The impact of forest residue removal and wood ash amendment on the growth of the ectomycorrhizal external mycelium

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Abstract

Intensive harvesting of forest residues for energy production may lead to the depletion of organic matter and mineral nutrients in the forest floor. In order to restore nutrient content wood ash has been suggested as a fertiliser. Ectomycorrhizal (EM) fungi are involved in the nutrient uptake of forest trees and this study investigates the influence of intensive harvesting and wood ash fertilisation on the external EM mycelium in forest soil. Nylon mesh bags filled with sand were buried in September 1997 in field plots which had or had not been intensively harvested. The effect of wood ash on the production of external EM mycelium was studied in mesh bags amended with wood ash. Mesh bags were retrieved in May and October 1998. The relative amount of fungal mycelia in the mesh bags was estimated with phospholipid fatty acid analysis. The fungi colonising the mesh bags were mainly (>90%) ectomycorrhizal. Fungal biomass in the mesh bags was low in the spring but high in the autumn. No significant effect on EM fungal biomass was observed in the mesh bags collected from intensively harvested plots compared with those from control plots, but wood ash amendment resulted in 2.4 times more EM fungal biomass (P < 0.05). The effect of external EM mycelium on the dissolution of wood ash was studied in mesh bags filled with wood ash, using mesh bags buried in soil isolated from roots as EM-free controls. The external EM mycelium had no effect on the dissolution rate of the wood ash. 80% of the potassium was lost from the wood ash within a month, whereas no phosphorus was lost during the experimental period (up to 13 months). © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

There is strong inducement to reduce the use of fossil fuels, which will result in an increasing need for renewable energy sources. Forest residues obtained after forest thinning and clear-cutting serve as a source of bioenergy. This intensive harvesting of forest residues has been shown to result in a thinner organic layer in forest soil [1], with a subsequent reduction in available mineral nutrients [2–4], which may lead to a reduction in forest growth in the future [5]. Growth reduction in young trees, which could be circumvented by nitrogen fertilisation, has been detected after intensive harvesting of forest residues [3,6]. In areas where the nitrogen deposition is high, such as southern Sweden [7], a reduction in growth due to nitrogen limitation seems unlikely. In these areas the combined effects of nitrogen deposition, soil acidification and intensive harvesting of forest residues may lead to a greater loss of base cations from the soil, which may have an effect on forest vitality [5].

Ectomycorrhizal (EM) fungi live in symbiosis with trees and are important for the uptake of mineral nutrients [8]. EM fungi have been shown to translocate nitrogen, phosphorus and potassium (reviewed in [9]) as well as magnesium [10] to trees, but the uptake of other mineral nutrients has also been suggested (reviewed in [9]). The nutrients are taken up mainly by the external mycelium and are translocated to the EM root tip [8]. The importance of bacteria in solubilising nutrients from soil minerals has also been recognised [11]. Since the microbiologically mediated release of nutrients from minerals might provide a means of compensating for the loss of nutrients through intensive harvesting, it is important to investigate how these organisms are influenced by intensive harvesting of forest residues.

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Total numbers as well as biomass of EM root tips have been found to decrease in the organic horizon in areas where forest residues had been harvested [1,2]. Due to its importance in nutrient absorption, the amount of external EM mycelium growing out from EM root tips is probably a better indicator of nutrient-absorbing capacity than the number of EM root tips themselves [12,13]. An increase in the production of EM external mycelium was found in laboratory experiments when growing EM pine seedlings under conditions of P deficiency [14]. A similar reduction in the availability of nutrients induced by the intensive harvesting of forest residues may also lead to an increase in the production of external EM mycelium resulting from increased carbon allocation to the EM fungi.

Recycling of wood ash has been proposed as a means of ameliorating the loss of nutrients due to intense harvesting of forest residues [6]. The effect of wood ash fertilisation on EM external mycelium has not been studied previously in the field, but Erland and Söderstroem [15] have reported on EM external mycelium has not been studied previously of forest residues [6]. The effect of wood ash fertilisation increased carbon allocation to the EM fungi. The influence of intensive harvesting of forest residues may also lead to an increase in the production of external EM mycelium resulting from increased carbon allocation to the EM fungi.

