Pedobiologia 59 (2016) 195–202

Contents lists available at ScienceDirect

Pedobiologia - Journal of Soil Ecology

journal homepage: www.elsevier.de/pedobi

Miscanthus biochar promotes growth of spring barley and shifts bacterial community structures including phosphorus and sulfur mobilizing bacteria

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ARTICLE INFO

Article history:
Received 22 March 2016
Received in revised form 22 July 2016
Accepted 25 July 2016

Keywords:
Hordeum vulgare
DGGE
Quantitative PCR
S K-edge XANES
Next generation sequencing
Multiple linear regression

ABSTRACT

Biochar has shown great potentials in plant growth promotion but its effect on soil bacteria that potentially support plant growth is less well understood. In this study, the effect of biochar soil amendment was investigated on the growth of spring barley, its rhizobacteria diversity and the abundance of sulfur (S) and phosphorus (P) mobilizing bacteria. Furthermore, the S oxidation status was determined in soil, feedstock and biochar. Soil amendment with biochar made from *Miscanthus x giganteus* resulted in significantly increased growth of barley and grain formation four to seven fold. Further significant increases were found for the soil pH and abundances of rhizosphere soil bacteria capable of growing with tri-calcium phosphate, phosphate-esters, phosphonates or aromatic sulfonates as sole source of S or P. A stepwise regression model attributed 74% of the variation in plant growth to the abundances of P and S mobilizing bacteria. 16S rRNA gene based fingerprint analysis revealed a significant shift in the bacterial community structure upon biochar amendment that correlated significantly with the above mentioned changes (pH, plant growth, bacterial abundances). Under biochar amendment, up to 100 times increases in genera *Brevundimonas* known for P cycling and *Arthrobacter* and *Cupriavidus* previously involved in sulfonate desulfurization were identified via quantitative PCR. S k-edge XANES confirmed that the key S source sulfonate was largely absent in the applied biochar, thus bacterial mobilization of sulfonate-S may have originated from the soil.

1. Introduction

Amendment of soils with biochar is often practised to sustainably improve soil fertility or to increase soil carbon stocks (Atkinson et al., 2010; Lehmann et al., 2006). Physical and chemical attributes of buried biochars are principally thought to be responsible for potential beneficial effects to soils (Jeffery et al., 2011), including greater surface area, porosity, higher water retention and lower bulk density (Downie et al., 2009; Sohi et al., 2010). Pyrolysis increases pore spaces in the parent material by over a thousand fold and these pores provide a habitat for microorganisms as well as offering protection from grazing predators (Thies et al., 2015; Warnock et al., 2007). Changes in physicochemical soil properties upon biochar amendment are very likely to shift microbial community structures (Anderson et al., 2011) and these changes were observed in the rhizosphere of ryegrass (Fox et al., 2014).

Despite the recalcitrant nature of many biochars (Lehmann et al., 2006), they may serve as a source of nutrients such as phosphorus (P) or act as a soil conditioner, improving soil nutrient access (Amonette and Joseph, 2009). Biochar P content may vary substantially depending on feedstock and pyrolysis ranging from 2.7 to 480 g kg^{-1} (Yin Chan and Xu, 2009). While very little to no P will be lost to the gas phase during pyrolysis, parts of the sulfur (S) in the feedstock may be lost to the atmosphere, depending on the pyrolysis conditions and feedstock type chosen (Di Blasi et al., 1999; Lang et al., 2005). Unlike nitrogen (N), macro-nutrients P and S are largely absent in the atmosphere and only found in smallest

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http://dx.doi.org/10.1016/j.pedobi.2016.07.003
0031-4056/

amounts in atmospheric depositions. Plants largely access P and S in the form of orthophosphate and sulfate from soil solution even though approximately 99 and 95% of P and S in soils are organically or inorganically bound (Autry and Fitzgerald, 1990; Randriamanantsoa et al., 2013). Thus, the role of soil microbes mobilizing P and S is of utmost importance in the adequate supply of these nutrients to support plant growth.

While some advancements have been made in biochar research when it comes to plant N supply and microbial cycling of N (Thies et al., 2015), advances in P and S cycling research in biochar amended soils are scarce. Soil P pools are dominated by monophosphate esters and inorganically bound P such as calcium phosphates (Shen et al., 2011; Turner et al., 2005). P availability in biochar amended soils can increase with the alterations in soil pH, enzyme efficiencies, organo-mineral complex formation and changes in the soil microbial community (DeLuca et al., 2015). Microbial mobilization of these bound forms is largely achieved through ester hydrolysis by a variety of esterase enzymes or through exudation of acids to solubilize inorganically bound P (Alikhani et al., 2006; Lim et al., 2007). Abundances of phosphate solubilizing, phytate and phosphonate utilizing bacteria may be increased (Anderson et al., 2011; Fox et al., 2014) and phosphatase enzyme activity may be enhanced under biochar amendment (Du et al., 2014; Ventura et al., 2014). Soil S pools are dominated by sulfonates while sulfate-esters are commonly the second most abundant form of S (Autry and Fitzgerald, 1990; Kertesz and Mirleau, 2004). Aromatic sulfonates appear to be particularly important and are mobilized by a functional bacterial guild (Schmalenberger et al., 2008). Recent biochar soil amendments identified higher abundances of sulfonate utilizing bacteria alongside ryegrass growth promotion in pots (Fox et al., 2014) and increased sulfatase activities in the field (Ventura et al., 2014).

