Short Term Effects of Compensatory Wood Ash Fertilization on Soil, Ground Vegetation and Tree Foliage in Scots Pine Stands

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Abstract

A wood ash experiment was set up in a 38-year-old Scots pine stand (forest type – *Pinetum vacciniosum*) growing on Arenosol. Raw, dry ash (fly ash, not stabilized) and nitrogen fertilizers were applied in the forest. There were 6 variants of the experiment: 1 – 1.25 t ash ha^{-1}; 2 – 2.5 t ash ha^{-1}; 3 – 5.0 t ash ha^{-1}; 4 – 2.5 t ash ha^{-1} and 180 kg N ha^{-1}; 5 – 180 kg N ha^{-1} and 6 – control (without ash and nitrogen). The primary effects (3 months–2 years after the treatment) of wood ash fertilization on soil, soil solution, soil microflora and biological activity, fine roots and mycorrhiza, ground vegetation diversity, tree foliage chemistry, physiological parameters and litterfall are presented in the paper.

The highest wood ash dose (5.0 t ha^{-1}) changed the chemistry of forest litter: the increased pH and total concentrations of most of the macronutrients were found after 2 years. In contrast, total N concentrations decreased due to ash application. Wood ash increased the number of ammonifying, denitrifying microorganisms and cellulose-decomposers in the forest litter 3 months after application.

Wood ash slightly reduced total length of fine roots and number of root tips 1 year after treatment. The highest degree of fine roots vitality was found in the plots treated with 2.5–5.0 t ha^{-1} of wood ash.

No changes in ground vegetation diversity were found after the wood ash and N application.

No changes of chlorophyll *a*, *b* were determined in the current year needles 5 months after application of wood ash. Wood ash decreased the content of the aminoacid proline and it has increased only after N addition.

Key words: wood ash, Scots pine, chemical composition, microflora, mycorrhiza, ground vegetation diversity, tree foliage

Introduction

Recently, an increase in utilization at logging residues for producing energy is widely noted in Lithuania. First of all, acceleration of wood residues use for bioenergy raises many ecological aspects (Kairiūkštis *et al*. 2005). In the nearest future, forest fuel resources could amount to 5 mill m^3 in Lithuania. The utilization of such an amount of forest fuel annually would create about 25–30 thousand tons of wood ash. From another point, the intensified forest fuel harvest would cause an additional export of nutrients from the forest ecosystems (Nilsson 2001). To obtain a sustainable utilization of biomass fuels, the nutrients containing ash have to be recycled back to the forests.

The chemical composition of wood ash differs because the concentrations of various elements vary widely: Ca – 72.3–300 g kg^{-1}, K – 5.3–74 g kg^{-1}, Mg – 9.45–74 g kg^{-1}, Cd –1.4–28.6 mg kg^{-1}, Cr – 9.5–225 mg kg^{-1}, Cu – 13–289 mg kg^{-1}, Pb – 4.5–100 mg kg^{-1}, Zn – 74–1500 mg kg^{-1} (Bååth and Arnebrant 1994, Fransman and Nihlgård 1995, Eriksson 1998, Hytönen and Kaunisto 1999, Levula *et al*. 2000, Saarsalmi *et al*. 2001 and Ozolincius *et al*. 2005). An exception is made for N that is usually lacking in wood ash.

Generally, wood ash has a strong neutralizing and buffering capacity on soils. This has been widely reported in a number of studies (Ohno and Erich 1994, Bramryd and Fransman 1995, Mälkönen 1996, Levula *et al*. 2000, Arvidsson *et al*. 2001 and Saarsalmi *et al*.

2001, *etc*.). The hydroxyl and bicarbonate ions formed as a result of dissolution of the oxides, hydroxides and carbonates in the ash neutralize the protons in the soil solution. Simultaneously the proton cation exchange sites are replaced by base forming cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}). The neutralization effects of applied wood ash become evident at a slower rate in the mineral soil than in the humus layer.

Differences in the average nutrient release of various chemical elements from ash have been found. For example, Ohno and Erich (1994) found that the average nutrient release from the ash to the soil was much lower for P compared to Ca, Mg and K. This can be attributed to the low solubility of P minerals in the ash.

Fewer findings have pointed out the wood ash influence on soil solution chemistry. Several times it has been stated that there were no differences in soil solution pH at 30 cm depth during a 5-year period after granulated ash application in central Sweden (Fransman and Nihlgård 1995). Arvidsson and Lundkvist (2001) found no treatment effects on pH in the soil water sampled at 50 cm depth after the application of hardened and crushed wood ash at four different locations in Sweden.

While the effect of wood ash application on soil solution pH is rather weak there are consistent observations of increased downward transport of base cations. Arvidsson and Lundkvist (2001) found higher concentrations of Ca, Mg and K in the soil solution at all of four different field experiments using two different crushed ash types. Rumpf *et al.* (2001) observed increased concentrations of both Ca and K at 0 cm, 10 cm, and 100 cm depth in the mineral soil.

Reduced soil acidity due to ash application affects the microbiological processes in the soil. According to Bååth and Arnebrant (1994), the total microbial activity and bacterial growth rates increase with increasing amount of wood ash (from 1.0 to 5.0 t ha^{-1}). However, the bacterial biomass appeared unaffected. In other experiments, positive effect on litter microflora was detected: the number of ammonifying bacteria, bacillus and oligotrophic microorganisms increased (Armolaitis *et al*. 2002).

The ash dose 7.5 t ha^{-1} was found to stimulate the root growth of spruce 4 months after planting (sample plot was fertilized by ash 12 months before planting), but the number of root tips was reduced (Erland and Söderström 1991). Contrary some authors (Clemensson-Lindell and Persson 1992) recorded the amount of live roots (diameter 0–1 mm) in spruce stands to be nearly twice as low as in the control 5 years after wood ash application (2.8 t ha^{-1}).