The aim of the present study was to investigate the impact of intensive harvesting of forest residues and wood ash amendment on the growth of EM external mycelium. For these purposes we used ingrowth mesh bags to estimate the growth of EM mycelium under field conditions, a method which has been described recently [18]. We also investigated the influence of EM mycelium on the dissolution of hardened wood ash in the field. Bacterial activity and biomass in the mesh bags were also estimated and related to the dissolution of wood ash.

2. Materials and methods

2.1. Study site

The study was conducted during 1997–1999 in Tönnersjöheden Experimental Forest, located in the south-west of Sweden (56°41’N, 4°57’E), where the annual mean temperature is 6.5°C and the annual precipitation 1000 mm. The soil type and texture have been classified as podzol with a sandy–silty till texture. The experimental forest consists of second-generation Norway spruce (*Picea abies* (L.) Karst.) planted in 1961 after clear-cutting.

Residues were removed from plots on four occasions: after clear-cutting in 1961 and during subsequent thinning in 1975, 1986 and 1992. This treatment will be referred to as intensive harvesting (IH). Control plots were thinned at the same time but residues were left on the forest floor. All plots were of the same size (10×15 m) and arranged in a randomised block design [19,20].

2.2. Experimental design

Estimates of EM external mycelium were obtained using ingrowth mesh bags [18]. The study was divided into two experiments.

Experiment 1: Mesh bags were filled with 200 g dw acid-washed sand (Silversand 90, Aschania, Sweden, grain size 0.90 mm) and buried in IH and control plots in three blocks in the experimental forest. To test the effect of wood ash, other mesh bags were filled with 200 g dw acid-washed sand mixed with 4 g dw ground hardened and granulated wood ash (Dahlia, Perstorpf Fjärvårme AB, Sweden, Table 1), particle size 0.050–0.63 mm. The mixture corresponds to a wood ash fertilisation rate of 4000 kg ha⁻¹. In September (autumn) 1997 six pairs of mesh bags, one containing sand and one amended with wood ash (10 cm separation), were buried in the organic layer in each plot (in total 72 bags), with the lower end in the mineral horizon to ensure that fungal mycelia present in the mineral horizon had the possibility to colonise the mesh bags. From each plot three pairs of mesh bags were retrieved in May (spring) and the remaining three pairs in October (autumn) 1998.

Experiment 2: In order to study the solubilisation rate of wood ash, mesh bags were filled with 2–5 g dw of ground hardened and granulated wood ash (Ljungbyverket, Sydkraft Värme Syd AB, Sweden, Table 1), particle size 0.050–0.63 mm. The mesh bags were marked in order to be able to estimate the weight loss and change in chemical composition of each mesh bag individually. Four mesh bags were buried in the organic horizon at a depth of 5 cm in April (spring) 1999 (Fig. 1) in each of six locations in the experimental forest, where no harvesting of forest residues had taken place. In order to obtain mycorrhizal-free controls at those six locations, soil trenching was carried out by inserting plastic tubes (20 cm long, diameter 15 cm) into the ground to cut off the mycorrhizal roots, preventing the growth of the external EM mycelium [18]. Inside each tube four mesh bags containing only ash were buried (Fig. 1). One mesh bag was retrieved from outside and one from inside the tubes at each location after 1, 3 and 5 months. Inside each tube, one mesh bag

<table>
<thead>
<tr>
<th>Element</th>
<th>Experiment 1 (Dahlian) (%)</th>
<th>Experiment 2 (Ljungbyverket) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>14.4</td>
<td>26.0</td>
</tr>
<tr>
<td>K</td>
<td>2.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Mn</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>P</td>
<td>1.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>
containing 2–5 g of acid-washed sand was also buried (Fig. 1) in order to estimate the colonisation by saprophytic mycelium.

