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Biological, physicochemical and plant health responses in lettuce and strawberry in soil or peat amended with biochar

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ABSTRACT

Biochar, a solid coproduct of biomass pyrolysis, has recently been proposed as soil amendment in agriculture. We studied the effect of biochar on soil and substrate physicochemical properties, plant growth, disease susceptibility and rhizosphere microbiology in two contrasting cropping systems, i.e., lettuce grown in field soil and strawberry grown in white peat. For both systems, changes in the physicochemical properties of the plant growth media were observed. In the lettuce bio-assay, biochar addition had no effect on crop growth, crop health (*Rhizoctonia solani* infection test) and rhizosphere microbiology. In contrast, in the strawberry bioassay, addition of 3% biochar to peat resulted in (i) a higher fresh and dry plant weight, (ii) a lower susceptibility for the fungal pathogen *Botrytis cinerea* on both leaves and fruits, and (iii) changes in the rhizosphere microbiology, analysed by Phospholipid Fatty Acid (PLFA) profiling and 16S rDNA amplicon sequencing. Biochar addition led to an increase of bacterial diversity and a shift in composition of the rhizosphere microbiota. Extra inorganic plant nutrition and lime added to the peat reduced these effects of biochar on the strawberry plants. We conclude that in certain plant growth media, biochar amendment can result in chemical changes that induce multiple responses in the plant, including shifts in the rhizosphere microbiome. Biochar can be beneficial for plant growth, especially in conditions of limited nutrient availability.

1. Introduction

Biochar is the by-product of pyrolysis of biomass for biofuel production (Gravel et al., 2013). Biochar has the potential to reduce the CO_2 release into the atmosphere and can further be used for environmental remediation (Barrow, 2012; Xie et al., 2015), although application in agriculture remains one of the most common uses of biochar. Addition of biochar may change the physicochemical and biological properties of soils and substrates, which in turn may affect crop growth and health (Elad et al., 2011; Jeffery et al., 2011).

Biochar has a typical porous structure, high surface area and affinity for charged particles (Keech et al., 2005; Steiner et al., 2008). Thanks to these properties, biochar addition to soil can lead to an increase in soil water permeability and soil water retention (Asai et al., 2009; Laird et al., 2010) and an increase in soil pH (Chan et al., 2007; Rondon et al., 2007). Other commonly reported effects of biochar addition to soil are retention of nutrients and an increase of the organic carbon content (Lehman et al., 2003; Chan et al., 2007; Nelissen et al., 2015). Considering all these effects, biochar would have the potential to improve plant productivity. Additionally, biochar could affect plant productivity due to its nutrient content (Graber et al., 2010).

Several pot and field trials showed that biochar addition to the soil can enhance productivity and performance of crops (e.g. Chan et al., 2007; Asai et al., 2009; Graber et al., 2010;). However, also neutral or even negative effects of biochar addition to the soil on crop growth have been reported (e.g.Gravel et al., 2013; Nelissen et al., 2015).

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Various types of biochars were reported to act in disease suppression against fungal foliar pathogens. Elad et al. (2010) showed less susceptibility of pepper and tomato plants to *Botrytis cinerea* and *Oidiopsis sicula* when biochar was added to soil. Likewise, biochar amendment to substrate reduced the severity of three foliar diseases caused by *B. cinerea*, *Colletotrichum acutatum* and *Podosphaera apahanis* on strawberry plants (Meller Harel et al., 2012). In these studies with foliar pathogens and biochar added to the soil or substrate, there is no direct toxicity effect of biochar on the pathogens. This suggests that biochar can affect the plant-wide systemic response (Jaiswal et al., 2014). It has been shown that biochar induces disease related genes linked to induced systemic resistance (ISR) (Meller Harel et al., 2012; Mehari et al., 2015; Huang et al., 2015). It is known that ISR can be promoted by the colonization of plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) (Mehari et al., 2015). These PGPR and PGPF are mainly present in the rhizosphere, i.e., the narrow zone of soil surrounding the plant root, and can therefore influence the growth, nutrition and health of plants (Philippot et al., 2013). Consequently, the positive effect of biochar on crop performance could also be indirect through the stimulation of plant beneficial rhizosphere microbes.

