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Research article

Impact of soybean stover- and pine needle-derived biochars on Pb and As mobility, microbial community, and carbon stability in a contaminated agricultural soil

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ABSTRACT

Biochar is gaining attention as a potential soil amendment to remediate and revitalize the contaminated soils. Simultaneous effects of biochar on metals mobility, microbial abundance, bacterial diversity and carbon storage in soil are scarcely addressed. This study assessed the effect of biochars on metal mobility, microbial abundance, bacterial community, and carbon storage in an agricultural soil contaminated with heavy metals. Biochars derived from soybean stover at 300 and 700 °C (S-BC300 and S-BC700, respectively) and pine needles at the same temperatures (P-BC300 and P-BC700, respectively) were used. A maximum reduction of Pb mobility by 95% was observed from a soil treated with S-BC700, associated with precipitation of chloropyromorphite and hydroxylpyromorphite. In contrast, As was desorbed from soil particles because of P competition. The abundance of Gram-positive and negative bacteria, fungi, actinomycetes, and arbuscular mycorrhizal fungi increased in the soils treated with biochar produced at 300 °C, possibly due to the high dissolved organic and active organic carbons. Microbial abundance in the soils treated with S-BC700 and P-BC700 was constant due to the existence of fixed or non-labile carbon. Changes to bacterial communities in the biochar-treated soils depended on feedstock type and pyrolysis temperature. *Actinobacteria* substantially increased whereas *Acidobacteria* and *Chloroflexi* decreased in the biochar-treated soils. The non-labile carbon fraction was ~25 fold higher in the biochar-treated soil than the control soil, indicating long-term carbon storage.

1. Introduction

Biochar is a C-rich solid obtained from pyrolysis or carbonization of biomass under limited oxygen conditions (Ahmad et al., 2013a; Sohi, 2012). Biochar is mainly produced from biomass or wastes such as crop residue, forest waste, animal litter, food processing byproducts, paper mill waste, sewage sludge etc.; therefore, converting these wastes materials to biochar provides a potential recycling solution to the waste disposal problems (Ahmad et al., 2014a).

Biochar has been suggested as a soil conditioner to ameliorate physicochemical and biological soil properties, such as soil aggregation, moisture retention, nutrient availability, and microbial/enzymatic activity (Awad et al., 2013; Verheijen et al., 2010). Biochar is also known as a valuable means of C storage, thus contributing to climate change mitigation by controlling greenhouse gases (Woolf et al., 2010). Recently, the application of biochar has been considered for remediating metal-contaminated soil (Ahmad et al., 2014c).

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The characteristics of biochar depend on the feedstock and pyrolysis temperature (Ahmad et al., 2013b). For instance, biochar contains a higher C content when derived from plant biomass (which contains lignocellulosic compounds) than when derived from animal biomass/waste (Cantrell et al., 2012). A relatively high pyrolysis temperature generally produces biochars having a high level of stable C (Singh et al., 2012). Various characteristics of biochars act as multifunctional substances in surrounding ecosystems.

Despite the detrimental effects of toxic metals on soil microbial communities (Bååth, 1989), the behavior of microbes in metal-contaminated soil is not predictable. Sheik et al. (2012) stated that metal toxicity destroys soil microbes whereas Jones and Lennon (2010) reported that microbes naturally resist metal toxicity by becoming dormant until favorable conditions return. Mutagenesis may also occur in soil microbial population because of toxic metals interaction, thus manifesting changes in their genome and functioning (Fedorova et al., 2007). However, it is well-known that the addition of C-rich amendments as energy sources helps maintain or enhance the soil microbial abundance even in metal contaminated soils (Fierer et al., 2003).

Bacteria are ubiquitous and have high diversity in soil. They perform a key role in soil biogeochemical processes such as nutrient cycling and decomposition of organic materials and pollutants (Acosta-Martinez et al., 2010). A recent study showed that the relative abundance of members of the phylum *Bacteroidetes* increased after biochar treatment of soil while the abundance of *Proteobacteria* decreased (Kolton et al., 2011). However, microbial richness and diversity are nutrients dependent emanating from the added soil amendments. Li et al. (2014) observed significant declines in bacterial diversity indices at the initial stages of soil reclamation, while bacterial diversity indices tended to be higher at later stages of soil reclamation. Therefore, it is worthwhile examining the changes in soil bacterial populations for determining the effects of soil treatments, especially in contaminated soils.

There is a need to identify exact role of biochar for specific purpose such as soil fertility, climate change mitigation, and contaminants remediation. Therefore, we proposed a comprehensive study on determining the impacts of biochars originating from different feedstocks and produced at different pyrolysis temperatures on metals mobility, microbial community and diversity, and C sequestration in a contaminated agricultural soil.