### 2.3. Analyses

To determine the proportion of mycorrhizal and saprophytic mycelia in the mesh bags in experiment 1 the δ^{13}C value of the mycelia was determined. This method is based on the higher values of δ^{13}C for fruit bodies from wood-decomposing fungi than that in fruit bodies of EM fungi [21]. The contents of selected mesh bags with sand (n = 4) and with wood ash amendment (n = 6) retrieved in the autumn regardless of IH treatment were mixed with water to make a slurry. Mycelia and rhizomorphs floated to the surface and could be collected on a nylon mesh. These were then dried at 70°C for 24 h. The mycelia were analysed for 12C and 13C abundance (at the Department of Forest Ecology, SLU, Umeå, Sweden) using an online, continuous-flow carbon and nitrogen analyser coupled to an isotope mass spectrometer. The results are expressed in the standard notation (δ^{13}C) in parts per thousand relative to the international standard Vienna Pee Dee Belemnite [21].

The phospholipid fatty acids (PLFAs) were extracted [22,23] from all mesh bags in experiment 1 and from the mesh bags containing sand in experiment 2. The PLFA 18:2ω6,9 was used as an indicator of fungal biomass [24] and the sum of the PLFAs i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, cy17:0, 17:0, 18:1ω7 and cy19:0 was used as an indicator of bacterial biomass [24,25]. The water content of the mesh bags was estimated by measuring the weight loss of subsamples of the contents after drying them for 2 days at 70°C. Bacteria were extracted from the mesh bags in experiment 1 [26,27] and the bacterial suspensions obtained were used for thymidine incorporation [27] and leucine incorporation assays to estimate of bacterial activity [28].

The elemental composition of the two kinds of wood ash used in the experiments was determined by the lithium borate method (ASTM method D3682) at the Department of Plant Ecology, Lund University, Lund, Sweden. Digestion by HNO₃ (as described below) was used to estimate the total contents of selected elements in the wood ash-containing mesh bags in experiment 1 and experiment 2. The solubilisation of Ca, K, Mn and P from wood ash was determined as the change in total contents. The total contents of these elements in the wood ash-amended bags (experiment 1) were further separated by estimating the fractions contained in the fungal mycelium and the exchangeable fractions obtained by extraction with BaCl₂ and sulfate fluoride as described below. The residual fraction of the total content when the mycelial and exchangeable fractions had been subtracted was viewed as an estimate of the more recalcitrant part of the wood ash.

Subsamples of 5 g from mesh bags amended with wood ash (experiment 1) and 0.5 g from mesh bags containing pure wood ash (experiment 2) were digested with concentrated HNO₃ for 1 week. The solutions were subsequently diluted 1:1 and the concentrations of Ca, K, Mn and P were analysed using inductively coupled plasma emission spectrometry (ICP-ES). The sand contained 76 μg g⁻¹ dw Ca, 38 μg g⁻¹ dw K, 8 μg g⁻¹ dw Mn and 13 μg g⁻¹ dw P. Since the sand was regarded as inert, those figures were subtracted from the measured HNO₃-digestible contents of mesh bags in experiment 1 and the sum was viewed as the total content of the element.

The amount of Ca, K, Mn and P in the mycelia in the wood ash-containing mesh bags (experiment 1) was calculated from the amount of PLFA 18:2ω6,9 in those mesh bags and the elemental content in mycelia collected from mesh bags containing pure sand (experiment 1). The mycelia were collected from subsamples of 11 mesh bags with pure sand and care was taken to remove all sand particles. After obtaining the dry weight, eight of the samples were digested with HNO₃ and analysed as described above, and the content of the PLFA 18:2ω6,9 was analysed on the remaining three samples.

The amount of exchangeable Ca, K and Mn in the mycelia in the wood ash amended with wood ash in experiment 1 and in acid-washed sand was estimated by BaCl₂ extraction [29]. Subsamples of 25 g were extracted with 100 ml 0.1 M BaCl₂ for 1 h. The pH of the solutions was measured and the concentration of the elements were analysed using ICP-ES.