While analyzing ground vegetation changes after wood ash application it is evident that due to morphological properties of bryophytes and lichen they are most susceptible to drastic changes in litter and soil pH and they often show immediate symptoms of damage after treatment (Mälkönen *et al*., 1980 and Mäkipää 1994). The short-term damage (discoloration) on bryophytes 3 months after application of loose ash and crushed ash, then granulated ash caused no visible damage (Kellner and Weibull 1998). Studies of wood ash effects on ground vegetation in pine, spruce and birch stands indicated the presence of more nitrophilic vascular plant species (Rühling 1996). No data about the effect on ground vegetation diversity were found.

Wood ash influence both physiology and the chemical composition of tree foliage. There is, however, hardly any information on changes of the pigments (chlorophylls, carotenoids) participating in photosynthesis, nor on changes of amino acids as stress indicators, for example, proline after the application of wood ash. However, for the indication of wood ash consequences at early stage, it may be rational to estimate the changes of these metabolites as they participate in many chemical reactions (Mandre and Korsjukov 2002 and Skuodienė 2005). Insignificant differences in the content of chlorophylls and carotenoids have been observed already 2 months after treatment in 19-year-old Scots pine stands (Mandre and Korsjukov 2000). It is recorded that wood ash application increased the content of chlorophylls and carotenoids, but not their ratios in young Scots pine trees (Mandre and Korsjukov 2002).

During ash application studies much attention has been paid to the chemical composition of foliage, especially needles. There were no effects on nutrient concentration in the needles four years after the application of a granulated wood ash (Rosengren-Brick 1994) and one year after the application of crushed wood ash (Arvidsson and Lundkvist 2002). However, the application of hardened wood ash increased the nutrient concentrations of P, K, and Ca in the current and first year needles after a period of five years. The concentrations of P and Ca were significantly higher only in the current year needles (Arvidsson and Lundkvist 2002 and Nilsson 2001).

The influence of wood ash fertilization in a Scots pine stand growing on nutrient poor sandy soil has been analyzed in the present study. The initial effects (5 months–2 years after the treatment) of wood ash fertilization on soil, soil solution, soil microflora, fine roots and mycorrhiza, ground vegetation diversity, tree foliage chemistry and physiological parameters, and litterfall are presented in the paper. We hypothesized that raw, dry wood ash applied on Arenosols intensively change the chemical parameters of the forest

litter and soil, intensify nutrient leaching and influence chemical composition of forest vegetation during the first 2 years after the treatment. Therefore, the changes in chemical soil condition would affect the number of microorganisms, also wood ash fertilization, as a stress factor, may cause the changes in the physiological processes in trees.

Materials and methods

Site description

An integrated field experiment was established in 2002 in the 38-year-old Scots pine stand. The soil was classified as Haplic Arenosols. The stand represented a typical Scots pine forests of *Pinetum vaccinio-sum* forest type for Lithuania. The average tree height was 14.8 m and the mean diameter at breast height was 14.3 cm at start of the experiment. Standing volume was 174.4 m^3 ha^{-1} and the stand production class determined to 5.3 m^3 ha^{-1} per year. The ground vegetation layer in the stand was dominated by different species of moss *Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., *Dicranum scoparium* Hedw. and *Hylocomium splendens* (Hedw.) Schimp. The coverage of vascular plants was low (~ 7%) and the most common species were *Festuca ovina* L. and *Calluna vulgaris* (L.) Hull. The total area of the experiment was 3.2 ha. Totally there were 24 plots (25x20 m) grouped into 4 blocks (replications) with 6 treatments in each block: 1 – 1.25 t of dry ash per 1 ha, 2 – 2.5 t ha^{-1}; 3 – 5.0 t ha^{-1}; 4 – 180 kg N ha^{-1}; 5 – 2.5 t together with 180 kg N ha^{-1} , and K – control. The raw (not hardened) wood ash was applied in June 2002. The wood ash applied in the field experiment had pH_{H2O} 11–12 and consisted of 2.1 g P kg^{-1}, 5.3 g K kg^{-1}, 72 g Ca kg^{-1}, 9.5 g Mg kg^{-1}, and also relatively small amounts of heavy metals (Cr, Cd, Pb, Ni, Cu, Zn) (for details – Ozolincius *et al*. 2005).

Sampling, analysis and data treatment

Soil sampling was carried out 2 years after wood ash application in September 2004. On each plot, soil subsamples were collected from 20 places of the forest litter (organic layer) and from the upper 0–5 cm mineral soil. One combined sample was produced per plot and per layer. Soil pH was measured with glass electrode on suspensions of soil in distilled water and 0.01 M Ca Cl_2. The contents of total N was analyzed by the Kjeldahl method, total Mg and Ca – with atomic absorbtion spectrophotometer (AAS), K – with flame photometer and P – using standard colorimetric methods (UN-ECE 2002).

Soil solution was sampled at 20 cm and 50 cm depths 15 months and 2 years after the application of wood ash and N fertilizer. The tension samplers (ceramic tension cups P80) were installed in all treatments. There were installed 144 tension lysimeters, systematically, with 6 in each plot. An underpressure of 70 kPa was used for sampling soil solution. The soil solution samples were analyzed for NO_3, P, K, Ca and Mg. NO_3 ions were measured spectrometrically, P – by the colorimetric method, K – with flame photometer, Ca and Mg – with AAS.

Soil microflora was studied 3 months after treatment. Composed forest litter samples (12 subsamples) from 0–10 cm layer (5subsamples) were collected (Raguotis and Kruopis 1961). Microfloric identification was carried out by sowing diluted suspensions of forest litters and soils on nutriculture media, distributing microorganisms into physiological and systematical groups. The decomposition of cellulose in the soil was also determined (Klupt 1962).

