Long-term effects of tree harvesting on ectomycorrhizal fungal communities in boreal Scots pine forests

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A B S T R A C T

Management of boreal forests with clear-cutting has a marked effect on forest biodiversity. One directly and severely affected group of species is ectomycorrhizal fungi (EMF), because of their dependence on living host trees. Key questions in nature conservation and potentially for fungal ecosystem services are whether EMF communities as regenerated stands age will develop into a similar composition as in natural stands, and whether forest regeneration method matters. We addressed these questions by analyzing EMF communities in (1) 157–174 year-old natural and about 50-year-old Scots pine forest stands regenerated by (2) clear-cutting and planting, or (3) shelterwood regeneration with naturally established seedlings. We identified 98 EMF species hypotheses (SHs) using IonTorrent sequencing of soil cores from nine forest stands. We compared these results with a sporocarp survey and an RFLP-analysis of ectomycorrhizal roots performed 18 years earlier.

When testing individual SHs (in the sequencing data 2013) 13 of the 20 most frequent EMF SHs in natural forest stands were present in similar frequencies in 50-year-old managed stands whereas 7 SHs were observed at different frequencies. The overall EMF community composition differed between natural and 50-year-old managed stands both when assessing sporocarp data from 1995 to 1998 and sequencing data from 2013. One individually tested SH was found to differ between 50-year-old shelterwood and clear-cut stands and no difference in EMF overall community composition was found between the two regeneration methods. Species richness was similar in all stands. The RFLP-analysis largely identified the same common species as the current study.

Our result suggests that timber harvest has a minor effect on frequent EMF in a 30–50 year perspective. However, both the current study of soil mycelia and the sporocarp survey imply the overall composition of EMF communities to be affected. A contributing factor is the higher presence of Norway spruce in the natural stands. Moreover, the majority of species were not sufficiently frequent to be statistically tested. The importance of surviving mycelia, spore bank and new spore deposition is discussed.

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1. Introduction

Industrialized forest management has led to transformation of forests into homogenous managed stands, which have impacted biodiversity negatively (Secretariat of the Convention on Biological Diversity, 2010). Clear-cutting followed by soil scarification and planting is the most widespread forest harvesting method being applied in Fennoscandian countries for more than half a century (Framstad et al., 2013; Lundmark et al., 2013; Ratnam et al., 2014).

One group of organisms directly and negatively affected by clear-cutting is ectomycorrhizal fungi (EMF) (Jones et al., 2003). They depend on their symbiosis with trees and largely disappear after clear-cutting as a consequence of the ceased transport of carbon from the cut trees (Harvey et al., 1980; Jones et al., 2003; Luoma et al., 2004). Observations within a few years after clear-cutting indicate that the composition of the surviving and re-establishing EMF community may differ from that of older stands (Byrd et al., 2000; Durall et al., 2006; Hartmann et al., 2012; Jones et al., 2003; Rao et al., 1997). The difference is mainly attributed to the strong reduction of living EMF mycelia, as a consequence of the harvested trees, but other contributing factors could be changes in the biological and chemical environment selecting for certain species as well as variation between different

EMF species in their ability to re-establish (Jones et al., 2003). The EMF community in boreal forests is species rich (Jonsson et al., 1999b; Taylor, 2002) with relatively few common species and many rare ones (Koide et al., 2005; Sterkenburg et al., 2015). As a regenerating stand develops and more belowground root biomass is created, the potential EMF species richness and activities of EMF increase, following the classical species richness vs. area relationship (Peay et al., 2007). Thus, it is essential to study disturbance responses of EMF communities in appropriate spatial and temporal scales. Studies of EMF communities done 30 years or more after harvest, using sporocarp surveys, mycorrhizal morphotyping or RFLP, show that EMF communities partly differ between previously clear-cut forest stands of different ages (Kranabetter et al., 2005; Palfner et al., 2005; Smith et al., 2002). However, the difference seems to be largest between young and older stands and less between older stands (Kranabetter et al., 2005). Wallander et al. (2010) studied long-term succession following clear-cutting using high throughput sequencing of EMF communities, based on extra radical mycelium, and reported *Tylospora fibrillosa* to totally dominate up to 30 years old stands of Scots pine but that species richness increased in 30–90-year-old stands.

