Open access • Journal Article • DOI:10.1111/PCE.12944

Enhanced growth of halophyte plants in biochar-amended coastal soil: roles of nutrient availability and rhizosphere microbial modulation. — Source link

Hao Zheng, Xiao Wang, Lei Chen, Zhenyu Wang ...+5 more authors

Institutions: Ocean University of China, Jiangnan University, University of Massachusetts Amherst

Published on: 01 Mar 2018 - Plant Cell and Environment (Wiley)

Topics: Biochar, Soil health, Soil fertility, Soil organic matter and Rhizosphere

Enhanced growth of halophyte plants in biochar-amended coastal soil: roles of nutrient availability and rhizosphere microbial modulation

Hao Zheng, Xiao Wang, Lei Chen, Zhenyu Wang, Yang Xia, Yipeng Zhang, Hefang Wang, Xianxiang Luo, Baoshan Xing

Enhanced growth of halophyte plants in biochar-amended coastal soil: roles of nutrient availability and rhizosphere microbial modulation

Hao Zheng[a] , Xiao Wang[a], Lei Chen[a], Zhenyu Wang[b,**], Yang Xia[a], Yipeng Zhang[a], Hefang Wang[a], Xianxiang Luo[a], Baoshan Xing[c,*]

[a] *College of Environmental Science and Engineering, and Key Laboratory of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China*

[b] *School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China*

[c] *Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA 01003, USA*

Running Title: *Enhanced plant growth in biochar amended soil*

*Corresponding authors: Dr. Baoshan Xing; phone: 413-545-5212; email: bx@umass.edu; Dr. Zhenyu Wang; email: wang0628@jiangnan.edu.cn

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pce.12944

Brief summary

Biochar alone or co-applied with fertilizer enhanced the growth (e.g. germination, root development, biomass) of two local halophyte plants, primarily attributed to the enhanced nutrients availability (i.e. NAE and PAE), the elevated microbial activities in rhizhosphere, and bacterial community shift towards the bacterial taxa responsible for C-stabilizing in soil, phosphate solubilizing and N-fixing. The co-application of biochar and fertilizer (≤ 5%) had greater benefits for the halophyte growth than the biochar or fertilizer alone. The biochar-enhanced plant growth and biomass in coastal wetlands could potentially buffer the negative effect of climate change, thus enhance soil health and food security. This is the first report on examining the rhizosphere microbial response (i.e., the shifts in bacterial community composition) to the biochar-enhanced nutrient bioavailability for halophytes growth.

ABSTRACT

Soil health is essential and irreplaceable for plant growth and global food production, which has been threatened by climate change and soil degradation. Degraded coastal soils are urgently required to reclaim using new sustainable technologies. Interest in applying biochar to improve soil health and promote crop yield has rapidly increased because of its multiple benefits. However, effects of biochar addition on the saline-sodic coastal soil health and halophyte growth were poorly understood. Response of two halophytes, Sesbania (*Sesbania cannabina*) and Seashore mallow (*Kosteletzkya virginica*), to the individual or co-application of biochar and inorganic fertilizer into a coastal soil was investigated using a 52-day pot experiment. The biochar alone or co-application stimulated the plant growth (germination, root development, biomass), primarily attributed to the enhanced nutrients availability from the biochar-improved soil health. Additionally, the promoted microbial activities and bacterial community shift towards the beneficial taxa (e.g., *Pseudomonas* and *Bacillus*) in the rhizosphere also contributed to the enhanced plant growth and biomass. Our findings showed the promising significance because biochar added at an optimal level (≤ 5%) could be a feasible option to reclaim the degraded coastal soil, enhance plant growth and production, and increase soil health and food security.

***Key-words**:* climate change; food security; salinity; growth; rhizosphere; plant nutrients

INTRODUCTION

Climate change may increase temperature (about 2-4°C globally) and frequency and severity of extreme droughts, elevate evapotranspiration (Stocker *et al.* 2013; Trenberth *et al.* 2014), result in more frequent and intense precipitation and flooding in temperate regions (Taylor *et al.* 2013), and even prolong the growing season for crops (Ray *et al.* 2015). These variable weather conditions and events could bring significant fluctuations in crop yields, and hence adversely affect global food security (Wheeler & von Braun 2013). Furthermore, the global population is projected to be 9.6 billion by 2050, 50% larger than the present and thus, the global food demand is projected to double (Godfray *et al.* 2010), which are major challenges in ways that do not compromise environmental integrity and public health (Godfray *et al.* 2010; Wheeler & von Braun 2013). Moreover, the effects of substantial climate changing on food production would exacerbate the growing competition for natural resources (soil, water and energy) and hinder humanity's efforts to provide adequate food for the increasing global population (Wheeler & von Braun 2013).

Healthy soil is essential for global food production and maintaining the climate sustainability (Koch *et al.* 2013). The detrimental consequences of climate change on agricultural productivity and food supply are attributed to the decline in soil functions, threatening the world soil health (Koch *et al.* 2013; Amundson *et al.* 2015). It is estimated that 25.1 million ha of farmland in China was suffered by droughts annually during 1991-2008, resulting in about 28.3 Mt of grain production loss (Ju *et al.* 2013). Additionally, the crops may experience a 9% shrink in productivity by 2050 and an unbearable level of 30% by 2050 in China, with risks of severe soil degradation under changing climate (Ye & Van

Ranst 2009). Therefore, effective strategies to ensure food security by sustainably managing and improving soil resources are becoming increasingly clear (Koch *et al.* 2013). Coastal ecosystem could provide substantial benefits for climate adaptation and resilience through wave attenuation, erosion prevention and sediment trapping (Howard *et al.* 2014), as well as high primary production and C storage capacity, which are increasingly referred to as ‘‘blue C’’ ecosystems (McLeod *et al.* 2011). Coastal soils may also hold a great potential for increasing global grain production and ensure food security (Novak *et al*. 2013; Stocker *et al*. 2013). Unfortunately, the coastal soils with an estimated 0.34-0.98 million ha degradation annually in the world (Sifleet *et al.* 2011) were stressed by a series of problems including nutrient deficiencies (e.g. soil organic carbon (SOC), nitrogen (N) and phosphorus (P)), and high-salt concentration, which consequently limited soil primary productivity (Amundson *et al.* 2015). Similarly, coastal cropland in the Yellow River Delta of China, rapidly decreased by 65.1 km^2 during 1986-2005 (Huang *et al.* 2012). The deterioration of soil health has become the critical limitations in restoring these degraded soils (Zhang *et al.* 2015), and exacerbate global climate change, thus threaten the food security (Wheeler & von Braun 2013). Therefore, new technologies or sustainable practices to reclaim the degraded coastal soils, restore vegetation and minimize the effect of climate change on soil production in these coastal ecosystems are urgently required.

As a promising soil amendment, biochar may be a potential solution because of its multiple benefits (Agegnehu *et al.* 2015; Guo *et al.* 2016). Enhanced plant growth and crop yields are two major promising benefits of applying biochar to soils (Genesio *et al.* 2015; Jeffery *et al.* 2011; Spokas *et al.* 2012; Vaccari *et al.* 2015; Zhang *et al.* 2016), which were

already demonstrated in acidic soils (Jeffery *et al.* 2011; Kammann *et al.* 2015). However, the biochar-enhanced plant growth does not always bring positive responses (Van Zwieten *et al.* 2010; Borchard *et al.* 2014; Vaccari *et al.* 2015; Zhang *et al.* 2016). The variable responses of crop growth to biochar additions were mainly attributed to types of soils, plant and biochars (Van Zwieten *et al.* 2010; Jeffery *et al.* 2011; Igalavithana *et al.* 2016; Sizmur *et al.* 2016) and the complicated interactions between them (Wang *et al.* 2015). Furthermore, the majority of these biochar studies focused on nonsalt-affected soils (e.g. acidic soils) (Khan *et al.* 2013; Xu *et al.* 2014; Zhang *et al.* 2016), limited attention was paid to the saline-sodic coastal soil quality and fertility (e.g. salt stress and nutrient bioavailability) and primary productivity (Wu *et al.* 2014). In the Yellow River Delta, previous studies documented that the peanut shell biochar application may enhance C sequestration (Luo *et al.* 2016c) and reduce net N mineralization through increasing C: N ratio and decreasing urease activity in coastal soils (Luo *et al.* 2016a). Still, uncertainty remains about the influence of biochar on the coastal soil in terms of soil health and primary productivity. We hypothesize that the biochar addition with or without supplementary fertilizer into the degraded coastal soil may enhance the local halophytes growth and increase their biomass, because 1) biochar may increase soil cation exchange capacity (CEC), soil organic matter (SOM) content, and soil surface area, thus improving the health of the degraded soil; 2) biochar may increase N and P availability in the soils and thus enhance their bioavailability; and 3) the joint application of biochar and fertilizers could perform better than biochar alone.

Due to the high sensitivity to soil environmental conditions (e.g. pH and substrates) in soils, soil microbial community composition and physiological activity could be affected by biochar additions (Lehmann *et al.* 2011; Gul & Whalen 2016). Song *et al.* (2014) found that low rate (e.g. 5%) application of cotton stalks biochar to a weakly alkaline soil significantly promoted growth of ammonia oxidizing bacteria (AOB), potentially resulting in an enhanced nitrification. Conversely, Wang *et al.* (2015) reported that a weakened nitrification process in an acidic orchard soil followed by peanut shell biochar amendments associated with a reduced abundance of AOB. Obviously, the exact effects of biochar on soil microbial activity and community responsible for plant nutrient availability in the saline-sodic soils were still needed to be clarified. We hypothesize that biochar could enhance microbial activity and shift the bacterial community towards the groups with high nutrient availability related to the halophytes growth.

Therefore, the specific objectives of this study were to (1) investigate the effects of the biochar additions with or without supplementary fertilizer into the degraded saline-sodic soil on growth and biomass of two local halophytes, (2) elucidate the mechanisms of biochar in affecting physico-chemical properties of the coastal soil, and (3) investigate the biochar-induced microbial response for enhancing bioavailability for halophytes growth.

MATERIALS AND METHODS

Soil sampling

The soil was collected from the Dongying Halophytes Garden (118.67°N, 37.42°E), located in the Yellow River Delta, China. The sampling field has been planted with okra (*Abelmoschus esculentus* L.) in the past years, and no fertilizer was used before. The soil samples were randomly collected from the topsoil (0-20 cm), air-dried, and ground to pass a 2-mm sieve and thoroughly homogenized. The soil was classified as a silty clay, and its properties are presented in Table 1.

Biochar preparation

A biochar sample was produced from peanut shell using a self-designed pyrolytic reactor, consisting of a heating tank and a cooling tank. Briefly, the peanut shell was charred at 350°C for 3 h in the reactor using slow pyrolysis as reported by Zheng *et al.* (2013b). The temperature of 350°C was selected to prepare the biochar, because of the lower pH, the higher production yield, and higher content of nutrients compared to the high temperature biochars (≥ 500°C) (Table S1). After charring, the biochar was milled to pass a 0.2-mm sieve prior to further analyses. The biochar properties are presented in Table 1 and Fig. S1.

