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Enzyme activities and wheat growth response in soils amended with coal ash from the UK and Tanzania

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Abstract

Due to the presence of essential plant nutrients and either acid or alkaline properties, coal ash can be a valuable amendment in improving characteristics and crop productivity of alkaline or acidic soils. This study aimed to evaluate the effects of coal ash application to woodland and arable soils on soil pH, selected soil enzyme activities (dehydrogenase, acid phosphatase, alkaline phosphatase, urease and β-glucosidase) and on vegetative growth of wheat. Two pot experiments were conducted in which wheat (*Triticum aestivum* var. Willow) was grown in woodland and arable soils amended with ash collected from either the UK or Tanzania, at concentrations of 0, 2, 4, 8 and 16% (w/w). Wheat was grown for 50 days. Soil amendment with UK ash at 0-16 % increased significantly ($p<0.001$) the pH of woodland and arable soils while amendment with Tanzanian ash at 0-16 % reduced the pH of both soils ($p<0.001$). Application of low concentrations (0-4%) of UK ash to both soils increased dehydrogenase and urease activities and wheat growth, but these ash concentrations didn't show any significant effect on alkaline and acid phosphatase activities. Glucosidase activity increased significantly ($p<0.001$) when woodland soil was amended with 2% of UK ash, then decreased significantly with increasing ash concentration. Application of 16% UK ash also inhibited acid and alkaline phosphatase activities. Application of the Tanzanian ash at low concentration did not have any significant effect on the activities of enzymes studied while application at 8-16% inhibited all enzyme activities. Tanzanian ash did not affect wheat growth parameters when applied to both soils while UK ash improved wheat growth. This study demonstrates that soil amendment with coal ash can either cause beneficial or detrimental effects, depending on the nature of the ash and soil characteristics thus, strategic agronomic use of coal ash is recommended.

Key words: *Coal ash; Enzyme activities; Soil amendment; Wheat growth*

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Introduction

Coal ash is one of the most abundant industrial waste materials generated globally. Numerous routes have been devised to reuse coal ash in an attempt to reduce its negative environmental and economic impacts. Due to the presence of essential plant nutrients and the alkaline nature of most coal ashes (Kolbe *et al.,* 2011; Gupta *et al.,* 2012; Singh *et al.,* 2014 and Ahmad *et al.,* 2021), it may provide a valuable amendment to improve soil characteristics and crop productivity.

However, coal ash also contains potentially toxic elements (PTEs) which may be detrimental to soil biota, including microorganisms (Wong *et al.,* 1986; Hattori, 1992). Several studies have reported the improvement of physical and chemical soil characteristics following soil amendment with coal ash (Adriano and Weber, 2001; Pathan *et al.,* 2003; Yunusa *et al.,* 2011; Singh *et al.,* 2014; Shaheen *et al.,* 2014, and Ahmad *et al.,* 2021); however, to date, few studies have investigated the effect of coal ash specifically on soil biological characteristics, particularly soil enzyme activities.
Soil enzymes are a small but very important fraction of soil organic matter, controlling all the biochemical reactions in the soil (Dahm *et al.,* 2011). Soil enzymes play an important role in regulating ecosystem processes by controlling nutrient cycling and fertilizer use efficiency; they reflect the overall microbiological functioning in soils and act as indicators of any changes in soil conditions (Dick and Wang 2000; Wang *et al.,* 2023). The primary source of soil enzymes is the microbiome, but enzymes may also originate from plants and animals (Dahm *et al.,* 2011). The sources of phosphatase enzymes in the soil, which hydrolyse organic P to inorganic P, are soil bacteria, plant roots and fungi (Kramer and Green 2000; Makoi and Ndakidemi 2008). Alkaline phosphatase is derived solely from microorganisms (Tabatabai, 1994) though acid phosphatase can be derived from either microbes or plants. The source of the enzyme dehydrogenase, which provides a direct measure of microbial activity in soil and an indication of soil health, is the microbes (Utobo and Tewari, 2014). Glucosidase is an enzyme that catalyses the hydrolysis of β-glucosides present in organic matter; it tends to originate from microbes, plants and animals (Utobo and Tewari, 2014). The main sources of urease, an enzyme that hydrolyses urea fertilizer to NH_3 and CO_2 and increases soil pH, are microbes and plants (Makoi and Ndakidemi, 2008).

Soil management by the application of coal ash has previously been noted to inhibit soil respiration and enzyme activities (Wong and Wong, 1986; Pitchel 1990; Pati and Sahu 2004). This inhibition has been linked to coal ash characteristics such as low availability of C and N, high pH and salinity, high concentration of soluble salts and the presence of PTEs in ashes. Therefore, studying the effect of coal ash on soil enzymes, an important index used in assessing the effects of soil contamination on microbial activities and fertility status of soils (Utobo and Tewari 2014), will help us to understand and manage the impact of coal ash amendments on soil biological health.

The present study was carried out with the aim of evaluating the effects of coal ash sourced from coal combustion plants in the UK and Tanzania on soil pH, selected soil enzyme activities (dehydrogenase, acid phosphatase, alkaline phosphatase, urease and β-glucosidase) and on vegetative growth of wheat grown in arable and woodland soils. The UK and Tanzanian coal ashes were chosen for this study based on variation of ash characteristics noted which in turn depend on factors such as the types (rank) of the feed coal, percentage of incombustible matter in the coal, sulphur content, the pulverisation process, furnace type, the efficiency of combustion process and storage and handling (Jala and Goya 2006; Tharaniyil, 2013; Alterary and Marei 2021). The specific objectives of this study were to evaluate the effects of increasing concentrations of the UK and Tanzanian coal ash i) on growth and biomass production of wheat and ii) on a range of soil enzyme activities (dehydrogenase, acid phosphatase, alkaline phosphatase, urease and β-glucosidase). iii)Also the study aimed to establish whether each coal ash exerted similar effects in two contrasting soils; arable and woodland soils.

Materials and methods

Description of the study area

The current study was conducted at Sutton bonington campus, University Nottingham (UK). The University lies between (52.830°N, 1.239°W) of Leicestershire, and is approximately 18 km south out of central Nottingham (West, *et al*., 2009). The mean annual rainfall of the area is 606 mm, which is evenly distributed throughout the year (The University of Nottingham Sutton Bonington Metrological Site) (West *et al*., 2009). The average annual high temperature in Nottingham is 13.8 °C (56.8 °F) and the average annual low temperature is 6.5 °C (43.7 °F). Soils at this area are classified as sandy loam (wick/arrow series).

Soil sample collection and preparation

The two experimental soils (arable and woodland) were collected from the University of Nottingham (UK) farm at the Sutton Bonington campus (52.830°N, 1.239°W). The soils were both sandy loam (Wick/Arrow series) but with contrasting land use and management histories. Topsoil (0-20 cm depth) were collected from different points within a woodland and an adjacent arable field. Samples from different points within each site were mixed together to make one composite sample, representative of either the arable field or the woodland. The aim of selecting soils from two adjacent sampling areas was to obtain soils with a common origin but with contrasting characteristics, due to the different vegetation types present and land management. In addition, the woodland soil was included to encompass the potential future application of coal ash in forestry. Soils were air dried and sieved (4 mm) to remove plant debris and gravel and to maintain the natural crumb structure. Sieved soils were stored in plastic bags and stored at 4˚C for further use in experiments.

