Low Impact of Stump Removal on Mycorrhization and Growth of Replanted *Picea abies*: Data from Three Types of Hemiboreal Forest

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Abstract

The objective was to investigate the impact of stump removal from clear-cuts on early mycorrhizal colonisation, seedling growth, and chemical properties of soil and needles of replanted *Picea abies*. The study included three forest types differing in soil conditions: *Hylocomiosa* (H), *Myrtillo-sphagnosa* (MS) and *Myrtillosa-mel.* (MM) forest types. Soil characteristic for H forest type is well-aerated dry podzol, while MS and MM comprise poorly-aerated gleyic, and respectively, wet and drained (dry) podzols. The clear-cuts were made in winter 2010-2011, stump removal accomplished during late spring – early autumn 2011, and the plantations established in April 2012. Prior to planting, each clear-cut was divided into discrete plots, subjected either to stump removal or, alternatively, to traditional soil preparation by disc trenching. After one growing season (in autumn 2012), seedling mycorrhization, shoot and root morphological parameters (length of the shoot, root collar diameter, mass of new roots), and chemical composition of needles and rhizosphere soil were determined. Seedling mycorrhization, chemical composition of needles and soil did not differ significantly between stump removal and trenching plots in any of the forest types. Also richness of mycorrhizal morphotypes and communities of root inhabiting fungi were similar. Fungi commonly detected in forest nurseries dominated fungal communities in roots, among which *Thelephora terrestris* was the most abundant composing 55.3 %. In each of the forest types, shoot length, root collar diameter and mass of new roots were either higher on stump removal plots or did not differ significantly. The only exception in this respect was the higher shoot height observed on trenching plots in H forest type. In conclusion, the study demonstrated that after one growing season stump removal has low or no impact on communities of mycorrhizal (and other soil) fungi, and performance of replanted *P. abies* seedlings on podzols with different mechanical composition, aeration and moisture.

Keywords: clear-cut, forest management, mycorrhiza, Norway spruce, reforestation, soil fungi, stump removal.

Introduction

In Latvia, the growing demand for biofuel makes the stump removal an attractive option to secure its supply. However, the potential effects of stump removal on below ground fungal communities, in particular ectomycorrhizal (ECM) ones, are generally poorly understood. ECM fungi in boreal forests enhance nutrient and water uptake to their hosts (Smith and Read 1997). Due to these effects, ECM fungi may promote establishment and growth of tree seedlings after their outplanting (Kropp et al. 1985, Menkis et al. 2007, Menkis et al. 2012). However, as silvicultural practices may affect communities of ECM fungi (Jones et al. 2003, Heinonsalo et al. 2004), the impact of stump removal in this respect should also be evaluated. For example, Pennanen et al. (2005) showed that even light soil preparation such as mounding can cause changes in the structure of ECM community. To date, only few studies investigated the impact of stump removal on belowground fungal communities associated with seedling roots (e.g. Page-Dumroese et al. 1998, Menkis et al. 2010, Kataja-aho et al. 2012); therefore, more studies are needed.

Stump removal may also impact forest regeneration on clear-cuts. Although the majority of available studies indicate improved seedling establishment and growth on stump removal sites, especially on dry and sandy soils (Vasaitis et al. 2008 and references therein), some studies indicate that stump harvesting may lead to increased biomass removal, which may result in a significant loss of nutrients, leading to potentially negative effects on future site productivity (Palviainen et al. 2010, Egnell 2011). Besides, stump removal in certain cases is associated with site disturbances, which may lead to alterations in soil chemical, physical and biological properties (Hope 2007). Therefore, in this respect more studies are needed, especially covering broader spectra of forest types and geographical regions.

The objective of the present study was to evaluate impact of stump removal on mycorrhization and growth of *Picea abies* seedlings one growing season after their outplanting on clear-cuts of *Hylocomiosa* (H), *Myrtillo-sphagnosa* (MS) and *Myrtillosa-mel.* (MM) forest types in Latvia, comprised of podzol soils differing in mechanical composition, aeration and moisture.

