European Journal of Soil Biology 70 (2015) 67–76

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China

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ARTICLE INFO

Article history:
Received 10 February 2015
Received in revised form
14 July 2015
Accepted 18 July 2015
Available online xxx

Keywords:
Salt stress
Biochar
Poultry manure
Pyroligneous solution
Enzyme activity
Microbial abundance
Microbial community structure

ABSTRACT

Salt stress has been increasingly constraining crop productivity in arid and semiarid lands of the world. In a previous study, salt stress was alleviated and maize productivity improved remarkably with soil amendment with biochar poultry-manure compost (BPC) in conjunction with pyroligneous solution (PS) in a saline soil from Central China Plain. In 2010, before maize sowing, BPC was incorporated into topsoil at 12 t ha^{-1} following surface spray of diluted PS solution at 0.15 t ha^{-1} one week in advance (BPC-PS2). Such an experiment was repeated in adjacent fields in 2011 (BPC-PS1). Both bulk and rhizosphere samples of these experiment plots were collected at the vegetative growth stage of maize in 2012. Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), and soil enzyme activity were measured. Based on 16S rRNA and 18S rRNA gene, bacterial and fungal community structure and abundance were respectively characterized using denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR (qPCR). With the amendment, C_{mic} and N_{mic}, and bacterial gene abundance were significantly and greatly increased in both bulk and rhizosphere samples, being greater under BPC-PS2 than under BPC-PS1. On contrast, smaller increase in fungal gene abundance was observed, along with a significant reduction in fungal diversity under BPC-PS2. In addition, two single bands belonging respectively to *Alphaproteobacteria* and *Deltaproteobacteria* emerged in the amended soil. Meanwhile, activities of urease, invertase and phosphatase in both bulk soils and rhizosphere soils were increased by 19–44% with the amendment except of urease in rhizosphere soils. Therefore, with the great enhancement of microbial growth and enzyme activities, combined use of biochar and poultry manure with pyroligneous solution could be a practical option to alleviate salt stresses on plant and soil microbial community in order to improve crop production in saline soils.

1. Introduction

Increasing salinity has been a critical problem in arid and semi-arid area of the world [1,2], which would challenge global food production in the mid-21st century with climate change [3]. Soil salinity constrains crop growth and development [3] with stresses of high osmotic potential on water and nutrient uptake by plants [4], restricts microbial growth and biochemical functioning [5]. Increased salinity could lead to reductions in soil microbial biomass, metabolical efficiency as well as deterioration of soil biophysical properties [6,7].

Biochar, produced via pyrolysis of biomass under limited oxygen, recalcitrant carbon-rich material with more or less nano-sized pore structure [8]. Generally, biochar soil amendment (BSA) helps improving soil structure, soil water retention and soil tilth [9,10]. Moreover, BSA is generally effective for promoting microbial

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http://dx.doi.org/10.1016/j.ejsobi.2015.07.005
1164-5563/

growth [11], improving nutrient availability, thus enhancing crop productivity [12]. BSA has been widely recommended for promoting carbon sequestration and mitigating N_2O emission from croplands [13,14]. However, they have been increasing studies showing biochar's role in enhancing resilience of biological systems, including enhanced microbial and plant health [15], enhanced systemic resistance to pathogens and diseases [16] as well as promoted seed germination and plant development [17,18]. Therefore, BSA could be potentially used for restore soil fertility and microbial activity in salt-stressed agricultural soils [19].

Pyroligneous solution (PS) is a mixture of volatilized substances captured during pyrolysis of plant residues [20]. In slightly acid reaction, PS is generally a mixture of reacting organic compounds with relatively small molecular weights (mainly organic acids and phenolic compounds) [21]. And PS has been shown beneficial for crop performance and the tolerance to pest and disease infection [22]. BSA supplemented with PS reduced salinity and soil pH, and increased crop yield in a salt-stressed soil [10]. Under this combined amendment, improvement was observed of plant growth and leaf biological activity of maize on the soil [23]. However, how this combined amendment affect microbial growth, activity and community composition in the treated salt-stressed soil has not yet been examined.

