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SHORT NOTE

Effects of stump and slash removal on growth and mycorrhization of *Picea abies* seedlings outplanted on a forest clear-cut

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Abstract The objectives of this study were to investigate impact of stump and slash removal on growth and mycorrhization of *Picea abies* seedlings outplanted on a forest clear-cut. Four non-replicated site preparation treatments included: (1) mounding (M), (2) removal of stumps (K), (3) mounding and removal of logging slash (HM) and (4) removal of logging slash and stumps (HK). Results showed that height increment of the seedlings was highest in K and lowest in M after the third growing season, and similar pattern remained after the fourth season. Ectomycorrhizal (ECM) colonisation of seedling roots was highest in M (96.6%) and lowest in K (72.3%), and even in HK (76.0%) and HM (76.3%). Morphotyping and sequencing of internal transcribed spacer of fungal ribosomal DNA revealed a total of 13 ECM species. Among those, *Thelephora terrestris* and *Cenococcum geophilum* were the most common, found on 27.4% and 26.3% of roots, respectively. The rest of species colonised 26.6% of roots. Richness of ECM species was highest in M (10 species) and lowest in K (three species). Consequently, stump and slash removal from clear-felled sites had a positive effect on growth of outplanted spruce seedlings, but negative effect on their mycorrhization. This suggests that altered soil conditions due to site disturbance by stump and slash removal might be more favourable for tree growth than more abundant mycorrhization of their root systems in less disturbed soil.

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Introduction

In order to satisfy increasing demand for forest biofuel, harvesting of tree stumps and logging slash (tree tops and branches) from clear-felled forest sites are expected to become common practices in northern Europe (Berglund and Åström 2007; Egnell et al. 2007). On the other hand, such silvicultural operations are usually associated with certain site disturbances which may lead to alterations in soil chemical, physical and biological properties (Hope 2007). A recent review had demonstrated, however, that in most cases, removal of stumps and slash from forest clear-cuts had a positive effects both on survival and growth of outplanted seedlings and on natural forest regeneration (Vasaitis et al. 2008, and references therein). Yet, as apparent from the review, to date, the majority of related investigations have been conducted in North America. In regard to European forest stands, data on stump and slash removal on seedling performance are available only from several long-term trials in Sweden (Kardell 1992, 1996, 2007, 2008; Kardell and Eriksson 2008). Nevertheless, taking into account current and future demand for forest biofuel, more such studies in the region would be desirable.

Even less is known about possible impacts that the related soil disturbances might have on communities of ectomycorrhizal (ECM) fungi, in particular on their potential to colonise seedling roots and on structure of their communities. From this perspective, ECM fungi are of high importance, as a number of studies had demonstrated that they promote establishment and growth of tree seedlings after their outplanting in the field (Kropp and Langlois 1990; Menkis et al. 2007; Stenström et al. 1990) and contribute to sustainability of a new forest stand (Pfleger and Lingerman 1994; Read et al. 1996). However, to date, only a handful of data are available on the impact of stump removal on mycorrhiza in subsequent seedling regeneration. Thus, an American study has reported negative effect of de-stumping on ectomycorrhizal number and diversity of 1-year-old *Pseudotsuga menziesii*, while *Pinus monticola* from this point of view was much less sensitive (Page-Dumroese et al. 1998). The present work has been carried out in Finland, and its objectives were to study possible effects of stump and slash removal on growth of outplanted *Picea abies* seedlings, ECM colonisation of seedling roots and ECM community structure.

Materials and methods

Study site and experimental design

The study site was located at the Hyytiälä Forestry Field Station of the University of Helsinki (N61º51′, E24º17′, 160 m a.s.l.) in central Finland. The area represented a boreal coniferous forest dominated by *P. abies* and *Pinus sylvestris* with admixture of *Betula pendula* and *Betula pubescens*. It was clear-felled in November 2004. Forest site type was partly *myrtillus* and partly *oxalis-myrtillus* type, and the soil was middle-coarse moraine, cryaquod spodosol (Mokma and Yli-Halla 2000). In May 2005, four plots each 20×30 m in size were established as different silvicultural site preparation treatments: (1) mounding (M), (2) stump removal (K), (3) mounding and slash removal (HM) and (4) removal of stumps and slash (HK). All those methods are commonly used in practical forestry in Finland (Finnish Statistical Yearbook of Forestry 2009). Stumps and slash were removed within days after timber harvesting. Mounding and stump removal were done with rounding excavator, which was equipped with a tool for stump lifting and mounding at the same time. In the mounds, 10–20 cm surface soil layer was turned upside down on the top of unhandled humus layer. Each treatment was separated from each other by the 5 m buffer zone. Planting was done in late May 2005 with 1-year-old nursery cultivated containerized (system PL81) seedlings of *P. abies* at the density of 1,800 seedlings per hectare. Seedlings were produced in Saarijärvi nursery and at the time of outplanting were 15 cm in height (seeds originated from seed orchard SV358). Prior to outplanting, ECM examination of seedling roots was not carried out.