The samples of fine roots were collected one year after wood ash treatment. With the A_o layer removed, the root samples were collected by using a 100 cm^3 metallic cylinder (H=4 cm, D=5.8 cm). Root samples were taken from forest litter at the depth of 4 cm, to some extent touching also the mineral soil. Samples were taken from both the control and the ash-fertilized sample plots of the fertilization dose 2.5 t ha^{-1} and 5.0 t ha^{-1} (totally from 12 sample plots). On each sample plot totally 24–26 samples were randomly taken over the crown projection area of average diameter trees. The samples were stored at the temperature +4°C. At the laboratory the root samples were carefully rinsed and analysed at first in a stereomicroscope (I Leica MZ–7.5) of the magnification 6.3. The mycorrhizal types were identified following the colour: white (having external hyphae and rhizomorphs), light yellow, dark, and pink. The samples with *Cenococcum sp*. were identified as well. The mycorrhiza vitality class was estimated. The mycorrhiza vitality was graded into 4 classes following a modified Göbl method (Göbl 1995). Vitality coefficient 1 stands for the highest vitality class, 4 – for the lowest. Roots of the diameter up to 3 mm were analysed. Comparison of the morphological indices of roots is done by using the software Win RHIZO 2002 C and the calibrated scanner EPSON STD-1600+. After scanning the root samples were dried for 48 h at +70°C to determine their mass.

Ground vegetation was studied before the experiment and up to 2 years after the treatment. Eight 1m^2 quadrates were systematically distributed on each experimental plot. In each quadrate species composition, layer and cover (%) were recorded (Tallent-Hansell 1994). The Shannon diversity index (Magurran 1987) was also calculated by the following equation:

$$H' = -\Sigma (n_i / N) \log_2 (n_i / N),$$

where n_i – cover of *i*-species; N – total cover.

Scots pine needles for the chlorophylls, carotenoids, aminoacid proline analyses and for chemical composition were sampled from 5 trees of the class II according to the Kraft classification. Current year and first year needles were sampled in October 2002 (after 3 months) and September 2004 (after 2 years). The needles were sampled from the 5–7th whorl from 1/3 upper part of the crown. Methods described by Lichtenthaler (1978) were used for chlorophylls and carotenoids assessment. Proline was determined by the method of Bates, Waldren and Teare (1973) modified for conifers (Skuodienė 1997). Total N in the needle samples was analysed by the Kjeldahl method, P – by the colorimetry, K – by flame photometry, and Ca and Mg – by AAS.

Litterfall has been monitored continuously since July 2002 until July 2005. Litterfall was sampled from 144 litter traps in Scots pine stand, 6 litter traps in each plot (surface area 0.25 m^2 at a height of 1 m above the ground). The traps made of wood and cotton bags were set up equidistantly and parallel in two rows in the plot (UN-ECE/ICP 1998). Litterfall was collected monthly in spring, summer and autumn-time and once in winter-time. The chemical composition was determined using the methods described above for the needle samples.

All chemical analyses were performed at the Center of Agrochemical Research of the Lithuanian Institute of Agriculture.

For statistical data analyses, ANOVA and *t*-tests were used to evaluate the significant effects of different treatments. The effects of the treatments were treated as significant with $p<0.05$.

Results and discussion

1. Chemical changes in forest soil and soil solution

Raw wood ash decreased the acidity of forest litter (organic layer) from pH 3.5 (the control) to pH 5.7 (5.0 t ha^{-1} of ash) 2 years after the application (Table 1). Meanwhile, no significant differences among the treatments were determined in mineral topsoil (0–5 cm) of Arenosols. An increase by 2.6 pH units in the forest litter has been indicated already 3 months after wood ash application (Ozolincius *et al*., 2005). The effects of dry wood ash treatment on soil acidity mainly depended on the contents of oxides of Ca, Mg and K, which will turn into hydroxides with rain water and then react with acids. Then the wood ash obviously caused the saturation of soil colloids with Ca ions.

Table 1. Average pH_{CaCl2} of the forest litter and the 0–5 cm layer of the mineral soil 3 months and 2 years after the application of wood ash and N fertilizers. Mean values and CI – 95% confidence intervals

Variant of experiment	Control		5 t ash ha^{-1}		180 kg N ha^{-1}		(2.5 t ash+180 kg N) ha^{-1}	
2002 (3 months)[a]								
Forest litter	3.44a*	+0.08 –0.07	6.03b	+0.26 –0.16	3.58a	+0.32 –0.18	4.61a	+0.45 –0.22
Mineral topsoil	4.23a	+0.10 –0.08	4.22a	+0.28 –0.17	4.28a	+0.22 –0.15	4.31a	+0.09 –0.08
2004 (2 years)								
Forest litter	3.49a	+0.27 –0.17	5.70b	+0.64 –0.25	3.47a	+0.52 –0.23	4.68a	+0.23 –0.15
Mineral topsoil	4.17a	+0.21 –0.14	4.25a	+0.16 –0.12	4.18a	+0.25 –0.16	4.24a	+0.21 –0.14

*Averages marked with the same letter within the soil layer are not significantly different at significance level $p<0.05$.
[a] The data for comparison are taken from Ozolincius *et al*., 2005.

The concentrations of total Ca significantly increased from 3.50±0.60 g kg^{-1} (the control) to 15.90±1.29 g kg^{-1} in the forest litter after 2 years in 5.0 t ash ha^{-1} treated plots (Table 2). Similarly, wood ash increased the concentrations of P by 1.4 fold, K– by 2.7 fold, Mg – by 3.5 fold compared with the control.

Contrary, N concentration decreased by 1.4 fold after wood ash application. The decreased soil acidity seemed to have intensified the microbial activity and nitrification. It possibly resulted in NO_3^- leaching to the deeper soil horizons, and ground vegetation and trees possibly have taken up a part of nitrates (Kahl *et al*. 1996 and Högbom *et al*. 2001).

The wood ash application together with N fertilizer significantly increased the concentrations of P, K and Mg, but had no influence on the N concentrations in forest litter (Table 2). Comparing with the results obtained 3 months after the treatment (Ozolincius *et al*. 2005), only very small differences between two measurements were indicated. The difference from the control for P, Ca and Mg was higher after 3 months when 5.0 t ha^{-1} of wood ash were applied to compare with the data obtained after 2 years (Table 2).

