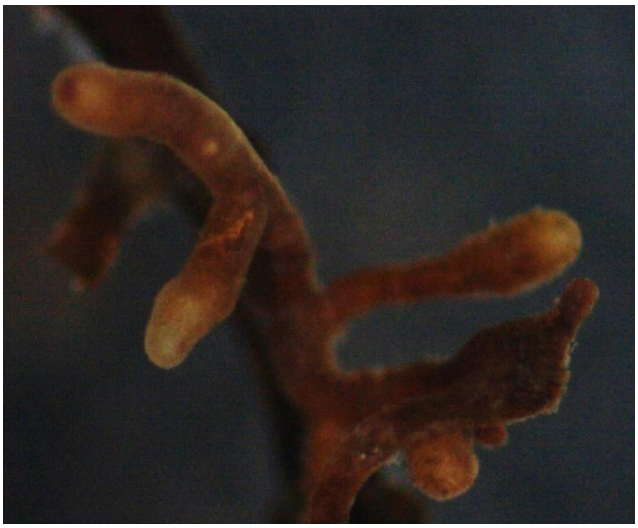

Figure 31. Sample of Hyaloscyphaceae sp. 1, edited

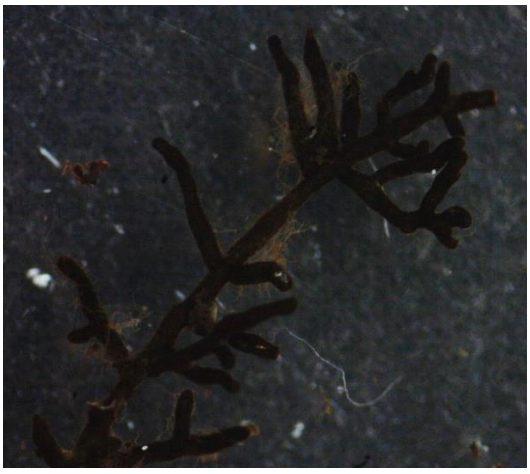

Figure 32. Sample of Hyaloscyphaceae sp. 2, edited

Figure 33. Sample of Hyaloscyphaceae sp. 3, edited

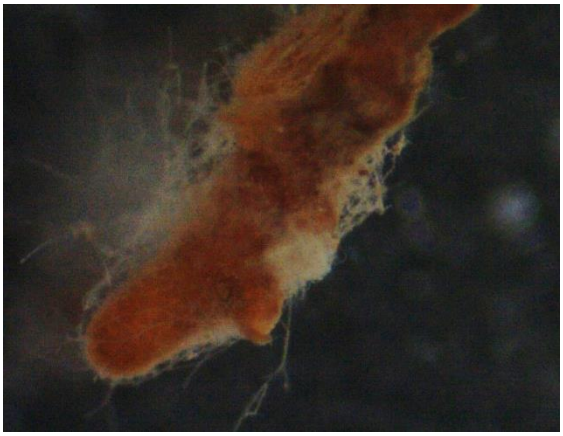

Figure 34. Sample of Tarzetta catinus, *edited*

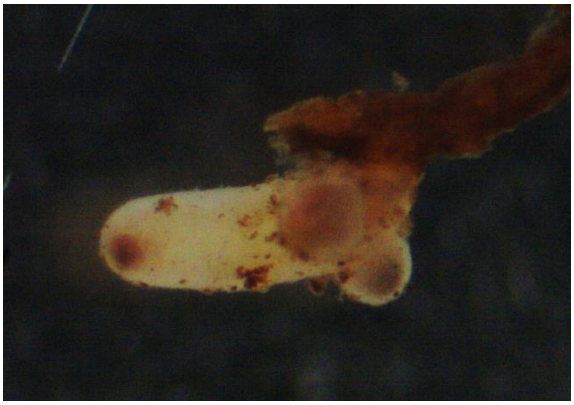

Figure 35. Sample of Clavulinaceae sp., edited

Figure 36. Sample of Hypholoma sublateritium*, edited*

Figure 37. Sample of Peziza depressa*, edited*

Pictures of the samples assigned to the species *Hygrophorus discoxanthus* by DNA analysis can be seen in Figure 12.

Another interesting aspect of the results of this work, is the high morphological variation of species that were identified to be of the same or closely related species by DNA analysis. Not only surface colour and texture varied, some samples even showed different exploration types (see Fig. 9 and 10). This observation gives us new hints regarding the functional adaptability of EM fungi, while we must also take into consideration that the sequencing result might have come from a contaminant whose DNA preferably paired with the chosen primers. Even though in the last decades new molecular methods have given us new insights into EM ecology, scientists warned of pitfalls which can lead to wrong conclusions (Lindahl et al., 2013). Pena et al. (2010) stated that their species assignments based on morphotyping tended to overestimate species numbers by around 30 %, Menkis et al. (2005) found a high morphological diversity of taxa leading to wrong results in morphotyping, while the frequent presence of non-EM fungi on root samples lead to misjudgments in DNA analysis. These aspects limit the validity and comparability of studies relying on just one method and highlight the importance of the combination of both morphological and molecular methods, with DNA analysis of several samples of an identified morphotype for verification.

When comparing the results of the current work to fungal data obtained from soil samples – not from tree root tips - using a high-throughput approach for DNA analysis from rightly before and after treatment establishment in the years 2015 and 2016 (Godbold et al., 2018), there are some major differences: in the data of the years 2015 and 2016, the most abundant EM taxa came from the families of Inocybaceae, which only ranked fourth in the present results of September, and were present only in low quantities in May. A striking finding in the years 2015 and 2016 was that no indication for the presence of species belonging to the Russulaceae could be found. This was rather untypical, as the genus *Russula* and *Lactarius* usually play a prominent role in temperate European mixed forests (Pena et al., 2010, Buée et al., 2005, Goldmann et al., 2015, Pena et al., 2017, Rosinger et al., 2018, Schirkonyer et al., 2013). Contrary to these results, in the current work three *Lactarius* species could be found on tree root tips, with *Lactarius blennius* being one of the most abundant species in May and September, whereas *L. rubrocinctus* and L*. helvus/pallidus* were only encountered in one block in September. The reason for their absence in the years 2015 and 2016 might lie in environmental factors. Additionally, their morphology, with a smooth surface and no emanating hyphae, may have made them difficult to detect in soil samples. This underlines the importance of including a morphological analysis of mycorrhizal tree root tips in studies of EM communities, as data solely coming from high-throughput molecular methods based on soil samples may not always reflect the mycorrhization patterns on tree roots.

7. Conclusions

The present work started with a short overview of the diversity of fungi and a classification of their roles within ecosystems. Concentrating on ectomycorrhizal fungi, which play a main role for net primary production in forest ecosystems, current knowledge was summarized and put into the context of disturbance regimes that increasingly threaten ecological equilibria and commercial revenues of temperate forests on the Northern hemisphere.

The initial objective of the practical part of this thesis, the comparison of EM communities on tree root tips between disturbed and control plots three years after disturbance treatment establishment, was not possible, as tree roots in treatment plots were encountered to be dead. Therefore, the focus of this thesis was shifted to the monitoring of EM community composition between two sampling dates in May and September. Additionally, in September young beech trees of treatment and control plots in one block were sampled to get an indication whether the mycorrhization rate and community composition differed between them and the communities from soil samples which were assumed to come from mature trees.

