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Microbial communities after wood ash fertilization in a boreal drained peatland forest

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ABSTRACT

Wood ash contains all the essential plant nutrients except nitrogen, and thus supports tree growth especially on peatlands where mineral nutrients often are in short supply. Here we report the effects of wood ash fertilization on the soil microbial community in a boreal drained peatland, characterized by phospholipid fatty acid (PLFA) composition, and more specifically the fungal community, characterized with PCR-DGGE fingerprinting and sequencing methods. We investigated the microbial communities in unfertilized and ash-fertilized sample plots (loose wood ash 5000 or 15 000 kg ha^{-1}) 6 years after fertilization. Microbial function measured as heterotrophic basal respiration increased over 30% in the surface peat layer after wood ash fertilization, yet the result was statistically insignificant. The higher amount of wood ash induced a shift in the microbial community in the surface peat layer (0–10 cm) along with an increase in pH. A higher relative proportion of fungal-specific PLFA 18:2ω6 and an increased fungal:bacterial ratio indicated that fungi benefited from the higher amount of wood ash in the topmost layer. Also, the lower amount of wood ash seemed to have stimulated actinobacteria. Our results indicated that higher pH and extra nutrients due to wood ash fertilization promoted the appearance of mycorrhizal and wood-decomposing fungi directly or via increased tree growth. We suggest that wood ash as a fertilizer would be a natural and economical choice to improve the nutrient status of drained peatlands used for forestry since it maintains the positive feedback between fungi and trees.

1. Introduction

Climate change has increased the demand for the use of renewable energy sources, such as forest residue combustion for biofuels, which generates large amounts of wood ash as a byproduct. The use of wood ash as fertilizer has increased considerably; e.g., currently ash is spread annually on nearly 10,000 ha of forest land (i.e. nearly one third of the total fertilized area) in Finland, mostly on drained peatland forests [1]. On peatland forests wood ash is utilized to increase tree growth for further biomass production since it contains considerable amounts of mineral nutrients and raises the soil pH, the cation exchange capacity and the base saturation in the soil [2–5]. Mineral nutrients, such as phosphorus (P) and potassium (K), often limit tree growth on peat soils as these generally are in short supply and not supplied by the weathering of minerals as on mineral soils [6]. Thus, the response of tree growth to ash fertilization is generally stronger in peatland forests than in mineral-soil forests, which often are limited by the soil nitrogen (N) supply and not directly affected by ash fertilization [2].

The soil microbial communities are essential in the regulation of the nutrient cycling in forests; they may both immobilize and release nutrients through the decomposition of organic matter and mycorrhizal interactions. Thus, it is crucial to understand responses of the microbial communities to ash fertilization and the consequent impacts on the nutrient cycling. Any changes in the microbial community may also have effects on the carbon (C) exchange and the greenhouse gas emissions from the soil. The increasing effect of pH, which also follows ash application, is suggested to be especially strong in organic soils [7], which may alone have a strong effect on the microbes, since pH has been detected as one of the most influential factors controlling microbial community composition across soils types [8–10].

The effects of wood ash on microbial activity or community have been extensively studied in mineral soils using several different techniques, e.g., SIR [11–13], PLFA [13,14], soil enzyme activities

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[15], fumigation-extraction [12,15] and bacterial growth rates as ^{3}H-thymidine incorporation [16]. However, the major constraints for ecosystem functioning - temperature, moisture, and nutrient regimes - as well as the soil physical, chemical and biological properties fundamentally differ between peat and mineral soils [e.g., 6]. Thus the microbial responses in organic soils may also differ from those in mineral soils. For example, the wood ash-induced changes in the biogeochemical cycles, e.g., an increase of soil organic matter mineralization in organic soils can last for a long time [2,7]. However, there are only a few studies which report the response of microbes, either community or activity, to ash fertilization in peatland forests.

The earlier studies from Finnish peatland forests revealed increased microbial activity after ash fertilization measured as soil respiration rates both in short term (1–4 years) and long term (13 years) [17,18], as increased decomposition rates of needles [19,20], and as increased decomposition of cellulose in the soil [21–24]. Consequently, these results indicate a faster C turnover rate in the soil ecosystem. More recently, various effects of wood ash application on the microbial community and its activity in peatland forests were reported from Southern Sweden without consistent results on PLFA composition, microbial biomass or processes [25]. However, the background conditions, e.g., the time since fertilization and the site nutrient status varied considerably. Thus, there is currently no comprehensive understanding of the effect of wood ash on the microbial communities that inhabit peatland forests soils.

It would be expected that wood ash directly or indirectly promotes those populations within of the microbial community, like mycorrhizal fungi, that are able to solubilize and transfer compounds originating from the wood ash for tree growth. Another feedback would likely occur when increased litter input due to enhanced tree growth in turn attracts those microbial community member groups that are responsible for decomposition. Also the wood-ash-induced increase in mineralization of organic matter influences the microbial community [16]. Yet, wood-ash-induced effects may be diverse since the observed responses may be linked to many factors, e.g., fertility of site, amount or type of wood ash (loose or hardened), time elapsed since wood ash fertilization, or specific microbial taxa. Therefore, there is a need for more knowledge about wood ash fertilization and its effects, especially in different types of peatland forests, before a consistent synthesis may be made, as also suggested by the authors of the recent review [2].