Materials and methods

Study sites and experimental design

The study sites comprised three forest clear-cuts (3.8 ha, 2.7 ha and 1.5 ha in size or 8 ha altogether) situated in central Latvia (Table 1). Each site represented different forest type including *Hylocomiosa* (H), *Myrtillo-sphagnosa* (MS) or *Myrtillosa-mel.* (MM). Characteristic for H forest type is well-aerated dry podzol, while MS and MM comprise poorly-aerated gleyic, respectively wet and drained (dry) podzols (Bušs 1997). In the area, the average air temperature during the survey period (growing season 2012) was 6.1 ºC and precipitation amounted 832 mm (Latvian Environment, Geology and Meteorology Centre), which in comparison were close to typical values.

In all sites, stands were clear-felled in winter 2010/2011 but stumps were left intact. Before planting in spring 2012, in each site stumps were removed from half of the area using a New Holland tractor (v. 5215B) equipped with a stump extractor MCR-500 (LSFRI "Silava" and Orvi Ltd., Salaspils, Latvia). For another half of each site, disc trenching was used as a soil preparation treatment using two-row disc trencher TTS Delta (LSFRI "Silava", Salaspils, Latvia). Distance between trenching rows was ca. 2.5 m. Trenching is a common forest soil scarification method in Latvia (Lazdiņš 2012). In the MS and H forest types, soil preparations (stump removal and trenching) were done in two replicates while in the MM type the treatments were not replicated. As a result, ten plots were established. Within each clear-cut, different treatments and replicates were separated from each other by a 20 m buffer zone.

Two-year-old containerized seedlings of *P. abies* (local forest nursery, Norupe, Latvia) were planted in April 2012. Seedlings were planted in rows at a density between ca. 918 and 2,773 seedlings per hectare. At outplanting, seedlings were ca. 12 cm in height (Lazdiņš 2012). Prior to planting, ECM status of the roots was not examined, but was assumed to be similar for all seedlings due to similar

Table 1. Position and characteristics of three clear-cuts reforested with *Picea abies*. The soil in each clear-cut was prepared using stump removal and disc trenching

Forest type of a clear cut	Position	Soil preparation treatment	Reforested area (ha)	No. of seedlings planted	No. of plants sampled	No. of root tips sampled	Former forest stand
Hylocomiosa (H) Dry podzolic mineral soil of good aeration	N56°53', E24°41'	Stump removal	0.95	1474	20	2000	Norway spruce, 60%, Scots pine, 20%, silver birch, 20%, common aspen and black alder in admixture. Age is 80 years
		Stump removal	0.95	1950	20	2000	
		Trenching	0.95	1837	20	2000	
		Trenching	0.95	1383	20	2000	
All H			3.8	6644	80	8000	
Myrtillo-sphagnosa (MS) Wet podzols or gleyic mineral soil of poor aeration	N56°51', E24°38'	Stump removal	0.675	620	20	2000	Scots pine, 70%, Norway spruce, 30%. Age is 100 years
		Stump removal	0.675	1257	20	2000	
		Trenching	0.675	714	20	2000	
		Trenching	0.675	807	20	2000	
All MS			2.7	3398	80	8000	
Myrtillosa mel. (MM) Drained gleyic mineral soil of poor aeration	N56°53', E24°39'	Stump removal	0.75	1760	20	2000	Norway spruce, 70%, Scots pine, 20 %, silver birch, 10%, common aspen and black alder in admixture. Age is 80 years
		Trenching	0.75	2080	20	2000	
All MM			1.5	3840	40	4000	
All clear-cuts			8.0	13882	200	20000	

growing conditions in the nursery. Previously, ECM status of seedling roots was shown to be similar in similar nursery cultivation systems (Menkis et al. 2005, Menkis et al. 2011).