A high variability of species and their abundances between sampling plots, blocks, seasons and tree roots of mature versus young trees, could be found. This suggests a high microsite diversity. As 27 of the 46 species were nevertheless encountered in more than one block without a statistically significant pattern for their occurrence, this might be an indication that all blocks share a common species pool with biotic and abiotic characteristics being the main factors behind microsite community composition. Especially the change of community composition between May and September is striking and seems to be due to an exceptionally dry and warm summer in the region that favored some species in comparison to others. The mechanisms behind these changes, whether they are triggered by different physiological optima and competition between EM or by processes initiated by their host plants, still need to be uncovered.

Mycorrhization patterns on young beech trees of disturbance treatment and control plots differed widely between them and also compared to communities on mature trees. Even though the young trees from disturbance treatment plots seem to have germinated already before treatment establishment, the observation of lower mycorrhization rates and the absence of species with Medium and Long Distance exploration types may be a result of their solitary positions with no access to mycorrhizal networks. As the sampling number of 4 trees from disturbance treatment plots and 4 trees of the control plot is low, the results can only be taken as indication and further research is necessary for verification.

Attention was also drawn to the fact that comparisons with other studies covering EM communities are difficult to impossible due to the high number of undescribed species. As many of them are difficult to culture and lack fruiting bodies, a common naming and standardized description might help to include them in regional or continental diversity studies. For these studies, also a combination of morphotyping and DNA analysis is suggested, as the single use of one of these methods can lead to the over-/underestimation of species numbers and the underrepresentation of families with restricted exploration patterns. A combination also has the advantage of giving us a better understanding of the morphological and functional variability of species.

Finally, this work provided evidence for the confirmation of the mycorrhizal status of species belonging to the families of Sebacinaceae, Hygrophoraceae, Pyronemataceae and Clavulinaceae. Mycorrhizal status of three species of Hyaloscyphaceae and *Hypholoma sublateritium* is suggested but will still need further proof for verification.

8. Perspectives

Even though scientists from all over the world regularly provide new insights into the fascinating lifestyles and enormous diversity of ectomycorrhizal fungi, our knowledge regarding their functions, interactions and relationships with their plant hosts, other microbes in the rhizosphere and among them is still rather basic.

Based on the focus of the present work the following questions still need to be answered:

- From which spatial extent do disturbances like clear-cutting result in a permanent loss of EM diversity?
- Is functional variability of species with long-distance propagation modes sufficient to step in when a large part of EM diversity was eliminated during large-scale disturbances?
- How long after a disturbance event are vital EM propagules present?
- Is there a timely succession among symbiotic fungal species after disturbances?
- Are there key species which are beneficial for a quick establishment of seedlings in forests after disturbances?
- Is an inoculation with selected fungal species beneficial for a quick establishment of seedlings in forests after disturbances?
- Which symbiotic fungal species are beneficial for the establishment of seedlings of selected tree species in calcareous mountain forest soils?
- Do similar ecosystems share over-regional EM species?

With increasing numbers and extent of disturbance events due to ecologically unfavorable forest management practices and changes in the global climate regime, a better insight into the roles and functional mechanisms of EM fungi will be necessary, to be able to develop guidelines for protection and battle the challenges of the present and future.

References

Agerer Reinhard (2001): Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza11: 107–114

Agerer Reinhard (2006): Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5: 67–107

Agerer R. & Rambold G. 2004–2018 [first posted on 2004-06-01; most recent update: 2011-01-10, accessed on 2018-06-28]. DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, Germany

Baber Kristin, Otto Peter, Kahl Tiemo, Gossner Martin M., Wirth Christian, Gminder Andreas, Bässler Claus (2016): Disentangling the effects of forest-stand type and dead-wood origin of the early successional stage on the diversity of wood-inhabiting fungi. Forest Ecology and Management 377: 161–169

Baldrian Petr and Kohout Petr (2017): Interactions of saprotrophic fungi with tree roots: can we observe the emergence of novel ectomycorrhizal fungi? New Phytologist 215: 511–513

Bässler Claus, Müller Jörg, Cadotte Marc W., Heibl Christoph, Bradtka Johannes H., Thorn Simon, Halbwachs Hans (2016): Functional response of lignicolous fungal guilds to bark beetle deforestation. Ecological Indicators 65 (2016) 149–160

BFW Bundesforschungszentrum für Wald (2018): Schadholzmengen durch Sturm, Schnee und Borkenkäferbefall. (https://bfw.ac.at/rz. https://bfw.ac.at/rz/bfwcms.web?dok=10380, Accessed on 2018-11-11)

Bonfante Paola and Genre Andrea (2010): Mechanisms underlying beneficial plant – fungus interactions in mycorrhizal symbiosis. *Nat. Commun.* 1:48 doi: 10.1038 / ncomms1046 (2010)

Brundrett Mark (2004): Diversity and classification of mycorrhizal associations. Biol. Rev. 79, pp. 473–495

Brundrett Mark (2009): Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320: 37-77

Brundrett Mark and Tedersoo Leho (2019): Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions. New Phytologist 221: 18–24

Buée Marc, Vairelles Dominique, Garbaye Jean (2005): Year-round monitoring of diversity and potential metabolic activity of the ectomycorrhizal community in a beech (Fagus silvatica) forest subjected to two thinning regimes. Mycorrhiza15: 235–245

Bueno C. Guillermo, Moora Mari, Gerz Maret, Davison John, Öpik Maarja, Pärtel Meelis, Helm Aveliina, Ronk Argo, Kuehn Ingolf, Zobel Martin (2017): Plant mycorrhizal status, but not type, shifts with latitude and elevation in Europe. Global Ecol Biogeogr. 26:690–699

Courty Pierre-Emmanuel, Franc Alain, Pierrat Jean-Claude and Jean Garbaye (2008): Temporal Changes in the Ectomycorrhizal Community in Two Soil Horizons of a Temperate Oak Forest. Applied and Environmental Microbiology Vol. 74, No. 18: 5792–5801

Desprez-Loustau Marie-Laure, Marcais Benoit, Nageleisen Louis-Michel, Piou Dominique, Vannini Andrea (2006): Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. 63: 597–612

Dickie Ian A., Alexander Ian, Lennon Sarah, Öpik Maarja, Selosse Marc-Andre, van der Heijden Marcel G. A. and Martin Francis M. (2015): Evolving insights to understanding mycorrhizas. New Phytologist 205: 1369–1374

Dickie Ian A., Martínez-García Laura B., Koele Nina, Grelet G.-A., Tylianakis Jason M., Peltzer Duane A., Richardson Sarah J. (2013): Mycorrhizas and mycorrhizal fungal communities throughout ecosystem development. Plant Soil 367:11–39

Ekblad, Wallander H., Godbold D. L., Cruz C., Johnson D., Baldrian P., Björk R. G., Epron D., Kieliszewska-Rokicka B., Kjøller R., Kraigher H., Matzner E., Neumann J., Plassard C. (2013): The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. Plant Soil 366:1–27

Fernandez Christopher W. and Kennedy Peter G. (2016): Revisiting the 'Gadgil effect': do interguild fungal interactions control carbon cycling in forest soils? New Phytologist 209: 1382–1394

