Fungal Communities in Roots of Scots Pine and Norway Spruce Saplings Grown for 10 Years on Peat Soils Fertilized with Wood Ash

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Abstract

The study was conducted in Scots pine (*Pinus sylvestris* L.) or Norway spruce (*Picea abies* (L.) H. Karst.) plots on peat soils, where fertilization trials with 0.5 to 2 kg m^{-2} wood ash had been conducted ten years previously. We examined the long-term effects of wood ash fertilization on fine root development (biomass and mycorrhizal colonization) and on communities of ectomycorrhizal (ECM) fungi and other soil microorganisms. Soil microorganisms were assessed by recording the number of colony forming units (CFU) from soil dilution series. ECM fungi in fine roots were evaluated by morphotyping and identified by sequencing of the fungal ITS region of rRNA genes. Soil chemical analysis indicated significant differences in soil pH between control and fertilized Scots pine plots, but soil pH did not differ among Norway spruce plots. Fine root biomass, numbers of living fine roots and numbers of dead fine roots did not differ significantly among wood ash treatments and the control for either pine or spruce. Relative abundance of living fine roots and its mycorrhizal colonization differed significantly among treatments and effects of wood ash were largely determined by tree species and amount of wood ash applied. Numbers of bacterial CFUs were higher in pine plots than in spruce plots and some differences were evident between the wood ash treatments and the control. Numbers of fungal CFUs were similar in all treatments. Of 21 fungal species identified on fine roots, the most common were *Amphinema byssoides*, *Agaricomycetes* sp., *Lactarius* sp. and *Tuber anniae*. Species composition of ECM fungi was mainly determined by the host species. However, principal component analysis and comparison of relative abundances of some species indicated differences in species composition among wood ash treatments. In conclusion, our data provided some evidence of a long-term effect of wood ash fertilization on soil pH, abundance of soil bacteria and diversity of ECM fungal community.

Key words: forest fertilization; liming; organic soils; fine root; ectomycorrhizal fungi, *Pinus sylvestris*, *Picea abies*

Introduction

Application of wood ash as a fertilizer can improve wood production and nutrient balance in managed forests (Aronsson and Ekelund 2004). Long-lasting positive effects of wood ash fertilization have been observed on shallow peat soils, rich in nitrogen (Hytönen 2003, Aronsson and Ekelund 2004) and on drained peatlands (Ferm et al. 1992, Moilanen et al. 2002, 2004, Haveraaen 2014, Rütting et al. 2014).

Most of the studies that have evaluated root parameters after wood ash treatment have been conducted on mineral soils (e.g. Taylor and Finlay 2003, Majdi and Viebke 2004) and only some have studied the effects on deep peat soils (e.g. Kakei and Clifford 2002). The effect of wood ash fertilizer on fine root development largely depends on the amount applied (Clemensson-Lindell and Persson 1995, Majdi and Viebke 2004, Augusto et al. 2008). The greatest adverse ecological effect of wood ash fertilization has been reported in acidophilic ecosystems, particularly regarding bryophytes, soil bacteria and ectomycorrhizal (ECM) fungal communities (Pitman 2006, Augusto et al. 2008).

Differences in ECM fungal community composition after liming have been explained by the increased forest soil capacity to neutralize soil acidity (Andersson and

Söderström 1995, Erland and Taylor 2002, Kjøller and Clemmensen 2009). Most ECM fungal species are sensitive to high pH (Hung and Trappe 1983) and liming of peat soil may reduce growth of ECM fungal mycelium of some species as e.g. *Piloderma* spp. (Erland et al. 1990). Recent study by Klavina et al. (2015) has demonstrated long-term effect of forest fertilization with wood ash on ECM fungal community. However, how different amounts of wood ash applied affects ECM community structure and species diversity associated with different tree species on peat soils is still poorly understood.

The aim of this study was to evaluate long-term effects of wood ash fertilization on fine root biomass and mycorrhizal colonization of Norway spruce and Scots pine and on communities of ECM fungi and other soil microorganisms.