In this study, we hypothesize that biotic/abiotic stresses to soil microbial community will be alleviated and thus microbial growth and activity improved in the salt stressed soil treated with soil amendment with biochar composted with poultry manure and supplemented with PS field experiments for years. This is now tested by characterizing the changes with the treatment in microbial biomass carbon and nitrogen by chemical assay, in gene abundance and in community structure by molecular biology assay and in enzyme activities by biochemical assay in this study. We aim to address improving soil biological quality and functioning and the recovery of salt-stressed soils for better crop productivity with biochar products.

2. Materials and methods

2.1. Experimental site

The field experimental site was the same as reported in previous work [10,23], and is situated in Kangzhuang Village (34°32′N, 115°30′E), Liangyuan District, Shangqiu Municipality, Henan, China. Located in the center of North China Great Plain, the local area has a semi-humid temperate monsoon climate. For the period of 2008–2012, the average mean annual temperature was 13.9 °C and total potential evaporation was 1735 mm. Annual precipitation was 770 mm and 785 mm in 2011 and in 2012, respectively. In addition, the area occupied a total sunshine time of 2510 h and 230 frost-free days annually. The soil was classified as Aqui-Entisol [24] formed on paleo-sediments of Yellow River. The soil is saline with a slightly alkaline reaction with a high cation exchange capacity while it is low in organic carbon content and moderately compacted (Table 1). Rotation of summer maize with wheat in winter has been practiced as conventional cropping system since the 1980s in the region.

2.2. Biochar and pyroligneous solution

Biochar used for the field experiment was produced through pyrolysis of wheat straw at 450 °C in a vertical kiln at Sanli New Energy Company in Shangqiu, China. Approximately 350 kg of biochar and 250 L of PS are produced per ton of wheat straw dry matter [20]. PS was obtained via condensation with cycling water of volatile organic compounds released in pyrolysis and stored in a closed underground tank. And PS was a mixture containing mainly ethylene, phenolic and ketones groups. The detail chemical composition of PS [21] and basic properties of the biochar [25] has already been reported. Here, Table 1 also contained some information. Prior to use for composting, biochar was ground to pass a 2-mm sieve and homogenized thoroughly. The PS was diluted 5-fold in distilled water before use.

2.3. Biochar poultry manure compost (BPC)

For production of biochar manure compost, poultry manure was collected from a local poultry farm and placed in open-air storage for a week under ambient conditions to air dry. The poultry manure (PM) was then mixed with biochar (BC) at a ratio of 1:3 (PM: BC, v/v) for composting for 6 weeks. The produced biochar poultry manure compost (BPC) was thoroughly mixed prior to its use as an amendment. The compost was a dark neutral loose organic material (pH 7.5 in water) containing 419.7 g kg^{-1} of organic carbon, 25.0 g kg^{-1} of total N, and 0.82 g kg^{-1} of alkaline-releasable N, 12.2 mg kg^{-1} of Olsen-P and 0.83 mg kg^{-1} of NH_4NO_3 exchangeable K (Table 1).

2.4. Experiment design

A field experiment using a combined amendment of BPC-PS was conducted on an abandoned salt-affected cropland in 2010. BPC was broadcast at 12 t ha^{-1} one week after spraying of the diluted PS at 0.15 t ha^{-1} on soil surface, after which maize was sown one week later. The broadcast BPC was thoroughly mixed with the topsoil by ploughing to a depth of 20 cm and then discing to homogeneity. A control with ploughing and discing but without this amendment was set up for comparison. A similar treatment was performed in an adjacent un-reclaimed soil in 2011 before wheat sowing (BPC-PS1) and was compared to the BPC-PS2 field amended in 2010. No more BPC but the same dosage of PS was applied to BPC-PS2 plots in 2011.

Maize cultivar Zheng Dan 958 was directly sown after the wheat harvest each year. A based application of phosphorus and potassium fertilizer was applied at the time of sowing. One third of total nitrogen fertilizer was applied as basal and the remaining was applied twice as a top dressing. The conventional practice in the region is to return the crop straw by ploughing it into the soil.

All of the treatments were performed in triplicate and the plots were arranged in a complete randomized block design. Each individual treatment plot had an area of 0.15 ha and was separated by surrounding border rows to avoid possible surface water fluxes across plots. The crop growth management was consistent across the plots. No irrigation was performed during maize production as the cropland was rain fed during this crop production period.

