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The effects of different biochars on microbial quantity, microbial community shift, enzyme activity, and biodegradation of polycyclic aromatic hydrocarbons in soil

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ABSTRACT

Plant-residue derived biochars from walnut shell, corn cob, corn straw, and rice straw obtained at three heat treatment temperatures (HTTs) (250 °C, 400 °C, and 600 °C) were applied in an incubation experiment to investigate how feedstocks, HTTs, and biochar properties affect the quantity of microorganisms, microbial community shift, enzyme activity, and biodegradation of polycyclic aromatic hydrocarbons (PAHs) in an aged contaminated soil. The microbial quantities (bacteria and fungi) and enzyme activities (catechol 2,3-dioxygenase and ligninolytic enzymes) generally decreased with the increase in HTTs. Microbial quantities had significantly positive correlations with the aliphatic carbon (C) ($p < 0.01$) but negative correlations with the aromatic C of biochars ($p < 0.01$). Similar findings were observed with enzyme activities, which had significantly positive correlations with microbial quantities ($p < 0.05$). Meanwhile, there were significantly positive correlations between C23O activity and bacterial quantity ($p < 0.01$) and between ligninolytic enzyme activities and the quantity of fungi ($p < 0.05$). These results indicate that the increase in recalcitrant aromatic C in biochars that occurs with the increase in HTT is unfavorable for the microbial growth and enzyme activity in the soil studied. Generally, application of biochars had little influence on the biodegradation rates of total PAHs. However, the effects of biochars on the biodegradation rates of major individual PAH (e.g., Nap, Phe, Pyr, and Chr) depended on both the types of biochars and the PAH properties such as benzene ring number and angular pattern of the ring linkage.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), a group of organic compounds with two or more fused aromatic rings, are mainly produced from incomplete combustion of energy fuels such as coal, plant biomass, gasoline, and diesel. When PAHs are widespread persistent organic contaminants in soils, they can pose risks to humans and the ecosystem because of their mutagenic, carcinogenic and teratogenic properties that have made their remediation a critical need (Kuśmierz et al., 2016; Oleszczuk and Koltowski, 2017). Among the techniques available, in situ bioremediation has been recognized as a cost-effective and environmentally friendly approach to remove PAHs from soil (Yu et al., 2011; Lladó et al., 2013; Rein et al., 2016). However, the biodegradation of PAHs solely utilizing native microorganisms under natural conditions is a very slow process. The potential solution to elevate the efficiency for the biodegradation of PAHs in soil is to implement bioaugmentation by introducing single strains or a consortium of microorganisms and/or biostimulation by providing a favorable environment or nutrients needed for the indigenous organisms (Sayara et al., 2011; Zhang et al., 2016a, 2016b; Rein et al., 2016). Some studies reported that bioaugmentation achieved a higher efficiency of PAH biodegradation than biostimulation, especially for high-molecular-weight PAHs (García-Delgado et al., 2015; Sun et al., 2012). Conversely, other studies revealed that biostimulation could effectively improve the PAH biodegradation, but bioaugmentation did not significantly enhance the biodegradation of PAHs (Sayara et al., 2011; Lladó et al., 2013; Rein et al., 2016).

Numerous studies on biostimulation have demonstrated that biochar can improve the soil's chemical properties, such as increasing the soil pH, organic carbon, nutrients, and cation exchange capacity that