Field measurements and sampling

In each treatment, the height increment of all *P. abies* seedlings (M—139; K—136; HM—140; HK—138) was measured as the total length of shoots after three and four growing seasons, respectively (measured in September 2007 and in May 2009). After 5 years since the plantation was established (May 2009), also root systems were sampled from 12 randomly selected plants (three from each treatment). Sampled roots were packed into the plastic bags, labelled, transported to the laboratory and kept at +4°C for a maximum period of 2 weeks.

Laboratory work

Roots were gently washed in tap water, and 100 single root tips from each plant were randomly collected from different parts of the root systems using forceps. ECM roots were identified by the presence of a mantle, external hyphae or rhizomorphs and the absence of root hairs. Macroscopic features were examined using a Carl Zeiss Stemi 2000-C dissection microscope (Oberkochen, Germany). ECM morphotyping was done as described by Menkis et al. (2005). For identification of fungal species, 1–3 randomly selected representatives of each distinct ECM morphotype from each root system were subjected to direct sequencing of internal transcribed spacer of fungal ribosomal DNA (ITS rDNA). More replicates were taken from more common morphotypes. DNA extraction and PCR were done as in a previous study (Menkis et al. 2005). Sequencing was performed by Macrogen Inc., Seoul, Korea, utilising ABI 3730 XL automated sequencers (Applied Biosystems, Foster City, CA, USA). Raw sequence data were analysed using the SeqMan version 5.01 software from DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit version 7.0.5.2 (Hall 1999).

Databases at GenBank (Altschul et al. 1997) and UNITE (Koljalg et al. 2005) were used to determine the identity of ITS rDNA sequences.

Statistical analyses

Analysis of the data was carried out using multivariate statistics. ECM community structure and possible treatment effects on plant growth and mycorrhization were analysed using Canonical Correspondence Analysis

(CCA) in CANOCO 4.5 (ter Braak and Smilauer 1998). Comparison of ECM community structure in different treatments was assessed by calculating qualitative (S_S) Sorensen similarity indices (Magurran 1988).

Results and discussion

The results demonstrated a positive effect of stump and slash removal on height increment of spruce regeneration on replanted clear-cut after three and four growing seasons. Thus, after three seasons, height increment of seedlings was highest in K treatment (20.1 cm), lowest in M treatment (17.1 cm) and intermediate in HM (19.1 cm) and HK (18.8 cm) treatments. Similar pattern in height increment was observed also after four growing seasons: highest in K (30.2 cm), followed by HM (29.5 cm), HK (28.6 cm) and M (26.6 cm). After three and four seasons, increment in K, HM and HK exceeded that in M by 9–15% and by 7–12%, respectively. In another Finnish study, shoot growth of *P. abies* seedlings in M treatment did not differ from growth in untreated control plots (Pennanen et al. 2005).

Positive effects on growth of regeneration on de-stumped clear-cuts were reported in many previous related studies. Thus, among 29 stump removal trials analysed (incl. different tree species, soil conditions and geographic localities), 13 had resulted in increased growth of subsequent tree regeneration, in ten low or no impact was observed and in only in six decreased tree growth has been reported (Vasaitis et al. 2008). For comparison, "whole-tree harvesting" trials in Sweden, height increment of planted *P. abies* and *P. sylvestris* after 7 years was, respectively, by 40–70% and by 15–20% higher on sites where stumps, and stumps and slash were removed, than on control sites with conventional stem harvesting (Kardell 1992). More recent studies of these trials had demonstrated that positive effect of stump removal on growth of subsequent regeneration might persist during 24–28 years (Kardell 2008; Kardell and Eriksson 2008). Faster height growth observed in K, HM and HK treatments of the current work could be probably attributed to rather extensive soil disturbance, which lead to increase in soil aeration, temperature, mineralisation and penetrability, reducing vegetation competition and soil pathogen inoculums, as also suggested in several other experiments where extensive soil treatments were applied prior to establishing forest plantations (Hope 2007; Morrison et al. 1991; Simard et al. 2003).