Pot experiment

Two common local halophytes, Sesbania (*Sesbania cannabina*) and Seashore mallow (*Kosteletzkya virginica*), widely used in restoring and remediating saline-sodic soil in the Yellow River Delta (Qin *et al.* 2015), were chosen as the tested plants in the pot experiment. Sesbania is an annual leguminous herb and often used as green manure with the optimal and tolerable level of soil EC at less than 1.18 dS m^{-1} and 2 dS m^{-1}, respectively, and tolerable

soil pH at 7.5-8.7 (Gopalakrishnan *et al.* 1996). Seashore mallow is a popular energy plant for making biodiesel because of the high content of protein and fat in the seeds, and its optimal and tolerable level of soil EC is 1.06-2 dS m^{-1} and 2.8 dS m^{-1}, respectively, and tolerable soil pH is 7.07-9.5 (Qin *et al.* 2015). The prepared biochar was incorporated into the selected soil at rates of 0%, 1.5%, 5% and 10% (w/w), hereafter referred to as CK, BC-1.5%, BC-5% and BC-10%, respectively. In addition, another portion of soil was treated with the same rates of biochar and a basal fertilizer (urea, 112.5 kg N ha^{-1}; calcium superphosphate,112.5 kg P_2O_5 ha^{-1}), hereafter referred to as CKF, BCF-1.5%, BCF-5% and BCF-10%, respectively. All plastic pots (10.5 cm × 10.5 cm × 9 cm) were filled with 400 g soil or mixture of soil and biochar, and were incubated for one week at 65% of maximum water holding capacity (WHC) to activate soil microbes before seed sowing. A total of nine Sesbania and six Seashore mallow seeds were sowed in each pot, respectively, and then thinned to the best three after germination. All pots were maintained at 65% of the maximum WHC of each treated soil using distilled water during the incubation (Table S2). Triplicates were set for each treatment and all the pots were randomly placed in a greenhouse. After 52 days, the shoots and roots of the two halophytes were separately harvested. The roots were lifted from the soils and gently shaken to collect the rhizosphere soil (soil adhering to roots) (Zheng *et al.* 2013a). The root-free soil samples were also collected, hereafter referred to as the non-rhizosphere soils.

Sample analysis

For the biochar sample, total C, N, H, O and S, pH, surface area, pore volume, CEC, WHC, zeta potential, contents of NH_4^+-N, NO_3^--N, Olsen-P and ash were measured as reported previously (details in the Supplementary Data) (Zheng *et al.* 2013b; Luo *et al.* 2016b). Soil pH was determined in a 1:2.5 (w/v) soil to water slurry using a pH-meter (AB150, Fisher Scientific, USA). Electrical conductivity (EC) was measured in a 1:5 soil to water slurry using a conductivity meter (Cond 3210, Germany). Soil bulk density was measured in the sampling field in situ using the cutting ring method without compaction treatment (Abu-Hamdeh 2003). SOM was measured using the potassium dichromate oxidation method. NH_4^+-N, NO_3^--N (extracted with 1 M KCl) and Olsen-P (extracted with 0.5 M $NaHCO_3$) were determined by a segmented continuous flow analyzer (Quaatro, Bran+Luebbe, Germany). CEC and exchangeable Na (Ex-Na) were determined by the compulsive exchange method with 1.0 M ammonium acetate extraction at pH 7.0 (Liang *et al.* 2006). Exchangeable sodium percentage (ESP) was calculated from Ex-Na content divided by the value of CEC. Soil surface area was determined from CO_2 (SA-CO_2) and N_2 (SA-N_2) adsorption isotherms using Quantachrome Autosorb-1 (Quantachrome, USA) (Zheng *et al.* 2013b).

Root morphology including length, surface area (SA), average diameter (AD) and tips, and leaf parameters including leaf surface area (LSA) and average leaf width (ALW) were analyzed using root scanners (Epson Scanning, Japan) and WinRHIZO software (Pro. 2005, Regent, Canada). Chlorophyll index was determined using a portable chlorophyll meter (CCM-200, OPTI-sciences, USA). TN contents in the plants were determined using an

elemental analyzer (FLASH-2000, Thermo Scientific, USA). Total phosphate (TP) content was measured using ICP-MS after microwave digestion (MARS5, CEM, USA) (0.1 g sample + 6 mL concentrated nitric acid).

Biolog analysis

The metabolic profile of microbial community was analyzed using a Biolog Microstation System (TM V4.2, Biolog Inc., Hayward, USA) (Rutgers *et al.* 2016). Briefly, 10 g fresh rhizosphere and non-rhizosphere soils collected after the plant growth were added into 90 mL phosphate buffer, and shaken at 70 rpm for 30 min. The supernatant was then diluted 1000-fold with the buffer, and 150-μL dilutions were directly added into the Biolog ECO plate. Then the plates were incubated at 28ºC in dark, and the color development at 590 nm was measured every 24 h for 7 days using a Microplate Reader (Multiskan Spectrum, Thermo Scientific, USA). Each soil was extracted and analyzed in triplicate. Average well color development (AWCD) versus incubation time reflected the development of soil bacterial community, and the metabolic diversity was determined (details in the Supplementary Data). Three diversity indices including Shannon–Weiner (*H´*) diversity index, Simpson's index (*D*) and Evenness (*E*) were used to highlight the overall effects of biochar-amendment on soil microbial diversity (details in the Supplementary Data).

DNA extraction, PCR amplification and high-throughput sequencing

Bacterial DNA was extracted using the TIANamp Soil DNA Isolation Kit (DP 336) (TIANGEN, China) according to the manufacturer's protocols. The V4-V5 region of the bacterial 16S ribosomal RNA gene (primer set 515 F/907 R) was amplified via PCR. PCR conditions included an initial denaturation stage of 98°C for 1 min followed by 30 cycles of

98°C for 10 s, 50°C for 30 s, and 30 s at 72°C, with final extension at 72°C for 5 min. The PCR amplification reactions were performed in triplicate and each 25-μL volume mixture consisted of 10 ng DNA, 0.2 mg ml^{-1}, 0.2 μM each primer, and 15 μL Phusion® High-Fidelity PCR Master Mix (New England Biolabs). PCR amplicons were extracted from 2% agarose gels, and purified using a Qiagen DNA Gel Extraction Kit (Qiagen, Valencia, CA). Purified amplicons were pooled in equimolar concentrations within each plot and were sequenced on a PacBio-RS II system (Pacific Biosciences, Menlo Park, CA) using C4 chemistry and standard protocols. PCR amplification was quantified in an iCycler IQ5 Thermocycler (Bio-Rad, Hercules, CA) by flourometric monitoring with SYBR Green 1 dye. After the assessment of sequencing libraries conducted on the Qubit@ 2.0 Fluorometer (ThermoFinnigan, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, USA), the PCR products were subjected to paired-end sequencing (2×250) on the Illumina HiSeq platform (Roche Diagnostics Corporation, Branford, CT, USA). The above operations were performed at Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

Processing of pyrosequencing data and analysis.

Data were processed and analyzed following the procedure described by (Smets *et al.*, 2016), where raw Fastq files were quality-filtered by QIIME (version 1.7.0) with the corresponding technological criteria. All sequences were then checked according to the process of UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html), and were taxonomically assigned using a RDP Classifier (version 2.2, http://sourceforge.net/projects/rdp-classifier/) with a bootstrap cutoff of 97% against the Greengenes database for 16S rRNA gene assemblages

using UPARSE (version 7.1, http://drive5.com/uparse/). Reads that could not be assembled were discarded. Representative sequences from each read were aligned using PyNAST, and the most abundant sequence in the OTU was selected as the representative sequence.

Statistical analysis

The significance of the various parameters was tested by one-way analysis of variance (ANOVA) using Duncan's multiple range test ($P = 0.05$) for the different soil treatments, and the least significant difference (LSD) ($P < 0.05$) based on a Student's t-test was used to illustrate the differences between the rhizosphere and non-rhizosphere soils by means of Statistical Product and Service Solutions Software (SPSS, version 20.0). The correlation was analyzed with the Pearson test (two-tailed) at $P = 0.01$ or 0.05 using SPSS 20.0.

RESULTS

Biochar impact on halophyte plant growth and biomass production

Biochar addition alone increased the seed germination rate of Sesbania and Seashore mallow by 275-395% and 44.4-48.4% compared to the CK treatment, respectively, and while the co-application of biochar and fertilizer had non-significant effect on both seeds germination compared to the CKF treatment (Fig. S2). The stem height and diameter of two halophytes seedlings showed an increasing trend (Fig. S3, 4), but generally without significant difference compared with the CK and CKF treatments, respectively, except that of Seashore mallow in BCF-1.5% and BCF-5% (Fig. S4). For both halophytes, all the amendments had little effect on leaf chlorophyll content (Fig. S5), but generally increased LSA and ALW at the higher biochar rates (≥ 5%) (Table 2).

Biochar addition alone had no significant effect on Sesbania root biomass at the lower rate of 1.5%, while it significantly promoted the root biomass by 113-190% at the higher rate (≥ 5%) (Fig. 1a). However, Sesbania shoot biomass was observably increased by 111-143% in all biochar alone treatments (Fig. 1a). The co-application had no consistent positive effect on the Sesbania root and shoot biomass, and the shoot biomass only significantly increased by 54.7 in BCF-1.5% and 60.0% in BCF-5% (Fig. 1b). Moreover, the biochar alone and co-application (except for BCF-10%) significantly increased the total biomass by 111-152% and 118-156%, respectively (Fig. 1a, b). For Seashore mallow, regardless of fertilizer added or not, the biochar addition increased the root biomass by 112% only in the BC-10% treatment, while had no significant effect in other treatments (e.g. BC-5% and BCF-5%) (Fig. 1c, d). The dose-response of biochar for shoot and total biomass was similar to that of Sesbania, and total biomass increased by 32.8-76.7% and 44.9-66.2% in the biochar alone and combined treatments, respectively (Fig. 1c, d). Moreover, the increased total biomass in the co-application of biochar and fertilizer treatments (e.g. BCF-5%) could produce more biomass for the two halophytes than that by biochar or fertilizer addition separately (Fig. S6).

Biochar impact on root morphology of halophyte plants

The halophytes roots were bigger in the biochar treatments than those in the CK (Fig. S3). For Sesbania, biochar addition alone significantly enhanced the root length, SA, AD and tips by 91.2-163%, 881-978%, 304-411% and 229-253%, respectively, but little significant difference was observed among the treatments of different rates (Table 2). However, the co-application of biochar and fertilizer had no consistent positive effect on root growth. For example, Sesbania root tips decreased by 76.6% in the BCF-10% treatment (Table 2). The

dose-response of biochar alone for root morphology of Seashore mallow was similar to those of Sesbania, which increased root length, SA, AD and tips by 59.7-138%, 3.28-143%, 2.13-6.38% and 15.6-110%, respectively. Co-application significantly increased root length, SA, and tips by 37.5-88.3%, 44.1-85.3% and 39.7-53.4%, respectively.

N and P bioavailability in the biochar-root-soil system

Two parameters were used here to evaluate the N and P bioavailability (Zheng *et al.* 2013a), namely N or P accumulation efficiency (NAE or PAE, ratio of TN or TP accumulated to total root length) and N or P utilization efficiency (NUE or PUE, ratio of produced biomass to unit of N or P uptake) (Fig. 2). For Sesbania, the NAE increased from 2.37 mg m^{-1} in CK to 3.46, 3.01 and 2.56 mg m^{-1} in the BC-1.5%, BC-5% and BC-10% treatments, respectively, and only the rate of 1.5% showed significant enhancement (Fig. 2a). However, the co-application had no significant effect on NAE (Fig. 2b). For Seashore mallow, the biochar addition alone had no significant effect on NAE (Fig. S7a), but the co-application significantly increased NAE by 33.6% in the BCF-1.5%, decreased NAE by 27.4% in the BCF-10% treatment (Fig. S7b). For both halophytes, the biochar alone significantly increased NUE in the BC-10% treatment, but had little effects in the BC-1.5% and BC-5% treatments (Fig. 2a and S7a). However, compared to the CKF treatment, the combined addition of biochar and fertilizer had no significant effects on NUE for both halophytes (Fig. 2b and S7b).