Fernandez Christopher W., McCormack M. Luke, Hill Jason M., Pritchard Seth G., Koide Roger T. (2013): On the persistence of Cenococcum geophilum ectomycorrhizas and its implications for forest carbon and nutrient cycles. Soil Biology & Biochemistry 65 (2013) 141e143

Food and Agriculture Organization of the United Nations (FAO) (2015): Global Forest Resources Assessment 2015. Country Report: Austria. (http://www.fao.org/3/a-az157e.pdf, Accessed on 2018-11-11)

Frey-Klett Pascale, Chavatte Michaël, Clausse Marie-Lise, Courrier Sébastien, Le Roux Christine, Raaijmakers Jos, Martinotti Maria Giovanna, Pierrat Jean-Claude and Garbaye Jean (2005): Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytologist (2005) 165: 317–328

Gerz Maret, Bueno Carlos Guillermo, Zobel Martin and Moora Mari (2016): Plant community mycorrhization in temperate forests and grasslands: relations with edaphic properties and plant diversity. Journal of Vegetation Science 27: 89–99

Glassman Sydney I., Peay Kabir G., Talbot Jennifer M., Smith Dylan P., Chung Judy A., Taylor John W., Vilgalys Rytas and Bruns Thomas D. (2015): A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. New Phytologist 205: 1619–1631

Godbold, D. L., Matthews B., Mayer, M., Katzensteiner, K., Schume, H., Gorfer, M., Berger, H., Kobler, J., Dirnböck, T., Rosinger, C., Iftekhar, U. A., Sandén, H., Rewald, B. 2018. Disturbance impacts on forest carbon dynamics in the calcareous Alps ("C- Alp") (Project Final Report)

Goldmann Kezia, Schöning Ingo, Buscot Francois, Wubot Tesfaye (2015): Forest Management Type Influences Diversity and Community Composition of Soil Fungi across Temperate Forest Ecosystems. Frontiers in Microbiology 6 Art. 1300: 1-11

Gómez-Aparicio Lorena, Ibánez Beatriz, Serrano María S., De Vita Paolo, Avila José M., Pérez-Ramos Ignacio M., García Luis V., Sánchez M. Esperanza and Maranón Teodoro (2012): Spatial patterns of soil pathogens in declining Mediterranean forests: implications for tree species regeneration. New Phytologist 194: 1014–1024

Greenacre, Michael (2010): Correspondence analysis of raw data. Ecology, 91(4): 958–963

Hagermann Shannon M., Jones Melanie D., Bradfield Gary E., Gillespie M., Durall D. M. (1999): Effects of clear-cut logging on the diversity and persistence of ectomycorrhizae at a subalpine forest. Canadian Journal of Forest Research 29, 1: 124-134

Hammer Øyvind, Harper David A.T., and Paul D. Ryan, 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, vol. 4, issue 1, art. 4: 9pp., 178kb.

Harvey A.E., Jurgensen M.F., and Larsen M.J. (1980). Clearcut harvesting and mycorrhizae: survival of activity on residual roots and influence of bordering forest stand in western Montana. Canadian Journal of Forest Research 10: 300-303

Hawkins B. J., Jones Melanie D., Kranabetter J. M. (2015): Ectomycorrhizae and tree seedling nitrogen nutrition in forest restoration. New Forests 46:747–771

Heilmann-Clausen, Jacob, Aude Erik and Christensen Morten (2005): Cryptogam communities on decaying deciduous wood – does tree species diversity matter? Biodiversity and Conservation 14: 2061–2078

Heinemeyer Andreas, Hartley Iain P., Evans Sam P., Carreira de la Fuente, José A., Ineson Phil (2007): Forest soil CO2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. Global Change Biology 13: 1786–1797

Hempel Stefan, Goetzenberger Lars, Kuehn Ingolf, Michalski Stefan G., Rillig Matthias C., Zobel Martin, Moora Mari (2013): Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. Ecology, 94 (6): 1389–1399

Hesslerová Petra, Huryna Hanna, Pokorný Jan, Procházka Jan (2018): The effect of forest disturbance on landscape temperature. Ecological Engineering 120: 345–354

Hobbie E. A. and Hobbie J. E. (2008): Natural Abundance of 15N in Nitrogen-Limited Forests and Tundra Can Estimate Nitrogen Cycling Through Mycorrhizal Fungi: A Review. Ecosystems 11: 815–830

Jones Melanie D., Durall Daniel M. and Cairney John W. G. (2003): Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytologist 157: 399–422

Kishchuk B.E., Thiffault E., Lorente M., Quideau S., Keddy T., and Sidders D. (2015): Decadal soil and stand response to fire, harvest, and salvage-logging disturbances in the western boreal mixedwood forest of Alberta, Canada. Canadian Journal of Forest Research 45: 141–152

Koide Roger T., Shumway Durland L., Xu Bing and Sharda Jori N. (2007): On temporal partitioning of a community of ectomycorrhizal fungi. New Phytologist 174: 420–429

Lang Christa, Seven Jasmin, Polle Andrea (2011): Host preferences and differential contributions of deciduous tree species shape mycorrhizal species richness in a mixed Central European forest. Mycorrhiza 21: 297–308

Leake, Jonathan R. (2001): Is diversity of ectomycorrhizal fungi important for ecosystem function? New Phytologist 152: 1–8

LeDuc Stephen D., Lilleskov Erik A., Horton Thomas R., Rothstein David E. (2013): Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia 172: 257–269

Lehmann Anika, Rillig Matthias C. (2015): Understanding mechanisms of soil biota involvement in soil aggregation: A way forward with saprobic fungi? Soil Biology & Biochemistry 88: 298-302

Likens, Gene E, Dresser, Brian K., Buso, Donald C. (2004): Short-Term Temperature Response in Forest Floor and Soil to Ice Storm. Northern Journal of Applied Forestry 21, 4; Natural Science Collection pg. 209-219

Lindahl Bjorn D., Nilsson R. Henrik, Tedersoo Leho, Abarenkov Kessy, Carlsen Tor, Kjøller Rasmus, Koljalg Urmas, Pennanen Taina, Rosendahl Søren, Stenlid Jan and Kauserud Havard (2013): Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytologist (2013) 199: 288–299

Liu Cindy M, Kachur Sergey, Dwan Michael G, Abraham Alison G, Aziz Maliha, Hsueh Po-Ren,Huang Yu-Tsung, Busch Joseph D, Lamit Louis J, Gehring Catherine A, Keim Paul, and Price Lance B (2012): FungiQuant: A broad-coverage fungal quantitative real-time PCR assay. BMC Microbiology 12:255

Lynch Michael D. J. and Thorn R. Greg (2006): Diversity of Basidiomycetes in Michigan Agricultural Soils. Applied and Environmental Microbiology Vol. 72, No. 11: 7050–7056

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

Martin F., Kohler A., Murat C., Balestrini R., Coutinho P. M., Jaillon O.,Montanini B., Morin E., Noel B., Percudani R. et al. (2010). Perigord black truffle genome uncovers evolutionary origins and mechanisms ofsymbiosis. Nature 464: 1033–1038.Marupakula Srisailam, Mahmood Shahid and Finlay Roger D. (2016): Analysis of single root tip microbiomes suggests that distinctive bacterial communities are selected by Pinus sylvestris roots colonized by different ectomycorrhizal fungi. Environmental Microbiology 18(5): 1470–1483