Seedling sampling and measurements

In October 2012, a half-year after planting, 20 Norway spruce seedlings were collected from each of ten plots, resulting in 200 sampled seedlings altogether (Table 1). In each plot, seedlings were sampled along the diagonal in a consecutive order. Seedlings were carefully excavated to preserve the fine roots, labelled, placed in plastic bags, transported to laboratory and kept at 4 °C for a maximum period of two weeks before further processing. For each seedling, total length of the present-year top shoot and root collar diameter was measured. Shoots were separated from the roots, dried for 12 h at 60 ºC and weighted. Roots of each seedling were washed in tap water and separated in two categories: roots originally present in a forest nursery container ("old roots" present in a peat lump); and, current year roots ("new roots") produced during the growing season 2012 (growing out from the peat lump). Old roots were directly placed into the oven and dried for 12 h at 60 ºC. New roots were stored at 4ºC for analysis of ECMs. Following analysis of ECMs (see below), different morphological parameters of these roots (root length, volume, biomass, surface area, number of root tips) were determined by using Epson Perfection V750Pro scanner (Epson, Tokyo, Japan) and WinRHIZO 2005 C (Regent instruments Inc., Canada) software. After scanning roots were dried for 12 h at 60 ºC and weighted.

Analysis of ectomycorrhizas

To evaluate mycorrhization of roots, new outgrown roots were placed in glass Petri dishes (14 cm in diameter) with a grid on the bottom (mesh size 7×7 mm) and examined using stereomicroscope (Leica MZ-7.5, Solms, Germany). Root tips in vicinity to the crossing points of the grid were systematically sampled. In total, 100 single root tips were sampled from each root system and their mycorrhization and ECM morphotypes were determined. ECM roots were identified by the presence of mantle, external hyphae or rhizomorphs and the absence of root hairs. ECM roots were divided into different morphotypes based on their colour, form and texture of the mantle and pattern of rhizomorphs and/or external mycelia (Agerer 1986-2006).

One to six root tips of each distinct ECM morphotype were sampled from each of 17 randomly selected seedlings of each sampling plot (170 seedlings altogether) and used for molecular identification of fungal taxa. Selected root tips were separately placed in 1.5 ml centrifugation tubes and stored at -20 ºC until used for molecular analyses. Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Inc., USA) was used for Direct PCR from root tips (Velmala et al. 2014). PCR amplification was performed using fungal-specific primer fITS7 (Ihrmark et al. 2012) and universal primer ITS4 (White et al. 1990), and Phire Hot Start II DNA polymerase (Thermo Fisher Scientific, Inc., USA). Sequencing was performed in one direction using ITS4 primer by Macrogen Europe Inc. (Amsterdam, Netherlands). Raw sequence data were analysed using the SeqMan version 5.07 software from DNASTAR package (DNASTAR, Madison, WI, USA). Databases at GenBank (Altschul et al. 1997) and UNITE (Kõljalg et al. 2005) were used to determine the identity of ITS rRNA sequences. The criteria used for identification were the following: sequence coverage > 80 %; similarity to taxon level 97–100 %, similarity to genus level 94–96 %. The sequences are available from GenBank under accession numbers KF954060-KF954092.

Chemical analysis of needles and soil

Soil samples used for chemical analyses comprised forest soil attached to seedling roots produced during the growing season 2012 (new outgrown roots). Substrate from the nursery was excluded. In total, 20 soil samples were collected from each of ten plots (see Table 1) and within each plot pooled together resulting in ten bulk samples. Soil was dried for a week at room temperature (ca. 21 ºC) and sieved (mesh size 2×2 mm) to separate larger fractions and roots which were discarded. Electrical conductivity (EC), pH and concentration of K, Ca, Mg, Zn, Mn, N, P, C and S were determined.

