



Is wood ash amendment a suitable mitigation strategy for N₂O emissions from soil?



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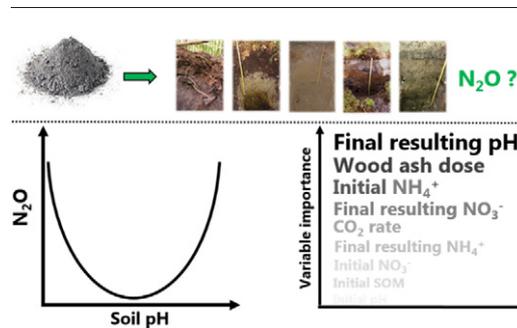
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HIGHLIGHTS

- Wood ash amended to different soil types affected the emission of N₂O.
- The final resulting soil pH was the most important factor affecting N₂O emissions.
- Wood ash applied at low rates (<9 t ha⁻¹) may mitigate N₂O emissions from soils with low pH and high SOM.

GRAPHICAL ABSTRACT



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ABSTRACT

Wood ash, the by-product of biomass combustion to energy, can return important nutrients back to the soil and counteract acidification. However, the application of wood ash may affect the emission of greenhouse gases. Here, the effect of wood ash application on nitrous oxide (N₂O) emissions from different soil environments were investigated in a 40 days incubation experiment comprising ten different soil types amended with five different wood ash concentrations (0, 3, 9, 20, and 54 t ash ha⁻¹). The emitted N₂O was measured continuously, and initial soil properties without ash application (carbon (C), nitrogen (N), ammonium (NH₄⁺), nitrate (NO₃⁻), and pH) and resulting soil properties (pH, NH₄⁺, and NO₃⁻) were measured prior and after the incubation period, respectively. The Random Forests (RF) model was used to identify which factors (initial and resulting soil properties, vegetation, management, wood ash dose, and respiration rate) were the most important to predict the development of emitted N₂O after ash application. Wood ash either increased, decreased, or had no effect on the amount of emitted N₂O depending on soil type and ash dose. The RF model identified the final resulting pH as the most important factor for the prediction of emitted N₂O. The results suggest that wood ash can mitigate N₂O emissions from soil, however, this effect depends on soil type where a mitigating effect of wood ash application was observed mainly in low pH soils with high soil organic matter whereas an increase in N₂O emissions was observed in mineral soils that had previously received N fertilization. This study emphasises the importance of pH manipulation in regards to N₂O emissions from soil.

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1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas contributing to radiative forcing. N₂O has a global warming potential 300 times that of carbon dioxide (CO₂), and it is now considered the third most influential greenhouse gas (Stocker et al., 2013). Today, emissions from agricultural and natural soils contribute 60% of the total N₂O emitted globally (Ciais et al., 2013).

The primary N₂O producing processes in soil are microbial nitrification and denitrification; which both utilize inorganic N compounds, ammonium (NH₄⁺) and nitrate (NO₃⁻), respectively (Firestone and Davidson, 1989) (Supplementary material one (S1), Fig. A). Nitrification is the most important source of N₂O fluxes from well-aerated soils (water-filled pore space <60%), while N₂O production in wet soils (water-filled pore space 60–90%) is predominantly derived from anaerobic denitrification (Skiba et al., 1997; Bateman and Baggs, 2005; Mathieu et al., 2006). Denitrification is the major biological process in soils that returns fixed nitrogen to the atmosphere, where N₂O is an obligatory intermediate and may under certain conditions also be the end product. N₂O from nitrification is formed during ammonia oxidation, where N₂O is formed by chemical decomposition of hydroxylamine (Hooper and Terry, 1979; Wrage et al., 2005; Frame and Casciotti, 2010). However, the formation of nitrite (NO₂⁻) is always the main pathway, and levels of nitric oxide (NO) and N₂O produced are several orders of magnitude lower (103–106) than those of NO₂⁻ (Arp and Stein, 2003). The NO₃⁻ produced by nitrification of NH₄⁺ is often directly denitrified. The process of denitrification coupled to nitrification involves distinct microorganisms (Arth et al., 1998; Avrahami and Conrad, 2002; Carrasco et al., 2004; Kool et al., 2010), and a patchy environment with mixed aerobic and anaerobic microsites. However, some ammonia oxidisers have the ability to perform nitrification and denitrification in one process called nitrifier denitrification (Wrage-Mönnig et al., 2018). The rate and end products of the N₂O producing processes are affected by soil properties and conditions such as soil organic matter (SOM), nitrogen (N) substrate form and availability, pH, and oxidative conditions. In S1 (Table A), soil conditions favouring N₂O production from different N₂O producing processes are summarized.

Previously, soil pH management has been proposed as a strategy to mitigate N₂O emissions from soils (Liu et al., 2010; Barton et al., 2013). Thus far, several authors have investigated the relationship between N₂O emissions and soil pH (Goodroad and Keeney, 1984; Yamulki et al., 1997; Stevens et al., 1998; Feng et al., 2003; Baggs et al., 2010; Cuhel et al., 2010; Barton et al., 2013) however, with conflicting results. Soil pH can be managed by applying different soil amendments (e.g. lime) or wood ash. Increasing amounts of wood ash are being produced as a by-product of biomass combustion to energy (Silva et al., 2019). Wood ash consists of the inorganic compounds from the harvested biomass, sand residues, and a very small fraction of not fully combusted organic material (Ingerslev et al., 2011). Hence, wood ash contains all inorganic plant macro- and micronutrients from the biomass except N (Rosenberg et al., 2010). During combustion different oxides develop and subsequent aeration will lead to the formation of carbonates in the wood ash, making the ash very alkaline with a pH between 8 and 13 (Augusto et al., 2008). As opposed to disposing of the ash in landfills, recycling the ash as a soil amendment will return important nutrients to the soil, and counteract acidification (Brunner et al., 2005; Pitman, 2006).