Coal ash sample collection and storage

Coal ash samples used in this study were collected from the UK and Tanzania. In the UK, ash samples were collected from a large coal-fired power plant in January 2015. In common with other commercial coal-fired power stations in the UK, this power plant burns pulverized coals sourced from various parts of the world, so the exact origin of this ash is unknown. In Tanzania, coal ash was collected from Morogoro region in April 2015. The coal combusted in this industry is bought from TANCOAL, a company which mines bituminous coal from the Ngaka coal field in the Ruhuhu Basin, Ruvuma region, Tanzania. Before analysis, both collected ash samples were well stored in cold room at 4˚C. Soil/coal ash sample storage in cold rooms scientifically does not affect the quality of data.

Soil and coal ash analysis

Before carrying out the experiments, the percentage moisture content of the soil and ash samples were determined gravimetrically; the pH of each soil and ash sample was determined by the method described by Rowell (1994). Total carbon and nitrogen in soil and ash samples were determined using a CN analyser (Thermo Scientific FlashEA 1112 Nitrogen and Carbon Analyser). Total extractable carbon (TC) and nitrogen (TN) were determined using a CN analyser (Model Shimadzu TOC-VCPH, PC-controlled high-sensitivity model) after extracting the ash or soil samples with 0.5 M K_2SO_4 solution in the ratio of 1:5 and diluting all soil or ash sample filtrates with ultra-pure water in the ratio of 1:10. Total concentrations of nutrients and PTEs in soil and ash samples were determined by digesting the samples using aqua regia. Multi-element analyses of diluted aqua regia digests were undertaken by ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany).

Experimental approach

Two pot experiments were conducted in which spring wheat (*Triticum aestivum* var. Willow) was grown in arable and woodland soils amended with ash. In the first experiment, soils were amended with the UK ash at concentrations of 0, 2, 4, 8 and 16% w/w; in the second experiment, soils were amended with the Tanzanian ash at the same concentrations. In both experiments, wheat plants were grown for 50 days (pre-grain development). Both experiments were designed to evaluate the effects of ash application in arable and woodland soils on soil enzyme activities (dehydrogenase, phosphatase, glucosidase, and urease) and wheat growth.

Experimental set up

Experiment 1: UK ash in woodland and arable soils

The experiment involved five concentrations of ash 0, 2, 4, 8 and 16 % w/w on a dry weight basis.

The equivalent fresh weights of 250 g dry weight of arable and woodland soils were mixed thoroughly with each concentration of ash and then the mixture was used to fill the required number of plant pots. Four replicates of each ash treatment plus soil mixture were prepared. All pots were placed in a growth room arranged in a randomized block design (with 4 replicate blocks), watered with deionized water and maintained at 20°C/18°C day/night, 16h/8h day/night duration (including 1-hour dawn and 1-hour dusk periods). Each pot was watered with deionized water and allowed to equilibrate for 24 hours before sowing the wheat seed. Six seeds of spring wheat were sown in each pot at a depth of about 1 cm. All plants were watered with deionized water and grown for up to 50 days, following normal agronomic requirements including thinning to 1 plant per pot after germination, removal of weeds and monitoring the occurrence of any disease and/or phytotoxic symptoms to the plant following the ash amendment. No fertilizer was added to these plant pots.

Experiment 2: Tanzanian ash in woodland and arable soils

The experiment was set up, arranged, sown, watered and monitored as for the UK ash experiment described above.

Plant harvesting and analysis

All plants from both experiments were harvested 50 days after the sowing date. Before harvesting, numbers of tillers and leaves were counted. The shoot biomass was determined by harvesting the shoots and oven drying at 60°C until constant weight and recording the weight of shoots per plant per pot. The roots were extracted from the soil, washed thoroughly with tap water, dried at 60°C until constant weight and root biomass recorded for each treatment.

Soil analysis after harvesting plants

After harvesting, all soils from each pot were homogenized and frozen at -20°C pending enzyme analysis. For the first experiment, soils were frozen for 18 months prior to enzyme analysis and for 4 months for the second wheat experiment. According to ISO 2009, storage of temperate soils for 3-12 months at -20, -80 or -180°C does not inhibit microbial activities. Kendeler and Gebger (1988) found no significant effect of freezing soil for 5 months on urease activities. Sample storage by freezing has also been recommended as a better method than air drying by Wallenius *et al.* (2010) due to its small effect on enzyme activities, particularly in clay loam and forest humus soils.

Before assaying enzyme activities in the experimental soils, all methods were tested by assaying urease, dehydrogenase, acid and alkaline phosphatases and glucosidase enzymes in fresh soil collected under actively growing grasses on the Sutton Bonington campus (results not shown). In addition to method testing, this enabled broad (order of magnitude) comparison with the data from the experimental soils to ensure that freezing had not affected the results and that comparisons between treatments were valid.

Enzyme assays were performed on the experimental samples after thawing in a cold room (4°C) for 2 days and then maintaining at approximately 20°C for 15 hours.

Determination of soil enzyme activities

The assay for urease activity in soil was performed following the procedure described by Kandeler and Gerber (1988). The assay for dehydrogenase activity was performed following the modified procedure by Thalman (1968). Phosphatase activities in soils were assayed following the original method of Tabatabai and Bremner (1969) modified by Eivazi and Tabatabai (1977). Glucosidase activities in soils were assayed following the modified method published by Hoffmann and Dedeken (1966).

Statistical analysis

Statistical analyses were performed using Genstat 17th Edition (VSN International, UK). A generalized two-way analysis of variance (ANOVA) was conducted on all parameters (soil pH, enzyme activities and plant growth data) using coal ash concentration and soil type as factors. Normality was tested by plotting residuals against expected normal quantiles and post-hoc comparisons between means were based on a least significant differences (LSD) test at 0.05 probability level.

Quality control

All analyses were performed following

previously published methods and the methods for enzyme assays were tested prior to application. All the experiments were carried out in controlled environment (growth rooms) and a randomized block design for each experiment was used to control any source of variation in these rooms. In each experiment, controls were included, and treatments were replicated 4 times to minimize variations between samples; this was verified by the relatively small calculated standard errors for data sets.

In pot experiments, plants were watered with deionised water to avoid soil contamination with minerals from tap water and, in all chemical analyses, ultra-pure water was used.

Results

Initial characteristics of soils and UK and Tanzanian ashes

The chemical characteristics and elemental composition of soils and coal ash used in both wheat experiments are presented in Table 1. The pH of the UK and Tanzanian ashes were 12.32 and 4.2, respectively. The two soils used were arable and woodland soils with pH values of 6.43 and 3.81, respectively. The lowest percentage total nitrogen (TN) was recorded in UK ash but, generally, the trend in TN followed the order woodland soil>Tanzanian ash>arable soil>UK ash. The highest percentage total carbon (TC) was recorded in Tanzanian ash and the trend in TC followed the order Tanzanian ash>UK ash>woodland soil>arable soil. The concentration of extractable C (mg kg^{-1}) in soils and ashes followed the order woodland soil>UK ash>arable soil>Tanzanian ash while the total extractable N followed the order arable soil>woodland soil>Tanzanian ash>UK ash.