For Sesbania, PAE presented the similar dose-response to the biochar addition alone, which only significantly increased by 69.1% in the BC-5% treatment (Fig. 2c, d). The PAE values for Seashore mallow had a similar trend compared with those of NAE in the amended treatments (Fig. S7). The PUE values for both halophytes showed decreased trends with increasing biochar addition (Fig. 2c, d and S7d), except for Seashore mallow in the biochar alone treatments (Fig. S7c).

N and P content in the biochar amended soils after plant growth

For Sesbania, biochar alone treatments significantly increased NH_4^+-N content by 34.9-55.3% in the rhizosphere soils, while decreased NH_4^+-N content by 10.8-50.8% in the non-rhizosphere soils (Fig. 3a). Moreover, NH_4^+-N contents in the rhizosphere soils were significantly higher than those in the non-rhizosphere soils (Fig. 3a). However, the co-application of biochar and fertilizer had no effect on NH_4^+-N content in the rhizosphere (except for BCF-10%) and non-rhizosphere soils (Fig. 3b). The biochar alone decreased NO_3^--N content by 62.7-68.6% and 63.9-89.3% in the rhizosphere and non-rhizosphere soils, respectively (Fig. 3c). In the fertilizer treatments, the biochar addition had no effect on NO_3^--N content in the rhizosphere soils (except for BCF-1.5%), but significantly decreased NO_3^--N content in the non-rhizosphere soils compared to the CKF treatment (Fig. 3d). For Seashore mallow, similar results were also found for NH_4^+-N and NO_3^--N content, but the difference between the rhizosphere and non-rhizosphere soils were clear (Fig. S8a, d). Contrary to the N contents, Olsen-P contents showed increasing trends with biochar additions in all treatments with or without fertilizer applied (Fig. 3e, f, and S8e, f). Additionally, for Sesbania, Olsen-P contents of the rhizosphere soils in BC-5% and BCF-5% were

significantly lower than those of the non-rhizosphere soils. In contrast, for Seashore mallow, Olsen-P contents of the rhizosphere soils in BC-5% and BCF-5% were significantly higher than those of the non-rhizosphere soils (Fig. S8e, f).

Biochar impact on soil properties

Regardless of the fertilizer added or not, the biochar addition significantly increased SOM content and C/N by 34.5-138% and 51.2-419%, respectively, but had no influence on Ex-Na content (Table 3). Similarly, biochar addition at high rates (e.g., 5%, 10%) increased soil TP content by 7.60-16.8% in the biochar alone treatments and 6.87-29.1% in the fertilizer treatments. However, the soil TN content decreased by 60.0-71.1% in the biochar alone treatments (except for BC-1.5%), and by 40.7-67.1% in the fertilizer treatments compared to the CKF treatment (Table 3). The CEC significantly increased by 12.0-14.7% in the biochar alone treatments than CK, while it was not affected in the fertilizer treatments compared to the CKF treatment (Table 3). In the biochar-amended treatments (except for BCF-1.5%), ESP values were significantly lower than those of CK and CKF treatments. Additionally, the biochar additions slightly decreased the SA-N_2 of soils by 4.45-6.40%, but largely increased SA-CO_2 by 28.0-46.8% (Table 3). For both halophytes, the biochar addition generally had little effect on the pH of rhizosphere and non-rhizosphere soils (Table 4). Moreover, no significant difference for pH values was observed between the rhizosphere and non-rhizosphere soils in the biochar alone treatments grown with both halophytes, but the pH values of rhizosphere soils were obviously lower than those of non-rhizosphere soils in the fertilizer treatments grown with Sesbania (Table 4). The biochar alone at rates of 1% and 5% significantly decreased EC values of the rhizosphere and non-rhizosphere soils for both

halophytes, but the addition at 10% had little effect on EC (Table 4). Similarly, biochar addition significantly increased EC in all the fertilizer treatments (except BCF-10% for Seashore mallow). For Sesbania, no significant difference was found for EC values between rhizosphere and non-rhizosphere soils (except for BC-5%), but for Seashore mallow, the rhizosphere soils had lower EC values compared with those of the non-rhizosphere soils.

Biochar impact on soil microbial activity and community

Microbial activities in the soils amended with and without biochar were estimated by AWCD values (Fig. 4). For rhizosphere soil, AWCD values in the BC-1.5% were significantly higher than those of the CK between 48-120 h (Fig. 4a). The AWCD values at 168 h had an order of CKF > BC-1.5% > BCF-1.5% ≈ CK, but no significant difference was observed among the treatments (Fig. 4a), similar to the AWCD values for non-rhizosphere soils (Fig. 4b). For the rhizosphere soils, the biochar addition alone significantly increased the values of *H´*, *D* and *E* by 57.1%, 23.6% and 16.5%, respectively, while it had no effect for the non-rhizosphere soils (Fig. S9). In the fertilized rhizosphere soils, the biochar addition had no effect on these values, but showed inconsistent impacts for the non-rhizosphere soils (Fig. S9). For example, the biochar addition increased *H´* value (Fig. S9a) but decreased *D* value (Fig. S9b) in the non-rhizosphere soil.

The significant increases of the bacterial community richness indices (i.e. OTUs, Chao and ACE) were found in all the biochar-amended rhizosphere and non-rhizosphere soils compared with the CK or CKF treatments (at a 3% distance) (Table S3), which was confirmed by the rarefaction curves (Fig. S10). Ten most abundant phyla were observed in the rhizosphere and non-rhizosphere soils, i.e., *Proteobacteria*, *Cyanobacteria*,

Actinobacteria, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, *Chloroflexi*, *TM7*, and *Verrucomicrobia*, accounting for 92.7-98.9% and 97.4-98.5% of the total bacterial taxa, respectively (Fig. 5, 6). In the rhizosphere soils, the biochar alone treatments increased the abundances of phyla *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Gemmatimonadetes*, *TM7* and *Verrucomicrobia* by 96.2%, 71.2%, 147%, 229%, 36% and 170%, respectively (Fig. 5). In the fertilizer treatments, the abundances of phyla *Proteobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Firmicutes* and *Verrucomicrobia* were promoted by biochar addition, companied with the decreases in the abundances of phyla *Actinobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Chloroflexi* and *TM7*. For the non-rhizosphere soils, the biochar addition alone increased the abundances of the phyla *Cyanobacteria*, *Bacteroidetes*, *Acidobacteria* and *Verrucomicrobia* (Fig. 6). However, the biochar alone had little influence on the abundance of phylum *Proteobacteria* in the non-rhizosphere soils, but decreased the phyla *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes*, *TM7* and *Verrucomicrobia* by 77.4%, 33.5%, 2.81% and 50%, 60.6% respectively. Compared with the non-rhizosphere soils, the biochar application to the fertilized and non-fertilized rhizosphere soils increased the abundances of phyla *Bacteroidetes* and *Firmicutes* (Fig. 5). At the class level, the abundances of *Alphaproteobacteria*, *Deltaproteobacteria* and *Cytophagia* were elevated up to 59.9-177% by the biochar addition in the rhizosphere soil without the fertilizer compared to the CK treatment, and up to 3.92-55.9% in the fertilized rhizosphere soil compared to the CKF treatment (Table S4). While the biochar additions decreased the classes *Gemmaproteobacteria* and *Bacilli* by 23.8% and 54.5%, and the classes *Oscillatoriophycideae* and *Synechococcophycideae* by 92.9% and 68.7%, in the non-fertilized

and fertilized non-rhizosphere soils, respectively (Table S4). Additionally, the two dominant classes in the rhizosphere soils, i.e., *Deltaproteobacteria* and *Bacilli*, were not observed in all the non-rhizosphere soils.

At the genus level, the biochar additions without or with the fertilizer increased the abundances of *Pseudomonas*, *Bacillus* and *Sphingomonas* by 23.4%, 243% 142%, and 51.5%, 164%, 260% in the non-fertilized and fertilized rhizosphere soils, respectively (Fig. 7a). However, in the non-rhizosphere soils, the abundances of the genera *Pseudomonas* and *Sphingomonas* were greatly elevated by the biochar addition up to 23.4% and 29.0%, respectively, but no obvious differences were observed between the BCF-1.5% and BC-1.5% treatments in the fertilized non-rhizosphere soils (Fig. 7b).

DISCUSSION

Response of plant growth to biochar amendments

Results from this study supported our hypothesis that the biochar alone or co-application promoted the halophytes growth (e.g. germination, root development and biomass). However, recent meta-analyses documented controversial effects of biochar on crop growth or yields response (Jeffery *et al.* 2011; Spokas *et al.* 2012). The inconsistent results could be ascribed to differences of soil properties (Borchard *et al.* 2014), biochar characteristics (Khan *et al.* 2013; Smider & Singh 2014), and crop types (Van Zwieten *et al.* 2010; Vaccari *et al.* 2015). That was why the individual biochar addition significantly increased the Sesbania root biomass, but had no influence on Seashore mallow root biomass (Fig. 1a, c), because Seashore mallow is more tolerant to salt stress than Sesbania (Gopalakrishnan & Jeevanand 1996; Qin *et al.* 2015), for which small mitigation of the salt stress (Table 4) could potentially

provide significant benefits for its growth. The increased seed germination in the biochar alone treatments was possibly ascribed to the improvements of soil characteristics (e.g., increased WHC, decreased EC, Table S2 and 3) and/or the increased nutrients availability. The co-application of biochar and fertilizer had little effect on seed germination compared to the CKF treatment, resulting from the increased seed germination due to the fertilizer addition (Fig. S2). The improvement of the halophytes performance was attributed to the following aspects. First, biochar could directly contribute nutrients (e.g. P and K) to plants (Zheng *et al.* 2013b), because of its inherent available nutrients (Table 1 and S1), which was why the content of Olsen-P in our soils (Table 1) significantly increased with biochar addition ($P < 0.05$, $r = 0.78$, Fig. 3e, f). The obviously lower content of available N in the biochar than the soil (Table 1) showed that the direct N contribution from the biochar could not be the primary reason for the improved plants growth. Second, biochar adsorbed more NH_4^+-N in soils (Fig. 3a) via acid functional groups (e.g. carboxyl and hydroxyl) (Fig. S1a), and weakened nitrification process due to reduced AOB abundance (Wang *et al.* 2015). Third, biochar-induced improvements of soil properties could be more favorable for plant growth (Gul & Whalen 2016), which was confirmed by the negative correlation between the Sesbania root biomass and soil EC values ($P < 0.01$, $r = - 0.83$), as well as the bigger roots in the biochar-amended soils (Table 2, Fig. S3), which ultimately can improve soil health and productivity (White & Kirkegaard 2010). Moreover, the positive correlation between the shoot biomass of Sesbania and NAE values ($P < 0.05$, $r = 0.72$), suggested that the elevated ability of acquiring nutrients by biochar application also contributed to the promoted Sesbania production. However, the root AD, tips, and total biomass of the two halophytes did

not always increase with increasing biochar addition (Table 2, Fig. 1), and Sesbania root tips decreased by 76.6% in the BCF-10% treatment (Table 2), suggesting that excessive biochar application may inhibit plant growth, possibly attributed to dissolution of soluble salts cations (e.g. K^+, Ca^{2+} and Mg^{2+}) from biochars (Smider & Singh 2014). Thus, biochar application rate should be kept at an optimal level (e.g. $\leq 5\%$). Notably, the increased total biomass (Fig. S6) agreed well with our hypothesis that the co-application of biochar and fertilizer could lead to better plant performance than the biochar or fertilizer alone (Fig. S6), demonstrating that that the ways of biochar application into soils could be important for its benefits in agricultural production (Agegnehu *et al.* 2015). The combined application could overcome nutrient deficiency (especially N) in biochars and soils (Sarkhot *et al.* 2012). Photosynthesis is the key physiological process to drive plant growth and general performance (Bloomfield *et al.* 2014). A few studies addressed the photosynthetic responses of plants to biochar amendments (Akhtar *et al.* 2014; Baronti *et al.* 2014; Xu *et al.* 2015). The enhanced peanut photosynthesis following biochar application was attributed to the high P input of biochar in P deficient red ferrosol (Xu *et al.* 2015). On the contrary, Kammann *et al.* (2011) suggested that biochar could weaken the photosynthetic activity of quinoa when soil moisture was kept constant. The leaf chlorophyll content, which is a good indicator of photosynthetic activity and a measure of plant response to environmental stress and nutritional status (Wu *et al.* 2008; Agegnehu *et al.* 2015), was not significantly affected by biochar amendments in this study (Fig. S5). This implied that biochar had no effect on the photosynthesis of the two halophyte plants. However, other studies have shown that application of biochar and compost with fertilizer significantly increased the leaf chlorophyll content of crops compared to fertilizer

alone in an acid soil (Agegnehu *et al.* 2015). Overall, the biochar-enhanced plant growth and biomass production in the degraded coastal soil hold a great potential for sustaining plant productivity in coastal wetlands, and thus soil health and food security could be ensured by sustainably managing and improving soil resources with the biochar strategy.

