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The contribution of ericoid plants to soil nitrogen chemistry and organic matter decomposition in boreal forest soil

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ABSTRACT

Nitrogen (N) availability restricts plant carbon assimilation from the atmosphere in N-limited boreal ecosystems. Boreal forest floor is covered with ericoid plants and in soil, ectomycorrhizal and ericoid mycorrhizal plant roots and fungal hyphae are intermixed. How different mycorrhizal plants affect N mobilization from soil organic matter (SOM) has yet to be elucidated. Here we compared the effects of ericoid plants (*Calluna vulgaris*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*), forming ericoid mycorrhiza (ERM), on soil N chemistry, plant N uptake and SOM decomposition and compared them with ectomycorrhizal (ECM) *Pinus sylvestris* in a microcosm experiment. Pine and ericoid plants affected soil N similarly, accessing both degradable and recalcitrant N pools. Both ericoid plants and pine took up N from organic source. Ericoid plant roots contained more phenolic compounds and condensed tannins than pine roots which together with lower pH under ericoids suggests substantial changes in soil chemistry and biology. This study underlines the plant stimulation of SOM degradation with special emphasis on ericoid plants. We point out that both ericoid and ectomycorrhizal plants and their interactions should be of interest when N uptake and SOM decomposition in boreal forests are studied.

1. Introduction

Soil organic matter (SOM) contains more carbon (C) than present in all the vegetation and atmosphere combined (Lehmann and Kleber, 2015). Any change in C release from SOM as CO_2 may have drastic global consequences for climate change. Ecosystem processes associated with the C cycle are constrained by C and N interactions (Tateno and Chapin, 1997). Boreal forests are regarded as N-limited at the ecosystem level (e.g. Magnani et al., 2007), even though the soil N content is high. Boreal forest soil N is mostly present in organic forms that are bound or complexed with soil compounds, including minerals and organic compounds such as polyphenols and polysaccharides (Korhonen et al., 2013; Schmidt et al., 2011). Thus, soil N must be released from these boundaries, depolymerized and mineralized to inorganic N before uptake by plants (Kieloaho et al., 2016; Schimel and Bennett, 2004). Alternatively, plants may short-cut this route taking up organic N forms directly (e.g. Adamczyk et al., 2010; Näsholm et al., 2009; Paungfoo-Lonhienne et al., 2008). In addition to soil microorganisms which depolymerize N compounds, soil fauna enhances the release of N by fragmenting organic matter and processing dead organic matter into a more available form for microbes. The most influential faunal group in boreal forest soils are worms of the family Enchytraeidae (Lappalainen et al., 2013).

The boreal forest humus layer is highly organic and rich in phenolic plant secondary compounds, especially tannins (Smolander et al., 2012). These polyphenols have the potential to directly interfere with organic N availability through the formation of recalcitrant complexes with proteins (see for review Hagerman, 2012), and potentially other organic N compounds such as arginine, chitin and polyamines (Adamczyk et al., 2011, 2013). High tannin contents in boreal forest soils may partially account for the

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phenomena of soil organic N recalcitrance. As tannins interfere also with enzymes (e.g. Adamczyk et al., 2009; Triebwasser et al., 2012), their influence on plant N uptake and SOM decomposition appears complicated.

To improve N uptake ground vegetation species, like the common heather (*Calluna vulgaris*), blueberry (*Vaccinium myrtillus*) and lingonberry (*Vaccinium vitis-idaea*), form intimate symbioses with ericoid mycorrhizal fungi (ERM) (Read, 1996), and trees like Scots pine (*Pinus sylvestris* L) form a symbiosis with ectomycorrhizal fungi (ECM). These fungi are capable of degrading and taking up organic N and sharing obtained N with the plant host (e.g. Heinonsalo et al., 2015; Read et al., 2004). The ability of some ericoid mycorrhizal and ectomycorrhizal fungi to take up N from recalcitrant sources, like tannin-protein complexes has been demonstrated (Bending and Read, 1996; Bennett and Prescott, 2004; Leake and Read, 1989; Wurzburger and Hendrick, 2009).

However, our understanding of the extent to which ERM and ECM plant-soil interactions affect N uptake and soil SOM pools in boreal forest remains limited. In contrast to ECM, of which ability and mechanisms to increase N availability and SOM decomposition have been acknowledged (Heinonsalo et al., 2012, 2015; Phillips et al., 2014; Shah et al., 2015a, 2015b), ERM are mostly studied in heathlands, especially on *Calluna vulgaris*, and their ERM fungi in pure cultures. These studies proved that ERM fungi are able to use amino acids, peptides, proteins or even chitin as a sole N source (Bajwa et al., 1985; Bajwa and Read, 1986; Leake et al., 1990; Leake and Read, 1990a, 1990b; Pearson and Read, 1973; Read, 1996). It was shown that ericaceous plants, through production of protein-binding phenolics, inhibit N mineralization, dominating heathland environments, as they restrict growth of competitors that lack the ability to utilize organic N sources (Jalal et al., 1982; Jalal and Read, 1983; Leake and Read, 1989). Studies of ERM in boreal forest have received less attention or the results have been somewhat contradictory. On one hand ERM increase N availability to host plant (Wurzburger and Hendrick, 2009), but on the other hand ERM appear to build-up recalcitrant SOM (Clemmensen et al., 2015; Mallik, 2003).