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are favorable for promoting microbial abundance or a shift in the microbial community composition in the soil (Lehmann et al., 2011; Xu et al., 2014; Steinbeiss et al., 2009; Lu et al., 2014). However, the feedstocks, heat treatment conditions, and technical processes of biochar production had a significant impact on the physicochemical and biological characteristics of the resultant biochars that may influence the microbial growth and composition and thus affect the biodegradation of PAHs in soil (Wang et al., 2015; Lehmann et al., 2011). For example, a previous study showed that biostimulation treatment of petroleum-contaminated soil by adding biochar increased the abundance of several bacterial classes, contributing to the effective biodegradation of aromatic fractions (Qin et al., 2013). In contrast, another recent study found that the addition of biochar to creosote-contaminated soil did not increase the effectiveness of PAH remediation due to the low bacterial and fungal development caused by the high C/N ratio and aromatic carbon content in the biochar (García-Delgado et al., 2015). With the increase in heat treatment temperatures (HTTs), the fused aromatic structure in the biochar changes from amorphous C to turbostratic C, and this may influence the microbial abundance and further affect the biodegradation of PAHs in contaminated soil (Keiluweit et al., 2010; Sayara et al., 2011). However, little is known about how HTTs and feedstocks affect the microbial abundance and community composition and their relation to the biodegradation of PAHs in aged contaminated soil.

The initial reactions of PAHs biodegradation by microorganisms are primarily ascribed to enzyme activities such as oxygenases, dehydrogenases and lignolytic enzymes (Yu et al., 2011; Wang et al., 2015). Some studies that examined the effect of biochar on enzyme activities suggested that the introduction of biochar into the soil contributed to a decrease in the production of some soil enzymes such as dehydrogenase and urease (Mierzwa-Hersztek et al., 2016). However, other studies indicated that the effect of biochar on enzyme activity was related to both the HTTs and the type of enzyme (Wang et al., 2015). Dioxygenase is the bacterial enzyme that is responsible for the biodegradation of PAHs by bacteria (Hadibarata and Kristanti, 2012). The fungal ligninolytic enzymes including laccase and peroxidases (manganese-dependent peroxidase and lignin peroxidase) have been demonstrated to have a positive effect on the biodegradation of PAHs in soil and other media by fungi, since these extracellular enzymes are able to diffuse effectively to the highly immobile high-molecular-weight PAHs (Yu et al., 2011; Hadibarata and Kristanti, 2012; Lee et al., 2015; Winquist et al., 2014). However, the effect of biochar types on these enzyme activities and their relation to PAH biodegradation in the soil is still unclear.

To address these gaps in knowledge, this study primarily aimed to investigate (1) the effect of biochar feedstock and HTTs on the microbial quantity, microbial community shift, and enzyme activity in an aged PAH-contaminated soil collected around an indigenous coking site; and (2) the effect of microbial community shift and enzyme activity on PAH biodegradation in biochar-treated soil.

2. Materials and methods

2.1. Soil collection and physico-chemical analysis

The surface soil (0–20 cm depth) for this study was collected from a former indigenous coking area that is unfavorable for the growth of some plants (in a hilly region in Lin Xian County, Lvliang City, Shanxi Province, China; 37°43′33″N, 110°56′27″E). Soil samples were air dried, homogenized, and sieved (< 2 mm). Particle size analysis was performed utilizing a particle size analyzer (clay: 0%, silt: 5.0%, sand: 95%, belonging to loamy sandy soil). The soil pH and electrical conductivity (EC) were determined in a suspension of soil in water (soil:water = 1:2.5, w/v) with a pH/EC meter (pH = 8.2, EC = 0.12 $ds \cdot m^{-1}$). Total organic carbon (TOC) was determined by a total organic carbon analyzer (Analysis Jena N/C 2100) after the soil was treated with 2 $mol \cdot L^{-1}$ HCl and dried in a drum wind-drying oven at 105 °C for 2 h. The TOC content was 24 $g \cdot kg^{-1}$. The soil total nitrogen (N, 0.39 $g \cdot kg^{-1}$) was determined by the Semi-micro Kjeldahl method according to the Chinese agricultural industry standard (NY/T 53-1987). The available N (22 $mg \cdot kg^{-1}$) was determined according to the Chinese forest industry standard (LY/T 1229-1999). The PAHs in the soil were determined by the gas chromatography–mass spectrometry (GC/MS) system as described in Section 2.6. The level of total PAHs (ΣPAHs) was 1193 $\mu g \cdot kg^{-1}$ in dry soil in which naphthalene (Nap, 498 $\mu g \cdot kg^{-1}$), phenanthrene (Phe, 436 $\mu g \cdot kg^{-1}$), pyrene (Pyr, 73 $\mu g \cdot kg^{-1}$), and chrysene (Chr, 80 $\mu g \cdot kg^{-1}$) were the most abundant individual PAHs.