Chemical analyses of soil solution showed leaching of chemical substances from the upper to the deeper soil horizons after the application of wood ash. Higher nutrient concentrations were detected in soil solution in below fine root zone (50 cm depth) compared with the rooting zone (20 cm depth), and this indicated a higher element output.

Even the highest wood ash dose did not significantly affect soil solution pH, still it has increased from 5.0 (the control) to 6.2 (5.0 t ash ha^{-1}) during the first 2 years after the treatment (data not shown). However, the main pH changes were determined during the second year after treatment when soil solution pH increased by 0.9–1.2 pH units compared with the control. N fertilizer insignificantly decreased soil solution

Table 2. Total concentrations of some macronutrients in forest litter 3 months and 2 years after the application of wood ash and N fertilizers. Mean values are followed by SE, n=4

Variant of experiment	N	P	K	Ca	Mg
	g kg^{-1}				
2002 (3 months)[a]					
Control	12.02±0.15	0.64±0.06	2.90±0.24	4.10±0.16	0.66±0.06
5 t ash ha^{-1}	9.45±0.58*	1.10±0.02*	7.40±0.58*	28.88±1.68*	4.15±0.36*
180 kg N ha^{-1}	11.91±0.55	0.66±0.04	2.18±0.11	4.60±0.71	0.62±0.07
(2.5 t ash+180 kg N) ha^{-1}	10.62±0.55	0.90±0.12*	4.78±0.35*	14.55±1.98*	1.96±0.17*
2004 (2 years)					
Control	12.73±0.13	0.81±0.03	0.66±0.02	3.50±0.60	0.48±0.06
5 t ash ha^{-1}	9.40±0.55*	1.11±0.12*	1.80±0.20*	15.90±1.29*	1.67±0.13*
180 kg N ha^{-1}	11.67±1.60	0.70±0.11	0.53±0.07	2.93±0.93	0.43±0.11
(2.5 t ash+180 kg N) ha^{-1}	11.07±0.61	0.89±0.02*	0.99±0.06*	9.30±0.68*	1.01±0.11*

* Significant difference from the control at significance level p<0.05
[a] The data for comparison are taken from Ozolincius *et al*., 2005.

pH, but the wood ash applied in combination with N fertilizers reduced acidifying impact of nitrogen at 20 cm depth, and gave no response at 50 cm depth (data not shown).

Wood ash had no effect on the NO_3^- concentrations in the soil solution neither 15 months nor 2 years following the treatment (Table 3). After the application of N fertilizers, however, the NO_3^- concentration increased by several folds at 20–50 cm depths indicating very fast nitrate leaching. The wood ash applied together with N fertilizers also increased the NO_3^- concentration by 5-folds both at 20 and 50 cm depths. Thus, our data could not confirm that wood ash application would cause higher NO_3^- concentrations in soil solution and thus increase the risk of leaching (Saarsalmi, Mälkönen 2001, Nilsen 2001, Nohrstedt 2001 and Gundersen *et al*. 2006). Otherwise, the amount of NO_3^- is reduced by active roots uptake and N immobilization by microorganisms (Vestgarden *et al*. 2003).

Table 3. The mean concentrations of NO_3^-, K^+, Ca^{2+} and Mg^{2+} at 20 and 50 cm depths after the application of wood ash and N fertilizers 15 months and 2 years after the treatment (n=4)

Variant of experiment	NO_3^-	K^+	Ca^{2+}	Mg^{2+}
	mg L^{-1}			
20 cm depth				
15 months after treatment				
Control	0.36±0.03	0.65±0.06	4.1±0.3	0.97±0.08
1.25 t ash ha^{-1}	0.33±0.01	1.36±0.18*	5.5±1.0	1.44±0.23
2.5 t ash ha^{-1}	0.34±0.01	0.47±0.14	5.3±0.4	1.47±0.19
5 t ash ha^{-1}	0.36±0.02	0.96±0.12	9.9±1.8*	2.24±0.37*
180 kg N ha^{-1}	0.89±0.51	0.27±0.20	3.3±0.3	0.76±0.09
(2.5 t ash +180 kg N)ha^{-1}	0.66±0.21	0.96±0.17	5.6±0.7	1.40±0.07
2 years after treatment				
Control	0.82±0.20	1.42±0.23	3.18±0.52	0.84±0.08
1.25 t ash ha^{-1}	0.88±0.23	1.03±0.11	3.70±0.39	1.08±1.05
2.5 t ash ha^{-1}	0.87±0.20	1.68±0.46	5.22±0.41*	1.53±0.21*
5 t ash ha^{-1}	1.37±0.43	0.96±0.40	5.17±1.70*	1.47±0.27*
180 kg N ha^{-1}	2.10±1.06	2.44±0.76	3.18±0.36	0.91±0.14
(2.5 t ash +180 kg N)ha^{-1}	4.35±1.33*	2.36±0.30	6.36±1.37*	1.73±0.18*
50 cm depth				
15 months after treatment				
Control	0.35±0.02	1.30±0.24	3.5±0.2	1.11±0.19
1.25 t ash ha^{-1}	0.37±0.03	1.22±0.25	3.6±0.3	1.11±0.07
2.5 t ash ha^{-1}	0.35±0.01	0.92±0.07	6.0±0.3*	1.59±0.23
5 t ash ha^{-1}	0.37±0.03	0.92±0.11	5.9±0.6*	1.90±0.24*
180 kg N ha^{-1}	1.11±0.51*	0.54±0.16	4.2±0.6	0.78±0.03
(2.5 t ash +180 kg N)ha^{-1}	0.92±0.31*	1.46±0.28	6.9±1.2*	1.62±0.29
2 years after treatment				
Control	0.86±0.15	0.85±0.31	2.81±0.22	0.83±0.12
1.25 t ash ha^{-1}	0.81±0.07	0.84±0.08	2.76±0.26	0.93±0.07
2.5 t ash ha^{-1}	1.59±0.53	0.80±0.15	4.33±0.92*	1.43±0.23
5 t ash ha^{-1}	1.12±0.33	0.83±0.34	3.80±0.32*	1.75±0.06*
180 kg N ha^{-1}	8.71±5.92*	1.10±0.48	7.49±4.52*	2.20±1.41*
(2.5 t ash +180 kg N)ha^{-1}	4.41±2.48*	1.46±0.28	7.51±1.87*	1.85±0.24*

