Assessment of organic compost and biochar in promoting phytoremediation of crude-oil contaminated soil using Calendula officinalis in the Loess Plateau, China (Postprint)

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Abstract

The Loess Plateau, located in Gansu Province, is an important energy base in China because most of the oil and gas resources are distributed in Gansu Province. In the last 40 a, ecological environment in this region has been extremely destroyed due to the over-exploitation of crude-oil resources. Remediation of crude-oil contaminated soil in this area remains to be a challenging task. In this study, in order to elucidate the effects of organic compost and biochar on phytoremediation of crude-oil contaminated soil (20 g/kg) by Calendula officinalis, we designed five treatments, i.e., natural attenuation (CK), planted C. officinalis only (P), planted C. officinalis with biochar amendment (PB), planted C. officinalis with organic compost amendment (PC), and planted C. officinalis with co-amendment of biochar and organic compost (PBC). After 152 d of cultivation, total petroleum hydrocarbons (TPH) removal rates of CK, P, PB, PC and PBC were 6.36%, 50.08%, 39.58%, 73.10% and 59.87%, respectively. Shoot and root dry weights of C. officinalis significantly increased by 172.31% and 80.96% under PC and 311.61% and 145.43% under PBC, respectively as compared with P ($P<0.05$). Total chlorophyll contents in leaves of C. officinalis under P, PC and PBC significantly increased by 77.36%, 125.50% and 79.80%, respectively ($P<0.05$) as compared with PB. Physical-chemical characteristics and enzymatic activity of soil in different treatments were also assessed. The highest total N, total P, available N, available P and SOM (soil organic matter) occurred in PC, followed by PBC ($P<0.05$). C. officinalis rhizospheric soil dehydrogenase (DHA) and polyphenol oxidase (PPO) activities in PB were lower than those of other treatments ($P<0.05$). The values of ACE (abundance-based coverage estimators) and Chao 1 indices for rhizospheric bacteria were the highest under PC followed by PBC, P, PB and CK ($P<0.05$). However, the Shannon index for bacteria was the highest under PC and PBC, followed by P, PB and CK (P<0.05). In terms of soil microbial community composition, Proteiniphilum, Immundisolibacteraceae and Solimonadaceae were relatively more abundant under PC and PBC. Relative abundances of Pseudallescheria, Ochroconis, Fusarium, Sarocladium, Podospora, Apodus, Pyrenochaetopsis and Schizothecium under PC and PBC were higher, while relative abundances of Gliomastix, Aspergillus and Alternaria were lower under PC and PBC. As per the nonmetric multidimensional scaling (NMDS) analysis, application of organic compost significantly promoted soil N and P contents, shoot length, root vitality, chlorophyll ratio, total chlorophyll, abundance and diversity of rhizospheric soil microbial community in C. officinalis. A high pH value and lower soil N and P contents induced by biochar, altered C. officinalis rhizospheric soil microbial community composition, which might have restrained its phytoremediation efficiency. The results suggest that organic compost-assisted C. officinalis phytoremediation for crude-oil contaminated soil was highly effective in the Loess Plateau, China.

Full Text

Assessment of Organic Compost and Biochar in Promoting Phytoremediation of Crude-Oil Contaminated Soil Using Calendula officinalis in the Loess Plateau, China

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Abstract

The Loess Plateau, located in Gansu Province, is an important energy base in China because most of the oil and gas resources are distributed in this region. Over the last 40 years, the ecological environment in this region has been severely degraded due to the over-exploitation of crude-oil resources. Remediation of crude-oil contaminated soil in this area remains a challenging task. In this study, to elucidate the effects of organic compost and biochar on phytoremediation of crude-oil contaminated soil (20 g/kg) by Calendula officinalis, we designed five treatments: natural attenuation (CK), planted C. officinalis only (P), planted C. officinalis with biochar amendment (PB), planted C. officinalis with organic compost amendment (PC), and planted C. officinalis with co-amendment of biochar and organic compost (PBC). After 152 days of cultivation, total petroleum hydrocarbons (TPH) removal rates for CK, P, PB, PC, and PBC were 6.36%, 50.08%, 39.58%, 73.10%, and 59.87%, respectively. Shoot and root dry weights of C. officinalis significantly increased by 172.31% and 80.96% under PC and by 311.61% and 145.43% under PBC, respectively, compared with P ($P<0.05$). Total chlorophyll contents in leaves of C. officinalis under P, PC, and PBC significantly increased by 77.36%, 125.50%, and 79.80%, respectively ($P<0.05$) compared with PB. Physical-chemical characteristics and enzymatic activity of soil in different treatments were also assessed. The highest total N, total P, available N, available P, and SOM (soil organic matter) occurred in PC, followed by PBC ($P<0.05$). C. officinalis rhizospheric soil dehydrogenase (DHA) and polyphenol oxidase (PPO) activities in PB were lower than those of other treatments ($P<0.05$). The values of ACE (abundance-based coverage estimators) and Chao 1 indices for rhizospheric bacteria were highest under PC, followed by PBC, P, PB, and CK ($P<0.05$). However, the Shannon index for bacteria was highest under PC and PBC, followed by P, PB, and CK ($P<0.05$). In terms of soil microbial community composition, Proteiniphilum, Immundisolibacteraceae, and Solimonadaceae were relatively more abundant under PC and PBC. Relative abundances of Pseudallescheria, Ochroconis, Fusarium, Sarocladium, Podospora, Apodus, Pyrenochaetopsis, and Schizothecium under PC and PBC were higher, while relative abundances of Gliomastix, Aspergillus, and Alternaria were lower under PC and PBC. According to nonmetric multidimensional scaling (NMDS) analysis, application of organic compost significantly promoted soil N and P contents, shoot length, root vitality, chlorophyll ratio, total chlorophyll, and abundance and diversity of rhizospheric soil microbial community in C. officinalis. A high pH value and lower soil N and P contents induced by biochar altered C. officinalis rhizospheric soil microbial community composition, which might have restrained its phytoremediation efficiency. The results suggest that organic compost-assisted C. officinalis phytoremediation for crude-oil contaminated soil was highly effective in the Loess Plateau, China.