Concentrations of Ca, Mg, P, B, and S were higher in the UK ash than in the two soils, while the concentration of these nutrients (except S) in the Tanzanian ash were lower than in the two soils. The concentrations of the PTEs Pb, Cu, Co, Cd, Ni, Zn and Se were higher in both ashes than in the two soils.

Table 1

Initial chemical properties and elemental composition of the UK and Tanzanian coal ashes and the experimental soils.

Parameter	UK1 ash	Tanzania1 ash	Arable soil	Woodland soil
pH	12.3 ±0.02	4.2 ±0.01	6.43 ±0.01	3.81 ±0.012
TC (%)	8.6 ±0.1	28.2±1.1	0.4±0.03	5.8 ±0.3
TN (%)	0.03±0.002	0.45 ±0.02	0.12 ±0.1	2.3 ±0.1
Extractable C *(mg kg^{-1})*	261.3 ±6	28.2 ±1.10	254.1 ±6	527 ±2
Extractable N (*mg kg^{-1})*	4.6 ±1.02	86.2 ±1.8	341 ±3	245 ±3
% moisture	9.4 ±0.03	3.6 ±0.02	16.7 ±0.1	22.5 ±0.1
Nutrients (mg kg^{-1})				
P	1023.4 ±14	237 ±6.3	801 ±94	411 ±3.85
K	893 ±7	837 ±19	1520 ±145.1	987 ±32.56
Mg	2774 ±42	201 ±9	1931 ±194	1807±53.11
Ca	14835 ±195	1054 ±51	2249 ±235	1326.4 ±58
S	449 ±18	1889 ±100	BDL	75.1 ±14
B	115.1 ±2	BDL	5.42 ±0.18	5.51 ±0.18
Mn	187.3 ±2.1	38.5 ±1.2	232 ±2 22	97.52 ±9.1
PTEs (mg kg^{-1})				
Zn	331.4±2.7	74.3 ±2.3	54.27 ±4.10	55.74 ±1.83
Cu	27 ±0.7	36 ±1.4	17.17 ±1.8	13.44 ±0.43
As	66 ±1	6 ±0.1	9.97 ±1	10.49 ±0.35
Co	8.3 ±0.1	20.6 ±0.3	4.25 ±0.11	3.31 ±0.13
Cd	16.7 ±0.5	0.5 ±0.02	0.27 ±0.01	0.17 ±0.00
Ni	24.2 ±0.2	31.1 ±0.6	9.08 ±0.61	9.57 ±0.47
Pb	546 ±15.2	17.7 ±0.6	39.01 ±1.6	59.22 ±2.11
Cr	23.2 ±0.3	11.1 ±0.2	13.85 ±1	11.31 ±0.28
Se	5.7 ±0.1	3.7 ±0.1	0.37 ±0.02	0.60 ±0.02

Note: Values given are means of four replicates ± standard errors. BLD=below detectable limit during ICP analysis. TN = total nitrogen; TC = total carbon.

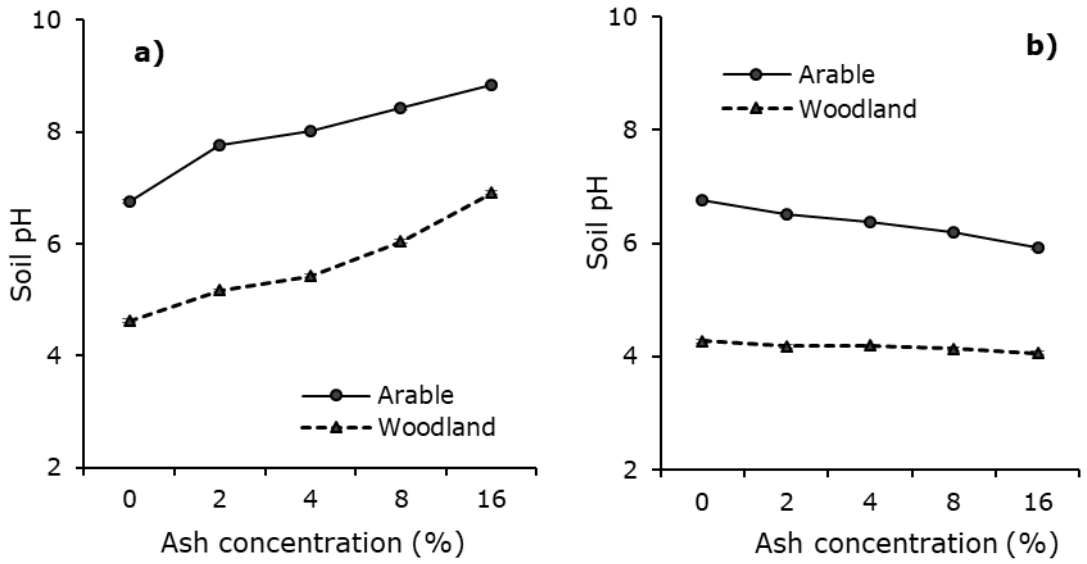
Effects of ash on soil pH

The effects of addition of the UK and Tanzanian ashes on soil pH are presented in Figure 1. The application of UK ash to both soils (arable and woodland) increased the soil pH (Figure 1a; ash concentration x soil type interaction; $p<0.001$). The pH in both soils increased with increasing ash concentration but the pH of the arable soil was higher than the woodland soil in all

treatments. Soil amendment with the Tanzanian ash reduced the pH of both soils (Figure 1b; ash concentration x soil type interaction; $p<0.001$) but the extent of this decrease was higher in the arable soil than in the woodland soil. The pH of the arable soil decreased from 6.76 to 5.92 and in the woodland soil from 4.27 to 4.06 following soil amendment with 0-16 % ash.

Figure 1

Effect of coal ash application on soil pH



Note: a) = The soil treated with the UK ash, LSD=0.095, SE=0.033 and b) = the soil treated with Tanzanian ash, LSD=0.068, SE=0.024. The individual error bars are based on the pooled variance estimate from the ANOVA with 30 degrees of freedom.

