Resources, Conservation & Recycling 134 (2018) 262–270

Contents lists available at ScienceDirect

Resources, Conservation & Recycling

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

Full length article

Phosphorus fertilising potential of fly ash and effects on soil microbiota and crop

Deborah Schönegger[a], María Gómez-Brandón[a,b], Thomas Mazzier[a], Heribert Insam[a], Roy Hermanns[c], Evert Leijenhorst[d], Tommaso Bardelli[a,e], Marina Fernández-Delgado Juárez[a,*]

[a] Institute of Microbiology, Universität Innsbruck, Austria
[b] Departamento de Ecología y Biología Animal, Universidad de Vigo, Vigo 36310, Spain
[c] OWI Oel-Waerme-Institut gGMbH, Aachen, Germany
[d] BTG Biomass Technology Group B.V., Enschede, The Netherlands
[e] Department of Agrifood and Environmental Science, University of Florence, Italy

ARTICLE INFO

Keywords:
Biomass fly ash
Soil microbial communities
phoD gene
Fast pyrolysis bio oil
Enzymatic activities
Phosphorus

ABSTRACT

The production of fast pyrolysis bio oil (FPBO) constitutes one of the newest technologies for gaining a liquid biofuel from woody biomass. During this process biomass fly ashes (FAs), rich in minerals and salts, are produced. However, FAs are often disposed in landfills and their fertilising potential has been underestimated. A greenhouse trial was set up to test the impact of FA on soil physico-chemical and microbiological properties with a special focus on phosphorus, one of the main limiting nutrients in terrestrial ecosystems. FA were added into an acidic grassland soil at a rate of 2% with wheat (*Triticum aestivum* subsp. *spelta*) used as test plant. Soil and plants were collected after an incubation period of 60 and 100 days. Ash application increased soil pH and electrical conductivity, and improved soil nutritional status by increasing soil total, inorganic, and plant available phosphorus over time. Accordingly, higher plant yields were observed in ash-treated soils. The effect of FA on microbial biomass, assessed as double stranded DNA content, was time dependent and increased significantly with plant presence. Acid phosphomonoesterase activity significantly decreased following ash addition. However, neither alkaline phosphomonoesterase (ALP) activity nor the abundance and composition of the ALP gene (*phoD*) harboured by bacteria were affected by FA application. On the whole, FA from FPBO production seems to improve soil nutrient status and plant growth without inheriting detrimental effects on soil microbial communities in the mid-term.

1. Introduction

Despite its relative abundance in soil, phosphorus (P) is one of the most limiting mineral nutrients in terrestrial ecosystems (Hammond and White 2008). According to Vitousek et al. (2010) the major mechanisms driving P-limitations in soil are (i) loss of inorganic and dissolved organic P via leaching; (ii) slow release of P from mineral forms; (iii) strong retention of P through sorption and precipitation; (iv) low-P parent material and (v) anthropogenic causes such as an enhanced supply of other resources, especially N.

The global P cycle can be highly affected by human activity. Indeed, P-fertilisers derived from phosphate rock mining have been used in intensive agricultural systems to ensure sufficient global food production (Bouwman et al. 2009; Cordell et al. 2009). However, this practice may lead to serious environmental concerns such as eutrophication when entering different water bodies (Withers and Haygarth 2007). Moreover, the application of this type of fertilisers into moderate to highly P-sorbing soils is often relatively inefficient (Simpson et al. 2011). Rock phosphate is a non-renewable resource and as pointed out by Cordell et al. (2009) the agricultural demand for P will outstrip global mineral P-resources within 50–100 years. As such, a more sustainable and environmentally friendly management of P resources and P-inputs is urgently required in agriculture.

Woody biomass can be used in a variety of ways for energy production, among them fast pyrolysis, during which biomass is rapidly heated in the absence of oxygen to obtain a liquid fuel that is known as Fast Pyrolysis Bio Oil (FPBO), that can be a substitute for fuel oil (Bridgwater et al. 1999). In the FPBO production process, not only oil, but also pyrolysis gas and charcoal arise. These valuable by-products are recycled to generate energy by combustion, resulting in the production of biomass fly ashes (FAs) containing most of the minerals originally present in the feedstock (Fernández-Delgado et al., 2016).

* Corresponding author.
E-mail address: marina.fernandez@uibk.ac.at (M. Fernández-Delgado Juárez).

https://doi.org/10.1016/j.resconrec.2018.03.018
Received 20 December 2017; Received in revised form 19 March 2018; Accepted 19 March 2018

FAs are recovered in a separate stream, so these by-products can be reutilized as a promising alternative for nutrient recycling (Cruz-Paredes et al. 2017; Knapp and Insam et al., 2011; Kuba et al. 2008). It is known that during combustion the organic matrix is mostly oxidised and nitrogen is mainly emitted as dinitrogen gas; however, essential macronutrients like P, K, Mg, Ca, S and micronutrients including Fe, Mn, Zn and Cu are retained in the ash (Fernández-Delgado Juárez et al., 2013; Knapp and Insam et al., 2011). Consequently, biomass ash addition may supply the soil with an ample range of mineral nutrients, among them P. In fact, several studies (Li et al., 2016; Ochecova et al. 2017; Schiemenz et al. 2011) have reported higher levels of plant-available P following ash application into soil. However, in several countries, the fate of biomass ashes is still their disposal in landfills or utilisation in cement industry. For instance, only in Austria in 2013, 58,300 tonnes of biomass ashes were landfilled and 39,400 tonnes were used in the cement industry (Walter et al. 2016). In this regard, the utilisation of biomass ashes retrieved from woody biomass combustion in agriculture constitutes a promising alternative. The properties of biomass ashes can vary considerably depending, not only on the production process, but also on its feedstock and the ash fraction (Maresca et al. 2017). Moreover, the conditioning of ashes (hardening, pelletizing, and outdoors storage) is also considered an important factor (Fernández-Delgado Juárez et al. 2015; Pesonen et al. 2017; Pitman 2006; Supancic et al. 2014) influencing their content of soluble salts and pH buffering capacity.

Although 0.2% of plant dry weight is made up of P, it is considered one of the most difficult nutrients for plants to uptake due to its low mobility in soil (Cruz-Paredes et al. 2017). The bioavailable forms of P for plants and microorganisms are the inorganic orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) which can be taken up directly by cell membrane transport systems of plant roots and microorganisms (Frossard et al. 1995). In particular, soil microorganisms play a crucial role through solubilisation of inorganic P and mineralisation of organic P via enzymatic processes such as the release of phosphatases (Richardson and Simpson, 2011).

In prokaryotes, genes encoding for phosphatases belong to the PHO-regulon, which includes those functional genes encoding for alkaline and acid phosphatases, orthophosphate-specific transporters, and other systems for P-mobilization (Ragot et al. 2016; Santos-Beneit 2015). Alkaline phosphatase (ALP) is a common enzyme in the environment, especially found in the bacterial kingdom, but also in fungi and archaea (Ragot et al. 2015). Specifically, the *phoD*-gene is considered the most representative for ALP in soil bacterial communities (Tan et al. 2013).

Although there exists previous studies dealing with the effects of mineral P fertilisers and organic fertilisers on the abundance and diversity of phosphatase harbouring bacterial communities (Fraser et al. 2015a,b; Ragot et al. 2016; Sakurai et al. 2008; Tan et al. 2013), to date little is yet known about how the addition of biomass ashes into soil influence this microbial group and the P cycle in general. Therefore, the main objective of this study was to evaluate, at a mesocosm level, the impact of FA recovered from the fast pyrolysis process on different fractions of soil-P (total, inorganic, plant available and microbial P), as well as on certain enzymatic activities related to the P cycle (alkaline and acid phosphomonoesterases). Furthermore, culture independent methods such as denaturing gradient gel electrophoresis (DGGE) fingerprint and real-time PCR were used to estimate the composition and abundance of *phoD* harbouring bacterial communities, respectively.