Mayer Mathias , Matthews Bradley, Rosinger Christoph, Sanden Hans, Godbold Douglas L., Katzensteiner Klaus (2017a): Tree regeneration retards decomposition in a temperate mountain soil after forest gap disturbance. Soil Biology & Biochemistry 115: 490-498

Mayer Mathias, Sanden Hans, Rewald Boris, Godbold Douglas L. and Katzensteiner Klaus (2017b): Increase in heterotrophic soil respiration by temperature drives decline in soil organic carbon stocks after forest windthrow in a mountainous ecosystem. Functional Ecology 2017, 31: 1163–1172

McGuire Krista L., Allison Steven D., Fierer Noah, Treseder Kathleen K. (2013): Ectomycorrhizal-Dominated Boreal and Tropical Forests Have Distinct Fungal Communities, but Analogous Spatial Patterns across Soil Horizons. PLoS ONE 8(7): e68278. doi:10.1371/journal.pone.0068278

Menkis Audrius, Vasiliauskas Rimvydas, Taylor Andrew F. S., Stenlid Jan, Finlay Roger (2005): Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16: 33–41

Moore Jessica A. M., Jiang,, Patterson Courtney M., Mayes Melanie A., Wang Gangsheng and Classen Aimee T. (2015): Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. Journal of Ecology 103: 1442–1453

Nakamura Noritaka, Tanaka Eiji, Tanaka Chihiro, Takeuchi-Kaneko Yuko (2018): Localization of helotialean fungi on ectomycorrhizae of Castanopsis cuspidata visualized by in situ hybridization. Mycorrhiza 28:17–28

Nicholson, Bailey A., Jones Melanie D. (2017): Early-successional ectomycorrhizal fungi effectively support extracellular enzyme activities and seedling nitrogen accumulation in mature forests. Mycorrhiza 27:247–260

O'Donnell K. *Fusarium* and its near relatives. In: Reynolds D R, Taylor J W, editors. The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics. Wallingford, Conn: CAB International; 1993. pp. 225–233.

O'Hanlon Richard and Harrington Thomas J. (2012): Similar taxonomic richness but different communities of ectomycorrhizas in native forests and non-native plantation forests. Mycorrhiza 22:371–382

Parsons W.F.J., Miller S.L. and Knight D.H. (1994): Root-gap dynamics in a lodgepole pine forest: ectomycorrhizal and nonmycorrhizal fine root activity after experimental gap formation. Canadian Journal of Forest Research 24: 1531-1538

Pena Rodica, Offermann Christine, Simon Judy, Naumann Pascale Sarah, Geßler Arthur, Holst Jutta, Dannenmann Michael, Mayer Helmut, Kogel-Knabner Ingrid, Rennenberg Heinz, and Polle Andrea (2010): Girdling Affects Ectomycorrhizal Fungal (EMF) Diversity and Reveals Functional Differences in EMF Community Composition in a Beech Forest. Applied and Environmental Microbiology Vol. 76, No. 6: 1831–1841

Pena Rodica, Schöning Ingo, Lang Christa, Lohaus Gertrud, Ammer Christian, Boch Steffen, Fischer Markus, Schall Peter, Polle Andrea (2017): Phylogenetic and functional traits ofectomycorrhizal assemblages in top soil from different biogeographic regions and forest types. Mycorrhiza 27: 233–245

Raaijmakers Jos M., Paulitz Timothy C., Steinberg Christian, Alabouvette Claude, Moenne-Loccoz Yvan (2009): The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321: 341-361

Rosinger Christoph, Sandén Hans, Matthews Bradley, Mayer Mathias and Godbold Douglas L. (2018): Patterns in Ectomycorrhizal Diversity, Community Composition, and Exploration Types in European Beech, Pine, and Spruce Forests. Forests 9, 445: 13-16

Santalahti Minna, Sun Hui, Jumpponen Ari, Pennanen Taina and Heinonsalo Jussi (2016): Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. FEMS Microbiology Ecology 92, 11: 1-12

Scharenbroch B. C., Bockheim J. G. (2008): The effects of gap disturbance on nitrogen cycling and retention in late-successional northern hardwood–hemlock forests. Biogeochemistry 87:231–245

Schelker J., Grabs T., Bishop K., Laudon H. (2013): Drivers of increased organic carbon concentrations in stream water following forest disturbance: Separating effects of changes in flow pathways and soil warming. Journal of geophysical research: Biosciences 118, 1814–1827

Schirkonyer Uwe, Bauer Christine, Rothe Gunter M. (2013): Ectomycorrhizal diversity at five different tree species in forests of the Taunus Mountains in Central Germany. Open Journal of Ecology 3: 66-81

Seidl Rupert, Schelhaas Mart-Jan, Rammer Werner, Verkerk Pieter Johannes (2014): Increasing forest disturbances in Europe and their impact on carbon storage. Nature Climate Change 4: 806-810

Silva Pedro Mariana, Rammer Werner, Seidl Rupert (2015): Tree species diversity mitigates disturbance impacts on the forest carbon cycle. Oecologia 177: 619–630

Simard Suzanne W., Perry David A., Jones Melanie D., Myrold David D., Durall Daniel M., Molina Randy (1997): Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388: 579-582

Song YY, Zeng RS, Xu JF, Li J, Shen X, et al. (2010) Interplant Communication of Tomato Plants through Underground Common Mycorrhizal Networks. PLoS ONE 5(10): e13324. doi:10.1371/journal.pone.0013324

Stern Eleanor R., Riva Federico and Nielsen Scott E. (2018): Effects of Narrow Linear Disturbances on Light and Wind Patterns in Fragmented Boreal Forests in Northeastern Alberta. Forests 9, 486: 1-13

Stuart-Haentjens Ellen J., Curtis Peter S., Fahey Robert T., Vogel Christoph S., Gough Christopher M. (2015): Net primary production of a temperate deciduous forest exhibits a threshold response to increasing disturbance severity. Ecology, 96(9): 2478–2487

Suz Laura M., Barsoum Nadia, Benham Sue, Dietrich Hans-Peter, Fetzer Karl Dieter, Fischer Richard, Garcia Paloma, Gehrman Joachim, Ofel Ferdinand Krist, Manninger Mikl Os, Neagu Stefan, Nicolas Manuel, Oldenburger Jan, Raspe Stephan, Sanchez Gerardo, Schröck Werner, Schubert Alfred, Verheyen Kris, Verstraeten Arne, Bidartondo Martin I. (2014): Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests. Molecular Ecology 23, 5628–5644

Taylor Lee D., Hollingsworth Teresa N., McFarland Jack W., Lennon Niall J., Nusbaum Chad, Ruess Roger W. (2014): A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs, 84(1): 3–20

Tedersoo Leho, Jairus Teele, Horton Bryony M., Abarenkov Kessy, Suvi Triin, Saar Irja and Kõljalg Urmas (2008): Strong host preference of ectomycorrhizal fungi in a Tasmanian wet sclerophyll forest as revealed by DNA barcoding and taxon-specific primers. New Phytologist 180: 479–49

Tedersoo Leho, May Tom W., Smith Matthew E. (2010): Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20:217–263

Tedersoo Leho and Smith Matthew E. (2013): Lineages of ectomycorrhizal fungi revisited: Foraging strategies and novel lineages revealed by sequences from belowground. Fungal Biology Reviews 27: 83-99

