Environmental and Experimental Botany 185 (2021) 104424

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

The complexity of wood ash fertilization disentangled: Effects on soil pH, nutrient status, plant growth and cadmium accumulation

Jesper Liengaard Johansen [a,*], Maiken Lundstad Nielsen [a], Mette Vestergård [a,b], Louise Hindborg Mortensen [a], Carla Cruz-Paredes [a,c], Regin Rønn [a], Rasmus Kjøller [a], Mads Hovmand [a], Søren Christensen [a], Flemming Ekelund [a]

[a] Center for Bioenergy Recycling – ASHBACK, Terrestrial Ecology Section, Department of Biology, University of Copenhagen, Universitetsparken 15, DK2100, Copenhagen, Denmark

[b] Department of Agroecology, AU-Flakkebjerg, Aarhus University, Forsøgsvej 1, DK4200, Slagelse, Denmark

[c] Department of Plant and Environmental Sciences. University of Copenhagen. Thorvaldsensvej 40, DK1871, København, Denmark

ARTICLE INFO

Keywords:
Cadmium
Circular economy
Plant growth
Plant nutrients
Soil pH
Sustainability
Wood ash

ABSTRACT

Wood ash is a by-product from energy production that can be recycled to forests to regain nutrients and prevent acidification. However, low concentrations of nitrogen (N) in wood ash may reduce its potential positive effect on plant growth. In addition, wood ash can have a high content of toxic heavy metals such as Cd, thus there are concerns that it may increase Cd accumulation in plants.

We grew *Deschampsia flexuosa* (Wavy hair-grass) in pots in acidic nutrient poor forest soil fertilized with different concentrations of wood ash (corresponding to field application of 0, 1.1, 3.3, 11 and 33 t ha^{-1}). Additionally, to disentangle the pH and nutrient effects of wood ash application, we included treatments with either CaO, to simulate pH effects of wood ash, or potassium (K) + phosphorus (P) fertilizer to mimic the nutrient effects. After 4.5 months of growth, we measured soil pH, plant biomass, Cd accumulation in shoots and N concentration in the various compartments of the system.

Wood ash addition stimulated plant growth, whereas CaO and K + P addition resulted in more moderate increases in biomass. Despite the low concentration of N in the wood ash, plant uptake of N increased in wood ash amendments, probably because wood ash stimulated mineralization of soil organic N. Plant Cd content significantly increased at the highest dose of wood ash.

Our results suggest that addition of wood ash significantly stimulates plant growth due to the combined effect of increased pH, elevated nutrient levels and increased N mineralization. Furthermore, despite the rather high Cd content in used wood ash (16.3 mg kg^{-1}), wood ash amendments up to 11 t ha^{-1} did not result in significantly increased plant uptake of Cd.

1. Introduction

Combustion of tree biomass for energy production has gained interest, as many countries wish to reduce their use and dependency of fossil fuels (Perkiomaki and Fritze, 2005). As a by-product, the incineration produces wood ash that consists of inorganic residues from the wood; thus most plant nutrients, notably phosphorus (P) and potassium (K) are preserved in the ash, whereas most nitrogen (N) is lost during incineration as NO_x compounds, and the remaining N is most likely strongly bound in organic residues and unavailable to organisms (Augusto et al., 2008; Demeyer et al., 2001). Further, wood ash is highly alkaline (pH > 12) due to its content of various metal oxides, mostly CaO, which is transformed to CaCO3 when the ash hardens. Wood ash may also contain considerable amounts of heavy metals, where the concentration of Cd (typically 1−20 mg kg^{-1}, but fly ashes can have higher concentrations) in this context is important (Bieser and Thomas, 2019; Maresca et al., 2017; Ring et al., 2006; Rosenberg et al., 2010).

In production forests with continuous harvest of tree biomass, soil acidification and nutrient depletion may present a serious problem for the long-term wood production (Huotari et al., 2015). Since wood ash is rich in both liming agents and plant nutrients it seems obvious to recycle it to the forest to mitigate these depletions (Arvidsson and Lundkvist,

* Corresponding author.
E-mail address: jljohansen@plen.ku.dk (J.L. Johansen).

https://doi.org/10.1016/j.envexpbot.2021.104424
Received 25 November 2020; Received in revised form 1 February 2021; Accepted 8 February 2021
Available online 20 February 2021

2003; Kjøller et al., 2017), and to improve the sustainability of the energy production by ensuring a circular nutrient flow. However, two major concerns may prevent wood ash recycling. Firstly, wood ash may be an insufficient fertilizer, since it contains little N. Secondly, it is unknown whether wood ash fertilization could lead to increased plant Cd accumulation and toxicity (Huotari et al., 2015). Therefore, the majority of wood ash is presently deposited as waste, which is costly and compromises the sustainability concept (Ingerslev et al., 2014).

Wood ash affects soil chemistry and biology in complex ways, due to primary effects, such as elevated pH and nutrient addition, and to derived effects such as changes in nutrient availability and interaction with biological processes (Bardgett, 2005). Plant growth is strongly affected by pH changes in the soil, mainly due to changes in availability of plant nutrients (Gough et al., 2000; Pärtel, 2002). Also, microorganisms and microbial processes are highly affected by soil pH (Rousk et al., 2009). Previous studies indicated that increased soil pH and nutrient concentration caused by wood ash application resulted in increased microbial mineralization of soil organic N into plant available N in the form of NH_4^+ and NO_3^- (Bang-Andreasen et al., 2017; Cruz-Paredes et al., 2017; Jäggi et al., 2004; Mortensen et al., 2020; Vestergård et al., 2018). This is particularly interesting since increased N mineralization might remedy the low concentration of N in wood ash.

The heavy metal content, especially of Cd, is a cause of concern in wood ash recycling (Ingerslev et al., 2014; McLaughlin and Singh, 1999). Cd is a non-essential element, and toxic to all organisms, which is actively taken up because it resembles the essential micro-nutrient zinc (Zn) (Clemens, 2006). Therefore, Cd may bioaccumulate in the food web and compromise ecosystem functioning and ultimately present a risk to human health (Godt et al., 2006; Järup and Åkesson, 2009). Plants in particular, could make up an important route of bioaccumulation as they take up Cd in the roots and translocate it to aboveground parts (Das et al., 1997; Kirkham, 2006). However, other research has shown that the bio-availability of Cd is generally low in soil (Johansen et al., 2019, 2018), and that it even decreases with increasing soil pH (Kindtler et al., 2019; Mortensen et al., 2018). Further, root symbionts, which are ubiquitous in natural ecosystems, reduce Cd accumulation in plants (Jiang et al., 2016; Rask et al., 2019).

The aim of this study was to disentangle the effect of wood ash application to soil, i.e. pH increase and nutrient addition, on plant growth, as well as to evaluate the potential problems with Cd accumulation in plants. We conducted a pot experiment with the perennial grass *Deschampsia flexuosa* (Wavy hair-grass) in a nutrient poor podzol soil. The pots were amended with different concentrations of either wood ash, CaO (to increase pH) or the mineral nutrients K + P to disentangle effects of pH and mineral nutrients. We used nutrient vector analyses to describe the effect of individual nutrients, which is often used to describe effects of complex fertilizers (Dumroese et al., 2018; Gale et al., 2017; Gale and Thomas, 2019; Omil et al., 2013). Furthermore, we evaluated the effects of wood ash application on Cd uptake in the plant shoots. We expected wood ash addition to increase pH, nutrient (mainly K and P) levels and N mineralization in the soil. We therefore hypothesised that wood ash fertilization would lead to increased plant growth. We expected the treatments with CaO and K + P to have a positive effect on plant growth, though not as pronounced as the wood ash treatment. Lastly, we hypothesised that Cd enrichment of the soil due to wood ash fertilization would lead to increased Cd concentration in plant tissue.