2.5. Soil sampling

Bulk and rhizosphere soils were collected at the vegetative growth stage of maize plants. Composite bulk topsoil (0–10 cm) was collected from six random locations in each plot using an *S*-shaped pattern. Cores were taken using an Eijkelkamp soil core sampler. At the same time, six random maize plants were excavated from each plot and a composite rhizosphere soil was obtained following the procedure used by Butler and co-authors [26]. Samples were sealed in plastic bags, stored on ice and shipped to laboratory within 24 h. Samples were stored at −20 °C for microbial community structure analysis and at 4 °C for soil enzyme activity and microbial C and N analyse, respectively. For DNA extraction soil samples were processed within one week of storage.

Table 1
Basic properties of the topsoil (0–20 cm), biochar and pyroligneous solution and biochar poultry manure compost before they were used for the experiment.

Sample	pH (H_2O)	TOC (g kg^{-1})	Total N (g kg^{-1})	Salt (g kg^{-1})	CEC (cmol kg^{-1})	Bulk density (g cm^{-3})
Top soil	8.25	5.13	0.70	12.68	21.26	1.33
Biochar	10.35	467	5.90	41.97	21.70	0.65
PS	9.37	3.87	0.55	ND	ND	ND
BPC	7.50	419	25.0	ND	ND	1.00

TOC total organic carbon; CEC cation exchange capacity, ND not determined.

2.6. Determination of microbial biomass carbon and nitrogen

Aliquots of the fresh soil samples were used for the determination of microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) with a modified fumigation extraction procedure as described by Vance and co-authors [27]. In brief, three subsamples of fresh moist soil (equivalent to 5.0 g dry soil) from each plot were fumigated for 24 h with ethanol-free chloroform ($CHCl_3$) at 25 °C in dark. The samples were shaken for 30 min with a rotatory shaker at 180 rpm. Unfumigated aliquots of three sub-samples from each treated plot were processed as control. After extraction, the samples were immediately filtered through a double-layered filter paper. For C_{mic} measurement, an aliquot of extract was analysed using an automated TOC Analyzer (Shimadzu, TOC-500, Japan). Total N_{mic} in extracted aliquots was determined using the Kjeldahl method. The extracted C_{mic} was calculated using values for extracted carbon (EC) of the fumigated sample subtracted from the value of the unfumigated sample, then a ratio of 0.45 was used to convert the measured C to C_{mic} as proposed by Wu and co-authors [28]. Similarly, microbial biomass nitrogen (N_{mic}) was estimated using a ratio of 0.5 proposed by Joergensen and Mueller [29].

2.7. Soil enzymes activity measurement

Soil activities of invertase, urease and phosphatase were analysed using the protocols described by Tabatabai [30]. For invertase activity, an aliquot of fresh moist soil (equivalent to 0.5 g dry soil) was added with sucrose solution as substrate and samples were incubated at 37 °C for 24 h. Incubated samples were vigorously shaken for 30 min on a rotatory shaker and then filtered in clean sterilized vials. Aliquots of the extracts were measured after dilution and addition of 3,5-dinitrosalicylic acid solution on a spectrophotometer at 508 nm for glucose content. Similarly, urease activity was assessed using a 10% urea solution as a substrate and the sample was incubated at 37 °C for 24 h. The extracted solution of 3 ml was further diluted up to 50 ml after addition of phenol sodium hypochlorite solution. Content of ammonium in the diluted solution was measured with a spectrophotometer at 578 nm. Phosphatase activity was measured using a 0.5% di-sodium phenol phosphate extract after incubation at 37 °C for 24 h. The phosphate activity was measured in extracted aliquots after addition of disodium phenyl phosphate solution and the diluted sample was measured with a spectrophotometer at 510 nm. Soil enzyme activities for urease, invertase and phosphatase blanks were analysed at the same time using soil samples without substrate.