So far, only a few studies have investigated the effect of wood ash application on N₂O emissions from soil. Klemmedtsson et al. (2010) found a decrease of 40–46% emitted N₂O, in the first year after wood ash application in a spruce forest on drained organic soil. They assigned the reduction of emitted N₂O to increased soil pH. Liimatainen et al. (2014) also found that granulated wood ash reduced N₂O production in peat soil. However, these authors assigned the reduced N₂O emissions to changes in the ion strength inhibiting N₂O production. Similarly,

Royer-Tardif et al. (2019) found that wood ash decreased N₂O production in a sugar maple forest two years after application. Contrarily, Maljanen et al. (2006a) found no effect of wood ash amendment on N₂O production in a podzolized spruce forest soil. However, in another study, the same authors observed a tendency towards both increased or decreased N₂O production from different long-term field experiments with peat soil (Maljanen et al., 2006b). Thus, depending on the N₂O producing processes dominating in the specific soil environment and initial soil properties, wood ash application may affect N₂O emissions differently. Thus, coupling specific soil properties to the effect of wood ash application on N₂O production is important to predict the potential effects of wood ash amendment to soil.

Here, the mitigating effect of wood ash application on N₂O production from a range of different soils was investigated. The aim was to examine the relative importance of different inherent and induced soil properties (SOM, pH, total C, total N, NH₄⁺, and NO₃⁻ content) and factors such as vegetation and fertilizer management to predict the development of N₂O production from soils amended with wood ash at different concentrations. Accordingly, an incubation study, including ten different temperate soils, was conducted. The soils differed in vegetation and land use (woodland, heath, and agriculture), fertilizer management (manure, NPK, or unfertilized), and basic soil properties. We hypothesized that the effect of wood ash application on N₂O production from soil depends on the initial soil properties where the initial and induced soil pH is an important factor.

2. Methods and materials

2.1. Wood ash

The wood ash was collected from Galten district heating plant, Galten, Denmark. The ash is a mix of bottom and fly ash originating from the combustion of wood chips of Norway spruce. To determine the chemical composition of the ash, samples were digested with nitric acid (HNO₃) and P, K, Ca, Mg, Na, Mn, Zn, Cu, Cd, Pb, Cr, and Ni were measured by inductively coupled plasma atomic emission spectroscopy ICP-OES (PerkinElmer, optima 3000 XL). pH was measured using a glass electrode in a 1:2.5 water to wood ash solution on a Phm240 pH/ION Meter (Meterlab, Radiometer Copenhagen). Wood ash properties are presented in S1 (Table B).

2.2. Soil sampling and analysis

Ten different soil types comprising plantation forests, peat, and agricultural soils were collected from various locations in Zealand, Denmark. Three woodland soils (W1, W2, and W3), three peat soils (P1, P2, and P3), and four agricultural soils (A1, A2, A3, and A4) were sampled from the top 1–8 cm beneath the litter layer and stored at 5 °C. More detailed descriptions of all soils are presented in S1 (Table C).

Soil bulk densities were determined using 100 cm³ volumetric rings on field soil. Estimates of field capacity were made as follows: Soils were air-dried for 48 h at 20 °C, sieved through a 2 mm mesh or homogenized by hand if the SOM content was high and subsequently compressed gently into 175 ml plastic cups with holes in the bottom. The samples were saturated with water, and the water content of the soil collected from 2 to 4 cm depth in the container was determined after 24 h. Soil pH was measured using a glass electrode in a 1:2.5 soil water extraction on a Phm240 pH/ION Meter (Meterlab, Radiometer Copenhagen). SOM was determined by loss of ignition as the weight % difference between samples oven-dried at 105 °C and samples combusted at 550 °C. Total C and N were determined by infrared absorption spectroscopy (IR) on a LECO TruSpec Carbon Nitrogen Determinator (Michigan, USA). Particle size distributions for the mineral soils (A1, A2, A3, and A4) were determined by laser diffraction using a Hydro 2000G, Malvern Mastersizer 200G. NH₄⁺ and NO₃⁻ were determined in a 1:5 soil water extraction and analysed colourimetrically on a 5000 FIASTAR flow injection analyser

(Höganäs, Sweden) using spectrophotometry. Initial soil properties are presented in Table 1.

2.3. Incubation experiment

The soils were sieved using a 2 mm sieve or homogenized by hand if the content of organic material was too high. Soil samples equivalent to 10 cm³ field soil were mixed with five different wood ash concentrations equivalent to 0, 3, 9, 20 and 54 t ash ha⁻¹, assuming that the wood ash was mixed in the top 5 cm soil. Mixed soil and wood ash samples were supplied with sterilized distilled water to obtain field capacity in 116-ml serum bottles and closed with airtight rubber stoppers. Samples were incubated in the dark at 15 °C for 40 days. The bottles were added 20 ml extra atmospheric air to secure an overpressure; hence, extraction of headspace gas samples was possible. Headspace gas samples were taken on day 12, 18, 28, and 36 using a syringe. Oxygen (O₂) and CO₂ levels were measured using a gas chromatograph with a TC detector (Thermal Conductor) and a Poropak Q column (1.8 cm × 3 cm) with Ar/CH₄ as carrier gas and N₂O levels were analysed on a Shimadzu 8A with an EC detector (Electron Capture) and a Poropak Q column (1.8 cm × 3 cm) with Helium (He) as carrier gas. When CO₂ levels in the control samples (no ash) reached >5%, all bottles were opened and aerated for 24 h to avoid oxygen depletion. After 40 days of incubation, soil pH, NH₄⁺ and NO₃⁻ were determined as follows: 70 ml distilled water was added to each incubation flask and shaken for one hour on a rotary shaker. Subsequently, the flasks were set to sediment for one hour, and 10 ml of the clarified supernatant was extracted for pH measurements, and 20 ml was centrifuged and filtered to determine NH₄⁺ and NO₃⁻ colourimetrically on a 5000 FIASTAR flow injection analyser (Höganäs, Sweden). The N₂O measured during the incubation period was calculated as the cumulative N₂O production for each incubation flask. Thus, the N₂O production will in the following refer to the cumulative N₂O production.

