



Recovery of the ectomycorrhizal community after termination of long-term nitrogen fertilisation of a boreal Norway spruce forest



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ABSTRACT

Ectomycorrhizal fungi (ECM) are a fundamental component of boreal forests promoting tree growth and participating in soil nutrient cycling. Increased nitrogen (N) input is known to largely influence ECM communities but their potential recovery is not well understood. Therefore, we studied the effects of long-term N-fertilisation on ECM communities, and their recovery after termination of N treatment. Fungal ITS sequencing data indicated that N-fertilisation ($34 \text{ kg N ha}^{-1} \text{ y}^{-1}$) for 46 y decreased the relative abundance of ECM species in the fungal community and suppressed originally dominating medium-distance fringe exploration types adapted to N-limited conditions, while the ECM diversity remained unaffected. In other plots, 23 y after termination of fertilisation at $73 \text{ kg N ha}^{-1} \text{ y}^{-1}$ for 23 y, the relative abundance of ECM species shifted closer to, but did not reach, control levels. These observations indicate only slow recovery of ECM community, likely due to a high soil N retention capacity.

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1. Introduction

Ectomycorrhizal fungi (ECM) are fundamental components of boreal forest ecosystems. As tree root symbionts, they improve capacities of their hosts to acquire water and nutrients by increasing their effective uptake surface area and soil exploration capacity. They also play important roles in soil organic matter decomposition (Lindahl and Tunlid, 2015), and both carbon (C) and nitrogen (N) sequestration (Högberg et al., 2014a; Clemmensen et al., 2015). However, different ECM taxa have different abilities to explore soil in search for nutrients and different capacities to utilize available substrates (Lilleskov et al., 2011). Therefore, the ECM community's composition is an important factor that strongly affects not only tree growth, but also soil organic matter and nutrient cycling.

Soil N availability is one of the most important factors affecting ECM community composition and functioning. Numerous investigators, using various measures of ECM abundance and community composition, have found that ECM abundance decreases in response to increases in N availability, either through N fertilisation or atmospheric N deposition (e.g. Arnolds, 1991; Lilleskov et al., 2002b; Treseder, 2004; Kjoller et al., 2012; Högberg et al., 2014a). However, there are a few exceptions (Wallenda and Kottke, 1997; Kalliokoski et al., 2009). Besides the local soil conditions, the magnitude of the ECM community response to elevated N probably depends on the N dose and duration of elevated N inputs (Hasselquist and Högberg, 2014).

The generally observed decline in abundance of ECM fungi after increase in soil N availability is likely driven by a reduction in the allocation of photosynthate C to the below-ground parts of trees and thus the ECM (Högberg et al., 2003, 2011; Hobbie, 2006; Janssens et al., 2010). However, plants allocate less photosynthate belowground only if no other nutrient (e.g. P) becomes limiting (Johnson and Gehring, 2007). When plant C allocation belowground decreases, ECM lose their main advantage in competition

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with other soil microorganisms (Högberg et al., 2003). These changes can be rapid: reductions in ECM sporocarp and extramatrical mycelium production have been observed within 5 y after commencement of N addition (Lilleskov et al., 2011).

Numerous studies have also reported negative effects of elevated N supply on ECM diversity (Lilleskov et al., 2001, 2002a, 2002b; Avis et al., 2003; Toljander et al., 2006; Cox et al., 2010; Kjoller et al., 2012; Hasselquist and Högberg, 2014). However, sensitivity of the ECM community to soil N enrichment is dependent on its taxonomic composition (Lilleskov et al., 2011). Some genera are generally sensitive to N additions and decrease in abundance, such as *Cortinarius* (Brandrud, 1995; Lilleskov et al., 2002a; Carfrae et al., 2006) and *Piloderma* (Lilleskov et al., 2002a; Cox et al., 2010), while others, such as *Lactarius* (Brandrud, 1995; Wiklund et al., 1995; Lilleskov et al., 2002a) and *Laccaria* (Termorshuizen, 1993), are generally regarded as N tolerant. The responses of many taxa to N additions also seem to depend on site characteristics, N enrichment parameters and temporal aspects of N addition (Lilleskov et al., 2011). The sensitivity of particular ECM taxa to soil N enrichment seems to be connected to their capacity to utilize complex N sources (e.g. proteins). Taxa growing effectively on protein-derived N sources are often reduced in abundance when soil N status is increased (Taylor et al., 2000; Lilleskov et al., 2002b, 2011).