Needles were separated from the oven dried shoots. From each seedling, 1 g of dry needles was taken and within each plot all sampled needles were pooled together resulting in ten bulk samples. Sampled needles were oven-dried at 60 °C for two weeks and then finely grounded using the A-11 analytical mill (IKA-Werke GmbH, Staufen, Germany), and concentration of K, Ca, Mg, Zn, Mn, P, N, C, and S was determined. Chemical analyses of soil and needles were done using established standard methods at LSFRI "Silava", the Laboratory of Forest Environment, Salaspils, Latvia.

Statistical analyses

Differences in seedlings morphological parameters, mycorrhization, richness of ECM morphotypes, chemical composition of needles and soil between different treatments of each study site were analysed by one-way analysis of variance (ANOVA) and Tukey's test, which provides confidence intervals for all pairwise differences between means (Chalmers and Parker 1989, Fowler et al. 1998). The statistics were computed using Minitab® v.16 (Minitab Inc., Coventry, UK). Richness of fungal taxa detected by ITS rRNA sequencing in different treatments of each site was compared by using nonparametric Chi-

square test (Mead and Curnow 1983). As root tips used for ITS rRNA sequencing were pre-selected during the morphotyping, the data from different replicates within each treatment and site were pooled and analysed together. Shannon diversity index and quantitative Sorensen similarity index were used to characterise diversity and composition of fungal communities in different treatments and sites (Shannon 1948, Magurran 1988). Fungal community structure and possible treatment effects were analysed using Principal Component Analysis (PCA) in CANOCO 4.5 (ter Braak and Smilauer 1998).

Results and Discussion

Results from our previous work (Menkis et al. 2007) demonstrated that already early mycorrhizal status has a profound long-term effect on growth of *P. abies* seedlings outplanted on mineral soil. The results of the present study highlight that stump removal, as compared with traditional trenching, has no effect on early mycorrhization of *P. abies* in forest types characterized by podzols differing in aeration and moisture ($p > 0.07$). Mycorrhization in the H-type was 84.0 % ± 2.1SE in stump removal treatment and 87.1 %±1.4SE in trenching treatment, in the MS-type amounted 81.7 % ± 1.7SE and 76.9 % ± 2.0SE, respectively, and in the MM-type made up 81.1 % ± 3.6SE and 80.4 % ± 3.4SE, respectively. Furthermore, richness of ECM morphotypes in each investigated forest type did not differ significantly between stump removal and trenching treatments ($p > 0.09$), which in the H-type was 3.3±0.1SE in stump removal treatment and 3.2 ± 0.1SE in trenching treatment, in the MS-type made 3.3 ± 0.1SE and 3.5 ± 0.1SE, respectively, and in the MM-type reached 2.4 ± 0.2SE and 2.9 ± 0.1SE, respectively. Amplification and direct sequencing of fungal ITS rRNA from ECM morphotypes showed that the number of fungal taxa in the H-type was 13 in stump removal treatment and 16 in trenching treatment, in the MS-type amounted to 14 and 19, respectively, and in the MM-type figured up to 7 and 8 (Table 2) , respectively. Consequently, richness of fungal taxa in each investigated forest type did not differ significantly between stump removal and trenching treatments ($p > 0.29$). The total fungal community was comprised of 33 taxa, among which 15 (45.5 %) were Ascomycetes and 18 (54.5 %) belonged to Basidiomycetes (Table 2). Among all fungi, 20 (60.6 %) were identified to taxon level, 11 (33.3 %) to genus level and 2 (6.1 %) remained unidentified. The most common taxa were *Thelephora terrestris* (55.3 %), *Wilcoxina* sp.1 (12.3 %), *Acephala macrosclerotiorum* (4.7 %), *Cenococcum geophilum* (4.0 %) and *Amphinema byssoides* (3.6 %) (Table 2). Results showed that in different treatments of each forest type the richness of ECM morphotypes and communities of root inhabiting fungi (in particular dominant) were largely the same and resembled fungi from the nursery (Table 2) (Menkis et al. 2005, Stenström et al. 2014). Such characteristic nursery fungi (e.g. *Thelephora terrestris*), which are also known to be better adapted to the specific conditions of the nursery (frequent disturbance and high amounts of mineral nutrients) (Marx et al. 1984), were likely benefiting from the alterations of soil properties as a result of site disturbance (Hope 2007). However, such effect is likely to be temporal as in the later years these fungi are gradually replaced by the indigenous ECM fungi present at a forest site (Menkis et al. 2007, Klavina et al. 2013). In the present study, *T. terrestris* dominated fungal communities in roots, showing higher abundances in stump removal treatments than in corresponding trenching treatments, while the remaining taxa altogether were less abundant that *T. terrestris* (Table 2).