Material and methods

Study area and experimental design

The study was conducted in experimental plots that were established in 2004 and 2005 in the eastern part of Latvia at the Forest Research station in the Kalsnava forest district (area is 0.1 ha, coordinates: 56º42,937'N, 25º50,834'E). The treatments were established in a drained grass fen with 15-year-old Norway spruce (*Picea abies* (L.) H. Karst.) at low density (Gaitnieks et al. 2005). The forest type was *Myrtillosa mel*. according to the Latvian classification (Bušs 1997). The soil at the start of the experiment was classified as a woody grass fen peat; the degree of decomposition was 47 % (Gaitnieks et al. 2005).

In May 2004, 2-year-old spruce and 1-year-old pine containerized seedlings were planted in rows in 2.7 m^2 square plots. Within each plot, seedlings were established at a 0.5×0.5 m spacing. In each plot, soil was prepared manually (the understory plants were removed and the top soil layer was turned over) and between 70 and 96 seedlings were planted (the density of seedlings was reduced after the first growing season by excavating between 30 and 40 seedlings in each plot for analyses). Wood ash was evenly dispersed over the soil surface a year prior to planting of seedlings. Two wood ash treatments were used, 0.6 kg m^{-2} and 1.2 kg m^{-2}, with replication of three replicate plots for pine and six for spruce. In addition, trials with pine seedlings and wood ash fertilization treatments of 0.5, 1.0, 1.5 and 2 kg m^{-2} directly after planting were established at the same time in four replicate plots, which were randomly arranged in 4 blocks. This study design resulted in 18 plots with Norway spruce and in 28 plots with Scots pine (Figure 1).

The plots were evaluated in June 2014. Height and stem diameter (dbh) were measured for all surviving saplings. There were two plots where no trees have survived.

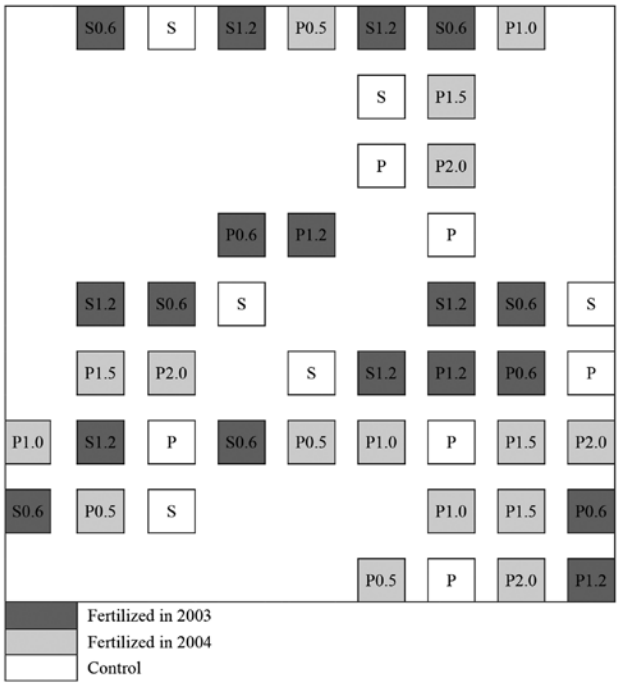

Figure 1. Schematic arrangement showing randomised placement of Norway spruce and Scots pine in wood-ash fertilized (shaded) and control (not shaded) plots fertilized with wood ash in 2003 and 2004. The tree species in each plot is denoted by the capital letters: P – pine and S – spruce. The numbers in the fertilized plots show the amount of the wood ash applied (0.5, 0.6, 1.0, 1.2, 1.5 or 2.0 kg m^{-2})

Soil chemical analysis

One soil sample was collected from each sample plot in late June 2014. A soil corer (3.5 cm in diameter) was used to take samples from 0–20 cm depth. In total, 44 soil cores were collected (samples were not collected from plots where no trees have survived). Chemical analyses of soil were conducted using established standard methods (International Organization for Standardization (ISO) standard). Samples were prepared for analysis according to ISO 11464:2005. Soil pH(H_2O) was measured potentiometrically in deionised water suspension according to LVS ISO 10390. Total N content was determined using a modified Kjeldahl method (ISO 11261:1995) and P was assayed spectrophotometrically using the ammonium molybdate method in 1 M HCl extract (LVS 398). Concentrations of K, Ca and Mg were determined by atomic absorption spectrophotometer with an acetylene-air flame in 1 M HNO_3 extract.