Agerer (2001) classified ECM into distinct ‘exploration types’ based on their ability to forage resources in the soil environment, and properties such as presence/absence of rhizomorphs, hydrophobicity and extramatrical mycelium branching patterns. The extramatrical mycelial growth of contact and short-distance (‘short’ hereafter) exploration types is limited to a few millimetres from the root tip, while ECM of medium- and long-distance exploration types capable of producing rhizomorphs may spread tens of centimetres from the root tip. Exploration type and susceptibility to N loading seem to be correlated, and long-distance, medium-distance fringe (medium fringe hereafter) and medium-distance mat exploration types seem to be the most sensitive (Lilleskov et al., 2011). These fungi are capable of producing long, hydrophobic rhizomorphs, which allow them to explore remote parts of the soil environment in searches for nutrients, and transport acquired resources over long distances. They also often have high capacity to utilize complex organic N sources (Finlay et al., 1992; Lilleskov et al., 2002b). Such attributes provide these ECM with potent advantages under N-limited conditions. However, production of rhizomorphs and the enzymes required to break down complex N sources is C- and energy-demanding, and thus disadvantageous in N-rich environments with reduced C supplies from trees. Under such conditions, less C- and energy-demanding contact and short ECM types may thrive.

Although N deposition rates have likely declined in Europe as a whole since 1990, there have been large temporal and spatial variations, and projections of future global N deposition do not indicate that levels will be decreasing in the near future (Galloway et al., 2004; Tørseth et al., 2012). Reductions in N deposition might result in recovery of N-saturated ecosystems, which could be accompanied by functional and structural changes of ECM communities. There have been few studies of such responses. Hasselquist and Högberg (2014) recently assessed the recovery of ECM communities from long-term high N loads in a boreal forest ecosystem (the Norrleden experimental site, Sweden). Three years after the treatment had ceased they found lower ECM sporocarp production and species richness in plots subjected to annual N additions (70 kg N ha⁻¹ y⁻¹) for 38 y compared to control plots. However, in plots that initially received a higher load (110 kg N ha⁻¹ y⁻¹ for 20 y) and subsequently have been left to recover for 23 y, sporocarp abundance and ECM species richness had returned to those of control

plots. Moreover, the role of ECM fungi in ecosystem N retention had been restored (Högberg et al., 2011, 2014b). To improve our understanding of the ability of ECM communities to recover structurally and functionally after termination of high N loading, additional similarly focused studies are needed.

The Stråsan experimental site in Sweden is ideal for such studies, as it allows comparison between experimental plots subjected to both ongoing and terminated N treatments and comparable, unfertilised control plots. Thus, we assessed changes in composition and diversity of ECM communities in these plots. For this purpose, we sequenced fungal internal transcribed spacer (ITS) amplicons obtained from soil DNA and assigned the detected ECM taxa to exploration types, in order to evaluate the functional potential of ECM communities in plots with different N loading histories. The primary aims of the study were to characterise responses of the ECM community in the N-limited Norway spruce boreal forest at Stråsan to N addition in detail, and evaluate its recovery 23 y after termination of annual N additions that had been ongoing for 23 y.

2. Materials & methods

2.1. Study site

E26A Stråsan (Tamm et al., 1974) is the site of a long-term fertilisation experiment, located in central Sweden (60°55' N, 16°01' E), in which Norway spruce (*Picea abies*) forest stands have been subjected to various fertilisation treatments (some ongoing and some now terminated). The stands were 55 y old when samples of soil, classified as a haplic podzol (Berggren Kleja et al., 1998), were collected from the site for this study (in 2013). The experiment has a randomized block design with two blocks of 30 m × 30 m experimental plots assigned to treatments, which are represented once within each block. The peripheral 5 m borders of the plots are treated as buffer zones, thus the inner 25 × 25 m areas are used for sampling and measurements. The treatments have included three levels of N addition (as NH₄NO₃ annually in May) since 1967: N0 – control with no N addition, N1 – ongoing fertilisation with 34 kg N ha⁻¹ y⁻¹ on average (hereafter called ‘fertilisation’), and N2 – fertilisation with 73 kg N ha⁻¹ y⁻¹ on average until cessation in 1990 (hereafter called ‘recovery’). The current rate of N deposition at the site is low at 3.2 kg N ha⁻¹ y⁻¹ (Rappe-George et al., 2013). For detailed information on N additions and soil chemistry see Table 1.

2.2. Soil sampling

The sampling was performed during spring (9th May) and autumn (11th October) of 2013. In total, 4 samples of the soil organic layer (25 cm × 25 cm) were collected for each plot per each sampling event. Thus in total, we collected 16 soil samples per each of the three treatments.

The samples were transported on dry ice to the laboratory, sieved through a 4 mm mesh and stored at –80 °C until preparation for analysis.

DNA was isolated from the samples using a FastDNA Spin Kit for Soil (MP Biomedicals, USA), including additional treatment with 5.5 M guanidinium thiocyanate (molecular grade; Sigma-Aldrich, USA) to remove polymerase chain reaction (PCR) inhibitors according to the manufacturer’s instructions. DNA was stored in 2 ml microtubes at –20 °C until analysed.

2.3. Illumina sequencing of ITS

LGC Genomics GmbH (Berlin, Germany) sequenced the ITS2 region in the DNA samples, including all of the preparation steps.

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