* Significant difference from the control at significance level p<0.05

The K^+ concentration at 20 cm depth increased from 0.65±0.06 mg L^{-1} (control) up to 0.96±0.12 mg L^{-1} (5.0 t ash ha^{-1}) 15 months after the treatment, yet no changes were found after 2 years (Table 3). Our data showed that K^+ could be leached more intensively than P, which confirmed earlier studies (Frank and Stuanes 2003, Saarsalmi *et al*. 2004 and Ozolincius *et al*. 2005). Wood ash, as well as N fertilizers increased the downward transport of exchangeable Ca^{2+} during the first year after the treatment (Ozolincius *et al*. 2005). The difference from the control by 1.4–1.6 fold was also evident after 2 years (Table 3). Similar tendencies of wood ash effects were indicated for Mg, which concentrations increased by 1.7–2.3 fold during the second year after treatment. Our findings reflect well the specified order K>Mg>Ca>P of the solubility and the potential plant availability of the macronutrients in the wood ash.

2. Changes of soil microflora and mycorrhiza

The mentioned chemical changes in forest litter and mineral topsoil, and intensified downward transport of some substances to the deeper soil horizons subsequently caused changes in biological parameters, such as soil microflora and mycorrhiza.

Significant quantitative changes in forest litter microflora were observed 3 months after wood ash application. The number of the ammonifying microorganisms increased from 6 (1.25 t ash ha^{-1}) up to 22 fold (5.0 t ash ha^{-1}) (Table 4). The increase in ammonifying microorganisms in the plots treated by pure N was, however, not significant. So, this phenomenon could probably be explained by the decreased acidity of forest litter (Table 1). The main changes caused by forest litter pH increase (alkalization) were determined

Table 4. The number of ammonifying microorganisms 3 months after the application of wood ash. Mean values are followed by SE, *n*=4

Variant of experiment	Number of microorganisms, mill g^{-1} (%)							
	Total		Bacteria		Actinomycetes		Micromycetes	
Control	9.4±0.8 (100)	a*	8.2±0.7 (87.2)	a	0.8±0.1 (8.5)	a	0.40±0.02 (4.3)	c
1.25 t ash ha^{-1}	57.6±3.9 (100)	c	51.8±3.9 (90.0)	c	5.4±0.7 (9.4)	c	0.35±0.02 (0.6)	b
2.5 t ash ha^{-1}	98.0±10.2 (100)	d	92.1±10.1 (93.9)	d	5.6±0.5 (5.8)	c	0.28±0.02 (0.3)	a
5 t ash ha^{-1}	204.5±12.3 (100)	e	196.8±12.3 (96.3)	e	7.4±0.8 (3.6)	d	0.27±0.02 (0.1)	a
180 kg N ha^{-1}	15.1±1.6 (100)	b	13.0±1.3 (86.5)	b	1.5±0.5 (10.2)	b	0.50±0.02 (3.3)	d
(2.5 t ash+180 kg N) ha^{-1}	118.5±8.7 (100)	d	109.5±9.9 (92.4)	d	8.7±1.6 (7.3)	d	0.31±0.02 (0.3)	b

* Values marked with the same letter within the treatments are not significantly different at significance level $p<0.05$.

for bacteria. The number of bacteria increased by 6–24 fold, while the number of actinomycetes increased by 7–10 fold. There were no changes in the number of micromycetes that usually are very tolerant to environmental changes.

Under the influence of wood ash the structure of microorganisms' communities had slightly changed. In 3 months the proportion of bacteria increased from 87% in the control up to 96% in the 5.0 t ash ha^{-1} plot. The proportion of micromycetes decreased from 4% (control) to 0.1% (5.0 t ash ha^{-1}). The percentage of actinomycetes did not change. Such a situation leads to the assumption that there is a possibility for the accumulation of incompletely decomposed organic material because bacteria usually participate only at the first stage of litterfall decomposition.

Actinomycetes and micromycetes, being able to decompose more complex substances, together showed a relative decrease. However, the reduced activity of these organisms could be compensated by sporic *bacteria bacilli*, which are able to decompose complex organic residues. The amount of these bacteria increased more than 10 fold (from 6 fold in the 1.25 t ash ha^{-1} plot up to 20 fold in the 5.0 t ash ha^{-1} plot) in the forest litter after wood ash application (Table 5).

Unexpectedly unusual abundance of non-sporic bacteria belonging to the *Pseudomonas herbicola* group (these bacteria characterized by yellow pigmentation on freshly dead organic material) was determined in the wood ash plots. The *Pseudomonas herbicola* group, being very sensitive to the acidity of substratum, were therefore not detected in the acid forest litter characteristic of the control and the variant 180 kg N ha^{-1} (Table 5).

The number of nitrifiers (nitrifying bacteria) in the forest litter was small in all plots. The largest number of them was found in the plots 5.0 t ash ha^{-1} and (2.5 t ash + 180 kg N) ha^{-1}. However, here also, the largest number of denitrifying bacteria was recorded. That leads to the assumption that a certain amount of N could be lost during the process of denitrification preconditioned by decreased soil acidity.

Practically, in all variants treated by wood ash the number of cellulose-decomposing bacteria in the forest litter increased by several folds, and they comprised 97–100% of all cellulose-decomposers, while in the control the bacteria comprised only about 50%.