2.8. DNA extraction and real-time quantitative PCR

Total DNA was extracted from both bulk and rhizosphere 0.25 g soil samples using a PowerSoil™ DNA isolation kit (Mo Bio laboratories Inc., CA) following the manufacture's protocols. The gene copy numbers for bacteria (16S rRNA) and fungi (18S rRNA) were determined using an iCycler IQ5 Thermocycler (Bio-Rad, Hercules, CA). The protocols for extracted gene and its amplification followed the criteria of MIQE guidelines [31] for evaluation of the qPCR assays. The thermal conditions and primers were those used by Fierer and co-authors [32]. The concentrations of DNA extracts were measured at 260 nm with a UV-spectrophotometer (Bio Photometer, Eppendorf, Germany), and were adjusted to a concentration of 10 ng μl^{-1}. For PCR, an aliquot of 1 μl of DNA was used in 20 μl volumes containing 0.4 μl of each primer (10 μmol L^{-1}) and 10 μl of SYBR premix Ex Tag TM (Takara Shuzo, Shiga, Japan). Melting curve analyses of the PCR products were conducted following each assay to realize the fluorescence signal from specific PCR products to avoid artifacts from primer-dimers. The PCR products were analysed by electrophoresis on 1.5% agarose gels in 1 × TAE buffer to confirm appropriate size. A plasmid standard containing the target region was generated for each primer set using the DNA extract for each sample. Purification of the amplified PCR products for the bacterial 16S rRNA gene and fungal 18S rRNA gene were performed using a purification kit (Takara, Japan) and ligated into pEASY-T1 cloning vector (Promega, Madison, WI) and cloned into *Escherichia coli* DH5. Correct inserts containing clones were chosen as the standards for qPCR. The DNA plasmids were isolated using a plasmid extraction kit (Takara, Japan), and concentrations were determined by the spectrophotometry. As the actual sizes of vector and PCR products were known, copies of plasmid DNA could be calculated, then copy numbers of 16S rRNA and 18S rRNA genes would be worked out. Standard curves were drawn using triplicate 10-fold dilution of plasmid DNA ranging from 3.35×10^4 to 3.35×10^9 copies for the bacterial 16S rRNA gene and 4.59×10^3 to 4.59×10^8 copies of template for fungal 18S rRNA.

2.9. Bacterial and fungal community analysis using DGGE

Extracted total DNA from each sample was amplified with the 968F-GC and 1401R using specific set of primers for bacteria [33], and with the Fung-GC and NS1 specific primers set for fungi [34]. PCR reactions were performed with an Eppendorf autothermer Cycler (Bio-Rad) using 25 μl reaction mixtures containing 12.5 μl GoTaq® Green Master Mix (Promega), 0.5 μl of DNA template, 0.5 μl of each primer (10uM). DGGE analysis of the PCR products was performed using 8% w/v polyacrylamide gels containing denaturing gradients of 35–65%, for bacteria, and 20–40%, for fungi, using the DCode universal mutation detection system (Bio-Rad). The 100% pure denaturant contained 7 M urea and 40% deionized formamide. DGGE was performed using 20 μl of the PCR products, in 1 × TAE buffer at 60 °C 200 V for 5 min followed by 120 V for 10 h for bacteria and 140 V for 8 h for fungi. Gels were stained for 30 min with SYBR Green I (Lonza, Rockland, ME, USA) and scanned using a gel document system (Bio-Rad, USA).

2.10. Phylogenetic analysis and gene sequencing

Dominant bands were excised from the DGGE gels. Bands with similar mobility in various lanes of the DGGE gels were excised in triplicate. Excised bands were left to diffuse passively for 24 h at 4 °C in 25 μl sterilized double deionized H_2O to elute DNA. The recovered DNA 2 μl was used as a template for PCR amplification under the same conditions. The PCR products were subjected to

DGGE again for confirmation and identification to ensure that all retrieved DGGE bands represented single bands. DNA was cloned into *E. coli* and selected for gene sequencing. The sequences were deposited in GeneBank under the accession numbers KF996222 to KF996254 (bacterial 16S rRNA gene) and KF996255 to KF996271 (fungal 18S rRNA gene).