2. Materials and methods

2.1. Soil sampling

We collected soil in Gedhus Plantation (Karup, Denmark, 56°16′38.72″ N 9°05′5.78″ E) in August 2014. The plantation is a second generation *Picea abies* L. (Norway spruce) plantation established on old heathland. *Deschampsia flexuosa* is the dominating vascular plant in the understory, which is otherwise dominated by mosses. The site has very low bioturbation, as the only earthworm present is the epigeic earthworm *Dendrobaena octaedra*, and that in low abundances. The soil is a podzol formed on coarse well drained sand with silt and clay each below 5% of the mineral soil. The O-horizon is approximately 5 cm deep followed by a 10 cm A-horizon (for detailed description of soil and humus composition, see Hansen et al. (2018) and Maresca et al. (2018). The annual precipitation is 781 mm. We sampled material from the O-horizon (soil organic matter (SOM) content = 869 g kg^{-1}; carbon/nitrogen (C/N) = 29.9; cation exchange capacity (CEC) = 12.8 cmol kg^{-1}) and the underlying A-horizon (SOM = 184 g kg^{-1}; C/N = 37.0; CEC = 4.9 cmol kg^{-1}) in separate containers. We separately sieved portions of the sandy A-horizon (8 mm) and homogenized the organic O-horizon by hand.

2.2. Experimental design

For the experiment, we constructed artificial soil profiles in cylindrical plastic pots (h = 27 cm; D = 9 cm), with a removable bottom with six holes, which ensured that water could drain freely. The bottom was covered with water repellent cotton. Each pot was then filled with 1000 g (859 g DW) of material from the sandy A-horizon (approximately a 10 cm layer) and topped with 250 g (88.01 g DW) material from the O-horizon (approximately a 5 cm layer). The experiment consisted of 13 treatments, i.e. one unamended control, four wood ash levels, four liming (CaO) levels and four K + P fertilizer levels (the four levels of Wood ash, CaO and K + P were labelled low (L), low intermediate (LI), high intermediate (HI), high (H), see Table 2). Each treatment was replicated five times, i.e. we had in total 65 pots.

For the ash treatments, we used wood ash from Ebeltoft power plant (Ebeltoft, Denmark, 56°11′4.45″ N 10°41′10.03″ E). The wood ash was residues from incineration of Norway Spruce (*Picea abies* L.). We used fly ash, which is known to have a higher concentration of problematic elements compared to bottom ash. The ash was highly alkaline (pH = 12.7; EC = 2785 mS m^{-1}). The concentration of elements relevant for this study are listed in Table 1, and for a comprehensive chemical analysis of the ash, we refer to Maresca et al. (2017) sample FA-2b. The four ash levels (0.7, 2.2, 7.1 and 21 g wood ash pot^{-1}) were chosen to mimic field application of 1.1, 3.4, 11.2 and 33.7 t ha^{-1}, where 3.4 t ha^{-1} corresponds to a normal field dosage. For the liming treatments, we used CaO (Sigma-Aldrich, Germany). The four CaO treatments (0.04, 0.97, 2.5 and 4.6 g CaO pot^{-1}) served to mimic the increase in pH caused by wood ash without concomitant nutrient addition. We conducted a pilot experiment to determine the approximate amounts of CaO needed to reach the same pH as caused by the four wood ash levels. To provide nutrients without a pH increase, we used KCl (Sigma-Aldrich, Germany) and NaH_2PO_4 (Sigma-Aldrich, Germany), respectively. The amounts and ratio of K and P (2.3 + 1.3, 6.9 + 3.9, 23 + 13 and 68 + 39 mg K + P pot^{-1}) were calculated as equivalents to those of the wood ash (Table 2). Wood ash and CaO were applied on the top of the soil column. After amendment with wood ash and CaO, we watered the cylinders with 30 mL ddH_2O whereas K + P were added in a 30 mL solution with ddH_2O. Unamended control pots were watered with 30 mL ddH_2O. Finally, the water content of each system was adjusted to 45 % (W/DW) corresponding to 60 % of water-holding capacity.

We then transferred eight pre-germinated seedlings of *Deschampsia flexuosa* to each pot. The pots were placed in a growth chamber at 17.5 °C and exposed to a 16 h light cycle (355 μmol m^{-2} s^{-1} for 4 h, 860 μmol m^{-2} s^{-1} for 8 h and 355 μmol m^{-2} s^{-1} for 4 h) and then 8 h of darkness. The pots were watered every second day and weighed once a week to ensure a water content of 45 % (DW). After two months of growth, the pots were fertilized with 12.7 mg N as NH_4NO_3 pot^{-1} two times with a weekly interval to avoid severe N deficiency.

2.3. Sampling

After 4.5 months of growth, we destructively harvested the pots. We separated shoots from roots, rinsed the roots and dried shoots and roots

Table 1
Concentration of plant nutrients and potentially problematic elements in the used wood ash. The wood ash was obtained from Ebeltoft power plant, and is a fly ash with Norway spruce (*Picea abies* L.) as parent material. The metals Cu, Ni and Zn are both micronutrients for plants and considered problematic in large amounts. Data is adapted from Maresca et al. (2017).

Macro/meso nutrients ($mg\ kg^{-1}$):						Potentially problematic elements ($mg\ kg^{-1}$):						
N	P	K	Ca	Mg	S	Cd	Cr	Cu	Co	Ni	Pb	Zn
1670	21400	51200	263000	30000	8490	16.3	60.6	115	5.79	22.4	55.3	924

Table 2
Amendments applied in a greenhouse pot experiment aimed to disentangle effects of wood ash amendment on pH increase and nutrient levels. *Deschampsia flexuosa* was grown for 4.5 months in artificial soil profiles amended with wood ash at four levels, corresponding to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1}. CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients (K + P) were added at four levels to obtain nutrient addition without increases in pH.

Label	Added to pot	Field amendment equivalent
Control	Unamended	Unamended
Low ash (L ash)	0.7 g wood ash	1.1 t wood ash ha^{-1}
Low Intermediate ash (LI ash)	2.2 g wood ash	3.4 t wood ha^{-1}
High Intermediate ash (HI ash)	7.1 g wood ash	11.2 t wood ha^{-1}
High ash (H ash)	21 g wood ash	33.7 t wood ash ha^{-1}
Low CaO (L CaO)	0.04 g CaO	pH equivalent of 1.1 t wood ash ha^{-1}
Low Intermediate CaO (LI CaO)	0.97 g CaO	pH equivalent of 3.4 t wood ha^{-1}
High Intermediate CaO (HI CaO)	2.5 g CaO	pH equivalent of 11.2 t wood ha^{-1}
High CaO (H CaO)	4.6 g CaO	pH equivalent of 33.7 t wood ash ha^{-1}
Low K + P (L K + P)	2.3 mg K + 1.3 mg P	K and P equivalent of 1.1 t wood ash ha^{-1}
Low Intermediate K + P (LI K + P)	6.9 mg K + 3.9 mg P	K and P equivalent of 3.4 t wood ash ha^{-1}
High Intermediate K + P (HI K + P)	23 mg K + 13 mg P	K and P equivalent of 11.2 t wood ash ha^{-1}
High K + P (H K + P)	68 mg K + 39 mg P	K and P equivalent of 33.7 t wood ash ha^{-1}

at 70 °C for 24 h. Next, we removed remnants of wood ash from the pot surfaces and divided the soil in upper O-horizon (0–1 cm), lower O-horizon (1–5 cm) and A-horizon (5–10 cm). Soil pH was measured on soil slurries (soil to water ratio was 1:5) with a glass electrode in the 0–1, 1–5 cm and 5–15 cm layers (PHM240 pH/ION METER, Meter-Lab). To analyse for NO_3^-, NH_4^+ and H_2PO_4- content, 15 g of fresh soil (0–5 cm) was mixed with 75 mL water, shaken for one hour at 200 rpm and filtered overnight at 3 °C. NO_3^-, NH_4^+ and PO_4^- concentrations were measured colorimetrically on a flow injection analyser (FIAstar 5000, Foss A/S, Denmark). We dried and homogenized soil fractions for determination of K, Ca, Zn and Cd content. Aliquots of 2 g of the organic 0–5 cm topsoil were digested in 40 mL 32.5 % HNO_3 using the "plant material" program on a CEM MARS microwave (CEM, North Carolina, USA) and concentration of the elements were measured by Atomic Absorption spectrometry (Perkin Elmer PinAAcle 900 T). Cd concentration was measured with the graphite furnace technique, and Zn, Ca and K with the acetylene flame technique.