Keywords: total petroleum hydrocarbons; soil physical-chemical characteristics; plant physiological parameters; soil enzyme; microbial community composition

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1 Introduction

Organic pollutants, especially crude-oil contaminants, severely affect soil structure, groundwater quality, natural ecosystems, and human health due to their high toxicity and environmental persistence (Bordoloi et al., 2012; Barati et al., 2017; Igun et al., 2019; Fadliah et al., 2020). In addition, the accelerated development of the global economy has rapidly increased crude-oil exploitation (Zhang et al., 2019). Consequently, environmental pollution of soil due to crude-oil contaminants released from underground storage tank leakage, accidental oil spills, and losses during extraction and transportation has aroused global attention (Bastida et al., 2016; Shen et al., 2018).

Bioremediation is broadly acknowledged as an effective approach to address issues concerning crude-oil contaminated soil (Hussain et al., 2018; Guo et al., 2020; Li et al., 2020). Bioremediation is also considered cheaper and more eco-friendly than physical-chemical remediation methods. Among the distinct bioremediation approaches, phytoremediation has emerged as the most eco-friendly, economically sustainable, and cost-effective method for biodegradation of soil pollutants (Tang et al., 2010; Igun et al., 2019). Phytoremediation can provide long-term rehabilitation of residual oil pollutants (Bordoloi et al., 2012; Sara et al., 2015). For example, plant species such as Festuca arundinacea, Medicago sativa, Axonopus compressus, Hordeum vulgare, Avena sativa, Bassia scoparia, Spartina anglica, and Lolium perenne have been successfully planted in crude-oil contaminated soil across the globe (Bordoloi et al., 2012; Cruz-Hernández et al., 2013; Moubasher et al., 2015; Panchenko et al., 2017; Zhang et al., 2019; Zhen et al., 2019). Phytoremediation involves the synergistic action of plants and associated rhizospheric microbial communities in removing soil pollutants (Khan et al., 2013; Muhammad et al., 2013; Wang et al., 2015; Hafida et al., 2019; Xu et al., 2019). Plants used in phytoremediation of crude-oil contaminated soil can substantially increase rhizospheric bacterial and fungal diversity (Zhang et al., 2010; Khan et al., 2013; Xu et al., 2019; Xu et al., 2020). Besides, these plants can increase the relative abundances of petroleum hydrocarbon-degrading microbes, including Acidobacteria, Sphingomonas, Gemmatimonas, Pseudomonas, Ohtaekwangia, Aquihabitans, Solimonas, Arenimonas, Mycobacterium, Hydrogenophaga, Pirellula, Opitutus, Thiobacillus, Proteiniphilum, Immundisolibacteraceae, Fusarium, Sarocladium, Podospora, Apodus, and Pyrenochaetopsis (Zhang et al., 2010; Chen et al., 2015; Kong et al., 2017; Igun et al., 2019; Zhang et al., 2019; Xu et al., 2020).

Organic compost improves plant growth, soil quality, and soil microbial community composition; furthermore, it enhances the activity of hydrocarbon-degrading bacteria (Taiwo et al., 2016; Hussain et al., 2018; Tan et al., 2018; Zhen et al., 2019). Organic compost has been commonly used for phytoremediation of petroleum hydrocarbon contaminated soil in the Loess Plateau, China. Numerous studies have shown that biochar not only increases soil fertility, nutrient retention, water holding capacity, and oxygen supply but also remediates soil contamination through surface adsorption, precipitation, partitioning, and sequestration (Kong et al., 2017; Shen et al., 2018). Besides, biochar remarkably mitigates wind erosion effects since it increases the coherence of surface particles, which decreases the detachment of soil particles (Feizi et al., 2019). However, most current studies on biochar application have centered on remediation of heavy metal-contaminated soil (Yang et al., 2021), and these studies mainly assessed the effects of biochar on pollutant adsorption, plant biomass, soil nutrient content, enzyme activity, and soil microbial community composition (Chintala et al., 2014; Cao et al., 2016; Han et al., 2016; Hussain et al., 2018; Guo et al., 2020; Li et al., 2020; Mehdizadeh et al., 2021). However, only a few studies have investigated biochar-assisted phytoremediation of crude-oil contaminated soil (Mohammad et al., 2021).