Effect of adding biochar on soil health

The improved soil properties in the biochar-amended soils (Table 3, 4) support our hypothesis that the biochar addition will improve the properties of degraded soil (e.g. SOM, CEC, and SA-CO_2). The biochar additions had little influence on soil pH (Table 4) due to the high buffering capacity of the saline-sodic soil. Notably, the enhanced root growth induced acidification of the rhizosphere soils (Table 4), which is beneficial for dissolution and activation of the less soluble nutrients (e.g. $Ca_2H_2P_2O_7$ in the biochar, Fig. S1b), because of organic acids (e.g. citric, oxalic and malic acids) exuded from the roots (Hinsinger *et al.* 2003). Another possible explanation for the pH decreases was the high CEC with the biochar (7.39 ± 0.15 cmol kg^{-1}, Table 1 and 3), which promoted absorption of cations (e.g. K^+, Ca^{2+} and Mg^{2+}) by plants, resulting in H^+ release to compensate charge balance (Hinsinger *et al.* 2003). This was confirmed by the reduced EC values in the biochar treatments with rates of 1.5-5% for the two halophytes (Table 4), as well as the reported increase of EC values with furfural biochar addition into the similar soil without plant grown (Wu *et al.* 2014). Especially, significant decreases in EC values in the rhizosphere soils relative to the non-rhizosphere soils (Table 4) demonstrated that the biochar-induced root growth play important roles in alleviating salt stress around root zone and ultimately providing more favorable habitats for root development (Nie *et al.* 2009; Downie *et al.* 2015). The increased

CEC in the biochar treatments (Table 3) was attributed to the increased exchange sites of soil colloids resulted from the biochar surface oxygen-containing functional groups (e.g. -OH and -COOH, Fig. S1a) and the increased soil SA (Table 3) (Liang *et al.* 2006). Therefore, the biochar addition reduced ESP values (a key parameter of saline soil evaluation) to a certain extent, but the Ex-Na content was not affected in all the treatments (Table 3), due to the introduced additional Na from biochar amendment while increasing the soil CEC and no leaching events occurred during the whole incubation time. SOC is one of several key indicators of soil health. As expected, the biochar contributed to the coastal soil C (SOM, Table 3) due to the higher content of biochar-C (55.5 ± 0.6%, Table 1), which greatly enhanced the "blue C" sinks in the coastal ecosystem (Luo *et al.* 2016c) because of the recalcitrant biochar-C (Fig. S1a). Additionally, although the higher rate of 10% resulted in stronger improvement in several soil properties (e.g. SOM, C/N ratio), but did not induce corresponding increases in total biomass of the two halophytes (Fig. 1), suggesting that the appropriate application rate of biochar needs to be maintained at an optimal level (e.g. ≤ 5%). Moreover, the improved soil porosity (SA-CO_2, Table 3) by biochar addition due to the abundant pores in the biochar (Fig. S1c), beneficial for air and water infiltration (Case *et al.* 2012), could be one of the reasons responsible for the enhanced root growth of the two halophytes.

Response of soil microbes to biochar addition

Significant higher AWCD values and obvious shifts in the bacterial community composition in the biochar amended soils (Fig. 4-7), are consistent with our hypothesis that biochar enhanced microbial activity and shift the bacterial community towards the groups with high nutrient availability related to the halophytes growth. Microbial responses to biochar addition could be primarily attributed to the altered substrate (e.g. C and N) availability due to the labile C input from biochar (Farrell et al. 2013; Whitman et al. 2016) and soil physico-chemical properties, including soil nutrient levels (e.g. available C, N and P) (Fig. 3 and S8) and salt stress (e.g. ESP, Table 3). The phylum *Acidobacteria* with a greater abundance in the biochar amended rhizosphere soils (Fig. 5), has been considered benefiting soil C storage via producing microbial mucilages and polysaccharides in favor of stabilizing soil aggregates (Trivedi *et al.* 2013; Gupta & Germida 2015). This could also account for the elevated SOM level followed by the biochar addition (Table 3). Herein, the biochar-mediated shifts in bacterial community may affect soil C cycling through enhancing soil C storage pathways (Whitman *et al.* 2016). These results are consistent with the higher abundances of the classes *Alphaproteobacteria* and *Cytophagia* in the soils amended with biochar than those without biochar (Table S4), which tended to directly utilize labile C (e.g. plant residue and root exudates) (Fierer *et al.* 2012). The results suggested that biochar additions could supply labile C resources for soil microbes to favor the best-adapted groups to thrive (Farrell *et al.* 2013). Similarly, the promoted phyla *Proteobacteria* in the biochar-amended rhizosphere soils (Fig. 5), classified as 'copiotrophic' bacteria with high growth rates under nutrient-rich conditions (Trivedi *et al.* 2013), implied that the more nutrients localized in the root zones for

plant growth. Consistently, the results were in line with the higher contents of NH_4^+-N and Olsen-P in the rhizosphere soils with biochar addition (Fig. 3a, e, f). Promotions of the phylum *Cyanobacteria* abundance in the biochar-amended soils (Fig. 5, 6) indicated that biochar could participate in the N fixation pathways *via* assimilating nitrite (NO_3^-) to ammonium (NH_4^+) (Nelson *et al*. 2016), probably weakening the nitrification process (Wang *et al.* 2015), perhaps one of the reasons responsible for the increased content of NH_4^+-N in the biochar-amended rhizosphere soils (Fig. 3a, d and S8a, b). At the genus level, the phosphate-solubilizing bacteria, *Pseudomonas* and *Bacillus*, were present more abundant in the biochar-amended soils (Fig. 7), indicating that the fixed P in the soil minerals or biochars could be solubilized or transformed into the available P form (e.g. Olsen-P) for plant uptake (Gul & Whalen 2016), consistent with the increased Olsen-P and PAE in the biochar-amended soils (Fig. 2c, d and 3e, f). The genus *Pseudomonas*, common inhabitants in non-saline-alkali soils (Egamberdieva *et al.* 2012), further confirmed the biochar-induced salt stress mitigation in the rhizosphere soils (Table 3, 4).

CONCLUSIONS

The biochar alone or co-applied with fertilizer generally promoted the halophytes growth in the coastal soil, which resulted from the improved soil health, enhanced nutrient availability, and elevated bacterial activities and abundances related to nutrient transformations. Moreover, the rates (e.g., ≤ 5%) and ways of biochar application into soils were crucial for its agronomic benefits, and the co-application of biochar with fertilizer could be the optimal option to maximize its potential benefits in reclaiming the degraded coastal soil. The enhanced halophyte plant growth and biomass yield in the degraded coastal soils could substantially benefit soil primary productivity, and thus promote global soil health and food security. To our knowledge, this is the first report on examining the biochar-induced rhizosphere microbial response (i.e. the shifts in bacterial community composition) to the biochar-enhanced nutrient bioavailability for halophytes growth in the degraded saline-sodic soil. Further experiments in the field will need to assess the effects of biochar on soil functions and environmental benefits in the coastal ecosystem under the changing climate.

ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (41406085, 41325013, 41573089), Foundation of Shandong Provincial Key Laboratory of Marine Ecology and Environment and Disaster Prevention and Mitigation (201512), Fundamental Research Funds for the Central Universities (201564016), Taishan Scholars Program of Shandong Province, China, and USDA NIFA McIntire-Stennis Program (MAS 00028).

REFERENCE

Abu-Hamdeh N.H. (2003) Compaction and subsoiling effects on corn growth and soil bulk density. *Soil Science Society of America Journal* 67, 1213-1219.

Agegnehu G., Bass A.M., Nelson P.N., Muirhead B., Wright G. & Bird M.I. (2015) Biochar and biochar-compost as soil amendments: effects on peanut yield, soil properties and greenhouse gas emissions in tropical North Queensland, Australia. *Agriculture, Ecosystems and Environment* 213, 72-85.

Agegnehu G., Bird M.I., Nelson P.N. & Bass, A.M. (2015) The ameliorating effects of biochar and compost on soil quality and plant growth on a Ferralsol. *Soil Research* 53, 1-12.

Akhtar S. S., Li, G., Andersen, M. N., & Liu, F. (2014). Biochar enhances yield and quality of tomato under reduced irrigation. *Agricultural Water Management* 138, 37-44.

Amundson R., Berhe A.A., Hopmans J.W., Olson C., Sztein A.E. & Sparks D.L. (2015) Soil and human security in the 21st century. *Science* 348, 1261071.

Baronti S., Vaccari F.P., Miglietta F., Calzolari C., Lugato E., Orlandini S., ... & Genesio L. (2014) Impact of biochar application on plant water relations in *Vitis Vinifera* (L.). *European Journal of Agronomy* 53, 38-44.

Bloomfield K.J., Farquhar G.D. & Lloyd J. (2014) Photosynthesis–nitrogen relationships in tropical forest tree species as affected by soil phosphorus availability: a controlled environment study. *Functional Plant Biology* 41, 820-832.

Borchard N., Siemens J., Ladd B., Möller A. & Amelung W. (2014) Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. *Soil and Tillage Research* 144, 184-194.

Case S.D., McNamara N.P., Reay D.S. & Whitaker J. (2012) The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil–the role of soil aeration. *Soil Biology and Biochemistry* 51, 125-134.

Conte P., Marsala V., De Pasquale C., Bubici S., Valagussa M., Pozzi A. & Alonzo G. (2013) Nature of water–biochar interface interactions. *Global Change Biology Bioenergy* 5, 116-121.

Downie H.F., Adu M.O., Schmidt S., Otten W., Dupuy L.X., White P.J. & Valentine T.A. (2015) Challenges and opportunities for quantifying roots and rhizosphere interactions through imaging and

image analysis. *Plant, Cell and Environment* 38, 1213-1232.

Egamberdieva D. (2012) Pseudomonas chlororaphis: a salt-tolerant bacterial inoculant for plant growth stimulation under saline soil conditions. *Acta Physiologiae Plantarum* 34, 751-756.

Farrell M., Kuhn T.K., Macdonald L.M., Maddern T.M., Murphy D.V., Hall P.A., ... & Baldock, J.A. (2013) Microbial utilisation of biochar-derived carbon. *Science of the Total Environment* 465, 288-297.

Fierer N., Leff J.W., Adams B.J., Nielsen U.N., Bates S.T., Lauber C.L., ... & Caporaso J.G. (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences* 109, 21390-21395.

Genesio L., Miglietta F., Baronti S. & Vaccari F.P. (2015) Biochar increases vineyard productivity without affecting grape quality: Results from a four years field experiment in Tuscany. *Agriculture, Ecosystems and Environment* 201, 20-25.