The amount of exchangeable P was estimated by sulfate fluoride extraction. Subsamples of 15 g from mesh bags amended with wood ash (experiment 1) or acid-washed sand were extracted for 1 h in 70 ml 0.05 M Na₂SO₄ and 0.02 M NaF. The solutions were filtered through a 25-mm RC filter (Lida Manufacturing, Windsor, UK) and were kept at −20°C until analysed. Phosphate concentration in the filtrate was determined using SnCl₂ as a reducing agent [30].
2.4. Statistical calculations

The effects of IH and wood ash amendment in experiment 1 were evaluated with a two-way analysis of variance (ANOVA) or Student’s t-test when testing for wood ash amendment only, using the software SPSS 10.0 (SPSS, Chicago, IL, USA). Only the autumn values were tested due to low values of mycelial production in the mesh bags in the spring. Heterogeneity of variance was detected in the values of fungal PLFA, bacterial PLFAs and thymidine incorporation, and the logarithmic values of the data were used in the ANOVA [31]. No significant interactions between IH and wood ash amendment were found.

3. Results

The mean value of δ13C for the mycelia collected from the wood ash-containing mesh bags in experiment 1 was $-26.2 \pm 0.2 \%e$, which did not significantly differ from the mean value for the sand mesh bags, $-25.4 \pm 0.3 \%e$, indicating that the mesh bags had been colonised by the same trophic group of fungi. In mesh bags containing sand retrieved from trenched soil (experiment 2), the amount of the PLFA 18:2ω6,9 was 0.05 nmol g$^{-1}$ dw and remained constant during the experimental period (presented as a line in Fig. 2A). The amount of mycelia that could be collected from these bags was much too small to allow an analysis of the δ13C value.

Colonisation of the mesh bags by EM fungi was higher in the autumn than in the spring (Fig. 2A). IH had no significant influence on the fungal colonisation of the mesh bags, but the addition of wood ash stimulated fungal ingrowth (Fig. 2A). In the autumn harvest, the fungal biomass had increased 2.4 times (ANOVA, $P < 0.001$) in mesh bags to which wood ash had been added compared with the bags containing pure sand.

Bacterial colonisation of the mesh bags was also higher in the autumn than in the spring (Fig. 2B). Samples collected in the autumn showed a significant increase in bacterial colonisation due to wood ash amendment in bags buried in IH plots ($P < 0.001$, Fig. 2B). The bacterial colonisation of the sand mesh bags from trenched soil (experiment 2) was 0.1 nmol g$^{-1}$ dw (presented as a line in Fig. 2B). The relative bacterial activity in the autumn, measured as the rate of thymidine incorporation, was $0.3 \pm 0.1$ fmol h$^{-1}$ g$^{-1}$ dw in the mesh bags with wood ash addition, which was significantly higher ($P < 0.001$) than the activity in the sand mesh bags ($0.04 \pm 0.01$ fmol h$^{-1}$ g$^{-1}$ dw). IH had no influence on bacterial activity in the mesh bags. Bacterial activity estimated as the incorporation rate of leucine (data not shown) gave the same pattern as that of the thymidine incorporation.

The pH was 6.7 ± 0.1 in the wood ash-amended mesh bags in the spring samples and 5.9 ± 0.1 in the autumn samples. The pH in the sand-containing mesh bags did not change over time and had an average value of 4.2 ± 0.0. Wood ash addition increased the water content of the mesh bags from 0.5 ± 0.0% to 0.9 ± 0.1% ($P < 0.001$).

IH did not affect the release of any of the elements in the wood ash in experiment 1 (Fig. 3). Neither had colonisation of EM mycelium any influence on the release of the elements in the wood ash in experiment 2 (values for K shown in Fig. 4).

