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„Exchange and distribution of carbon and soil nutrients in common mycorrhizal networks of European beech (*Fagus sylvatica*)"

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1. GENERAL INTRODUCTION

Almost all land plants around the world are associated with mycorrhizal fungi. In this symbiosis, plants allocate photosynthetic carbon (C) to mycorrhizal fungi living in their roots in exchange for nutrients the fungi take up from the soil. The ability of the fungi to grow extraradical mycelium considerably increases the surface area available for nutrient uptake beyond the range of roots and therefore increases the access to nutrients sources in the soil. This bidirectional transfer of nutrients is the central part of the symbioses (Smith and Read 1997) and has been shown to increase plant growth and fitness, impacting plant diversity, nutrient cycling and ecosystem productivity. As a result, they are amongst the most important mutualists that are capable of improving plant nutrient uptake. Additionally, mycorrhizal fungi impact C balance at ecosystem level as they can represent either a C sink, by acting as a belowground C storage, or be involved in C losses, by acting as decomposers. Either way, their role in the carbon global cycle is often under estimated (Talbot et al. 2008). Until today, the terms in which host plants and their fungal partners trade nutrients remains unclear, in particular in ectomycorrhiza (ECM) networks, as most studies have been focusing on arbuscular mycorrhiza (AM).

There are two main types of mycorrhizal association which represent most of this symbiotic association, arbuscular mycorrhiza and the ectomycorrhiza. They both differ in their morphology and physiology, as well as in their interaction with host plants and strategies for nutrient acquisition. In both cases, different mycorrhizal fungi can be associated with different host plants, connecting their root system belowground via extraradical mycelium network. This connection, called Common Mycorrhizal Networks (CMNs), have been suggested to provide a belowground pathway for transferring carbon, nitrogen and phosphorus from one plant to another as well as water and herbivore-induced defense signals. As a result, CMNs can influence competition among plants positively or negatively, impacting plant community structure and ecosystem productivity (Walder et al. 2012). More recently, the role of both types of mycorrhizal association in the decomposition of soil organic matter (SOM) has been increasing due to the consequences for how soil C stocks will be affect in response to global warming (Talbot et al. 2008).

Arbuscular Mycorrhiza evolutionary history traces back 400 Ma and AM is considered to be the mutualistic partner that helped the first terrestrial plants to colonize land (Redecker et al. 2000). Up to 80% of all living plants have this type of association which is predominantly

found in agricultural ecosystems and tropical biomes. In this association, all fungal partners belong to the Glomeromycota clade and are unable to complete their life cycle without intracellular colonization of a host plant (obligate biotrophic organisms) (Kiers and van der Heijden 2006). AM fungi are able to take up N and P in organic and inorganic forms by accessing these nutrients beyond the root extension area (Walder et al. 2012). These two nutrients are demanded in large amounts by plants and their availability in natural soils is often a constraint for plants, hence the importance of AM association for ecosystem services. They are known to having limited extracellular enzymatic capability leading to a low capacity to access organic compounds in the soil. Therefore, it has been suggested that they rely on other soil microbes such as Bacteria and/or saprotrophic fungi to mineralize soil organic matter (SOM) and then acquire mineral nutrients released in the soil (Smith and Smith 2011). Studies provide evidences that the establishment of AM is highly dependent on N and P availability and promoted when at least one of these two major nutrients is limited in soils. In return for the nutrients, the host plants transferred C to the fungi in two major forms, sugars and fatty acids (FAs). In case of the first, photosynthetically C is transferred via phloem to the root system and it is unloaded into the plant's tissue as sucrose. The sucrose must be cleaved by the host cell into hexoses and finally transferred as glucose at the plant-fungal interface into the fungal tissue. The precise mechanisms by which fungi take up the C at the cellular level remains unknown. Regarding the lipids, they are the major compounds in which AMF store C and are directly involved in the spore formation and hyphal growth. The lack of genes encoding the production of a fatty-acid synthetase complex (FAS 1) required for the FAs biosynthesis in different AMF is suggested as one of the reasons for the biotrophic nature of these fungi (Wipf et al. 2019).

In contrast to AM fungi, Ectomycorrhizal fungi are facultative biotrophs belonging to the phyla Basidiomycetes and Ascomycetes and establish a symbiosis with around 6.000 plants species mainly in boreal and temperate forests (Martin et al. 2016). Despite of the fact that they colonize a relative small number of plants species in comparison to AM fungi, the number of ECM fungi is estimated to be much higher, around 20.000 species (Tedersoo et al. 2012). Their importance is related to their widely spread distribution of the terrestrial land surface and also to the economic value of truffles and porcini (Smith and Read 1997).

Their role in N uptake is well established in the literature as they are capable of taking up organic and inorganic forms of N and therefore help plants to overcome the strong N limitation in these environments (Tamm 1991; Mello and Balestrini 2018). Normally in terrestrial ecosystems, only a small fraction of N is available in the inorganic form, mainly as ammoni-

um or nitrate, which are accessible for plants. Therefore, ECM play an important role in the decomposition of organic N sources from the soil organic matter and the transfer of accessible forms of N to their host plants (Tamm 1991). These fungi are able to produce chitinases and phosphatases to break down complex organic matter and access nitrogen and phosphorus locked up in these compounds. However, it has been suggested that ECM fungi are also capable of decomposing plant litter in order to mobilize glucose and some amino acids, a niche that has been attributed to saprotrophic fungi (Lindahl and Tunlid 2015). The ability of ECM fungi to obtain nutrients from non-living organic material is related to the fact that ECM symbiosis has evolved independently from saprotrophic groups several times throughout the evolutionary history. Though they are normally spatially separated from saprotrophs, which are present mostly in the fresh litter layer (Lindahl et al. 2007), molecular studies have shown that ECM have a reduced gene repertoire responsible for extracellular enzymes that degrade plant cell walls than saprotrophic fungi. During the evolutionary history ECM fungi have partially lost their capability of degrading lignocellulose during their exploration of SOM (Martin et al. 2016). Still, they possess a much larger molecular machinery to produce extracellular enzymes to degrade SOM in comparison to AM fungi (Read and Perez-Moreno 2003).

ECM fungi occupy a dual niche, having access to nutrients provided by their host plants or scavenging themselves from the soil (Martin et al. 2016). In their studies, Talbot et al.(2008) suggest three hypotheses by which mycorrhizal fungi may promote soil C decomposition. The first, named “Plan B”, the authors suggests that the decomposition of organic C by mycorrhiza happens as an alternative to obtain carbon due to low photosynthesis rates and therefore a decrease in C allocation from the host plants. In the second hypothesis, named “Coincidental Decomposer”, the mobilization of organic C is a secondary process that happens due to the breakdown of compounds targeting the release of mineral nutrients, such as phosphorus and nitrogen, not the C itself. The third hypothesis, named “Priming Effect”, would involve a high C allocation from the host plants that accelerates the decomposition of organic C by soil microbes due to the high input of labile C source as an energy source. The understanding of C decomposition by ECM fungi and the mechanisms behind offer a better view on how above and belowground diversity might affect the global C balance.

The vast majority of ECM fungi share three fundamental anatomical traits (Martin et al. 2016): a mantle of fungal tissue surrounding the root tips which explores the rhizosphere and the nearby soil, an intraradical hyphal that grows between the epidermal and cortical plant cells (the Hartig Net) without promoting intracellular penetration and an outwardly growing

hyphal system connecting soils and the fruit bodies (Smith and Read 1997). Although these conserved anatomical traits are shared among different species of ectomycorrhizal fungi, they present a vast phenotypic variation. Agerer (2001) categorized different types of mycelium systems based on the amount of emanating hyphae, presence and/or differentiation of rhizomorphs. They can be classified as close contact, short, medium or long distance species and these anatomical differences might lead to different foraging strategies. It has been suggested that nutrient uptake and transport are performed by the most distal parts of the fungal mycelium (Unestam and Sun 1995) and the storage and exchange between symbionts are performed in the mantle (Smith and Read 1997). The extension of these morphological differences could be of functional advantage to the plants, as by hosting different fungal species with different exploitation strategies could increase the access to nutrients in the soil.

By principle, the idea of fungus-plant specificity has application for laboratory studies but does not hold ecologically (Van der Heijden 2016). Many studies have shown that many different tree species are colonized by different fungal species and vice-versa (Van Der Heijden and Horton 2009 for review). In temperate and boreal forests for example, there is a dominance of a few tree species such as pines and oaks while the biggest biodiversity is actually located belowground, where several hundreds of different ECM fungi species co-exist (Martin et al. 2016). The extraradical mycelium produced by these fungi might promote not only access to patchy nutrient hotspots but also a connection between different fungal species located at the same and/or different root systems. These belowground networks, so called Common Mycorrhizal Networks (CMNs) might also have an impact on the nutrient acquisition by the host plants as different fungi metabolize and take up different nutrients by accessing different pools in soil and producing different enzymes (Selosse et al. 2006).

CMNs affect soil structure, move nutrients from one plant to another between far distances and might influence plant community composition by facilitating or suppressing growth between neighboring plants. The interconnection with bigger plants could influence the establishment of seedlings either by amplifying or alleviating the competition for nutrients as it allows intra and interspecific transference of resources between plants (Simard et al. 1997b; Klein et al. 2016) .

The idea of a C transfer between plants via hyphal pathway has been debated for many years and most of the literature available concerns CMNs formed by AM fungi. In a laboratory experiment, Finlay and Read (1986) provided evidences of a ^{14}C transfer between pine plants via EM mycelium connection and that the polarity of movement was influenced by shading one of the seedlings. Only in 1997, Simard et al. (1997b) provided the first evidence of a bidi-

rectional C transfer between different seedling species via ectomycorrhizal CMN in a field experiment. By using reciprocal labelling (^{13}C and ^{14}C) in the field they were able to show the net carbon transfer between two ectomycorrhizal plant species was higher when the seedlings were subjected to deep shading. The results show a bidirectional transfer of 4% of the total isotope fixed by both species together in the first year of experiment and increased up to 7% in the second year. In a greenhouse experiment, Philip et al. (2010) compared the belowground C transfer between CMN and soil pathways between paper-birch (*Betula papyrifera*) and Douglas-fir (*Pseudotsuga menziesii*) ectomycorrhizal seedlings. Their main findings showed a bidirectional movement between plants, and that CMNs transferred 3 times more C than the soil pathway. Interestingly, the C was transferred even when the CMNs were severed with a razor blade and still higher than the soil pathway, suggesting that ectomycorrhizal connection between root systems played a significant role in facilitating C transfer between plants.

The first experiments showing a C transfer via CMN connecting forest trees, not seedlings, were shown by Klein et al. (2016). By using a tall canopy crane to label Norway spruces tree with $^{13}CO_2$ for 5 years they were able to detect the ^{13}C in fine roots of unlabeled neighboring plants bellowing to different taxa. They also traced the ^{13}C into fruiting bodies of ECM fungi but not in the biomass of saprotrophic fungi that degrade litter and do not form mycorrhizal association nor in the AM fungi associated with neighboring herbs. The authors claimed a yearly transfer of 280kg carbon per hectare between different tree species in a mixed forest in Switzerland and suggested evidences that ectomycorrhizal fungi acted as carbon conducts between these trees.

All these studies are important to elucidate what are the trading rules behind this symbiosis. The mechanisms by which C is transferred from the hosts to CMNs and how different mycorrhizal fungi move and distribute nutrients within plant communities are still poorly understood.

2. MANUSCRIPT

“Exchange and distribution of carbon and soil nutrients in common mycorrhizal networks of European beech (*Fagus sylvatica*)”

2.1 Introduction

Mycorrhizal fungi form associations with almost all land plants around the world and the mycorrhizal symbiosis has been suggested to have played a major role in the colonization of terrestrial habitats and diversification of early land plants (Hoysted et al. 2018). The association is based on the exchange of essential nutrients between both partners, with the host plant providing photosynthetically assimilated carbon (C) in exchange for soil nutrients, such as nitrogen (N) and phosphorus (P) acquired by the fungi. The fungal mycelium, which extends far into the soil, promotes access to nutrients that are not directly accessible to the roots themselves, influencing nutrient cycling as well as plant fitness and distribution (Smith and Read 1997). Mycorrhizal mycelia can also establish belowground plant-to-plant interconnections, called Common mycorrhizal networks (CMNs) where nutrients can be moved among different fungal and host plants partners (Gilbert and Johnson 2017).

Ectomycorrhizal association (EM) is established between fungal taxa belonging to Ascomycetes and Basidiomycetes and most of angiosperm and gymnosperm trees (Bonfante 2010). Mycorrhizal fungi of this association colonize the tip of lateral roots forming an anatomically complex organ composed by the tissue of both symbionts. Despite morphological differences among species, similar anatomical traits are shared: a mantle of fungal tissue that encloses the root tips, hyphae that grow between epidermal and cortical plant cells forming the so called 'Hartig Net' and an extraradical mycelium extending into the rhizosphere and the soil beyond the root influence zone (Balestrini and Kottke 2016). EM are dominant in boreal and temperate forests as they can support plants to overcome the N deficiency typical of these environments (Tamm 1991; Mello and Balestrini 2018). They possess genes encoding for extracellular enzymes that degrade labile and recalcitrant soil organic matter (SOM) in order to mobilize nutrients that are bound in the organic matter.

This symbiosis has been established as one of the most fruitful relationships as both organisms involved usually benefit from this relation. It has been suggested that the photosynthetically fixed C delivered by the plants is "luxury good" as its costs are almost negligible to the plants (E. Toby Kiers & Heijden, 2006). EM fungi benefit from labile C provided by the host plant allowing them to grow and extend their hyphal network into the soil and forage for nutrients (Fellbaum et al. 2014), which in return, benefits the host plants. In addition, by hosting different species of mycorrhizal fungi with different nutrient acquisition strategies, plants benefit from physically expanding their area, by accessing different soil compartments or regions by the extraradical mycelia extension (Agerer 2001).

At a forest level, plants do not grow isolated from each other. Different plant species co-exist and they also host different fungal species within their root systems forming intra or interspecific mycelial connections where nutrients can be moved (Simard et al. 1997b). Since the early 1980, many studies have been done in order to understand the role of CMNs in carbon transfer between plants. At first, these studies were performed exclusively in laboratory conditions due to the fragile and microscopic nature of extraradical mycelia. The use of ^{13}C or ^{14}C isotopic labelling allowed tracing the uptake from a donor seedling and transfer to a neighboring receiver seedling. Since then, many studies claimed to have proven a translocation of ^{13}C via CMNs from one mycorrhizal plant to another, even between different plants species (Finlay and Read 1986; Philip et al. 2010; Weremijewicz and Janos 2013; Weremijewicz et al. 2016) but only a few of them have been done in field conditions (Simard et al. 1997b; Klein et al. 2016).

A CMN typically connects a number of different plants with a variety of different fungi. That means each plant hosts several fungal partners, while every fungi colonizes several plant partners. The 'terms of trade' under which nutrients are exchanged in such a network of 'n:n' partners are complex and likely driven by a combination of inherited plant and fungal species strategies, the ability to recognize and reward beneficial partners (Kiers et al. 2011, 2016; Fellbaum et al. 2012), and by supply and demand of the traded nutrients in the network (Werner et al. 2014; Wyatt et al. 2014; Whiteside et al. 2019). In practice, however, the actual mechanisms of nutrient trading between plants and fungi in CMNs still remain unclear. For example, by using differences in natural abundances of $^{13}C/^{12}C$ between C_3 and C_4 plants and ^{15}N and ^{33}P labelling Walder et al. (2012) found a strong asymmetry in the nutrient trading via CMNs of arbuscular mycorrhizal (AM) fungi. The plant species which provided most C into the mycelial network received up to 90% less N and P in return, in comparison to its competitor plant, who invested less C to the fungal partners. This result strongly suggests a 'non-fair' trade of nutrients, with the CMN acting in favor of the weaker plant partner. On the other hand, Fellbaum et al. (2014) and Weremijewicz et al. (2016) showed that CMNs formed by AM fungi preferentially allocated more resources to full-sun large host plants who delivered most C into the fungal network and penalized plants that had been shaded and thus provided less C into the network. This, by contrast, hints to a 'fair' exchange of resources, which as a consequence reinforced the advantage of full-sun plants over their competitors. These findings suggest that CMNs can potentially amplify or alleviate plant competition by moving resources across sink-source gradients in different ways. However there is still a lack of understanding the mechanisms behind this nutrient transfer and how plant-to-plant dynam-

ics are affected by CMNs (Van Der Heijden and Horton 2009). Additionally, much less is known about nutrient exchange in EM networks, in contrast to AM networks.