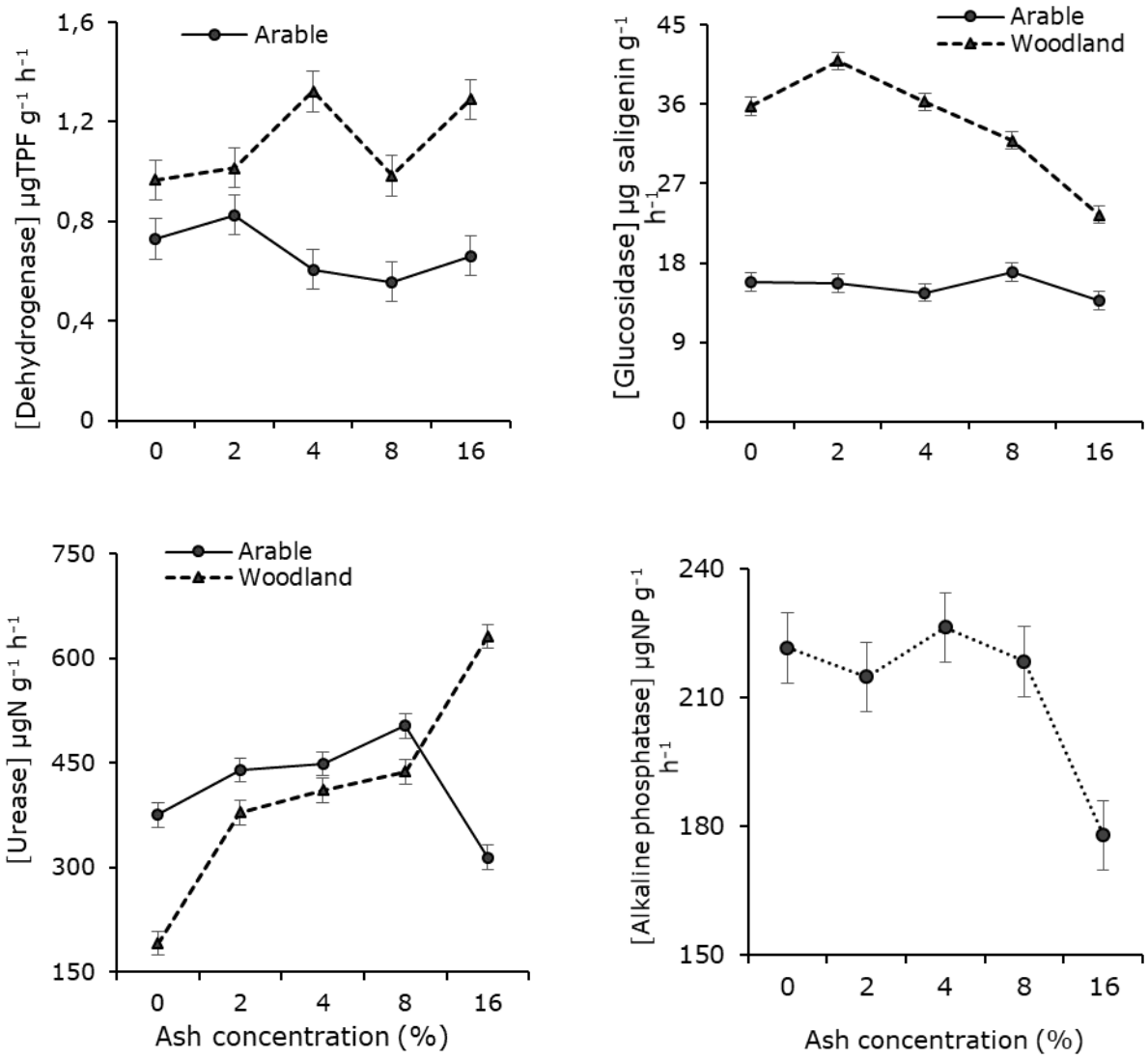
Enzyme activities in soils amended with the UK ash

In Experiment 1, application of the UK ash in both soils influenced the activities determined for all the enzymes (Figure 2). In woodland soil, application of UK ash from 0-4 % and at 16 % significantly increased dehydrogenase activity in comparison to the control, but this activity decreased significantly at 8% ash concentration (Figure 2; ash concentration x soil type interaction; $p=0.023$). Amendment of the arable soil with this ash did not significantly affect dehydrogenase activity. Glucosidase activity increased significantly when the woodland soil was amended with 2% UK ash but decreased significantly with increasing ash concentration from 4-16 % (Figure 2; ash concentration x soil type interaction; $p<0.001$). Application of the UK ash to the arable soil did not significantly affect glucosidase activity. Urease activity in the woodland soil increased significantly with increasing concentration of the UK ash (Figure 2; ash concentration x soil type interaction; $p<0.001$). A similar trend was noted in the arable soil amended with this ash from 0-8%, but at 16% ash concentration the urease activity in the arable soil decreased significantly (Figure 2; ash concentration x soil type interaction; $p<0.001$). Fly ash concentration as an individual factor did not produce any significant effect in alkaline phosphatase activity when both soils were amended with 0-8% ash, but the alkaline phosphatase activity decreased significantly in soils amended with 16% ash concentration (Figure 2; $p=0.002$). Soil type as an individual factor also influenced the alkaline phosphatase activity; the activity was significantly higher in the arable soil than in woodland soil (arable soil=262.4 and woodland soil=161.2 $\mu g\ NP\ g^{-1}\ h^{-1}$; $p<0.001$ data not shown).

Acid phosphatase activity was determined only in the woodland soil (which was very acidic) and not in the arable soil (which was slightly acidic/near neutral) due to the predominance of acid phosphatase in acid soils and alkaline phosphatase in neutral or alkaline soils (Dick and Tabatabai, 1984; Dick *et al.,* 2000).

Figure 2

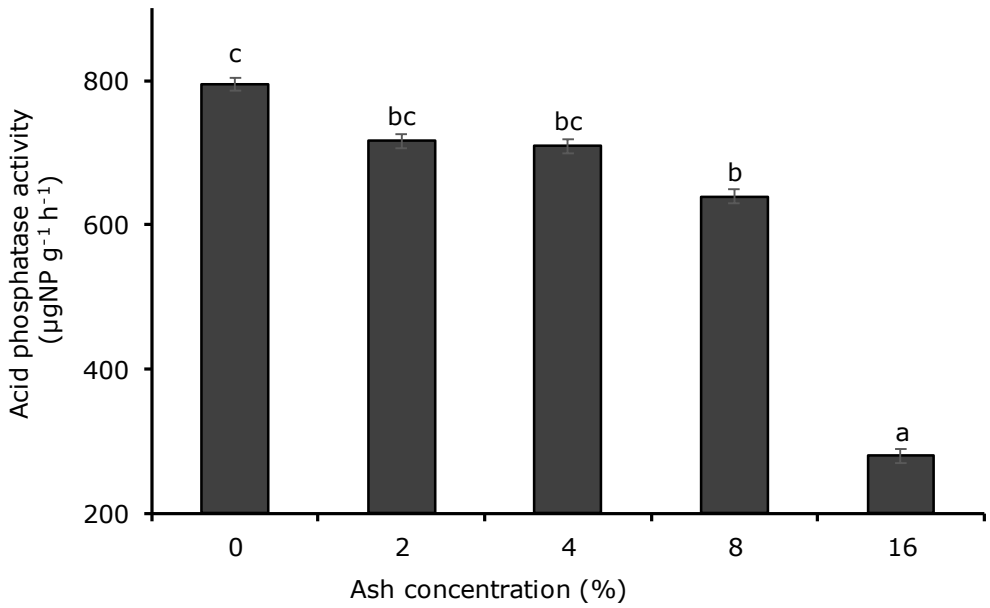
Enzyme activities in UK ash amended soils, under wheat plants for 50 days



Note: LSD for dehydrogenase = 0.261, glucosidase =2.97, urease =50.4, and alkaline phosphatase =23.52. The individual error bars are based on the pooled variance estimate from the ANOVA with 30 degrees of freedom. Please note the scale differences on the Y-axes. For alkaline phosphatase, data are means pooled across the woodland and arable soils.