This study aimed to identify correlations between biochar based growth promotion of barley, bacterial community structures, bacterial utilization of calciumphosphate, phytate, phosphonate and sulfonate and abundances of selected bacterial genera. The hypothesis was that in a biochar based barley growth promotion scenario, bacterial community structures will be shifted and that these changes in the bacterial community includes in particular P and S mobilizing bacteria. Furthermore it was hypothesised that these shifts in bacterial communities associated with P and S mobilisation are responsible for a portion of the plant-growth promotion under biochar amendment.

2. Materials and methods

2.1. Pot experiment

Biochar from *Miscanthus x giganteus* biomass was prepared as described previously and contained 2.44 mg P g^{-1} DW and 0.96 mg S g^{-1} DW (Fox et al., 2014). For the pot trial, soil was taken from the long-term cowlands experiment conducted at Johnstown Castle research centre (Teagasc) in the south east of Ireland (52° 16′N,06° 30′W), free of P fertiliser since 1968 (Tunney et al., 2010). The soil type is a poorly drained gley soil, with a loamy topsoil (18% clay) with a pH of 6 (Mollic Histic Stagnosol; WRB, 2006), 46.1 g kg^{-1} carbon (Griffiths et al., 2012), 2.8 g kg^{-1} N, 0.34 g kg^{-1} S and 0.32 g kg^{-1} P (FW; Lancrop Laboratories Ltd., York, UK). Water extractable phosphate and sulfate (biochar, soil) was determined via ion chromatography using a Dionex ICS1100 with an AS23 column and a carbonate mobile phase as recommended by the manufacturer (Sunnyvale, CA).

Pots were established with the soil mixed 1:1 with distilled H_2O washed sand (900 g per pot, replicates of eight). A top layer (300 g) was kept biochar-free to exclude an effect of the biochar on seed germination. The middle layer (300 g, M layer) was amended with 3 or 6% biochar (1% and 2% per pot; biochar particles <1 cm in diameter; referred to as 1 and 2% amendment from hereon), while the bottom layer was again biochar free (300 g) as published recently (Fox et al., 2014). Each pot was planted with four seedlings of spring barley (*Hordeum vulgare* var. SY Taberna) and thinned to two plant shoots after a period of two weeks. After a growth period of 80 days in a greenhouse at ambient temperatures (Irish summer) and natural lighting, plants had fully developed heads that lost their green colour. Pots were harvested and plant shoot heights, weights and soil pH were recorded as described previously (Fox et al., 2014). Barley shoots were analysed for elemental composition at Lancrop Laboratories Ltd. (employing atomic absorption spectroscopy, inductively coupled plasma spectrometry, titrations, and spectrophotometry). Seed numbers and weights (DW) were recorded. Total seed N was quantified in a Vario EL Cube elemental analyser (Elementar, Goettingen, Germany) and crude protein subsequently calculated. Starch content was measured in the seeds using a Megazyme (Bray, Ireland) total starch assay kit as recommended by the manufacturer. Where replicate measures were taken, results were subjected to univariate analysis (Tukey HSD in SPSS 20, IBM, Armonk, NY).

2.2. Quantification of P and S mobilizing bacteria

Bacteria were extracted from rhizosphere associated soil from the M layer of each pot (replicates of eight) as follows. After destructive disassembly of the plant pots, roots of 3 g with loosely attached soil (and biochar therein for the 1 and 2% treatment) were added to 50 ml conical tubes with 20 ml of sterile saline (NaCl 0.85% [wt vol^{-1}]) solution and rotated at 75 rpm (RM-2 mixer) for 30 min at 4 °C. 0.1 ml of the obtained suspension was used for serial dilution and subsequent cultivation. The remainder without plant roots was centrifuged at 4500 rpm for 15 min at 8 °C and the pellet was stored at −20 °C. Cultivable bacteria capable of mobilizing P from phytate (phosphate-esters, MM2Phy) and phosphonoacetic acid (MM2PAA) and S from toulenesulfonate (MM2TS) as sole source of P or S respectively, were quantified through a most probable number (MPN) approach in microtiter plates (Fox et al., 2014). Colony forming units (CFU) were established to determine the cultivable bacteria solubilizing P from tri-calcium phosphate agar plates as indicated by a zone of clearance around the colonies (Rondon et al., 2007). Both CFU and MPN data were normalized (log_{10}) for univariate analysis (Tukey HSD in SPSS 20).

2.3. Soil DNA extraction, amplification and analysis

DNA was extracted using the ULTRA CLEAN™ soil DNA kit (MO BIO Laboratories, Cupertino, CA) and subjected to amplification of 16S rRNA gene fragments (replicates of eight). Amplicons were used individually for denaturing gradient gel electrophoresis (DGGE). PCR was performed with primers GC-341F and 518R (Muyzer et al., 1993) and a touch-down protocol as described in the Supplementary materials (SM1). Gel electrophoresis was performed in a TV-400 DGGE system (Scie-Plas, Cambridge, UK) with 200 × 200 × 1 mm gels, with a polyacrylamide gel strength of 10% (vol vol^{-1}) in 1x TAE and a denaturing gradient of 30–70% made from urea and formamide at 63 V for 16 h at 60 °C. Gels were stained with SYBR Gold as recommended by the manufacturer (Invitrogen, Carlsbad, CA). DGGE profiles were translated into a binary gel image matrix (Phoretix advanced 1D; Nonlinear Dynamics, Newcastle, UK) for canonical correspondence analysis (CCA) biplots and permutation tests (Monte-Carlo) using forward selection and 9999 replicates (CANOCO 4.5; Microcomputer Power Inc., Ithaca, NY).

PCR reactions for next generation sequencing (NGS) were conducted with the universal primer pair 16SF and 16SR to target