Here, we examined C and N flow through an EM mycelium network connecting pairs of young beech trees with the following research questions: (1) Is there a plant-to-plant carbon movement between beech trees via CMNs, in particular if trees have unequal access to C from photosynthesis? (2) How are plant photosynthates distributed belowground within rhizosphere soil and the mycorrhizal network, and is this distribution influenced by the size of the network? (3) Do CMNs formed between beech trees amplify or alleviate belowground competition for nutrients? To address these points, young beech trees were grown inside perforated pots covered with a double layer of meshes sized 121 µm and 49 µm which prevent root but not hyphae growth into the surroundings of the pots. The same two meshes were used to enclose approximately 8 grams of ^{15}N labeled peat, thus providing a hyphal-exclusive N source (hereby referred as 'peat bags'), which were buried in each pot. Always two plant pots were placed inside of one plastic box and the interstitial space was filled with quartz sand. Two treatments were applied in a fully factorial design: 1) Allowing/preventing the establishment of a CMN between the pots (some pots were turned around at a regular interval to prevent the establishment of CMNs) and 2) inequality of access to photoassimilated C (in part of the boxes one of the two plants was shaded). While in the 'no CMN' treatment each plant had mycorrhizal access only to their own 'native' ^{15}N labelled peat bag, in the 'CMN' treatments, plants could theoretically also access the peat bag N resource of their plant neighbor's pot via their mycorrhiza. In a combined ^{13}C-CO_2 and ^{15}N labelling approach, we subsequently traced the label assimilated by one plant of each tree pair (i.e. the 'donor' plant) into belowground pools of both donor and 'receiver' plants (i.e. the unlabeled neighboring plants), as well as into the receiver plant itself, by isotope ratio mass spectrometry (EA-IRMS) and ^{13}C phospholipid fatty acid (PLFAs) analysis (GC-IRMS).

We hypothesized the following: (i) The presence of a CMN will amplify inequalities between pairs of neighboring plants caused by different access to light. We expect that plant pairs that invest equal amounts of C into the mycorrhizal network will receive equal amounts of N from their peat bag, if they are growing separated, or from the shared resource of both peat bags, when they are connected by a CMN. If plant pairs invest unequal amounts of C to their mycorrhizal partners (because one of them is shaded, which we assume will have a negative effect on plant photosynthesis), we expect that, when growing separated, the shaded plant will receive less N in return from its mycorrhizal partner leading to a decrease of plant biomass relative to its non-shaded neighbor. We hypothesize that the presence of a CMN will amplify

this inequality between the neighboring plants, because more of the N from the shared resources will be directed towards the full-sun plants by the fungal network. (ii) We further hypothesized that plant photoassimilates will be preferable allocated within the realm of the plant's 'home' pot when plants have equal access to C (i.e. none of them is shaded). This is because obtaining nutrients from the nearer area of the home pot would be associated with less C costs compared to obtaining nutrients from the neighboring pot, which requires hyphal growth through the sand barrier and competition with the neighbor's mycorrhiza. However, under unequal C supply, we expect that full-sun donor plants will allocate more C into the shaded plant peat bag in exchange for its N. (iii) In this sense, we also hypothesize that photoassimilated carbon will be transferred from the full-sun plants to the shaded plant mycorrhiza when they are connected via CMNs. The transferred C, however, will stay in the fungal tissue of the mycorrhizal roots of receiver plants and not be transferred to the plant itself (it helps sustaining fungal colonization of shaded plants).

2.2 Material and methods

Sampling and experimental setup

Young beech trees (20 - 25 cm high), all from the same cohort growing up in a clearing of the forest were collected from the Vienna Woods (Schottenwald) in October 2016. Soil was sampled from the uppermost 10 cm and sieved through a 4 mm mesh. Beech litter from the site was cut into 1-2 cm pieces by hand and uniformly mixed with the soil. The litter made up only small fraction of the total soil volume and was added to increase the amount of mycorrhizal hyphae and spores in the soil to ensure a good (re-)colonization of the plants after their transfer to pot. Perlite was mixed with the soil in order to provide aeration and improve water drainage.

The plants were transferred into pots filled with the mycorrhiza-enriched soil. Each pot (9 cm top diameter x 7 cm height x 6 cm bottom diameter) had been perforated 33 times 1 cm holes on the side and 6 times 1 cm holes on the bottom. The inner part of each pot was covered with a double layer of meshes sized 121 and 49 µm, in order to prevent fine root growth but not hyphae growth outside the pots. In addition, the same two mesh sizes were used to enclose approximately 8 grams of ^{15}N labeled peat ($\delta^{15}N$= 400‰), thus providing a Mycorrhiza-exclusive N source buried in each pot. The natural abundance control plants received mesh bags containing unlabeled soil instead of labeled peat to provide the same handling as the other plants, but to avoid a source with a different isotopic signature (also the unlabeled peat had slightly different isotopic signatures compared to the soil).

^{15}N Peat labeling

A total amount of 0.2 g of ^{15}N labeled algal amino acid mix (98 atom % ^{15}N) was added to 1.8 g of unlabeled amino acid mix, resulting in a total of 2 g ^{15}N labeled amino acid mix (10 atom % ^{15}N). This was mixed with 1 kg of fresh peat (Immergrün Gartentorf, Raiffeisen Ware Austria Aktiengesellschaft, Vienna, Austria) and 1 liter ultra-purified water (Milli-Q), resulting in homogeneous slurry left air-drying for 10 days. After that, the peat was frozen at -20°C overnight, thawed and further filled into mesh bags (11 x 12 cm) (Figure 1a,b). We assumed that microbes in the peat will readily take up the added amino acids and incorporate them into complex molecules in their biomass. Freezing and thawing was carried out in order to kill a proportion of living cells, thereby promoting the transfer of ^{15}N from microbial

biomass to dead organic matter in the peat. Labelled peat was kept at 4°C for 12 days until the setup of the experiment.

Pot set up

The plant pots were positioned in pairs at a distance of 6 cm bottom to bottom in plastic boxes (22 x 14 x8c m) filled with quartz sand (Figure 1c). Two holes were punched in the bottom of the plastic boxes, and pots were fitted in to keep them equally spaced, whilst allowing external drainage (i.e. to avoid accumulation of water draining from the bottom holes of the pots inside the plastic box). Two treatments were applied in a fully factorial design (Figure 3): 1) 'CMN Treatment': Allowing connection or no connection between the two plant pots in a box via mycorrhizal mycelia (some pots were turned around to prevent the establishment of mycorrhizal connections in the sand between the pots) and 2) 'Shading treatment': equality or non-equality of access to photoassimilated C (in part of the boxes one of the two plants was shaded). Regarding the 'CMN' treatment, for six months half of the boxes (*n=15*) were kept untouched in order to allow hyphae to spread and establish mycelial connections between the two neighboring pots or plants ('CMN' treatment). On the other half (*n=15*) the pots were manually rotated every second day in order to prevent CMN establishment between the two neighboring plants ('no CMN' treatment). The pots serving as natural abundance controls (5 pots in 3 boxes because 1 plant died) were also rotated every two days. Concerning the 'Shading' treatment, in six boxes from each the 'CMN' and 'no CMN' treatments *(n=12*) one of the two plants was partially shaded for 5 months by attaching a woven, white polyester shade cloth that blocked above 90% of UV rays (Floracord Balkonsonnensegel) around a cylindrical, flexible fence, loosely closed on top.
Plants were left in this setting for around 6 months in an open-roof-top glasshouse, which provides natural conditions, such as sunlight and temperature, except in case of snow or rain where a movable ceiling would close. All pots were watered daily.

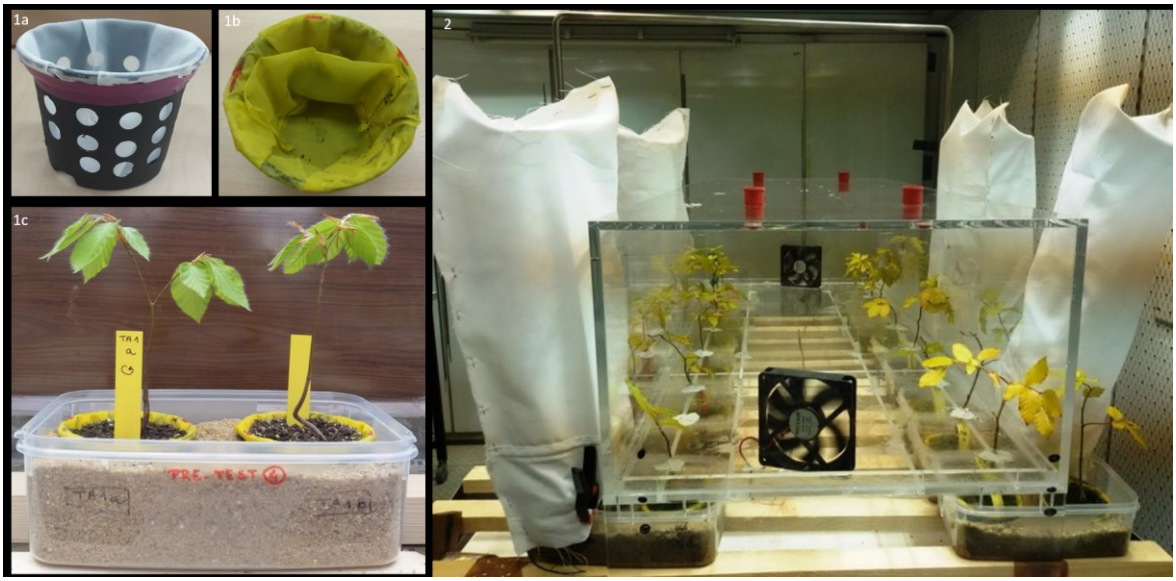

Figure 1: Experimental set up. (a) Perforated pots wrapped in a coarse (121µm) and fine mesh (49µm) to allow hyphae but not root growth. (b) Mycorrhiza-exclusive ^{15}N-labelled peat in double layer of a coarse (121µm) and fine mesh (49µm) mesh bags buried in each pot. (c) Pots positioned in pairs in plastic boxes filled with sand, to allow mycorrhizal connections to establish between them.

Figure 2. Labelling chamber where the aboveground plant parts were incubated in a ^{13}C-CO_2 enriched atmosphere (approximately 90 atom% ^{13}C).

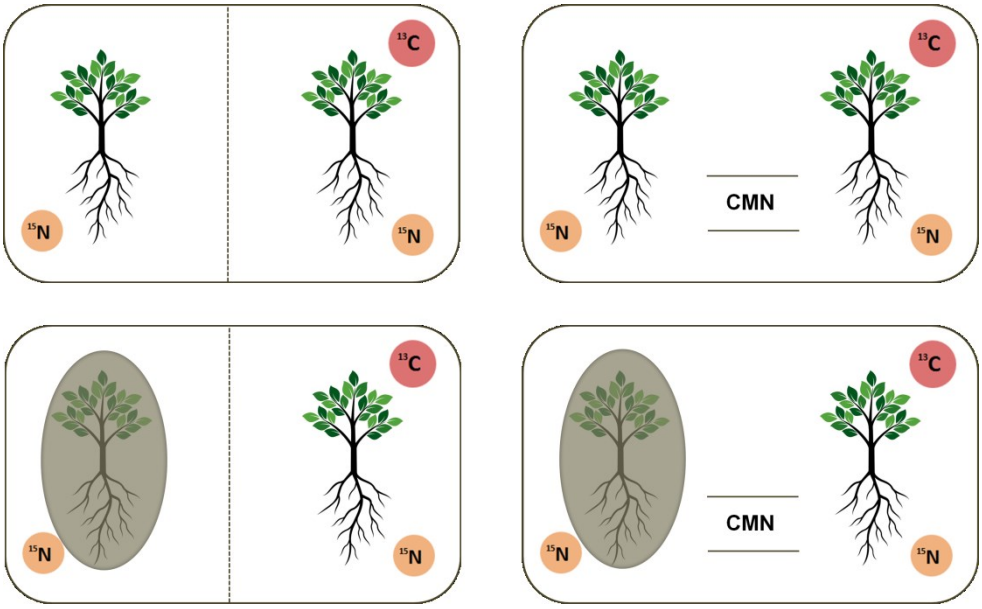

Figure 3: Fully factorial experimental design varying 2 factors: presence/absence of 'CMN' and equal/unequal conditions (i.e. shading of one tree). The full setup was replicated 6 times, and the 'CMN' and 'no CMN' treatments (both without shading) was replicated an additional 3 times, making a total of 30 boxes (4 treatments x 6 replicates + 2 treatments x 3 replicates).

$^{13}CO_2$ labeling

After the growing period (6 months), and four weeks before harvesting the plants the $^{13}CO_2$ labeling started in August 2017. The labeling was performed in a transparent plexiglass incubation chamber (1,350 × 545 × 320 cm) which was placed inside of a climatic chamber where day and night conditions were simulated. In the climatic chamber, plants had total of 14,5 hours of light and 9,5 hours of darkness, temperate varied between 16°C to 25°C, simulating ambient conditions for the month of August. The Plexiglass incubation chamber was manufactured in a way allowing the upper part of the plants (shoots) to be inside the chamber, and the lower parts (roots and soil) to be outside. The bottom of the chamber consists of sideway slidable parts allowing the plants to be fitted in at stem height into 1 cm width open gaps which can then be closed by sliding in the bottom plates (Gorka et al. 2019). Gaps between the stem and the remainder of the 1 cm holes were closed with a malleable sealant (Terostat IX) to prevent the diffusion of the labeled gas from the chamber into the soil. The dimensions of the slideable bottom plates of the chamber were tailor-made to fit the sizes of the plastic boxes in a way to allow us to place only one of the two trees of a box inside the chamber whereas the partner tree could stay outside (Figure 2). The chamber has an approximate volume of 235 cm^3 and its atmosphere was filled with $^{13}CO_2$ (app. 90 atom %). 16 plants can be placed in the chamber at a time therefore two days were necessary to complete one round of labelling, each day with 15 plants inside of the chamber. To prevent a systematic bias during labelling, the plants were sorted according to the treatments, therefore always the same number of plants belonging to the same treatments were inside the labelling chamber at the same time. During the labelling period, the shoots of one of the two neighboring plants ("donor plant") of each box were regularly incubated inside the labelling chamber (8 hours once a week for four weeks). The neighboring plants, which were never placed inside the $^{13}CO_2$ chamber, are referred to as "receiver plants". In every day of labeling, the chamber was connected with an infrared gas analyzer (EGM-4, PP systems, Hitchin, UK) to monitor the CO_2 concentrations. We performed a pre-experiment to determine the rate of CO_2 uptake by the plants. After closing the chamber, the CO_2 concentration was measured by the infrared gas analyzer and the results showed a depletion of 60 ppm per hour. Thus, in each day of the labelling, the $^{13}CO_2$ injections started approximately two hours after the chamber had been sealed. When the CO_2 dropped to 400 ppm due to photosynthetic activity after closing the chamber, the 8-hour labelling period started, and 28 ml of ^{13}C-CO_2 (99

atom%, Sigma-Aldrich, Vienna, Austria) corresponding to 120 ppm, was injected with a gas tight syringe via built in septa. Follow-up injections were done every two hours resulting in four injections per day of labeling to ensure a highly enriched ^{13}C-CO_2 atmosphere inside the chamber. Right before and right after each new injection a gas samples was taken later analyzed by a headspace gas sampler (GasBench II, Thermo Fisher Scientific, Bremen, Germany) interfaced to continuous-flow isotope ratio mass spectrometry (Delta Advantage V, Thermo Electron, Bremen, Germany) to determine total CO_2 concentration and ^{13}C enrichment in the chamber during the course of the experiment. Two hours after the last injection and by the end of the 8 hours labeling the lights were switched off to prevent any photosynthetic activity and only then the plants were removed from the chamber.

The ^{13}C assimilated by the donor plants was subsequently traced into belowground pools of both donor and receiver plants (i.e. the unlabeled neighboring plants), as well as into the receiver plant itself, by isotope ratio mass spectrometry (EA-IRMS) and ^{13}C phospholipid fatty acid (PLFAs) analysis (GC-IRMS).

Harvest

The harvest took place in five days. On the first day only the natural abundance controls were harvested in order to prevent any contamination by the labeled plants. The other plants were harvested in four consecutive days. Within these four days, each of the two batches of 15 boxes that had always been labelled together, was harvested at four consecutive days, respecting an interval of six days after the last $^{13}CO_2$ labeling for each batch to ensure similar time for ^{13}C assimilation by the plants.

Respiration measurements

In each day of harvest, the plants were removed from the plastic boxes and gas samples of the soil plus plant respiration and also microbial peat bag respiration were measured. In order to do so, each plant pot was placed inside of a closed dark chamber (1.410 ml) to prevent any photosynthetic activity, with a fan attached inside to ensure a homogeneous atmosphere. Two 15 ml gas samples were drawn, the first one immediately after closing the chamber and the second one after 15 minutes. To prevent an under pressure in the system, immediately after the first sample taken, 15 ml “ambient CO_2 concentration” (approximately 200 ppm) was injected in the chamber. After that, the peat bags were harvested and placed inside of 100 ml incubation jars and again two gas samples were drawn following the same procedure as

described before. In order to determine the total CO_2 concentration and also the ^{13}C enrichment in the respiration the samples were analyzed by a headspace gas samples (GasBench II, Thermo Fisher Scientific, Bremen, Germany) interfaced to continuous flow isotope ratio mass spectrometry (Delta Advantage V, Thermo Electron, Bremen, Germany).