Application of 0-4% of UK ash to the woodland soil did not have any significant effect on acid phosphatase activity, while further increase in ash concentration from 8-16 % decreased the activity of this enzyme significantly in comparison to the control (Figure 3; p=<0.001).

Figure 3
Acid phosphatase in woodland soil amended with different concentrations of the UK ash



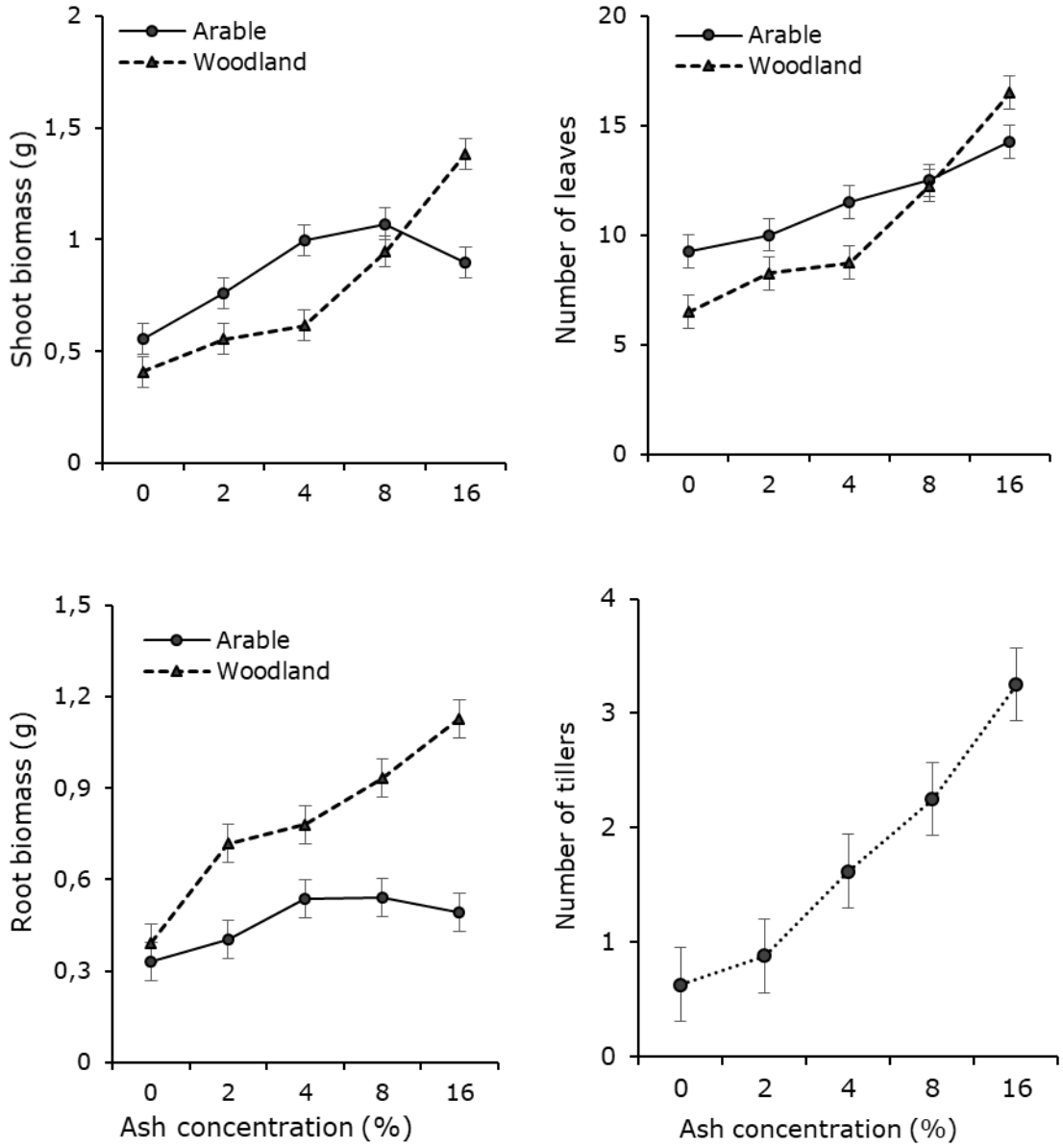
Note: Columns with the same letters are not significantly different according to Tukey's multiple comparison test, $p<0.001$.

Effect of the UK ash on wheat growth

Soil amendment with the UK ash improved all growth parameters of spring wheat (Figure 4). In woodland soil, the shoot biomass and number of leaves increased significantly with increasing concentration of the ash from 4-16%. In the arable soil, shoot biomass and leaf number increased significantly for the plants grown in 0-4 % ash and thereafter (from 8-16% ash) there were no further significant increases in these parameters (Figure 4; ash concentration x soil type interaction; $p<0.001$ for shoot biomass and $p=0.01$ for number of leaves). In woodland soil, the root biomass increased with increasing concentration of the UK ash while in the arable soil a significant increase in the root biomass was noted in plants grown in soil amended with 4% ash; there was no further significant increase in root biomass with the 8-16% UK ash amendments (Figure 4; ash concentration x soil type interaction; $p=0.002$). UK ash as a single factor also influenced tiller formation in plants grown in both soils; the number of tillers increased significantly with an increasing concentration of ash from 4-16 % (Figure 4; $p<0.001$).

Figure 4

The effect of the UK ash on wheat growth parameters after 50 days



Note: LSD for shoot biomass=0.2017, Number of leaves=2.138, root biomass=0.1807, and number of tillers=0.923. The individual error bars are based on the pooled variance estimate from the ANOVA with 30 degrees of freedom