ECM colonisation of individual plants varied between 63% and 100% of roots and was most abundant in M treatment (290 ECM tips among 300 examined, or 96.6%). In other three treatments, ECM colonisation of roots was lower by 20.3–24.3%: K (217 out of 300, or 72.3%), HK (228 of 300, or 76.0%) and HM (229 of 300, or 76.3%). Consequently, stump and slash removal from clear-felled sites had a positive effect on growth of outplanted spruce seedlings but negative effect on their mycorrhization. This suggests that despite positive effects that mycorrhiza is expected to have on seedling viability (Kropp and Langlois 1990; Menkis et al. 2007; Stenström et al. 1990), soil conditions altered due to site disturbance by stump and slash removal might be more favourable for tree growth than more abundant mycorrhization of their root systems in less disturbed soil. Consequently, stump and slash removal from clear-felled sites is likely to increase soil mineralization and hence nutrient availability at the same time decreasing the need for seedlings to support mycorrhizas for nutrient uptake as nutrients are more readily available. Related American study also reported adverse effect of stump removal on ECM colonisation of replanted seedlings, but in contrast to our results, it also reported reduced seedling growth on de-stumped sites (Page-Dumroese et al. 1998).

Differently from the above discussed experiments, other methods of intensive mechanised site preparation involving removal of the forest floor (sieving, scarification, mounding, scalping) were reported to have little impact on abundance of ECM colonisation. However, those treatments might have pronounced impact on ECM community structure by facilitating species that establish on seedlings in the nursery vs. indigenous forest ECMs which dominate on undisturbed sites (Jones et al. 2003; Lazaruk et al. 2008; Pennanen et al. 2005). In the present work, ECM species richness was highest in M treatment (ten species) and lowest in K treatment (three species). Five and four ECM species were detected in HM and HK treatments, respectively. Thus, at the least disturbed site, there was highest richness on ECM species after 4 years. In comparison, abundant colonisation of seedling roots by indigenous ECMs was already observed after the first growing season following plantation establishment on abandoned former agricultural land (Menkis et al. 2007).

The overall ECM community identified from 1200 root tips of *P. abies* seedlings consisted of 13 distinct species (Table 1). Among those *Thelephora terrestris* and *Cenococcum geophilum* were the most common and colonised 27.4% and 26.3% of all seedling roots, respectively. Remaining species all together colonised 26.6% of all seedlings roots (Table 1). *T. terrestris* and *C. geophilum* were the only species detected at different frequencies in all four treatments (Table 1.) The CCA showed 11.1% variation in the ECM community structure that can be attributed to M, K, HK and HM treatments (Fig. 1). Ordination showed that individual plants of each treatment were in close proximity on axis 1, indicating high intraspecific similarity in ECM community structure within the treatments. Larger differences in ECM community structure were notable between different treatments in

Table 1 Frequency of ectomycorrhizal species (percent of plants colonised/percent of mycorrhizal roots colonised) on root tips of *Picea abies* seedlings under different site preparation treatments

Fungal species	GenBank accession no.	Treatments				
		M	K	HM	HK	All
Amphinema byssoides	GU550106	–/–	–/–	–/–	33.3/4.0	8.3/1.0
Boletus edulis	GU550107	–/–	–/–	33.3/17.3	–/–	8.3/4.3
Cadophora finlandica	GU550108	33.3/4.3	33.3/3.0	–/–	–/–	16.6/1.8
Cenococcum geophilum	GU550109	100/15.0	100/27.0	100/23.7	100/39.7	100/26.3
Clavulina cf. *amethystina*	GU550110	–/–	–/–	–/–	33.3/3.3	8.3/0.8
Cortinarius flexipes var. *flabellus*	GU550111	33.3/3.3	–/–	–/–	–/–	8.3/0.8
Elaphomyces muricatus	GU550112	33.3/9.0	–/–	–/–	–/–	8.3/2.3
Lactarius camphoratus	GU550113	33.3/2.7	–/–	–/–	–/–	8.3/0.7
Piloderma fallax	GU550114	33.3/1.0	–/–	–/–	–/–	8.3/0.3
Thelephora terrestris	GU550115	66.6/47.3	100/42.3	33.3/10.0	33.3/10.0	58.3/27.4
Tylospora asterophora	GU550116	33.3/1.0	–/–	–/–	–/–	8.3/0.3
Tylospora fibrillosa	GU550117	33.3/1.7	–/–	–/–	66.6/19.3	25.0/5.3
Wilcoxina sp. Hy16	GU550118	33.3/11.3	–/–	66.6/25.0	–/–	25.0/9.1

