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

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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).

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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.