2.4. Statistical analysis

The Random Forests (RF) model was used to identify the relative importance and interactions of soil properties on the N₂O production following wood ash application. The RF model belongs to a newer assemblage of machine learning models. The advantage of these models is that they can capture complex nonlinear relationships between predictor and response variables more effectively than a linear regression model (Pearson, 2016). We constructed the RF model based on the variables listed in Table 2. The full dataset can be viewed in the supplementary material two (S2). The RF model constructs single decision trees from a subset of observations from the data input. The variables computing each particular tree are randomly selected and by default comprise one-third of the total number of variables (Liaw and Wiener, 2015). The ensemble of decision trees comprises a decision forest. For a single data point, each tree in the forest will calculate an average

Table 2
Variables used in the random forests model.

Dependent variable	Unit
Accumulated N ₂ O production in a 36 days incubation	log(μg N-N ₂ O m ⁻²)
Independent variables	Unit
Initial soil pH	pH
Initial soil organic matter (SOM)	%
Initial soil NH ₄ ⁺	mg kg ⁻¹
Initial soil NO ₃ ⁻	mg kg ⁻¹
Initial soil C	%
Initial soil N	%
Wood ash application	t ha ⁻¹
Final resulting soil pH	pH
Final resulting NH ₄ ⁺	mg kg ⁻¹
Final resulting NO ₃ ⁻	mg kg ⁻¹
Average CO ₂ rate during the incubation	μg C-CO ₂ m ⁻² h ⁻¹
Vegetation	1 Deciduous forest, 2 Spruce/fir forest, 3 Heather 4 Agriculture
Management	1 Natural 2 Agriculture + NPK, 3 Agriculture no NPK 4 Agriculture + Manure

based on the subset of observations. Consequently, the average that gets the most “votes” determines the final prediction. The RF model was constructed using the R software version 3.3.0. The randomForest () function in the randomForest package was used to construct the model (Liaw and Wiener, 2015). The number of trees was set to 5000, and the default settings for the number of variables included in each tree (i.e. $\frac{V}{3}$, where V is the total number of variables) was applied.

The rfPermute package with the functions rfPermute() and rp.importance() was used to calculate variable importance (Archer, 2016). Finally, the rfUtilities package holding the rf.significance() function was used to test for model significance (Jeffrey et al., 2016). The partial dependence plots were produced using the partial dependence plot function in the randomForest package.

One way analysis of variance ($P < .05$) and Tukey's test were performed for the N₂O production for each soil type. Normality of the data was evaluated visually and by using the Shapiro-Wilks test, and Levene's test was performed to confirm the homogeneity of variance before the ANOVA test. All analysis were performed in R software version 3.3.0.

3. Results

3.1. Soil types

As expected, the collected soil types differed greatly in initial soil properties. The three woodland soils (W1, W2, and W3) had initially

Table 1
Initial soil properties ±SD (n = 3), SOM (n = 4).

Soil type	Bulk density g cm ⁻³	pH (H ₂ O)	SOM %	Total C %	Total N %	NH ₄ ⁺ mg kg ⁻¹	NO ₃ ⁻ mg kg ⁻¹
W1 Arenosol	0.23 (0.10)	3.9 (0.05)	76 (3.0)	45 (0.000)	1.8 (0.006)	16 (1.0)	63 (1.1)
W2 Podzol	0.25 (0.13)	4.0 (0.03)	83 (1.6)	41 (0.071)	1.5 (0.002)	13 (1.5)	1 (0.1)
W3 Alisol	1.00 (0.11)	4.4 (0.02)	34 (3.1)	19 (0.566)	1.0 (0.026)	40 (1.0)	35 (2.8)
P1 Histosol low pH	0.18 (0.01)	4.0 (0.03)	96 (0.9)	53 (0.141)	1.0 (0.009)	20 (0.8)	22 (1.3)
P2 Histic Planosol	0.75 (0.06)	5.8 (0.14)	46 (0.6)	14 (0.071)	1.1 (0.001)	0.2 (0.02)	67 (0.4)
P3 Histosol high pH	0.24 (0.02)	6.5 (0.05)	77 (0.8)	38 (0.071)	2.9 (0.004)	0.1 (0.02)	42 (1.2)
A1 Luvisol high pH	1.30 ^a	7.2 (0.02)	11 (0.7)	8 (0.170)	0.3 (0.002)	0.2 (0.04)	166 (0.4)
A2 Luvisol unfertilized	1.40 ^a	7.0 (0.07)	5 (0.02)	1 (0.038)	0.1 (0.009)	0.1 (0.03)	31 (1.6)
A3 Luvisol manure	1.40 ^a	6.0 (0.02)	4 (0.03)	1 (0.001)	0.1 (0.000)	0.2 (0.004)	39 (4.4)
A4 Luvisol NPK	1.40 ^a	6.6 (0.03)	4 (0.004)	2 (0.083)	0.2 (0.003)	0.2 (0.003)	32 (5.0)

^a Values used based on personal communication with farmers.

low pH ≤ 4.4 and high SOM content 34–83% (Table 2). However, soil W3 had slightly higher pH and lower SOM content compared to soils W1 and W2, due to the difference in vegetation (spruce/fir vs. deciduous forest) and parent material (sand vs. till) (Table 1). The peat soils (P1, P2, and P3) differed in initial pH (Table 2) and the degree of decomposition of the organic material (Table 1). The agricultural soils A2–4 had similar properties as they were collected from the same location and mainly differed in their management history i.e. unfertilized, manure, or NPK. Soil A1 was a different soil type than soils A2–4 and had a high initial pH due to a naturally high content of CaCO_3 and a naturally high Fe-content (Tables 1 and 2).

3.2. Random Forests model

The RF regression model was significant at $p < 0001$ (Fig. 1). The extracted measure of variable importance indicated that the final resulting pH, wood ash dose, initial NH_4^+ , and final resulting NO_3^- content were the most important variables for the prediction of N_2O production from the different soils. Hence these parameters will be the focus of discussion. In contrast, total C, total N, and management added the least explanation to the prediction model (Fig. 1).