After the respiration measurements, the plants were taken apart and split into leaves, stem, coarse and fine roots. Fine roots were cut into smaller pieces and frozen at -20°C. The soil and peat were homogenized by sieving through a 4mm mesh and aliquots were taken for measurements. From the sand a subsample of approximately 20 g was sampled with a spoon from the area in the middle of the box between the two neighboring pots and immediately frozen at -20°C. Later on, samples (except sand) were freeze-dried, ground and had their $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios measured with an elemental analyzer (EA 1110 elemental analyzer, CE Instruments, Milan, Italy) coupled to an isotope-ratio mass spectrometer (Finnigan MAT DeltaPlus, Thermo Finnigan, Bremen Germany).

Root tip sampling

Immediately after the harvest, fine roots were split into five sections of similar sizes and analyzed under stereo microscopes in order to access mycorrhizal colonization. Individual root tips of different mycorrhizal morphotypes as well as non-mycorrhizal tips were collected and later had their enrichment in ^{13}C and ^{15}N measured via isotope ratio mass spectrometry coupled to an elemental analyzer (EA-IRMS). Some of the root tips were sampled and frozen at -80°C for downstream molecular analysis. Additionally, some samples have been stored in liquid Nitrogen for future NanoSIMS analysis.

^{13}C, Carbon and Nitrogen in Microbial Biomass and Dissolved Pools

In order to determine Dissolved organic carbon (DOC), Total dissolved nitrogen (TDN) and also the C and N assimilated in the microbial biomass, around 1,5g of fresh soil and peat were extracted with 15 ml 0.5M K_2SO_4 before and after Chloroform fumigation extraction (CFE). For the CFE, samples were fumigated for 48 hours after harvest under vacuum and dark conditions. After being stored at -20°C, aliquots of the extracts were analyzed by a TOC/TN analyzer (TOC-VCPH Total organic carbon analyzer, Shimadzu, Japan) and the ^{13}C was analyzed by a High Performance Liquid Chromatography System (HPLC – Dionex

Sunnyvale, USA) coupled to a IRMS (Finnigan Delta-V, Thermo Electron, Germany) via an LC-Isolink (Thermo Electron, Germany).

The microbial biomass C and N were calculated by the difference between fumigated and unfumigated samples as "µg g^{-1} dry weight". The ^{13}C enrichment was calculated based on the difference in relative isotopic enrichment between labeled and unlabeled controls in atom% excess.

Phospholipid fatty acid (PLFAs) extraction

In order to access the microbial community and its incorporation of the ^{13}C in the microbial biomass, PLFAs were extracted according to Buyer and Sasser (2012) with some modifications. After the harvest, samples were stored at -20°C as described in Schnecker et al. (2012). The extraction was performed on peat, soil, roots and in the sand between the two neighboring plants.

0.5 - 2 g of freeze-dried soil and peat, 0.1 g of freeze-dried roots and 6 g of freeze-dried sand were extracted with a mixture of chloroform, methanol and citrate buffer (1:2:0.8, v/v/v) overnight. After the phase separation with chloroform and citrate buffer (1:1, v/v) the lower organic phase was collected and dried under a constant flow of N_2 to prevent the oxidation of the fatty acids. The lipids were later fractionated by solid phase extraction (SPE) using a 96-well SPE plate containing 50 mg of silica per well (Phenomenex, Torrance, CA, USA). In this step, each well was pre-conditioned with methanol (2x 1.5ml) then with chloroform (2x 1.5ml) before receiving the samples dissolved in 1ml chloroform. Nonadecanoic acid (FAME 19:0) was used as internal standard for further quantification and added to the samples prior methylation.

The lipids were eluted in consecutive order into neutral lipids (1.5 ml chloroform), glycolipids (1.5 ml acetone) and phospholipids (0.5 ml of 5:5:1 methanol:chloroform:H_2O). Each fraction was collected into 1.5 ml glass vials (Multi-Tier microplate, E&K Scientific, Santa Clara, CA, USA) and dried under a constant flow of N_2. The PLFAs were converted to methyl esters via alkaline methanolysis. Samples were re-dissolved in 100µl Isooctane and subsequently measured with gas chromatography-mass spectrometry (Trace GC Ultra, Thermo Scientific) coupled to a mass spectrometer (ISQ, Thermo Scientific) for identification of the biomarkers and on a Trace GC-Ultra coupled to a Delta V Advantage

isotope ratio MS via a GC Isolink and Conflo IV (all instruments by Thermo Scientific) for quantification and determination of ^{13}C abundance.

The assignment of PLFA markers was done as described by Ruess and Chamberlain (2010). The PLFAs i15:0, a15:0, i16:0, i17:0, a17:0 and i19:0 were used as indicators for Gram-positive bacteria, 16:1ω5, 16:1ω7, cy17:0, and cy19:0 for Gram-negative bacteria, 15:0 and 17:0 and the sum of the above for bacteria in general, cis18:1ω9, trans18:1ω9 and 18:2ω6,9 for fungi, and 14:0, 16:0 and 18:0 for general PLFAs.

The isotopic enrichment for each PLFA was calculated by subtracting the ^{13}C content of unlabeled controls from the ^{13}C enrichment of labeled samples, expressed as atom% ^{13}C excess. For the calculation of the relative ^{13}C content for each microbial group (i.e. bacteria, fungi), the relative ^{13}C-enrichment was weighted by the mean abundance (in µg C in PLFAs g^{-1} dry soil) of individual PLFAs representative for this group.

Calculating N uptake from the peat

The fraction of nitrogen in leaves or root tissue that was derived from the peat bags versus that was derived from the soil was calculated using a Two-pool mixing model with the ^{15}N signatures of peat and soil as end-members. The contribution of N from the peat bags to the leaves was calculated by:

$$N_{peat} = \frac{(\text{atom\% } ^{15}N_{leaves} - \text{atom\% } ^{15}N_{soil})}{(\text{atom\% } ^{15}N_{peat} - \text{atom\% } ^{15}N_{soil})}$$

as well as to the roots:

$$N_{peat} = \frac{(\text{atom\% } ^{15}N_{roots} - \text{atom\% } ^{15}N_{soil})}{(\text{atom\% } ^{15}N_{peat} - \text{atom\% } ^{15}N_{soil})}$$

Where N_{peat} and N_{soil} represents the fraction of total N in the target tissue (roots or leaves) that stems from peat or soil (each has a value between 0 and 1, with $N_{peat} + N_{soil} = 1$).

Statistical analysis

All statistical analyses were carried out in R (R 3.6.0), with package 'ggplot2' for plotting (Wickham 2009) and package 'vegan' for multivariate analyses (Oksanen et al. 2019). All

data, except PLFAs, were analyzed by two-way analysis of variance (ANOVA) with ‘CMN’ and shading as factors. Before ANOVA, heteroscedasticity of data was examined by Levene’s test and normality by Shapiro-Wilk’s test. PLFA data were analyzed by non-parametric tests (Kruskal-Wallis rank sum test optionally followed by Wilcoxon signed-rank’s post-hoc test of multiple comparisons with Benjamini-Hochberg procedure for multiple-sample comparisons) as any data transformation improved the analyses.

2.3 Results

Plants take up the majority of N from the mycorrhiza accessible peat bags

Our data show that the $\delta^{15}N$ signature of leaves and roots are much more similar to the $\delta^{15}N$ signature of the peat, than to the natural abundance $\delta^{15}N$ signature of the soil (Tables 1 and 2) indicating that the mycorrhiza-exclusive peat bag was the preferential source of nitrogen in the system. Applying a two-pool mixing models with the initial ^{15}N signatures of peat and soil as end members showed that on average across all treatments, 63% of the plant biomass N originated from the ^{15}N-labelled peat bags whereas only 37% were soil derived (Figures 4, 5). In absolute values, average of 3 mg N derived from the peat were allocated in the leaves and 4.8 mg N in the roots, indicating a mobilization and uptake of as much as 31% of the ca 25 mg N that were initially present in each peat bag. In comparison, only around 2.4 mg N and 1.74 mg N in leaves and root biomass stemmed from the soil, which initially comprised a N pool of 240 mg (Table 1), showing that this pool lost only 1.7% by plant uptake. Since for the plant N that originates from the natural abundance source it cannot be distinguished whether it was incorporated within the experimental period or before, and the plants had already considerable biomass when we planted them together with the peat bags in the pots, it is likely that much more than 63% of the plant N was obtained from peat-bags during the experimental period. More of the new N taken up from peat was incorporated in roots compared to leaves (Figure 6).

No proof that CMN amplifies or alleviates belowground competition for N

Over the course of 5 months, neither CMN nor shading did show any statistically significant effect in the amount of N transferred from the peat bags to the plant tissues (Figure 6). Average of plant biomass was 0.85g ± 0.06 and 0.90g ± 0.06 dry weight for donors and receivers, respectively and no significant effect of treatments were found in the plant biomass (see Supplementary Material, Figure S1). According to our initial assumptions, we expected that shading would have a negative effect on plant biomass and N content, leading to inequality between neighboring plants. We further expected that this inequality would be amplified if there was a CMN connection between shaded plants and full-sun plants, as N would be preferentially directed to full-sun plants by the fungal network. However, since the assumption of the negative effect of shading was not met in the first place our results cannot prove whether the presence of a 'CMN' amplified or alleviated the belowground competition for nutrients.

We also found no treatment effects on relative ^{15}N enrichments (atom% excess) of different plant tissues (roots, leaves and mycorrhizal root tips), apart from a trend of increased ^{15}N enrichment in leaves of donor plants in the presence of a CMN (Figure 4). When analyzing the relative ^{15}N enrichment at single root tips scale, colonized tips of donors and receivers had similar ^{15}N enrichment and even though no statistically significant differences were found, the results provide hints that some differences between treatments might have occurred (Figure 4). The mycorrhizal tips of donors in presence of shaded neighbors had a highest enrichment in ^{15}N when their respective shaded neighbors had the lowest enrichment and this seemed to be enhanced by the absence of CMN. When the donors were grown in the absence of a shaded partner, they did not seem to be affected by sharing or not a connection via CMN. On the receiver side, the CMN treatment had a stronger effect on the ^{15}N content. As expected, the lowest ^{15}N was found in the colonized tips of shaded receivers. However, the biggest difference in the ^{15}N enrichment was found between shaded and non-shaded receivers in the absence of CMN. These findings suggest that shading and disruption of CMNs might have influenced how different EM fungi morphotypes acquire N at the root tip scale.

Table 1. Total C and N budget of donor and receiver plants across all experimental two-plant mesocosms (boxes). Shown are total amounts of C and N, and total ^{13}C and ^{15}N excess in g or ng *per pot,* as well as the distribution of the excess ^{13}C and ^{15}N within each pot across the different pools (leaves, root, soil, peat), given (for example for C) as $\%^{13}C$ excess of total ^{13}C excess. In addition, $\delta^{13}C$ $\delta^{15}N$ and C/N ratio are given for each pool. Data is presented by means across all boxes (including all treatments) with standard errors shown in brackets; n = 30.

Donor plants (98% of total ^{13}C)

Pool	C [g]	^{13}C excess [ng]	$\%^{13}C$ of total ^{13}C	$\delta^{13}C$	N [g]	^{15}N excess [ng]	$\%^{15}N$ of total ^{15}N	$\delta^{15}N$	C/N
Leaves	0.17 (0.01)	476.83 (40.64)	40.21 (3.75)	234.36 (14.24)	0.01 (0.0004)	6.33 (0.97)	7.32 (1.05)	306.19 (35.31)	32.14 (0.75)
Roots	0.24 (0.02)	467.45 (87.31)	27.17 (2.67)	161.73 (32.54)	0.01 (0.0004)	8.81 (0.78)	10.84 (0.89)	379.56 (16.69)	39.79 (1.52)
Plant biomass	**0.41 (0.03)**	**928.39 (116.55)**	**67.38 (2.68)**		**0.01 (0.001)**	**14.93 (1.53)**	**18.17 (1.65)**		
Soil	4.62 (0.03)	457.96 (75.48)	28.05 (2.03)	-18.62 (1.52)	0.22 (0.001)	28.18 (1.23)	35.27 (1.40)	32.81 (1.44)	20.66 (0.10)
Peat	0.65 (0.01)	34.08 (4.48)	4.57 (1.57)	-22.86 (0.63)	0.02 (0.0003)	36.92 (0.24)	46.57 (0.87)	444.35 (6.71)	28.74 (0.43)
Belowground	**5.28 (0.03)**	**492.04 (79.17)**	**32.62 (2.68)**		**0.25 (0.002)**	**65.09 (1.24)**	**81.83 (1.65)**		
Total Donors	**5.68 (0.05)**	**1420.43 (187.19)**	**100**		**0.26 (0.002)**	**80.03 (1.48)**	**100**		

Receiver plants (2% of total ^{13}C)

Pool	C [g]	^{13}C excess [ng]	$\%^{13}C$ of total ^{13}C	$\delta^{13}C$	N [g]	^{15}N excess [ng]	$\%^{15}N$ of total ^{15}N	$\delta^{15}N$	C/N
Leaves	0.17 (0.01)	0	0	-27.72 (1.03)	0.01 (0.0004)	5.77 (0.67)	6.85 (0.68)	269.24 (19.28)	30.94 (0.66)
Roots	0.25 (0.02)	0	0	-27.61 (0.17)	0.01 (0.0005)	9.13 (0.81)	10.93 (0.84)	358.57 (13.54)	37.53 (1.40)
Plant biomass	**0.41 (0.03)**	**0**	**0**		**0.01 (0.001)**	**14.90 (1.35)**	**17.78 (1.34)**		
Soil	4.76 (0.03)	15.43 (3.18)	50.13 (3.66)	-25.91 (0.05)	0.23 (0.001)	30.92 (0.99)	38.24 (1.52)	34.46 (1.15)	20.27 (0.05)
Peat	0.65 (0.01)	12.08 (0.35)	49.87 (3.66)	-27.42 (0.07)	0.02 (0.0003)	37.41 (0.48)	43.97 (1.76)	442.77 (5.99)	28.19 (0.26)
Belowground	**5.23 (0.16)**	**26.59 (3.23)**	**100**		**0.26 (0.002)**	**67.08 (1.63)**	**82.22 (1.34)**		
Total Receivers	**5.64 (0.17)**	**26.59 (3.23)**	**100**		**0.27 (0.002)**	**81.99 (2.13)**	**100**		

Table 2. Total C, N, $\delta^{13}C$, $\delta^{15}N$ and C/N ratio of natural abundance soil and peat before the experimental setup. Data is presented by means with standard errors shown in brackets; n = 6.

Pool	% C	% N	$\delta^{13}C$	$\delta^{15}N$	C/N
soil	8.55 (0.54)	0.42 (0.01)	-27.69 (0.08)	-1.42 (0.16)	20.41
peat	25.54 (5.48)	0.98 (0.19)	-27.07 (0.25)	0.48 (0.70)	28.13

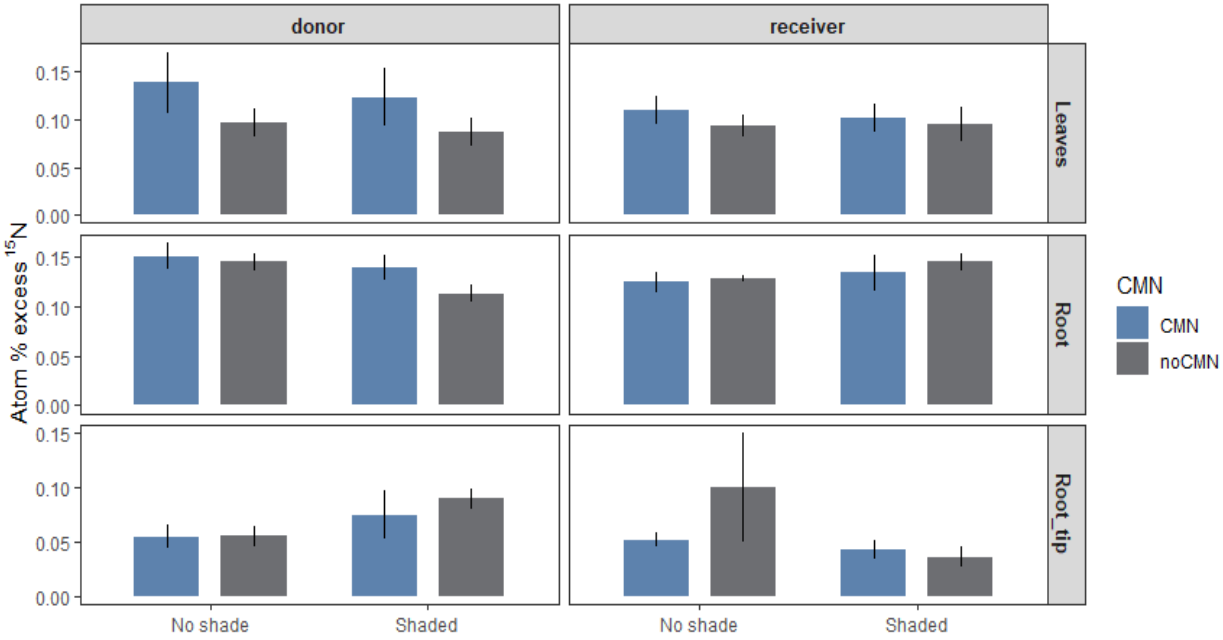

Figure 4 – Nitrogen content in leaves, roots and single ectomycorrhizal root tips in atom % excess ^{15}N. No significant differences between the treatments were found, however the higher variability in ^{15}N in the root tip suggests that shading and disruption of CMNs might have influenced how different EM fungi morphotypes acquire N at the root tip scale. All pools were significantly different from natural abundance control plants ($p < 0.05$, ANOVA), no significant effect of treatments were found. Error bars represent standard error; n = 6 for shaded and n=9 for not shaded treatments.