The soil in the Loess Plateau, China has high $CaCO_3$ content, an arid and saline texture, and layer stratification, making it highly susceptible to wind erosion (Ajami et al., 2016; Guo et al., 2018; Lyu et al., 2020). Crude-oil contaminated soil in the Loess Plateau is commonly deficient in nutrients and organic matter, substantially restricting plant growth and hampering phytoremediation efficiency (Hussain et al., 2018). Although the positive effects of biochar have been demonstrated in heavy metal remediation, biochar-assisted phytoremediation of crude-oil contaminated soil in the Loess Plateau remains unexplored. Thus, to further our understanding of bioremediation mechanisms for crude-oil contaminants, this study aimed to assess the phytoremediation efficiency of two organic amendments—organic compost and biochar—using Calendula officinalis.

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2 Materials and Methods

2.1 Soil Sampling and Preparation of Compost and Biochar

This study was conducted at the No. 2 oil production plant of Changqing Oilfield (35°54 33 N, 107°31 19 E), located in Qingyang City, eastern Gansu Province, China. The crude-oil contaminated soil collected from different production areas of the No. 2 oil production plant was a mixture of ground and tank crude oil with TPH (total petroleum hydrocarbons) concentrations of 10.04% ($±1.97±2.41±$1.65%), and it was uniformly marked as 20.00% throughout the study. We determined the average organic composition of crude oil as 58.67% saturated hydrocarbons, 19.86% aromatic hydrocarbons, and 11.94% resins and asphaltenes using the method described by Wang et al. (2015). The soil physical-chemical properties in this study are shown in Table 1 .

Organic compost used in the study was a commercial product from Shuanggang Agricultural Science and Technology Trade Co. Ltd., Jingzhou City, China. The compost contained cow dung, cabbage leaf, and sawdust in varying proportions and was fermented for 6 weeks. The pH, electrical conductivity (EC), and contents of organic matter (OM), total nitrogen (TN), and total phosphorus (TP) of organic compost samples were determined as described by Taiwo et al. (2016). Biochar used in the study was a commercial product from Xi' an Huanfa Biological Technology Co. Ltd., Xi' an City, China. Biochar was derived from maize straw, which was subjected to 500°C for 3 h in a furnace under hypoxic conditions. Carbon (C), hydrogen (H), N, and sulfur (S) element contents were determined using an elemental analyzer (Euro EA3000, Euro Vector, Milano, Italy) (Han et al., 2016). Ash content was measured at 600°C for 6 h. Biochar was dissolved in deionized water (1% w/v) and shaken for 24 h at 200 r/min before measuring pH (Han et al., 2016). BET surface area (BET SA) and total pore volume (TPV) of biochar were determined using an ASAP 2020/Tristar 3000 (ASAP 2020, Micromeritics Inc., USA) automatism isothermal adsorption instrument equipped with a surface area analyzer (Micromeritics, Norcross, USA) (Kong et al., 2017). Ground biochar (<2 mm) was added to the tested soils. The physical-chemical properties of organic compost and biochar are shown in Table 1.

2.2 Plant Preparation

C. officinalis seeds were purchased from the Gansu Academy of Agricultural Sciences, Lanzhou City, China. C. officinalis is a wild and ubiquitous plant widely distributed across northwestern China that promotes petroleum degradation. In our previous study, the TPH removal rate of C. officinalis was found to be 59.98% ($±1.37±2.38°C)andameanrelativehumidityof61.90±$1.30%). The daily photoperiod was characterized by 14 h of daylight. After 30 days of cultivation, 5 plants with relatively uniform growth (plant height approximately 10 cm) were transplanted into pots containing the tested soil. We watered the pots based on visual inspection (approximately 100 mL every alternate day), and plants that died within 10 days were replaced with healthy plants (Bordoloi et al., 2012).

2.3 Experimental Design

The crude-oil contaminated soil was transported to the laboratory. Stones and other large particulates were removed from the soil before further use. Each plastic pot with a diameter of 250 mm and a height of 200 mm was filled with 2.5 kg of crude-oil contaminated soil. The crude-oil contaminated soil was amended either with biochar (5%) or with compost (5%) as described previously by Khan et al. (2013) and Zhen et al. (2019). The amendments in this study are shown in Table 2 . A total of 15 pots were cultured in the greenhouse under simulated natural illumination conditions. Starting from the date of transplant, the total experiment duration was 152 days. Each amendment was tested in triplicate.

2.4 Analytical Methods

2.4.1 Removal Rates of TPH, Alkanes (ALK), and Aromatic Compounds We measured TPH, ALK, and aromatic compounds according to previously described procedures (Li et al., 2018). The removal rates (%) of TPH, ALK, and aromatic compounds were determined using the following equation:

Removal rate = $(C_0 - C_{152}) / C_0 \times 100$

where C_0 is the amount of TPH, ALK, or aromatic compounds on day 0 (mg/kg), and C_{152} is the amount after 152 days of incubation (mg/kg).