Enzyme activities in soils amended with Tanzanian ash

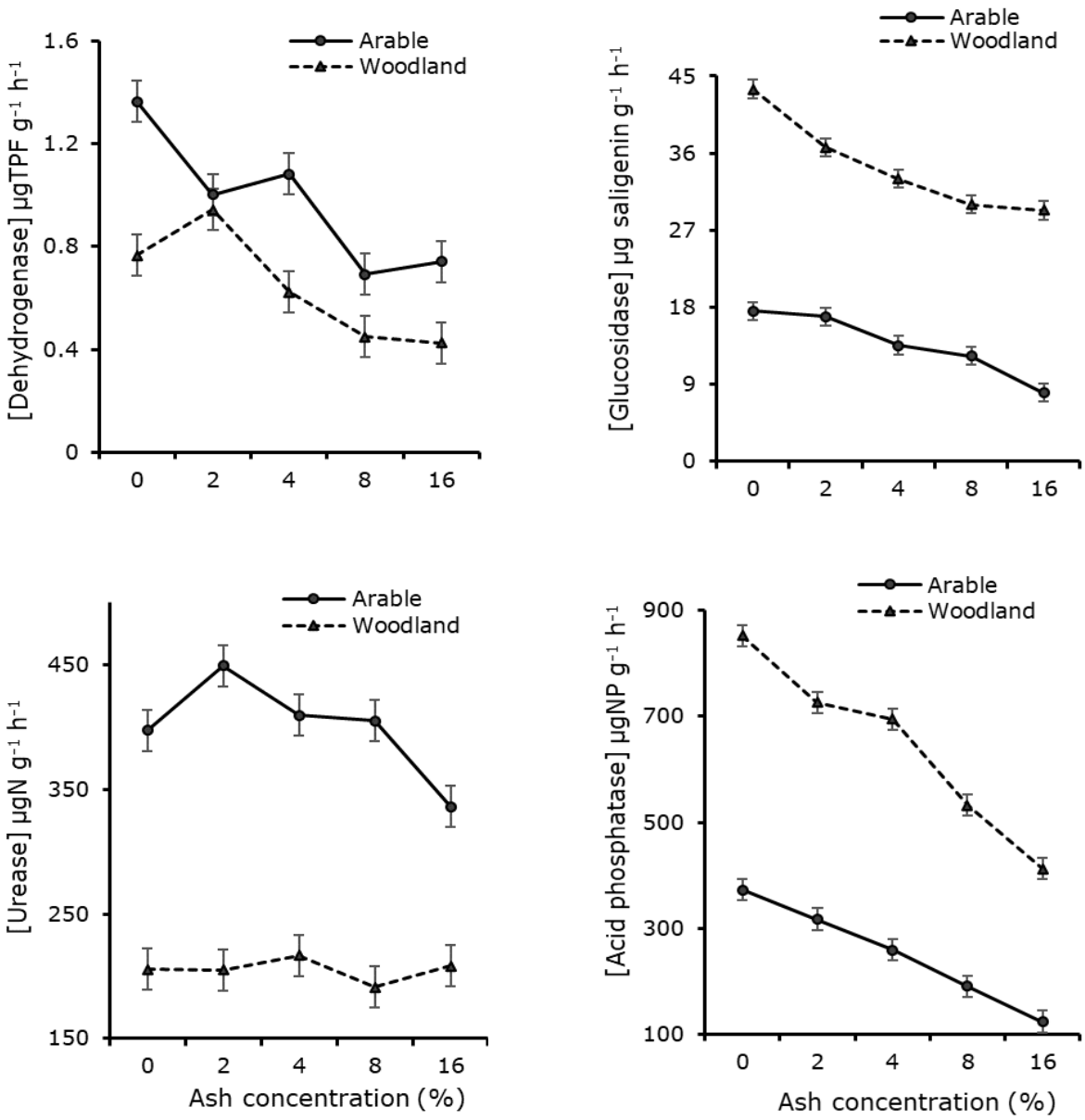
In Experiment 2, soil amendment with the Tanzanian ash (TZ) also influenced the activities of all enzymes measured (Figure 5). In woodland soil, a slight increase in dehydrogenase activity was noted when the soil was amended with 2% of the TZ ash, but higher ash concentrations up to 16% decreased dehydrogenase activity significantly (Figure 5; ash concentration x soil types interaction; p=0.024). In the arable soil,

there was an overall decrease in dehydrogenase activity with increasing ash concentration, although this trend fluctuated (Figure 5; ash concentration x soil types interaction; $p=0.024$).
Glucosidase activity in the woodland soil decreased significantly with increasing ash concentration from 0-8% but higher ash concentrations up to 16% did not result in further significant decreases in activity (Figure 5; ash concentration x soil types interaction; $p=0.008$). However, in the arable soil, glucosidase activity decreased significantly with increasing ash concentration from 4-16% (Figure 5; ash concentration x soil types interaction; $p=0.008$). Soil amendment with the TZ ash did not affect urease activity in the woodland soil while in the arable soil, urease activity increased significantly in the soil amended with 2% ash concentration (Figure 5 and; ash concentration x soil types interaction; $p=0.024$). There was no further increase in urease activity in the arable soil amended with 4-8% ash and 16% TZ ash decreased urease activity in the arable soil (Figure 5; ash concentration x soil types interaction; $p=0.024$).

A decreasing trend in acid phosphatase activity in both soils was observed following ash amendment but, in the woodland soil, the activity decreased significantly with addition of 2-16% TZ ash in comparison to the control, while in the arable soil, activity decreased significantly with 4-16% TZ ash amendment (Figure 5; ash concentration x soil types interaction; $p<0.001$).

Figure 5

Enzymatic activities in soils amended with the Tanzanian ash under wheat plants after 50 days



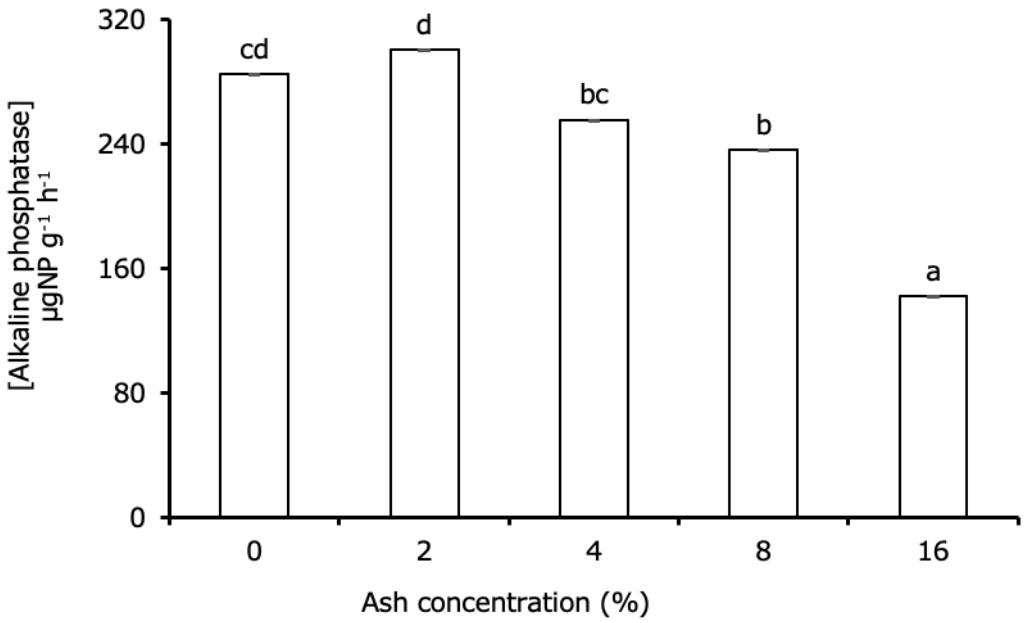
Note: LSD for dehydrogenase=0.232, glucosidase=3.1, urease=47.96, and acid phosphatase=57.89. The individual error bars are based on the pooled variance estimate from the ANOVA with 30 degrees of freedom.