2.4. Plant analysis

We first determined dry weight of shoots and roots. For Cd, Zn, Ca and K analyses of shoots, samples of approximately 1 g were digested with 10 mL 16 % HNO_3 using the "plant material" program in a CEM MARS micro wave (CEM, North Carolina, USA). Samples were diluted with 10 mL ddH_2O and analysed for Cd content by Atomic Absorption spectrometry (Perkin Elmer PinAAcle 900 T) with the graphite furnace technique, and for Zn, Ca and K with Atomic Absorption spectrometry using the acetylene flame technique. Furthermore, 25 mg shoot material was digested with 7.5 mL HCl and total N and P contents were measured colorimetrically on a flow injection analyser (FIAstar 5000, Foss A/S, Denmark).

2.5. Data analyses

In Figs. 1–4, we use box plots to present the data. The box (Interquartile range, IQR) represents the third to first quartile with the median marked as a line. The whiskers mark the data range, and possible outliers are marked with a circle. For all measured parameters (Tables 3 and 4 and Figs. 1–4), we analysed the data with a one-way ANOVA with the measured parameters as dependent variable and the 13 treatments (categorical) as independent variable. In case of significant effects ($p < 0.05$) in the dataset, we used a Tukey's Honest Standard Deviation (HSD) test to separate and categorize means ($p < 0.05$). In Fig. 5, we constructed nutrient vector diagrams for the measured nutrients N, P, K, Zn and Ca. The diagrams were constructed by plotting the relative concentration, relative content and relative biomass for each nutrient in each treatment as compared to the control treatments, as in Haase and Rose (1995). One-way ANOVA and Tukey's HSD test were performed in SAS Enterprise Guide 6.1, while figures and nutrient vector diagrams were made in SigmaPlot 13.0.

3. Results

3.1. Effects on pH and element concentrations in soil

Amendments with wood ash and CaO resulted in a significant pH increase in both the uppermost 0–1 cm and in the lower 1–5 cm of the organic soil (Fig. 1). However, we observed no significant pH increases in the 5–15 cm mineral soil layer. The CaO treatment did not result in a pH increase at plant harvest as strong as expected from our initial experiments. Apparently, the alkaline component in CaO interacted with the soil in a different manner than the alkaline components in the wood ash during the 4.5 month experiment. The fertilizer treatment with K + P had no significant effect on pH (Fig. 1). It is not surprising that the strongly alkaline substances increase soil pH, however it is noteworthy that the effect is limited to the very top layers of the soil, which was also observed by Hansen et al. (2017).

The soil element concentrations (after removal of visible ash residues) increased significantly in those treatments where the particular element was added as part of the application i.e. soil Cd, Zn, Ca, P and K increased in the wood ash treatments, whereas only soil Ca increased in the CaO treatments, and only soil K and P increased in the K + P treatments (Table 3).

3.2. Plant biomass and element composition

Aboveground biomass of *Deschampsia flexuosa* increased significantly in all three treatment types, most markedly in wood ash treatments, where it increased with up to 270 % the biomass of the control treatment (Fig. 2). The CaO and K + P amendments resulted in moderate increases of respectively 110 % and 130 % in shoot biomass (Fig. 2). Plant concentration of K, P and Zn did not increase with wood ash application (Table 4). The CaO treatment reduced plant P concentration,

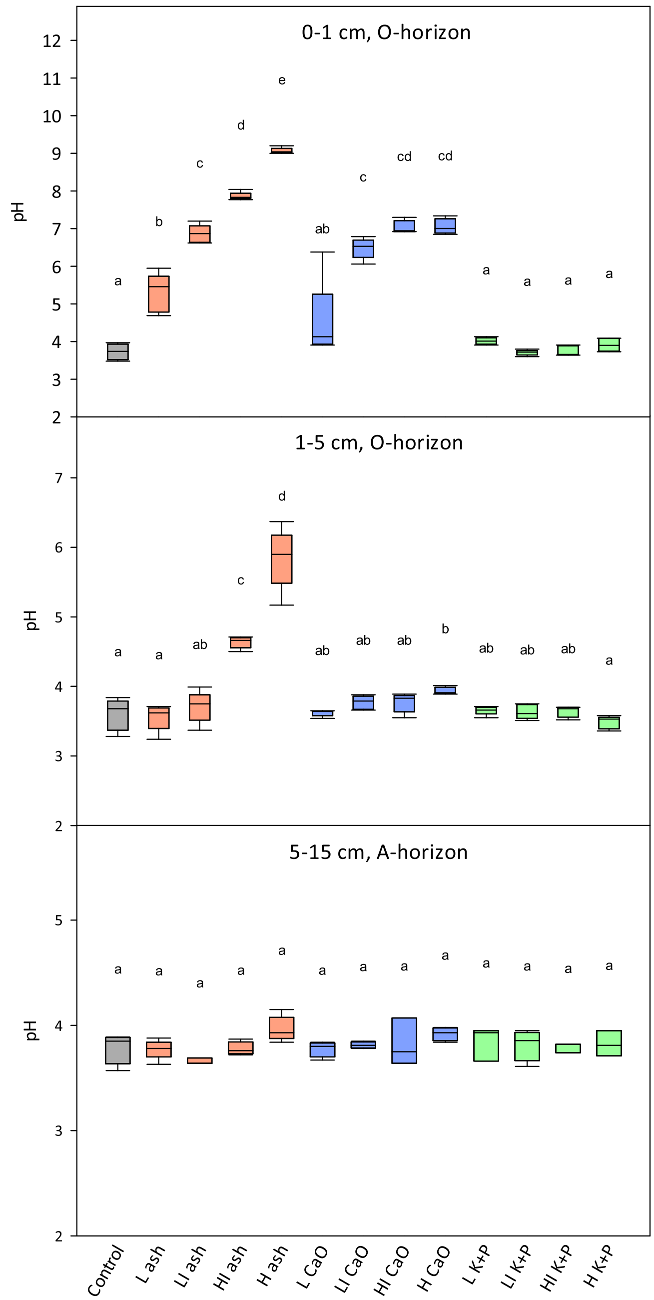

Fig. 1. Box plots (see section 2.6) of pH in the upper 0-1 cm O-horizon (top), the 1-5 cm O-horizon (middle) and the 5-15 cm A-horizon (bottom) from a greenhouse pot experiment, where *Deschampsia flexuosa* was grown for 4.5 months in artificial soil profiles amended with wood ash, corresponding to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH, Wood ash (orange), CaO (blue) and K + P (green), unamended control (grey). We found significant pH changes in the 0-1 cm and 1-5 cm layers ($p < 0.001$; one-way ANOVA), but not in the 5-15 cm layer. Treatment means are separated by letters ($p < 0.05$, Tukey HSD test) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

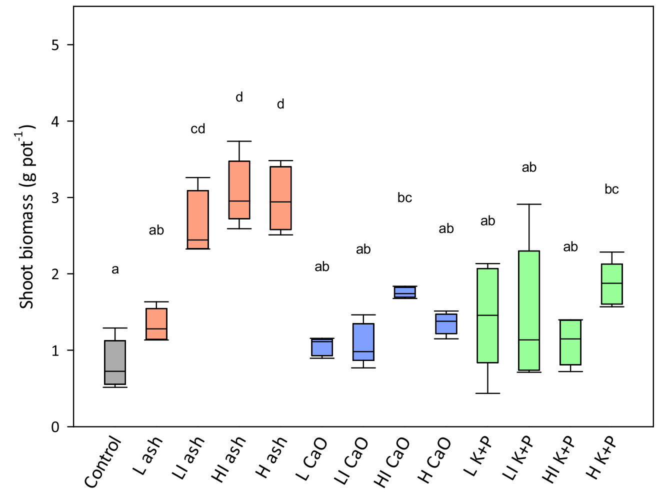

Fig. 2. Box plots (see section 2.6) of shoot biomass as affected by the four levels of wood ash (orange), CaO (blue) and K + P (green) as compared to the control (grey). Wood ash amendments corresponded to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH. Treatments differed significantly ($p < 0.001$, one-way ANOVA). Treatment means are separated by letters ($p < 0.05$, Tukey HSD test). For further details see Table 2 and Fig. 1 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