Among all taxa, eight of them were exclusively present in stump removal treatments, twelve of them occurred in trenching treatments and thirteen of them were common to both treatments (Table 2). The Shannon diversity index of fungal communities in different treatments and study sites was between 1.01 and 2.39 (Table 2). The quantitative Sørensen similarity index of fungal communities between stump removal treatment and trenching treatment was 0.41 in the H-type, 0.61 in the MS-type and 0.67 in the MM-type (data not shown). The principal component analysis (PCA) of fungal communities explained 76.1 % variation on Axis 1 and 10.7 % variation on Axis 2. PCA showed that stump removal and trenching treatments were in close proximity on Axis 1 (which explained most of the variation and the treatment effect on fungal communities) for the H-type and the MS-type but slightly more distant for the MM-type (Figure 1). In PCA, different forest types (the H, MS and MM types) were more or less well separated from each other (Figure 1). In the present study, the majority of the dominant fungi were often shared between different treatments of each forest type (Table 2). As a result, PCA showed a rather proximal aggregation on Axis 1 of both treatments within each MS- and H-types at the same time showing that stump removal had little impact on fungal community structure (Figure 1). A more distal placement of different treatments within the MM-type was largely determined by abundance of *T. terrestris* while composition of fungal communities was similar (Table 2). PCA also showed certain specificity of fungal communities present at each forest type as these were more or less well separated from each other (Figure 1) thereby indicating the relative importance of rare taxa (probably indigenous) present at each site. In PCA, Axis 1 and Axis 2 regression coefficients for the three most common fungi were the following: *T. terrestris* 25.9 and -3.2, respectively, *Wilcoxina* sp. 1 -4.4 and -20.5, respectively, and *A. macrosclerotiorum* 6.9 and -15.9, respectively. Quantitative Sørensen simi-

Table 2. Frequency of fungal taxa shown as percentage of ECM roots colonised of *Picea abies* seedlings under stump removal and trenching site preparation treatments. The number of ECM root tips used is shown in the parentheses