2.4.2 Shoot Length, Root Vitality, Chlorophyll Content, and Plant Biomass Shoot length of plants was determined using a tape measure after completion of remediation. Plants were air-dried at room temperature in the interior until a constant weight was attained, and plant weight was measured (Shen et al., 2018). Fresh plant roots were rinsed until all soil was removed, and 0.5 g of root was used to measure root vitality using the triphenyltetrazolium chloride method (Xiao et al., 2018). We quantified chlorophyll a and b contents according to Zhen et al. (2019).

2.4.3 Physical-Chemical Properties and Enzymatic Activity of Plant Rhizospheric Soil Soil pH was measured in a 1.0:2.5 soil-deionized water slurry using a digital pH meter (PT-10, Sartorius, Gottingen, Germany). TN was determined using an elemental analyzer (Elementar Vario-EL, Langenfeld, Germany) (Yang et al., 2019). The alkaline hydrolysis diffusion method was employed to determine soil available nitrogen (AN) (Lu et al., 1999). Soil TP and available phosphorus (AP) were assayed using HF-$HClO_4$ digestion and sodium bicarbonate extraction methods (molybdenum blue), respectively (Taiwo et al., 2016). The Walkley-Black wet oxidation method, which measures dichromate ions consumed in organic matter oxidation in the soil, was used to quantitate organic carbon. Organic matter (%) was calculated by multiplying the value of organic carbon by 1.729 as described previously by Taiwo et al. (2016). Dehydrogenase (DHA) and polyphenol oxidase (PPO) activities were measured using the methods reported by Cao et al. (2016). We measured urease (URE) activity based on the method reported by Hu et al. (2014), and alkaline phosphatase activity (APA) was estimated using the method described by Jin et al. (2016).

2.4.4 Soil Bacterial and Fungal Community Analysis We extracted total DNA from soil samples using a PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA) according to the manufacturer's instructions. Partial 16S rRNA genes and ITS amplicons were generated for each DNA sample using barcoded primers. Primers 338F (5 -ACTCCTACGGGAGGCAGCA-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3) (Yang et al., 2019) were used to amplify the V3-V4 hypervariable regions of the bacterial 16S rRNA gene. Primers ITS1F (5 -CTTGGTCATTTAGAGGAAGTAA-3) and ITS2R (5 -GCTGCGTTCTTCATCGATGC-3) (Xu et al., 2019) were used to amplify fungal genes. The PCR program was set as follows: 95°C for 2 min followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and 72°C for 10 min. PCR products were purified using a Qiagen PCR Purification Kit and pooled at equimolar concentrations. High-throughput sequencing was performed by Biomarker Technologies, Beijing, China using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). Furthermore, sequencing and library construction were performed by Beijing Biomarker Technologies Co. Ltd., Beijing, China. Bioinformatics analysis was performed on the biomarker biocloud platform (www.biocloud.org). To obtain high-quality sequences, we filtered all raw reads with QIIME (Zhang et al., 2018). The datasets thus obtained were clustered into operational taxonomic units (OTUs) at 97% sequence similarity. The data volume of reads, richness (ACE and Chao 1) and diversity (Simpson and Shannon) indices for each sample were calculated using the biomarker biocloud platform (www.biocloud.org) (Zhen et al., 2019).

2.5 Data Analysis

Statistical analysis was performed using SPSS version 19.0 (SPSS, Inc., Chicago, USA). One-way analysis of variance with Duncan multiple range tests was performed to test significant differences ($P<0.05$) in analyzed parameters among different amendments. To elucidate soil microbial community composition differences among amendments, we ordinated bacterial and fungal communities using non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity measurement (Shi et al., 2014). To unravel the correlation between microbial community and environmental factors, we fitted soil variables in this study as vectors (a total of 17 vectors including 6 vectors of soil physical-chemical variables, 4 vectors of soil enzyme variables, 3 vectors of pollutant removal rates, and 4 vectors of soil microbial community α-diversity) onto NMDS plots using the envfit function from the vegan library of the R package (Shi et al., 2017). The relationship between microbial community and environmental factors was tested using a Mantel test with measurement of Bray-Curtis distance. Vectors significantly correlated to soil microbial community composition ($P<0.05$) were fitted onto NMDS plots. NMDS axes were rotated to ensure that the first axis of each ordination could largely explain variations in community dissimilarity (Shi et al., 2017).

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3 Results

3.1 Removal Rates of TPH, ALK, and Aromatic Compounds

The removal rates of TPH, ALK, and aromatic compounds after 152 days of incubation are shown in Figure 1. PC showed the highest removal rate of TPH (73.10%), followed by PBC (59.87%), while removal rates under P and PB were 50.08% and 39.58%, respectively (Fig. 1a [Figure 1: see original paper]). The lowest removal rate under PB indicated poor effects of biochar amendment on C. officinalis phytoremediation of crude-oil contaminated soil. P (36.67%), PC (39.87%), and PBC (36.89%) treatments showed the highest removal rates of ALK, with no significant differences among them ($P>0.05$; Fig. 1b). Removal rates of ALK under PB and CK treatments were 22.77% and 3.71%, respectively. Furthermore, the highest removal rate of aromatic compounds occurred under PC (20.06%), followed by P (9.42%), PB (8.07%), and PBC (12.51%), with insignificant differences among them (P>0.05; Fig. 1c). These results indicated that organic compost was more effective than biochar for phytoremediation of crude-oil contaminated soil planted with C. officinalis.