We hypothesised that the addition of biomass fly ashes into soil results in: (i) an increase over time in the different plant available, organic, and inorganic soil P fractions; (ii) higher plant yields due to an improved nutrient status of the soil; (iii) an increase in alkaline phosphatase activity due to the liming effect of the ashes; and (iv) microbial community encoding *phoD* genes changes in terms of abundance and diversity.

Table 1
Chemical composition, electrical conductivity and pH of the fly ashes and the soil used in the experiment. All data are on a dry weight basis (average ± s.d.; n = 3).

Parameters	Fly ash	Soil
DM [%]	99.74 ± 0.27	68.78 ± 0.22
pH (H_2O)	12.51 ± 0.03	6.17 ± 0.05
EC[a] [mS cm^{-1}]	19.10 ± 0.20	12.90 ± 0.49[b]
C [%]	4.40 ± 0.05	7.22 ± 0.25
H [%]	0.11 ± 0.01	1.26 ± 0.06
N [%]	0.13 ± 0.00	0.71 ± 0.02
S [%]	1.15 ± 0.03	0.22 ± 0.03
O [%]	8.59 ± 0.07	12.13 ± 0.20
Ca [g/kg]	122.2 ± 1.86	4.98 ± 0.05
K [g/kg]	51.1 ± 0.94	5.92 ± 0.49
Mg [g/kg]	32.8 ± 1.14	7.35 ± 0.18
P [g/kg]	9.11 ± 0.09	0.75 ± 0.02
Zn [g/kg]	1.99 ± 0.07	0.06 ± 0.00
As [mg/kg]	5.48 ± 0.98	136.1 ± 5.35
Ni [mg/kg]	53.53 ± 3.31	122.9 ± 78.8
Cd [mg/kg]	9.41 ± 0.27	0.18 ± 0.01
Cr [mg/kg]	169.5 ± 7.19	61.6 ± 19.06
Cu [mg/kg]	545.3 ± 10.5	18.75 ± 6.85
Pb [mg/kg]	231.4 ± 3.90	25.17 ± 1.02

[a] EC: electrical conductivity.
[b] Units is µS cm^{-1}.

2. Material and methods

2.1. Soil sampling and experimental set up

The soil used in this study was collected from a grassland in Tirol (Austria; 47°04′57.7″ N 11°25′46.8″ E) in May 2016, and classified as eutric Cambisol (IUSS Working Group WRB 2015). It had a slightly acidic pH (5.23 ± 0.02) and was a lime free sandy-loamy soil (sand 58%, clay 8.5%, silt 33.5%), which had not received any kind of amendment in the last 7 years. The biomass ashes used as a soil amendment were FA resulting from the FPBO production from untreated wood chips as described by Solantausta et al. (2012) and Leijenhorst et al. (2016). An overview of the physico-chemical properties of the ashes and the soil are shown in Table 1.

A greenhouse trial was set up to evaluate the fertilising effects of the aforementioned ashes in soil. Perspex columns (Ø = 11 cm, length 20 cm) with a tight mesh (250 µm) in the bottom were used and filled with 2000 g soil each (fresh weight, fw). Biomass ash application took place at the beginning of the experiment by homogenising the ashes with the soil: 2 g of ash plus 98 g of soil (2% w/w; fresh weight basis). This amount is equivalent to 100 kg ash per ha and year, which is the dose recommended for agricultural soils according to the Guidelines for the use of biomass ash in Austria (BMLFUW, 2011) considering a soil bulk density of 1 g cm^{-3} and an influence depth of 5 cm. A control treatment without the addition of ashes was also included. A regional wheat variety (Tiroler Früher Dinkel; *Triticum aestivum* subsp. *spelta*) was used to test the effects of ash on plant growth. The columns were arranged in triplicate in a randomised block design and they were destructively sampled after 0, 60 and 100 days (t0, t60 and t100, respectively). Three extra columns with soil were used as humidity control, which was adjusted when necessary. The six columns referred as t0, with and without ashes (n = 3), were left for an equilibration period of 2 days at 4 °C prior to analyse. Soil was homogenised and sieved (< 4 mm) and all visible roots were removed before the beginning of the experiment. After each sampling, soil samples were sieved (< 2 mm) and stored at −20 °C and 4 °C for molecular and physico-chemical analyses, respectively.

2.2. Physico-chemical analyses

Soil pH was measured in 0.01 M $CaCl_2$ extracts (1:2.5, w/v) using a pH Meter Metrohm 744 (Germany). Electrical conductivity (EC) was measured in distilled water extracts (1:10, w/v) by using a conductivity Meter LF330 WTW (Weilheim, Germany). Total C (TC) and total N (TN) were determined in oven-dried samples using a CN analyser (TrueSpec CHN; LECO, Michigan, U.S.A.). Volatile solids (VS) content was determined from the weight loss following ignition in a muffle furnace (Carbolite, CWF1000) at 550 °C for 5 h. Soil moisture and water holding capacity (WHC) were assessed according to Öhlinger et al. (1996a). Total, inorganic P and plant available P were determined as described by Illmer et al. (1996). Microbial P was determined by using the fumigation extraction method (Öhlinger et al., 1996b), with some minor modifications as described by Amaral and Abelho (2016): after the fumigation step, soil samples were agitated (1 h, 150 r.p.m) using 0.5 M $NaHCO_3$ and decanted (1 h) followed by filtration with phosphate-free filters. Then, 1.5 mL of 10 M hydrochloric acid was added to soil extracts and agitated again followed by a second filtration step in order to remove organic compounds such as humic acids. For the determination of nutrient and heavy metal contents, the initial ash and soil samples were treated (if needed) with a disc vibrating mill (Fritsch Pulverisette 9) containing an agate disc, as well as an ultracentrifugal mill (Retsch UZM 200) and milled to an average particle size of < 200 µm, dried in a cabinet dryer at 40 °C and stored in a desiccator over silica gel. The elemental analysis of C, H, N, S and O was carried out utilising an ELEMENTAR Macro Vario EL (O2 mode, WLD). The digestion for ICP-analysis (Horiba-Jobin Yvon ULTIMA 2) for determining the heavy metal content was performed using analytical grade HNO_3 and HCl at a ratio of 8:3 (aqua regia) in a microwave oven (MLS Start 1500) over a time period of 2 h.

2.3. Soil enzyme activities

Acid - and alkaline phosphomonoesterase activities were determined in duplicate from all the soil samples by a heteromolecular exchange procedure as described by Fornasier and Margon (2007) using a lysozyme solution (3%) as desorbant and a bead-beating agent to disrupt soil aggregates and lyse microbial cells. Enzymes were quantified fluorometrically using fluorescent, 4-methyl-umbelliferyl- (MUF) and 4-amido-7-methyl-coumarine (AMC) substrates. The activities were expressed as nanomoles of MUF (or AMC) min^{-1} g^{-1} dry soil.

2.4. Plant yields

To test for a possible delay derived from the addition of ashes, 10 seeds of *T. aestivum* were placed in the corresponding columns at the beginning of the set up and left 15 days for germination. Afterwards, three strong wheat plants in each soil column were left to develop and harvested after 60 and 100 days. Both the aboveground and root biomass were determined after oven-drying at 60 °C for 48 h, and re-weighing so as to determine their dry weight. The dry weight of wheat ears was also determined.

2.5. Molecular analyses

2.5.1. Soil microbial biomass index (dsDNA)

Whole community DNA was extracted from soil samples (0.20 g, fw) as described by Fornasier et al. (2014); afterwards crude double strand DNA (dsDNA) was quantified by using PicoGreen fluorescent dye (Life Technologies).