Tedersoo Leho, Bahram Mohammad, Puusepp Rasmus, Nilsson R. Henrik and James Timothy Y. (2017): Novel soil-inhabiting clades fill gaps in the fungal tree of life. Microbiome: DOI 10.1186/s40168-017-0259-5

Teste Francois, Simard Suzanne W., Durall Daniel M., Guy Robert D., Jones Melanie D., Schoonmaker Amanda L. (2009): Access to mycorrhizal networks and roots of trees: importance for seedling survival and resource transfer. Ecology, 90(10): 2808–2822

Tisdall Judith M., Nelson Sam E., Wilkinson Kevin G., Smith Sally E., McKenzie Blair M. (2012): Stabilisation of soil against wind erosion by six saprotrophic fungi. Soil Biology & Biochemistry 50: 134-141

Twieg Brendan D., Durall Daniel M., Simard Suzanne W., Jones Melanie D. (2009): Influence of soil nutrients on ectomycorrhizal communities in a chronosequence of mixed temperate forests. Mycorrhiza 19: 305-316

Valásková Vendula, Snajdra Jaroslav, Bittner Britta, Cajthaml Tomás, Merhautová Vera, Hofrichter Martin, Baldrian Petr (2007): Production of lignocellulose-degrading enzymes and degradation of leaf litter by saprotrophic basidiomycetes isolated from a Quercus petraea forest. Soil Biology & Biochemistry 39: 2651–2660

van der Heijden Marcel G. A., Martin Francis M., Selosse Marc-Andre and Sander Ian R. (2015): Mycorrhizal ecology and evolution: the past, the present, and the future. New Phytologist (2015) 205: 1406–1423

van der Wal Annemieke, Klein Gunnewiek Paulien, de Hollander Mattias, de Boer Wietse (2017): Fungal diversity and potential tree pathogens in decaying logs and stumps. Forest Ecology and Management 406: 266–273

Voriskova Jana, Brabcova Vendula, Cajthaml Tomas, and Baldrian Petr (2014): Seasonal dynamics of fungal communities in a temperate oak forest soil. New Phytologist 201: 269–278

Wang Lixia, Otgonsuren Burenjargal, Godbold Douglas L. (2017): Mycorrhizas and soil ecosystem function of co-existing woody vegetation islands at the alpine tree line. Plant Soil 411:467–481

ZAMG Zentralanstalt für Meteorologie und Geodynamik (2019). Historical Instrumental Climatological Surface Time Series of the Greater Alpine Region. http://www.zamg.ac.at/histalp/datasets.php [Accessed on 18 March 2019]

Zehetgruber Bernhard, Kobler Johannes, Dirnböck Thomas, Jandl Robert, Seidl Rupert, Schindlbacher Andreas (2017): Intensive ground vegetation growth mitigates the carbon loss after forest disturbance. Plant Soil 420:239–252

Appendix

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Pictures (original) without editing

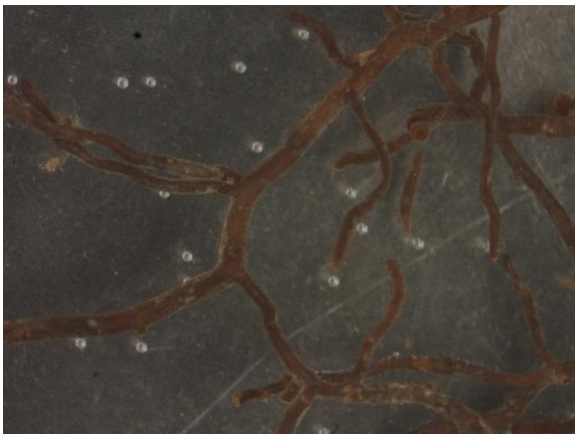

Figure 3. Dead root tips from May Block 3, treatment clear-cut with debris

Figure 4. Dead root tips from May Block 1, treatment girdling

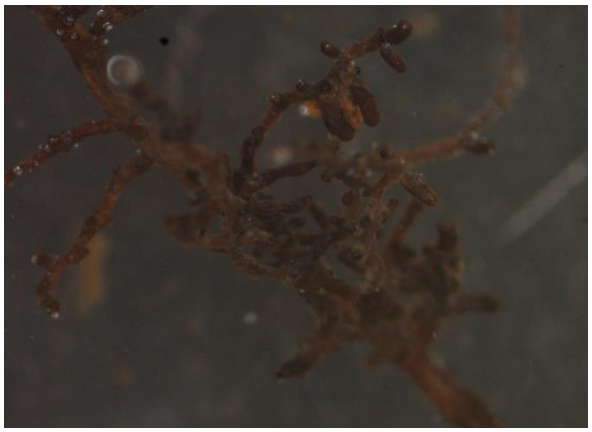

Figure 5. Dead root tips with recognizable EM from May Block 2-2, treatment girdling

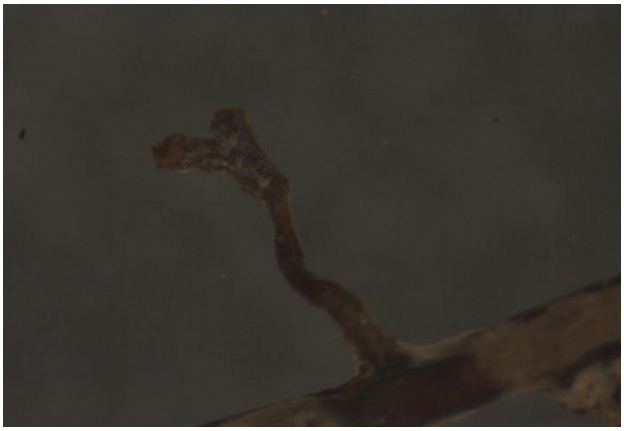

Figure 6. Dead root tips with recognizable EM from May Block 2-2, treatment clear-cut