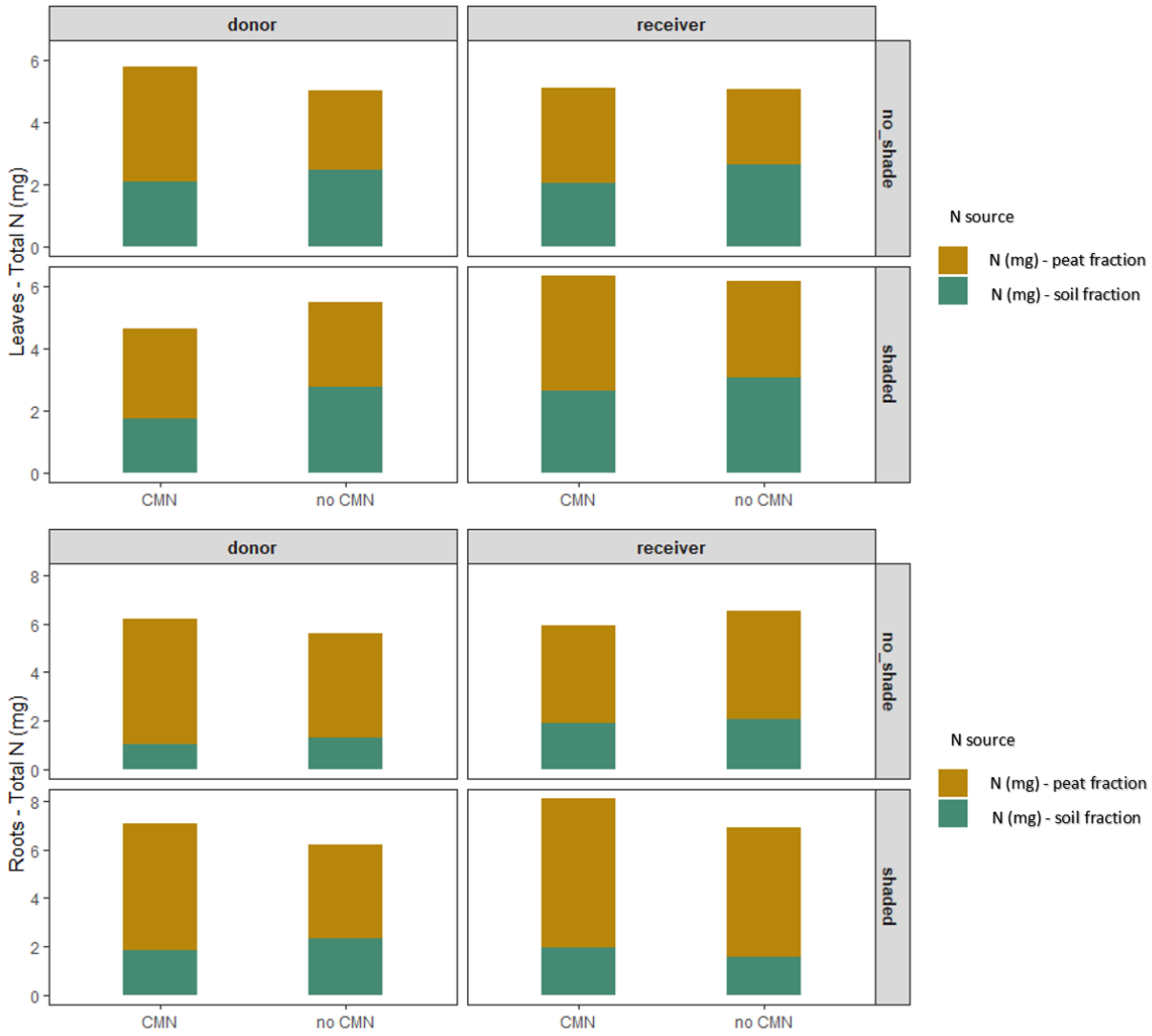

Figure 5 – Two-pool mixing model showing the N allocation in the leaves and in the roots (in mg N) derived either peat bags or soil. Results indicate that host plants relied mainly on their fungal partners to acquire nutrients as on average 63% of total plant N derived from the peat bags, a mycorrhiza-exclusive N source, rather than the soil. No significant differences were found between treatments by ANOVA. Error bars represent standard error; n = 6 for shaded and n=9 for not shaded treatments.

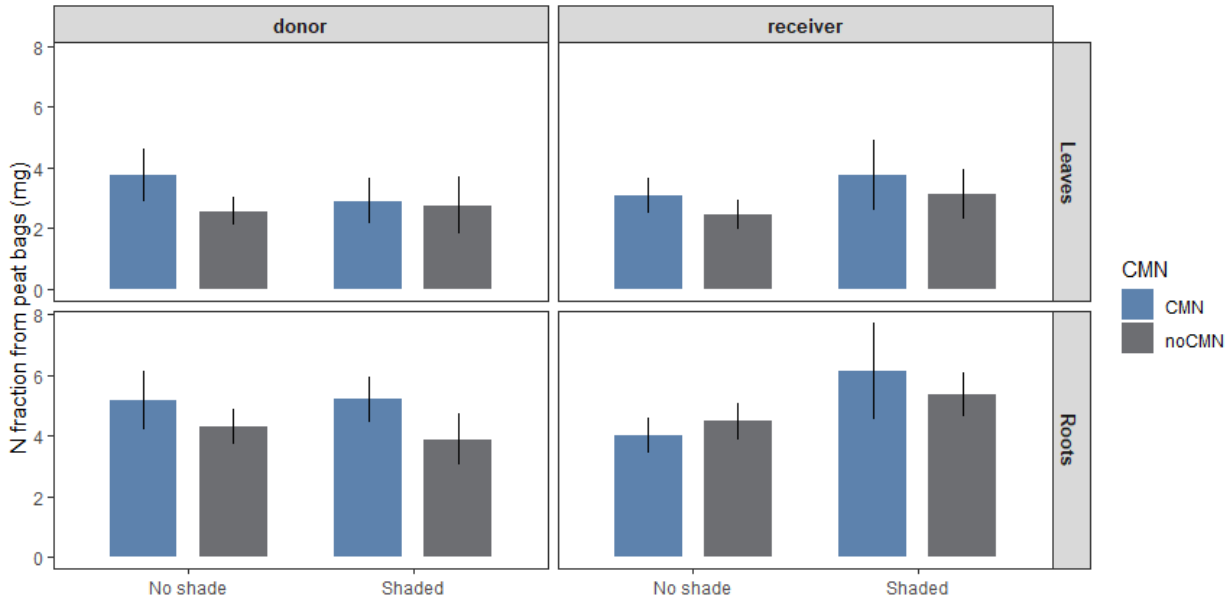

Figure 6 – Total N allocation in the leaves and in the roots (in mg N) originated from the peat bags did not vary across treatments, showing no significant effect of CMN nor shading on the belowground competition for nutrients. Results by ANOVA, calculated based on Two-pool mixing model. Error bars represent standard error; n = 6 for shaded and n=9 for not shaded treatments.

Distribution of ^{13}C in the two-plant box systems

Donor plants were highly enriched in ^{13}C in the above and belowground pools, accounting for 98% of the total ^{13}C in the system (Table 1). The remaining 2% of the ^{13}C photoassimilated by donors was found in the soil and peat bags of receiver plants, suggesting a belowground transfer via fungal hyphae between the two neighboring plant pots. Both peat and bulk soil in the receiver pots were significantly enriched in ^{13}C (p= <2e-16 for peat and p=0.0535 for soil) in comparison to natural abundance controls. There was no significant enrichment of ^{13}C in the roots or leaves of the receiver plants, proving that accidental direct plant assimilation by receivers from a potential cross-contamination with $^{13}CO_2$ during the labelling process was negligible, but also ruling out a significant C transfer and incorporation to the plant tissue by belowground transfer (Figure 7).

Plant photoassimilates were preferentially transferred into mycorrhiza-exclusive peat bags of the own and the remote partner pot

Within donor plants, 67% of the total ^{13}C were detected in the leaves and roots and 33% in the belowground pools. From the belowground allocation, 5% of the ^{13}C went into peat bags while bulk soil had the majority of it, 28%. In contrast, the receiver plants show a different pattern. Even though the soil and peat of donor plants received different amounts of total ^{13}C excess in absolute values (average of 34.08 ± 4.5 ng per pot for peat and 457.96 ± 75.48 ng per pot for soil) similar amounts were transferred to the system of their neighboring plants (average of 12.08 ng ± 0.35 for peat and 15.01 ng ± 3.28 for soil) (Table 1). However, on a per gram DW basis, photoassimilated ^{13}C allocated in the peat bags was higher and statistically different from the soil (p= 0.00724 for donors and p = <2e-16 in receiver pots) (see Supplementary Material, Figure S2). Similarly, the relative enrichment in Atom% excess ^{13}C also show a higher enrichment in the peat rather than soil in the receivers. (Figure 8). In absolute numbers, ^{13}C enrichment in the receiver peat bags was almost as high as 40% of the amount of ^{13}C found in the 'home' peat bag of the donor plant (Figure 7). This demonstrates a significant belowground C transfer between the two neighboring plant pots, with a targeted C transfer to the mycorrhiza-exclusive N source in the remote plant pot that bridged the sand and also the soil layer in between, which were far less enriched.

This finding was confirmed and even stronger pronounced in the PLFA data (Figures 9 and 10). Although the abundance of microbes per g DW was higher in soil than in peat (Figure 10), more photosynthetic ^{13}C was found to be allocated to fungal and bacterial biomarkers in peat rather than in the soil (Figure 9).

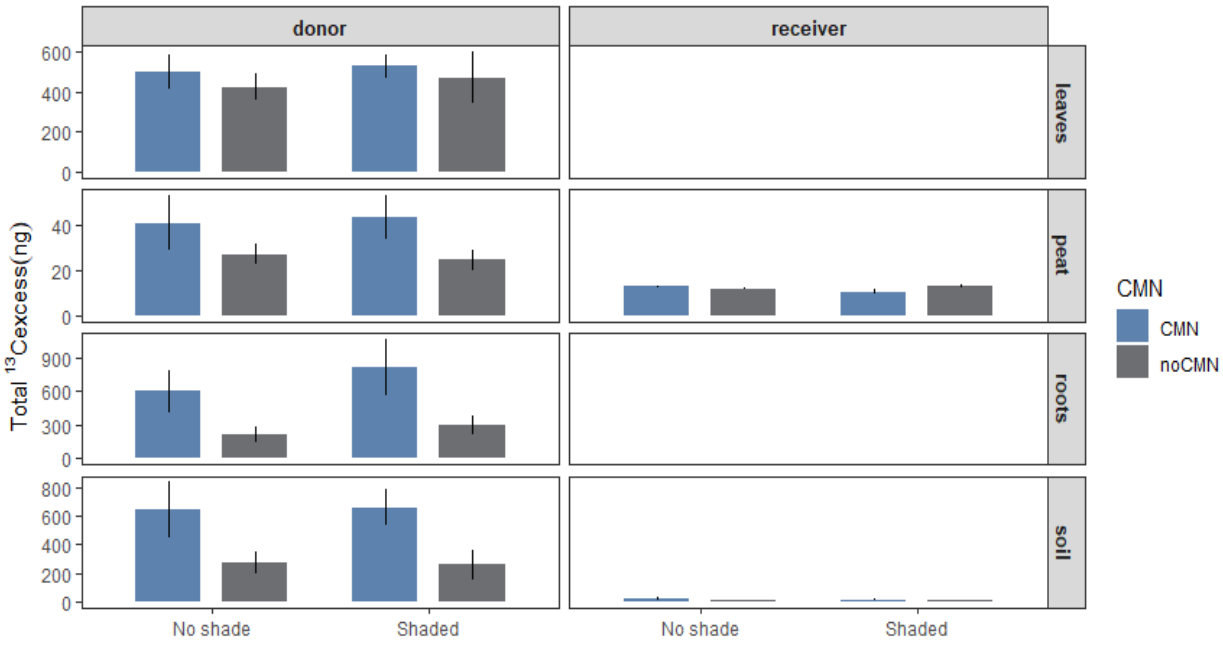

Figure 7 – Absolute ^{13}C enrichment in all pools expressed in Total ng ^{13}C excess. Donors from the intact CMN treatment had higher ^{13}C-CO_2 uptake by leaves and higher belowground C allocation when compared to the donor plants which had the CMN disrupted. However, similar amounts of ^{13}C were transferred to receiver plants, with a preferential allocation of ^{13}C to the peat bags, a mycorrhiza exclusive N-source rather than soil. Belowground pools were significantly different from natural abundance control plants in donor and receivers but leaves and roots only in donor plants ($p < 0.05$). In the donors, peat, roots and soil were significantly affected by the CMN treatment (p= 0.0824, p= 0.0121 and p=0.0128 respectively) but not leaves. In the receiver pots only peat was significantly affected by CMN and shading (p= 0.0133). Results by ANOVA; n = 6 for shaded and n=9 for not shaded treatments. Error bars represent standard error.

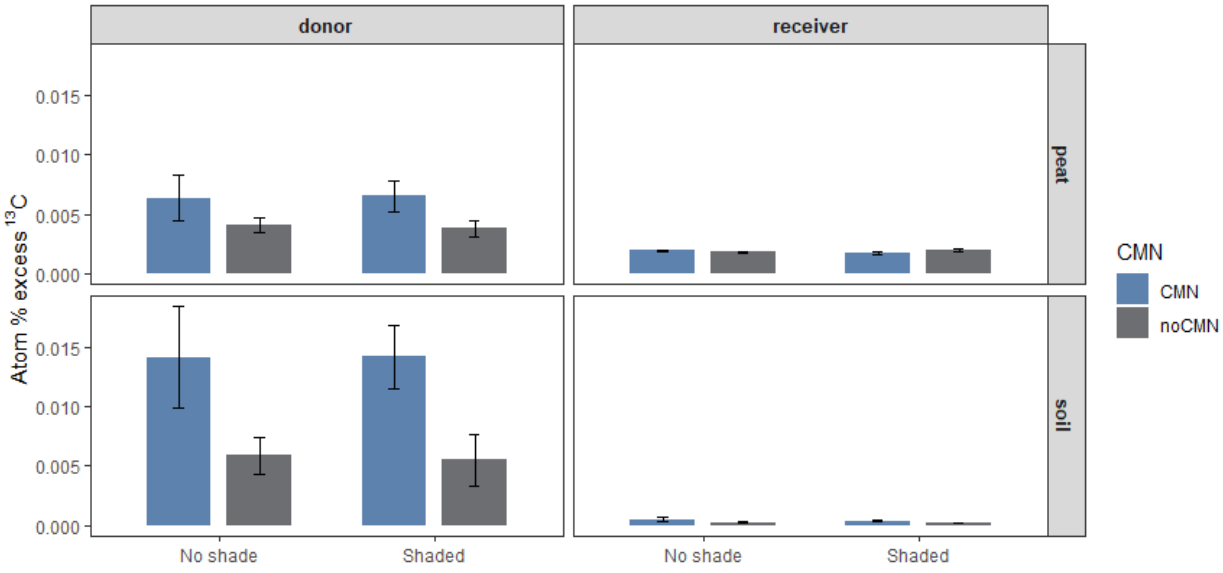

Figure 8 – Relative ^{13}C enrichment between belowground pools expressed in Atom% excess ^{13}C showing a preferential allocation to the peat bags, a mycorrhiza exclusive N-source rather than soil. Both pools were significantly different from natural abundance control plants ($p < 0.05$) and from each other in donor ($p = 0.0104$) and receivers ($p < 2e\text{-}16$). Only donor pools were significantly affected by CMN ($p = 0.0829$ in the peat and $p = 0.0114$ in the soil), not receivers. Results by ANOVA; n = 6 for shaded and n=9 for not shaded treatments. Error bars represent standard error.

The PLFA analyses also confirm that peat bags were hotspots for ^{13}C delivery to microbes, notwithstanding if they were closed to the plant or separated by a 6 cm sand barrier. The microbial community in the peat bags became highly enriched in ^{13}C compared to the microbes in the surrounding soil (Figure 9). PLFA biomarkers (fungi and bacteria) in both the nearby (i.e. the peat bag buried under the donor plant) and the distant peat bags (i.e. the peat bag buried under the receiver plant) were significantly enriched in ^{13}C indicating a hyphal transfer of photoassimilated C not only in the belowground realm of their host plants, but also over longer distances into the realm of their neighbors.