Previous studies examined generally only one or two of the above mentioned effects of biochar on agricultural properties (e.g. soil physicochemical and biological properties, crop productivity and performance, plant health or rhizosphere microbiology). However, we believe that, in order to define biochar as a positive or negative operator on the crop-soil/substrate system, all these individual factors need to be integrated in order to estimate the overall impact and to understand the underlying mechanism. Therefore, in this study, we selected two target crop systems for our bio-assays: lettuce and strawberry. Lettuce is typically well adapted for growth in field-soil, representing a complex environment for the plant roots. Strawberry grows well in a soilless system such as white peat, a well standardised environment with a low nutritional and microbial background. Lettuce is known to be responsive to nutrients (Upadhyay et al., 2014) and *Rhizoctonia solani* was chosen as pathogen system, as it is the predominant pathogen causing basal rot on lettuce (Van Beneden et al., 2009). Strawberry was infected with *Botrytis cinerea,* known as a serious pathogen reported to cause fruit losses up to 50% (Jarvis, 1962). However, the leaves are also very important in the infection cycle, as infection of leaves by *B. cinerea* may lead to increased inoculum production when leaves are senescing in a perennial growing system (Braun and Sutton 1988; Sutton and Peng, 1993). Biochar is expected to affect the composition of the plant growth media, such as pH, carbon content, nutrient availability, microbiology and water management and availability. It has previously been suggested that the effect of biochar on crop productivity would be dose and crop dependent (Gravel et al., 2013), but we realize that it should also be soil or substrate dependent. Therefore, in the present study the plant growth media were well characterized before and after the plant growth tests. The used field soil had an optimal pH and stored relevant nutrient concentrations for growth of lettuce. The white peat of the strawberry was confirmed to be low in plant nutrients and microbial diversity, and had a low pH. Based on previous reports, we expected that biochar has a neutralising effect on the peat pH and a fertilising effect for the plant (Carter et al., 2013). Effects beyond these two factors were tested by also combining biochar mixed in white peat with liming and extra addition of plant nutrient compounds.

This study aims to increase our understanding of the effect of biochar on the relation between the physicochemical properties of the plant growth media, crop growth, disease susceptibility and the rhizosphere microbial community. This kind of information is needed to fully appreciate the role of biochar as a soil or substrate amendment for agriculture.

2. Materials and methods

2.1. Biochar and plant growth medium

Biochar was prepared from holm oak at 650 °C for 12–18 h and was kindly provided by Proininso S.A. (Malaga, Spain). This biochar consists of 72.4% dry matter (DM) (%/fresh), 77.8% organic matter (%/DM) and 74.2% C (%/DM) and was previously used and fully characterized by Vandecasteele et al. (2014, 2016),

Field soil used in the lettuce assay was sampled from the arable layer 0–20 cm of an ongoing field experiment at ILVO (BOPACT; D'Hose et al., 2016, 225) and its chemical properties at the beginning and end of the experiment were measured as described below (Sections 2.2 and 2.3) and are listed in Supplemental (S) Table S1a and Table S1b, respectively. This sandy loam soil (pH-KCl = 5.79; clay = 5.3%; silt = 37.7%; sand = 57.0%) was sieved (1 cm), air-dried (99% dry matter/fresh), and stored at room temperature until use.

Peat used in the strawberry assay was NOVOBALT white peat 100% (AVEVE Lammens, Wetteren, Belgium). The chemical properties of the 'NOVOBALT peat' at the beginning (week 1) and end (week 13) of the experiments are listed in Supplemental Table S1c and Table S1d, respectively.

2.2. Chemical characterization of soil and amended soil

Methods for the chemical characterisation of soil and peat are based on European Standards developed by the European Committee for standardization (CEN) or by the International Organization for Standardization (ISO). European Standard EN numbers or ISO numbers refer to the specific standards.

Soil was sampled at the start and the end of the lettuce experiment for chemical analysis. At the start of the experiment, 1 l of thoroughly mixed soil was sampled after one week of pre-incubation. At the end of the experiment the soil that remained after sampling for rhizosphere microbiology (see Section 2.6 and 2.7) was used (± 1 l).

Prior to chemical analysis, the soil samples were thoroughly mixed and divided into three sub-samples. The first sub-sample was used immediately for pH-KCl, Electrical Conductivity (EC) and soil mineral N (NO_3^--N + NH_4^+-N) determination. Soil dry matter (DM) content was determined by oven drying at 105 °C. The pH was measured potentiometrically in a 1:5 soil:KCl (1 M) extract according to ISO 10390. The EC was measured by means of a temperature compensating conductivity meter (E SK 10B electrode, 25 °C) in a 1:5 soil:H_2O extract according to EN 13038. Soil mineral N was determined in a 1 M KCl extract according to ISO TS14256-1:2003 with a Skalar San++ mineral N analyzer. The second and third sub-sample were oven dried at 45 °C and 70 °C, respectively. The samples were ground in a mortar and passed through a 2 mm and 250 μm sieve, respectively, prior to analysis of chemical soil properties. Ammonium lactate (AL) extractable elements were assessed on the second sub-sample by extracting plant-available concentrations of P, K, Ca, Mg, Fe, Mn and Na with ammonium lactate (extraction ratio 1:20) in dark polyethylene bottles, shaken for 4 h (Egnér et al., 1960). The suspension was filtered in dark polyethylene bottles that were stored at 4 °C until analysis. Elements were analysed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian Vista-Pro) with an axial torch. Total organic carbon (TOC) was measured on the third sub-sample by dry combustion at 1050 °C using a Skalar Primacs SLC TOC analyser (ISO 10694).