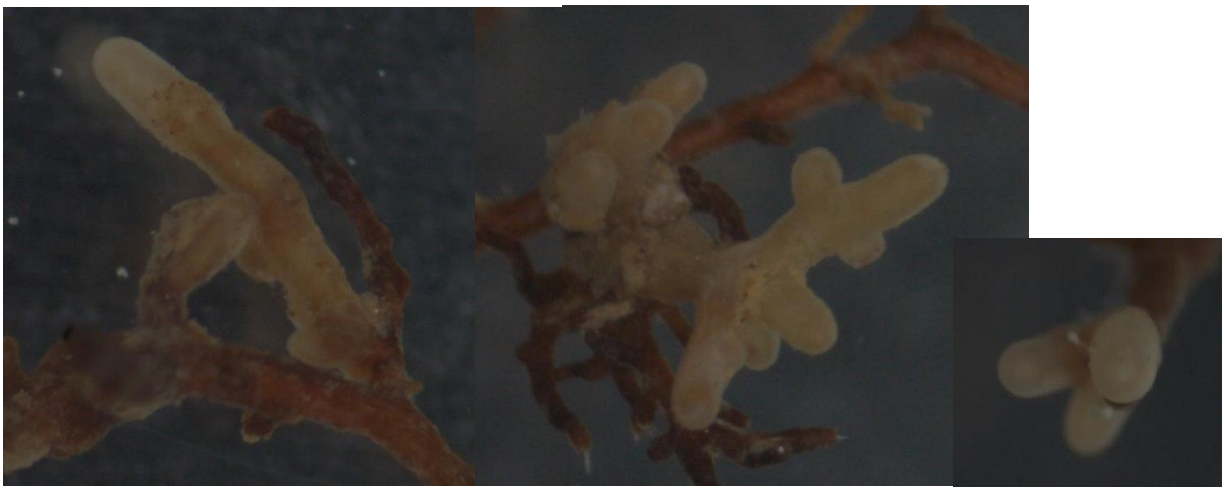

Figure 8. Morphotypes of Lactarius helvus/pallidus

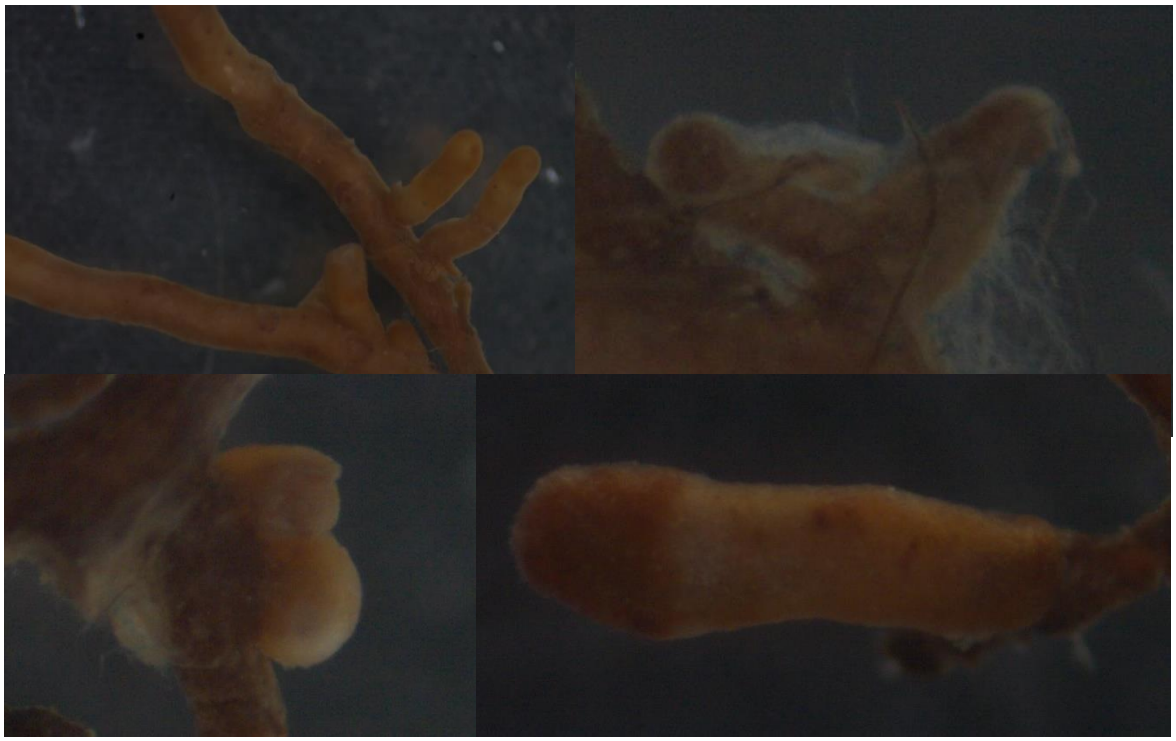

Figure 9. Morphotypes of Lactarius rubrocinctus

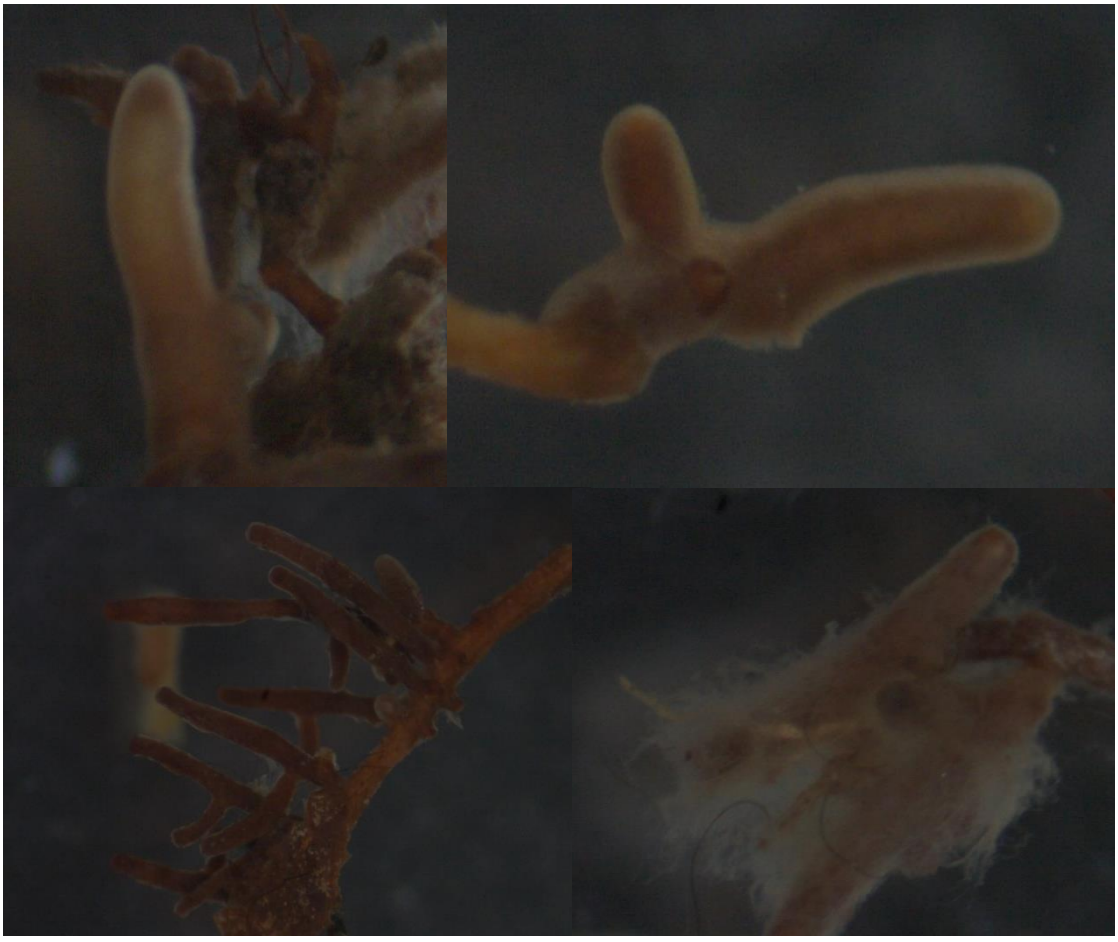

Figure 10. Morphotypes of Lactarius blennius

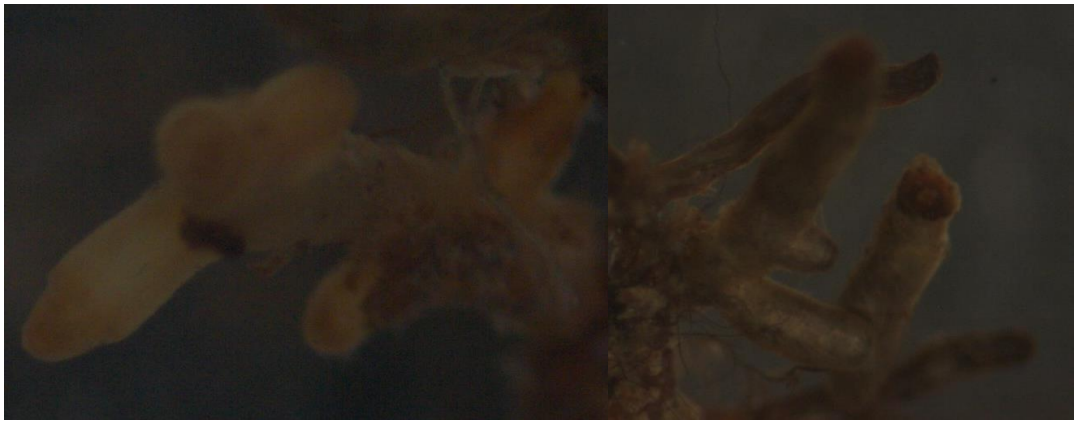

Figure 11. Morphotypes of Sebacina aff. incrustans

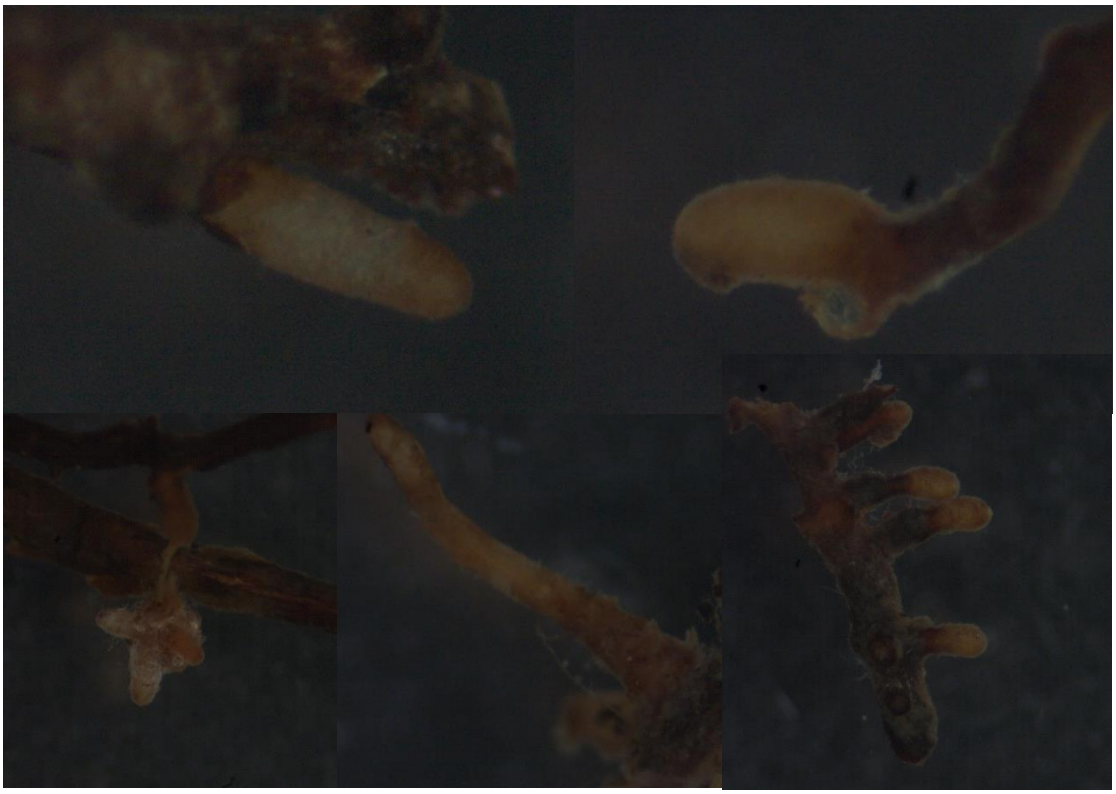

Figure 12. Morphotypes of Hygrophorus discoxanthus