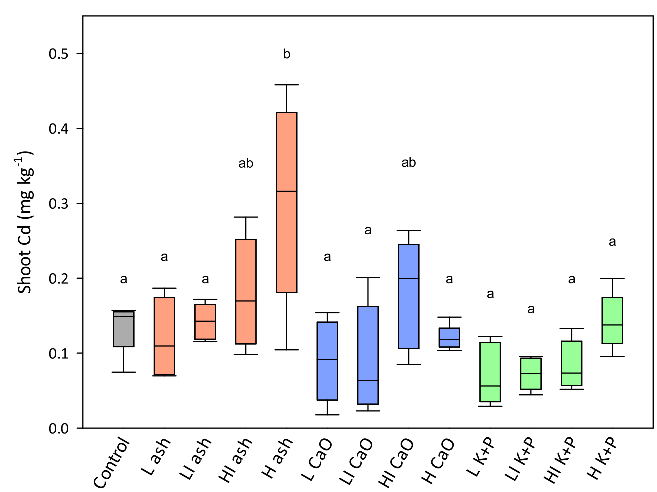

Fig. 3. Box plots (see section 2.6) of shoot Cd concentration as affected by the four levels of wood ash (orange), CaO (blue) and K + P (green) as compared to the control (grey). Wood ash amendments corresponded to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH. Treatments differed significantly ($p < 0.001$, one-way ANOVA). Treatment means are separated by letters ($p < 0.05$, Tukey HSD test). For further details see Table 2 and Fig. 1 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

whereas the K + P treatment affected plant uptake of P positively (Table 4). Plant Cd concentration increased with wood ash amendment, but the concentration was only significantly higher than the control in the highest (H) wood ash treatment (Fig. 3). The plant Ca concentration did not vary significantly between treatments, although the levels appeared elevated in wood ash and CaO treatments.

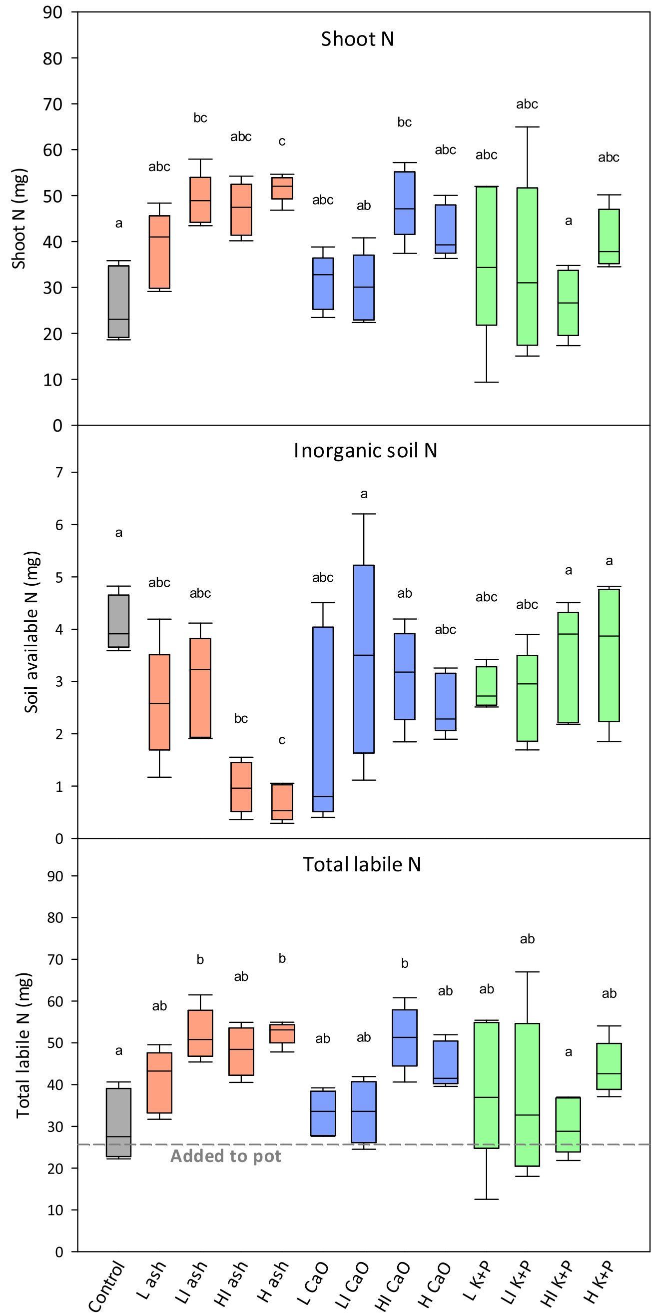

Fig. 4. Box plots (see section 2.6) of total shoot N (top), inorganic soil N (NH_4^+ + NO_3^-) (middle) and total labile N of the pots (shoot N + inorganic soil N) (bottom). The graphs show the effect of the four levels of wood ash (orange), CaO (blue) and K + P (green) as compared to the control (grey). Wood ash amendments corresponded to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH. The amount of added N fertilizer is marked as a dotted line on the bottom graph. Treatments differed significantly ($p < 0.001$, one-way ANOVA). Treatment means are separated by letters ($p < 0.05$, Tukey HSD test). For further details see Table 2 and Fig. 1 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

3.3. Nitrogen content in soil and plant

Plant uptake of N increased in the wood ash and CaO treatments, though only significantly in the LI and H ash treatments, and in the HI CaO treatment (Fig. 4 top). The inorganic soil N, which mostly consisted of NH_4^+ and only little NO_3^- (Table 3), decreased with increasing wood ash amendment, while CaO and K + P amendment had no significant effect on soil inorganic N (Fig. 4 middle). Total labile N in the system (shoot N + soil inorganic N) increased with ash and CaO treatments (Fig. 4 bottom). We found a significant positive linear correlation ($p < 0.001$; $r^2 = 0.58$) between labile N of the pots and shoot biomass, which suggests that mineralized N is very important to for plant growth in this system.

3.4. Nutrient vector analyses

We constructed nutrient vector analysis diagrams to evaluate the foliar nutritional status in the treatments compared to the control treatment (Fig. 5). Nutrient vector analysis diagrams are plots of relative nutrient concentration, relative nutrient content and relative biomass, for each nutrient (N, P, K, Zn, and Ca) in each treatment compared to the control treatment. The analyses revealed that in all treatments, increased biomass relative to the control coincided with increased N content and N concentration, signifying N limitation (Haase and Rose, 1995). The foliar concentration of K and P increased markedly in the K + P treatments, with moderate increases in biomass, which suggests some degree of K and P limitation (Haase and Rose, 1995). In wood ash treatments, K and P concentrations did not increase with increasing biomass, indicating K and P sufficiency. In CaO treatments, K and P concentrations appeared to decline with increasing biomass, indicating that K and P availability was also sufficient in CaO treatments. Foliar concentrations of Ca increased with biomass increase in wood ash and CaO treatments, and thus appeared limiting for plant growth in these treatments. However, the Ca concentrations decreased with biomass increase in the K + P treatments. Zn availability appeared to be sufficient in all treatments.

4. Discussion

4.1. Experimental design and limitations

Wood ash is a complex compound that contains several components, which may affect various soil parameters, and hence plant growth, in several ways. In our experimental design, we attempted to disentangle two basic characteristics of wood ash addition; its pH increasing effect and its increase of the two macro-nutrients K and P. We did this using separate amendments with CaO - to increase pH, and amendments with the two macro nutrients K and P. These treatments have some inherent challenges and limitations compared to wood ash treatments, and there are aspects of wood ash treatments that the CaO and K + P treatments do not encompass. Thus, the CaO treatments affected not only soil pH, but also the concentration and plant-availability of Ca. K and P were amended as salts and therefore Na and Cl were also included in these treatments. Finally, wood ash contains a number of micro-nutrients, such as Mg, B, S, Zn and Cu, the contents of which were only manipulated in the wood ash treatments.