3.3. Effect of wood ash amendment on the N_2O production from soil

The effects of wood ash application on the N_2O production differed between soil types. In forest soil W1 and peat soil P1, wood ash application significantly decreased the N_2O production whereas high doses of wood ash (54 t ha^{-1}) significantly increased the N_2O production in the agricultural soils A1, A3, and A4 compared to the control samples (Fig. 2). The results were more variable for the woodland soils W2 and W3, where wood ash application either increased or decreased the

N_2O production depending on the ash dose (Fig. 2). Finally, wood ash application did not significantly affect the N_2O production in agricultural soil A2 and peat soils P2 and P3 compared to the control samples. Still, there was a tendency that wood ash caused an increase in N_2O production (Fig. 2). The partial dependence plot showed that the general relationship between wood ash dose and the N_2O production was negative when wood ash was applied at low doses ($0\text{--}9 \text{ t ha}^{-1}$) and positive at high doses ($>20 \text{ t ha}^{-1}$) (Fig. 3).

3.4. Relationship between key predictor variables, wood ash application, and N_2O production

In all soil types, wood ash amendment resulted in a pH increase (Fig. 2). The highest wood ash dose (54 t ha^{-1}) increased pH between 1.6 and 3.6 pH units, depending on soil type, compared to control samples. The partial dependence plot revealed that the relationship between the N_2O production and final soil pH was negative in the pH range from 4.0–5.3 and positive in the pH range from 8.0–9.0. Whereas, pH did not influence the N_2O production in the pH range from 5.3–8.0 (Fig. 3).

The initial NH_4^+ content was highest in the natural soils with initially low pH (W1, W2, W3, and P1), whereas the agricultural soils and natural soils with pH close to neutral had approximately 100 times less initial content of NH_4^+ (Table 1). The partial dependence plot revealed that the relationship between initial NH_4^+ content and N_2O production was overall positive (Fig. 3).

There was a general trend towards an increase in the final soil NO_3^- content following wood ash application. However, in soils W1, P1, and A2, the NO_3^- content did not increase at the highest wood ash dose (54 t ha^{-1}). Furthermore, the NO_3^- content was not notably affected by wood ash application in soils A1 and W2 (Fig. 4). According to the

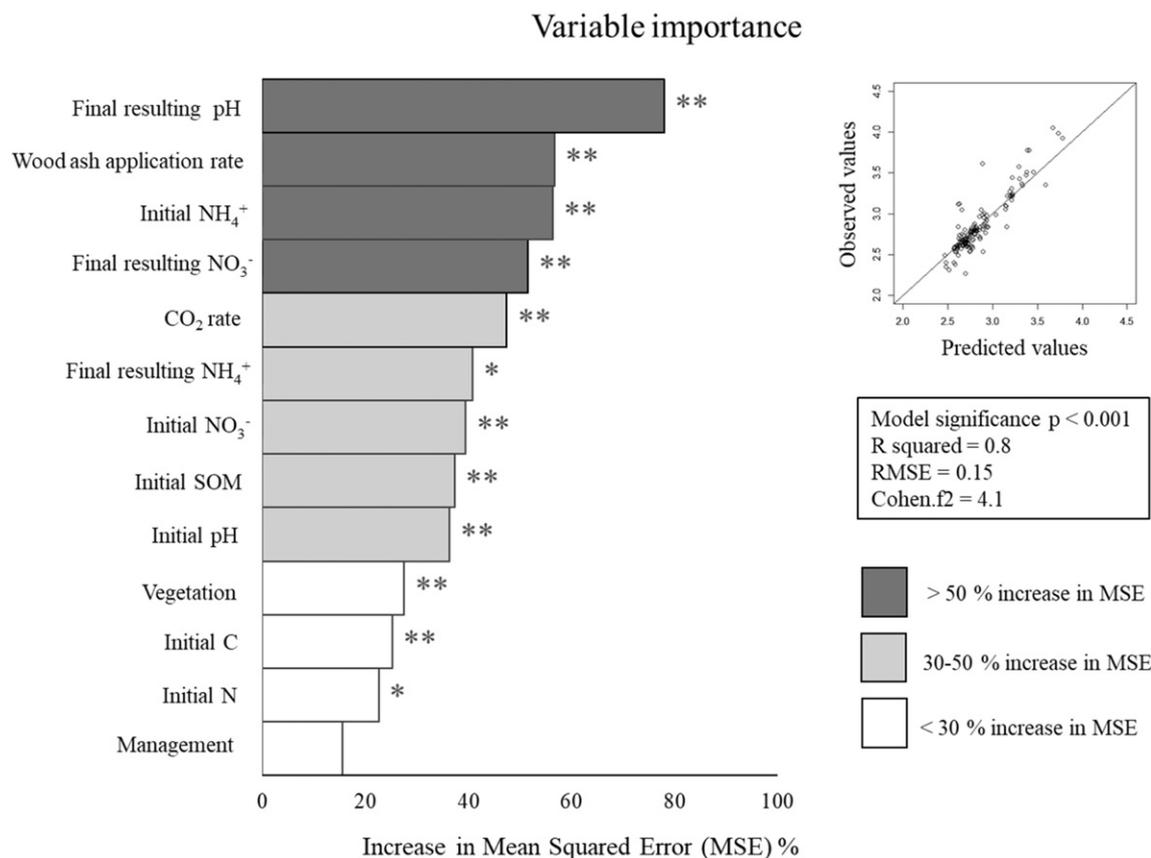


Fig. 1. Variable importance determined from the random forests model. Variable importance is defined as the percentage increase in the model Mean Squared Error (MSE) if a particular variable is excluded from the model. Significance level for independent variables ** = $p < .01$, * = $p < .05$. Predicted values are plotted against actual values of the accumulated N_2O in the top right corner.