2.5.2. DNA extraction

Whole community DNA was extracted using NucleoSpin Soil kit (Macherey-Nagel, Düren, Germany) with some modifications: 0.35 g (fw) of soil were weighed into the bead beating tubes and the initial sample lysis-step was done by using the lysis buffer 1 (SL1). DNA was finally eluted by adding 60 µL of Buffer SE. DNA-concentration and quality were determined by fluorescence-based nucleic acid quantification with Quantus™ Fluorometer specific for ds DNA (Promega GmbH, USA). Purified DNA was stored at −20 °C in low-DNA binding tubes (Genuine Axygen Quality 1.7 mL Maximum recovery, Axygen Inc., USA) for downstream analyses.

2.5.3. Denaturing gradient gel electrophoresis (PCR-DGGE)

Microbial community level endpoint PCR was carried out to amplify the bacterial *phoD* gene by using the primer pair ALPS-F730/ALPS-R1101 as described by Sakurai et al. (2008). A GC- clamp was attached to the reverse primer to prevent complete denaturation during electrophoresis. The PCR reaction was prepared each with 1 µL DNA in 25 µL solution containing a final concentration of 1X MyTaq reaction buffer (Bioline GmbH, Germany); 0.2 µM of each primer; 0.4 mg mL^{-1} bovine serum albumin (BSA; Sigma, Austria); 1X Enhancer (VWR Internation GmbH, Germany); 0.035U μL^{-1} MyTaq DNA polymerase (Bioline GmbH, Germany) and distilled water (RNase/DNase free).

For DGGE fingerprint, 60 ng of PCR products were loaded on an 8% (w/v) polyacrylamide gel with a denaturing gradient of 30–60% according to Hupfauf et al. (2016). Gene ruler 50 bp (Thermo Scientific, 0.5 ug/uL) was used as marker. Gels were run in an INGENYphorU System (Ingeny International BV, The Netherlands), stained with silver nitrate using the Hoefer automated Gel Stainer (Amersham Pharmacia Biotech, Germany), dried and scanned for subsequent image analysis. The quality of the images was optimised using the program Fiji (Schindelin et al. 2012). Similarities among soil microbial communities following ash addition were estimated based on Ochiai similarity coefficient using the GelCompar II software (version 4.0, Applied Maths, Ghent, Belgium) as described by Smalla et al. (2001).

2.5.4. Quantitative real-time PCR

phoD gene was quantified using DNA from *Pseudomonas aeruginosa* PA01. Specifically, fragments of the correct size were excised, suspended in distilled water (RNase/DNase free) and further amplified in a subsequent PCR. Products were purified with a NucleoSpin Extract II kit (Macherey- Nagel), quantified via QuantiFluor® dsDNA Dye and sequenced by Eurofins MWG Operon (Ebersberg, Germany). The obtained sequence was validated by alignment via NCBI Blast tool. Standard curves for qPCR were constructed using the sequenced fragment with the primer pair ALPS-F730/ALPS-R1101 as described by Fraser et al. (2017), with the following modification: an annealing temperature of 59 °C was used in this study. Standard curves were generated using 10-fold dilutions of the above-mentioned sequence. The 20 µL- reactions contained: 10 µL of 1X Sensimix™ SYBR® Hi-rox (Bioline, USA), 0.5 µL of each 10-µM forward and reverse primers, 0.4 mg mL^{-1} BSA, distilled water (RNase/DNase free). To the PCR reactions, 2 µL of each, standards and soil DNA-extracts (1:10 diluted) were added (reactions were run in duplicate). PCR conditions were 10 min at 94 °C followed by 40 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s; following a melting analysis starting from 60 °C to 95 °C with temperature increments of 0.25 °C, to check the specificity of the obtained products. PCR was conducted on Rotorgene 6000 Real Time Thermal Cycler (Corbett Research, Sydney, Australia) in combination with the Rotor-Gene Series Software 1.7. In addition, the amplified products purity was confirmed by the presence of a single band of the expected length on a 1% agarose gel.

2.6. Statistical analyses

The impact of FA on the different soil properties was evaluated over time and in the presence and absence of plants by a factorial analysis of variance (ANOVA). Whenever it was necessary, data were transformed to meet the normality assumptions, followed by pairwise comparison tests (Tukey HSD test) when differences were significant. Kruskal-Wallis

Table 2
Physico-chemical properties of the control and the ash-treated soils at the three incubation times (0, 60 and 100 days) in the presence and absence of plants (P and NP respectively). Values are means (n = 3) with the standard deviations in brackets. Data are expressed on a dry weight basis.

		pH ($CaCl_2$)			EC [µS cm^{-1}]			VOS [%]			WHC [%]			Carbon to Nitrogen ratio		
		Sampling time (days)														
Treatment		0	60	100	0	60	100	0	60	100	0	60	100	0	60	100
Control	P	na	4.50 (0.02)	4.61 (0.15)	na	13.1 (1.32)	29.2 (2.71)	na	12.2 (0.43)	12.3 (0.57)	na	36.2 (4.79)	45.5 (2.25)	na	13.5 (1.67)	13.0 (0.53)
	NP	5.23 (0.02)	4.55 (0.06)	5.07 (0.08)	12.9 (0.60)	16.0 (1.13)	54.5 (2.75)	10.1 (0.26)	11.9 (0.36)	11.0 (0.07)	44.1 (0.70)	51.6 (101)	40.5 (1.48)	17.2 (4.70)	12.6 (0.19)	13.2 (0.79)
Ash	P	na	7.22 (0.15)	7.15 (0.09)	na	365 (18.0)	368 (13.5)	na	11.0 (0.34)	11.5 (0.27)	na	20.9 (2.17)	25.8 (2.50)	na	11.6 (0.68)	14.2 (1.37)
	NP	7.46 (0.07)	7.38 (0.01)	7.30 (0.02)	305 (4.58)	355 (59.2)	340 (27.0)	11.9 (0.20)	11.3 (1.01)	11.9 (0.17)	46.2 (0.14)	58.9 (3.82)	45.5 (2.84)	14.9 (3.80)	13.4 (0.31)	14.8 (2.08)

		Total phosphorus [µg g^{-1}]			Inorganic phosphorus [µg g^{-1}]			Available phosphorus [µg g^{-1}]			Microbial phosphorus [µg g^{-1}]		
		Sampling time (days)											
Treatment		0	60	100	0	60	100	0	60	100	0	60	100
Control	P	na	641 (33)	684 (18)	na	272 (94)	180 (26)	na	9.45 (0.67)	4.87 (0.88)	na	77.1 (14.8)	253.9 (76.7)
	NP	676 (123)	662 (25)	638 (21)	263 (62)	214 (16)	206 (13)	4.91 (0.10)	10.02 (1.25)	5.03 (0.56)	25.3 (3.03)	167.0 (16.01)	85.2 (19.2)
Ash	P	na	1050 (84)	1060 (24)	na	666 (67)	675 (63)	na	55.9 (1.9)	47.6 (1.3)	na	94.6 (6.1)	177.6 (18.7)
	NP	1035 (23)	1064 (68)	1031 (20)	620 (10)	650 (29)	629 (33)	55.1 (3.1)	46.8 (2.3)	51.4 (1.6)	32.1 (5.46)	101.2 (17.9)	205.7 (24.5)

EC: electrical conductivity.
WHC: water holding capacity.
VOS: volatile organic solids.
na: not available.

test were performed when data did not have a normal distribution. Non-metric multidimensional scaling (NM-MDS) on log-normalised data based on Bray-Curtis distance indices was used to visualize the shifts in microbial abundance, diversity and enzyme activities in the differently treated soils in relation to the soil properties. The arrows length is indicative of the relevance of the soil parameters for sample differentiation. This test was performed using PAST software, version 2.17 (Hammer et al. 2001).