4.2. pH and nutrient status of the soil

The aim of wood ash application in forestry is to counteract acidification and nutrient depletion of the soil in order to maintain productivity (Augusto et al., 2008; Rosenberg et al., 2010; Stupak et al., 2007). Our results suggest that wood ash application does prevent nutrient depletion as we recorded increased levels of K, P, Ca and Zn in the top soil (Table 3); in accordance with previous studies (Arvidsson and Lundkvist, 2003; Ohno and Erich, 1990). Moreover, wood ash increased

Table 3
Heavy metals and plant nutrients in 0-5 cm organic top soil in a greenhouse pot experiment, where *Deschampsia flexuosa* was grown for 4.5 months in artificial soil profiles amended with wood ash at four levels, corresponding to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH. Values are means ±1 SE (n = 5). All parameters showed significant treatment effects ($p < 0.001$; one-way ANOVA). Values with different letters differ significantly ($p < 0.05$; Tukey HSD) between treatments.

		Control	L	LI	HI	H
Cd (mg kg^{-1})						
	Wood ash		–	0.344 (±0.023) ^{b}	–	0.818 (±0.036) ^{c}
	CaO	0.286 (±0.005) ^{ab}	–	0.258 (±0.003) ^{a}	–	0.276 (±0.014) ^{a}
	K + P		–	0.289 (±0.006) ^{ab}	–	0.242 (±0.003) ^{a}
Zn (mg kg^{-1})						
	Wood ash		–	27.7 (±1.5) ^{a}	–	57.7 (±3.7) ^{b}
	CaO	24.2 (±0.4) ^{a}	–	22.5 (±0.9) ^{a}	–	23.7 (±1.6) ^{a}
	K + P		–	23.5 (±0.7) ^{a}	–	22.1 (±0.7) ^{a}
Ca (mg kg^{-1})						
	Wood ash		–	2459 (±115) ^{b}	–	5850 (±274) ^{d}
	CaO	1181 (±37) ^{a}	–	1978 (±152) ^{b}	–	4017 (±234) ^{c}
	K + P		–	1253 (±30) ^{a}	–	1153 (±42) ^{a}
K (mg kg^{-1})						
	Wood ash		–	353 (±29) ^{bc}	–	2651 (±248) ^{d}
	CaO	271 (±10) ^{ab}	–	237 (±17) ^{a}	–	233 (±19) ^{a}
	K + P		–	489 (±83) ^{c}	–	2971 (±217) ^{d}
PO_4^{3-} (mg kg-1)						
	Wood ash		28.0 (±2.2) ^{abc}	58.3 (±6.9) ^{cd}	134.4 (±9.9) ^{e}	48.9 (±12.7) ^{bcd}
	CaO	16.0 (±5.3) ^{ab}	24.4 (±12.6) ^{ab}	10.3 (±5.9) ^{a}	6.1 (±1.8) ^{a}	7.6 (±3.17) ^{a}
	K + P		15.9 (±1.9) ^{ab}	28.9 (±3.0) ^{abc}	86.2 (±11.7) ^{de}	406.9 (±27.6) ^{f}
NH_4^+ (mg kg^{-1})						
	Wood ash		28.4 (±5.5) ^{abc}	32.8 (±5.0) ^{abc}	10.3 (±2.5) ^{ab}	5.9 (±1.5) ^{a}
	CaO	44.2 (±2.6) ^{c}	19.1 (±9.6) ^{abc}	36.2 (±10.2) ^{bc}	32.7 (±4.8) ^{abc}	24.0 (±2.4) ^{abc}
	K + P		30.7 (±1.9) ^{abc}	28.5 (±4.5) ^{abc}	37.8 (±5.4) ^{c}	40.5 (±6.6) ^{c}
NO_3^- (mg kg-1)						
	Wood ash		1.12 (±0.22) ^{ab}	0.81 (±0.21) ^{ab}	0.88 (±0.31) ^{ab}	1.59 (±1.04) ^{ab}
	CaO	2.59 (±0.17) ^{abc}	3.45 (±0.94) ^{bc}	3.00 (±0.31) ^{ab}	2.69 (±0.37) ^{abc}	5.02 (±0.82) ^{c}
	K + P		2.10 (±0.58) ^{ab}	2.69 (±0.96) ^{abc}	0.94 (±0.27) ^{ab}	0.22 (±0.06) ^{a}

Table 4
Plant parameters of *Deschampsia flexuosa* from a greenhouse experiment, where it was grown for 4.5 months in artificial soil profiles amended with wood ash at four levels, corresponding to field amendment with 1.1, 3.4, 11.2 and 33.7 t wood ash ha^{-1} (low (L), low intermediate (LI), high intermediate (HI), high (H)). CaO was applied at four levels to mimic the pH increase caused by the wood ash, without concomitant addition of mineral nutrients, whereas the mineral nutrients were added at four levels to obtain nutrient addition without increase in pH. Values are means ±1 SE (n = 5). All parameters except shoot Ca showed significant treatment effects ($p < 0.001$; one-way ANOVA). Values with different letters differ significantly ($p < 0.05$; Tukey HSD) between treatments.

		Control	L	LI	HI	H
Root biomass (g)						
	Wood ash		0.55 (±0.07) ^{a}	0.87 (±0.19) ^{ab}	1.31 (±0.07) ^{b}	0.79 (±0.23) ^{ab}
	CaO	0.32 (±0.11) ^{a}	0.43 (±0.05) ^{a}	0.69 (±0.14) ^{ab}	0.63 (±0.03) ^{a}	0.47 (±0.05) ^{a}
	K + P		0.37 (±0.12) ^{a}	0.41 (±0.14) ^{a}	0.33 (±0.06) ^{a}	0.54 (±0.06) ^{a}
Shoot K (g kg^{-1})						
	Wood ash		16.9 (±0.8) ^{ab}	15.3 (±1.0) ^{ab}	18.2 (±1.7) ^{ab}	22.3 (±1.1) ^{ab}
	CaO	15.4 (±1.4) ^{ab}	9.9 (±1.7) ^{ab}	8.6 (±1.4) ^{a}	10.4 (±0.8) ^{ab}	11.5 (±0.6) ^{ab}
	K + P		17.3 (±1.3) ^{ab}	20.4 (±2.0) ^{ab}	23.0 (±0.9) ^{ab}	33.0 (±1.3) ^{b}
Shoot P (g kg^{-1})						
	Wood ash		1.9 (±0.1) ^{ef}	1.3 (±0.1) ^{cde}	1.3 (±0.1) ^{bcd}	2.0 (±0.3) ^{fg}
	CaO	1.6 (±0.2) ^{def}	1.1 (±0.05) ^{bcd}	0.9 (±0.1) ^{abc}	0.7 (±0.03) ^{a}	0.9 (±0.04) ^{ab}
	K + P		2.8 (±0.1) ^{gh}	4.0 (±0.2) ^{h}	6.9 (±0.6) ^{i}	18.7 (±0.9) ^{j}
Shoot Ca (mg kg^{-1})						
	Wood ash		616 (±58)	707 (±59)	858 (±89)	939 (±89)
	CaO	490 (±28)	421 (±66)	442 (±97)	767 (±97)	832 (±77)
	K + P		362 (±48)	339 (±16)	380 (±35)	438 (±37)
Shoot Zn (mg kg^{-1})						
	Wood ash		30.0 (±2.7) ^{ab}	25.4 (±2.8) ^{ab}	27.6 (±3.8) ^{ab}	36.3 (±0.9) ^{b}
	CaO	29.5 (±3.1) ^{ab}	23.5 (±4.5) ^{ab}	20.2 (±3.8) ^{a}	32.5 (±3.2) ^{ab}	28.6 (±4.4) ^{ab}
	K + P		21.5 (±2.0) ^{ab}	22.3 (±0.14) ^{ab}	25.1 (±1.8) ^{ab}	29.4 (±2.8) ^{ab}