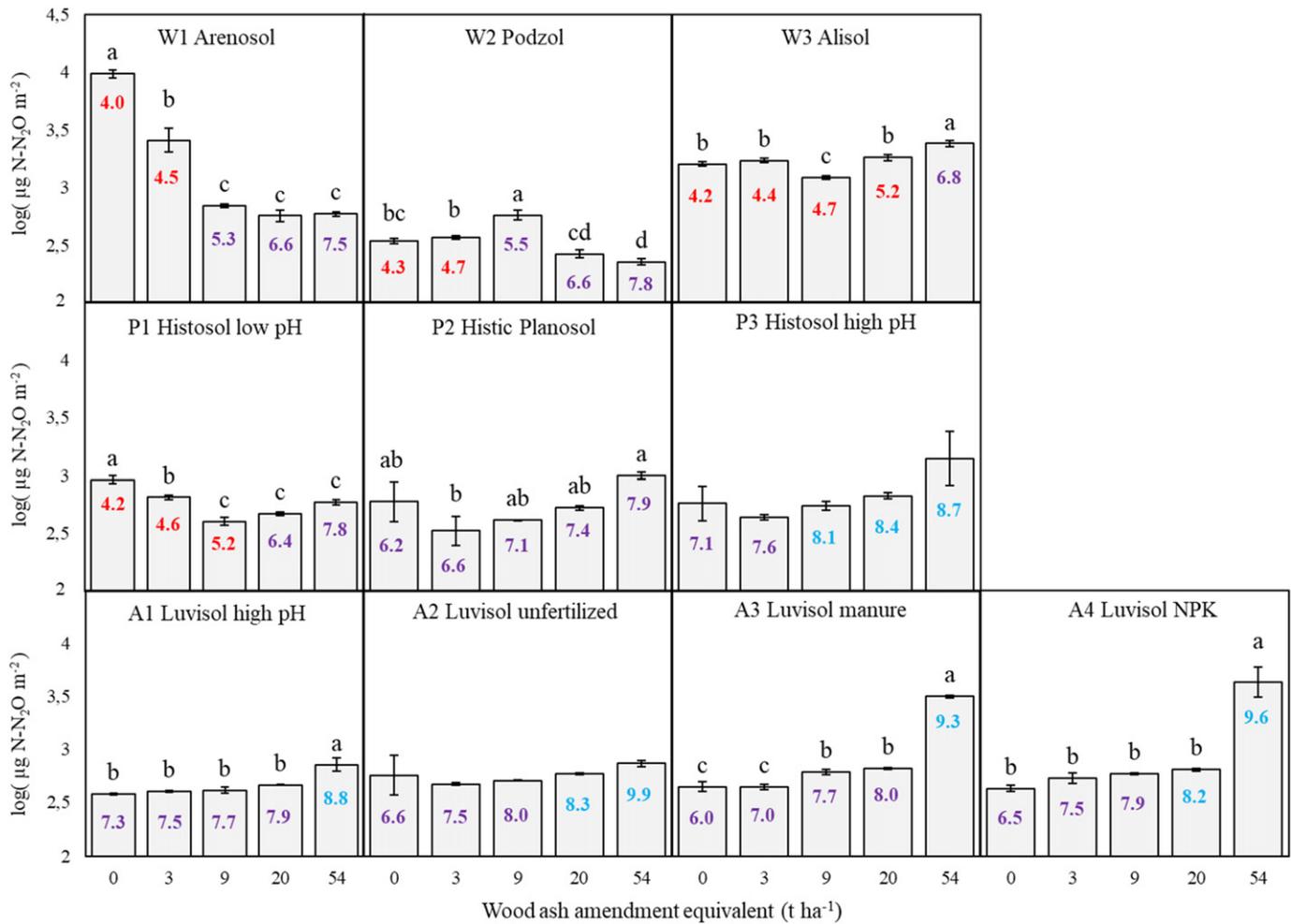


Fig. 2. Accumulated N_2O during a 36 day incubation period in an experiment including ten soils of different soil type and/or land uses. Soils were amended with an array of different wood ash concentrations, equivalent to field application of 0–54 t ha⁻¹. W = woodland soils on mineral parent material, P = peat soils, and A = agricultural soils on mineral parent material. See Tables 1 and S1 (Table C) for more details of the different soils. Error-bars indicate 1 SE. Different letters indicate significantly different N_2O accumulation for the particular soil (One way ANOVA ($p < .05$) followed by Tukey 2-way comparison). Colour of values at each bar indicates level of soil pH: Red: pH = 3–5.3, purple: pH = 5.3–8, blue: pH = 8–10.

partial dependence plot, the relationship between N_2O production and final soil NO_3^- content was positive from 0 mg kg⁻¹ to approximately 50 mg kg⁻¹, whereas there was no clear relationship between the two parameters at higher concentrations (Fig. 3).

4. Discussion

4.1. pH effects on the N_2O production from soil

As expected wood ash amendment increased the pH in all soils. Correspondingly, the RF model suggested that the final resulting soil pH was the strongest predictor for the N_2O production from the different soil types (Fig. 1). In addition, the RF model specified that the relationship between N_2O production and the final resulting pH was not straightforward, as the relationship was U-shaped (Fig. 3). This picture was somewhat, but not completely reflected in the relationship between wood ash application and N_2O production (Fig. 3). Hence, the effect of the ash amendment depends to some extent on the initial soil properties, as the final resulting pH is a combined effect of basic soil properties (affecting the buffer capacity) and wood ash dose. Therefore, the final resulting pH rather than wood ash application rate was the better predictor for the N_2O production. In essence, the RF model results indicate that wood ash applied at low doses (3–9 t ha⁻¹), with less effect on soil pH (pH < 8), reduced or had no significant effect on the N_2O production from a wide range of different soil environments. In contrast,

there was a tendency towards increased N_2O production when the soils became alkaline (pH > 8) which was achieved at the highest wood ash amendment rates for some soils (Figs. 2, 3 and 5).

In general, increasing pH in acidic soils stimulates the microbial activity and modulates the composition of the soil microbial community (Fierer and Jackson, 2006), which in turn can affect the rate and end products of the N_2O producing processes (Šimek et al., 2002). Accordingly, the microbial activity, continuously measured as the O_2 consumption, increased in all soils amended with wood ash, except for the initially alkaline soil A1 (S1, Fig. A). We attribute this phenomenon to the combined effect of nutrient addition and pH increase by the wood ash.