Godfray H.C.J., Beddington J.R., Crute I.R., Haddad L., Lawrence D., Muir J.F., ... & Toulmin C. (2010) Food security: the challenge of feeding 9 billion people. *Science* 327, 812-818.

Gopalakrishnan S. & Jeevanand H.R. (1996) Physiological characteristics of fast growing *Rhizobium sp.* of Sesbania. *Annals of Agriculture Biology Research* 1, 113-119.

Griffin D.E., Wang D., Parikh S.J. & Scow K.M. (2017) Short-lived effects of walnut shell biochar on soils and crop yields in a long-term field experiment. *Agriculture, Ecosystems and Environment* 236, 21-29.

Gul S. & Whalen J.K. (2016) Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. *Soil Biology and Biochemistry* 103, 1-15.

Guo M., Uchimiya S.M., & He Z. (2016) Agricultural and environmental applications of biochar: Advances and barriers. In *Agricultural and Environmental Applications of Biochar: Advances and Barriers* (eds M. Guo, Z. He & S. M. Uchimiya), pp. 495-504. Soil Science Society of America Inc., San Diego.

Gupta V.V. & Germida J.J. (2015) Soil aggregation: Influence on microbial biomass and implications for biological processes. *Soil Biology and Biochemistry* 80, A3-A9.

Hinsinger P., Plassard C., Tang C. & Jaillard B. (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and Soil* 248, 43-59.

Howard J., Hoyt S., Isensee K., Telszewski M. & Pidgeon E. (2014) Coastal Blue Carbon: methods for assessing carbon stocks and emissions factors in mangroves, tidal salt marshes, and seagrass meadows.

Conservation International, Intergovernmental Oceanographic Commission of UNESCO, International Union for Conservation of Nature, Arlington, Virginia, USA.

Huang L., Bai J., Chen B., Zhang K., Huang C. & Liu P. (2012) Two-decade wetland cultivation and its effects on soil properties in salt marshes in the Yellow River Delta, China. *Ecological Informatics* 10, 49-55.

Igalavithana A.D., Ok Y.S., Usman A.R., Al-Wabel M.I., Oleszczuk P., & Lee S.S. (2016) The effects of biochar amendment on soil fertility. In *Agricultural and Environmental Applications of Biochar: Advances and Barriers* (eds M. Guo, Z. He & S. M. Uchimiya), pp. 123-144. Soil Science Society of America Inc., San Diego.

Jeffery S., Verheijen F.G., van der Velde M. & Bastos A.C. (2011) A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems and Environment* 144, 175-187.

Jones D.L., Rousk J., Edwards-Jones G., DeLuca T.H. & Murphy D.V. (2012) Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biology and Biochemistry* 45, 113-124.

Ju H., van der Velde M., Lin E., Xiong W. & Li Y. (2013) The impacts of climate change on agricultural production systems in China. *Climatic Change* 120, 313-324.

Kammann C.I., Schmidt H.P., Messerschmidt N., Linsel S., Steffens D., Müller C., ... & Stephen J. (2015) Plant growth improvement mediated by nitrate capture in co-composted biochar. *Scientific Reports* 5.

Khan S., Chao C., Waqas M., Arp H.P.H. & Zhu Y. (2013) Sewage sludge biochar influence upon rice (*Oryza sativa L*) yield, metal bioaccumulation and greenhouse gas emissions from acidic paddy soil. *Environmental Science and Technology* 47, 8624-8632.

Koch A., McBratney A., Adams M., Field D., Hill R., Crawford J., ... & Angers D. (2013) Soil security: solving the global soil crisis. *Global Policy* 4, 434-441.

Kolton M., Graber E.R., Tsehansky L., Elad Y., & Cytryn E. (2016) Biochar-stimulated plant performance is strongly linked to microbial diversity and metabolic potential in the rhizosphere. *New Phytologist* 213, 1393-1404.

Lehmann J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C. & Crowley D. (2011) Biochar effects on soil biota–a review. *Soil Biology and Biochemistry* 43, 1812-1836.

Liang B., Lehmann J., Solomon D., Kinyangi J., Grossman J., O'neill B., ... & Neves E.G. (2006) Black carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal* 70,

1719-1730.

Luo X., Chen L., Zheng H., Chang J., Wang H., Wang Z. & Xing B. (2016a) Biochar addition reduced net N mineralization of a coastal wetland soil in the Yellow River Delta, China. *Geoderma* 282, 120-128.

Luo X., Liu G., Xia Y., Chen L., Jiang Z., Zheng H. & Wang Z. (2016b) Use of biochar-compost to improve properties and productivity of the degraded coastal soil in the Yellow River Delta, China. *Journal of Soils and Sediments* 17, 780-789.

Luo X., Wang L., Liu G., Wang X., Wang Z. & Zheng H. (2016c) Effects of biochar on carbon mineralization of coastal wetland soils in the Yellow River Delta, China. *Ecological Engineering* 94, 329-336.

Mcleod E., Chmura G.L., Bouillon S., Salm R., Björk M., Duarte C.M., ... & Silliman B.R. (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO_2. *Frontiers in Ecology and the Environment* 9, 552-560.

Nelson M.B., Martiny A.C. & Martiny J.B. (2016) Global biogeography of microbial nitrogen-cycling traits in soil. *Proceedings of the National Academy of Sciences* 113, 8033-8040.

Nie M., Zhang X., Wang J., Jiang L., Yang J., Quan Z., ... & Li B. (2009) Rhizosphere effects on soil bacterial abundance and diversity in the Yellow River Deltaic ecosystem as influenced by petroleum contamination and soil salinization. *Soil Biology and Biochemistry* 41, 2535-2542

Novak J.M. & Busscher W.J. (2013) Selection and use of designer biochars to improve characteristics of southeastern USA Coastal Plain degraded soils. In *Advanced biofuels and bioproducts* (ed. L.W. James), pp. 69-96. Springer Press, New York.

Qin P., Han R., Zhou M., Zhang H., Fan L., Seliskar D.M. & Gallagher J.L. (2015) Ecological engineering through the biosecure introduction of *Kosteletzkya virginica* (seashore mallow) to saline lands in China: A review of 20 years of activity. *Ecological Engineering* 74, 174-186.

Ray D.K., Gerber J.S., MacDonald G.K. & West P.C. (2015) Climate variation explains a third of global crop yield variability. *Nature Communications* 6.

Rutgers M., Wouterse M., Drost S.M., Breure A.M., Mulder C., Stone D., ... & Bloem J. (2016) Monitoring soil bacteria with community-level physiological profiles using Biolog™ ECO-plates in the Netherlands and Europe. *Applied Soil Ecology* 97, 23-35.

Sarkhot D.V., Berhe A.A. & Ghezzehei T.A. (2012) Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics. *Journal of Environmental Quality* 41, 1107-1114.

Sifleet S., Pendleton L. & Murray B.C. (2011) State of the Science on Coastal Blue Carbon. A Summary for Policy Makers. *Nicholas Institute Report* 11-06.

Sizmur T., Quilliam R., Puga A.P., Moreno-Jiménez E., Beesley L., & Gomez-Eyles J.L. (2016) Application of biochar for soil remediation. In *Agricultural and Environmental Applications of Biochar: Advances and Barriers* (eds M. Guo, Z. He & S. M. Uchimiya), pp. 295-324. Soil Science Society of America Inc., San Diego.

Smets W., Leff J.W., Bradford M.A., McCulley R.L., Lebeer S. & Fierer N. (2016) A method for simultaneous measurement of soil bacterial abundances and community composition via 16S rRNA gene sequencing. *Soil Biology and Biochemistry* 96, 145-151.

Smider B. & Singh B. (2014) Agronomic performance of a high ash biochar in two contrasting soils. *Agriculture, Ecosystems and Environment* 191, 99-107.

Song Y., Zhang X., Ma B., Chang S. & Gong J. (2014) Biochar addition affected the dynamics of ammonia oxidizers and nitrification in microcosms of a coastal alkaline soil. *Biology and Fertility of Soils* 50, 321-332.

Spokas K.A., Cantrell K.B., Novak J.M., Archer D.W., Ippolito J.A., Collins H.P., ... & Lentz, R.D. (2012) Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *Journal of Environmental Quality* 41, 973-989.

Stocker T.F., Qin D., Plattner G.K., Tignor M., Allen S.K., Boschung J., ... & Midgley P.M. (Eds.) (2013) IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Taylor R.G., Scanlon B., Döll P., Rodell M., Van Beek R., Wada Y., ... & Konikow L. (2013) Ground water and climate change. *Nature Climate Change* 3, 322-329.

Trenberth K.E., Dai A., van der Schrier G., Jones P.D., Barichivich J., Briffa K.R. & Sheffield J. (2014) Global warming and changes in drought. *Nature Climate Change* 4, 17-22.

Trivedi P., Anderson I.C. & Singh B.K. (2013) Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiology* 21, 641-651.

Vaccari F.P., Maienza A., Miglietta F., Baronti S., Di Lonardo S., Giagnoni L., ... & Valboa G. (2015) Biochar stimulates plant growth but not fruit yield of processing tomato in a fertile soil. *Agriculture, Ecosystems and Environment* 207, 163-170.

Van Zwieten L., Kimber S., Morris S., Chan K.Y., Downie A., Rust J., ... & Cowie A. (2010) Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant and Soil* 327, 235-246.

Wang Z., Zong H., Zheng H., Liu G., Chen L. & Xing B. (2015) Reduced nitrification and abundance of ammonia-oxidizing bacteria in acidic soil amended with biochar. *Chemosphere* 138, 576-583.

Wheeler T. & Von Braun J. (2013) Climate change impacts on global food security. *Science* 341, 508-513.

White R.G. & Kirkegaard J.A. (2010) The distribution and abundance of wheat roots in a dense, structured subsoil–implications for water uptake. *Plant, Cell and Environment* 33, 133-148.

Whitman T., Pepe-Ranney C., Enders A., Koechli C., Campbell A., Buckley D.H. & Lehmann J. (2016) Dynamics of microbial community composition and soil organic carbon mineralization in soil following addition of pyrogenic and fresh organic matter. *The ISME Journal* 10.

Wu C., Niu Z., Tang Q. & Huang W. (2008) Estimating chlorophyll content from hyperspectral vegetation indices: Modeling and validation. *Agricultural and Forest Meteorology* 148, 1230-1241.

Wu Y., Xu G., Sun J. & Shao H. (2014) Does thermal carbonization (Biochar) of organic material increase more merits for their amendments of sandy soil?. *Solid Earth Discussions* 6, 535-558.

Xu C., Hosseini-Bai S., Hao Y., Rachaputi R.C., Wang H., Xu Z. & Wallace H. (2015) Effect of biochar amendment on yield and photosynthesis of peanut on two types of soils. *Environmental Science and Pollution Research* 22, 6112-6125.

Xu H., Wang X., Li H., Yao H., Su J. & Zhu Y. (2014) Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. *Environmental Science and Technology* 48, 9391-9399.

Ye L. & Van Ranst E. (2009) Production scenarios and the effect of soil degradation on long-term food security in China. *Global Environmental Change* 19, 464-481.

Zhang T., Wang T., Liu K., Wang L., Wang K. & Zhou Y. (2015) Effects of different amendments for the reclamation of coastal saline soil on soil nutrient dynamics and electrical conductivity responses. *Agricultural Water Management* 159, 115-122.

Zhang X., Luo Y., Müller K., Chen J., Lin Q., Xu J., ... & Wang H. (2016) Research and Application of Biochar in China. In *Agricultural and Environmental Applications of Biochar: Advances and Barriers* (eds M. Guo, Z. He & S. M. Uchimiya), pp. 377-408. Soil Science Society of America Inc., San Diego.