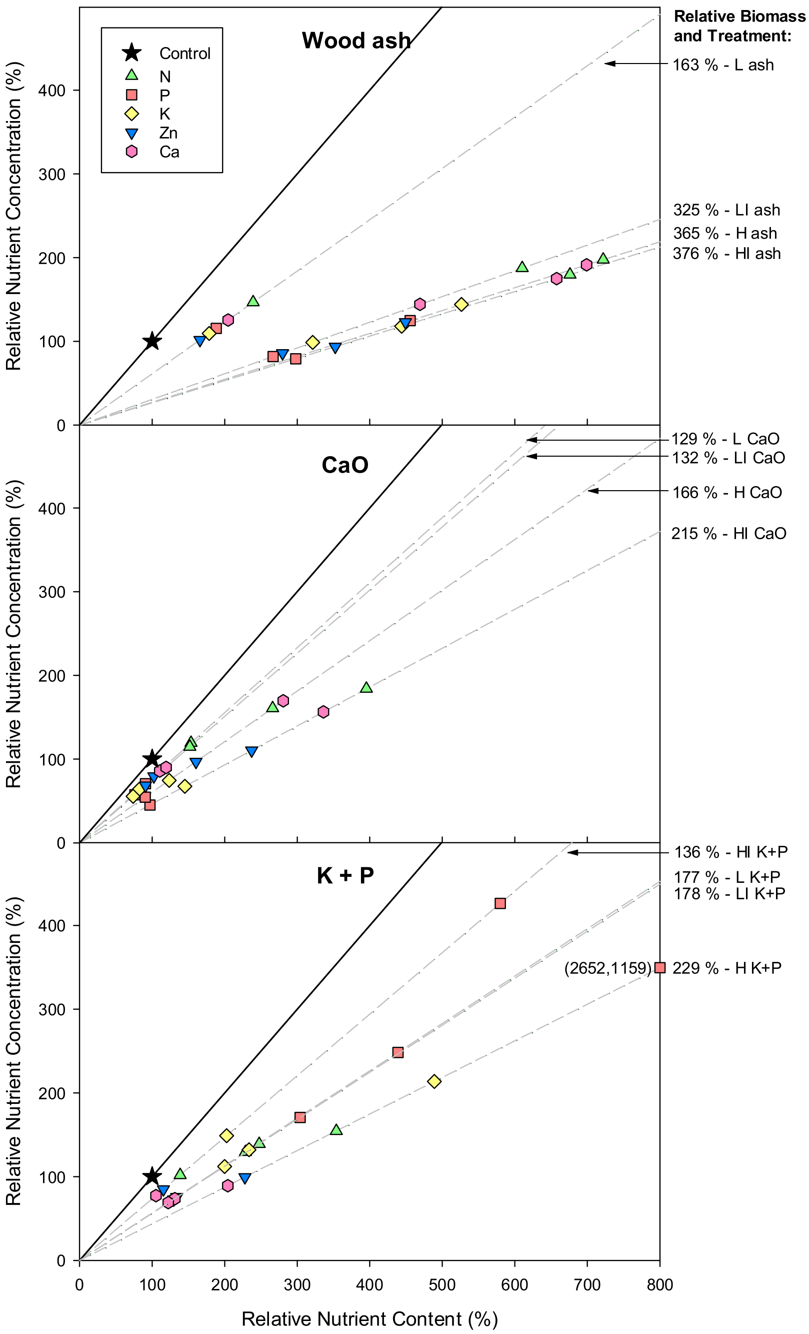

Fig. 5. Nutrient vector diagrams of the measured elements N, P, K, Zn and Ca in the four treatments L, LI, HI and H of each treatment type - Wood ash (top), CaO (middle) and K + P (bottom).The plot is a depiction of the relative concentration and relative content of each nutrient in each treatment compared to the Control treatment. The diagonal axis is the relative biomass of the treatment compared to the Control treatment (solid line is 100 % relative biomass). Each point represent a vector from the Control reference point.

pH in the 0−1 cm top soil layer from 3.7 to 9.0, and from 3.6 to 5.8 in the 1−5 cm organic layer, though not in the lower 5−15 cm bottom layer (Fig. 1). Apparently, the pH effects are limited to the uppermost layers, even in long-term experiments (Hansen et al., 2018), though this effect is probably limited to soils where there is no appreciable bioturbation or cryoturbation of soils. Jokinen et al. (2006) and Weber et al. (1985) similarly reported on mitigating effects on acidification and increases in the level of important plant soil nutrients.

4.3. Nitrogen mineralization and uptake

Wood ash contains little N (Table 1), as most of it is lost during the incineration (Ingerslev et al., 2014), but the wood ash may stimulate microbial processes that lead to mineralization of N from soil organic matter. The test plant *Deschampsia flexuosa* is known to take up small organic N compounds (Näsholm et al., 1998; Persson et al., 2003; Weigelt et al., 2005), and if the treatments increased the availability of these compounds, it may have contributed to the N consumption by the plants. We did not measure the organic N compounds in the soil, but such compounds taken up by the plant are included in the plant N measurements. We observed that total labile N increased with up to 25 mg N pot^{-1} (Fig. 4 bottom) after wood ash and CaO amendment. This suggests that mineralization of organic N was stimulated by increases in soil pH. Mortensen et al. (2019) demonstrated that wood ash stimulates decomposition of recalcitrant organic matter, which can lead to increases in N mineralization (Jäggi et al., 2004; Mortensen et al., 2020; Vestergård et al., 2018).

We found that wood ash fertilization actually decreased the soil concentration of inorganic and plant available N (Fig. 4 middle), probably due to efficient plant uptake. Yet, we observed a large increase in labile N in the system. This observation was only possible because our experimental pot system allowed assessment of all compartments. Thus this detailed insight into gross N flow from the soil to plants provides a valuable supplement to field experiments, where the flow of N is not easily assessed.

We found no indications that wood ash fertilization will lead to increased leaching of inorganic N, despite the increased mineralization. Likewise, ash application did not affect inorganic N leaching at field scale (Hansen et al., 2018). The effects of wood ash were limited to the uppermost layers shown by the steep pH profile (Fig. 1), which is also reported from an experiment designed to study this (Hansen et al., 2017). In our experiment, mineralized N was quickly taken up by plants, and the amount that could potentially be leached actually decreased with wood ash amendment.

4.4. Plant growth

We observed increases in growth of *Deschampsia flexuosa* in all three treatment types compared to the control, but wood ash treatments yielded by far the largest increases in biomass (up to 270 %) (Fig. 2). It is not surprising that wood ash is the best fertilizer, since each of the two other treatments only partially contribute the presumed plant growth promoting factors of wood ash, i.e. K + P and pH increase, respectively. This is underlined by the conclusions from nutrient vector analyses diagrams (Fig. 5), where growth of the CaO and K + P treated plants seem to be limited by the availability of Ca and N and the macronutrients K and P, respectively. In addition, the treatments with wood ash may have benefitted from nutrients that was not assessed in this experiment (e.g. Mg, S, Cu, B). This stresses the complementary effects caused by the chemically complex wood ash.

In field studies, similar effects have been recorded; application of 8 and 16 t ash ha^{-1} increased wood production of scots pine 13 and 17 times, respectively (Moilanen et al., 2002). Arvidsson and Lundkvist (2003)found that a smaller dose, 3 t wood ash ha^{-1}, had limited effect on Norway spruce growth over 5 years but that the coverage of *D. flexuosa* was slightly increased.

K and P addition will alleviate depletion of these nutrients, whereas the effect of increased pH is more complex. Generally, a pH increase will stimulate plant growth in acidic soil as the pH optimum for most plants is around 6.5–7, because nutrients like P and K in particular are more available at neutral pH (Raven et al., 2005) and because the microbial activity will facilitate nutrient mineralisation (e.g. inorganic N) (Mortensen et al., 2020; Vestergård et al., 2018).