3. Results

3.1. Effect of biomass fly ash on soil physico-chemical parameters

An overview of the physico-chemical parameters is given in Table 2. Overall, the pH in the ash-amended soils was approximately 2 units higher compared to the control ($H_{1,36}$ =26.31, $p < 0.0001$), irrespective of the sampling time and plant presence. Likewise, higher soil EC values were recorded over time following ash addition ($H_{1,36}$ = 26.29, $p < 0.0001$) and regardless of the plant presence. VS content was also significantly higher following ash addition ($F_{1,14}$ = 5.58, $p = 0.03$) at the beginning of the experiment; however, no significant differences relative to the control treatment were recorded for the remaining sampling times (60 and 100 days), resulting in a significant interaction between ash treatment and time ($F_{2,24}$ = 32.50, $p < 0.0001$). Overall, FAs amendment led to a reduction in the C/N ratio relative to the control ($F_{1,24}$ = 5.73, $p = 0.02$) for all of the sampling points in the presence and absence of plants. Moreover, a significant decrease in this ratio was observed after 60 days in the presence of plants ($F_{1,24}$ = 3.97, $p = 0.03$), while no further changes were recorded until the end of the experiment. No significant differences between ash-amended and control soils were observed for the soil maximal WHC over time. Nevertheless, plant presence induced a significant decrease in this parameter ($H_{1,36}$ = 8.30, $p = 0.004$) after 60 and 100 days in the ash-treated soils, while in the control soils such a decrease was only observed after 60 days. Total P was, for each sampling time and regardless of the crop presence, approximately 1.5-times higher in the ash-treated soils than in the control ($H_{1,36}$ = 26.29, $p < 0.0001$). Likewise, the ash-amended soils had a higher inorganic P content ($H_{1,36}$ = 26.29, $p < 0.0001$), being 3-times higher than in the control soils for all of the three sampling points. A significant increase in plant available P was also detected following ash addition ($H_{1,36}$ = 26.29, $p < 0.0001$), being 10-times higher relative to the control at the beginning of the trial and after 100 days and 5-fold higher after 60 days in the presence and absence of plants. Nevertheless, microbial P was neither influenced by the ash addition nor by the presence of plants. Significant differences were, however, found over time ($H_{2,36}$ = 25.90, $p < 0.0001$), showing a 5-fold increase in the control soils and a 3-fold increase in the ash-treated soils after 60 and 100 days compared to the beginning of the experiment.

3.2. Effect of biomass fly ash on soil microbiological properties and plant yield

Acid phophomonoesterase activity was significantly reduced in the ash-amended soils regardless of the presence of plants ($H_{1,36}$ = 26.29, $p < 0.0001$), being approximately 2-times lower at the beginning of the experiment and 4-times lower after 60 and 100 days in comparison with the control treatment (Fig. 1a). FA amendment did not have a significant effect on alkaline phosphomonoesterase activity, even though it was close to significance ($H_{1,36}$ = 3.36, $p = 0.07$). However, significant differences were detected over time ($H_{2,36}$ = 9.67, $p = 0.008$) and the highest alkaline phosphomonoestrerase activity was found after 100 days in the ash-treated soils in the absence of plants (Fig. 1b).

A higher soil microbial biomass (assessed as double-stranded DNA, dsDNA) was found in the ash-treated soils (1.3 times higher than in the control) at the beginning of the experiment (Fig. 1c); while the opposite trend was observed after 100 days ($F_{2,24}$ = 10.77, $p < 0.001$). Plant presence resulted in higher dsDNA values irrespective of the sampling time ($F_{1,24}$ = 6.28, $p = 0.02$; Fig. 1c). Despite there were no significant differences in the bacterial *phoD* gene abundance with ash addition, a significant increase was recorded over time in both control and ash-amended soils ($H_{2,36}$ = 25.37, $p < 0.0001$), being around 4 times higher after 100 days than at the beginning of the experiment, regardless of the plant presence (Fig. 1d).

DGGE profiles of the bacterial *phoD* gene showed a higher diversity, assessed by the Shannon Index (H'), in the control than in the ash-amended soils at the beginning of the experiment (H´ = 2.26 and 1.76, respectively) and after 60 days (H´ = 2.21 and 1.69 respectively), while no significant differences were observed after 100 days resulting in an

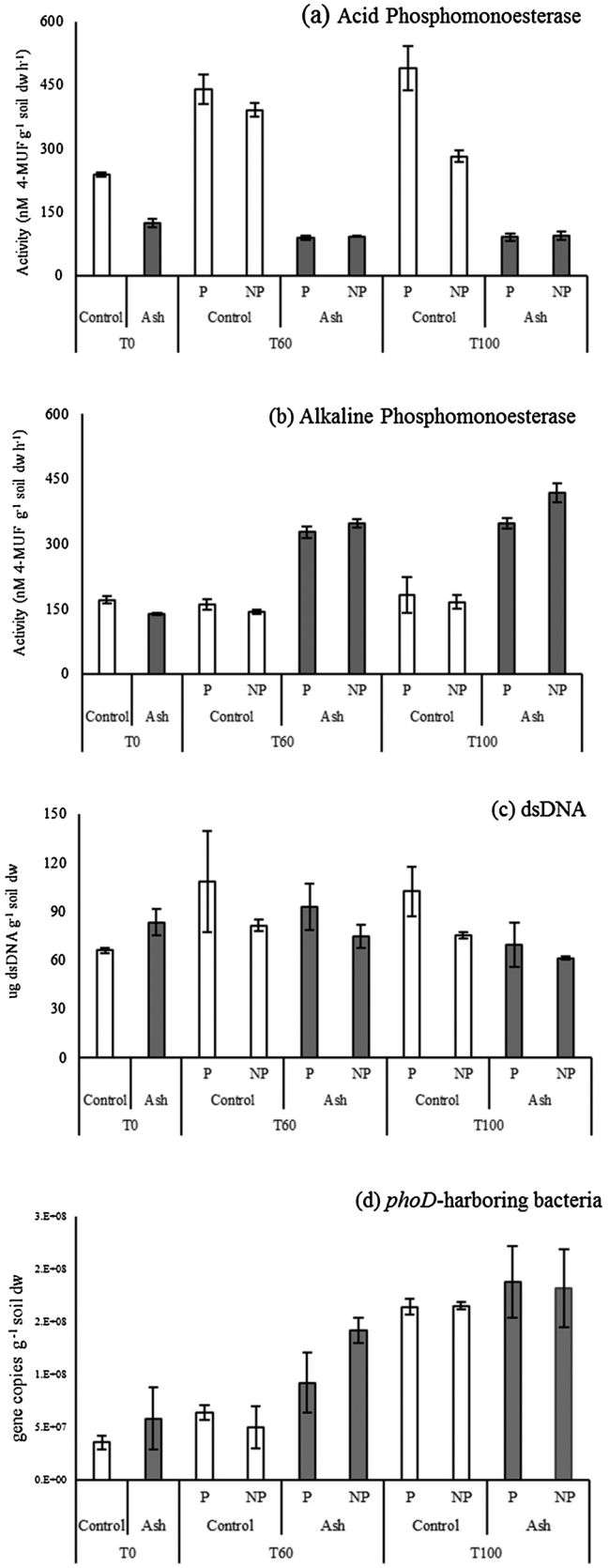

Fig. 1. Acid (a) and alkaline (b) phosphomonoesterases potential activities; (c) soil microbial biomass assessed as dsDNA; and (d) phoD gene abundance determined by qPCR in the control and the ash-treated soils in the presence (P) and the absence (NP) of plants at the beginning of the experiment (T0), and after 60 and 100 days (T60 and T100, respectively). Data show mean values ± standard deviation.

interaction between ash treatment and time ($F_{2,24} = 4.512$, $p < 0.02$). Plant presence did not have a significant impact on the diversity of this group. Moreover, DGGE patterns showed that *phoD* bacterial communities clustered into two main groups according to the sampling time (Fig. 2). The first group comprised all the soil samples collected after 100 days plus the ash-treated soils from day 60 in the absence of plants. The second group comprised the control and the ash-treated soils from the beginning of the experiment (day 0) and those from day 60 where the crop was present, respectively.