Taxa	Genbank accession No.	*Hylocomiosa* (H)		*Myrtilliosa-sphagnosa* (MS)		*Myrtilliosa mel* (MM)		Total
		Stump removal (101)	Trenching (134)	Stump removal (171)	Trenching (169)	Stump removal (63)	Trenching (44)	(682)
Ascomycetes								
Acephala macrosclerotiorum	KF954060	3.0	4.2	7.1	4.1	-	6.9	4.7
Cadophora sp.	KF954063	0.1	-	-	0.2	-	-	0.1
Cenococcum geophilum	KF954064	2.6	5.1	3.3	0.5	1.8	19.4	4.0
Elaphomyces sp.	KF954066	0.5	-	-	-	-	-	0.1
Hydnotrya bailii	KF954069	-	-	-	0.8	-	-	0.2
Ilyonectria radicicola	KF954082	-	2.0	-	-	-	-	0.3
Meliniomyces bicolor	KF954070	1.7	0.6	3.4	0.1	0.6	14.7	2.5
Meliniomyces sp.	KF954071	0.8	-	3.1	0.9	-	-	1.2
Meliniomyces variabilis	KF954084	-	-	2.4	-	-	-	0.7
Phialocephala fortinii	KF954086	-	4.7	0.4	2.2	4.7	0.5	1.9
Tuber sp.	KF954075	-	-	-	1.2	-	-	0.3
Wilcoxina mikolae	KF954078	-	5.9	-	-	-	-	1.0
Wilcoxina sp.1	KF954079	24.7	34.8	1.6	4.1	-	20.9	12.3
Wilcoxina sp.2	KF954080	-	2.1	-	2.2	0.6	-	0.9
Unidentified sp. A	KF954077	-	0.5	-	-	-	-	0.1
All Ascomycetes		33.5	59.9	21.4	16.1	7.7	62.3	30.2
Basidiomycetes								
Amphinema byssoides	KF954061	10.7	1.5	2.6	0.1	0.4	13.0	3.6
Amphinema sp. 1	KF954062	0.1	-	3.0	3.6	-	0.5	1.8
Amphinema sp. 2	KF954081	-	-	-	1.8	-	-	0.4
Clavulina sp.	KF954065	-	6.3	-	9.5	-	-	3.2
Cryptococcus magnus	KF954092	10.6	-	-	-	-	-	1.5
Hebeloma sp.	KF954068	-	0.6	-	0.2	-	-	0.2
Inocybe napipes	KF954083	-	-	0.3	-	-	-	0.1
Paxillus involutus	KF954085	-	-	2.2	0.4	-	-	0.7
Russula cf. emetica	KF954087	-	0.4	-	-	-	-	0.1
Russula densifolia	KF954088	0.4	-	-	-	-	-	0.1
Russula cf. firmula	KF954089	-	0.2	-	-	-	-	0.04
Russula cf. velenovskyi	KF954072	-	-	-	2.8	-	-	0.6
Sebacina sp.	KF954073	8.2	-	-	-	-	-	1.1
Thelephora terrestris	KF954074	36.5	30.4	69.4	62.9	91.1	24.2	55.3
Tomentella stuposa	KF954076	-	-	0.2	-	-	-	0.1
Tylospora asterophora	KF954090	-	0.6	-	-	-	-	0.1
Tylospora fibrillosa	KF954091	-	-	-	2.6	0.8	-	0.7
Unidentified sp. B	KF954067	-	-	1.0	-	-	-	0.3
All Basidiomycetes		66.5	40.1	78.6	83.9	92.3	37.7	69.8
Total no. of taxa		13	16	14	19	7	8	33
Shannon diversity index		2.15	2.39	1.95	2.17	1.01	1.64	

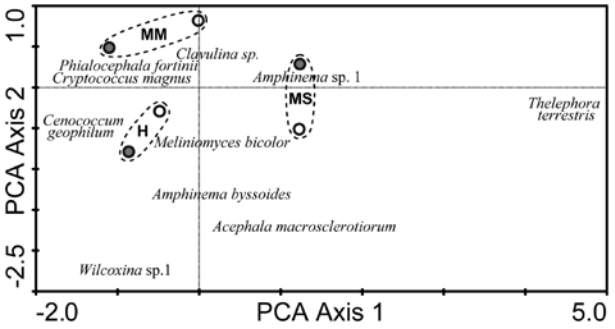

Figure 1. Ordination diagram based on a principal component analysis of fungal communities in roots of *Picea abies* seedlings outplanted on *Hylocomiosa* (H), *Myrtillo-sphagnosa* (MS) and *Myrtillosa mel.* (MM) forest types in which soil was prepared using stump removal (open circles) and disc trenching (filled circles). Taxonomic names correspond to a position (centred) in the ordination and represent ten most common taxa of the present study

larity indices were between moderate and high, repeatedly suggesting that fungal communities detected in this study were similar and thus generally unaffected by the stump removal treatment. The Shannon diversity indices of fungal communities were relatively low (Table 2) and of magnitude reported from forest nurseries (Flykt et al. 2008, Stenström et al. 2014), indicating that diversity of fungal taxa remained largely unchanged and thus generally unaffected by the stump removal treatment.