Potassium was rapidly lost from the mesh bags containing wood ash in both experiments 1 and 2 (Figs. 3A and 4). As little as 20% remained in the mesh bag after 1 month in the forest soil in experiment 2 (Fig. 4). The release of Ca and Mn from the wood ash showed opposite trends in the two experiments. In experiment 1 (where the wood ash was mixed with sand), Ca and Mn were rapidly lost from the ash (Fig. 3B,C), while in experiment 2 (pure wood ash) the contents of both these elements remained constant in the wood ash throughout the experiment (data not shown). The amount of P remained constant in the mesh bags throughout both experiments (Fig. 3D, data...
not shown for experiment 2). The total mass of the wood ash was reduced to 80% of the original mass within a month in experiment 2, but remained constant throughout the rest of the experiment.

The exchangeable fractions of Ca and K were high in the original mixture in experiment 1 compared to the field sample, whereas the exchangeable fractions of Mn and P were higher in the field samples (Fig. 3). The exchangeable fractions of the elements were at the same level in both the spring and autumn (Fig. 3). After acid wash 17 \( \text{g} \cdot \text{g}^{-1} \) Ca, 0.40 \( \text{g} \cdot \text{g}^{-1} \) K and 0.33 \( \text{g} \cdot \text{g}^{-1} \) Mn could be extracted from the sand with BaCl\(_2\) and 0.21 \( \text{g} \cdot \text{g}^{-1} \) P with sulfate fluoride. Sand only contributed with a minor part (<5%) to the exchangeable K and Ca in the original mixture of wood ash and sand, whereas all exchangeable Mn and P in the original mixture could be ascribed to the sand. The EM mycelium contributed significantly to the total contents of K and P (13–30% of the total content) in the mesh bags amended with wood ash in the autumn, whereas the mycelium contributed very little to the total contents of Ca and Mn (Fig. 3).

### 4. Discussion

The values of \( \delta^{13} \)C for the mycelia in the mesh bags correspond to those obtained from ectomycorrhizal fruit bodies (−25.7 ± 0.6‰), while they are different from those obtained from saprophytic fruit bodies (−23.6 ± 0.3‰) in the same forest [18]. The values of \( \delta^{13} \)C for the mycelia in this study were also in the same range as those recently reported for EM fruit bodies from other areas [21,32]. This shows that the majority of the mycelium in the mesh bags was of EM origin. Three EM fungi (\textit{Amanita citrina}, \textit{Cortinarius} sp. 1 [1] and an unidentified fungus) were identified by molecular analysis of selected rhizomorphs collected from the same mesh bags (S. Mahmood, personal communication). Wood ash amendment did not significantly influence the proportion
of saprophytic or mycorrhizal mycelia in the mesh bags, based on the δ13C values.

The amount of fungal biomass in mesh bags containing sand collected from trenched soils in experiment 2 was of a similar level as the background values for production of saprophytic mycelium in trenched soil found by Wallander et al. [18]. Since it remained constant even in the autumn samplings, when the production of EM mycelium is largest, this shows that the mesh bags collected from the trenched soil (experiment 2) could be regarded as EM-free controls. The background production was 10% of the mycelial production found in the autumn samples in experiment 1. This suggests that 90% of the mycelium in the mesh bags was of EM origin at the autumn sampling.

Mesh bags collected in the spring (experiment 1) contained low amounts of fungal biomass compared with samples collected in the autumn. The low fungal biomass in the spring led us to focus on the autumn samples when evaluating the effects of wood ash amendment and forest residue harvesting on the growth of external EM mycelium in the present study.

Intensive harvesting of forest residues had no influence on the production of EM mycelium in the present study, but earlier studies have shown that the numbers of mycorrhizal root tips are reduced in IH plots [1]. The results can be explained either by an increased flow of carbon over the mycorrhizal roots where forest residues have been harvested, or by a higher production of root tips in the mineral soil following intensive harvesting leading to a sustained production of EM mycelium.