Amending the soil with ashes led to an increase in the plant above ground biomass ($H_{1,12} = 8.31$, $p = 0.004$), being around 4 times higher than that in the control regardless of the sampling time (Fig. 3). A similar trend following ash addition was recorded for the root dry mass ($F_{1,8} = 29.70$, $p < 0.001$; Fig. 3). Moreover, this latter parameter also varied significantly over time ($F_{1,8} = 8.18$, $p = 0.02$), showing the highest values after 60 days in both the control and the ash-treated soils. Accordingly, a significant increase in the grain dry biomass was detected following ash addition ($F_{1,8} = 119.55$, $p < 0.0001$) with approximately 4-fold higher values in comparison with the control after 60 and 100 days (Fig. 3). The highest grain biomass was recorded at the end of the experiment ($F_{1,8} = 31.03$, $p < 0.001$; Fig. 3).

3.3. Non-metric multidimensional scaling (NMDS) analysis

All the control soils (negative side) grouped differently from the ash-treated soils which mostly clustered on the positive side of the first ordination axis (Fig. 4). EC ($r = 0.76$), pH ($r = 0.68$), together with the different soil-P fractions (*Pinorg*: $r = 0.62$; *Pav*: $r = 0.57$; *Ptot*: $r = 0.54$; *Pmic*: $r = 0.42$) were the major determinants for the clustering of the soil samples along this axis, with higher values following ash addition (Fig. 3). A differentiation along the second ordination axis was seen as a function of time (0, 60 and 100 days) for the control soils and, to a lesser extent, for the ash-treated soils (Fig. 4). Available P ($r = 0.67$) and pH ($r = 0.67$) were the most influencing parameters for this differentiation. Moreover, microbial P ($r = 0.51$) was negatively correlated with this axis indicating higher levels after 60 and 100 days than at the beginning of the experiment (Fig. 4). The presence or absence of plants was not determinant for the clustering of the different treated soils (Fig. 4).

4. Discussion

Our study reveals that the biomass FAs generated from the production of FPBO from untreated wood chips has a potential use as a soil amendment as, in general, positive or no harmful effects were observed in soil properties from a chemical and microbiological viewpoint. As expected, there was a pH rise in the ash-amended soils which indicates that the alkaline FA (pH = 12.5) used in the current study can be a replacement for lime in order to reduce soil acidity to a level suitable for agriculture (Pandey and Singh 2010; Siddaramappa et al. 1994). Indeed, the liming effect of biomass ashes is well documented (Bougnom et al. 2009) and its alkalinity is linearly associated with the CaO content and/or the CaO/SO_4 ratio (Ram and Masto 2014), as well as with the ageing and stabilization of the applied biomass ashes (Huotari et al. 2015; Maresca et al., 2017; Pesonen et al. 2017; Supancic et al. 2014). Soil pH is considered one of the most important parameters as it can influence nutrient and heavy metal availability and uptake, as well as the composition and activity of microbial communities in soil (Kemmitt et al. 2006; Lauber et al. 2009). For most crops, the optimum pH for the availability of essential nutrients (e.g. P, K, Ca and Mg) is 6.5 to 7.5, which is in line with the pH values of our ash-amended soils. The electrical conductivity is also an important parameter to evaluate in ash-treated soils because it reflects the degree of salinity and suitability for plant growth (Fernández-Delgado Juárez et al., 2013). Biomass FAs are rich in soluble salts and as such, their application usually leads to an increase in soil EC (Jala and Goyal 2004; Matsi and Keramidas 1999) as

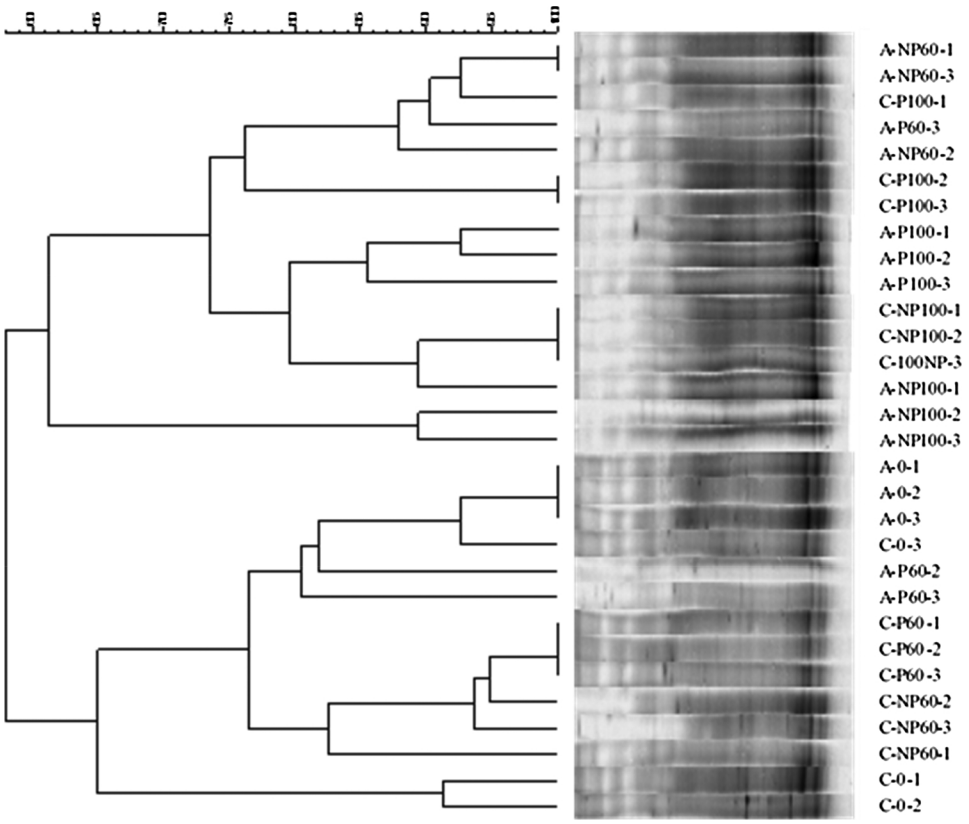

Fig. 2. Hierarchical cluster analyses of DGGE fingerprints of*phoD* bacterial communities in the control (C) and the ash-treated (A) soils in the presence (P) and the absence (NP) of plants at the beginning of the experiment (T0), and after 60 and 100 days (T60 and T100, respectively).

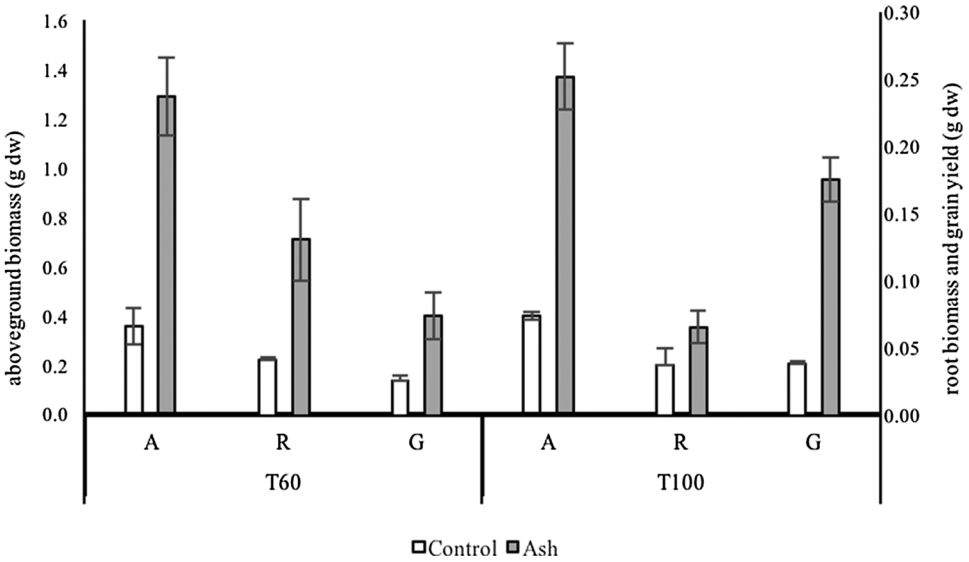

Fig. 3. Effects of ash application on the aboveground (A) and root (R) dry biomass and on the grain yield (G) of the Tirolean wheat variety (*Triticum aestivum subsp. spelta*) after 60 and 100 days. Data show mean ± standard deviation.