Wood ash had no influence on microflora of the mineral topsoil (0–10 cm) (data not shown). The main increase in the ammonifying microorganisms occurred

Table 5. Number of microorganisms in forest litter 3 months after the application of wood ash. Mean values are followed by SE, *n*=4

Variant of experiment	Number of microorganisms, thou. g^{-1}									
	Bacilli, mill. g^{-1}		*Pseudomonas herbicola*, mill. g^{-1}		Nitrifiers		Denitrifiers		Cellulose-decomposing (number of bacteria, %)	
Control	0.0±0.0	a*	0.0±0.0	a	0.22±0.13	a	4±1	a	0.7±0.3 (51.1)	a
1.25 t ash ha^{-1}	6.0±1.1	c	6.4±1.9	b	0.22±0.13	a	11±3	b	12.8±1.2 (96.6)	a
2.5 t ash ha^{-1}	11.3±1.6	d	9.8±1.3	b	0.75±0.29	b	19±5	c	18.0±2.2 (99.8)	b
5 t ash ha^{-1}	19.5±2.3	e	25.1±2.4	c	1.39±0.56	bc	167±55	d	39.4 ±5.8 (100.0)	c
180 kg N ha^{-1}	0.9±0.2	b	0.0±0.0	a	0.18±0.12	a	25±10	c	0.8±0.2 (66.8)	a
(2.5 t ash+180 kg N) ha^{-1}	14.8±2.4	d	13.3±2.1	b	1.51±0.40	c	230±20	d	35.0±5.6 (97.6)	c

* Values marked with the same letter within the treatments are not significantly different at significance level $p<0.05$

mostly due to the changes in bacteria quantity, while the amount of actinomycetes and micromycetes remained unchanged. Positive influence of wood ash on the topsoil microorganisms of other studied group was revealed only after the highest wood ash dose application (5.0 t ash ha^{-1}). Here, the number of bacilli increased by about 30%, *Pseudomonas herbicola* bacteria – by several folds.

It was presumed that due to the changes in the topsoil chemical composition and in the number of soil microorganisms and their activity after the application of wood ash would influence the tree growth, and first of all, fine root growth and mycorrhiza. Special attention was paid to plots treated with 2.5 t ha^{-1} and 5.0 t ha^{-1} of wood ash. Morphological indices of fine roots and mycorrhiza one year after the application of wood ash showed no differences in fine root morphology except total length and number of root tips. These two parameters were insignificantly reduced after 5.0 t ash ha^{-1} application (Table 6). Our results corresponded with the data of authors who have recorded that the applications of wood ash can cause the reduction of root tips (Erland and Söderström 1991) and small (diameter 0–1 mm) live roots (Clemensson-Lindell and Persson 1992).

Table 6. The morphological indices, mycorrhiza and vitality of Scots pine fine roots one year after the application of 2.5 and 5.0 t ha^{-1} of wood ash. Mean values are followed by SE

Indices	Variant of experiment		
	Control	2.5 t ash ha^{-1}	5.0 t ash ha^{-1}
Morphological indices of fine roots			
Total length, cm	314.05±13.85	321.78±20.85	291.20±23.70
Surface area, cm^2	52.28±1.48	56.38±3.12	52.68±3.94
Volume, cm^3	0.78±0.03	0.79±0.03	0.77±0.06
Number of tips*	1074.38±103.79	1029.45±69.44	949.38±75.15
Mass, g*	0.31±0.02	0.33±0.02	0.32±0.03
Mycorrhiza (occurrence, % of total)			
White	44.25±7.44	41.00±2.89	32.25±7.66
Light-yellow	12.50±2.84	22.25±8.00	12.50±3.28
Dark	18.00±8.14	31.75±6.09	31.00±6.56
Pink	26.25±9.45	23.75±10.96	30.75±10.73
Coralloid	23.75±5.66	47.25±11.25	42.25±9.15
Cenococcum sp.	33.50±13.23	43.00±19.67	29.33±11.86
Vitality class	3.63±0.10	3.43±0.15	3.20±0.07

* Mean value per middle root sample

Clusters of coralloid mycorrhiza were identified in 42–47% of the samples after the application of 2.5–5.0 t ash ha^{-1}, while it amounted to only 23% in the control. Light-yellow mycorrhiza having smooth thick mantle was identified in 22% of the samples in the plots treated with 2.5 t ash ha^{-1}. However, in the control and the plots treated with 5 t ash ha^{-1}, the light-coloured mycorrhiza made u[12% of the samples analysed. A number of authors hold the light-coloured mycorrhiza to be more "efficient" (Göbl 1963, 1967 and Шемаханова 1962). However, there are indications that the dark-coloured mycorrhiza is superior (Dominik 1965). The dark-coloured mycorrhiza was found in 31–32% of the samples in 2.5 and 5.0 t ash ha^{-1}, while for the control this index was 18 %. The proportion of *Cenococcum sp*. was lower (29% of the samples) in 5.0 t ash ha^{-1} plot, compared with the control and 2.5 t ash ha^{-1}, which was 29–34%. Regarding the occurrence of *Cenococcum sp*., the data of this study agreed with the results obtained in studying the mycorrhization of pine container stock. These authors found the proportion of *Cenococcum sp*. to be higher in the control as compared with the stock planted out in ash-fertilized plots (fertilization dose 7.5 t/ha) (Erland and Söderström 1991).

To adequately describe the fertilization effect, apart from root morphological traits, it is essential to assess root vitality and mycorrhization. The assessment of the vitality of fine roots showed the highest average vitality degree 3.2 for the fertilization dose of 5 t ash ha^{-1}; 3.4 for dose of 2.5 t ash ha^{-1}, and 3.6 for the control.

