Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

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

Biochar chemistry defined by ^{13}C-CPMAS NMR explains opposite effects on soilborne microbes and crop plants

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ARTICLE INFO

Keywords:
Beneficial microbes
C/N and H/C ratios
^{13}C CPMAS NMR
Disease suppression
Organic amendment
Phytotoxicity
Rhizoctonia solani
Soilborne pathogens

ABSTRACT

Numerous recent studies have demonstrated that biochar may significantly reduce the incidence of plant diseases caused by airborne and soilborne pathogens, although contrasting results have also been reported. In this work, we investigated how biochar affects crop plant and soilborne microbe growth. Aims of this study were: i) to analyze the chemical changes occurring in four organic feedstocks (e.g. wood chips, organic urban waste, *Zea mays* residues, and *Medicago sativa* hay) when pyrolyzed at 300 °C and 550 °C by using ^{13}C NMR spectroscopy and SEM (Scanning Electron Microscopy); ii) to assess how biochar affects growth of five bacteria, nine fungi, and three crop plants; and iii) clarify the relationships between biochar chemistry and its effect on target species.

As pyrolyzation temperature increased, organic matter chemistry of all products changed significantly, with a progressive loss of *O*-alkyl C, di-*O*-alkyl C, and methoxyl and *N*-alkyl C, coupled with an enrichment in aromatic C types. Untreated urban waste and *Medicago* hay severely inhibited *Lepidium*, *Lactuca* and *Solanum* root growth, whereas no inhibitory effects were found for the other feedstocks. However, these phytotoxic effects largely decreased after pyrolyzation. In contrast to the crop plants, fungi and bacteria thrive on most of the unprocessed organic materials but showed reduced growth and development or complete growth inhibition on biochars obtained at 300 °C and 550 °C. Soilborne microbes demonstrated remarkably similar correlation patterns between their growth to the organic feedstock and biochar chemical components. This work demonstrates that defining organic matter quality by ^{13}C NMR extends our understanding of the impact of biochar on crop plants and key components of the soil food-web.

1. Introduction

Biochar is the heterogeneous material generated through pyrolysis, a thermal process carried out at temperatures ranging from 250 °C to > 900 °C and under limited oxygen availability. The International Biochar Initiative defines biochar as "a solid material obtained from the thermo-chemical conversion of biomass in an oxygen limited environment" (IBI, 2012). Biochar, basically, is distinguished from charcoal because of its final use in agricultural and environmental management, instead of the use as a fuel and energy source (Lehmann and Joseph, 2009).

The positive effects of biochar on crop production have been known since ancient times. The Pre-Columbian populations of Amazonia developed the so called "*terra preta*" or "*dark earth*" soils by repeating cycles of fire and cultivation, i.e. the slash-and-burn cultivation system (Steiner et al., 2004). However, scientific investigations on the benefits of this ancient agricultural practice comes only in the past decade with a research boom on this topic (Biederman and Harpole, 2013). Due to its demonstrated capacity to increase soil quality, and subsequently crop production, nowadays, biochar is largely used as a soil amendment and commercialized worldwide (Jirka and Tomlinson, 2015). The meta-analysis of Jeffery et al. (2011) reported that, on average, crop yields increased 10% after biochar applications, and only few cases of negative effects on crop performances have been noted (Wisnubroto et al., 2010; Calderón et al., 2015). The beneficial effects of biochar on crop productivity has been related to different mechanisms. Biochar has a consistent liming effect that is especially important in acidic soils (Biederman and Harpole, 2013). Moreover, biochar has the capability of increasing soil water retention capacity (Novak et al., 2012; Barnes et al., 2014), as well as adsorbing phytotoxic organic molecules including xenobiotics and natural allelopathic compounds (Beesley et al., 2011; Oleszczuk et al., 2012). Finally, biochar has been shown to

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https://doi.org/10.1016/j.apsoil.2017.11.027
Received 16 November 2016; Received in revised form 21 November 2017; Accepted 26 November 2017

stimulate the activity of mycorrhizal fungi (Warnock et al., 2007), and plant-growth-promoting microbes (Graber et al., 2010; Kolton et al., 2011) in bulk soil as well in the rhizosphere (Thies et al., 2015).

In recent years, several reports indicated that biochar applications have also provided an effective suppression of diseases caused by both airborne (Harel et al., 2012; Graber et al., 2014) and soilborne plant pathogens (Elmer and Pignatello, 2011). Some of these studies attributed an ability of biochar to induced systemic resistance in the plant, which consequently reduced the disease incidence (Elad et al., 2010; Zwart and Kim 2012; Mehari et al., 2015). Others found that biochar can act as a carrier for several biological control agents such as *Bacillus*, *Pseudomonas*, and *Streptomyces* (Postma et al., 2013). However, some studies reported no significant effects (Bonanomi et al., 2015a,b), even noting an increase of disease incidence after biochar application to soil (Knox et al., 2015). The variable effect of biochar on plant pathogens, limits the application of this material as a general method for effective plant disease control.

The variable effect of biochar can be related to the species-specific response of different plant-pathogen systems, as well as to the complex relationship between biochar chemistry, soil microbes and plant health. In fact, until now biochar has been tested only in a few pathosystems (Elad et al., 2012). Moreover, biochar chemical diversity can be very high in relation to the quality of the initial organic feedstock and pyrolysis conditions. In this regard, the use of ^{13}C-cross-polarization magic-angle spinning nuclear magnetic resonance (^{13}C-CPMAS NMR) spectroscopy (Knicker, 2007) allows a detailed monitoring of the chemical changes occurring in plant biomass following pyrolyzation. Several studies reported a consistent and progressive loss of *O*-alkyl C and di-*O*-alkyl C, associated to carbohydrates, and a corresponding increase of the aromatic carbons with increasing temperature of pyrolyzation (Krull et al., 2009). Other studies, instead, considered the H/C ratio as a reliable indicator of biochar quality because this parameter showed a consistent decrease as pyrolysis temperature increased (Xiao et al., 2016). The link between chemical changes occurring during biomass pyrolysis and the effects on soilborne microbes and crops plants is far from being understood.