M mounding; *K* stump removal; *HM* logging residues removal and mounding; *HK* logging residues removal and stump removal

which samples were more or less well separated from each other (Fig. 1). The latter was also indicated by estimates of qualitative (S_S) Sorensen similarity indices which were moderate varying 0.40–0.57 between the treatments (Fig. 1). In the ordination, vector arrows pointed into virtually different directions showing little or no association between height growth and mycorrhization. As a result, growth was mostly favoured in K, HM and HK treatment while mycorrhization in M treatment. Furthermore, results suggest that ECM species composition was strongly affected by the treatments. Consequently, *C. geophilum* and *Boletus edulis* were more frequent in K, HM and HK treatments. On the other hand, *T. terrestris*, *Cortinarius flexipes var. flabellus*, *Elaphomyces muricatus*, *Tylospora asterophora*, *Piloderma fallax* and *Cadophora finlandica* were more often found in M treatment.

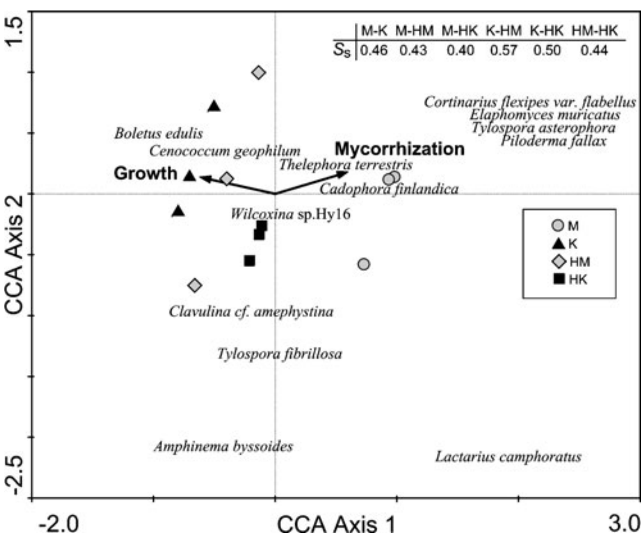

Fig. 1 Ordination diagram based on CCA of ectomycorrhizal communities in roots of *P. abies* seedlings from different site preparation treatments (*M*, *K*, *HM* and *HK*). The diagram shows the part of the variation within the communities (11.1%) that can be attributed to the treatments along CCA axes 1 and 2. *Arrows* indicate the relative importance of environmental variables (plant growth and mycorrhization). *Points* represent individual seedlings of the treatments. *Taxonomic names* correspond to a position in the ordination. Community structure is compared by calculating qualitative (S_S) Sorensen similarity indices

In conclusion, the current work indicated positive impact of stump and slash removal on height increment of replanted seedlings through four subsequent growing seasons, simultaneously revealing the adverse effects on both frequency of ECM colonisation and ECM species richness in seedling roots. However, due to not replicated treatments, one must be aware of limitations of the present study, and obtained results, therefore, should be interpreted with caution. Yet, the work is first of the kind performed in European forests. In order to obtain more comprehensive picture of the processes of forest regeneration on sites extensively disturbed due to harvesting of forest biofuel, more related studies are needed in the region, encompassing wide range of soil conditions and tree species.

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Berglund H, Åström M (2007) Harvest of logging residues and stumps for bioenergy production—effects on soil productivity, carbon budget and species diversity. Report available at: http://www.balticforest.net/index.asp?pg=128&lng=SV

Egnell G, Hyvönen R, Högbom L, Johanson T, Lundmark T, Olsson B, Ring E, von Sydow F (2007) Miljökonsekvenser av stubbskörd—en sammanställning av kunskap och kunskapsbehov. Summary: Environmental aspects of stump-harvest—compilation of knowledge and knowledge gaps. p. 154 Energimyndigheten Rapport ER 2007:40. Statens Energimyndighet, Eskilstuna (In Swedish with English summary)

Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucl Acid S 41:95–98

Hope GD (2007) Changes in soil properties, tree growth, and nutrition over a period of a 10 years after stump removal and scarification on moderately coarse soils in interior British Columbia. Forest Ecol Manag 242:625–635. doi:10.1016/j.foreco.2007.01.072