3. Changes in ground vegetation diversity, tree foliage and litterfall

Following the primary data (Ozolinčius *et al*., in press), wood ash and N fertilizers significantly decreased the cover of bryophyte in the period from 2002 to 2004. The cover of the bryophyte decreased from 98% to 90% in the plots treated with 5.0 t ash ha^{-1}, and from 99% to 92% in the N-fertilized plots. No changes of vascular plant cover were found. Despite the changes of the bryophyte cover, the Shannon diversity index for all treatments has varied from H□=0.7 to 1.2. But no significant differences among treatments were indicated (Fig. 1). The given results showed that neither wood ash nor N fertilizers changed the ground vegetation diversity in the first 2 years experiment period.

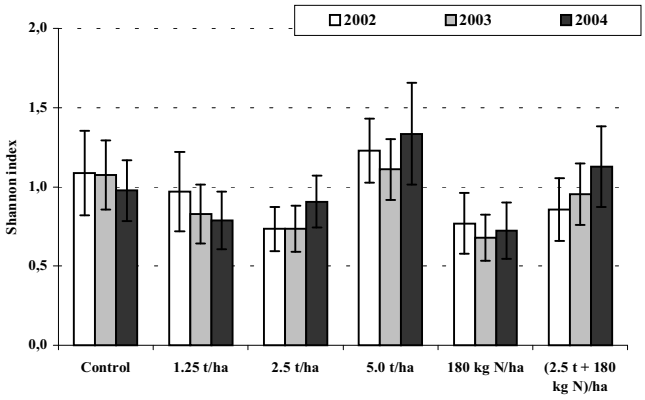

Figure 1. Shannon diversity index of ground vegetation before the experiment (2002), one (2003), and two (2004) years after the application of wood ash and N fertilizers (*n*=4).

Most often analyzing the consequences of wood ash, i.e. the changes in the content of mineral nutrients in the soil and plants, effects on growth, etc., the mechanisms through which wood ash influence plants is still little understood (Mandre and Korsjukov 2002). Initially, the nutrient imbalances or changes of nutrient concentrations as stress factors may cause significant changes on the physiological processes in trees. The concentrations of chlorophyll *a* varied in a narrow range of 1.2–1.3 mg g^{-1}, chlorophyll *b* – 0.3–0.4 mg g^{-1}, carotenoids – 0.40–0.45 mg g^{-1} (Table 7), and calculated ratios of chlorophylls (*a*+*b*)/carotenoids was about 3.5–3.6.

In this study, a significant increase in the chlorophylls was detected in current year needles 3 months after the application of 180 kg N ha^{-1}: the concentration of chlorophyll *a* increased by 0.18 mg g^{-1} and chlorophyll *b* – by 0.15 mg g^{-1} (Table 7). Not significant but still an increase in chlorophyll *a* was indicated in the current year needles when the wood ash in doses of 2.5–5.0 t ha^{-1} was applied, and also in a combination of wood ash together with N fertilizers treatment. There were no significant changes in chlorophylls *a* and *b* in the first year needles (Table 7). Our results partly confirm earlier results received in Estonian studies when wood ash has direct response on the concentrations of the chlorophyll increment (Mandre and Korsjukov 2002). Generally, wood ash comprised of essential plant macronutrients (K, Mg, Ca, P) is treated as the amendment to the soils (Table 2) but at early stage some of these elements signify the pigment synthesis in tree (Mandre and Korsjukov 2002 and Skuodienė 2005).

The concentrations of aminoacid proline significantly increased by 1.5 fold after the application of wood ash together with N fertilizers in the current year needles. Similarly, the pure N treatment also increased this content by 10% (Table 7). However, the concentration of proline decreased by 15% in the plots treated with different (1.25–5.0 t ha^{-1}) wood ash doses.

Wood ash had no significant effect on needle nutrient concentrations, except that a 1.2–1.3 fold increase in the N and Ca concentrations was found in the needles after N fertilizers addition (data not shown).

It is interesting to mention that the concentrations of all chemical elements investigated (N, P, K, Ca), except Mg, were higher in the needles in the plots treated with 180 kg N ha^{-1}. A significant increase by 1.2 fold was similarly detected for Zn in the current year needles. The values of other elements varied within the uncertainty range.

The litterfall study showed neither significant wood ash nor N fertilizer influence on the amount of litter, and its different fractions, and there was no change in the seasonal variations during a 3-year period after the treatment (Fig. 2). The maximum amount of litterfall was formed during an active vegetative period from May to July and comprised about 25–30%

Table 7. The concentrations of chlorophyll *a*, chlorophyll *b*, carotenoids, and amino acid proline in the current and first year Scots pine needles 5 months after the application of wood ash and N fertilizers. Mean values are followed by SE, *n*=4

Variant of experiment	chlorophyll *a*		chlorophyll *b*		Carotenoids		Proline
	current year	first year	current year	first year	current year	first year	current year
	mg g^{-1} DW						mg 100 g^{-1} DW
Control	1.27±0.04	1.29±0.05	0.32±0.03	0.37±0.04	0.42±0.03	0.45±0.02	3.10±0.03
1.25 t ash ha^{-1}	1.28±0.09	1.23±0.09	0.32±0.06	0.30±0.05	0.45±0.03	0.47±0.01	2.70±0.23
2.5 t ash ha^{-1}	1.29±0.05	1.36±0.05	0.30±0.03	0.36±0.04	0.45±0.02	0.46±0.02	2.60±0.02*
5.0 t ash ha^{-1}	1.30±0.03	1.31±0.07	0.32±0.02	0.34±0.04	0.45±0.01	0.46±0.00	2.70±0.50
180 kg N ha^{-1}	1.45±0.04*	1.36±0.06	0.47±0.04*	0.41±0.05	0.44±0.01	0.43±0.03	3.40±0.10*
(2.5+180 kg N) ha^{-1}	1.30±0.05	1.21±0.14	0.34±0.04	0.33±0.08	0.44±0.02	0.45±0.01	4.40±0.40*

* Significant difference from the control at significance level $p<0.05$.