Morphotyping and molecular analysis of ectomycorrhizas

For root analysis, five soil samples in each plot were collected in June 2014 to a depth of 20 cm using a 2.8 cm corer. Collected samples from each plot were pooled

and analysed as one bulk sample. Samples were stored at + 4 ºC for up to two weeks prior to processing.

All woody roots were picked from soil samples and rinsed under tap water. Coarse roots (larger than 2 mm in diameter) were discarded. Non-conifer roots, identified under a stereomicroscope Leica MZ-7.5 (Leica Microsystems, Wetzlar, Germany), were also discarded. The remaining fine roots were cut into 1 cm segments and evenly spread in Petri dishes with water. The number of living and dead root tips was determined, living root tips were sorted into ECM and non-ECM, and all ECM root tips were morphotyped. These analyses were done using a stereomicroscope. The distinction between the live and dead roots was made by evaluating the colour and elasticity of the central cylinder of fine roots (Vogt and Persson 1991). ECM fungal morphotypes were identified visually according to colour, form and texture of ECM root tips and the presence of rhizomorphs or external mycelia (Agerer 1986–2006).

One to five single root tips of each distinct morphotype per sample plot were separately placed in 1.5 ml centrifugation tubes and stored at -20 ºC for molecular identification of fungal taxa. The Phire Plant Direct PCR Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for direct PCR amplification from root tips (Velmala et al. 2014). To amplify the fungal ITS region of rRNA genes, primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) were used. Sequencing was performed by Macrogen Europe Inc. (Amsterdam, The Netherlands).

Raw sequence data was analysed using the SeqMan version 5.07 software from the DNASTAR package (DNASTAR, Madison, WI, USA) and BioEdit v. 7.0.9.0 (Hall 1999). Databases at GenBank (Altschul et al. 1997) and UNITE (http://unite.ut.ee/) (Kõljalg et al. 2013) were used to determine the identity of sequences. The criteria used for identification were the following: sequence coverage > 80%; similarity to species level 97–100 %, similarity to genus level 94–96 %. The sequences are available from GenBank under accession numbers KT182903-KT182923.

Soil microbiological analysis

Soil samples used for microbiological analysis were collected in August 2014. A soil core 2.8 cm in diameter was used to obtain the samples down to 20 cm depth. Two replicate cores were collected from each sample plot, pooled and analysed as one bulk sample. Soil samples were collected from the same plots as for root analysis. In addition, two soil samples were taken from Scots pine plots where no trees had survived (resulting in 46 samples, in total). Samples were immediately transported to the laboratory and stored at + 4 ºC.

For each sample ten grams of soil were suspended in 90 ml of sterile water in 500-ml Erlenmeyer flasks by shaking at room temperature at 150 rpm for 30 minutes (Alef and Nannipieri 1998). 0.1 ml aliquots from the serial dilutions of the suspensions were plated on the following media: peptone yeast extract media (5 g of peptone, 3 g of yeast extract, 15 g of agar, 1 l of distilled water) for bacteria; malt media (malt extract (d = 1.028), 18 g of agar, 1 l of distilled water) for maltose degrading bacteria and fungi. The assay was conducted in three replicates of prepared Petri dishes incubated at room temperature (20 ± 2 ºC) for three days. After incubation, the numbers of colony forming units (CFU) were counted (Vanderzant and Splittstoesser 1992) and their abundance was calculated per one gram of soil (Alef and Nannipiri 1988). Dominant fungal species in each Petri plate were identified to genus level according to their morphological characteristics, which were examined under a microscope Leica DFC 490 (Leica Microsystems, Wetzlar, Germany).