The wood-ash-induced increases in plant biomass was achieved without addition of N fertilizer, which have been a concern with the use of wood ash as fertilizer, since N is commonly a limiting factor for plant growth. The limited effect of K + P fertilization on plant biomass (Table 4, Fig. 2) underlines that the high content of these macro nutrients in wood ash cannot account for enhanced biomass production alone. The additional effect on soil pH and other plant nutrients, makes wood ash a more complex and better fertilizer, as these factors have both a direct effect on plant growth and an effect on microbial processes such as N mineralization (Bang-Andreasen et al., 2017; Cruz-Paredes et al., 2017; Mortensen et al., 2020; Vestergård et al., 2018).

In our pot experiment, N mineralization was the sole supply of N for the plants, and it appears to be a limiting factor, as seen in the nutrient vector diagrams (Fig. 5). The site where we collected the soil for the experiment has a very large pool of organic matter (O-horizon SOM = 869 g kg^{-1}, C/N = 29.9; A-horizon SOM = 184 g kg^{-1}; C/N = 37.0), and the stimulation of N mineralization may continue for a long time, thus sustaining plant growth. In addition, this site receives N from atmospheric deposition, which is the case in many parts of the world. However, the supply of N must be taken into consideration when choosing sites for wood ash recycling, as it may otherwise have limited effects (Huotari et al., 2015; Stupak et al., 2007)

Application rates above 11.2 t ash ha^{-1} (HI ash) did not increase the biomass of *D. flexuosa* further, which may suggest that plant growth was no longer limited by the nutrients available in wood ash. The saturation pattern could however also be due to a reduced availability of N since plant N concentration decreased with larger ash amendments (see below). Nitrogen concentrations in the plant shoots decreased from 1.4 to 1.1 % (DW/DW, Fig. 4B and Fig. 2) when ash dose increased from 3 to 11.2 t ash ha^{-1}, which could be the reason for a smaller plant growth response with the largest ash amendments.

4.5. Cd accumulation

Another concern about the usage of wood ash as fertilizer is the Cd content. We saw that wood ash amendment did increase Cd concentration in leaves of *Deschampsia flexuosa*, but that only very high ash concentrations caused this response, despite the high Cd content (13.9 mg kg^{-1}) in the ash we used (Maresca et al., 2017). At the highest wood ash amendment (H ash), corresponding to field amendment of 33.7 t wood ash ha^{-1}, plant concentration of Cd significantly increased compared to the unamended control (from 0.14 mg kg^{-1} in the control treatment to 0.30 mg kg^{-1} in the H ash treatment; Fig. 3). However, in practice wood ash amendments above 10 t ha^{-1} are not relevant to consider. This amount would be too much with regard to mass balance and would compromise guidelines that define soils with more than 0.5 mg kg^{-1} soil as polluted soils (Ingerslev et al., 2014). Though Cd content in *Deschampsia flexuosa* increases at the highest ash application level, the concentrations are still well below EU recommended limits for leafy vegetables of 0.2 mg kg^{-1} fresh weight (EU, 2006). Thus, about the same as the highest value we measured (Fig. 3), but we measured in dry weight which is less than 10 % of fresh weight. Other studies have similarly concluded that application of wood ash in forest ecosystems is not a concern with regard to the bio-availability of Cd for soil microorganisms (Cruz-Paredes et al., 2017; Mortensen et al., 2018), Cd accumulation in the humus layer (Perkiomaki and Fritze, 2005) or with respect to Cd accumulation in berries and mushrooms (Moilanen et al., 2006).

5. Conclusion

We studied wood ash recycling to acidic forest soil as a strategy to achieve efficient nutrient recirculation within biomass-based energy production, secure long term biomass production and prevent acidification. Based on a greenhouse pot experiment with *Deschampsia flexuosa*, we conclude that wood ash fertilization increases pH and nutrient levels in the top soil. This, in combination with increased N mineralization from organic soil N, resulted in large increases in plant biomass. Cd concentration in shoots increased at the highest wood ash addition, which is unwanted. However, this level of wood ash application was unrealistically high based on current legislation (10 times higher), and the shoot Cd concentration is still within EU recommendations for foodstuff.

CRediT authorship contribution statement

Jesper Liengaard Johansen: Writing - review & editing, Data curation, Writing - original draft, Conceptualization, Methodology, Visualization, Investigation. **Maiken Lundstad Nielsen:** Writing - review & editing, Data curation, Writing - original draft, Visualization, Investigation. **Mette Vestergård:** Writing - review & editing, Data curation, Writing - original draft, Conceptualization, Methodology, Supervision. **Louise Hindborg Mortensen:** Writing - review & editing. **Carla Cruz-Paredes:** Writing - review & editing. **Regin Rønn:** Writing - review & editing, Data curation, Writing - original draft, Conceptualization, Methodology, Supervision. **Rasmus Kjøller:** Writing - review & editing. **Mads Hovmand:** Writing - review & editing, Supervision. **Søren Christensen:** Writing - review & editing, Data curation, Writing - original draft, Conceptualization, Methodology. **Flemming Ekelund:** Writing - review & editing, Data curation, Writing - original draft, Conceptualization, Methodology, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This research is part of the ASHBACK project funded by the Danish Council of Strategic Research (DSF-12-132655) and HedeDanmark. Flemming Ekelund, Jesper Liengaard Johansen and Mette Vestergård were funded by Danish Council for Independent Research (DFF-4002-00274). The comments and suggestions of two anonymous reviewers improved the manuscript considerably.

References

Arvidsson, H., Lundkvist, H., 2003. Effects of crushed wood ash on soil chemistry in young Norway spruce stands. For. Ecol. Manage. 176, 121–132. https://doi.org/10.1016/S0378-1127(02)00278-5.

Augusto, L., et al., 2008. Wood ash applications to temperate forest ecosystems - potential benefits and drawbacks. Plant Soil 306, 181–198. https://doi.org/10.1007/s11104-008-9570-z.

Bang-Andreasen, T., et al., 2017. Wood ash induced pH changes strongly affect soil bacterial numbers and community composition. Front. Microbiol. 8, 1400. https://doi.org/10.3389/fmicb.2017.01400.

Bardgett, R., 2005. The Biology of Soil: a Community and Ecosystem Approach. Oxford University Press.

Bieser, J.M., Thomas, S.C., 2019. Biochar and high-carbon wood ash effects on soil and vegetation in a boreal clearcut. Can. J. For. Res. 49, 1124–1134. https://doi.org/10.1139/cjfr-2019-0039.

Clemens, S., 2006. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie. 88, 1707–1719. https://doi.org/10.1016/j.biochi.2006.07.003.

Cruz-Paredes, C., et al., 2017. Using community trait-distributions to assign microbial responses to pH changes and Cd in forest soils treated with wood ash. Soil Biol. Biochem. 112, 153–164. https://doi.org/10.1016/j.soilbio.2017.05.004.

Das, P., et al., 1997. Studies on cadmium toxicity in plants: a review. Environ. Pollut. 98, 29–36. https://doi.org/10.1016/S0269-7491(97)00110-3.

Demeyer, A., et al., 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour. Technol. 77, 287–295. https://doi.org/10.1016/S0960-8524(00)00043-2.

Dumroese, R.K., et al., 2018. Biochar can be a suitable replacement for sphagnum peat in nursery production of Pinus ponderosa seedlings. Forests 9, 232. https://doi.org/10.3390/f9050232.

EU, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Union. 364.

Gale, N.V., Thomas, S.C., 2019. Dose-dependence of growth and ecophysiological responses of plants to biochar. Sci. Total Environ. 658, 1344–1354. https://doi.org/10.1016/j.scitotenv.2018.12.239.

Gale, N.V., et al., 2017. Comparative responses of early-successional plants to charcoal soil amendments. Ecosphere 8, e01933. https://doi.org/10.1002/ecs2.1933.

Godt, J., et al., 2006. The toxicity of cadmium and resulting hazards for human health. J. Occup. Med. Toxicol. 1, 22. https://doi.org/10.1186/1745-6673-1-22.

Gough, L., et al., 2000. Vascular plant species richness in Alaskan arctic tundra: the importance of soil pH. J. Ecol. 88, 54–66. https://doi.org/10.1046/j.1365-2745.2000.00426.x.