We investigated the microbial communities in unfertilized and ash-fertilized plots (loose wood ash 5000 or 15 000 kg ha^{-1}) in the 0–50 cm surface peat layer of a mesotrophic peatland forest 6 years after fertilization. We used two different cultivation-independent techniques to detect microbial community changes. The analysis of PLFAs of the cell membranes was used to characterize the total microbial community [26]. PLFAs of bacteria as well as fungi were detected as group-specific indicators of the microbial community. Fungi are suggested to be the most important organism groups in oxic peat soils, mostly because of their diverse ability to decompose a variety of peat-derived recalcitrant compounds [27]. Therefore, a PCR-DGGE (denaturing gradient gel electrophoresis) fingerprinting method with partial fungal 18S rRNA gene as a marker was further used to analyze the fungal community. In order to identify the members of the fungal community, dominant fungal DGGE bands from each of the representative control and sample plots were excised and sequenced. The objective of our study was to detect whether wood ash fertilization induces changes in 1) the activity and structure of the total microbial community and more specifically 2) the soil fungal community of a boreal drained peatland forest site.

2. Material and methods

2.1. Site description and sampling

The study site was located on a drained peatland in Muhos, Pelso (64° 29′ 32″N, 26° 18′ 34″E, ca. 110 m above sea level). The site that in its pristine state had been a mesotrophic 'herb-rich sedge birch-pine fen' [28] had initially been drained in the 1950s followed by supplementary drainage in 1994. Consequently, the site had developed in its post-drainage succession towards a '*Vaccinium myrtillus* II' forest site type (MtkgII) [29,6]. The total N concentration in the surface peat was accordingly relatively high, ranging from 2.7 to 3.2% (of dry mass), and therefore the site was classified as relatively fertile. The peat layer thickness exceeded 100 cm. Scots pine (*Pinus sylvestris* L.) seedlings had been planted on the site in 1960. During the following 50 years, the pines were interspersed by naturally regenerated downy birch *(Betula pubescens* Ehrh.) (<20% of the stand volume). Water-levels at sampling time were over 75 cm below the surface throughout the site.

The experimental design followed the principles of randomized blocks and consisted of control (C) plots and two wood ash fertilization treatments, 5000 (A5) and 15 000 kg ha $^{-1}$ (A15). Both the control and the treatment plots had four replications. The plot size was 30 × 30 m. The ash was spread manually in May–June 1997. The loose wood ash applied originated from Metsä-Botnia's Äänekoski pulp mill. To give an overview of the chemical changes that had taken place over four years in the soils of the treatment plots as compared to the control plots, the soil element contents determined from the plots in 2001 are presented in Supplementary data (Table S1). Growth of the tree stand had been monitored since 1994 and in the sampling year 2003, it was 3.7, 10.7 and 10.9 m^3 ha^{-1} yr^{-1} for the C, A5 and A15 plots, respectively.

Soil sampling for microbial analyses in this study was conducted in October 2003, 6 years after the ash fertilization. Soil cores (7 × 7 cm) were extracted down to the depth of 50 cm in all four replicate plots of C, A5 and A15. One core per plot was taken at 3 m in a random direction from the center point of the plot. The green living or fresh litter containing surface was removed before the cores were divided into 10 cm sections by depth (L1, 0–10 cm; L2, 10–20 cm; L3, 20–30 cm; L4, 30–40 cm; L5, 40–50 cm); these sections will be called "samples". The final number of samples was 60 including the control and two ash fertilized plots (n = 3), replicate soils cores (n = 4), and sampled peat depths (n = 5). Each sample was divided into sub-samples for specific analyses. One set of sub-samples were frozen (−20 °C) for DNA analyses. The sub-samples for PLFA analysis were stored at +4 °C. The sub-samples for basal respiration measurements, which were conducted within one week, were stored at +14 °C. The water content of the soil was determined by drying one set of sub-samples at 105 °C overnight. The soil pH was determined in distilled water (soil:water 1:3, vol/vol).

2.2. Basal respiration measurement and PLFA analyses of peat soil

The basal respiration (BR) rates were measured from 6 ml of fresh peat soil (put into a 10-ml cut-end syringe and inserted into a 120-ml incubation bottle) as the amount of CO_2 evolved after 24 h and 48 h as described earlier [30].

The phospholipid extraction and analysis of PLFAs were carried out as described earlier [26]. Briefly; 1 g of fresh peat was extracted with a chloroform:methanol:citrate buffer mixture (1:2:0.8) and the lipids were separated into neutral lipids, glycolipids and phospholipids on a silic acid column. The phospholipids were subjected to mild alkaline methanolysis and the fatty acid methyl esters were detected by gas chromatography using a flame