Data analysis

Stem parameters, numbers of fine roots and their biomass, soil chemical parameters and numbers of CFUs of soil microorganisms were tested for normality using the Shapiro and Wilk test (Royston 1982). All soil chemical parameters (soil pH, total C, total N, P, K, Ca, Mg) were normally distributed. Other variables (fine root biomass and sapling stem parameters) met this assumption only when analysed separately for pine and spruce plots; for numbers of fine roots and numbers of CFUs of soil microorganisms normality was obtained after logarithmic transformation and separation of pine and spruce plot data. One-way analysis of variance (ANOVA) and Tukey's tests (Fowler et al. 1998) were performed to compare all those parameters among wood ash and control treatments analysing pine and spruce plots together (in case of soil parameters) or separately for other parameters. A Tukey's test was performed at confidence level $\alpha = 0.05$. The Wilcoxon test (Hollander and Wolfe 1999) was used to compare fine root biomass, number of fine roots, stem parameters and numbers of CFUs of soil microorganisms between Scots pine and Norway spruce plots.

Significant differences in mycorrhizal colonization of fine roots, relative abundance of living fine roots and ECM fungal species between wood ash treatments and the control were tested by chi-square (χ^2) analysis calculated from the actual number of observations (Mead and Curnow 1983). As the total number of root tips analysed was large, the χ^2 test was performed at confidence level $\alpha = 0.0001$. Data were statistically analysed using the R program (Vienna, Austria) (R Development Core Team 2011).

Species richness and the Shannon diversity index (Shannon 1948) were calculated for each sample plot in ComEcoPaC (Drozd 2010). ECM fungal community structure was analysed using principal component analysis (PCA) in CANOCO 4.5 (ter Braak and Smilauer 1998).

The initial experimental design for pine included variants with similar amounts of wood ash applied (0.5 and 0.6; 1.0 and 1.2 kg m^{-2}); to unify experimental design in pine and spruce we analysed data from those pine plots together.

Results

Of the examined soil parameters, only pH differed significantly among treatments (Table 1). It showed higher values in pine plots fertilized with 1.0 to 2.0 kg m^{-2} of wood ash than in control plantings of both tree species and spruce plantings fertilized with 1.2 kg m^{-2}. However, no significant differences in soil pH were observed among spruce plots. No significant differences among the treatments were observed also in total nitrogen (mean 27 g kg^{-1}), carbon (434 g kg^{-1}) and phosphorus (1.6 g kg^{-1}), and plant available potassium (0.35 g kg^{-1}), calcium (40.4 g kg^{-1}) and magnesium (1.58 g kg^{-1}).

Scots pine saplings, in comparison to Norway spruce saplings were significantly taller (mean 3.7 ± 0.1 m versus 2.8 ± 0.1 m) and had larger stem diameter (dbh) (mean 3.3 ± 1.8 cm versus 2.0 ± 0.1 cm). No significant differences in height and diameter of pine saplings were observed among wood ash and control treatments after ten growing seasons (Table 1). Spruce tree height was significantly

Table 1. Soil pH, fine root morphological parameters and abundance of soil microorganisms in samples from wood ash fertilized and control plots of *Pinus sylvestris* and *Picea abies* ten years after the application of wood ash (±SE)