Calculations have shown that 60–70% of the store of base cations was lost between 1927 and 1984 at Tönnersjöheden [33]. Nevertheless, the increase in the production of EM mycelium in the mesh bags induced by wood ash amendment is probably not a result of growth limitation by base cations or phosphorus in the forest soil, since soil microorganisms are generally regarded as being carbon-limited [34]. In addition, there was no stimulation of mycelial production in wood ash-amended mesh bags collected from IH plots, where one can expect a lower availability of base cations and phosphorus [4], compared to the wood ash-amended mesh bags from control plots. Some EM fungi are able to respond to locally amended wood ash patches by forming a dense mycelium through changing the allocation pattern of carbon within the fungus [17]. A similar response has been found when EM fungi grow in patches with increased pH [35] or colonise patches of organic matter in the soil [36–38]. A dense mycelium is believed to promote the release and uptake of nutrients [39]. If the wood ash-amended mesh bags are to a larger extent colonised by EM fungi forming dense mycelia, this could explain the increased mycelial production of those bags. A community study of the mycelia found in the mesh bags would reveal if this is the case. The increase in the water-holding capacity due to wood ash amendment may also make the bags more favourable for fungal colonisation during dry periods than the mesh bags containing only sand [40].

The presence of EM mycelium did not influence the dissolution rate of wood ash in the field. Potassium was found to be rapidly lost from the wood ash, irrespective of the presence of EM mycelium. Potassium released from wood ash may still be effectively absorbed by the EM mycelium, and it is likely that a functional EM mycelium is important in reducing the loss of K to the groundwater when wood ash is used as a fertiliser. EM fungi have been found to translocate relatively high amounts of K to plants growing under K limitation [41] and it may therefore be recommended that wood ash be spread during the autumn when the growth of the EM mycelium is most active.

The phosphorus content of the mesh bags amended with wood ash remained constant in both experiment 1 and experiment 2. Phosphorus is present in hardened wood ash mainly in the form of hydroxyapatite [42], which makes it recalcitrant to solubilisation. Earlier field experiments on wood ash dissolution have shown no or small decreases in total P content [43] and even slight increases in P content [44] during the first few years after use. In laboratory systems the weathering rate of apatite due to EM fungi has been estimated to be 1% per year [45]. The experiments in this study were therefore too short to detect any significant reduction in the phosphorus content of wood ash induced by EM mycelium.

The contrasting results for Ca and Mn release (net release in experiment 1 and no release in experiment 2) may be due to a higher pH in pure wood ash in experiment 2 than in the sand/ash mixture used in experiment 1. At a pH of 10–11, Ca readily precipitates in complex with phosphate or carbonate [46]. The different release patterns could also be due to some chemical or physical difference between the two batches of wood ash used.

The decrease in the total contents of K, Ca and Mn in wood ash-amended mesh bags in experiment 1 was greater than the BaCl2-extractable fractions of each element in the original mixture of wood ash and sand (Fig. 3). The loss of these elements from the wood ash must thus have been due to solubilisation of wood ash, rather than an exchange of easily available ions.

Bacterial activity and colonisation of the mesh bags seem to be an effect of high colonisation by fungal mycelium. The ratio of fungal to bacterial PLFAs in the mesh bags in experiment 1 is 0.8, which is high compared with the more normal value of 0.3 for forest soils [24], whereas in the mesh bags containing sand from the trenched soil (experiment 2) the production of mycelium was low as was bacterial colonisation (Fig. 2). Since there was no difference in the dissolution rate of wood ash between trenched and untreated soil (experiment 2), this also shows that bacteria do not influence the dissolution of wood ash either. Therefore, it appears that the short-term dissolution of wood ash is of more chemical than biological origin.
This study has shown that intensive harvesting of forest residues does not affect the production of EM mycelium in the forest soil. Amendment of wood ash locally stimulated the production of EM external mycelium in those patches, although the mycelium did not participate in the solubilisation of the ash (up to 13 months). Growth of EM mycelium is most pronounced during the autumn, which suggests that this is the best time to spread wood ash in forests to avoid leakage of easily solubilised elements such as potassium.

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rates in pure culture at different pH values compared to growth rates in symbiosis with the host plant. New Phytol. 115, 683–688.