3.2 Physiological Parameters of Plant Growth

Physiological parameters of C. officinalis growth with amendments are shown in Figure 2 [Figure 2: see original paper]. Shoot length under PC and PBC significantly increased by 62.08% and 31.91%, respectively, while that under PB significantly decreased by 26.55% (P<0.05) compared with P (Fig. 2a). Shoot length was 54.68% and 44.32% shorter under PB than under PC and PBC, respectively, indicating that PC and PBC amendments significantly promoted shoot length, whereas PB significantly decreased it (P<0.05). Root vitalities under PC and PBC were significantly increased by 117.11% and 112.14%, respectively, but did not change significantly under PB (Fig. 2b) compared with P.

Plant leaf total chlorophyll contents under P, PC, and PBC significantly increased by 77.36%, 125.50%, and 79.80%, respectively (P<0.05) compared with PB (Fig. 2c). P and PC showed the highest plant leaf chlorophyll ratio, followed by PBC, while total chlorophyll content was lowest under PB (P<0.05), indicating that biochar inhibited total chlorophyll content and its ratio in C. officinalis for phytoremediation of crude-oil contaminated soil. C. officinalis plant biomass assessment showed that shoot and root dry weights followed similar trends across amendments. Compared with P, shoot dry weights under PC and PBC significantly increased by 172.31% and 311.61%, respectively (P<0.05), whereas root dry weights under PC and PBC significantly increased by 80.96% and 145.43%, respectively (P<0.05). However, no significant differences were observed between P and PB (P>0.05) (Fig. 2e and 2f). The application of organic compost significantly increased plant biomass, whereas biochar application significantly decreased it. These results indicated that, compared with biochar, organic compost promoted shoot length, root vitality, plant leaf chlorophyll ratio, total chlorophyll content, and plant biomass of C. officinalis for phytoremediation of crude-oil contaminated soil.

3.3 Soil Physical-Chemical Characteristics and Enzyme Activity

PC and PBC showed the highest TN and AN, followed by P and PB, whereas CK showed the lowest TN and AN (P<0.05; Table 3), indicating that addition of organic compost amendments significantly improved soil TN and AN contents compared with other amendments. TP was highest under PC, followed by PBC and P, whereas it was lowest under CK and PB (P<0.05). Soil TP content under CK was lowest among all amendments. However, addition of biochar to crude-oil-contaminated soil did not increase soil TP content (P>0.05). Moreover, soil TP under P, PC, and PBC significantly increased by 66.67%, 145.45%, and 60.61%, respectively (P<0.05) compared with PB. Soil AP content was highest under PC, followed by PBC, P, PB, and CK. Soil AP content under PB significantly decreased by 70.33% compared with PC. These results demonstrated that organic compost amendment significantly improved soil TP and AP contents, while biochar showed no significant effects on soil TP and AP contents.

Regarding soil organic matter (SOM), PB showed the lowest SOM content, which did not vary significantly compared with CK ($P>0.05$). In contrast, the highest SOM content was observed under PC, followed by PBC and P ($P<0.05$), showing that organic compost significantly increased SOM in crude-oil contaminated soil compared with biochar. Furthermore, soil pH across amendments followed the order PB>CK>PBC>P>PC ($P<0.05$), indicating that organic compost amendment to crude-oil contaminated rhizospheric soil can significantly lower soil pH compared with biochar amendment. These results suggested that contents of soil TN, AN, TP, AP, and SOM under PB were significantly lower than those under P, whereas they were relatively higher under PC, indicating that biochar amendment did not positively affect soil nutrients compared with organic compost.

Significant differences in soil enzymatic activities among amendments are shown in Figure 3. Compared with CK, soil DHA activity significantly increased by 76.83%, 55.91%, 261.01%, and 139.81% under P, PB, PC, and PBC, respectively ($P<0.05$; Fig. 3a). Additionally, soil PPO activity significantly increased by 87.38%, 42.11%, 174.34%, and 110.48% under P, PB, PC, and PBC, respectively ($P<0.05$; Fig. 3b) compared with CK. These results demonstrated that biochar amendment alone did not improve C. officinalis rhizospheric soil DHA and PPO activities. The highest soil URE and APA activities were observed under PC ($P<0.05$; Fig. 3c and 3d). Compared with CK, URE activity significantly increased by 128.13%, 115.63%, 212.50%, and 175.00% under P, PB, PC, and PBC, respectively ($P<0.05$; Fig. 3c). Similarly, soil APA activity significantly increased by 58.51%, 59.57%, 172.35%, and 139.36% under P, PB, PC, and PBC, respectively, compared with CK ($P<0.05$; Fig. 3d).