Species	Spruce			Pine			
Amount of wood ash applied	**Control**	**0.6 kg m^{-2}**	**1.2 kg m^{-2}**	**Control**	**0.5-0.6 k g m^{-2}**	**1.0-1.2 kg m^{-2}**	**1.5-2.0 kg m^{-2}**
Sapling growth							
No. of trees	107	105	129	31	29	19	23
Average height, m	2.6 ± 0.1 a	2.7 ± 0.1 ab	3.0 ± 0.1 b	3.7 ± 0.2	3.6 ± 0.3	3.4 ± 0.2	3.4 ± 0.2
Average DHB, cm	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	3.1 ± 0.3	3.2 ± 0.4	2.9 ± 0.4	3.1 ± 0.4
Soil chemical and fine root analysis							
No. of samples	6	6	6	6	5	7	8
Soil pH (H_20)	5.7 a	5.9 ± 0.1 ab	5.7± 0.1 a	5.7 ± 0.1 a	5.9 ± 0.1 ab	6.1 ± 0.2 b	6.2 ± 0.1 b
C org. / total	442 ± 5	405 ± 18	414 ± 13	450 ± 6	445 ± 18	433 ± 15	444 ± 11
P (g/kg)	1.64 ± 0.03	1.97 ± 0.18	1.92 ± 0.08	1.46 ± 0.10	1.45 ± 0.15	1.53 ± 0.15	1.52 ± 0.13
K (g/kg)	0.27 ± 0.02	0.22 ± 0.06	0.35 ± 0.01	0.43 ± 0.03	0.37 ± 0.04	0.41 ± 0.04	0.32 ± 0.06
Ca (g/kg)	41 ± 2	46 ± 2	33 ± 2	38 ± 1	41 ± 2	43 ± 3	44 ± 1
Mg (g/kg)	1.3 ± 0.1	1.9 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2	1.8 ± 0.1
Fine root biomass (kg m^{-2}) * 10^{-2}	5.6 ± 1.2	4.4 ± 1.3	3.9 ± 0.8	1.8 ± 0.4	2.1 ± 0.2	1.6 ± 0.4	1.9 ± 0.2
No. of dead fine roots per m^2 * 10^4	7.3 ± 2.5	5.8 ± 2.4	6.2 ± 2.1	2.2 ± 0.6	2.1 ± 0.4	2.3 ± 0.3	2.3 ± 0.5
No. of living fine roots per m^2 * 10^4	8.5 ± 1.7	6.0 ± 1.0	3.0 ± 0.8	4.7 ± 0.3	6.6 ± 1.5	3.7 ± 0.4	2.6 ± 0.8
Relative abundance of living fine roots (%)	53.7 b	50.9 a	58.0 c	68.7 e	51.5 a	61.3 d	53.5 b
Mycorrhization (%)	89.8 ab	92.6 c	88.2 ab	90.3 ab	88.0 a	89.8 ab	91.2 bc
Soil microbial analysis							
No. of samples	12	12	12	12	14	14	16
Bacteria (CFU per g) * 10^6	3.7 ± 0.4 a	7.3 ± 0.9 b	8.7 ± 1.0 b	39.5 ± 14.3 cd	12.1 ± 3.8 c	40.8 ± 14.7 cd	42.3 ± 11.3 e
Maltose degrading bacteria (CFU per g) * 10^5	3.9 ± 0.7	6.0 ± 1.3	6.6 ± 0.6	6.8 ± 1.5	7.3 ± 3.1	6.9 ± 1.3	19.8 ± 8.6
Actinobacteria (CFU per g) * 10^5	1.7 ± 0.4 a	5.5 ± 0.7 b	8.1 ± 1.9 ab	12.3 ± 5.0	5.6 ± 1.3	4.6 ± 0.8	9.9 ± 2.5
Filamentous fungi (CFU per g) * 10^4	3.8 ± 1.1	4.8 ± 1.1	4.6 ± 0.9	4.5 ± 0.9	4.2 ± 0.9	4.4 ± 0.9	5.4 ± 0.8

* Within each row, different letters next to mean values indicate significant differences between treatments. If no significant differences are found among treatments, no letter is given.

greater in the treatment with 1.2 kg m^{-2} of wood ash, compared with that in the control (2.9 ± 0.1 m and 2.6 ± 0.1 m, respectively), while stem diameter (dbh) did not differ significantly. In general, spruce saplings showed higher survival rate than pine saplings. No trees survived in two plots of Scots pine treated with 0.5 kg m^{-2} of wood ash.

Fine root biomass and numbers of fine roots were significantly lower in pine versus spruce plots ($p < 0.05$). Fine root biomass, numbers of living fine roots and numbers of dead fine roots did not differ significantly among wood ash treatments and the control for either pine or spruce (Table 1). For both tree species, relative abundance of living fine roots was lower in sites fertilized with wood ash in low amounts (0.5 to 0.6 kg m^{-2}). Spruce plots of this treatment had higher levels of mycorrhizal colonization than other treatments, while pine saplings showed the opposite effect – lower mycorrhizal colonization in plots fertilized with low amounts of wood ash.