Here we compared the effect of ERM-forming ground vegetation plant species i.e. *Calluna vulgaris*, *Vaccinium myrtillus*, *V. vitis-idaea* (later called "ERM-plants") with the effect of ECM-forming Scots pine (later called "ECM-pine") on soil N chemistry, plant organic N uptake and SOM degradation. These species were chosen on the basis of their dominance at the study site from where the soil was taken. Seedlings were grown in microcosms containing homogenized organic soil originating from a Scots pine forest. We measured plant uptake of ^{15}N from organic sources, soil concentrations of NO_3-N, NH_4-N, the total amino acid pool, total N, degradable and recalcitrant N pools, enzyme activities, condensed tannin and total phenolic compound concentrations, differences in SOM using infrared spectroscopy, and abundance of enchytraeid worms. Our hypotheses were: 1) ECM-pine seedlings increase but ERM-plants substantially decrease the mobilization of soil N, 2) ERM and associated plants have significantly greater access to organic N in recalcitrant forms compared to ECM-pine 3) tannin-rich ERM-plants differ functionally from ECM-pine in affecting N cycling.

2. Materials and methods

2.1. Experimental design

Soil for this study was collected in the vicinity of the SMEAR II station of Helsinki University at Hyytiälä (61°84′N, 24°26′E) in southern Finland (see for site details Hari et al., 2013; Ilvesniemi et al., 2000). The soil was haplic podzol and Scots pine (*Pinus sylvestris* L.) was the dominant tree species. *Vaccinium myrtillus*, *V. vitis-idaea* and *Calluna vulgaris* were the dominating ground vegetation species. Soil was taken from the organic layer (Ofh) and after removal of visible roots was homogenized and sieved through a 4 mm mesh.

Calluna vulgaris seedlings were germinated naturally from sieved humus. *Vaccinium* (*V. myrtillus* and *V. vitis-idaea*) berries were collected from Southern Finland, and separated seeds were washed and dried. Collected seeds were surface sterilized with H_2O_2 (30% for 30 s) and planted into the sieved humus germination pots. Due to very slow growth, ericoid plants were approximately 6 months old before transfer to microcosms (see below). Scots pine (*Pinus sylvestris*) seeds were obtained from tree breeding seed collection (lot M29-92-0059 Sv. Ullanristi) and after surface sterilization with H_2O_2 (30% for 30 s) they were germinated on glucose agar (3.0 g glucose, 1.5 g agar in 1000 ml) to detect any microbial contaminants and to verify sterility. Thereafter, seedlings were grown aseptically in glass tubes on Brown-Wilkins growth medium for 2.5 months (Heinonsalo et al., 2015) prior to transplantation to microcosms.

With the use of above described soil, Perspex®microcosms (height 30 cm x width 20 cm) were filled with a thin organic layer (4 mm) in 5 treatments: *C. vulgaris*, *V. vitis-idaea*, *V. myrtillus*, *P. sylvestris* and humus with no plants. When transplanting to microcosms ericoid seedlings were 6 months old and pine 2.5 months old; all seedlings were at that time about the same size. One seedling was planted individually into each microcosm, every treatment was replicated 14 times. Microcosms were placed vertically in growth chambers having transparent lids. Roots were protected from the light and the soil was regularly watered by spraying deionized water in order to maintain soil moisture. After an establishment period of 3 months in microcosms, a seven week wintering period was simulated in December–January by shutting off the artificial light and lowering temperature levels to +5 °C. After a gradual elevation of temperature and light, the microcosms were kept in standard growth conditions for 11 months. Then, seedling shoots were exposed to 160–220 μmol s^{-1} m^{-2} light intensity, 18 °C temperature during the 18 h-long day and 14 °C during the 6 h-long night. Due to different pre-growth period and length of the harvesting period (several weeks), the total duration of the experiment was 540 days for *V. myrtillus*, 547 days for *C. vulgaris*, 582 days for *V. vitis-idaea*, and 412 days for *P. sylvestris*. However, it is noteworthy that the aim was to have similar size seedlings rather than similar age seedlings as both cannot be achieved simultaneously. The biomass data is presented as growth increment per day (biomass divided by number of days) due to differences in the length of the growing periods.

Soil samples were collected from microcosms at harvest at the end of the experiment. In a long experiment with natural forest soil, it is presumed that by the soil is colonized and enriched by root-associated bacteria and fungi. Soil samples were vacuum-dried (Christ LSC plus) and pulverized using a mechanical ball-grinder (2000-230 Geno/Grinder, SpexSample Pred, US) at 28 rpm, for 30 s. For some measurements fresh soil was used (Ammonium-N, nitrate-N, total amino acid pool, enzyme activity measurements).

2.2. Uptake of labeled ^{15}N from fungal biomass

Fungal biomass (basidiomycete wood rotting fungi *Dichomitus squalens*, strain code FBCC 312; Fungal Biotechnology Culture Collection, FBCC) was cultivated on Hagem's liquid medium (Heinonsalo et al., 2015) where 10% of required NH_4Cl was replaced with 99% $^{15}NH_4Cl$ (Larodan, Solna, Sweden). After cultivation fungal biomass was autoclaved and fresh material (corresponding to 13 mg DW) was put into 50 μm mesh bags, which allow fungal, but