Zheng H., Wang Z., Deng X., Herbert S. & Xing B. (2013a) Impacts of adding biochar on nitrogen

retention and bioavailability in agricultural soil. *Geoderma* 206, 32-39.

Zheng H., Wang Z., Deng X., Zhao J., Luo Y., Novak J., ... & Xing B. (2013b) Characteristics and nutrient values of biochars produced from giant reed at different temperatures. *Bioresource Technology* 130, 463-471.

Table 1. Selected chemical and physical characteristics of the soil and biochar samples

Soil		Biochar	
pH	7.98±0.03	pH	9.43±0.04
EC/dS m^{-1}	1.00±0.01	EC/dS m^{-1}	5.53±0.45
Ex-Na/cmol kg^{-1}	1.60±0.16	C/%	55.5±0.6
ESP/%	59.0±3.6	H/%	2.38±0.00
SOM/g kg^{-1}	9.75±0.31	N/%	1.54±0.01
TN/g kg^{-1}	0.45±0.21	O/%	12.3±0.1
NH_4^+-N/mg kg^{-1}	22.2±2.0	S/%	0.34±0.03
NO_3^--N/mg kg^{-1}	7.12±0.27	NH_4^+-N/mg kg^{-1}	8.81±0.21
Olsen-P/mg kg^{-1}	11.2±2.0	NO_3^--N/mg kg^{-1}	1.54 ±0.41
CEC/cmol kg^{-1}	2.72±0.32	Olsen-P/mg kg^{-1}	77.2±6.9
WHC/%	42.4±0.6	CEC/cmol kg^{-1}	7.39±0.15
Sand /%	2.20	WHC/g g^{-1}	1.04±0.15
Silt/%	79.3	Zeta potential/mV	-36.8±0.2
Clay/%	18.5	Ash/%	28.5±0.8
		Water-extractable nutrients	
		organic C (g kg^{-1})	3.85±0.14
		N (mg kg^{-1})	2.05±0.37
		P (mg kg^{-1})	128±1.23
		Ca (mg kg^{-1})	88.2±3.05
		Mg (mg kg^{-1})	47.3±2.15
		S (mg kg^{-1})	2.12±0.22
		S_{BET}/m^2 g^{-1}	9.18
		Micropore area/m^2 g^{-1}	6.99
		Mesopore area/m^2 g^{-1}	5.20
		Total pore volume/cm^3 g^{-1}	0.03
		Average pore width/nm	14.7

Table 2. Growth indices of the two halophytes in the pot experiment

Treatments		Root				Leaf	
		Length cm	SA[β] cm^2	AD mm	Tips	LSA/cm^2	ALW/cm
Sesbania	CK[α]	260±69c[γ]	37±10e	0.46±0.00a	219±73d	185±210b	4.19±1.59b
	BC-1.5%	497±70b	363±12b	2.35±0.27c	721±74a	389±211ab	5.50±2.01ab
	BC-5%	575±20ab	371±2b	2.06±0.07b	740±24a	489±23a	6.62±0.35a
	BC-10%	683±51ab	399±11ab	1.86±0.09b	772±78a	496±12a	6.81±0.32a
	CKF	522±85ab	380±11b	2.35±0.31c	587±62b	227±49b	4.42±0.85b
	BCF-1.5%	319±74b	168±11c	1.72±0.25b	430±55c	464±127a	6.56±0.81a
	BCF-5%	690±92a	418±27a	1.94±0.15bc	675±29ab	541±61a	6.72±1.07a
	BCF-10%	555±238ab	88±20d	0.55±0.15a	651±147ab	459±81a	7.00±0.83a
Seashore mallow	CK	417±68c	61±8c	0.47±0.02b	430±178b	199±50c	7.70±1.51ab
	BC-1.5%	407±84c	63±11c	0.50±0.05ab	497±127b	278±25bc	7.90±0.63ab
	BC-5%	666±268b	100±40b	0.48±0.01b	680±243ab	299±104b	8.08±0.83ab
	BC-10%	993±44a	148±7a	0.47±0.03b	905±123a	345±42ab	8.61±0.75a
	CKF	429±83c	68±15bc	0.50±0.03ab	697±318ab	244±59bc	7.10±0.49b
	BCF-1.5%	590±55bc	98±9bc	0.53±0.04a	672±111ab	424±44a	8.12±0.47ab
	BCF-5%	721±170b	117±26ab	0.52±0.01ab	1048±180a	396±44ab	7.85±0.43ab
	BCF-10%	808±103ab	126±23ab	0.50±0.04ab	974±383a	313±55b	7.15±0.50b

[α] CK, BC-1.5%, BC-5% and BC-10% indicate that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively;

[β] SA: surface area; AD: average diameter; LSA: leaf surface area; ALW: average leaf width;

[γ] Different small letter behind the values indicated significant difference between different treatments ($P < 0.05$).

Table 3. The properties of the biochar-amended soils after plant growth for 52 days

	SOM (g kg^{-1})	C/N	TN (g kg^{-1})	TP (g kg^{-1})	CEC (cmol kg^{-1})	Ex-Na (cmol kg^{-1})	ESP (%)	SA-N_2 (m^2 g^{-1})	SA-CO_2 (m^2 g^{-1})
CK[α]	11.1±0.4e[β]	12.3±0.6ef	0.45±0.03a	184±7.7a	2.72±0.32b	1.60±0.16a	61±1a	23.61	50.8
BC-1.5%	15.0±0.6d	18.6±5.0e	0.47±0.06a	198±4.7a	3.05±0.08a	1.55±0.33a	44±5c	22.56	65
BC-5%	17.0±1.2c	37.1±4.4c	0.18±0.00b	208±3.8b	3.13±0.03a	1.54±0.09a	51±1bc	22.42	71.5
BC-10%	21.2±0.8b	51.8±1.6a	0.13±0.08c	215±7.7b	3.12±0.07a	1.64±0.17a	55±1b	22.1	74.6
CKF	10.0±0.6e	11.1±1.6f	0.76±0.13a	189±5.6a	3.05±0.06a	1.86±0.13a	63±4a	ND[γ]	ND
BCF-1.5	14.0±1.8d	26.3±7.7d	0.45±0.06b	202±5.6b	3.07±0.14a	1.70±0.19a	59±2ab	ND	ND
BCF-5%	21.0±0.0b	45.0±0.3b	0.25±0.07d	216±7.6b	3.18±0.03a	1.54±0.29a	53±3b	ND	ND
BCF-10%	23.9±1.5a	57.6±2.0a	0.42±0.03c	244±10.3b	3.14±0.16a	1.76±0.13a	55±1b	ND	ND

[α]CK, BC-1.5%, BC-5% and BC-10% indicate that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively; CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively;

[β]Different small letters behind the values in the same column indicate significant difference between different treatments ($P < 0.05$);

[γ] ND indicate that the data was not detected.

Table 4. pH and EC values of the rhizosphere and non-rhizosphere soils with Sesbania and Seashore mallow

	Treatments	Sesbania		Seashore mallow	
		Rhizosphere	Non-rhizosphere	Rhizosphere	Non-rhizosphere
pH	CK[α]	7.77±0.11a	7.87±0.01ab	7.74±0.01a	7.75±0.02ab
	BC-1.5%	7.78±0.07a	7.82±0.02bcd	7.70±0.06a	7.78±0.08a
	BC-5%	7.84±0.05a	7.85±0.03abc	7.70±0.05a	7.72±0.07abc
	BC-10%	7.79±0.07a	7.91±0.04a	7.68±0.09a	7.70±0.10abc
	CKF	7.64±0.09b	7.87±0.01ab*	7.68±0.05a	7.64±0.04bc
	BCF-1.5%	7.58±0.04b	7.78±0.09d*	7.66±0.05a	7.62±0.10c
	BCF-5%	7.63±0.03b	7.78±0.02cd*	7.55±0.05b	7.76±0.01ab*
	BCF-10%	7.77±0.04a	7.86±0.03ab*	7.71±0.04a	7.81±0.03a*
EC dS m^{-1}	CK	0.95±0.02b	1.06±0.09ab	0.94±0.04a	1.11±0.07ab*
	BC-1.5%	0.78±0.02c	0.80±0.02e	0.81±0.09bc	0.81±0.08d
	BC-5%	0.76±0.02c	0.91±0.02de*	0.64±0.06d	0.88±0.06cd*
	BC-10%	0.80±0.15bc	1.04±0.04abc	0.87±0.04ab	0.98±0.04bc*
	CKF	1.02±0.08a	1.16±0.08a	0.94±0.07a	1.25±0.08a*
	BCF-1.5%	0.82±0.05bc	0.93±0.04cde	0.72±0.02cd	0.99±0.13bc*
	BCF-5%	0.72±0.16c	0.92±0.05cde	0.75±0.07c	0.82±0.02d
	BCF-10%	0.94±0.01b	1.03±0.13bcd	0.87±0.02ab	1.02±0.09bc*

[α]CK, BC-1.5%, BC-5% and BC-10% indicated that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively; CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively;

[β]Different small letters behind the values in the same column indicate significant difference between different treatments ($P < 0.05$);

[γ]Asterisks indicate significant difference between the rhizosphere and non-rhizosphere soils ($P < 0.05$).

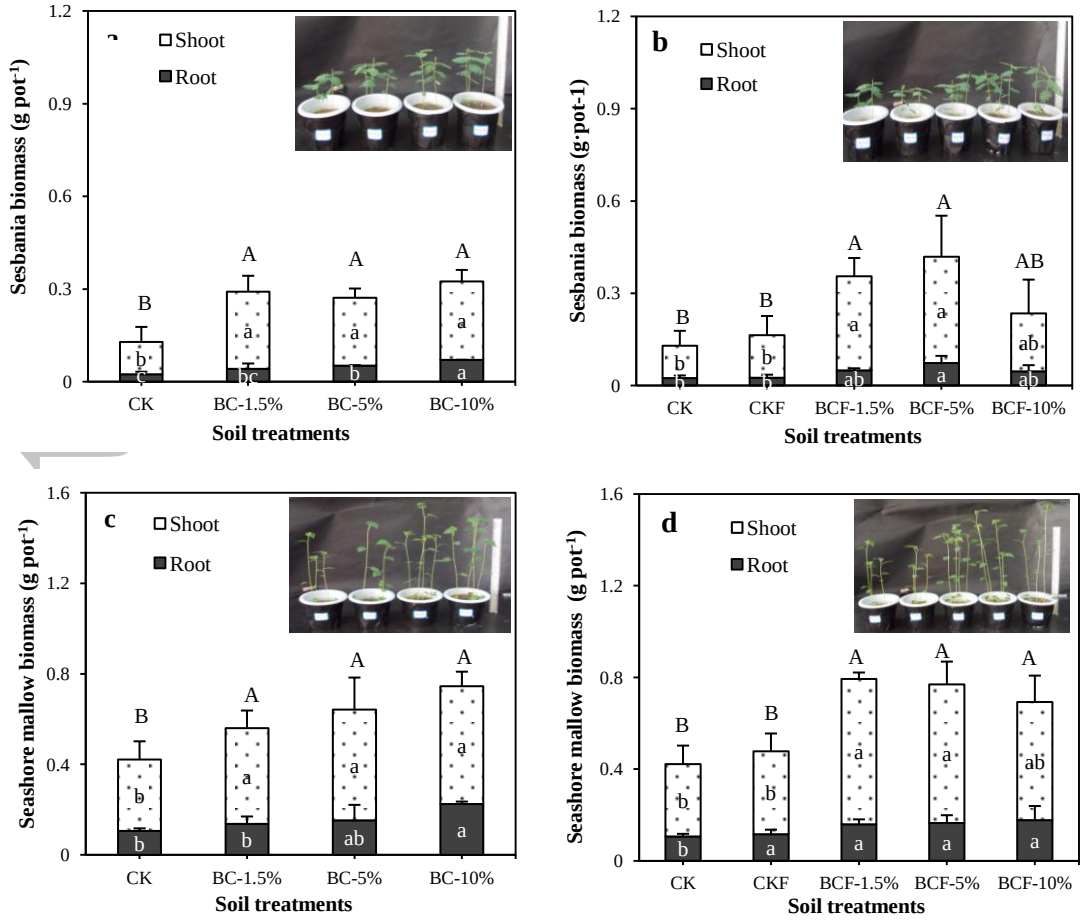

Figure 1. Effect of biochar addition on biomass of Sesbania (a, b) and Seashore mallow (c, d). CK, BC-1.5%, BC-5% and BC-10% indicate that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. Different small and capital letters indicate significant difference between different treatments ($P < 0.05$). The inserted photos showed the plants growth at day 52, and a 30-cm ruler was used as a reference.