3.4 Richness and Diversity Indices of Soil Microbial Community

ACE and Chao 1 indices of bacterial diversity followed the order PC>PBC>P>PB>CK ($P<0.05$). The highest Shannon value was observed under PC and PBC, followed by P and PB, with the lowest Shannon value under CK ($P<0.05$; Table 4). These bacterial diversity results indicated that crude-oil contaminated soil with organic compost amendments (PC and PBC) had relatively higher bacterial diversity than biochar amendment (PB). Shannon values were lower under PB and P than under PC and PBC but higher than CK (Table 4), indicating that biochar amendment had no significant effects on soil bacterial diversity. Abundances of soil fungal community were not significantly different among P, PB, and PBC, but they were significantly higher than CK ($P<0.05$). These results suggested that amendments, except for organic compost amendment, had no significant effects on abundances of soil fungal community. Organic compost amendment remarkably increased the diversity of soil fungal community in crude-oil contaminated soil.

3.5 Soil Microbial Community Composition

To determine microbial community response to crude-oil contamination stress under different phytoremediation amendments, we examined rhizospheric soil bacterial and fungal community compositions (genus level) under CK, P, PB, PC, and PBC (Fig. 4 [Figure 4: see original paper]). Based on Bray-Curtis dissimilarity measurement, we divided bacterial community composition in crude-oil contaminated soil with different amendments into four groups: group 1 (PB and PBC), group 2 (P), group 3 (PB), and group 4 (CK). Alcanivorax, Nocardioides, and Galbitalea in group 4 were dominant genera with relative abundances of 56.90%, 4.92%, and 1.34%, respectively (Fig. 4a), indicating these genera are dominant indigenous bacteria in crude-oil contaminated soil.

Compared with CK, Marinobacter exhibited a higher abundance of 31.52% in group 3, while Proteiniphilum, Nocardioides, Immundisolibacteraceae, Solimonadaceae, Pseudoxanthomonas, Saccharimonadales, Promicromonospora, Sphingomonas, Galbitalea, and Marinobacterium exhibited lower relative abundances of 1.14%, 0.51%, 0.58%, 0.77%, 0.29%, and 1.58%, respectively ($P<0.05$). Proteiniphilum, Nocardioides, Immundisolibacteraceae, and Solimonadaceae in group 2 were not identified, while relative abundances of Pseudomonas, Nocardioides, Sphingomonas, Arthrobacter, and Mycobacterium exhibited higher abundances of 32.90%, 7.54%, 4.21%, 7.24%, and 3.97%, respectively, compared with PB ($P<0.05$). In group 1, bacterial and fungal community composition was identical to that in other groups, but their abundances significantly differed from other groups (Fig. 4a).

The fungal community composition under different amendments aligned with soil bacterial community composition (Fig. 4b). Soil fungal community composition of C. officinalis in group 2 was significantly different from other amendments. Higher relative abundances of Gliomastix (35.81%), Fusarium (16.20%), and Sarocladium (4.22%) and significantly lower relative abundances of Pseudallescheria, Cladosporium, Alternaria, Staphylotrichum, Mortierella, Podospora, Apodus, Pyrenochaetopsis, and Schizothecium were found in group 2 compared with other amendments ($P<0.05$). Higher relative abundances of Pseudallescheria (21.51%), Gliomastix (1.58%), Aspergillus (2.69%), and Alternaria (5.41%) were observed, whereas Cladosporium, Alternaria, Staphylotrichum, Niesslia, Mortierella, Sarocladium, Podospora, Apodus, Pyrenochaetopsis, and Schizothecium exhibited lower abundance in group 3 ($P<0.05$). Soil fungal community composition in group 1 was similar. Relative abundances of Pseudallescheria, Ochroconis, Fusarium, Sarocladium, Podospora, Apodus, Pyrenochaetopsis, and Schizothecium in group 1 were relatively higher, whereas relative abundances of Gliomastix, Aspergillus, and Alternaria were lower ($P<0.05$). Relative abundances of Gliomastix, Ochroconis, Cladosporium, Alternaria, Staphylotrichum, Mortierella, Sarocladium, Podospora, Apodus, Pyrenochaetopsis, and Schizothecium were significantly higher under PC than under PBC, while relative abundance of Pseudallescheria was lower (45.23%; $P<0.05$).

3.6 Soil Microbial Community Composition

NMDS with Bray-Curtis dissimilarity measurement was employed to elucidate the response of soil microbial community composition to different amendments and its relationship with various soil environmental factors. NMDS results revealed that composition of soil bacterial and fungal communities varied among amendments (Fig. 5a [Figure 5: see original paper] and 5b); community composition under CK and PB differed from those of P, PC, and PBC, while community composition under P was distinct from PC and PBC (Fig. 5).

Ordination plots of soil environmental factors showed that soil bacterial community was significantly correlated (Mantel test, P=0.05) to PAH (removal rate of aromatics), pH, PPO, DHA, SOM, AN, and AP, as well as Shannon and Chao 1 indices. As shown in Figure 5 and Table 5 , soil bacterial communities under P, PC, and PBC were remarkably distinct. PB and CK strongly affected PAH, PPO, DHA, SOM, AN, AP, Shannon, and Chao 1 indices of soil bacterial community. However, soil bacterial community under PB was significantly different from other amendments as it was substantially affected by soil pH. In addition, soil fungal community under PB and CK was strongly affected by soil AN, TN, Simpson and Chao 1 indices, URE, and TPH. Soil fungal community under PB differed from other amendments and was also strongly affected by soil pH (Fig. 5 and Table 5).