N_2O production under acidic soil conditions is usually caused by denitrification and heterotrophic nitrification (Šimek and Cooper, 2002; Mørkved et al., 2007; Zhang et al., 2015a). In contrast, autotrophic nitrification performed by bacteria is sensitive to low pH and will mainly contribute to N_2O production at neutral and high pH (S1, Table A) (Baggs et al., 2010). Thus, changing the soil pH may affect which processes are mainly responsible for the N_2O production. In line with our findings, several studies have suggested that raising pH by e.g. liming may reduce N_2O emissions from acidic soil environments (Yamulki et al., 1997; Barton et al., 2013; Wang et al., 2018). On the other hand, elevated pH may lead to an increase in the rate of nitrification and thus a higher production of N_2O (Baggs et al., 2010). However, the exact relationship between pH and N_2O emissions from soil is complex,

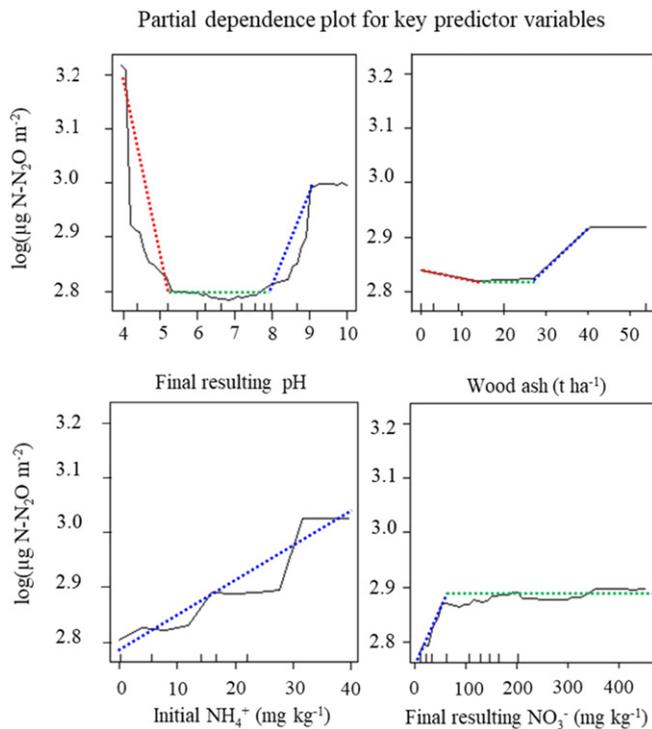


Fig. 3. Partial dependence plot obtained from the random forests model for the four most important variables (see Fig. 1) for the prediction of N_2O production, based on incubation experiments including ten different soils (Tables 1 and S1 (Table C)). The y-axes represent the mean of the predicted values of the response variable in $\log(\mu\text{g N-N}_2\text{O m}^{-2})$ corresponding to the particular specific independent variables on the X-axis. Red dotted lines indicate a negative relationship between the response variable and the independent variable, green dotted lines indicate that there is no clear relationship between the response and independent variable, and blue dotted lines indicate a positive relationship between the response and independent variable.

and the underlying mechanistic effects of pH on N_2O emissions are continuously debated (Goodroad and Keeney, 1984; Šimek et al., 2002; Šimek and Cooper, 2002; Baggs et al., 2010; Cuhel et al., 2010; Liu et al., 2010; Mcmillan et al., 2016; Kim et al., 2017; Wang et al., 2018). In this study, the investigated soils fell into three different subgroups, i.e. ash amendment increased, decreased, or had no effect on the N_2O production compared to the unamended controls (Fig. 5). Two soils (W2 and W3) overlap in the groups, as the N_2O production was found to either increase or decrease depending on the ash dose.

Ash-induced increases in N_2O production was mainly observed in the cultivated mineral soils with near-neutral pH and low SOM content (A1, A3, and A4) (Figs. 2, 5 and Table 2). Specifically, the highest doses of wood ash application (54 t ha^{-1}) significantly increased the N_2O production from cultivated soils that had received NPK or manure (A1, A3, and A4), yet not from the unfertilized agricultural soil A2 (Figs. 2 and 5). Higher N_2O production, due to higher substrate availability, from soils amended with conventional NPK fertilizers or manure, has previously been reported (Meng et al., 2005; Ding et al., 2013). The ash induced increase in pH may have resulted in a higher nitrification rate and thus increased the N_2O production from this process. However, a significant increase in the N_2O production from NPK or manure treated soils was only observed at very high ash applications that raised the soil pH to 8.8–9.6 pH. Such high soil pH is not considered beneficial from an agricultural perspective due to restricted nutrient availability of nutrients such as phosphorus (P) (Brady and Weil, 2008). Thus, our results suggest that moderate ash applications (3 t ha^{-1}) do not pose a great risk for short-term enhancement of N_2O emissions even from the cultivated mineral soils.