Figure 13. Morphotype of Hyndobolites sp.

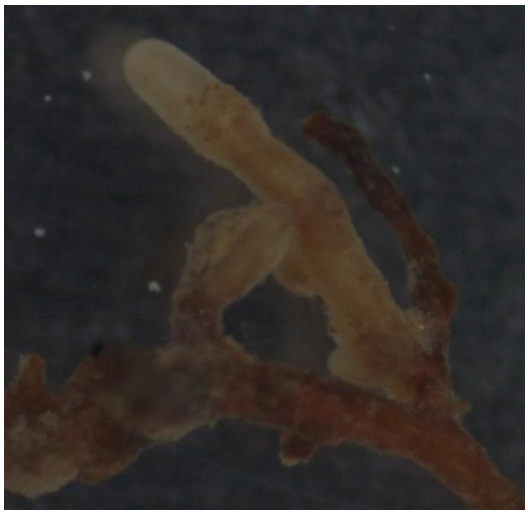

Figure 14. Morphotype of Lactarius pallidus/helvus

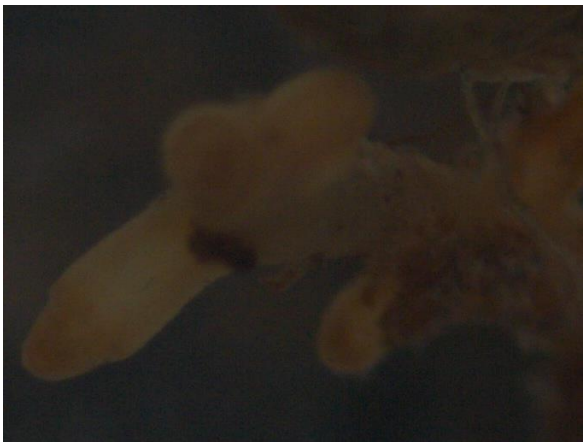

Figure 15. Morphotype of Sebacina aff. incrustans

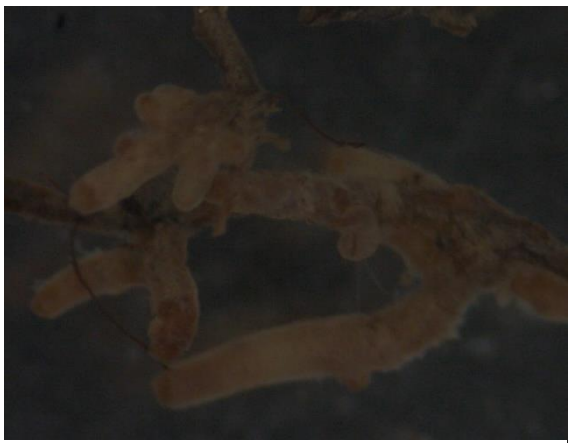

Figure 16. Morphotype of Inocybe hirtella *var. bispora*

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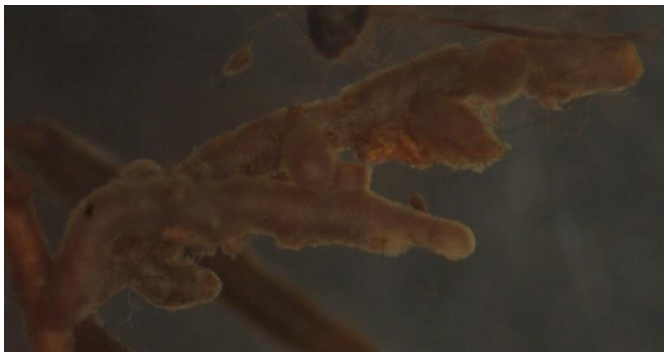