2. Materials and methods

2.1. Biochar production and characterization

Soybean stover biomass (S-BM) and pine needles biomass (P-BM) were collected from an agricultural field in Chungju-si, Korea and a forested area at Kangwon National University, Korea, respectively. Each biomass sample was dried at 60 °C and crushed to <1.0 mm particle size using a mechanical grinder. The powdered samples were pyrolyzed at a heating rate of 7 °C/min under a limited oxygen supply, using a muffle furnace (MF 21GS, Jeio Tech, Korea). Two peak temperatures of 300 and 700 °C were maintained for 3 h. The derived biochars were thoroughly mixed, cooled in a desiccator and stored in air-tight containers. The biomass (S-BM and P-BM) and their derived biochars at 300 and 700 °C (i.e. S-BC300, S-BC700, P-BC300, and P-BC700) were characterized according to the methods described by Ahmad et al. (2013b). Briefly, the moisture content was determined by heating a sample (1.5–2.0 g) at 105 °C in a drying oven for 24 h. The volatile matter and ash contents were measured at 450 °C in a covered crucible for 1 h and at 750 °C in an open-top crucible for 1 h, respectively. The contents of resistant matter were calculated from the differences in moisture, ash, and volatile matter, according to the following equation:

$$\text{Resistant matter}(\%) = 100 - [\%(\text{moisture} + \text{volatile matter} + \text{ash})]$$

The elemental compositions (C, H, N, S, and O) of the biomass and biochars were determined by an elemental analyzer (EA1110, CE Instruments, Italy). The C, H, N, and S elements were determined simultaneously by combustion in a quartz tube at 1020 °C under a continuous helium flow; while for measuring O, a separate oxygen assembly was installed, and the samples were combusted in a pyrolyzer at 1060 °C crossed by helium stream. A thermal conductivity detector (TCD) was used to detect the resulting combustion gases after separating through a gas chromatographic column. Finally, the elemental composition was calculated from the resulting signals proportional to the amount of eluted gases. The molar H/C and O/C ratios were also calculated. The values of pH and electrical conductivity (EC) were measured in 1:5 solid/water (w/v) ratio. The specific surface areas of the biomass and biochars were determined by N_2 adsorption onto previously degassed samples using a gas sorption analyzer (NOVA-1200, Quantachrome Corp., USA). The Brunaur–Emmett–Teller equation was employed to calculate the specific surface area.

2.2. Soil collection and analysis

Soil was collected from an agricultural land adjacent to the abandoned Poong-Jeong mine in Gangwon-do, Korea. Air-dried soil was sieved through a 2.0-mm sieve and was then characterized for selected physicochemical properties. Soil texture was determined by the hydrometer method (Gee and Or, 2002), and pH and EC were measured in 1:5 soil/water (w/v). To measure the exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}), soil was extracted in 1.0 M ammonium acetate and the filtrate was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7300 DV, Perkin Elmer, USA). The soil cation exchangeable capacity (CEC) was calculated from cation and hydrogen ion concentrations following Brown's method (NIAST, 2000). The total metal contents of Pb, Cu, Zn, and As were analyzed using an ICP-OES after acid digestion of soil samples in *aqua regia* in a microwave-assisted digestion unit (MARS, HP-500 plus, CEM Corp., USA), following the method 3051A (da Silva et al., 2014; USEPA, 1998).

Total C contents in the treated and untreated soils were analyzed with an elemental analyzer (EA1110, CE Instruments, Italy) as described in section 2.1, while the active organic C (AOC) (or oxidizable organic C) were measured following the procedure described by Weil et al. (2003). The fraction of AOC was considered as the labile C, and the non-labile C fraction was calculated as the difference between total C and AOC, following the method of Blair et al. (1995).

2.3. Soil incubation

A soil incubation experiment was conducted by mixing 100-g soil with 10% by weight of biomass and biochars in high-density polyethylene bottles, and maintaining the water content at 70% of the water holding capacity. The bottles were kept in an automated incubator (MIR-554, SANYO, Japan) in the dark at 25 °C for 90 d. Each treatment was applied in triplicate. At the end of incubation, a portion of fresh soil was freeze-dried for microbial analysis, while the remaining soil was air-dried for 72 h for various chemical analyses. General soil properties were analyzed as described in section 2.2. The bioavailable or exchangeable forms of Pb and As were measured by extracting soil with 1.0 M ammonium acetate and analyzing with an ICP-OES. The toxicity characteristics leaching