Conversely, ash application reduced the N_2O production in soils W1, W2, W3, and P1 (Figs. 2 and 5). These were all natural soils with similar properties such as initially low pH and high SOM content (Tables 1, 2). In general, when soil pH increases, the rate of denitrification and nitrification also increase, due to higher microbial activity and thus substrate availability (i.e. C, NO_3^- , and NH_4^+) (Šimek and Cooper, 2002; Baggs et al., 2010). However, for denitrification, the emitted N_2O relative to N_2 decreases with increasing pH. Thus, the emitted N_2O from denitrification is generally higher at acidic conditions (Šimek and Cooper, 2002; Mcmillan et al., 2016; Shaaban et al., 2018). Therefore, an increase in soil pH induced by wood ash amendment may have resulted in reduced N_2O emissions from denitrification in the acidic soils. The particular mechanisms controlling the product ratio ($N_2O:N_2$) from denitrification at shifting pH is still up for debate. Especially the relationship between soil pH and the relative abundance of bacteria possessing the *nosZ* gene encoding the reductase of N_2O to N_2 has drawn much attention (Philippot et al., 2009; Liu et al., 2010; Zhang et al., 2015b). Accordingly, it has been suggested that the N_2O reductase enzyme is sensitive to low pH (Šimek and Cooper, 2002; Liu et al., 2010). However, based on experiments with denitrifying bacteria *Shewanella loihica*, Kim et al. (2017) suggested that enhancement of NO_2^- reduction to N_2O rather than a decrease in N_2O reduction to N_2 was the actual cause of high N_2O accumulation in acidic environments. However, N_2O production from denitrification is not solely controlled by pH. Hence, other environmental factors such as the NO_3^- content, the $\text{NO}_2^-:\text{NO}_3^-$ ratio, and the C:N ratio have been reported to overarch the effect of pH (Blackmer and Bremner, 1978; Yoon et al., 2015; Kim et al., 2017). Recently, Senbayram et al. (2019) found that the addition of NO_3^- to soil inhibited the reduction of N_2O to N_2 resulting in an increase in N_2O emissions regardless of soil pH. Accordingly, the authors proposed that the NO_3^- content and not soil pH was the major factor controlling the product ratio ($N_2O:N_2$) from denitrification. In this study, ash application increased the NO_3^- content in most soils (Fig. 4) (except in soil A1 and W2), possibly due to increased nitrification at elevated soil pH. Similar increases in soil NO_3^- content have previously been reported for wood ash amended soils (Kahl and Fernandez, 1996; Arshad et al., 2012; Vestergård et al., 2018). However, a tendency towards a positive relationship between N_2O production and the final resulting NO_3^- content was only observed in the low range of approximately 0–50 $\text{mg NO}_3^- \text{ kg soil}^{-1}$ (Fig. 3). Thus, there was no indication that an increase in the NO_3^- content overarched the effect of pH changes on the N_2O production. However, it is possible that the application of wood ash in combination with NO_3^- could counteract the mitigating effect of wood ash on N_2O emissions from acidic environments, hence such interactions need to be further investigated.

Besides from denitrification, heterotrophic nitrification may significantly contribute to N_2O production in acidic to neutral soils. The main N substrate for this process is organic N compounds (Zhang et al., 2015a). Accordingly, Zhang et al. (2015a) found that the gross heterotrophic nitrification rate positively correlated with the soil C:N ratio and that heterotrophic nitrification contributed substantially to nitrification in most acidic forest soils with low pH, and thus, also to the emitted N_2O . Furthermore, they estimated a rough threshold of pH 6 for the significance of heterotrophic nitrification in forest soils. Thus, high N_2O production at acidic soil conditions may also result from heterotrophic nitrification by the direct production of N_2O or by supplying NO_3^- to the denitrification process. However, to our knowledge, the effect of elevated pH on the gross heterotrophic nitrification rate and related N_2O emissions from soil has not been investigated. Therefore, such investigations would give important insight into the effects of pH management and thus wood ash amendments to natural soils with low pH.

Finally, in soils W2 and W3, ash application either increased or decreased the N_2O production depending on the ash dose (Figs. 2 and 5). This emphasises that the underlying mechanisms controlling the N_2O outlet from soils is highly complex and depends on various environmental factors besides pH. Nitrification and denitrification may occur

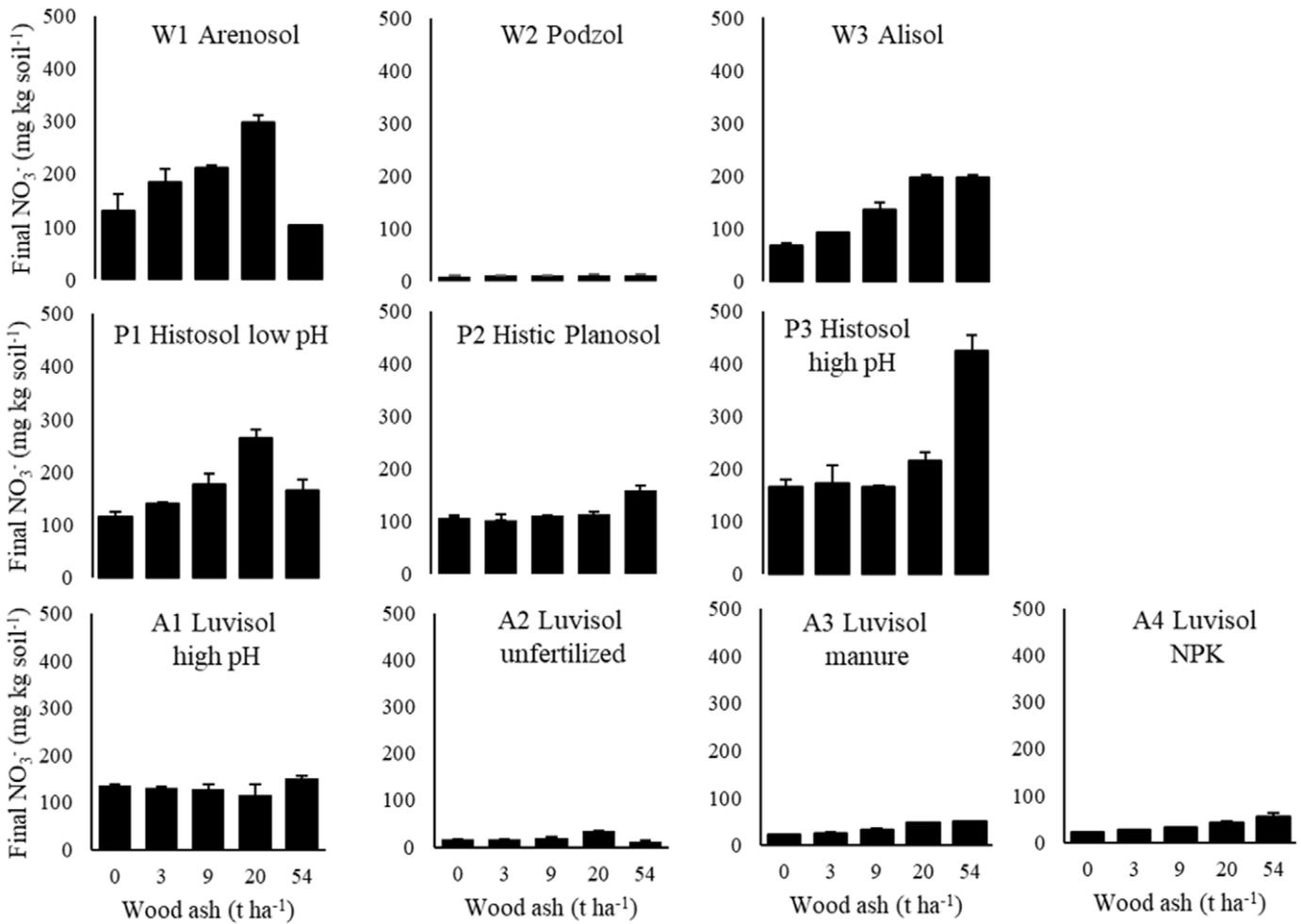


Fig. 4. Final water-extractable NO₃⁻ content in the soil incubations ±S.D. (n = 3).