2.2. Biochar preparation and characterization

Biochars were produced from walnut shells (WS), corn cobs (CC), corn stems (CS), and rice straw (RS). Each feedstock was washed and dried in an oven at 80 °C. To produce biochar, the materials were placed in a muffle and pyrolyzed under oxygen-limited conditions at different HTTs (250 °C, 400 °C, and 600 °C) for 4 h, with the heating rate of 15 $°C \cdot min^{-1}$ to the target temperature. The biochar was cooled to room temperature inside the furnace. The biochars were milled to pass through a 0.15-mm sieve before further use. The prepared biochars were referred to as WS2, WS4, WS6, CC2, CC4, CC6, RS2, RS4, RS6, CS2, CS4, and CS6, respectively.

The pH and EC of the biochar were measured in a suspension of soil in water (biochar:water = 1:10, w/v) with a pH/EC meter. The pretreatment of biochars for this characterization can be found in our previous study (Zhang et al., 2011). The element composition (C, H, and N) was determined with an elemental analyzer (Flash EA 1112). The ash content was measured by heating the biochars in a muffle at 750 °C for 4 h. The oxygen content was calculated from the mass difference. The N_2-BET surface areas (SA) were determined by utilizing an ASAP-2020 surface area analyzer (Micromeritics Instrument Corporation, US). The surface chemistry of the biochar was provided by a Nicolet iS10 FT-IR spectrometer (Thermo Nicolet Corporation, US) that recorded the spectra from 4000 cm^{-1} to 400 cm^{-1} with a resolution of 4 cm^{-1}. The solid-state cross-polarization magic angle spinning ^{13}C NMR spectra of the biochars were obtained utilizing a Bruker DRX-400 NMR spectrometer operated at a ^{13}C frequency of 100.36 MHz and a magic angle-spinning rate of 8.0 kHz.

2.3. Soil incubation experiment

According to the preliminary experiment via incubation of untreated soil for 30 d at 28 °C in the dark, the concentrations of all the target individual PAHs (Nap, 260 $\mu g \cdot kg^{-1}$; Phe, 250 $\mu g \cdot kg^{-1}$; Pyr, 50 $\mu g \cdot kg^{-1}$; and Chr, 70 $\mu g \cdot kg^{-1}$) decreased to below the standard limit based on Environmental Quality Standards for Soils (GB15618-2008). Consequently, to investigate the effect of biochar on the soil microbial community shift, enzyme activity, and PAH biodegradation, an incubation experiment was conducted for 30 d at 28 °C without light in an artificial climate box (BSG-250/300/400, Shanghai Boxun Industry & Commerce Co. Ltd., Shanghai, China). A total of 1500 g of air-dried soil collected from an indigenous coking contaminated site was weighed into a plastic container (top diameter of 18 cm, bottom diameter of 15 cm, and height of 13 cm). Biochars were added separately at 0% and 2.5% (w/w, equivalent to 39 $t \cdot ha^{-1}$ based on soil density of 2.6 $g \cdot cm^{-3}$ and depth of 6 cm) by weight to the soil and mixed thoroughly. The control sample was referred to as S, whereas the samples treated with corresponding biochars were referred to as S + WS2, S + WS4, S + WS6, S + CC2, S + CC4, S + CC6, S + RS2, S + RS4, S + RS6, S + CS2, S + CS4, and S + CS6. Each treatment was incubated in duplicate using two containers. All treatments were not fertilized during the entire incubation period. The humidity of the soil mixtures was maintained at 60% of the water holding capacity by weighing the pots