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422

Kardell L (1992) Vegetationsförändring, plantetablering samt bärproduktion efter stub- och ristäkt. Institutionen för Skoglig Landskapsvård, Rapport 50. Sveriges Lantbruksuniversitet, Uppsala. (In Swedish)

Kardell L (1996) Stubbrytningsförsöken i Piteåtrakten 1979–1990. Institutionen för Skoglig Landskapsvård, Rapport 63. Sveriges Lantbruksuniversitet, Uppsala. (In Swedish)

Kardell L (2007) Vegetationseffekter efter stubbrytning. Analys av några försök 1978–2006. Institutionen för Skoglig Landskapsvård, Rapport 100. Sveriges Lantbruksuniversitet, Uppsala. (In Swedish)

Kardell L (2008) Stubbrytning och schaktning. Skogsenergiförsöken i Vindeln 1979–2004. Institution för skoglig landskapsvård. Report 102. Sveriges Lantbruksuniversitet, Uppsala. (In Swedish)

Kardell L, Eriksson L (2008) Stubbrytningsförsöken i Bergslagen 1977–2007. Institution för skoglig landskapsvård. Report 103. Sveriges Lantbruksuniversitet, Uppsala. (In Swedish)

Koljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166:1063–1068. doi:10.1111/j.1469-8137.2005.01376.x

Kropp BR, Langlois CG (1990) Ectomycorrhizae in reforestation. Can J For Res 20:438–451

Lazaruk LW, Macdonald SE, Kernaghan G (2008) The effect of mechanical site preparation on ectomycorrhizae of planted white spruce seedlings in conifer-dominated boreal mixedwood forest. Can J For Res 38:2072–2079. doi:10.1139/X08-035

Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton

Menkis A, Vasiliauskas R, Taylor AFS, Stenlid J, Finlay R (2005) Fungal communities in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation systems, assessed by morphotyping, direct sequencing and mycelial isolation. Mycorrhiza 16:33–41. doi:10.1007/s00572-005-0011-z

Menkis A, Vasiliauskas R, Taylor AFS, Stenlid J, Finlay R (2007) Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi—impact on plant performance and ectomycorrhizal community. Mycorrhiza 17:337–348. doi:10.1007/s00572-007-0110-0

Metsätilastollinen Vuosikirja (2009) (Finnish Statistical Yearbook of Forestry 2009). Report: Finnish Forest Research Institute, Vantaa, Finland

Mokma DL, Yli-Halla M (2000) Keys to soil taxonomy for Finland. USDA Natural Resources Conservation Service, Washington, DC

Morrison D, Merler H, Norris D (1991) Detection, recognition and management of Armillaria and Phellinus root diseases in the Southern Interior of British Columbia. Canada—British Columbia Partnership Agreement on Forest Resource Development: FRDA II, Report 179, Victoria, British Columbia

Page-Dumroese DS, Harvey AE, Jurgensen MF, Amaranthus MP (1998) Impacts of soil compaction and tree stump removal on soil properties and outplanted seedlings in northern Idaho, USA. Can J Soil Sci 78:29–34

Pennanen T, Heiskanen J, Korkama U (2005) Dynamics of ectomycorrhizal fungi and growth of Norway spruce seedlings after planting on a mounded forest clearcut. Forest Ecol Manag 213:243–252. doi:10.1016/j.foreco.2005.03.044

Pfleger FL, Lingerman RG (eds) (1994) Mycorrhizae and plant health. APS, St Paul

Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) (1996) Mycorrhizas in ecosystems. CAB, Wallingford

Simard SW, Jones MD, Durall DM, Hope GD, Stathers RJ, Sorensen NS, Zimonick BJ (2003) Chemical and mechanical site preparation: effects on *Pinus contorta* growth, physiology, and microsite quality on steep forest sites in British Columbia. Can J For Res 33:1495–1515

Stenström E, Ek M, Unestam T (1990) Variation in field response of *Pinus sylvestris* to nursery inoculation with four different ectomycorrhizal fungi. Can J For Res 20:1796–1803

ter Braak CJF, Smilauer P (1998) Canoco reference manual and user's guide to Canoco for Windows: software for canonical community ordination, Version 4. Microcomputer Power, Ithaca

Vasaitis R, Stenlid J, Thomsen IM, Barklund P, Dahlberg A (2008) Stump removal to control root rot in forest stands. A literature review. Silva Fenn 42:457–483