



## Stimulation of soil organic nitrogen pool: The effect of plant and soil organic matter degrading enzymes



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### ABSTRACT

The majority of nitrogen (N) in boreal forest soils is bound to soil organic matter (SOM) in forms not readily available to plants. Northern boreal forest ecosystems are often N limited, despite atmospheric N deposition, and the utilization of organic N from SOM is of crucial importance to the site productivity. The effect of microbial produced oxidative SOM degrading enzymes (laccase and manganese peroxidases) and proteases on soil N forms and availability was studied in a pot experiment with or without a Scots pine (*Pinus sylvestris* L.) seedling. The combination of SOM degrading enzymes and proteases decreased the total soil N content and increased the N losses significantly in the absence of the Scots pine seedlings. The total soil N content also decreased in the presence of the Scots pine seedlings, irrespective of the enzyme treatment. Most of the other N parameters studied were not sensitive to enzyme additions, and differed only between planted and non-planted treatments. Our results show that the alkylamine content of boreal forest soil are at the same levels as nitrate. We showed that SOM decomposition, stimulated by oxidative enzyme additions, is a key step in soil organic N utilization, while proteases alone do not increase N use from SOM. Plants stimulate N losses from SOM highlighting the importance of rhizosphere processes in soil C and N cycling.

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### 1. Introduction

A large part of total soil nitrogen (N) of boreal forest soil is in organic forms (Korhonen et al., 2013). The total N quantities in such ecosystems can be surprisingly high, as was shown by Korhonen et al. (2013) who quantified the N balance of an entire Scots pine (*Pinus sylvestris* L.) forest stand. They found that the total soil N pool was 2070 kg N ha<sup>-1</sup> and the annual uptake by trees amounted to 50 kg N ha<sup>-1</sup>. Mineral N concentrations are usually low, despite the large total N pool, compared to the plant uptake and the ecosystem productivity is limited by the availability of N (Korhonen et al., 2013) indicating poor N availability of organic N for plants and microbes (Schulten and Schnitzer, 1998; Knicker, 2011; Korhonen

et al., 2013). Nitrogen is integrated to SOM via stabilization processes that form either chemically recalcitrant N, or protect N physically from microbial utilization (Schulten and Schnitzer, 1998; Rillig et al., 2007). The majority of soil organic N is proteinaceous material (ca. 40%) or heterocyclic N including purines and pyrimidines (cs. 35%) (Schulten and Schnitzer, 1998). Decomposition of SOM and SON is regulated by many processes and factors including molecular structure, condensation reactions, fire residues, rhizosphere inputs, physical disconnection, soil depth, freezing-thawing and microbial products, as highlighted by Schmidt et al. (2011). In all, multiple processes may preserve but also release N from SOM and significantly affect soil C and N cycling.

The release of the chemically recalcitrant N from SOM and the formation of more readily available forms of N are of crucial importance for ecosystem productivity in N limited forest ecosystems (Schimel and Bennet, 2004; Knicker, 2011). The release of N from the large recalcitrant organic N pool in boreal forest soil SOM is enhanced by the presence of degrading enzymes such as laccase

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and manganese peroxidases (MnP) (Von Lütow et al., 2006; Kleber, 2010; Dungait et al., 2012). Additional biochemical processes are responsible for transformation of larger macromolecules to dissolved organic nitrogen (DON), including peptides or amino acids, which can be taken up by plants or microbes (Schimel and Bennet, 2004). Finally, peptides and amino acids are mineralized via microbial ammonification and nitrification–denitrification processes to inorganic nitrogen, ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) (Schimel and Bennet, 2004; Fig. 1). The depolymerization of proteinaceous material and the release of monomers and oligopeptides (e.g. amino acids) has been proposed to be the key step in regulating N uptake by plants and microbes in boreal forest ecosystems (Schimel and Bennet, 2004).

The formation of a symbiotic relationship between trees and ectomycorrhizal (ECM) fungi is widespread in boreal ecosystems. The fungal symbionts improve nutrient uptake of the host plant, especially from organic sources (Smith and Read, 2008). In exchange, plants allocate a significant portion of the C assimilated in photosynthesis to the ECM fungi and associated microflora (Smith and Read, 2008). Fungal symbionts increase N availability by stimulating SOM decomposition via root exudation and the excretion of extracellular enzymes (Talbot and Treseder, 2010; Drake et al., 2011; Phillips et al., 2011; Kaiser et al., 2011; Lindén et al., 2014). Recently it has been shown that manganese peroxidase (MnP)-like peroxidases, enzymes essential in the degradation of lignin-compounds, may be produced by the common ECM genus *Cortinarius* (Bödeker et al., 2009). Furthermore, class II peroxidase-encoding genes have been identified in a wide range of ECM fungi (Bödeker et al., 2009). ECM fungi are also known to degrade SOM using a Fenton-reaction based mechanism in a similar way as brown-rot fungi (Rineau et al., 2012). A non-specific oxidative enzyme, laccase, is produced by many fungi, including ECM fungi (Chen et al., 2003; Luis et al., 2004; Heinonsalo et al., 2012). Several laccase genes are encoded in the genome of the common ECM genus *Piloderma* sp. (Kohler et al., 2015) and laccase gene expression was related to N availability (Chen et al., 2003). Talbot and Treseder (2010) suggested that the symbiotic relationship between trees and their ECM fungi is the key factor in stimulating SOM decomposition and the subsequent release of N for use by plants and microbes.