Numbers of bacterial CFU were higher in plots with Scots pine than in those with Norway spruce ($p < 0.05$), and in plots, where wood ash was applied in higher amounts (Table 1). Abundance of maltose degrading bacteria did not differ among wood ash treatments and control plots. In spruce plots, actinobacteria were more abundant in wood ash fertilized plots than in control plots, while in pine plots no significant differences between wood ash treatments and control were observed (Table 1). Abundance of filamentous fungi CFU in soil samples was similar in all treatments. Taxanomical identification indicated dominance of *Penicillium*, *Mortierella* and *Verticillium* fungal genera in all soil samples. *Colletotrichum* and *Paecilomyces* tended to be more abundant in fertilized plots of both tree species. *Cladosporium* was approximately five times more abundant on spruce sample plots, while *Trichoderma* species were approximately ten times more abundant in pine sample plots. Species from genera *Botrytis*, *Fusarium*, *Geotrichum* and *Pythium* were identified in some samples.

Sequencing of the ITS region of fungal rRNA genes from 80 root tips representing each of 14 observed morphotypes resulted in 50 sequences of basidiomycetes (11 species) and 30 sequences of ascomycetes (10 species) (Table 2). Most sequenced species were ectomycorrhizal fungi, but some species, such as two unidentified ascomycetes and one species from the family *Nectriaceae* were most probably saprotrophic species. The most frequently sequenced ectomycorrhizal species were the basidiomycetes *Amphinema byssoides* and *Lactarius* cf. *deliciosus* var. *deterrimus* (each one constituted 12.5 % of all sequences). The truffle species *Tuber anniae* was also common (11 % of all sequences).

The number of ECM species did not differ significantly between different treatments or between different host species. Most of the ECM species were represented by more than one sequence and occurred in both control and fertilized sample plots. None of these species was found exclusively in control plots. However, some species, such as *Tuber* sp., *Tomentella coerulea* and *Tomentella* sp. 2, were found exclusively in fertilized plots. Total numbers of fungal species were similar between host species (13 on pine and 15 on spruce), but only five ECM species (*Agaricales* sp., *Cenococcum* sp., *Inocybe sindonia*, *Tomentella* sp. 2 and *Tuber anniae*) were found in both host species.

Shannon diversity index of root-associated fungal species for both host species was higher in plots with a medium amount (0.5 kg m^{-2}) of wood ash fertilization. The dominant species *Agaricomycetes* sp. and *Amphinema byssoides* were less abundant in fertilized sites, while unidentified *Lactarius*, *Amphinema* and *Agaricales* species were more abundant.

The relative abundance of fungal species differed significantly between Norway spruce and Scots pine. Ectomycorrhizal fungi *Amphinema byssoides* and *Lactarius* cf. *deliciosus* var. *deterrimus* were dominant on spruce, while *Cadophora finlandica* and *Agaricomycetes* sp. were dominant on pine. The relative abundance of some ECM species differed significantly between fertilized and control plots: *Cadophora finlandica* and an unidentified *Cenococcum* species were more abundant in control samples, while an unidentified *Tuber* species and *Wilcoxina rehmii* were more abundant in wood ash fertilized sites. Principal component analysis (PCA) clearly showed these trends (Figure 2). The first principal component of the PCA ordination explained 75.3 % of the variation in root-associated fungal community composition and separated communities on pine and spruce (Figure 2). The second component explained 14.0% of the variation in root-associated fungal community composition and showed a certain difference between the ash and control treatments for each tree species.

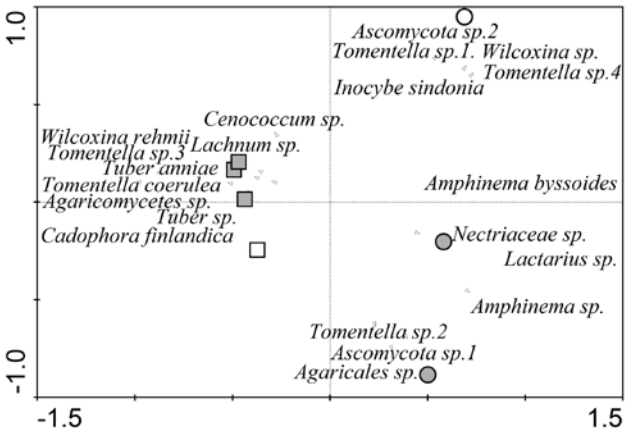

Figure 2. Principal component analysis (PCA) of fungal taxa in wood ash fertilised and control plots of Norway spruce and Scots pine. Symbols indicate treatments and the tree species: circle – Norway spruce; square – Scots pine; not filled – control; grey filled – wood ash fertilized plots