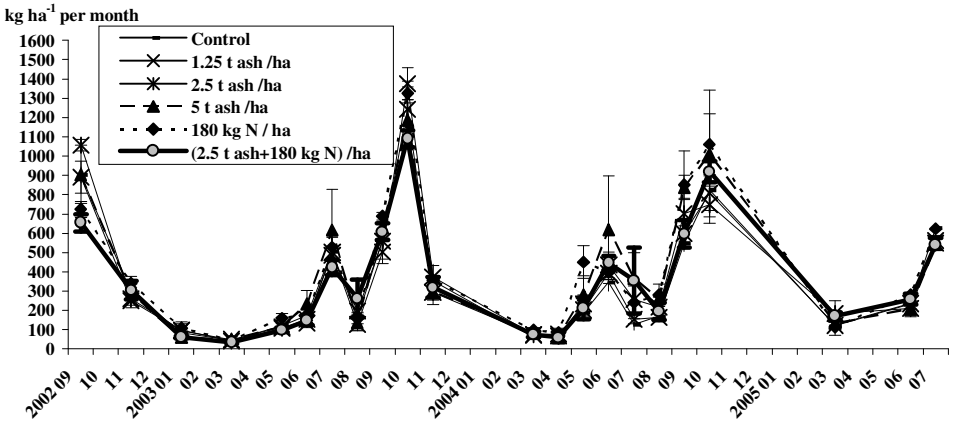

Figure 2. Seasonal changes of litterfall mass (kg ha^{-1} per month) in Scots pine stand (*n*=4)

of total annual litterfall, and in autumn – about 55–65%. This corresponded well with the studies of Vaičys *et al*. (1979), Helmisaari (1992) and Thelin (2000). The total annual litterfall amounted to 3.1 t ha^{-1}, and the needle part comprised about 2.6 t ha^{-1}. Despite the fact that there were no significant differences from the control, the following changes were obtained: 1.25–2.5 t ash ha^{-1} increased total litterfall by 5–9%, 5 t ash ha^{-1} – by 24%, N fertilizers – by 27%, and wood ash applied together with nitrogen – by 8%.

Neither was found any wood ash nor N fertilizer effects on nutrient concentrations in litterfall needles.

Conclusions

1. Wood ash significantly increased soil pH and total concentrations of the most of the macronutrients after 2 years. However total N concentrations decreased due to ash application.

2. Wood ash increased the number of ammonifying, denitrifying microorganisms and cellulose-decomposers in the forest litter 3 months after application. Positive influence of wood ash on the mineral topsoil microorganisms (*bacilli*, *Pseudomonas herbicola* bacteria and denitrifying microorganisms) was determined after the application of the 5.0 t ash ha^{-1}.

3. Wood ash slightly reduced total length of fine roots and the number of root tips one year after treatment. To compare with control, the highest degree of fine roots vitality was found in the plots treated with 2.5–5.0 t ha^{-1} of wood ash.

4. The Shannon diversity index of ground vegetation for wood ash and N fertilizers treatments has varied within the range of H'=0.7–1.2, but no differences in ground vegetation diversity were indicated.

5. The slight increase in chlorophyll *a* was determined in the current year needles 5 months after application of wood ash, but the main increase in chlorophylls was found after the N fertilizer application. Wood ash decreased the concentration of aminoacid proline but it was increased by N addition.

6. Wood ash had no significant effect on the chemical composition of needles. Increased N concentrations in both current and first year needles were determined after the application of N fertilizers.

7. The obtained results showed that the recycling of untreated (raw and dry) wood ash to the forest ecosystem could have primary deleterious effects on soil, ground vegetation and tree foliage.

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ПЕРВИЧНЫЙ ЭФФЕКТ УДОБРЕНИЯ ДРЕВЕСНОЙ ЗОЛОЙ НА ПОЧВУ, ЖИВОЙ НАПОЧВЕННЫЙ ПОКРОВ И ДРЕВЕСНУЮ ЛИСТВУ В ДРЕВОСТОЯХ СОСНЫ ОБЫКНОВЕННОЙ

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Резюме

Эксперимент по удобрению древесной золой был заложен в 38-летнем сосновом древостое (тип леса – *Pinetum vacciniosum*) на песчаной почве. В лесу высыпана сыпучая (нестабилизированная) древесная зола и азотные удобрения. Эксперимент составляет 6 вариантов: 1) 1,25 т га$^{-1}$; 2) 2,5 т га$^{-1}$; 3) 5,0 т га$^{-1}$;4) 2,5 т га$^{-1}$ + 180 кг N га$^{-1}$; 5) 180 кг N га$^{-1}$; 6) контроль. В статье предоставлен первичный эффект удобрения леса древесной золой (3 мес. – 2 года после удобрения) на почву, почвенный раствор, почвенную микрофлору и биологическую активность, мелкие корни и микоризу, разнообразие живого напочвенного покрова, химический состав древесной листвы, физиологические параметры и опад.

Максимальная доза древесной золы (5,0 т га$^{-1}$) изменила химический состав лесной подстилки: через 2 года после удобрения повысилась величина *pH* и суммарные концентрации многих микроэлементов. Однако суммарные концентрации *N* после удобрения снизились. Через 3 месяца в удобренных древесной золой площадках установлена повышенная численность аммонифицирующих, денитрифицирующих и целлюлозоразлагающих микроорганизмов. Древесная зола через 1 год незначительно снизила длину мелких корней и численность корешков. Наивысшая жизнеспособность корешков установлена на участках, удобренных 2,5 – 5,0 т га$^{-1}$ дозой золы.

Разнообразие напочвенного покрова после внесения древесной золы и азота не изменилось. Через 5 мес. после удобрения древесной золой не изменились и концентрации хлорофила *a* и *b*. Зола снизила количество аминокислоты пролина, тогда как азотные удобрения его повысили.

Ключевые слова: древесная зола, сосна обыкновенная, химический состав, микрофлора, микориза, разнообразие напочвенного покрова, древесная листва.