The microbial nitrogen turnover processes may also result in a small fraction of nitrogen being lost to the atmosphere in the form of nitrogen monoxide (NO), nitrous oxide ( $\text{N}_2\text{O}$ ), molecular nitrogen ( $\text{N}_2$ ), or volatile organic nitrogen (e.g. amines) (Fig. 1). Very little information is available on the formation, production rates, or function of the volatile organic nitrogen compounds, such as amines, in boreal forest soil. However, as amines have been

suggested to be formed via aminification processes (Yan et al., 1996; Yu et al., 2002), such as the decarboxylation of amino acids (Dudareva et al., 2013), it is possible that during depolymerization of SON amine formation is also induced. Amines are reactive compounds in the atmosphere and even in very low concentrations they participate in chemical reactions leading to formation and growth of aerosols in the atmosphere (Kurten et al., 2008; Almeida et al., 2013; Kurten et al., 2014). In addition to their importance to the atmospheric chemistry, amines may also have important and currently unknown functions in the cycling of N in the soil (Schulten and Schnitzer, 1998; Vranova et al., 2011; Warren, 2013). However, due to technical challenges in measuring amines in the atmosphere and in soils, there are few studies reporting amine concentrations in the atmosphere (Sellegri et al., 2005; VandenBoer et al., 2012; Kieloaho et al., 2013; You et al., 2014) and especially in soils (Yu et al., 2002).

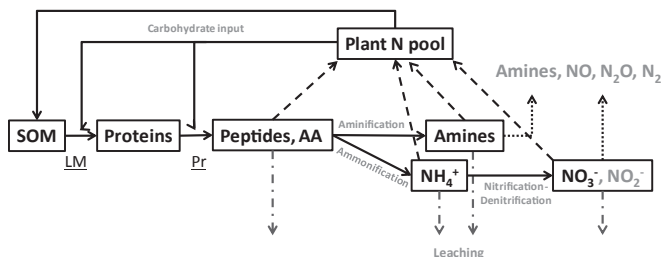
The aim of the experiment was to study the effect of SOM degrading enzymes laccase (L) and manganese peroxidase (M) and protein degrading enzymes (proteases, Pr) on soil N cycling with the presence or absence of a Scots pine seedling (*P. sylvestris* L.). The concentrations of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , amino acids, alkylamines, total N, recalcitrant N, degradable and proteinaceous N, plant biomass, enzyme activities and ECM fungal root tip numbers were quantified after the enzyme treatments. The schematic presentation of hypothetical boreal forest soil N cycling, effective points of enzyme treatments and the measured N forms are shown in Fig. 1. We hypothesize that 1) the addition of SOM and protein degrading enzymes increase SOM decomposition and the proportion of N forms which are available to the plant, 2) the presence of a plant stimulates SOM decomposition and N availability in the soil, 3) increased protein degradation in protease treatments (Pr) induces amine synthesis and amine concentration in the soil.

## 2. Materials and methods

### 2.1. Experimental setup

The boreal forest soil used in the experiment was collected in May 2011 in the vicinity of SMEAR II station of Helsinki university at Hyytiälä (61°51'N, 24°17'E) in Southern Finland (Hari and Kulmala, 2005). The soil at the site is Haplic podzol and the stand is dominated by Scots pine (*P. sylvestris* L.) and occasional Norway spruce (*Picea abies* (L.) H. Karst.), Silver birch (*Betula* spp. L.), or European aspen (*Populus tremula* L.) could be found mainly in the understory. A detailed site description can be found in Ilvesniemi et al. (2009). Only the organic soil layer (mixed F/H horizon, later called 'soil') was used in the experiment. Large roots were removed, the fresh soil was homogenized and sieved through a 4 mm mesh, and stored at +4 °C for one month until the experiment was established. The total N content of the soil was 6.9 mg-N g<sup>-1</sup> DW.

The experimental setup is shown in Fig. 2. The experiment consisted of six treatments. Three of the treatments were planted with one-year-old nursery grown Scots pine seedlings (N = 13) and three were non-planted treatments (N = 5). Altogether the experiment consisted of 54 pots, 39 with and 15 without Scots pine seedlings. Within the planted and non-planted treatments, three enzymatic treatments were created: 1) soil amended with BSA protein only (Bovine Serum Albumin, BSAS 0.01, Bovogen, Australia; in total 205.6 mg) was regarded as control treatment (Con). 2) Protease treatment (Pr) was created by adding two kinds of proteases: 2.16 mg protease 1 (*Streptomyces griseus*, Sigma–Aldrich, P5147, 0.84 activity units per mg), and 112.5 mg protease 2 (*Rhizopus* sp., Sigma–Aldrich P0107, 0.2 activity units per mg). 3) Laccase, manganese peroxidase and protease (LMPr) treatment was created by adding a mixture of enzymes including 90 mg laccase



**Fig. 1.** A schematic representation of N cycling in boreal forest ecosystems, modified from Schimel and Bennet (2004). Boxes: compounds in the soil or plant, solid arrows: microbial and plant-driven processes, dashed arrows: plant N uptake or N loss from the soil via leaching or volatilization. Black color: quantities measured in the experiment, gray color: quantities not measured but are part of N cycle. Underlined LM and Pr refers to reactions stimulated by enzyme treatments in this study: LM = laccase and manganese peroxidase, and Pr = protease additions, respectively.

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