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4 Discussion

Removal rate of soil petroleum hydrocarbon pollutants provides the most direct evidence of phytoremediation efficiency, which can be increased through synergistic effects of plants, rhizospheric microbes, and organic amendments (Wang et al., 2015; Hussain et al., 2018). The present study aimed to further understand phytoremediation efficiency of TPH-contaminated soil using Calendula officinalis along with two amendments. Many studies have reported that biochar plays a crucial role in phytoremediation of petroleum hydrocarbon contaminated soil (Tang et al., 2010; Oleszczuk et al., 2012; Quilliam et al., 2013; Tan et al., 2018; Zhang et al., 2019; Zhen et al., 2019). Pore spaces in biochar remarkably enhance water-holding capacity, air retention, and nutrient absorption, which increase survival rates of soil microbes and petroleum hydrocarbon degradation (Barati et al., 2017; Wang et al., 2017). However, in the present study, removal rates of petroleum hydrocarbon pollutants with biochar amendment were significantly lower than those of organic compost amendment or biochar and compost co-amendment. To clarify why organic compost amendment was superior to biochar amendment in C. officinalis-mediated phytoremediation of crude-oil contaminated soil, we analyzed plant parameters, soil physicochemical properties, enzyme activity, and soil microbial community composition across amendments.

Plants under organic pollutant stress exhibit reduced plant growth and root vitality (Cao et al., 2016; Han et al., 2016; Hussain et al., 2018). Petroleum products can permeate root or membrane structures and destroy root duct tissue, resulting in reduced root vitality (Xiao et al., 2018). Leaves serve as crucial components of plants in resisting organic pollutant stress (Kollárová et al., 2018). However, under this stress, plant photosynthesis is inhibited and photosynthetic pigment content in leaves is reduced (Hernández-Vega et al., 2017). Therefore, shoot length, root vitality, chlorophyll ratio, total chlorophyll, and biomass are leading indicators for assessing plant capability to resist organic pollutant stress. In the present study, we observed that addition of biochar to TPH-contaminated soil did not increase plant shoot length, root vitality, chlorophyll ratio, total chlorophyll content, or plant biomass of C. officinalis (Fig. 2).

Biochar and organic compost are the most widely used organic amendments for phytoremediation of crude-oil contaminated soil (Han et al., 2016; Barati et al., 2017; Wang et al., 2017). According to other studies, biochar amendment can have several adverse effects on soil, including toxicity to plants and soil microbes (Oleszczuk et al., 2012; Quilliam et al., 2013). Additionally, biochar can strongly adsorb soil nutrients and organic matter, blocking pores and subsequently reducing pore size and surface area of soil (Joseph et al., 2010). A key problem of biochar is its pore size, which can remarkably affect its function. Smaller pores of biochar can prevent molecule adsorption and transport, while larger pores, which are important for nutrient absorption, can increase adsorption of toxic substances such as petroleum hydrocarbons (Saum et al., 2018). Kong et al. (2017) reported that increasing pyrolysis temperature for biochar from 300°C to 500°C increased total pore volume (TPV) from 0.004-0.006 to 0.033-0.039 m^3/g. Higher biochar pyrolysis temperature increased biochar toxicity to plants and soil microbes. This aligns with a phytoremediation study using ryegrass by Han et al. (2016), which demonstrated that biochar hampered petroleum hydrocarbon degradation by reducing nutrient availability, thereby inhibiting plant growth and abundance and diversity of bacterial and fungal communities in soil (Han et al., 2016). In our study, biochar was a commercial product produced from maize straw at a relatively high pyrolysis temperature (500°C) with a TPV of 0.035 ($±0.001)m^{3}$/g. Biochar application might have suppressed physiological plant parameters of C. officinalis and reduced soil nutrient availability, resulting in reduced abundance and diversity of soil bacterial and fungal communities.

Phytoremediation of crude-oil contaminated soil can supply soil nutrients and promote growth and activity of plants and soil microbes (Taiwo et al., 2016). In the present study, we observed that organic compost amendment in crude-oil contaminated soil increased nutrient availability and thus promoted shoot length, root vitality, chlorophyll ratio, total chlorophyll, and plant biomass of C. officinalis. Organic compost also increased abundances and diversity of C. officinalis rhizospheric soil bacterial and fungal communities, resulting in improved phytoremediation efficiency.