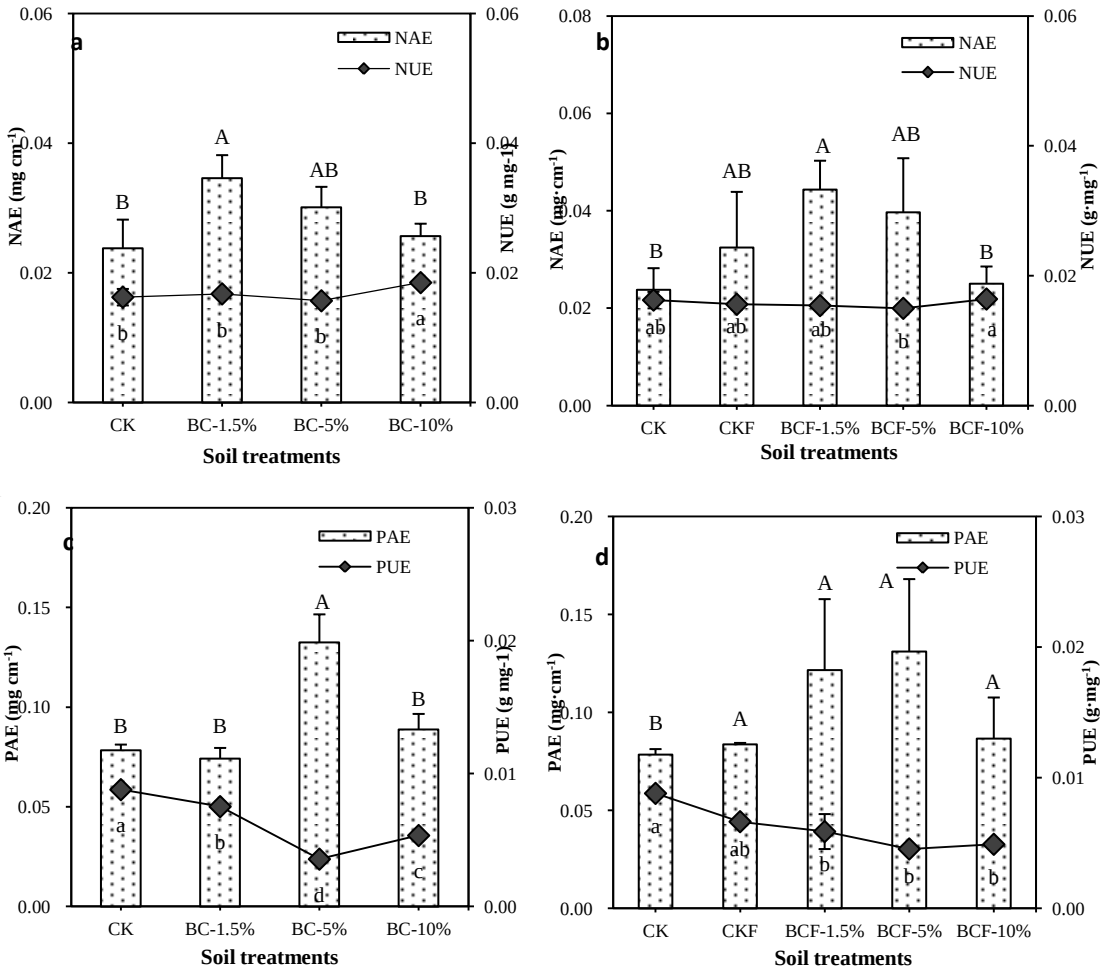

Figure 2. Effect of biochar addition on accumulation and utilization efficiency of N (a, b), and accumulation and utilization efficiency of P (c, d) for Sesbania. NAE/PAE: N/P accumulation efficiency, the amount of N/P intake per unit of root length. NUE/PUE: N/P utilization efficiency, the amount of biomass produced by per unit of N/P. CK, BC-1.5%, BC-5% and BC-10% indicate that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. Different small and capital letters indicate significant difference between different treatments ($P < 0.05$).

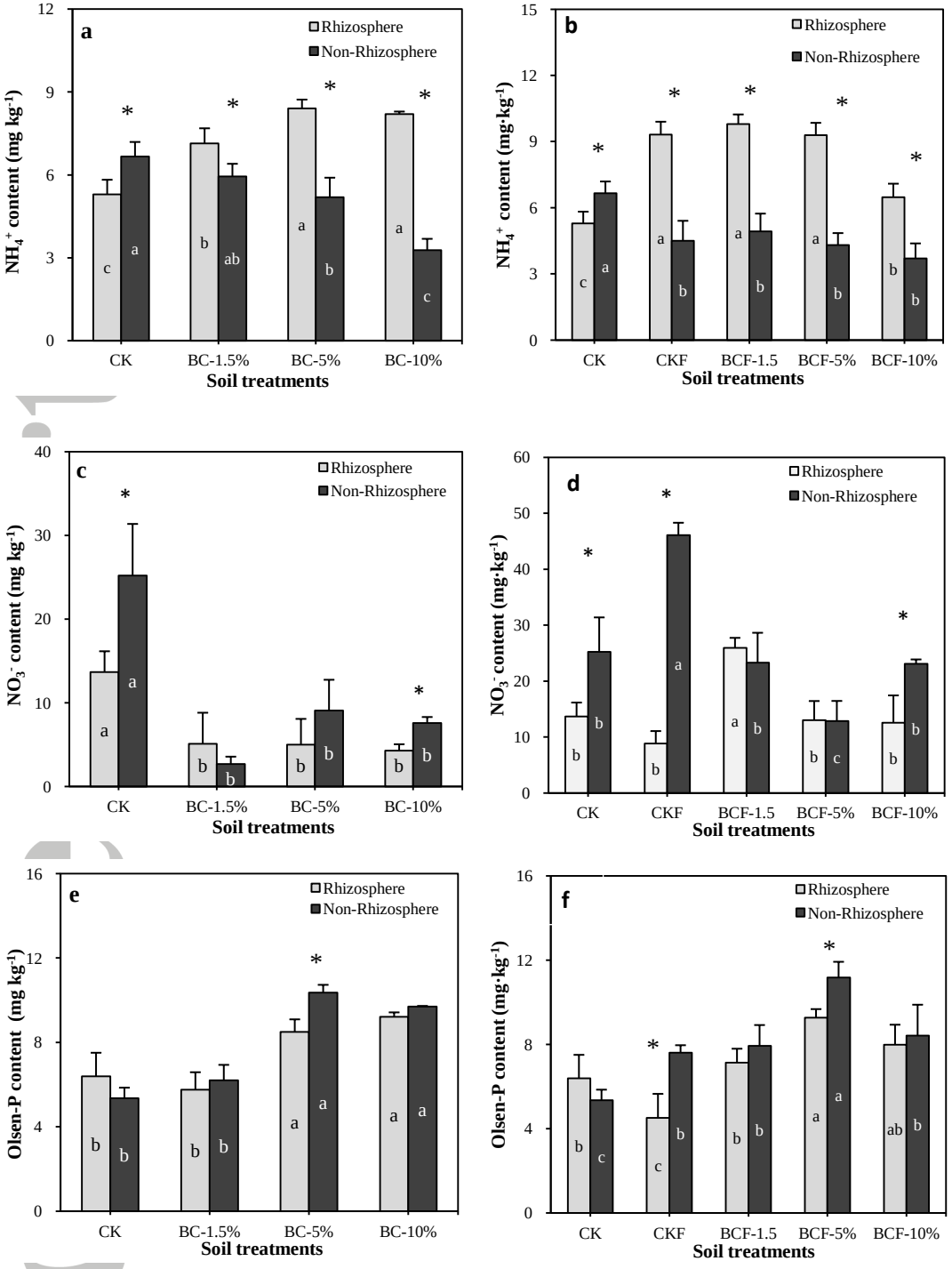

Figure 3. Effect of biochar addition on N and P availability in the soils with Sesbania. CK, BC-1.5%, BC-5% and BC-10% indicate that the soil was amended with the biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. CKF, BCF-1.5%, BCF-5% and BCF-10% indicate that the soil was amended with the fertilizer and biochar at rates of 0%, 1.5%, 5% and 10% (w/w), respectively. Different small letters indicate significant difference between the soil treatments, and asterisks indicate significant difference between the rhizosphere and non-rhizosphere soils ($P < 0.05$).

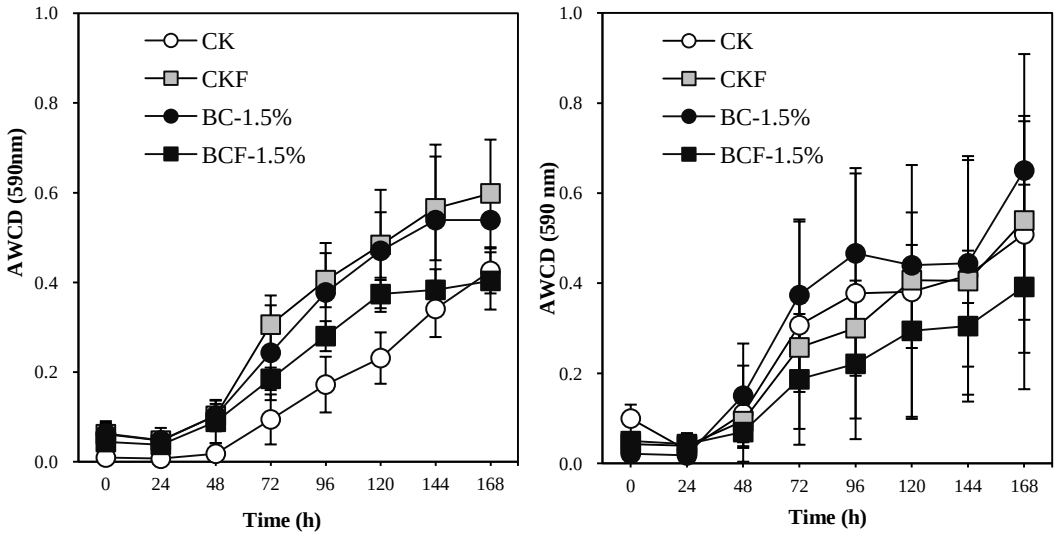

Figure 4. Average well color development (AWCD) of metabolized substrates in Biolog ECO plates for microbial community in the (a) rhizosphere and (b) non-rhizosphere soils grown with Seashore mallow based on 168-h incubation ($n = 3$). CK and BC-1.5% indicate that the soil was amended with the biochar at rates of 0% and 1.5% (w/w), respectively. CKF and BCF-1.5% indicate that the soil was amended with the fertilizer and biochar at rates of 0% and 1.5% (w/w), respectively.