Although overall ^{13}C enrichment of bulk samples is higher in soil compared to peat bags (Figure 8: soil ca 0.01, peat ca 0.005 APE) in donor pots, microbial biomass (fungi and bacteria) is about double as high enriched in peat bags compared to soil (Figure 9: soil fungi 0.2, peat fungi 0.6 APE). Moreover, the soil PLFAs were significantly enriched only in the donor pots, once more supporting the hypothesis of a preferential C allocation via hyphae to the enclosed N source in the peat bags and excluding a ^{13}C transfer via water. The relative enrichment (in Atom% excess ^{13}C) of fungal markers was 10 times greater than the in bacterial markers. In the receiver peat bag, fungal markers were as high as the bacterial markers in the

donor peat bags, representing a substantial C transfer and allocation into the fungal microbial biomass of the distant peat (Figure 9).

Interestingly, the translocation of C into the receiver peat bags happened to the same degree also in the 'no CMN' treatment, where CMNs has been disrupted every two days by turning the pots (Figure 8).

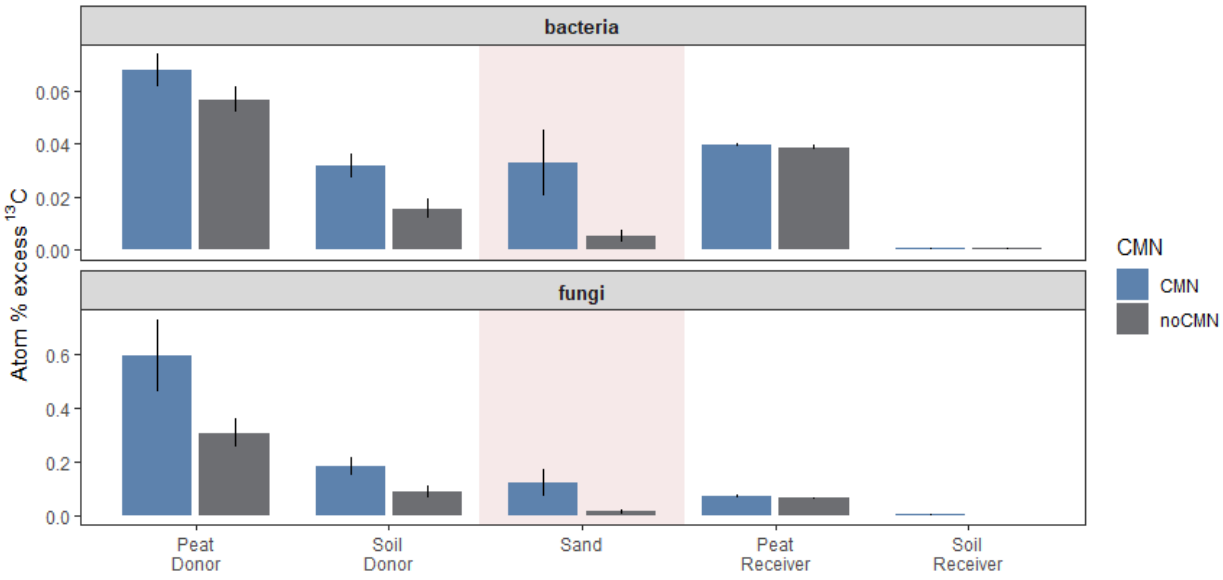

Figure 9 - Enrichment of ^{13}C in fungal-specific (18:1ω9 and 18:2ω6,9) and bacteria-specific PLFAs in peat, soil and sand. All microbial groups were significantly enriched compared to natural abundance controls in peat and sand but in the soil, only donors were significantly enriched. The CMN treatment significantly affected atom% excess ^{13}C in bacterial and fungal biomarkers in the soil of donor pots as well as in the sand ($p<0.05$), but not in peat of donor pots and not in the receiver compartments. Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Wilcoxon signed-rank's post-hoc test of multiple comparisons with Benjamini-Hochberg correction (adj. $p <0.05$); n = 15. Error bars represent standard error.

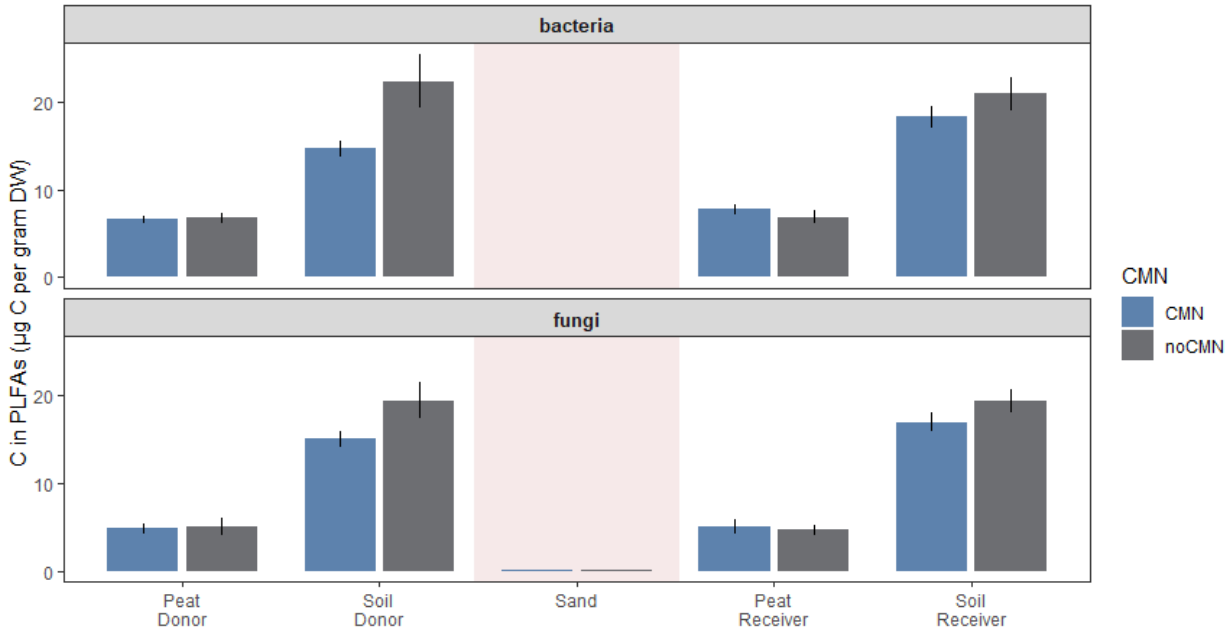

Figure 10 - C in fungal-specific (18:1ω9 and 18:2ω6,9) and bacteria-specific PLFAs used as proxy for microbial biomass in peat, soil and sand. The presence of CMN significantly affected the abundance of fungal markers (p= 0.03) but not the bacterial markers (p= 0.1) in the sand. No significant differences were found in the other pools. Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Wilcoxon signed-rank's post-hoc test of multiple comparisons with Benjamini-Hochberg correction (adj. $p < 0.05$); n = 15. Error bars represent standard error.

The presence of CMN increased assimilation and belowground transfer of ^{13}C by donor plants

Contrary to our expectations, donor plants were more positively affect by the presence of CMN than receivers. We hypothesized that the CMN treatment would mainly have an impact receivers with a diminished photosynthetic capability caused by shading, but in fact, across all ^{13}C data, the biggest effect of the presence of a CMN was found in the donor rather than receiver plants. Donors from the intact CMN treatment showed higher amounts of ^{13}C in all above and belowground pools (leaves, roots, peat and soil) when compared to the donor plants which had the CMN disrupted (Figure 7). The average of total ng ^{13}C excess in the system was 1884.55 ± 302.46 for CMN and 956.31 ± 151.35 for disrupted CMN treatment, indicating that donor plants assimilated about twice as much ^{13}C from the ^{13}C-labelled CO_2 source when having an intact hyphal connection to a neighboring plant. The CMN had a significant effect on donors (p= 0.0105) but not on receivers (p= 0.152).

The total ng^{13}C excess per gram DW soil and peat of donor plants were both affected by the presence of CMN (p = 0.0128 for soil and p = 0.0764 for peat). The donor plants also allocated significantly more ^{13}C to bacterial and fungal PLFAs in the soil (Figure 9) and had higher respiration rates with more ^{13}C respired when they were grown in boxes that allowed the establishment of a CMN (Figure 11). However, these differences were not translated into a higher C transfer to their respective neighbors. In the receivers, neither the presence nor absence of CMN had a strong effect on the transferred C (Figures 7, 8 and 9).

The positive effect of CMNs on ^{13}C assimilation in the donor plant systems was also reflected in the plant and peat respiration measurements, which were higher with the CMN treatments (Figure 11). Average of plant respiration was 6.57 ± 0.51 and 7.05 ± 0.91 (nmol CO_2/hour/sample) while in the peat bags it was 0.73 ± 0.06 and 0.94 ± 0.07 (nmol CO_2/hour/sample) for donors and receivers, respectively. The respired ^{13}C was significantly different from the natural abundance control plants only in the donor plants (p=2.7e-06) and donor peat bag microbes (p=6.6e-06). However, even not being statistically significant, the ^{13}C found in the peat bag respiration data shows once more a C transfer to distant peat bags, incorporation by the microbes and a release of excess ^{13}C in the respiration.

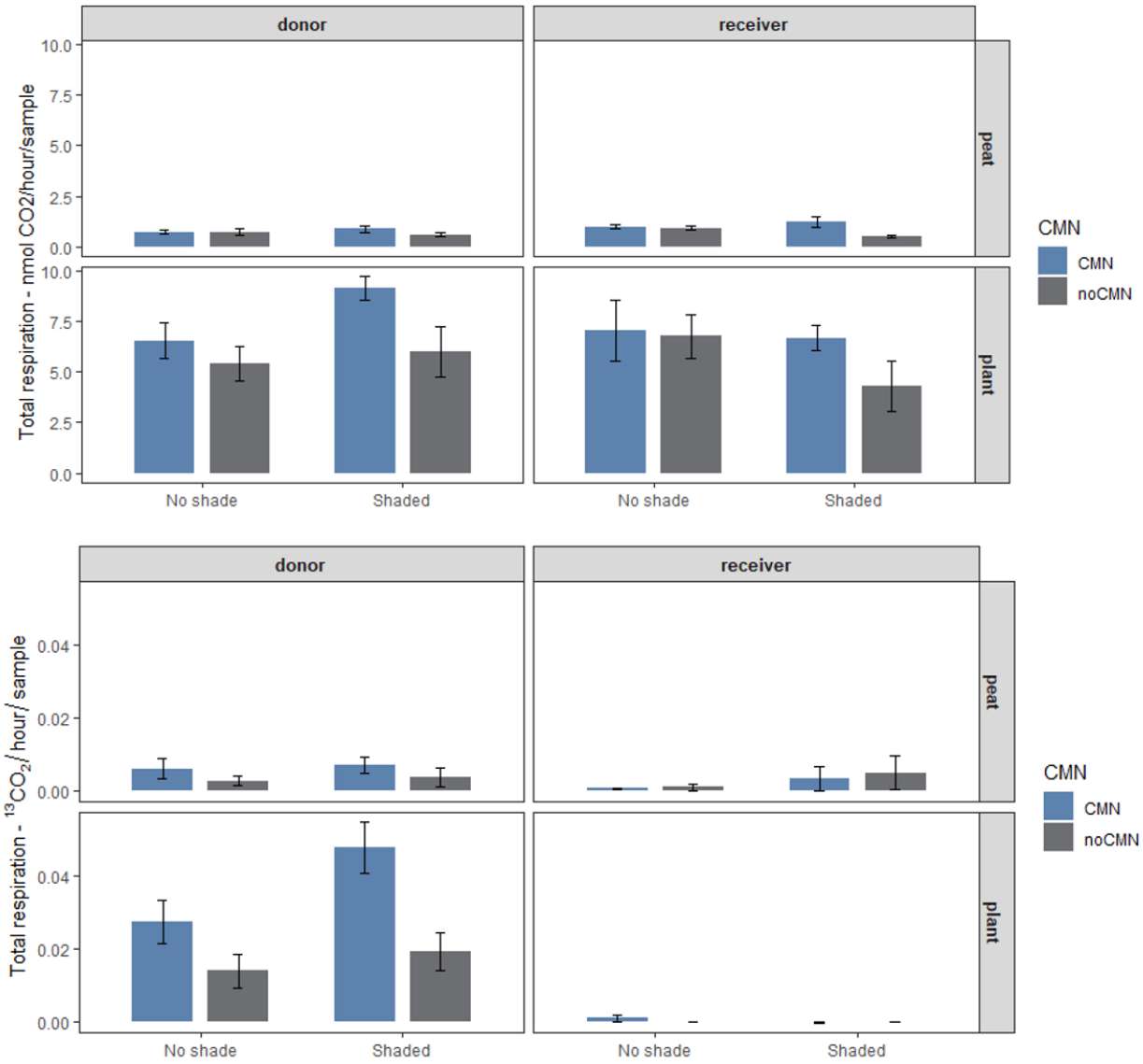

Figure 11 – Total plant system and peat bag respiration expressed in nmol CO_2/hour/sample and nmol $^{13}CO_2$/hour/sample in the respiration of plant system and peat bag microbes. Only donors were significantly different from natural abundance control ($p < 0.05$). Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Wilcoxon signed-rank's post-hoc test of multiple comparisons with Benjamini-Hochberg correction (adj. $p < 0.05$); n = 6 for shaded and n=9 for not shaded treatments. Error bars represent standard error.

Delivery of ^{13}C to receiver peat bag was independent of CMN

Driven by the fact that the peat bags of the receiver plants in the absence of CMN treatment were also enriched, PLFAs of the sand in between the two neighboring plants were also analyzed (Figures 9, 12). The relative enrichment (Atom% excess ^{13}C) in both bacterial and fungal markers were significantly different from the natural abundance control sand (p= 0.037

for both microbial groups) and not different from each other (p=0.59), showing that both microbial groups had access to the ^{13}C from the donor plant pots. The access to the labeled C occurred even when the CMN was not present, although in smaller amounts, with fungal markers always higher in enrichment when compared to Bacteria. There was a significant positive effect of CMN on the abundance of fungi, and ^{13}C enrichment of both fungi and bacteria in the sand (Figure 12). In the presence of CMN, average of the enrichment in Bacteria was 0.03 ± 0.01 and Fungi was 0.11 ± 0.05. In the no CMN treatments, average of the enrichment in Bacteria was 0.006 ± 0.002 and Fungi 0.01 ± 0.008.

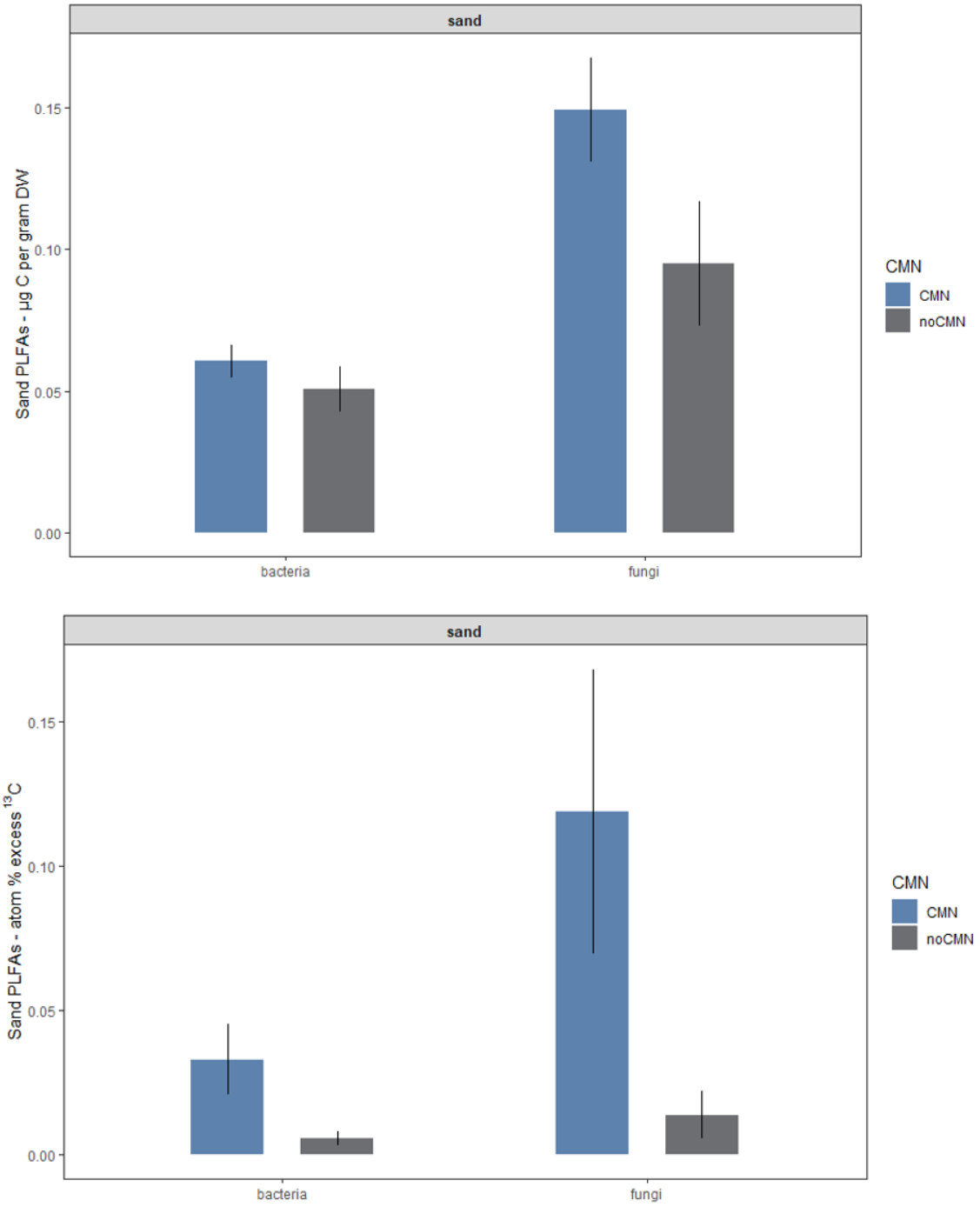

Figure 12 - C per gram DW used as proxy for microbial biomass in sand and ^{13}C enrichment (in atom% excess) in fungal-specific (18:1ω9 and 18:2ω6,9) and bacteria-specific PLFAs. The presence of CMN significantly affected the abundance of fungal markers (p= 0.03) but not the bacterial markers (p= 0.1) in the sand. Both microbial groups were significantly enriched in ^{13}C compared to natural abundance controls. CMN had a significant effect on atom% excess ^{13}C in bacterial and fungal biomarkers (p= 0.017). Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Wilcoxon signed-rank's post-hoc test of multiple comparisons with Benjamini-Hochberg correction (adj. $p < 0.05$); n = 15. Error bars represent standard error.