parallel in different microsites and both processes may be affected by ash-induced changes to pH and nutrient addition. Thus, pH cannot solely explain the development of N₂O emissions from soil, however,

in this study, the final resulting pH was a good overall indicator of the effect of wood ash application on N₂O emissions from contrasting soil environments.

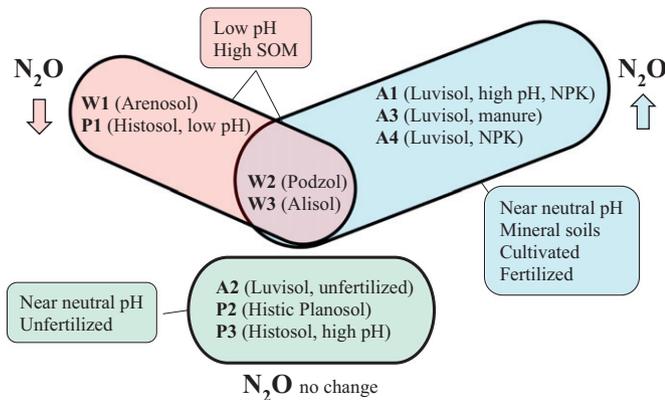


Fig. 5. The ten soils included in the incubation experiment (Tables 1 and S1 (Table C)) grouped according to their response to wood ash application with regard to the cumulative N₂O production. Important soil properties are linked to the different groups. Red group: wood ash significantly ($p < .05$) decreased the N₂O production, blue group: wood ash significantly ($p < .05$) increased the N₂O production, and yellow group: wood ash had no significant effect ($p > .05$) on the N₂O production at any wood ash application rate. Two soils overlap in the groups (W2 and W3) as wood ash either increased or decreased the N₂O depending on application dose in these soils.

4.2. Wood ash amendment as an N₂O mitigation strategy?

Wood ash amendment was mainly beneficial with a reduction in N₂O emissions or unaltered N₂O emission rates depending on soil type. The greatest mitigating effect on the N₂O outlet of wood ash was observed at an application rate of 9 t ha⁻¹, whereas higher applications did not further reduce the emitted N₂O from most soils (Fig. 2). To date, the legislative regulations of ash applications are limited to applications of only 2–7.5 t ha⁻¹ 100 y⁻¹ in Europe (Silva et al., 2019). Based on our observations, such relatively low applications of wood ash will reduce the emitted N₂O from most soil environments in the short-term or cause no changes in the emitted N₂O; albeit this positive effect should be considered in relation to the effects on other greenhouse gases (CO₂ and CH₄). Furthermore, Maljanen et al. (2006b) found no significant effects of wood ash application (5–8 t ha⁻¹) on N₂O emissions from five boreal forests soils 14–50 years after application, indicating that the long-term effects of relatively low wood ash applications on N₂O emissions are negligible. Finally, wood ash contains all macro- and micronutrients except for N. Therefore, wood ash application combined with N application is considered as a management strategy on N poor sites (Saarsalmi et al., 2006). As soils with a history of N fertilizers resulted in an increase in N₂O emissions, more research is needed to identify potential negative interactions between wood ash and N fertilizers and the effect on N₂O emissions from different soil environments.

4.3. Random Forests (RF) model prediction applicability and interpretation

The RF model has proven more accurate in prediction modelling, compared to more traditional linear regression models (Pearson, 2016) and is used in many different scientific disciplines including prediction of N₂O emissions from soil (Philibert et al., 2013; Perlman et al., 2014). Yet, to our knowledge, this is the first usage of this tool to analyse ash-induced effects on a soil service. Corresponding to our findings, other modelling tools have equally emphasised soil pH as the most important factor explaining regional differences in N₂O emissions (Wang et al., 2018). However, Philibert et al. (2013) also used the RF model to determine variable importance for the prediction of N₂O emissions from cultivated soils with different N inputs using data from published experiments. They, on the other hand, found that the N rate and crop type were the most important predictors for N₂O emissions from the investigated soils. Likewise, Perlman et al. (2014) used the RF model to evaluate variable importance for the prediction of N₂O emissions from wheat and maize agricultural fields. However, they used computed and not empirically collected N₂O emission data and found that soil organic carbon (SOC) was the most important predictor variable. Consequently, differences in scale, data input, experimental design, and general scope of the studies using the RF model to predict N₂O emissions from soils complicates comparison between the studies. Thus, interpretation of the model output should always be restricted to the specific experimental design, and correct interpretations rely on a thorough ecological understanding of the investigated processes. Although, the RF model output represents a generalization of the effects of wood ash amendment to a wide range of different soil environments the results are valuable as a tool to generate guidelines to the use of wood ash in soil management.

5. Conclusion

Wood ash application at different rates had various effects on the emitted N₂O from contrasting soil environments. The final resulting soil pH rather than the wood ash amendment rate was the key predictor for N₂O production from different soils. The relationship between the final resulting pH and the N₂O production was U-shaped. Thus, the soil N₂O production was highest at low pH (<5.3 pH) and high pH (>8.0 pH). Therefore, wood ash application at low rates <9 t ha⁻¹ may mitigate N₂O production from acidic soils with high SOM in the short-term. Still, the underlying mechanisms controlling the effects of elevated soil pH induced by wood ash amendment on N₂O production needs to be further investigated. Likewise, interactions between wood ash amendment and N application should be considered when using wood ash as a soil management tool.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.136581>.

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