Alkaline phosphatase activity was only determined in the arable soil (which was slightly acidic/neutral) and not in the woodland soil (which was very acidic) due to the predominance of alkaline phosphatase in neutral or alkaline soils and acid phosphatase in acidic soils (Dick and Tabatabai, 1984; Dick *et al.,* 2000). Application of the TZ ash (ash as an individual factor) from 0-4% did not affect alkaline phosphatase, while 8-16% ash addition resulted in decreased alkaline phosphatase activity compared to the control (Figure 6; $p=<0.001$).

Tanzanian (TZ) ash did not affect any of the measured wheat growth parameters when applied to either the arable or the woodland soil.

Figure 6

Alkaline phosphatase in the arable soil amended with different concentrations of the Tanzanian ash



Note: Columns with the same letters are not significantly different according to Tukey's multiple comparison test, $p<0.001$, $F_{4, 15} = 42.41$.

Discussion

Effects of UK coal ash on soil pH

Soil amendment with the UK ash increased the pH of both soils, which reflects the high pH (12.3) of this ash. The 'liming' effect of this ash may be associated with the presence of considerable amounts of CaO and MgO in coal ashes reported previously (Adriano *et al.*, 1980; Pati and Sahu, 2004).

Effects of UK coal ash on soil enzyme activities

In Experiment 1, application of the UK ash at lower concentrations (0-4 %) increased dehydrogenase activity in the woodland soil but not in the arable soil, which suggests a beneficial effect of applying alkaline coal ash in improving microbial activity in very acidic soil. However, at higher ash concentrations (8-16%) there were no clear trends of dehydrogenase activity in the woodland soil. Similar findings were reported by Sarangi (2001) and Pati and Sahu (2004) who observed a positive influence of ash on dehydrogenase activities in soils amended with 2.5% ash but an inhibitory effect at ash concentrations greater than 2.5%. Wong and Wong (1986) also noted a positive effect on dehydrogenase activity in soil amended with 10% coal ash but an inhibitory effect at ash concentrations greater than 10%; this was linked to the ability of ash to supply nutrients to microbes to perform their metabolic activities and the toxicity of ash to microbes when applied at higher concentration. Since soil pH has been suggested as the best predictor of soil

dehydrogenase activities (Moeskops *et al.,* 2010), the variation in dehydrogenase activity between the woodland and arable soils after being amended with UK ash is probably associated with changes in the soil pH. Siddaramappa *et al.* (1994) and Pati and Sahu (2004) also associated the influence of ash on soil enzyme activity with its effect on soil pH because the activities of all enzymes are strongly pH dependent. However, even though the pH of both soils here increased following soil amendment with the UK ash, this increase did not have any effect on dehydrogenase activity in the arable soil. Variation in dehydrogenase activities between the soils before and after amendment with ash might also be linked to differences in organic matter (OM) content between the soils; the woodland soil had a higher OM content than the arable soil. OM in the soils provides a substrate for microbial biomass and is likely to increase enzyme activities (Yuan and Yue, 2012). Indeed, glucosidase activity was higher in woodland than in arable soil across all ash concentrations, although increasing ash content lowered the enzyme activity. This suggests that nutrient availability in woodland soil was sufficiently high to drive biotic decomposition of the organic matter, although production of phosphatase indicated that P may have been limiting. High ash concentrations in woodland soil lowered glucosidase and phosphatase activities, suggesting that ash amendment resulted in a toxic effect (perhaps because of PTE addition) or that N became a limiting factor, which is evidenced by increased urease production with high concentrations of the UK ash. Plant growth benefited from addition of high ash amendments to the woodland soil, again suggesting that nutrients were not limiting and were likely to have been released in plant- and microbe-available forms. It is known that nutrient elements affect decomposition of soil organic matter and that N, P and C cycles are coupled (Galloway *et al.,* 2008). However, the key effect of adding the UK ash to woodland soil was that of enhancing the soil pH. It is known that microbial biomass is lower in acidic soils and that a positive relationship between pH and soil microbial activity exists (Treseder, 2008). That plant biomass was not increased by high additions of UK ash to arable soil signifies a more complex scenario; in this case it is possible that PTEs adversely affected growth. If this is the case, it is feasible that the higher organic matter content of the woodland soil limited PTE availability due to increased cation exchange capacity. Xiao *et al.* (2017) demonstrated that organic matter addition to paddy fields reduced Cd transfer to rice grain, but not As.

The increase in dehydrogenase activity in woodland soil may also be linked to the increase in the root biomass and associated rhizosphere microbes. Urease activity in control soil was lower in the woodland soil than in the arable soil, which might be linked to the history of fertilizer application to the arable soil (which is not known) and the lower pH of the woodland soil since urease activity tends to increase with increasing soil pH (Makoi and Ndakidemi 2008). Soil amendment with the UK ash increased the urease activity at all concentrations (0-16%) in the woodland soil and from 0-8% in the arable soil. The increase in urease activity might be linked to the increase in soil pH and Ca content in soils (Blonska, 2010) following soil amendment with this ash (Table 1). A similar finding regarding the positive effect of ash on urease activities was noted by McCarty *et al.* (1994); in their study, increased urease activity was also linked to the soil liming effect of the ash. The decline in urease activity in the arable soil amended with 16% ash might be due to the high pH of the ash which probably created unfavourable conditions for microbial activities (Lai *et al.,* 1999). This reduction might also be due to the accumulation of potentially toxic elements in ash amended soil which tend to inhibit soil enzymatic activities (Sarangi *et al.,* 2001; Pati and Sahu 2004; Yang *et al.,* 2006). Since the urease enzyme originates from soil microbes and plants (Burns, 1986; Makoi and Ndakidemi 2008), the increase in activity of this enzyme in ash-amended soil coincided with the increase in plant growth (root and shoot biomass), suggesting a beneficial effect of the applied ash on nutrient cycling, particularly that of N.

Application of the UK ash at low concentrations did not affect phosphatase activity in comparison with the control soil, although at high ash concentration (16%), phosphatase activity declined significantly. Since the source of alkaline phosphatase in the soil is the microbiome (Tabatabai, 1994), application of this ash to both soils probably did not provide any beneficial effect to the microbes producing this enzyme and they were, therefore, inhibited at high ash

concentration. The inhibition of this enzyme at higher ash concentration may be attributed specifically to the accumulation of PTEs in the soil amended with ash (Lai *et al.,* 1999). Acid phosphatase, which was only determined in the woodland soil due to the preference of this enzyme for acidic soil (Dick and Tabatabai, 1984; Dick *et al.,* 2000), was also unaffected by low ash concentrations, but declined significantly with higher ash amendments (8-16%). Despite the increase in the root biomass seen in plants grown in woodland soil, which could be another source of acid phosphatase in addition to that produced by microbes (Makoi and Ndakidemi 2008), acid phosphatase activity was still inhibited in soil amended with the high ash concentration. The reduction of phosphatase activities (acid and alkaline) in both soils is probably linked to the high pH, low availability of P and the toxicity of PTEs from fly ash (Pan and Yu, 2011; Sanchez *et al.,* 2015).