The higher ^{13}C enrichment found in the peat and sand rather than soil PLFAs is also shown by the multivariate correspondence analysis (CA) of atom% excess ^{13}C in PLFAs, where the sand microbial groups were more closely related to the peat community rather than soil communities. The CA also show that the fungal markers 18:1ω9 and 18:2ω6,9 are clustered closer to donor plants rather than receivers due to the high enrichment in ^{13}C (Figure 13). When analyzing how the microbial communities were distributed based on the PLFA C per gram DW basis the correspondence analysis also shows some distinctions between microbial communities from the soil, peat and sand (Figure 14). The soil microbial community was separated from the peat and sand, most likely due to the higher amount of PLFAs in this pool (Figure 10). The CA also shows that the high abundance of the fungal marker 18:2ω6,9 might have driven peat and sand communities to cluster together. When analyzing the microbial community composition in each pool separately no significant effects of the treatments were found except in the sand, where microbes affected by the presence or absence of CMN (see Supplementary Material, Figure S3).

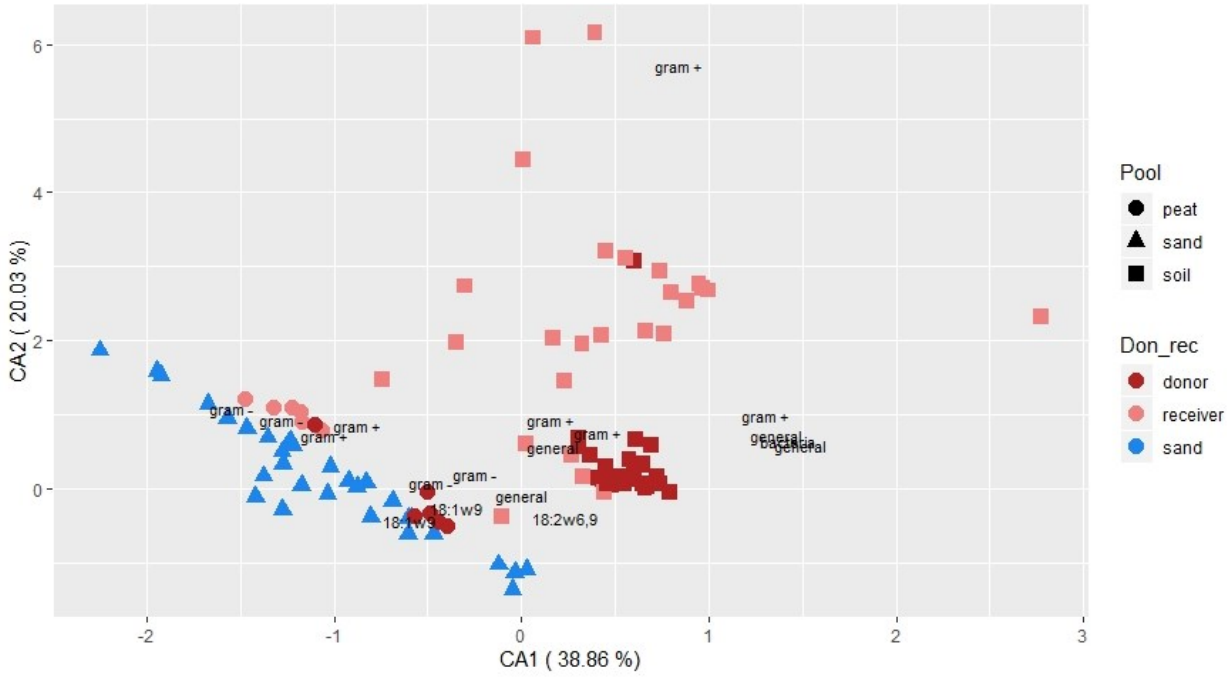

Figure 13 – Correspondence analysis of the relative ^{13}C enrichment (Atom% excess) in the soil, peat and sand. Sand microbial community is more closely related to the peat microbes rather than soil. Also, the fungal markers 18:1ω9 and 18:2ω6,9 are clustered closer to donor plants rather than receivers due to the high ^{13}C enrichment.

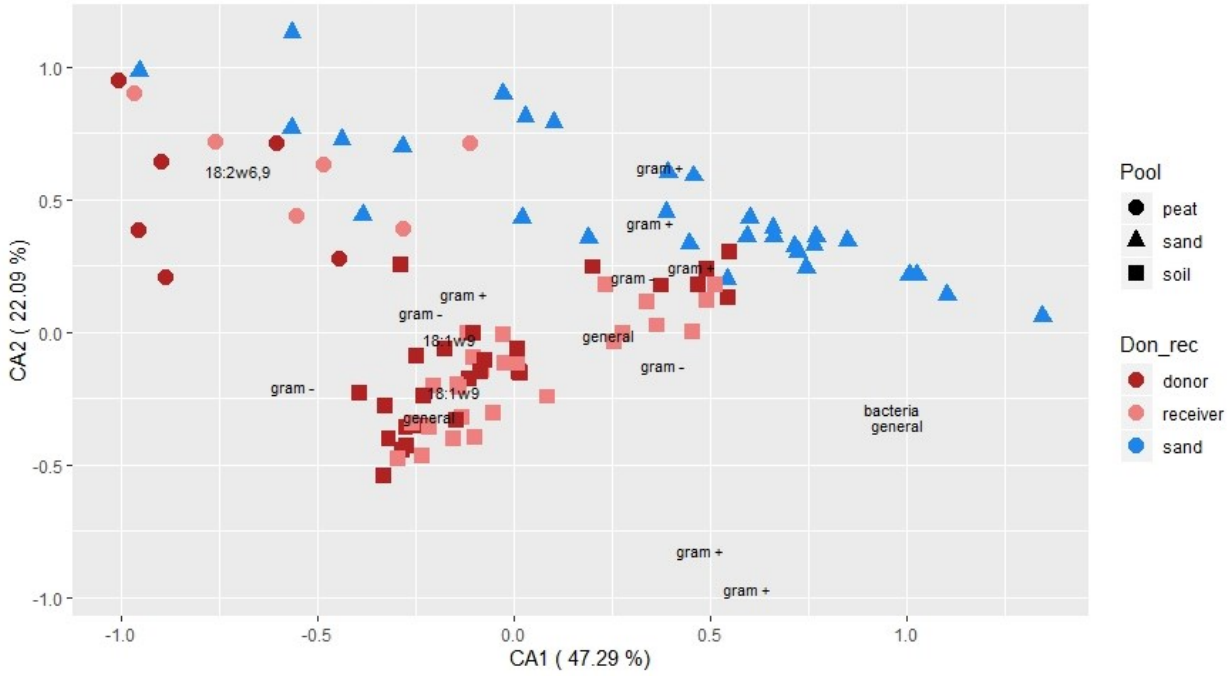

Figure 14 – Correspondence analysis of C in PLFAs (µg C per gram DW) in the sand, peat and soil. It shows a separation of soil microbial community, most likely due to the higher amount of PLFAs in this pool. Peat and sand are more closely related due to the abundance of the fungal marker 18:2ω6,9.

Transfer of ^{13}C to receiver plant roots was facilitated by CMN

Fungal PLFA biomarkers extracted from the roots of the receiver plants were slightly, but significantly enriched in ^{13}C only when a CMN was present (Figure 15). This may indicate a translocation of photoassimilated C from the donor plant into the root system of the receiver plant (see Supplementary Material, Figure S4). Whether this C stayed in the fungal tissue or was transferred into plant cells is however unknown.

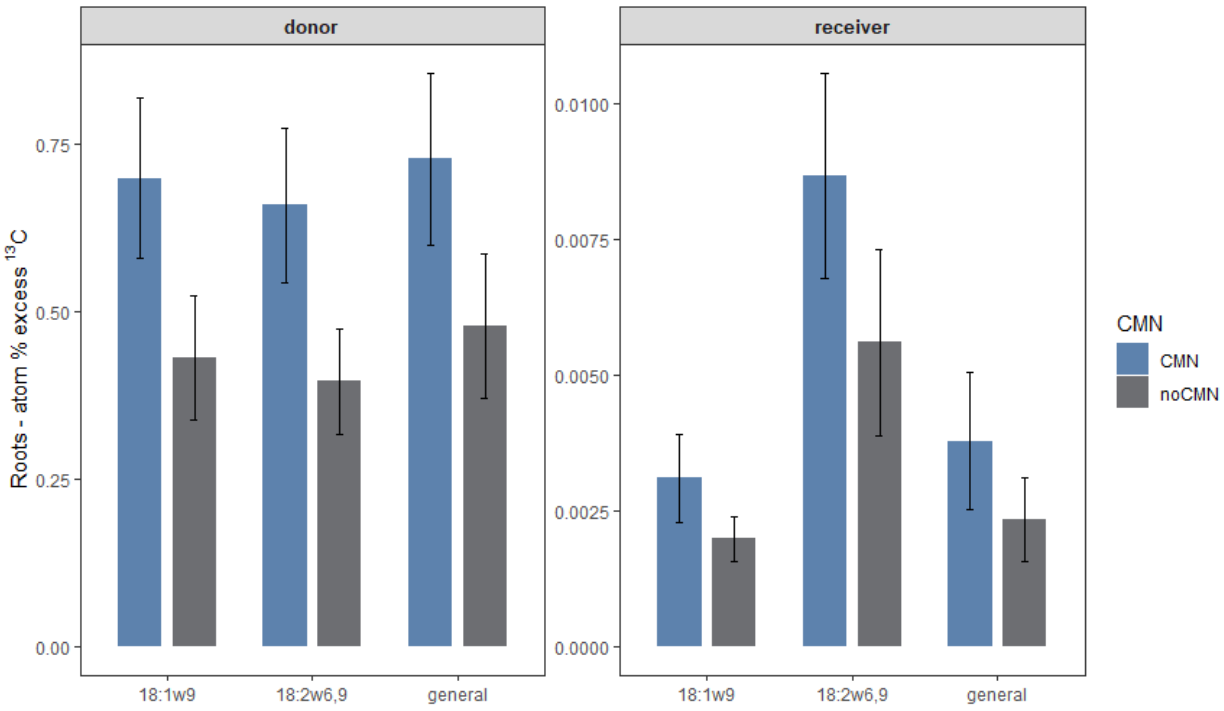

Figure 15 - Relative ^{13}C enrichment in fungal-specific and general PLFAs in roots of donor and receiver plants expressed as Atom% excess ^{13}C. Donors were significantly enriched in ^{13}C compared to natural abundance but in the receivers only one fungal-specific biomarker (18:2ω6, 9) was statistically significant (p= 0.03), suggesting a ^{13}C transfer via ECM hyphae. No effect of treatments were found. Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests were followed by Wilcoxon signed-rank's post-hoc test of multiple comparisons with Benjamini-Hochberg correction (adj. p <0.05); n = 15. Error bars represent standard error.

2.4 Discussion

The mycorrhizal symbiosis is based on bidirectional exchange of resources, where photoassimilated C is transferred to the fungi in return for nutrients scavenged from the soil (Smith and Read 1997). The lack of host-symbiont specificity is demonstrated by the formation of CMNs, where different fungal partners can interact with different host plants forming hyphal connections between different plant species. It has been widely suggested that C transfer between plants and their fungal partners happens on a fair trade basis where most C is allocated to the fungal partner that delivers most nutrients in exchange (Kiers and van der Heijden 2006; Hammerstein and Noë 2016) however, the rules of resource exchange in CMNs might be complex. In a review, Van Der Heijden and Horton (2009) analyzed costs and benefits of nutrient exchange in CMNs and suggested three possible scenarios for the relationship between symbionts; first where costs and benefits are positively correlated, second where the relation is variable, depending on the species involved and environmental conditions and third where they are negatively correlated. In order to understand how these scenarios would develop in small Beech trees, we setup an experiment to investigate whether CMNs amplify or alleviate competition between plants, and which effect they have on the distribution of plant photosynthates within the belowground network. The aim was to analyze the impact of CMNs on nutrient allocation between beech seedlings when sink-source gradient would be manipulated by shading and disrupting hyphal linkage between the plants.

Effect of CMNs on nutrient allocation in Beech trees

Greater differences in N allocation between the neighboring beech trees were expected to be found when a full-sun donor plant would supply more C into the mycorrhizal network than its shaded neighboring plant. The impact of unequal nutrient allocation to donors and receivers would be seen by differences in plant biomass and also plant biomass N. Our results, however, did not support this assumption, as our experiment could not distinguish a clear unbalance in the sink-source gradient as the shading treatment did not lead to a decrease of plant biomass or plant N. However, the high plant biomass N derived from the hyphal-exclusive compartment demonstrates that host plants relied on their fungal partners to access nutrients even though they could have been directly taken up by roots from the soil. This suggests that the maintenance of the fungal partners might not have been costly for the hosts and they could benefit from them, even when their photosynthetic capacity was reduced by shading. Additionally, we argue that the high N availability in the system present in hyphae-exclusive nu-

trient source might also have stimulated hosts to allocate C to the belowground pools. On the other hand, colonizing roots of C-stressed plants might also bring advantages for the fungal partners. Having access to minimum amounts of labile C might be a trade-off that represents a better cost-benefit rather than obtaining C by decomposing soil organic matter. Moreover, it might bring an advantage to be well positioned in case of temporary shading Weremijewicz et al. (2016). Studies aiming to show C transfer between plants via CMN manipulate the sink-source gradient by shading plants, depleting nutrient of the growing substrate or growing plants with different sizes in the same pot (Finlay and Read 1986; Simard et al. 1997b; Weremijewicz and Janos 2013; Weremijewicz et al. 2016). In our experiment, it was expected that shading would have a strong impact on the photosynthetic capacity of the plants, and therefore a diminished amount of C allocated to the fungi belowground. However, the overall C and N data does not show a pronounced effect of shading. The reason might have been due to the fact that Beech trees are shade-tolerant species and seedlings are naturally growing in the forest understory (Robson et al. 2009). In contrast to our findings, Simard et al. (1997b) showed that a bidirectional C transfer via hyphal connection was highly influenced by shading one of the plants in an two years experiment with two ectomycorrhizal seedlings grown in a forest. The differences in the results were greater in the second year when the increase in nutrient demands by full-sun plants led to a greater effect of the shading in the neighbor plant. The non-shaded ones had twice as much foliar N, 4 times higher photosynthetic rates in comparison to deep shade plants and therefore led to greater differences in C transfer between the seedlings. In a AM study, Weremijewicz and Janos (2013) also showed that interconnected plants by CMNs were, on average, 15% larger and had 32% greater size inequality than those in the disrupted CMN treatment. Using a compilation of published studies, Van Der Heijden and Horton (2009) explored whether CMNs have a positive, negative or neutral effect on individual plants and in plant communities. They found 60 cases in the literature where seedlings were grown together with larger plants with or without mycorrhizal fungal networks. In 48% of them were positively affected, as CMNs promoted seedling growth, 25% were negatively affected and 27% were neutral. When performing separated analyses for different mycorrhizal associations, CMNs formed by EM fungi were beneficial to the seedlings in 75% of the cases while the remaining 25% were neutral. In contrast, CMNs formed by AM fungi responded positively in 42% of the cases, neutral in 29% and negatively affected in 33% of them.

Effect of CMNs on photoassimilated ^{13}C by donor plants

Interestingly the presence of an intact CMN had a strong positive effect on the donor plants, but not on the receivers. The total ^{13}C in the donor system was almost twice as high when the CMN was present suggesting that having a belowground connection between plants might have increased plant photosynthetic activity. There was never a pronounced difference across the ^{13}C data in the receiver plants, indicating that although the mycorrhizal connection to the neighboring plant pot seemed to have triggered a significant increase of plant $^{13}CO_2$ assimilation, the excess C was allocated mainly to above and belowground pools within the same pot and not transferred to the larger mycorrhizal network.