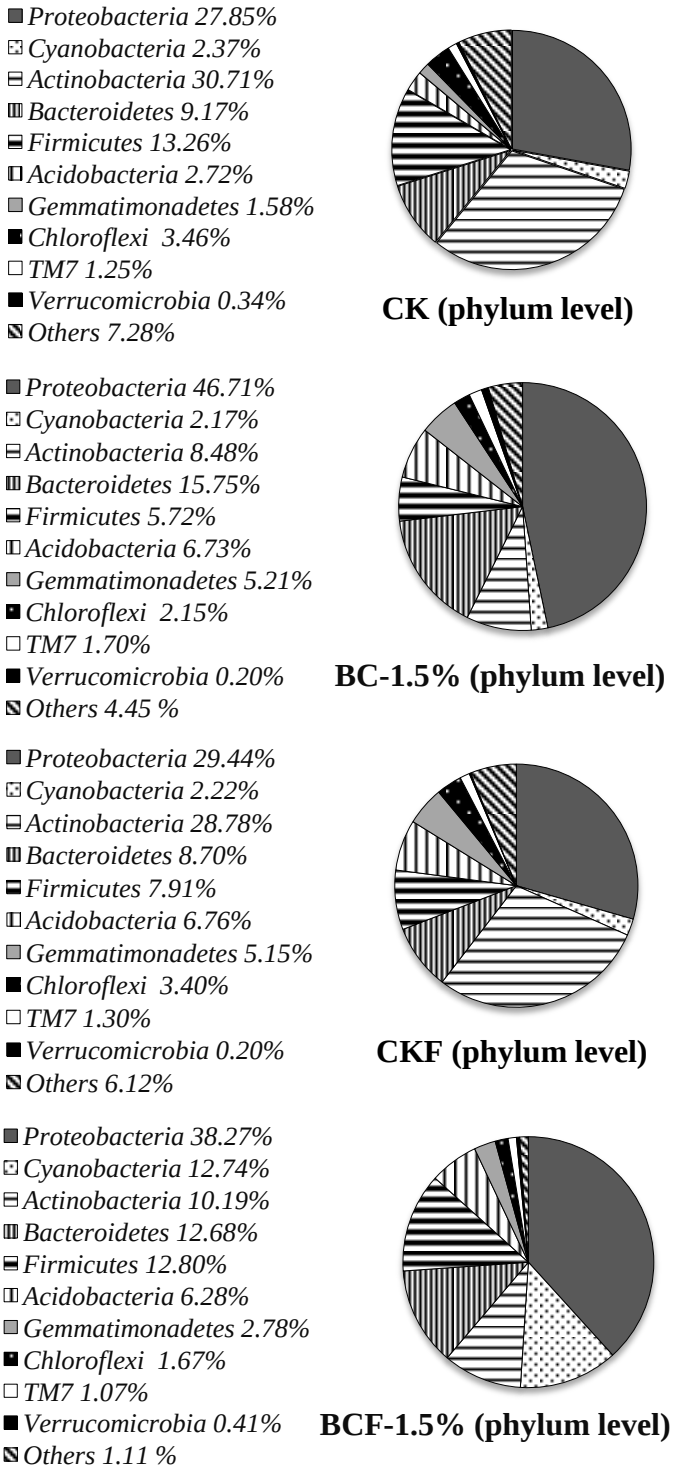

Figure 5. Taxonomic classification of the pyrosequencing results from the bacterial communities in the rhizosphere soils grown with Seashore mallow at the phylum levels. CK and BC-1.5% indicate that the soil was amended with the biochar at rates of 0% and 1.5% (w/w), respectively. CKF and BCF-1.5% indicate that the soil was amended with the fertilizer and biochar at rates of 0% and 1.5% (w/w), respectively. The phyla accounted for less than 1% of the total composition in each library were represented by "others".

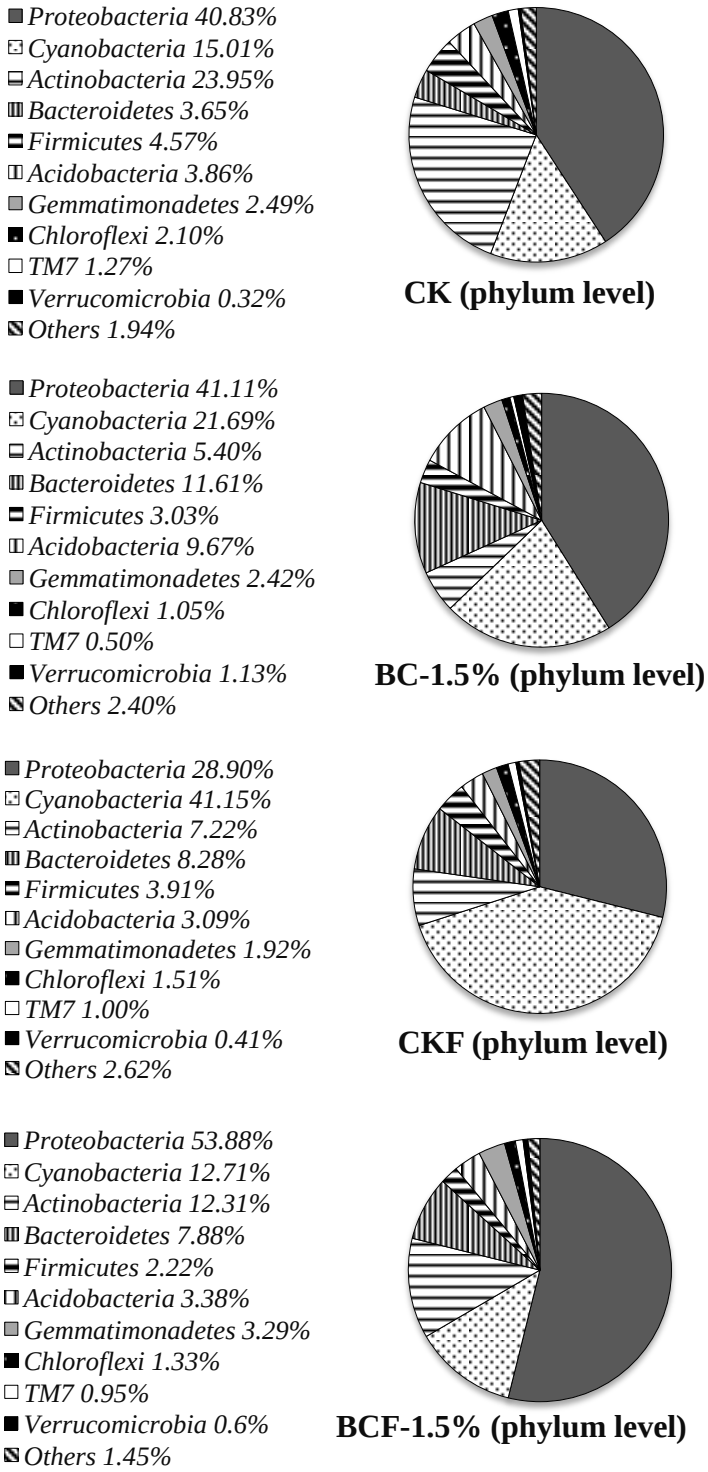

Figure 6. Taxonomic classification of the pyrosequencing results from the bacterial communities in the non-rhizosphere soils grown with Seashore mallow at the phylum levels. CK and BC-1.5% indicate that the soil was amended with the biochar at rates of 0% and 1.5% (w/w), respectively. CKF and BCF-1.5% indicate that the soil was amended with the fertilizer and biochar at rates of 0% and 1.5% (w/w), respectively. The phyla accounted for less than 1% of the total composition in each library were represented by "others".

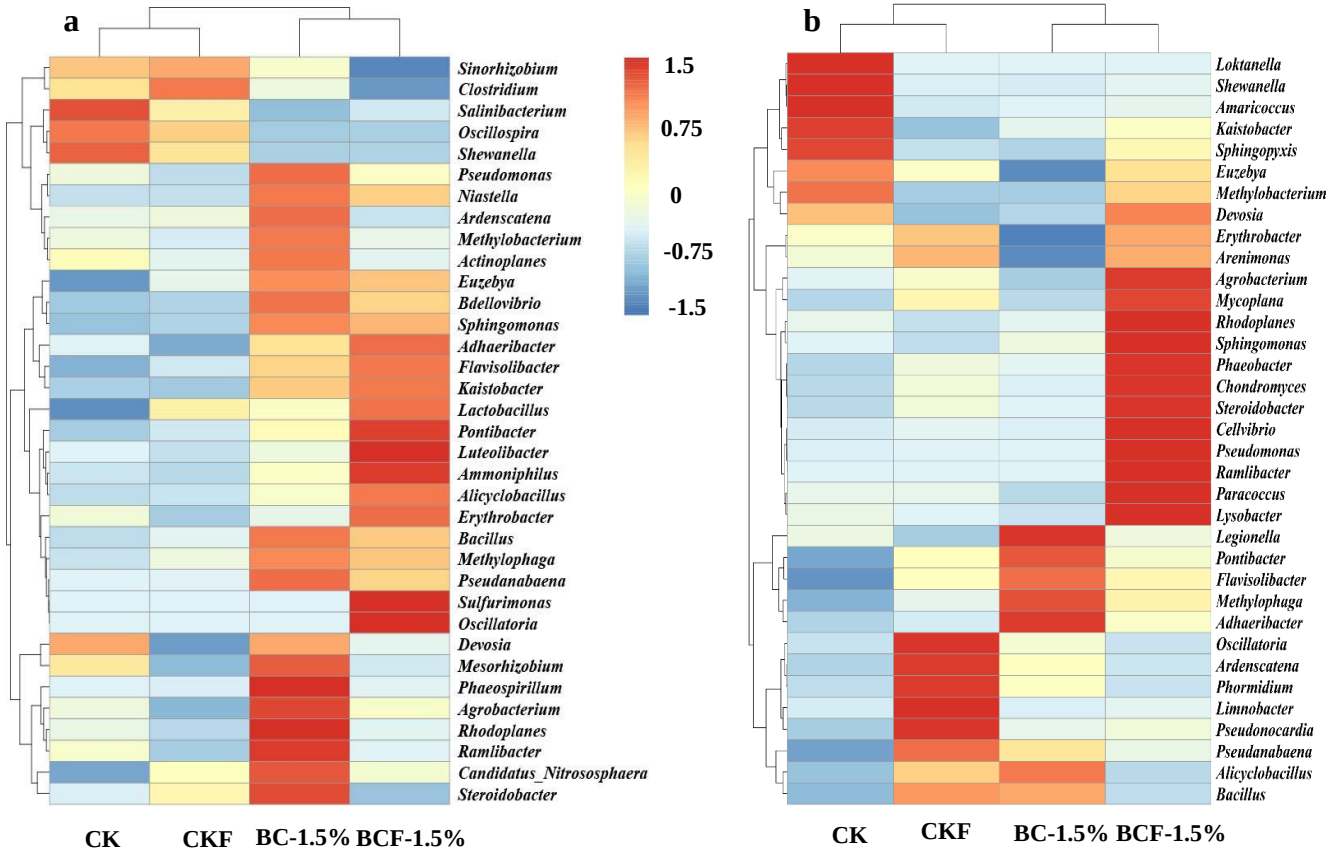

Figure 7. Hierarchical cluster analysis of the 35 most abundant genera in the rhizosphere (a) and non-rhizosphere soils (b) with Seashore mallow. The relationship among samples was determined using the Bray-Curtis distance and the complete clustering method. The color intensity of the scale demonstrated the relative abundance of each genus. Relative abundance was defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%). CK and BC-1.5% indicate that the soil was amended with the biochar at rates of 0% and 1.5% (w/w), respectively. CKF and BCF-1.5% indicate that the soil was amended with the fertilizer and biochar at rates of 0% and 1.5% (w/w), respectively.