occurred in our study. However, due to carbonisation processes, biomass ash addition to soils did not show any influence on soil EC when it was combined with organic amendments (Bougnom et al. 2009; Fernández-Delgado Juárez et al., 2013). It has also been shown that ions contributing to soil EC such as K, Cl and Mg are more easily leached from ashes than others like Ca (Fernández-Delgado Juárez et al. 2018; Maresca et al. 2017; Nieminen et al., 2005), and thus soil irrigation can influence ions availability and affect plant growth. For reasons of plant health soil conductivity should be not higher than 4 mS cm^{-1} (Haynes 2009), a threshold that was never reached in this study. Another positive effect relies on the fact that the addition of biomass FAs led to an improved nutrient status of the soil by increasing the different P fractions (total, inorganic and plant-available P) in a short-term (i.e., 60 days) which is in agreement with the findings from Schiemenz and Eichler-Löbermann (2010) who reported higher soil P-pools after the addition of different biomass ashes. Microbial P increased as a function of time, being such increase less pronounced in the ash-amended than in the control soils. Along these lines, we also

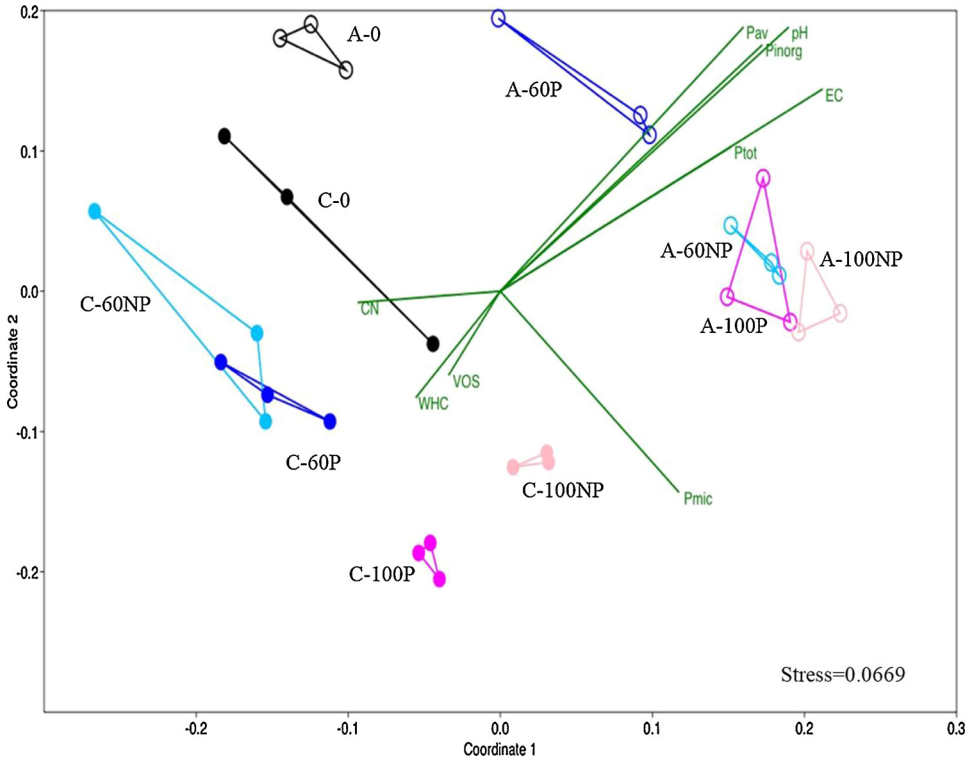

Fig. 4. Non-metric multidimensional scaling (NMDS) of the physicochemical and microbiological parameters from the control (C; full symbols) and ash-treated soils (A; empty symbols) at the beginning of the experiment (T0; black), after 60 days (T60, blue) and 100 days (T100, pink) in the presence (P; bold print) and the absence (NP; light print) of plants. The variables analysed were: electrical conductivity (EC); pH, volatile solids (VOS), water holding capacity (WHC), C:N ratio (CN), microbial phosphorus (Pmic), total phosphorus (Ptot), inorganic phosphorus (Pinorg) and plant available phosphorus (Pav). The length of the arrows indicates the significance of the physico-chemical parameters for sample differentiation (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

observed that amending the soil with FA resulted in a higher above ground and root biomass along with a higher grain yield, corroborating our first two hypotheses. In fact, the positive effects of FA on plant growth and crop yields have already been documented in previous studies (Khan and Kahn, 1996; Katiyar et al. 2012; Li et al., 2016; Masu et al. 2016).

It is known that enzyme activities show a quick response to soil management practices and are important dynamic indicators of soil quality (Sharma et al. 2015). Phosphomonoesterases are known to play an important role in soil as they are responsible for the mineralisation of organic P into phosphate by hydrolysing phosphoric monoester bonds under acid and alkaline conditions, respectively (Nannipieri et al. 2011). As expected, the addition of FA to soil led to a reduction in acid phosphomonoesterase activity irrespective of the sampling time probably due to the rise in soil pH as a result of the liming effect of ash; however, and contrary to our third hypothesis, the ashes did not induce an increase in alkaline phosphomonoesterase activity. This could be due to the fact that in the present study the highest content of available P was recorded in the ash-amended soils; indeed, an increase in P-acquiring enzyme activities would be expected in case of P deficiency (Fraser et al. 2015b). Another plausible explanation could be that an application rate of 2% was not high enough to enhance the activity of alkaline phosphomonoesterase in the short-term. It has been reported that doses up to 10% of FA may exert a boosting effect on phosphomonoesterases (Roy and Joy 2011). Accordingly, we neither observed an increase in the abundance and diversity of *phoD* harbouring bacteria following FA addition. In fact, the DGGE profiles showed that *phoD* harbouring bacterial communities mainly clustered as a function of time.

In our study the impact of biomass FAs on microbial biomass (using dsDNA) was time dependent. The initial increase in soil microbial biomass in the ash-amended soils might be explained by the easily available nutrient contribution that boosted microbial growth; however, the lack of C and N in the ashes inhibited microbial growth towards the end of the experiment (Perucci et al. 2006). On the one hand, Nayak et al. (2014) found that soil microbial biomass carbon and carbon mineralization were not affected up to a 2.5% of FA application. Likewise, García-Sánchez et al. (2015) did not observe any changes in total microbial biomass assessed as the total amount of phospholipid fatty acids following FA addition. On the other hand, there exist a few studies reporting an increase in microbial biomass after addition of FA alone or in combination with other treatments (Chandrakar et al. 2015; Singh et al. 2016).

5. Conclusion

Over the last few decades biomass fly ash has received increasing attention as an alternative to lime or micronutrient fertilizers. Indeed, this study showed that biomass fly ash from FPBO production may constitute an environmentally friendly alternative to non-renewable mineral P fertilisers. They may help in sustaining soil P levels, specifically plant available P, rising pH, and increasing crop yields. Moreover, biomass fly ash application did not appear to be detrimental to ALP gene (*phoD*) harbouring bacterial communities. All in all this suggests its potential use as a valuable additive in acidic grassland soils. Nevertheless, it would be of future interest to evaluate, at a field scale, the long-term effects of biomass fly ashes recovered from FPBO production and to determine their optimal application rate.