Observed non-significant differences in mycorrhization were probably one of the reasons of low differences in seedling growth. Yet in a number of cases seedling morphological parameters in stump removal treatments were either significantly higher or did not differ significantly from those in corresponding trenching treatments in each of investigated forest types (Table 3). A single exception was seedling shoot height at the H-type, which was significantly higher in trenching treatment than in stump removal treatment. The latter demonstrated that stump removal in comparison to conventional trenching treatment on each of investigated forest types had in general low but sometimes slightly positive impact on growth of *P. abies* seedlings. Such initial impact appears to be lasting since similarly better growth of the trees has also been observed 3 – 28 years after their outplanting (Kardell 2008, Kardell and Eriksson 2008, Menkis et al. 2010, Kataja-aho et al. 2012). However, Egnell and Leijon (1999) reported that after 15 years the height increment of *P. abies* was lower following the whole-tree harvesting. This could probably be explained by natural variation imposed by specific conditions present at different forest types. For example, in the present study only shoot height of the seedlings was

Table 3. *Picea abies* seedling morphological parameters after first growing season (2012) in *Hylocomiosa, Myrtillo-sphagnosa*, *Myrtillosa mel.* forest types in which soil was prepared using stump removal and disc trenching. Values are shown as the mean ± *SE* (standard error)

Morphological Parameter	*Hylocomiosa* (H)		*Myrtillo-sphagnosa* (MS)		*Myrtillosa mel.* (MM)	
	Stump removal (n=40)	Trenching (n=40)	Stump removal (n=40)	Trenching (n=40)	Stump removal (n=20)	Trenching (n=20)
Shoot height, cm	26.3±0.9	29.1±0.8*	28.4±0.8	29.3±0.6	28.0±1.3	31.2±1.3
Shoot biomass, g	7.3±0.5	7.1±0.3	6.9±0.3	6.4±0.3	7.4±0.5	7.0±0.5
Root collar diameter, cm	0.7±0.01**	0.6±0.02	0.6±0.02	0.6±0.01	0.7±0.03	0.6±0.02
Old root biomass, g [a]	3.1±0.2	2.9±0.1	3.3±0.2**	2.6±0.1	3.1±0.3	2.6±0.2
New root biomass, g [b]	0.9±0.1	0.8±0.1	0.7±0.1*	0.5±0.1	0.7±0.1*	0.4±0.1
Total root biomass, g [a+b]	4.0±0.2	3.6±0.2	4.0±0.2**	2.9±0.2	3.8±0.3*	3.0±0.2
Root length, cm [b]	1108.8±89	1035.9±68	1036.5±75*	835.4±51	954.0±107	723.6±91
Root surface area, cm^2 [b]	230.0±20	214.4±16	196.3±17	162.7±11	203.8±24*	134.7±18
Root volume, cm^3 [b]	3.9±0.4	3.6±0.3	3.0±0.3	2.6±0.2	3.5±0.5*	2.0±0.3
Number of root tips [b]	2877±231	3398±200	2612±156	2289±158	2349±295	1913±246
Mycorrhization, % [b]	84.0±2.1	87.1±1.4	81.7±1.7	76.9±2.0	81.1±3.6	80.4±3.4

Significantly greater at: * - $p < 0.05$; ** - $p < 0.01$.

[a] Roots produced during seedling cultivation in a forest nursery.

[b] Roots produced after seedling outplanting on a clear-cut (growing season 2012).

significantly lower in stump removal treatment as compared to trenching treatment of the H-type, but not in the MS- or MM-types where it did not differ significantly between the treatments of these forest types (Table 3). Root collar diameter, by contrast, was significantly higher in stump removal treatment as compared to trenching treatment of the H-type while the remaining morphological parameters did not differ significantly between those treatments (Table 3).