Several studies show higher extramatrical mycelium production by ECM fungi in response to the increase in plant belowground C allocation due to higher carbon fixation by plants promoted by elevated CO_2 concentrations (Fransson et al. 2005; Alberton et al. 2005; Alberton and Kuyper 2009). In some morphotypes, the carbohydrate stored in the hyphae increases and differences in mantle thickness can be seen (Turnau et al. 2001). The nutrient acquisition promoted by the fungi may as well vary under elevated CO_2. In a study with twice-ambient CO_2 concentration with nitrogen amendment, Weigt et al. (2011) show that neither of the two ECM species colonizing Norway spruce seedlings increased their capacity of N uptake in proportion to the rise of CO_2. Despite the fact that there was an increase in length and fungal biomass production, their results suggest that high C increase the degree of mycorrhization but not necessarily promoting a better nutrient supply to the hosts.

In agreement with the pronounced response of donor plants to elevated CO_2 concentrations, differences in the ^{15}N enrichment of single root tips shown by our data suggest that at this level of root architecture, different access to nutrients might also have occurred. Mycorrhizal tips of donors in presence of shaded neighbors had highest enrichment in ^{15}N when their respective shaded neighbors had the lowest enrichment and this was enhanced by the absence of CMN. It seems that even though donors had a higher belowground ^{13}C in the system, the exchanged N was similarly delivered by the ECM fungi across all treatments but different amounts of N were held back within the fungal tissue leading to these differences in enrichment at root tip scale. However, whether these differences happened due to a response to the treatments applied in our experiment or due to physiological differences of the ECM fungi species remains unclear.

Preferential C allocation via fungal hyphae

Our results show a preferential C allocation to the distant hyphal-exclusive nutrient patches buried in the realm of a receiver plant via fungal hyphae. This is corroborated by the fact that soil in the receiver pots had a significant lower isotopic enrichment at the bulk level in comparison to the peat bags. Additionally, the significantly high abundance and ^{13}C enrichment of the PLFAs 18:2ω6,9 and 18:1ω9 in donor and receiver systems suggests that fungi were the main mobilizers of nutrients in our system. Both markers are frequently used as indicators of fungal biomass (Frostegård et al. 2011; Schnecker et al. 2012; Gorka et al. 2019) and 18:2ω6,9 has been shown to have good correlations between ectomycorrhizal colonization of root tips and soil samples from ECM dominated forest (Kaiser et al. 2010). Yet, they have also been described to occur in plant tissue (Laczko et al. 2004). Since in our experiment roots were prevented to grow outside of the pots as well as inside of the peat bags by a mesh we exclude the root-borne contribution of these markers in our PLFA results.

In our experiment we could not rule out the presence of saprotrophic fungi by using PLFAs as proxy to assess the fungal community but we argue that there was a niche separation between them and ECM fungi. The high C transfer to the peat microbial community not only of donor plants but also to the neighbors suggests that the C might have been preferentially shared among fungal species that occupy similar niches. If C, as "luxury good", would have been allocated without regard, most of it would have stayed in the belowground pools of donor pots, shared by ECM fungi and saprotrophs and not transferred to the realm of the neighbor plant. However, our data show an unbalanced C allocation with peat being always higher in the ^{13}C enrichment than soil, at the bulk level and in the microbial assimilation. This is even more pronounced in the receiver PLFAs, as soil microbes were not even significantly enriched. Hence, we suggest that most ^{13}C was allocated to the ECM fungi by donor plants and preferentially transferred to the peat bags in the receivers, most likely shared among similar ECM fungal morphotypes. By using mesh bags buried in soils to estimate extramatrical mycelium (EMM), Kjøller (2006) and Wallander et al. (2011) show a niche separation between ECM fungi and saprotrophs. The results of DNA analyses showed that, even though saprotrophic fungi could colonize the mesh bags, they were dominated by ECM fungi. In temperate and boreal forests the niche separation is more clear, the fresh litter are mostly dominated by saprotrophs (Lindahl et al. 2007) and ECM fungal communities more strongly separated across vertical soil layers as more recently shown by meta-analysis by Bahram et al. (2014).

Mechanisms behind the C transfer via disrupted hyphal linkage

The fact that ^{13}C was transferred between neighboring plant systems even when the CMN was disrupted indicates that ECM fungi played a significant role in facilitating the transfer. Given the fact that the soil microbial biomass of the receiver pots did not show a significant enrichment in ^{13}C but the microbial peat did, we assume that the C transfer between pots happened by fungal hyphae. Surprisingly, our data indicates that fungi were able to bridge the adjacent pots to promote the C movement between them even when the CMN was disrupted by turning the pots 360 degrees. Philip et al. (2010) found similar results where a bi-directional C transfer between paper birch and Douglas fir seedlings happened even when preventing the CMN formation by razor blade between the root pouches. Their assumption was that the high similarity of ECM morphotypes colonizing the root systems by both species facilitated the transfer between the seedlings. In our system, we suggest three mechanisms to explain how the ^{13}C could have reached the receiver pots in the disrupted CMN treatment. The first one would occur upon death and release of the ^{13}C present in the fungal hyphae due to physical break down caused by rotating the pots. In this case, ECM fungal hyphae from the receiver plants could have accessed the C released in the sand between the pots when expanding extraradical mycelium and mining for nutrients. This would explain why the ^{13}C enrichment in the microbial biomass of sand was higher than in the soil microbes from the receiver pots. In the second mechanism, roots would have been colonized by fast growing fungi capable of emanating long distance exploration hyphal type (Agerer 2001). Different ECM fungi vary not only morphologically but also physiologically, with differential abilities to acquire and metabolize the C from the host plants. As consequence, the percentage of root colonization and mycelial growth rate would as well be different between fungal species (Simard et al. 1997a). The patchy N source buried in each plant pot could have stimulated ECM fungi to fast grow hyphae in order to access the nutrient source. Laboratory studies suggest that ECM fungi hyphal growth to be rapid, between 9-15 cm^2 per day (Finlay and Read 1986) but this might not reflect the conditions in natural forests. In natural systems, the rates of extramatrical mycelium (EMM) of ectomycorrhizal fungi in ca. 140 sites were estimated but the results present a great variation due to site and year of sample collection, differences between methods used to assess mycelial biomass and different factors driving the EMM production (Ekblad et al. 2013). The third mechanism by which the ^{13}C might have reached the receiver pot is due to formation of hyphal anastomosis. Fungal vegetative hyphal fusion allows a bidirectional communication of water, nutrients and chemical signals within different parts of

their hyphal network (Nuti et al. 2003). The ability to anastomose disrupted hyphae and reestablish a network has been suggested to be the mechanism allowing AM fungi to persist in frequently disturbed events like drought or tillage in agricultural soils (Chagnon 2014). Likewise, the constant rotation of the pots in our experiment severely damaged the hyphal connections but we argue that the high nutrient availability and the high C allocation in the belowground pools stimulated the reestablishment of the connection between the fungal communities present in each of the pots. Still, while turning the pots did not alter the C transferred to the receiver plant's peat bag, allowing the establishment of a CMN by *not* turning the pots (which was confirmed by larger fungal abundance and ^{13}C enrichment in the sand) still had a large positive effect on ^{13}C assimilation of the donor plant, and it also increased ^{13}C transfer into mycorrhizal roots of the receiver plants. Together this indicates that while an undisturbed CMN greatly affects assimilation and belowground distribution of C, there still may be ways for mycorrhizal fungal hyphae to bridge constant disruptions.

Conclusion

Mycorrhizal symbiosis is present in almost all land plants around the world and the below-ground network formed by the root-associated fungi has been shown to increase plant growth and fitness, impacting plant diversity, nutrient cycling and ecosystem productivity. Yet, the dynamics and mechanisms behind this interaction remain poorly understood (Van Der Heijden and Horton 2009). Our results suggest that the C transfer between plant systems via fungal networks is more strongly related to the distribution of nutrient-rich patches in the soil rather than differences in photosynthetic capacity of the host plants. When connected by a CMN, plants showed higher CO_2 assimilation and directed more of this C into the fungal network, without promoting a clear belowground competition for the N resources.

2.5 Supplementary material

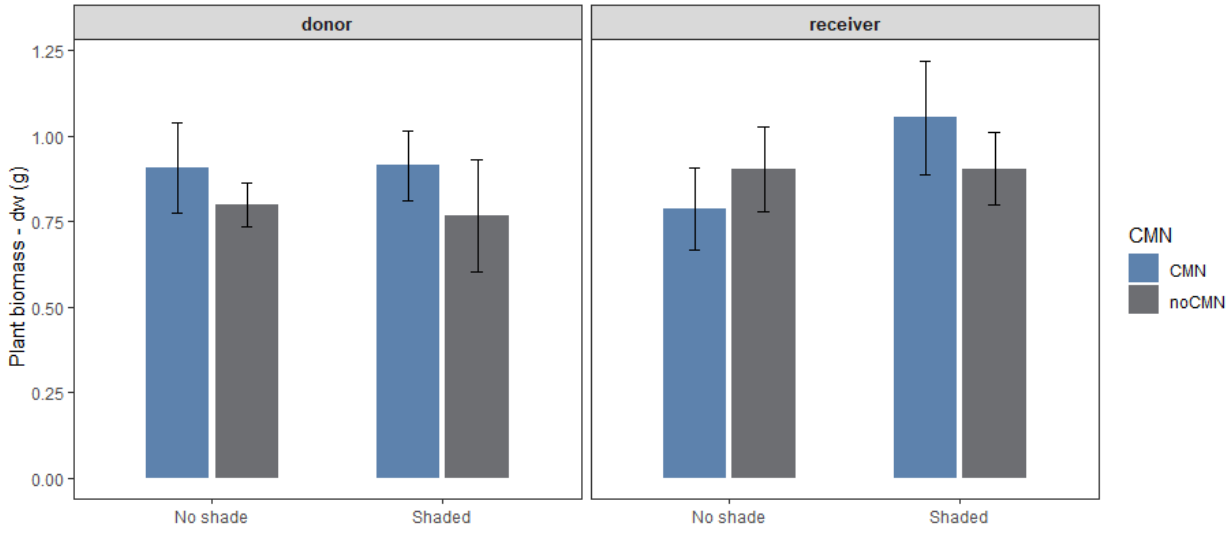

Figure S1 – Total plant biomass (sum of leaves and roots) of donor and receiver plants was not significantly affected by CMN nor shading treatments by ANOVA. n = 6 for shaded and n=9 for not shaded treatments. Error bars represent standard error.

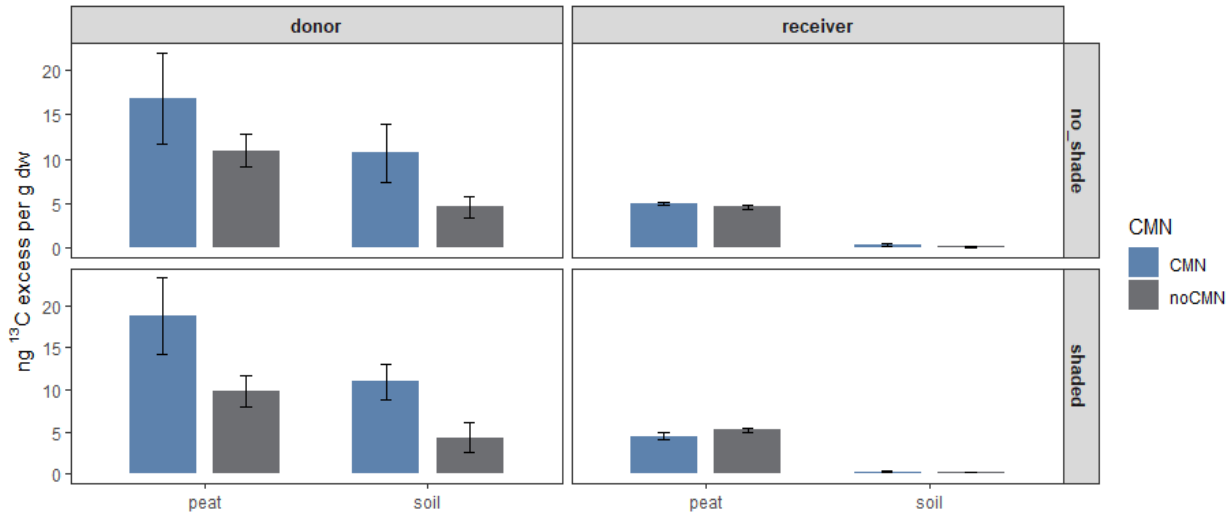

Figure S2 – Comparison between enrichment in ^{13}C in the belowground pools expressed in Atom% excess ^{13}C per gram dw. The results show a preferential allocation to the peat bags, a mycorrhiza exclusive N-source rather than soil. Both pools were significantly affected by the CMN treatment in the donor plants (p=0.0128 for soil and p= 0.0764 for peat). In the receivers, no significant effect of CMN nor shading were found. However, both pools were different from each other (p= 0.00724 for donors and p= <2e-16 for receivers, results by ANOVA); n = 6 for shaded and n=9 for not shaded treatments. Error bars represent standard error.

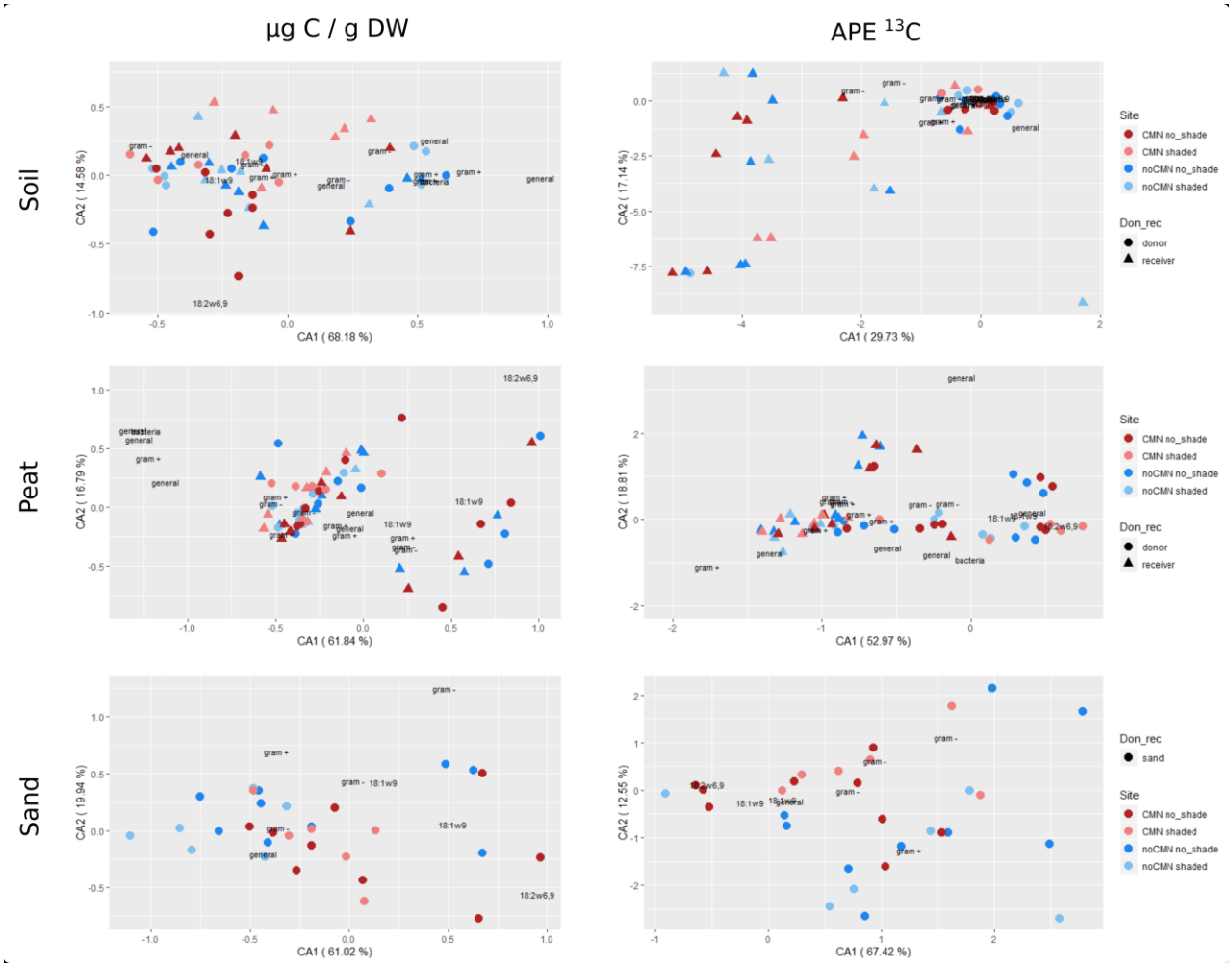

Figure S3 – Correspondence analysis of C in PLFAs (µg C per gram DW) and relative ^{13}C enrichment (Atom% excess) in the soil, peat and sand. No clear effects of treatments were found in the microbial community composition, except in the sand where the community was affected by the presence of CMNs. In soil and peat, different ^{13}C enrichment between donors and receivers led to a separation of the microbial communities.