Acknowledgements

The authors thank Christian Partl from the Amt der Tiroler Landesregierung for supplying the seeds for the trial; Monika Leitner for providing a soil sampling area; as well as Daniela Pirkebner and Peter Schönswetter from the Institute of Botany at the University of Innsbruck for kindly providing greenhouse space. T. Bardelli has been funded by a PhD grant from the University of Florence and by the Doctoral Scholarship for Junior Researchers (Vizerektorat für Forschung) at the University of Innsbruck. M. Gómez-Brandón acknowledges support by the Programa Ramón y Cajal (RYC-2016-21231; Ministerio de Economía y Competitividad). The Residue2Heat project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No. 654650.

References

Amaral, F., Abelho, M., 2016. Effects of agricultural practices on soil and microbial biomass carbon, nitrogen and phosphorus content: a preliminary case study. Web Ecol. 16, 3–5.

Bougnom, B.P., Maier, J., Etoa, F.X., Insam, H., 2009. Composts with wood ash addition: a risk or a chance for ameliorating acid tropical soils? Geoderma 153, 402–407.

Bouwman, A.F., Beusen, A.H., Billen, G., 2009. Human alteration of the global nitrogen and phosphorus soil balances for the period 1970–2050. Global Biogeochem. Cy. 23, 1–16.

BMLFUW: Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft, 2011. Richtlinie für den sachgerechten Einsatz von Pflanzenaschen zur Verwertung auf Land- und Forstwirtschaftlich genutzten Flächen. pp. 74. (accessed 4 December 2017). https://www.bmlfuw.gv.at/service/publikationen/umwelt/richtlinie_fuer_den_sachgerechten_einsatz_von_pflanzenaschen_zur_verwertung_auf_land-und_forstwirtschaftlich_genutzten_flaechen.html.

Bridgwater, A.V., Meier, D., Radlein, D., 1999. An overview of fast pyrolysis of biomass. Org. Geochem 30, 1479–1493.

Chandrakar, T., Jena, D., Dash, A.K., Jena, S.N., Panda, N., Monica, M., 2015. Soil microbial activity as influenced by application of fly ash and soil amendments to maize crop in acidic alfisols. Int. Res. J. Agric. Sci. Soil Sci. 5, 120–128.

Cordell, D., Drangert, J.O., White, S., 2009. The story of phosphorus: global food security and food for thought. Global Environ. Chang. 19, 292–305.

Cruz-Paredes, C., López-García, Á., Rubæk, G.H., Hovmand, M.F., Sørensen, P., Kjøller, R., 2017. Risk assessment of replacing conventional P fertilizers with biomass ash: Residual effects on plant yield, nutrition, cadmium accumulation and mycorrhizal status. Sci. Total Environ. 575, 1168–1176.

Fernández-Delgado Juárez, M., Waldhuber, S., Knapp, A., Partl, C., Gómez-Brandón, M., Insam, H., 2013. Wood ash effects on chemical and microbiological properties of digestate-and manure-amended soils. Biol. Fertil. Soils 49, 575–585.

Fernández-Delgado Juárez, M., Gómez-Brandón, M., Knapp, A., Stöhr, D., Insam, H., 2015. Chemical and microbiological properties of alpine forest soils: effects of pelletized ashes in a short-term trial. For. Ecol. Manage. 357, 42–49.

Fernández-Delgado, M., Gómez-Brandón, M., Insam, H., Hermanns, R., 2016. Residue2Heat: Residential Heating With Fast Pyrolysis Bio-oil. Biobased Future - Mitteilungsblatt über Biomasse für Energie und Industrie in einer nachhaltigen Wirtschaft 6 - Juli 2016. pp. 28.

Fernández-Delgado Juárez, M., Mostbauer, P., Knapp, A., Müller, W., Tertsch, S., Bockreis, A., Insam, H., 2018. Biogas purification with biomass ash. Waste Manage. 71, 224–232.

Fornasier, F., Margon, A., 2007. Bovine serum albumin and Triton X-100 greatly increase phosphomonoesterases and arylsulphatase extraction yield from soil. Soil Biol. Biochem. 39, 2682–2684.

Fornasier, F., Ascher, J., Ceccherini, M.T., Tomat, E., Pietramellara, G., 2014. A simplified rapid, low-cost and versatile DNA-based assessment of soil microbial biomass. Ecol. Indic. 45, 75–82.

Fraser, T., Lynch, D.H., Entz, M.H., Dunfield, K.E., 2015a. Linking alkaline phosphatase activity with bacterial *phoD* gene abundance in soil from a long-term management trial. Geoderma 257, 115–122.

Fraser, T.D., Lynch, D.H., Bent, E., Entz, M.H., Dunfield, K.E., 2015b. Soil bacterial *phoD* gene abundance and expression in response to applied phosphorus and long-term management. Soil Biol. Biochem. 88, 137–147.

Fraser, T.D., Lynch, D.H., Gaiero, J., Khosla, K., Dunfield, K.E., 2017. Quantification of bacterial non-specific acid (*phoC*) and alkaline (*phoD*) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields. App. Soil Ecol. 111, 48–56.

Frossard, E., Brossard, M., Hedley, M.J., Metherell, A., 1995. Reactions controlling the cycling of P in soils. In: Tiessen, H. (Ed.), Phosporus in the Global Environment: Transfers, Cycles and Management. Wiley, Chichester pp. 107–137.

García-Sánchez, M., Siles, J.A., Cajthaml, T., García-Romera, I., Tlustoš, P., Száková, J., 2015. Effect of digestate and fly ash applications on soil functional properties and microbial communities. Eur. J. Soil Biol. 71, 1–12.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1–9.

Hammond, J.P., White, P.J., 2008. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. J. Exp. Bot. 59, 93–109.

Haynes, R.J., 2009. Reclamation and revegetation of fly ash disposal sites - challenges and research needs. J. Environ. Manage. 90, 43–53.

Huotari, N., Tillman-Sutela, E., Moilanen, M., Laiho, R., 2015. Recycling of ash – For the good of the environment? For. Ecol. Manage. 348, 226–240.

Hupfauf, S., Bachmann, S., Fernández-Delgado Juárez, M., Insam, H., Eichler-Löbermann, B., 2016. Biogas digestates affect crop P uptake and soil microbial community composition. Sci. Total Environ. 542, 1144–1154.

Illmer, P., 1996. Total, organic, inorganic and plant-available phosphorus. In: Schinner, F., Kandeler, E., Öhlinger, R., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin Heidelberg pp. 412–416.

Jala, S., Goyal, D., 2004. Fly ash as a soil ameliorant for improving crop production - a review. Bioresour. Technol. 97, 1136–1147.

Katiyar, D., Singh, A., Malaviya, P., Pant, D., Singh, P., Abraham, G., Singh, S.K., 2012. Impact of fly-ash-amended soil on growth and yield of crop plants. Int. J. Environ. Waste Manag. 10, 150–162.

Kemmitt, S.J., Wright, D., Goulding, K.W., Jones, D.L., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biol. Biochem. 38, 898–991.

Khan, M.R., Kahn, M.W., 1996. The effect of fly ash on plant growth and yield of tomato. Environ. Pollut. 92, 105–111.

Knapp, B.A., Insam, H., 2011. Recycling of biomass ashes: current technologies and future research needs. In: Knapp, B.A., Insam, H. (Eds.), Recycling of Biomass Ashes. Springer, Berlin Heidelberg pp. 1–16.

Kuba, T., Tschöll, A., Partl, C., Meyer, K., Insam, H., 2008. Wood ash admixture to organic wastes improves compost and its performance. Agric. Ecosyst. Environ. 127, 43–49.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75, 5111–5120.