Despite some slight variations, the amounts of respective chemical elements in either needles or soil did not differ significantly among the samples from stump removal vs. trenching treatments of each investigated forest type (Table 4), suggesting that the alterations in soil physical properties following stump removal can be the cause of the observed root growth responses in *P. abies* seedlings (Table 3). On the other hand, significantly higher values of new root biomass, root length, root surface area and root volume observed in stump removal plots of the MS- and/or MM-types might well be influenced by the fact that old root biomass on those plots was higher than in trenching plots (Table 3).

Conclusions

The study demonstrated that stump removal on clear-felled forest sites representing forest site types comprising podzol soils of wide range of moisture and aeration had in general low but sometimes slightly positive impact on growth of replanted *P. abies* seedlings. At the same time, seedling mycorrhization and communities of root inhabiting fungi remained largely unchanged and therefore generally unaffected by the stump removal treatment.

Table 4. Amounts of different chemical elements in needles of *Picea abies* and soil after the first growing season (2012) in *Hylocomiosa*, *Myrtillo-sphagnosa*, and *Myrtillosa-mel.* forest types in which soil was prepared using stump removal and disc trenching. Values are shown as the mean ± SE (standard error)

Chemical element	*Hylocomiosa* (H)		*Myrtillo-sphagnosa* (MS)		*Myrtillosa mel.*(MM)	
	Stump removal	Trenching	Stump removal	Trenching	Stump removal	Trenching
Needles						
N, g/kg	22.5±0.5	21.5±0.5	23.0±1.0	21.0±3.0	25.0	23.0
C, g/kg	529.5±1.5	551.0±12.0	532.0±9.0	554.0±6.0	540.0	549.0
S, g/kg	1.15±0.1	0.9±0.1	1.05±0.1	0.95±0.1	1.0	0.9
P, g/kg	1.3±0.1	1.2±0.1	1.3±0.1	1.2±0.1	1.4	1.2
Ca, g/kg	4.4±0.5	3.7±0.1	3.4±0.2	3.4±0.7	3.4	3.4
K, g/kg	6.5±0.2	6.2±0.1	7.3±0.3	6.6±0.4	7.3	5.9
Mg, g/kg	1.8±0.1	1.8±0.1	1.7±0.1	1.6±0.1	1.5	1.6
Mn, g/kg	0.5±0.1	0.25±0.1	0.3±0.1	0.25±0.1	0.4	0.4
Fe, g/kg	0.4±0.2	0.35±0.1	0.3±0.1	0.3±0.1	0.3	0.4
Soil						
Absolute moisture of dry soil, %	3.1±0.1	4.1±2.4	6.2±2.2	7.3±2.4	2.0	3.3
pH(KCl)	3.5±0.1	4.0±0.3	3.0±0.1	3.1±0.3	3.2	3.3
EC, μS cm^{-1}	288.5±36.5	262.0±74.0	331.0±11.0	365.0±104.0	173	380
N, g/kg	3.2±0.3	2.8±1.3	5.1±1.8	5.8±1.4	2.3	3.7
C, g/kg	84.5±12.5	72.0±20.0	147.0±84.0	147.5±55.5	156.0	337.0
S, mg/kg	210.0±12.0	155.5±55.5	346.0±220.0	314.0±106.0	357.0	744.0
P, mg/kg	101.5±57.5	127.5±33.5	49.5±4.5	44.5±15.5	21.0	39.0
K, mg/kg	67.0±11.0	66.0±6.0	133.5±56.5	122.5±3.5	139.0	196.0
Mn, mg/kg	29.4±9.8	54.8±28.0	7.3±1.2	29.0±23.9	15.9	13.2
Mg, g/kg	0.12±0.1	0.19±0.1	0.17±0.1	0.17±0.04	0.2	0.3
Ca, g/kg	0.48±0.2	1.29±1.2	1.0±0.7	1.0±0.7	0.7	0.8
Zn, mg/kg	6.6±0.2	4.5±0.6	9.3±2.8	4.7±1.8	14.1	15.2

Note: In the MM, only one replicate was available.

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