This body of evidence suggests that biochar chemistry is a key factor to explaining the variable responses of prokaryotes, fungi and higher plants to such organic material. Numerous previous studies provided detailed assessment of biochar chemistry (Baldock and Smernik, 2002; González-Pérez et al., 2004; Xiao et al., 2016), but the research did not investigate the effect on higher plants, bacteria and fungi simultaneously. The approach of studying contemporarily different food-web components would increase our capability to clarify the effects of biochar on agro-ecosystems. In this study, we combined organic feedstock and biochar characterization by using ^{13}C-cross-polarization magic-angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy (Kögel-Knabner, 2002) and scanning electron microscope (SEM), with a multi-species bioassay approach to analyze the relationship between biochar chemistry and the response of bacteria, fungi and higher plants to the different compounds. The effect of four organic feedstocks and eight biochar types, spanning a wide range of chemical qualities, was assessed on the growth of fourteen microbes and three plant species. Our hypothesis is that as organic matter is pyrolyzed, its chemistry changes because of a progressive depletion of easily degradable C sources while, at the same time, there is an increase of recalcitrant compounds (e.g. aromatic C fractions). The dynamics of biochemical changes can drive different, but contrasting responses of higher plants and saprotrophic microbes, which result in a decrease in bacterial and fungal growth with organic matter pyrolyzation. Furthermore, we hypothesize that the effect of organic matter on plant root growth can shift from negative to positive with pyrolyzation, probably due to the progressive reduction of phytotoxic compounds.

Specific aims of the study were to:

(1) assess the effect of four organic feedstocks and eight biochar types on the growth of five bacteria, nine fungi and three crop plants;
(2) explore the relationships between organic feedstocks and biochar chemistry, as defined by ^{13}C-CPMAS NMR spectroscopy, and growth of target species;
(3) compare ^{13}C-CPMAS NMR data with elemental chemical analysis (pH, C, N, C/N and H/C ratios) to characterize functional indicators of organic feedstocks and biochar chemistry in relation to target species performances.

2. Materials and methods

2.1. Organic feedstocks and biochar production

Four organic feedstocks were selected to represent a wide range of organic matter chemistry: i. *Medicago sativa* hay, ii. *Zea mays* stalks, iii. wood chips from a sawmill, and iv. the organic fraction of municipal solid waste (F.O.R.S.U.; contains only food waste remains i.e. vegetables, meat and fish). Dried samples of each organic feedstock were subject to pyrolysis at two temperatures (300 °C and 550 °C) for five hours in a muffle furnace to obtain eight biochar types (treatments included four feedstocks × two temperatures, plus four thermally untreated feedstocks as controls). All materials were ground in a blender to obtain a powder of < 2 mm particles, then the material was stored in air-tight containers.

2.2. Plant bioassay

Plant bioassays, hereafter the “seed germination” experiment, were used to assess the effects of the four thermally untreated feedstocks and the eight biochars on the growth of three plant species. *Lepidium sativum* was selected as a target plant species because of its recognized sensitivity to phytotoxic compounds (Bonanomi et al., 2011a, b), while *Lactuca sativa* and *Solanum lycopersicum* were chosen for their economic importance in horticulture. To obtain water extracts of the feedstocks and biochars, polverized material was passed through a 2 mm – mesh sieve, then added at 5% w/v (50 g l^{-1}), with distilled water, to a beaker and agitated for 5 h. The suspensions were centrifuged (2395*g* for 10 min), then filter-sterilized (0.22-μm pore) to obtain a test solution of each compound, and stored at −20 °C until use.

For the seed germination experiment, twenty-five seeds of each species were placed in Petri dishes (90 mm diameter) lined with sterile filter paper (Whatman Grade 1) wetted with 4 ml of the test solution. Each solution plus a water control were replicated five times, for a total of 4875 seeds (3 species × 25 seeds × 12 extracts × 5 replicates plus the untreated control) tested for each plant species. Petri dishes were arranged in a growth chamber, in a completely randomized design at +24 °C, in the dark. After germination, the seedling root length was measured for *Lepidium* after 36 h, and after five days for *Lactuca* and *Solanum*.

2.3. Microbial bioassay

A microbial bioassay was performed to assess the effects of untreated feedstocks and biochar on the saprotrophic growth of nine different fungi, with varying ecological/biological roles: *Aspergillus niger*, *Botrytis cinerea, Fusarium oxysporum, Fusarium oxysporum* f. sp. *radicis-lycopersici, Ganoderma lucidum, Penicillium italicum, Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Trichoderma harzianum* (Table S1). All strains, available in the mycology laboratory of the Department of Agricultural Sciences, University of Naples, were maintained on potato dextrose agar (PDA, Oxoid) medium. Assays were conducted with the test solutions, with or without the addition of potato dextrose broth (PDB; serving as an external source of organic carbon and nutrients) to determine if fungal growth was inhibited by biochar constituents, i.e. presence of toxic compounds, or to the lack of suitable carbon sources.

A spore germination bioassay was conducted with six fungi (*A. niger*,