Table 2. Fungal species and their relative abundance (%) in wood ash fertilized and control plots of Norway spruce and Scots pine in wood ash fertilized and control plots ten years following addition of wood ash

Species	GenBank accession No.**	Spruce			Pine				Total
Amount of wood ash applied		**Control**	**0.6 kg m^{-2}**	**1.2 kg m^{-2}**	**Control**	**0.5-0.6 kg m^{-2}**	**1.0-1.2 kg m^{-2}**	**1.5-2.0 kg m^{-2}**	
No. of root tips analysed		5616	4124	4280	1945	2602	2867	2329	23763
Shannon diversity index		1.85	2.27	1.30	1.67	2.18	1.70	1.34	2.94
Ascomycota									
Ascomycota sp. 1 (SH214904.07FU)*	KT182903	-	0.8	-	-	-	-	-	0.1
Ascomycota sp. 2 (SH202705.07FU)	KT182904	0.5	-	-	-	-	-	-	0.1
Cadophora finlandia (SH214265.07FU)	KT182905	-	-	-	7.7 c***	2.2 b	1.8 b	0.5 a	1.1
Cenococcum sp. (SH214459.07FU)	KT182906	0.6 a	0.3 a	0.1 a	1.5 b	0.2 a	0.5 a	4.9 c	0.9
Nectriaceae sp. (SH182800.07FU)	KT182907	-	-	0.6	-	-	-	-	0.1
Lachnum sp. (SH201605.07FU)	KT182908	-	-	-	-	-	3.5	-	0.4
Tuber cf. *anniae* (SH202491.07FU)	KT182909	0.2 a	0.3 a	2.1 b	0.3 a	2.5 b	9.0 c	1.0 b	2.0
Tuber sp. (SH216303.07FU)	KT182910	-	-	-	-	2.7 b	-	0.2 a	0.3
Wilcoxina sp. (SH194157.07FU)	KT182911	6.7 b	-	1.0 a	-	-	-	-	1.8
Wilcoxina rehmii (SH211927.07FU)	KT182912	-	-	-	1.3 a	8.1 b	3.0 a	7.6 b	2.1
Basidiomycota									
Agaricales sp. (cf. *Hebeloma leucosarx*) (SH215995.07FU)	KT182913	-	13.2 b		2.0 a	-	-	-	2.5
Agaricomycetes sp. (SH206583.07FU)	KT182914	-	-	-	68.0b	60.3a	70.1c	77.8d	28.2
Amphinema byssoides (SH197943.07FU)	KT182915	64.9b	46.7a	74.5c	-	-	-	-	36.9
Amphinema sp. (SH197945.07FU)	KT182916	0.4 a	4.0 b	0.2 a	-	-	-	-	0.8
Inocybe sindonia (SH176685.07FU)	KT182917	2.5 b	-	0.5 a	-	4.2 c	-	-	1.1
Lactarius cf. *deliciosus* var. *deterrimus* (SH220113.07FU)	KT182918	4.5 b	10.4 c	2.3 a	-	-	-	-	3.3
Tomentella coerulea (SH177784.07FU)	KT182919	-	-	-	-	7.3 b	-	2.7 a	1.1
Tomentella sp. 1 (SH177803.07FU)	KT182920	0.7	-	-	-	-	-	-	0.2
Tomentella sp. 2 (SH177794.07FU)	KT182921	-	4.6 b	-	-	-	1.1 a	-	0.9
Tomentella sp. 3 (SH189390.07FU)	KT182922	-	-	-	-	-	-	3.4	0.3
Tomentella sp. 4 (SH177825.07FU)	KT182923	5.3 b	-	1.4 a	-	-	-	-	1.5
Unidentified morphotypes		13.6	19.7	17.2	19.3	12.6	11.1	1.9	14.3

* Reference species hypothesis (Kõljalg et al. 2013) is given in parenthesis.