Studies have found that organic compost can improve soil quality and foster activity and growth of microbes involved in hydrocarbon degradation (Bastida et al., 2016; Hussain et al., 2018). In the present study, we observed that biochar amendment substantially increased soil pH, whereas organic compost amendment increased soil nutrients (Table 3). These results align with other studies reporting increased soil pH and reduced nutrient availability in crude-oil contaminated soil following biochar application (Cornelissen et al., 2005; Farrell et al., 2013; Schulz et al., 2013; Ameloot et al., 2014; Chintala et al., 2014; Xu et al., 2014; Saum et al., 2018). Wang et al. (2018) reported that crude-oil contamination increased soil pH, which in turn significantly decreased soil nutrient availability and α-diversity of soil microbial community. Thus, biochar amendment-induced high soil pH could significantly affect phytoremediation efficiency, soil nutrient content, and abundance and diversity of soil microbial community (Fernandez et al., 2014; Ghosh et al., 2014; Fernanda et al., 2017; Wei et al., 2017; Hussain et al., 2018). In the present study, co-amendment of biochar and compost resulted in significant increases in soil nutrient content, OTU number, OTU abundance, and diversity indices of soil microbial community, but soil pH decreased compared with biochar amendment alone. The decrease in soil pH might be due to acids generated from biodegradative activities of soil microbes during nitrification processes in organic compost-amended crude-oil contaminated soil (Murray et al., 2011; Taiwo et al., 2016).

Based on the above analysis and discussion, we concluded that higher pH and lower soil nutrient content and microbial community abundance and diversity induced by biochar application alone could explain the relatively poor phytoremediation efficiency.

Soil enzymatic activity is also a sensitive indicator of contaminant decomposition during phytoremediation (Dindar et al., 2015; Zhen et al., 2019). As validated in the present study, phytoremediation can significantly stimulate enzymatic activity (Fig. 3). Soil DHA and PPO are important oxidoreductases related to biodegradation of alkanes and aromatics (Tang et al., 2010; Wei et al., 2017). Increased soil DHA and PPO activities indicate enhanced soil microbial activity (Wang et al., 2015), as demonstrated by abundance and diversity of soil bacterial and fungal communities (Table 4). URE and phosphatase govern dynamics of soil nitrogen and phosphorus cycling, and these elements are essential for plants and microbes (Zhang et al., 2019). In the present study, organic compost amendment significantly improved soil URE and APA activities (Fig. 3), and accordingly, relatively higher soil N and P contents occurred under PBC and P (Table 3). Increased soil nutrients due to organic compost amendment might have increased N and P availability for microbes compared with nutrients absorbed by biochar, increasing activities of URE and phosphatase in this study.

Soil microorganisms are the most important biological environmental factor determining phytoremediation efficiency of crude-oil contaminated soil, since plant rhizosphere creates a conducive microenvironment for growth and development of soil microbes (Wang et al., 2015; Han et al., 2016; Kolton et al., 2017; Zhang et al., 2018; Xu et al., 2019; Zhang et al., 2019). Previous studies have shown that organic amendments to crude-oil contaminated soil can drive shifts in bacterial and fungal community composition (Oleszczuk et al., 2012; Zhang et al., 2019), improve soil quality, and foster activity and growth of hydrocarbon-degrading microbes (Hussain et al., 2018). In this study, organic compost amendment increased relative abundances of petroleum-degrading bacteria and hydrocarbonoclastic fungi (genus level) (Fig. 4). In a study by Zhen et al. (2019), planting Spartina anglica in petroleum hydrocarbon contaminated soil increased relative abundances of Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Gemmatimonadetes, Parcubacteria, Immundisolibacteraceae, Solimonadaceae, and Pseudallescheria. Moreover, Shen et al. (2018) investigated bacterial community response to phytoremediation in petroleum-contaminated soils in northwestern China, and found Pseudomonas, Nocardioides, Marinobacterium, Pseudallescheria, Ochroconis, Fusarium, Sarocladium, and Podospora to be dominant bacterial genera in rhizospheric soil of Agropyron cristatum and Cynodon dactylon. According to previous studies, Pseudomonas, Nocardioides, Galbitalea, Marinobacterium, Pseudallescheria, Solimonadaceae, and Proteiniphilum were dominant bacteria in petroleum-contaminated soil, and most were PAH and alkane-degrading bacteria (Zhang et al., 2010; Kong et al., 2017; Shi et al., 2017; Igun et al., 2019; Zhang et al., 2019; Xu et al., 2020). Besides, previous studies have reported that Pseudallescheria, Fusarium, and Ochroconis were hydrocarbonoclastic fungi (Yemashova et al., 2007; Isola et al., 2013), indicating that organic compost addition enhanced petroleum hydrocarbon-degrading microbial community in the rhizosphere. Moreover, NMDS results also validated that higher pH and lower soil nutrition content induced by biochar application alone drove changes in soil microbial diversity and community composition (Fig. 5), probably leading to low phytoremediation efficiency.

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5 Conclusions

We conclude that organic compost-assisted C. officinalis phytoremediation for crude-oil contaminated soil was highly effective in the Loess Plateau, China. Crude-oil contaminated soil amended with organic compost showed lower pH, higher removal rates of TPHs, alkanes, and aromatics, higher soil dehydrogenase and PPO activities, and relatively higher abundance of PAH and alkane-degrading bacteria. Additionally, organic compost amendment significantly increased shoot length, root vitality, shoot and root dry weights of C. officinalis, and SOM, TN, AN, TP, and AP contents compared with biochar amendment. Thus, organic compost is highly effective in increasing phytoremediation efficiency of C. officinalis for crude-oil contaminated soil.

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Note: Figure translations are in progress. See original paper for figures.