Trees and other mycorrhizal host plants left at harvest enable EMF mycelia to survive into the new, regenerating forest (Amaranthus and Perry, 1987; Rosenvald and Lõhmus, 2008). An interesting question is whether the choice of forest regeneration method affects the survival of EMF after forest harvest. At present, the majority (78%) of the Swedish forests are clear-cut and planted or seeded compared to 20% that are naturally regenerated using seed trees (Skogsstyrelsen, 2014). In seed tree regeneration 50–150 seed trees per hectare are temporarily retained at harvest and cut when the seedlings are naturally established, around 10 years later (Karlsson and Örlander, 2004). By then, a portion of the naturally regenerated seedlings may potentially be colonized by EMF mycelia that were life-boated by the seed trees. Seedlings planted on clear-cut areas instead introduce a subset of EMF naturally established in the nurseries (Menkis et al., 2016). In theory, the use of seed trees should thus be more favorable for maintaining the EMF community in a harvested forest than clear-cutting and planting. A natural regeneration method similar to regeneration with seed trees is shelterwood regeneration, in which more trees are left (Karlsson and Örlander, 2004) and gradually harvested over a longer period of time aiming to increase regeneration success by reinforcing the effects of seed trees. This method is, however, not used in Sweden, but chosen since it is more contrasting to clear-cutting than seed tree regeneration.

The objective of this study was to investigate long-term effects on EMF communities by forest management using soil DNA sequencing in 50-year-old forest regeneration field experiments. We hypothesized (1) that effects of forest harvesting on the community composition and species richness of EMF to last for at least 50 years and, (2) that shelterwood regeneration has smaller effects than clear-cutting, due to differing levels of biological legacies of EMF during the early tree regeneration phase. We emphasize the community composition since a shift in community composition could potentially have functional effects on the ecosystem but also investigate species richness. We examined three stands that had been clear-cut and planted and three stands that were self-regenerated under shelterwood trees as part of experiments on regeneration methods initiated around 1960 and compared these with nearby natural stands. We also hypothesized (3) that EMF communities 2013 would be similar to those recorded at the same sites 18 years earlier. Finally, we examined (4) the potential of soil DNA sequencing to detect, not only widespread and frequent EMF, but also more rare species. The latter two questions were addressed by comparing the sequence data from the present study

with previous results from an RFLP analysis of root tips and a sporocarp survey performed 18 years earlier in the same stands (Kårén, 1997).

2. Material and methods

2.1. Study site and sampling design

The study was conducted in Siljansfors Experimental Forest (1500 ha), located in west central Sweden, in a Scots pine dominated area within the boreal zone at an elevation of 210–425 m above sea level (N 60°54′, E 14°23′) (Fig. 1). Mean annual temperature is +3 °C and mean annual precipitation 674 mm. The annual deposition of atmospheric nitrogen was in the range of 2–4 kg N/ha (data from 1995 to 2014, Swedish Environmental Protection Agency, 2016). Management and production of the stands are well documented – management experiments have been conducted at Siljanfors since the start of the experimental park in 1921. Prior to experimental manipulation, the forests were extensively used for selective logging, cattle grazing and firewood harvesting during several centuries. Six experimental Scots pine (*P. sylvestris*) dominated stands with similar site index (an index used in Swedish forestry to indicate potential tree productivity) were selected from a larger regeneration experiment running 1940–1960. The stands are located on podzolised tills and have a field-layer dominated by *Vaccinium myrtillus, Vaccinium vitis-idea* and *Calluna vulgaris* (Table A3). Three stands were regenerated around 1960 by clear-cutting and planting, hereafter denoted C1–3, and three were regenerated by natural regeneration under shelterwood, S1–3 (Table 1). As reference, three natural stands with similar characteristics were chosen, N1–3 (aged 157–174 years, Table 1). These natural forest stands had a structure and dynamic not significantly affected by humans (Rouvinen and Kouki, 2008). The natural forests had higher presence of Norway spruce compared to the managed forests (Table 1), which is inevitable in natural succession of Scots pine forests (Linder et al., 1997). The experimental and reference stands ranged from 0.11 to 12 ha (Table 1). The first cutting in the shelterwood (S1–3) management (1940s) resulted in 120 trees ha^{-1} left, a second cutting (1950s) in 40–70 trees ha^{-1} remaining, where after all mature trees were cut 10 years later (1960s) (Table A3). The clear-cut stands (C1–3) were all cut in the 1960s and planted 1–6 years later (Table 1). The stands C1, S1, and N2 have a history of frequent wildfires occurring in 1733, 1777, 1824 and 1857. None of the plots have been fertilized or limed.

2.2. Sampling and sample preparation

In September 2013, 25 soil cores (3 cm diameter, 5 cm depth) were collected at the center of each stand in a grid pattern with 5 m distance between samples (Fig. 1), resulting in a total of 225 samples. Litter was discarded and the humus (H) layer was collected in falcon tubes and frozen at −18 °C within 8 h. Sample preparation followed the procedure described by Clemmensen et al. (2015). The samples were freeze-dried and homogenized using a ball mill (Retsch GmBH, Haan, Germany) and DNA was extracted from about 400 mg of each sample using the NucleoSpin Soil kit (Macherey-Nagel Düren, Germany) and DNA concentration was checked using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, USA). Milli-Q water was used for negative controls. Two samples from which no DNA could be extracted were excluded from the analysis. The fungal-specific internal transcribed spacer 2 (ITS2) region was PCR amplified with 50ul reaction volume and 3 min at 95 °C, 25–30 cycles of (30 s at