Leijenhorst, E.J., Wolters, W., Van de Beld, L., Prins, W., 2016. Inorganic element transfer from biomass to fast pyrolysis oil: Review and experiments. Fuel Process. Technol. 149, 96–111.

Li, X., Rubæk, G.H., Sørensen, P., 2016. High plant availability of phosphorus and low availability of cadmium in four biomass combustion ashes. Sci. Total Environ. (557), 851–860.

Maresca, A., Hyks, J., Astrup, T.F., 2017. Recirculation of biomass ashes onto forest soils: ash composition, mineralogy and leaching properties. Waste Manage 70, 127–138.

Masu, S., Jurj, N.L., Lixandru, B., Burtica, G., 2016. Application of fly ash as amendment in agricultural field of Avena sativa. Environ. Eng. Manage. J. 15, 2681–2688.

Matsi, T., Keramidas, V.Z., 1999. Fly ash application on two acid soils and its effect on soil salinity, pH, B, P and on ryegrass growth and composition. Environ. Pollut. 104, 107–112.

Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of phosphatase enzymes in soil. In: Bünemann, E., Oberson, A., Frossard, E. (Eds.), Phosphorus in Action. Springer, Berlin, Heidelberg pp. 215–243.

Nayak, A.K., Kumar, A., Raja, R., Rao, K.S., Mohanty, S., Shahid, M., Tripathy, R., Panda, B.B., Bhattacharyya, P., 2014. Fly ash addition affects microbial biomass and carbon mineralization in agricultural soils. Bull. Environ. Contam. Toxicol. 92, 160–164.

Nieminen, M., Piirainen, S., Moilanen, M., 2005. Release of mineral nutrients and heavy metals from wood and peat ash fertilizers: field studies in Finnish forest soils. Scand. J. For. Res. 20, 146–153.

Ochecova, P., Mercl, F., Košnář, Z., Tlustoš, P., 2017. Fertilization efficiency of wood ash pellets amended by gypsum and superphosphate in the ryegrass growth. Plant Soil Environ. 63, 47–54.

Öhlinger, R., 1996a. Maximum water holding capacity. In: Schinner, F., Kandeler, E., Öhlinger, R., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin Heidelberg pp. 385–386.

Öhlinger, R., 1996b. Biomass-P by fumigation extraction technique. In: Schinner, F., Kandeler, E., Öhlinger, R., Margesin, R. (Eds.), Methods in Soil Biology. Springer, Berlin Heidelberg pp. 412–416.

Pandey, V.C., Singh, N., 2010. Impact of fly ash incorporation in soil systems. Agric. Ecosyst. Environ. 136, 16–27.

Perucci, P., Monaci, E., Casucci, C., Vischetti, C., 2006. Effect of recycling wood ash on microbiological and biochemical properties of soils. Agron. Sustain. Dev. 26, 157–165.

Pesonen, J., Kuokkanen, T., Rautio, P., Lassi, U., 2017. Bioavailability of nutrients and harmful elements in ash fertilizers: Effect of granulation. Biomass Bioenerg. 100, 92–97.

Pitman, R.M., 2006. Wood ash use in forestry – a review of the environmental impacts. Forestry 79, 563–588.

Ragot, S.A., Kertesz, M.A., Bünemann, E.K., 2015. *PhoD* alkaline phosphatase gene diversity in soil. Appl. Environ. Microbiol. 81, 7281–7289.

Ragot, S.A., Kertesz, M.A., Mészáros, É., Frossard, E., Bünemann, E.K., 2016. Soil *phoD* and *phoX* alkaline phosphatase gene diversity responds to multiple environmental factors. FEMS Microbiol. Ecol. 93, 1–15.

Ram, L.C., Masto, R.E., 2014. Fly ash for soil amelioration: a review on the influence of ash blending with inorganic and organic amendments. Earth-Sci. Rev. 128, 52–74.

Richardson, A.E., Simpson, R.J., 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. Plant Physiol. 156, 989–996.

Roy, G., Joy, V.C., 2011. Dose-related effect of fly ash on edaphic properties in laterite cropland soil. Ecotoxicol. Environ. Saf. 74, 769–775.

Sakurai, M., Wasaki, J., Tomizawa, Y., Shinano, T., Osaki, M., 2008. Analysis of bacterial communities on alkaline phosphatase genes in soil supplied with organic matter. Soil Sci. Plant. Nutr. 54, 62–71.

Santos-Beneit, F., 2015. The Pho regulon: a huge regulatory network in bacteria. Front. Microbiol. 6, 1–13.

Schiemenz, K., Eichler-Löbermann, B., 2010. Biomass ashes and their phosphorus fertilizing effect on different crops. Nutr. Cycl. Agroecosys 87, 471–482.

Schiemenz, K., Kern, J., Paulsen, H.M., Bachmann, S., Eichler-Löbermann, B., 2011. Phosphorus fertilizing effects of biomass ashes. In: Knapp, B.A., Insam, H. (Eds.), Recycling of Biomass Ashes. Springer, Berlin Heidelberg pp. 1–16.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682.

Sharma, S., Thind, H.S., Singh, Y., Singh, V., Singh, B., 2015. Soil enzyme activities with biomass ashes and phosphorus fertilization to rice–wheat cropping system in the Indo-Gangetic plains of India. Nutr. Cycl. Agrosys. 101, 391–400.

Siddaramappa, R., Wright, R.J., Codling, E.E., Gao, G., McCarty, G.W., 1994. Evaluation of coal combustion byproducts as soil liming materials: their influence on soil pH and enzyme activities. Biol. Fertil. Soils. 17, 167–172.

Simpson, R.J., Oberson, A., Culvenor, R.A., Ryan, M.H., Veneklaas, E.J., Lambers, H., Lynch, J.P., Ryan, P.R., Delhaize, E., Smith, F.A., Smith, S.E., Harvey, P.R., Richardson, A.E., 2011. Strategies and agronomic interventions to improve the phosphorus-use efficiency of farming systems. Plant Soil 349, 89–120.

Singh, K., Pandey, V.C., Singh, B., Patra, D.D., Singh, R.P., 2016. Effect of fly ash on crop yield and physico-chemical, microbiological and enzyme activities of sodic soils.

Environ. Eng. Manag. J. 15, 2433–2440.
Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., Berg, G., 2001. Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Appl. Environ. Microbiol. 67, 4742–4751.
Solantausta, Y., Oasmaa, A., Sipilä, K., Lindfors, C., Lehto, J., Autio, J., Jokela, P., Alin, J., Heiskanen, J., 2012. Bio-oil production from biomass: steps toward demonstration. Energy Fuels 26, 233–240.
Supancic, K., Obernberger, I., Kienzl, N., Arich, A., 2014. Conversion and leaching characteristics of biomass ashes during outdoor storage – results of laboratory tests. Biomass Bioenerg. 61, 211–226.
Tan, H., Barret, M., Mooij, M.J., Rice, O., Morrissey, J.P., Dobson, A., Griffiths, B., O'Gara, F., 2013. Long-term phosphorus fertilisation increased the diversity of the total bacterial community and the *phoD* phosphorus mineraliser group in pasture soils. Biol. Fertil. Soils 49, 661–672.
Vitousek, P.M., Porder, S., Houlton, B.Z., Chadwick, O.A., 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. Ecol. Appl. 20, 5–15.
Walter, B., Mostbauer, P., Karigl, B., 2016. Biomasse-Aschesträme in Österreich. Umweltbundesamt - Perspektiven für Umwelt und Gesellschaft. accessed 8 December 2017. http://www.umweltbundesamt.at/fileadmin/site/publikationen/REP0561.pdf.
Withers, P.J.A., Haygarth, P.M., 2007. Agriculture, phosphorus and eutrophication: a European perspective. Soil Use. Manage. 23, 1–4.