** The homology of our sequences to the GenBank reference sequences was 97–100 %.

*** Different letters next to relative abundance of each species indicate significant differences among treatments (chi-square test p-value < 0.0001).

Discussion and conclusions

The observed differences in soil pH between control and fertilized plots of pine plots 10 years after wood ash application indicate a long-lasting neutralizing effect of wood ash in the organic soil layer, as described by other authors (Bramryd and Fransman 1995, Saarsalmi et al. 2001, Moilanen et al. 2002). In contrast to results previously reported by Arvidsson and Lundkvist (2003), no significant difference in soil pH was observed between treatments in spruce plots. This could be explained by a higher survival rate and density of spruce in experimental plots resulting in greater litter input in comparison with pine. It could also be related to species differences associated with the significant impact of Norway spruce litter on soil properties that have been described by other authors (Bonifacio et al. 2008). The lower stem growth rate and higher fine root biomass and abundance in spruce plots might also be due to higher tree density in spruce plots compared to pine plots.

Increased abundance of bacteria and actinobacteria and no effect on abundance of fungi after liming has also been described by other authors (Frostegård et al. 1993). We observed a higher number of bacterial CFUs in pine plots than in spruce plots. This can be explained by higher soil pH in fertilized pine sites, as higher soil pH can promote bacterial growth (Frostegård et al. 1993; Bååth and Anderson 2003). The higher abundance of *Trichoderma* species in pine sites and fertilized plots could also be associated with increased soil pH (Jackson et al. 1991).

We observed significant differences in ECM community composition between host species, which is in accordance with previous studies (e.g. Klavina et al. 2013). The observed differences in fungal species abundance between fertilized and control sites are consistent with previous studies showing species-specific changes in ECM fungal community composition after forest liming (Jonsson et al. 1999, Kjøller and Clemmensen 2009) and wood ash application (Klavina et al. 2015). *Amphinema byssoides* and *Tuber* species are favoured by soil conditions with more neutral pH, and they can thus dominate after liming or wood ash treatment (Qian et al. 1998, Erland and Taylor 2002, Kjøller and Clemmensen 2009, Klavina et al. 2015). However, our data indicate that abundance of those species may strongly depend on amount of wood ash applied. Greater abundance of *Tomentella coerulea* and *Hebeloma* cf. *leucosarx* in wood ash treated plots than in control was observed in this and previous studies (Klavina et al. 2015). In contrast, *Cadophora finlandia* in both studies was tend to be more abundant in control sites.

Application of wood ash amounting up to 1 kg m^{-2} are mostly used in experiments and practical forestry (Augusto et al. 2008). Application of wood ash in amounts lower than 1 kg m^{-2} has been observed to be the best treatment for promoting stem growth of pine saplings, when evaluated one year after treatment (Gaitnieks et al. 2005). Our study also indicated that effects of wood ash application in low amounts (0.5 to 0.6 kg m^{-2}) are positive, especially regarding Shannon diversity of ECM communities. This might be due to reduced abundance of dominant species *Amphinema byssoides* (on spruce) and *Agaricomycetes* sp. (on pine) in those plots. In addition, a higher colonization of roots by mycorrhizal fungi on spruce saplings was observed in plots fertilized with wood ash 0.6 kg m^{-2}. Since *Amphinema byssoides* is a frequent colonizer of dead wood substrates (Veerkamp et al. 1997), its high abundance on fine roots of other treatments might be related saprotrophic activity and not only to formation of mycorrhizal symbioses. Some *Agaricomycetes* sp. reference sequences in the GenBank database were also assigned to the genus *Amphinema* and therefore the ecology of these species hypothetically might be similar to that of *A. byssoides*.

In conclusion, our data provide some evidence of a long-term effect of wood ash fertilization on soil pH, ECM community composition and abundance of soil bacteria in both Scots pine and Norway spruce plots, at the same time suggesting that the treatment might have effects on the functioning of forest ecosystems.

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