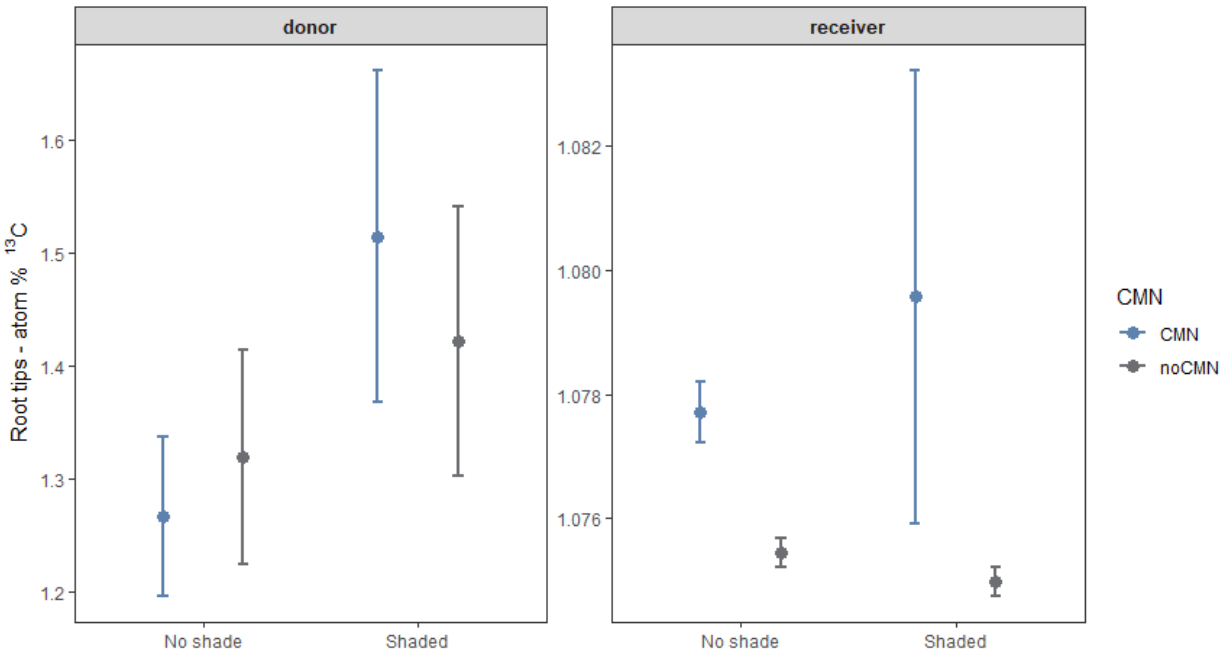

Figure S4 – Relative enrichment in single ectomycorrhizal root tips in atom % ^{13}C. Only mycorrhizal tips from donor plants were significantly different from natural abundance control plants ($p < 0.05$, ANOVA). However, the high variability in ^{13}C in the root tip of receiver plants suggests that shading and disruption of CMNs might have influenced how different EM fungi morphotypes acquire C at the root tip scale. Error bars represent standard error; n = 6 for shaded and n=9 for not shaded treatments.

3. SUMMARY

3.1 Summary

Mycorrhizal symbiosis is present in almost all land plants around the world. Plants allocate photosynthetic carbon (C) to their fungal partners present in the roots in exchange for nutrients and water from the soil. This association is present in up to 80% of all living plants around the world and has been suggested to have played a major role in the colonization of terrestrial habitats and diversification of early land plants. Mycorrhizal fungi can promote plant-to-plant belowground interconnections via mycelium, called Common mycorrhizal networks (CMNs) where nutrients can be moved among different fungal and host plants partners. These connections have been shown to impact plant growth, fitness and diversity by influencing nutrient cycling at the ecosystem level. However, until today, the terms in which host plants and their fungal partners trade nutrients remain poorly understood.

This work focuses on carbon and nitrogen exchange via mycelia connections between European beech trees and ectomycorrhizal fungi (EM fungi). This symbiosis is predominant in boreal and temperate forests where EM fungi help plants to overcome the strong N limitation in these environments. We set up an experiment in order to elucidate how resources can be moved along hyphal connections between plants with the following research questions: (1) Is there a plant-to-plant carbon movement between beech trees via Common mycorrhiza networks, in particular if trees have unequal access to C from photosynthesis? (2) How are plant photosynthates distributed within the belowground network? (3) Do CMNs amplify or alleviate belowground competition for nutrients? To address these points, young beech trees were grown inside perforated pots covered with a double layer of meshes sized 121 and 49 μm to prevent root growth but not hyphae outside of the pots. The same two meshes were used to enclose approximately 8 grams of ^{15}N labeled peat, thus providing a hyphal-exclusive N source (hereby referred as 'peat bags') buried in each pot. Two plant pots were placed inside of plastic boxes and the interstitial space was filled with quartz sand. Two treatments were applied in a fully factorial design: 1) Allowing/preventing the establishment of a CMN between the pots (some pots were turned around at a regular interval to prevent the establishment of CMNs) and 2) inequality of access to photoassimilated C (in part of the boxes one of the two plants was shaded). In a combined ^{13}C-CO_2 and ^{15}N labelling approach, we subsequently traced the label assimilated by one plant of each tree pair (i.e. the 'donor' plant) into belowground pools of both donor and 'receiver' plants (i.e. the unlabeled neighboring plants),

as well as into the receiver plant itself, by isotope ratio mass spectrometry (EA-IRMS) and ^{13}C phospholipid fatty acid (PLFAs) analysis (GC-IRMS).

We hypothesized the following: (i) The presence of a CMN will amplify inequalities between pairs of neighboring plants caused by different access to light, because more of the N from the shared resources will be directed towards the full-sun plants by the fungal network. (ii) Plant photoassimilates will be preferable allocated within the realm of the plant's 'home' pot when plants have equal access to C (i.e. none of them is shaded). However, under unequal C supply, we expect that full-sun donor plants will allocate more C into the shaded plant peat bag in exchange for its N. (iii) Photoassimilated carbon will be transferred from the full-sun plants to the shaded plant's mycorrhiza when they are connected via CMNs. The transferred C, however, will stay in the fungal tissue of the mycorrhizal roots of receiver plants and not be transferred to the plant itself (it helps sustaining fungal colonization of shaded plants);

Our results show that plants relied mostly on their mycorrhizal partners to acquire nutrients as on average across all treatments, as most of the plant biomass N originated from the ^{15}N-labelled mycorrhiza-exclusive peat bags rather than soil. According to our initial assumptions, we expected that shading would have a negative effect on the plants, shown by lower biomass and N content compared to full sun plants. However, since these assumptions were not met in the first place we cannot prove with our results that the 'CMN' amplified or alleviated the competition between plants. We argue that having a source of N available only to fungal hyphae uptake might have stimulated host plants to allocate C to the belowground pools even when their photosynthetic capacity was reduced by shading. On the other hand, hosting roots of C-stressed plants might have been a trade-off that represents a better cost-benefit rather than obtaining C by decomposing soil organic matter.

The results also show that air-borne ^{13}C was highly incorporated into the biomass of donor plants and allocated to the belowground pools. 2% of the total ^{13}C in the system was found in the belowground pools of receiver pots, almost exclusively within the microbial community of the peat bags, especially in the fungal markers. This result suggests that fungi were the main mobilizers of nutrients in our system, and that the resources were transported over long distances preferentially to the mycorrhiza-exclusive N source. Because the C translocation happened even when CMNs were disrupted in similar amounts as the intact CMN plants we argue that the patchy N source buried in each plant pot combined with high belowground C allocation by host plants stimulated ECM fungi to fast grow hyphae in order to reestablish the connection between the pots. Still, the presence of intact CMN significantly increased $^{13}CO_2$ assimilation by the donor plant as well as ^{13}C allocation to belowground pools in the donor

pot. The total ^{13}C in the donor system was always higher when the CMN was present suggesting that having a larger belowground mycelium might have increased plant photosynthetic activity.

We conclude that the C transfer between plant systems via fungal networks is more strongly related to the distribution of nutrient-rich patches in the soil rather than differences in photosynthetic capacity of the host plants. When connected by a CMN, plants showed higher CO_2 assimilation and directed more of this C into the fungal network, without promoting a clear belowground competition for the N resources.

3.2 Zusammenfassung

Beinahe alle Landpflanzen weltweit leben in Symbiose mit Mykorrhizapilzen. Pflanzen verteilen an ihre in den Wurzeln lebenden Pilzpartner durch Photosynthese gewonnenen Kohlenstoff (C) im Austausch für aus dem Boden aufgenommene Nährstoffe und Wasser. Es scheint, dass diese Lebensgemeinschaft, die von bis zu 80% der Pflanzen weltweit eingegangen wird, eine wichtige Rolle in der Besiedelung terrestrischer Lebensräume und der Diversifizierung der frühen Landpflanzen gespielt hat. Mykorrhizapilze können die Pflanzen durch so genannte „Common Mykorrhiza Networks“ (CMNs), unterirdisch miteinander verbinden, wobei Nährstoffe zwischen verschiedenen Pilz- und Wirtspflanzenpartnern über das Netzwerk ausgetauscht werden können. Studien haben gezeigt, dass dieses Netzwerk Einfluss auf Wachstum, Fitness und Diversität von Pflanzen hat, indem es den Nährstoffkreislauf auf Ökosystemebene beeinflusst. Nichtsdestotrotz sind die Bedingungen, unter denen Wirtspflanzen und ihre Pilzpartner Nährstoffe tauschen, bis zum heutigen Tag unzureichend bekannt.

Diese Arbeit legt ihren Fokus auf Austausch und Verteilung von Kohlenstoff und Stickstoff in einem Netzwerk von ektomykorrhizierenden Pilzen (EM Pilzen) zwischen Rotbuchen. Ektomykorrhiza ist eine Form der Mykorrhiza die in borealen und temperaten Wäldern vorherrscht. EM Pilze helfen den Pflanzen, die starke Stickstofflimitierung in diesen Lebensräumen zu überwinden. Um aufzuklären, wie Resourcen zwischen Pflanzen entlang von Hyphenverbindungen bewegt werden können, führten wir ein Experiment mit folgenden Forschungsfragen durch: (1) Findet ein Transfer von Kohlenstoff zwischen Rotbuchen, vorallem unter solchen Bäumen, die ihre Photosyntheseleistung unterschiedlich ausschöpfen können, durch Mykorrhizapilznetzwerke statt? (2) Wie werden Pflanzenphotosynthate im unterirdischen Netzwerk verteilt? (3) Verstärken oder schwächen CMNs den unterirdischen Wettbewerb um Nährstoffe? Um diese Fragen beantworten zu können, ließen wir junge Buchen in perforierten Töpfen wachsen, welche mit Netzen zweier Maschengrößen (121 und 49µm) umgeben waren. Dies soll einerseits Wurzelwachstum außerhalb der Töpfe verhindern und andererseits Hyphenwachstum ermöglichen. Aus den gleichen Netzen fertigten wir Beutel an, die wir mit ungefähr 8 Gramm ^{15}N-markiertem Torf füllten und als ausschließlich für Hyphen zugängliche Stickstoffquelle in allen Töpfen vergruben (im folgenden Text beziehe ich mich auf diese als “Torfbeutel”). Wir stellten je zwei Töpfe in eine Plastikkiste und füllten den Zwischenraum mit Quarzsand. Wir wandten zwei Behandlungen in einem faktoriellen Design an: 1) Wir erlaubten oder verhinderten (indem wir manche Töpfe in

regelmäßigen Intervallen drehten) die Bildung eines CMN zwischen den beiden Töpfen, und 2) wir ermöglichten den Bäumen ungleichen Zugang zu photoassimiliertem C (indem in manchen Boxen eine Pflanze beschattet wurde). Mithilfe eines kombinierten ^{13}C-CO_2 and ^{15}N Markierungsansatzes, verfolgten wir die von einer der beiden Pflanzen des Baumpaares assimilierte Markierung (dh. der Spenderpflanze) in unterirdische Pools von sowohl Spender- und Empfängerpflanze (dh. die Nachbarpflanze, die nicht markiert wurde), als auch in die Empfängerpflanze selbst. Dies alles erfolgte mittels Isotopenverhältnis-Massenspektrometrie (EA-IRMS) und ^{13}C Phospholipid Fettsäuren (PLFAs) Analyse (GC-IRMS).

Wir stellten folgende Hypothesen auf: (i) Das Bestehen von CMN verstärkt die durch ungleiche Lichtverhältnisse bedingten Ungleichheiten zwischen benachbarten Pflanzen, da das Pilznetzwerk der Pflanzen mit vollem Lichtzugang mehr Stickstoff von den geteilten Vorräten zukommen lässt. (ii) Pflanzen Photoassimiliate werden bevorzugt im eigenen Topf verteilt, wenn Pflanzen gleichen Zugang zu C haben (dh. keine von beiden wird beschattet). Unter ungleicher C Versorgung allerdings, nahmen wir an, dass unbeschattete Pflanzen im Tausch für N dem Torfbeutel im Topf der beschatteten Pflanze mehr C zukommen lassen würden. (iii) Photoassimilierter C wird von den unbeschatteten Pflanzen zu der Mykorrhiza der beschatteten Pflanze übertragen, solange beide durch CMNs verbunden sind. Der übertragene C verbleibt jedoch im Pilzgewebe der mykorrhizierten Wurzel und kommt nicht der Pflanze selbst zu (dies hilft die Kolonisierung von beschatteten Bäumen durch Pilze aufrecht zu erhalten).

Unsere Ergebnisse zeigen, dass die Nährstoffgewinnung der Pflanzen über alle Behandlungen hinweg vor allem von ihren Mykorrhizapartnern abhängt, da der Großteil des N in der Pflanzenbiomasse, nicht aus der Erde, sondern aus dem mit ^{15}N markiertem Torf aus dem nur für den Pilz zugänglichen Torfbeutel stammte. Bezugnehmend auf unsere anfänglichen Annahme, dass sich Beschattung negativ auf Pflanzen auswirken würde, erwarteten wir niedrigere Biomasse und N Gehalt von beschatteten Pflanzen im Vergleich zu nicht beschatteten Pflanzen. Da wir diese negative Auswirkungen nicht beobachten konnten, können wir keine Aussage darüber treffen, ob das CMN den Wettbewerb zwischen Pflanzen verstärkt oder abgeschwächt hat. Wir nehmen an, dass die N Quelle, die alleine für die Pilzhyphen zugänglich war, Wirtspflanzen angeregt haben könnte, C unterirdischen Pools zuzuweisen, selbst wenn ihre Photosynthesekapazität durch Beschattung eingeschränkt war. Die Kolonisierung der Wurzel einer C-limitierten Pflanze könnte sich dennoch als vorteilhafter herausstellen, als die Erlangung von C aus der Zersetzung von organischem Material.

Unsere Ergebnisse zeigen weiters, dass ^{13}C aus der Luft in hohem Maße in die Biomasse der Spenderpflanzen eingebaut und unterirdischen Pools zugeordnet wurde. 2% des gesamten ^{13}C des Systems wurde in den unterirdischen Pools der Empfängerpflanze gefunden, der Großteil allerdings wurde in der mikrobiellen Gemeinschaft des Torfbeutels, vorallem den Pilzmarkern, festgestelllt. Dies lässt darauf schließen, dass Pilze die wichtigsten Nährstoffmobilisierer in unserem System waren, und dass Resourcen über lange Distanzen, vorallem in die den Hyphen vorbehaltenen Torfbeutel, transportiert wurden. Da C Translokation in Systemen, in denen CMNs unterbrochen wurden, in ähnlichem Ausmaß stattfand wie in denen, wo CMNs ausgebildet waren, gehen wir davon aus, dass EM Pilze von der zusätzlichen N Quelle (Torfbeutel) zusammen mit hoher Zufuhr von C durch Wirtspflanzen, zu schnellem Hyphenwachstum angeregt wurden, um die Verbindung zwischen den Töpfen wieder herzustellen. Die Anwesenheit intakter CMNs erhöhte die $^{13}CO_2$ Assimilierung durch die Spenderpflanzen als auch die ^{13}C Zuteilung in unterirdische Pools der Töpfe der Spenderpflanze. Die Beobachtung, dass der Gesamt-^{13}C-Gehalt im Spendersystem in Anwesenheit von CMN immer höher war, könnte darauf hindeuten, dass ein größeres Myzel die Photosyntheseaktivität der Pflanzen unterstützt hat.

In conclusio, C Austausch zwischen Pflanzensystemen über Pilznetzwerke hängt stärker von der Verteilung von nährstoffreichen Bereichen im Boden ab, als von den Unterschieden in Photosynthesekapazität der Wirtspflanzen. Pflanzen wiesen höhere C Assimiliation auf und verteilten mehr desselben an das Pilznetzwerk, wenn sie durch CMN verbunden waren, ohne dabei den unterirdischen Wettbewerb um N Vorräte zu verstärken.

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