In this study, the activity of β-glucosidase increased in woodland soil amended with 2% of the UK ash in comparison to the unamended soil but, above this concentration, this enzyme's activity declined significantly. However, application of the UK ash to the arable soil did not have any significant effect on the glucosidase activity. The higher glucosidase activity in the unamended woodland soil compared to the arable soil (controls) could be attributed to the presence of higher organic matter in the woodland soil as this enzyme catalyses the hydrolysis and biodegradation of various β-glucosides found in decomposing organic matter (Makoi and Ndakidem 2008; Sing *et al.,* 2016). The increase in glucosidase activity in the woodland soil with 2% ash amendment was probably due to the higher carbon content of the ash (Table 1); however, inhibition of glucosidase activity at higher ash concentrations might be linked to the sensitivity of this enzyme to the changes in pH brought about by application of the ash (Acosta-Martinez and Tabatabai (2000). The reduction of β-glucosidase activities may also be associated with the high pH and lower availability of C from the ash (Sanchez *et al.,* 2015), as well as the adverse effect of trace elements in ash amended soil (Fang *et al.,* 1998).

Effects of UK coal ash on wheat growth

Besides the variation in soil enzymatic activities in both arable and woodland soils amended with the UK ash, wheat growth responded positively to this amendment. Almost all the growth parameters determined (shoot biomass, number of leaves, root biomass and number of tillers) increased with increasing ash concentration, but the rate of increase was higher in the woodland soil than in the arable soil. This might be linked to the increase in dehydrogenase activity, which is an indicator of gross soil biological activity (Lai *et al.,* 1999; Makoi and Ndakidemi 2008) and high organic matter in this soil. In the arable soil, the root biomass increased significantly only when the soil was amended with 0-4% ash; there was no further significant increase when ash concentration was greater than 4%. Improvement in wheat dry matter yield (shoot and root biomass) might also be linked to the liming effect of ash (Lai *et al.,* 1999; Fang *et al.,* 1998 and Pati, 2004) and the nutrient-supplying ability of the ash (Garg *et al.,* 2005; Tripathi *et al.,* 2009; Tsadilas *et al.,* 2014).

Effects of Tanzanian coal ash on soil pH

Soil amendment with the Tanzanian ash decreased the pH of both soils due to the relatively low pH (4.2) of the applied ash. Soil acidification due to the application of this ash may be associated with the presence of low concentrations of basic cations (e.g. Ca) and the high S content of this ash (Table 1). When soil S content is increased, soil acidification is often linked to the negative relationship between [S] and soil pH (Basu *et al.,* 2009; Singh *et al.,* 2016).

Effects of Tanzanian coal ash on soil enzyme activities

The higher dehydrogenase activity in the arable than in woodland soil might be due to the pH of arable soil which was slightly acidic (pH 6.8), thus within the optimum pH range of 5.17 to 7.27 reported for dehydrogenase activities (Brzezińska *et al.,* 2001; Natywa *et al.,* 2011). Besides the presence of organic matter in the woodland soil and the positive correlation between the organic matter and dehydrogenase activities reported earlier (Yuan and Yue, 2012), low dehydrogenase activity in this soil could be associated with its lower pH (3.81).

Higher glucosidase and acid phosphatase activities in the woodland soil than in the arable soil might be due to higher organic matter in the

former since β-glucosidase is involved in cycling C by catalysing the conversion of disaccharides to glucose (Moeskops *et al.,* 2010). The lower pH of the woodland soil favoured acid phosphatase because this activity tends to dominate in acidic soils (Shaw and Read, 1989; Dick *et al.,* 2000). Soil amendment with ash at lower concentrations (<10%) has been noted by others to have no significant effect, or even a positive effect, on soil enzyme activities and at higher concentrations to inhibit enzyme activities (Lai *et al.,* 1999; Pati and Sahu, 2004; Sanchez *et al.,* 2015). However, in this study almost all enzymes were inhibited even by lower concentrations of the Tanzanian ash. Since application of the Tanzanian ash decreased the pH in both soils (from 6.76 to 5.93 in arable soil and from 4.27 to 4.06 in the woodland soil), inhibition of enzyme activities in both soils may be linked to soil acidification. Even though the pH in woodland soil amended with Tanzanian ash changed by <1 pH unit (from 0-16% ash), this change was statistically significant and its impact on almost all enzyme activities was apparent. The decrease in dehydrogenase activities in both soils amended with Tanzanian ash might be associated with inhibition of this enzyme in acidic soils (Levyk *et al.,* 2007). Alkaline phosphatase tends to dominate in neutral or alkaline soils (Dick *et al.,* 2000), thus soil acidification due to ash application probably inhibited activity.

Microbes are the main source of urease in the soil and N is the major nutrient required to synthesise urease in microbial cells (Singh *et al.,* 2016). Therefore, soil acidification due to ash application (which tends to inhibit some microbial activities) and the lower content of N in coal ash might together explain the reduction in urease activity in the arable soil.

Despite the dominance of acid phosphatase in acidic soils (Shaw and Read, 1989; Dick *et al.,* 2000) and the increase of its activity in soils in which inorganic P supply is limiting (Makoi and Ndakidemi, 2008), further acidification of both soils by addition of high concentrations of the Tanzanian ash inhibited acid phosphatase activity. Moreover, the dramatic decrease in enzyme activities in both soil types amended with Tanzanian ash might also be attributed to increased concentrations of potentially toxic elements in the soil (Table 1). Pan and Yu (2011) associated reduced enzyme activities in soil contaminated with heavy metals to the interaction between metals and the enzyme-substrate complex, denaturation of enzymes and the effect of the metals on enzyme synthesis by microbial cells. Fang *et al.* (1998) and Sanchez *et al.* (2015) also associated the reduction of soil enzyme activities to accumulation of PTEs in soil following soil amendment with coal ash.

Effects of Tanzanian coal ash on wheat growth

Soil amendment with the Tanzanian ash did not have any effect on wheat growth up to the stage when the wheat plants were harvested (50 days), despite the ability of coal ash to supply nutrients (Tripathi *et al.,* 2009; Tsadilas *et al.,* 2014). This may be linked to inhibition of soil enzyme activities which plays an important role in the cycling of nutrients in the soil.

Conclusion

This study has shown that soil amendment with coal ash can either cause a beneficial effect akin to 'liming' acidic soils or a detrimental effect in which acidic soils are further acidified. However, these effects will depend on the pH and the concentration of the applied ash. Application of the UK ash increased dehydrogenase activity in woodland soil and urease activities in both soils, suggesting a beneficial 'liming' effect of ash on acidic soils (due to high pH of this ash), which in turn created favorable conditions for microbes and a positive growth response of wheat. Since some enzymes were inhibited in soils amended with high concentrations of this ash, despite its potentially beneficial liming effect, only concentrations of <4% of alkaline ashes (similar to that of the UK ash) may be recommended. However, application of acidic ash like the Tanzanian ash to acidic soils is not recommended due to its effect on soil acidification and inhibition of microbial activities. In practice, it is necessary to tailor any applications of coal ash as an agricultural soil amendment to ensure that the nature and quantity of ash applied is compatible with the soil being amended.

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