Figure 28. Sample of Hygrophorus unicolor

Figure 29. Sample of Hygrophorus unicolor

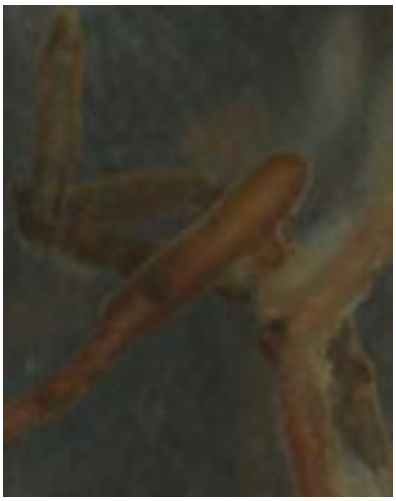

Figure 30. Sample of Hygrophorus unicolor

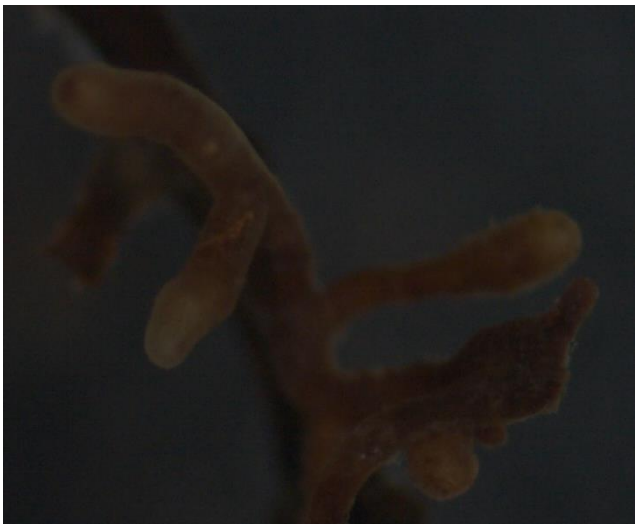

Figure 31. Sample of Hyaloscyphaceae sp. 1

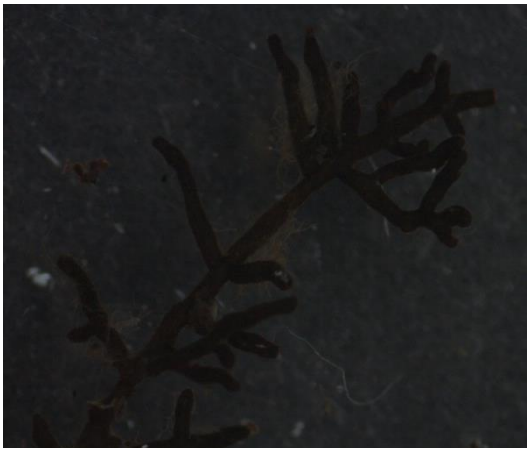

Figure 32. Sample of Hyaloscyphaceae sp. 2

Figure 33. Sample of Hyaloscyphaceae sp. 3

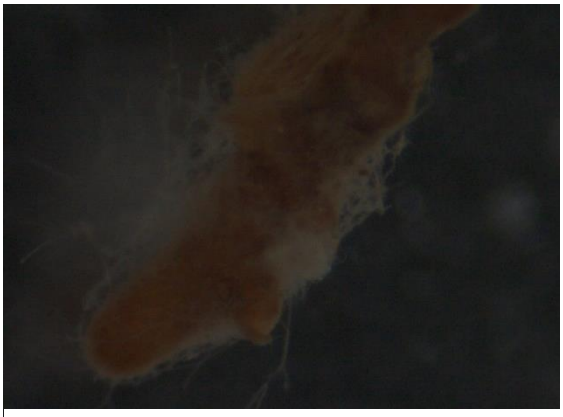

Figure 34. Sample of Tarzetta catinus

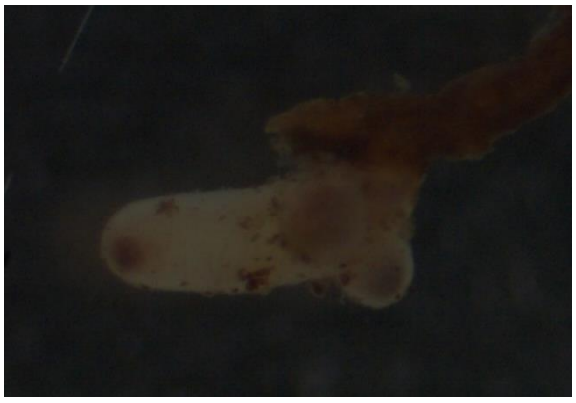

Figure 35. Sample of Clavulinaceae sp.

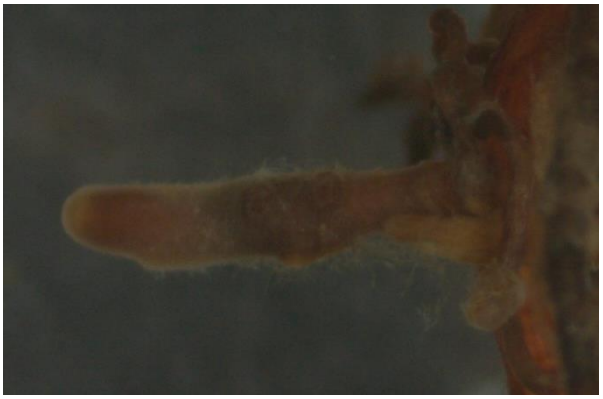

Figure 36. Sample of Hypholoma sublateritium

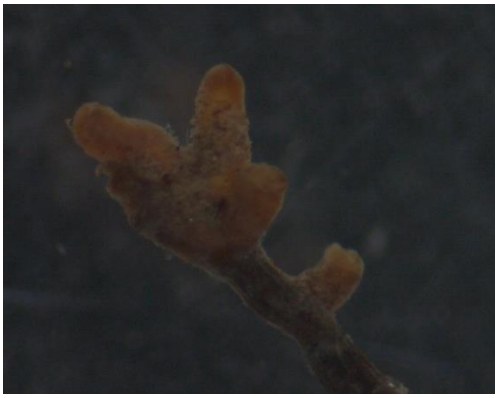

Figure 37. Sample of Peziza depressa

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt, keine anderen als die angegebenen Hilfsmittel benutzt und alle aus ungedruckten Quellen, gedruckter Literatur oder aus dem Internet im Wortlaut oder im wesentlichen Inhalt übernommenen Formulierungen und Konzepte gemäß den Richtlinien wissenschaftlicher Arbeiten zitiert, durch Fußnoten gekennzeichnet bzw. mit genauer Quellenangabe kenntlich gemacht habe.

Für Elena, Gregor, Moritz

und Robert