Haase, D., Rose, R., 1995. Vector analysis and its use for interpreting plant nutrient shifts in response to silvicultural treatments. For. Sci. 41, 54–66. https://doi.org/10.1093/forestscience/41.1.54.

Hansen, M., et al., 2017. Micro vertical changes in soil pH and base cations over time after application of wood ash on forest soil. For. Ecol. Manage. 406, 274–280. https://doi.org/10.1016/j.foreco.2017.09.069.

Hansen, M., et al., 2018. Effects of ash application on nutrient and heavy metal fluxes in the soil and soil solution in a Norway spruce plantation in Denmark. For. Ecol. Manage. 424, 494–504. https://doi.org/10.1016/j.foreco.2018.05.005.

Huotari, N., et al., 2015. Recycling of ash - For the good of the environment? For. Ecol. Manage. 348, 226–240. https://doi.org/10.1016/j.foreco.2015.03.008.

Ingerslev, M., et al., 2014. Effects of wood chip ash fertilization on soil chemistry in a Norway spruce plantation on a nutrient-poor soil. For. Ecol. Manage. 334, 10–17. https://doi.org/10.1016/j.foreco.2014.08.034.

Jäggi, M., et al., 2004. Wood ash treatment affects seasonal N fluctuations in needles of adult Picea abies trees: a 15 N-tracer study. Trees 18, 54–60. https://doi.org/10.1007/s00468-003-0280-0.

Järup, L., Åkesson, A., 2009. Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol. 238, 201–208. https://doi.org/10.1016/j.taap.2009.04.020.

Jiang, Q.-Y., et al., 2016. Can arbuscular mycorrhizal fungi reduce Cd uptake and alleviate Cd toxicity of Lonicera japonica grown in Cd-added soils? Sci. Rep. 6, 1–9. https://doi.org/10.1038/srep21805.

Johansen, J.L., et al., 2018. Toxicity of cadmium and zinc to small soil protists. Environ. Pollut. 242, 1510–1517. https://doi.org/10.1016/j.envpol.2018.08.034.

Johansen, J.L., et al., 2019. Wood ash decreases cadmium toxicity to the soil nematode Caenorhabditis elegans. Ecotoxicol. Environ. Saf. 172, 290–295. https://doi.org/10.1016/j.ecoenv.2019.01.092.

Jokinen, H.K., et al., 2006. Exploring the mechanisms behind elevated microbial activity after wood ash application. Soil Biol. Biochem. 38, 2285–2291. https://doi.org/10.1016/j.soilbio.2006.02.007.

Kindtler, N.L., et al., 2019. Wood ash effects on growth and cadmium uptake in Deschampsia flexuosa (Wavy hair-grass). Environ. Pollut. 249, 886–893. https://doi.org/10.1016/j.envpol.2019.03.098.

Kirkham, M., 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137, 19–32. https://doi.org/10.1016/j.geoderma.2006.08.024.

Kjøller, R., et al., 2017. Ectomycorrhizal fungal responses to Forest liming and wood ash addition: review and meta-analysis. Soil Biological Communities and Ecosystem Resilience, pp. 223–252. https://doi.org/10.1007/978-3-319-63336-7_14.

Maresca, A., et al., 2017. Recirculation of biomass ashes onto forest soils: ash composition, mineralogy and leaching properties. Waste Manag. 70, 127–138. https://doi.org/10.1016/j.wasman.2017.09.008.

Maresca, A., et al., 2018. Column leaching from a Danish forest soil amended with wood ashes: fate of major and trace elements. Biomass Bioenergy 109, 91–99. https://doi.org/10.1016/j.biombioe.2017.12.014.

McLaughlin, M.J., Singh, B.R., 1999. Cadmium in soils and plants. Cadmium in Soils and Plants. Springer, pp. 1–9. https://doi.org/10.1007/978-94-011-4473-5_1.

Moilanen, M., et al., 2002. Effects of wood-ash on the tree growth, vegetation and substrate quality of a drained mire: a case study. For. Ecol. Manage. 171, 321–338. https://doi.org/10.1016/S0378-1127(01)00789-7.

Moilanen, M., et al., 2006. Does wood ash application increase heavy metal accumulation in forest berries and mushrooms? For. Ecol. Manage. 226, 153–160. https://doi.org/10.1016/j.foreco.2006.01.033.

Mortensen, L.H., et al., 2018. Bioaccumulation of cadmium in soil organisms - with focus on wood ash application. Ecotoxicol. Environ. Saf. 156, 452–462. https://doi.org/10.1016/j.ecoenv.2018.03.018.

Mortensen, L.H., et al., 2019. Ash application enhances decomposition of recalcitrant organic matter. Soil Biol. Biochem. 135, 316–322. https://doi.org/10.1016/j.soilbio.2019.05.021.

Mortensen, L.H., et al., 2020. Effect of ash application on the decomposer food web and N mineralization in a Norway spruce plantation. Sci. Total Environ. 715, 136793 https://doi.org/10.1016/j.scitotenv.2020.136793.

Näsholm, T., et al., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914–916. https://doi.org/10.1038/31921.

Ohno, T., Erich, M.S., 1990. Effect of wood ash application on soil pH and soil test nutrient levels. Agric. Ecosyst. Environ. 32, 223–239. https://doi.org/10.1016/0167-8809(90)90162-7.

Omil, B., et al., 2013. Soil and tree responses to the application of wood ash containing charcoal in two soils with contrasting properties. For. Ecol. Manage. 295, 199–212. https://doi.org/10.1016/j.foreco.2013.01.024.

Pärtel, M., 2002. Local plant diversity patterns and evolutionary history at the regional scale. Ecology 83, 2361–2366. https://doi.org/10.1890/0012-9658(2002)083[2361:LPDPAE]2.0.CO;2.

Perkiomaki, J., Fritze, H., 2005. Cadmium in upland forests after vitality fertilization with wood ash - a summary of soil microbiological studies into the potential risk of cadmium release. Biol. Fertil. Soils 41, 75–84. https://doi.org/10.1007/s00374-004-0816-5.

Persson, J., et al., 2003. Nitrogen acquisition from inorganic and organic sources by boreal forest plants in the field. Oecologia 137, 252–257. https://doi.org/10.1007/s00442-003-1334-0.

Rask, K.A., et al., 2019. Differences in arbuscular mycorrhizal colonisation influence cadmium uptake in plants. Environ. Exp. Bot. 162, 223–229. https://doi.org/10.1016/j.envexpbot.2019.02.022.

Raven, P.H., et al., 2005. Biology of Plants. Macmillan.

Ring, E., et al., 2006. Soil-solution chemistry in a coniferous stand after adding wood ash and nitrogen. Can. J. For. Res. 36, 153–163. https://doi.org/10.1139/x05-242.

Rosenberg, O., et al., 2010. Effects of wood-ash application on potential carbon and nitrogen mineralisation at two forest sites with different tree species, climate and N status. For. Ecol. Manage. 260, 511–518. https://doi.org/10.1016/j.foreco.2010.05.006.

Rousk, J., et al., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl. Environ. Microbiol. 75, 1589–1596. https://doi.org/10.1128/AEM.02775-08.

Stupak, I., et al., 2007. Sustainable utilisation of forest biomass for energy—possibilities and problems: policy, legislation, certification, and recommendations and guidelines in the Nordic, Baltic, and other European countries. Biomass Bioenergy 31, 666–684. https://doi.org/10.1016/j.biombioe.2007.06.012.

Vestergård, M., et al., 2018. The relative importance of the bacterial pathway and soil inorganic nitrogen increase across an extreme wood-ash application gradient. Gcb Bioenergy 10, 320–334. https://doi.org/10.1111/gcbb.12494.

Weber, A., et al., 1985. Microbial activities in a histosol: effects of wood ash and NPK fertilizers. Soil Biol. Biochem. 17, 291–296. https://doi.org/10.1016/0038-0717(85)90063-X.

Weigelt, A., et al., 2005. Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia 142, 627–635. https://doi.org/10.1007/s00442-004-1765-2.