2.11. DGGE profile analysis

DGGE profiles of bacterial 16S rRNA and fungal 18S rRNA genes were performed for all replicated plot (sub-samples). Diversity indices, Shannon (H) and Richness (S) index were quantified and calculated on the bases of DGGE profile, respectively. Detected bands of each replicate were defined as specific phylotypes. The pixel intensity of each band was measured using software *QUANTITY ONE* (Version 4.0, Bio-Rad) for identification of position of different lanes of the gel and intensity of bands. Briefly, the Shannon index (H) was calculated using following equation:

$$H = -\sum_{i=1}^{S}(N_i/N)\ln(N_i/N) \quad (1)$$

Here, *H* is the Shannon index, *Ni* is the abundance of the *i*th phylotype, *N* showed the total abundance of all phylotype (lane of the DGGE gel) in the sample and *S* is the number of phylotype.

2.12. Statistical analysis

All analytical data were expressed as mean plus/minus one standard deviation. Data processing was performed with Microsoft Excel 2003. Statistical analysis was done with SPSS, version 16.0 (SPSS Institute, USA, 2001). Significance of differences between the treatment means was examined by one-way analysis of variance (ANOVA), with a probability defined at 0.05. And the procedure of Ducan was used as a post-hoc test.

3. Results

3.1. Soil microbial carbon and nitrogen, and soil enzyme activity

Contents of soil microbial carbon (C_{mic}) and nitrogen (N_{mic}) in both bulk and rhizosphere soils from treated plots increased as compared to values measured for control soils for each year of the experiment (Table 2). C_{mic} was increased by 75–169% for bulk samples and 70–158% for rhizosphere samples, and N_{mic} increased by 79–108% for bulk soil and 65–88% for rhizosphere soil. Thus, treatment resulted in a higher C_{mic}/N_{mic} ratio in soils of treatment BPC-PS2. The concentration of ammonium (NH_4^+) increased by 19–35% in bulk soil and had no difference in rhizosphere soil; whereas nitrate (NO_3^-) decreased by 13–22% and 51% respectively in bulk and rhizosphere soil.

A significant increase in enzyme activity was observed for both bulk and rhizosphere soil under BPC-PS treatments over the control except urease in the rhizosphere soil (Table 3). Significantly higher activities of urease, invertase and phosphatase were observed by 30–44%, 19–31% and 25–36% in bulk soil respectively under BPC-PS over the control, while 30–38% for invertase and 24–34% for phosphatase in rhizosphere soil.

3.2. Bacterial and fungal gene abundance and community structure

Bacterial 16S rRNA gene and fungal 18S rRNA gene abundances in bulk and rhizosphere soils were presented in Fig. 1. A significant increase in bacterial 16S rRNA gene abundance from 127% to 173% in bulk soil was observed in the BPC-PS1 and BPC-PS2 treatments, respectively as compared to the control, while in rhizosphere soil, increase of 217% only under BPC-PS2 over the control. Fungal 18S rRNA gene abundance increased by 98–190% in the bulk soil and 79–262% in the rhizosphere soil under BPC-PS over the control, and the fungal abundance under BPC-PS1 was higher than BPC-PS2 in both bulk and rhizosphere soils.

The DGGE profile of bacterial community structure for bulk and rhizosphere soils together with a cluster analysis was shown in Fig. 2. Communities associated with soils under different treatments were readily distinguished (Fig. 2B). Clearly seen from band intensity, Shannon index and richness of bacteria in bulk soil were significantly increased under BPC-PS over the control, while in rhizosphere soil, only bacterial Shannon index was higher under BPC-PS2 than the control (Table 4).

The DGGE profile of fungal community was shown in Fig. 3A, and cluster analysis of DGGE bands was different between treatments (Fig. 3B). However, fungal DGGE bands both of bulk soil and rhizosphere soil under BPC-PS2 was similar to those under control As shown in Table 4, there were significant decreases in Shannon index and richness of fungal community in rhizosphere soil under BPC-PS2 treatment over the control.

3.3. Phylogenetic analysis

A total of 33 bands representing bacteria were excised from DGGE profile and cloned for 16S rRNA gene sequencing. Sequences were compared with the GenBank database. Major sequences of bacterial phyla and detected bands included bands of 4, 5, 6, 8, 20, 27, 32 and 33, which represented Acidobacteria and Proteobacteria in BPC-PS amended soil (Fig. 4). Sequences analysis of DGGE bands showed band 5, 19 and 32 belonged to Gammaproteobacteria. Interestingly, two new single bands represented two special phyla that occurred in soils under BPC-PS1 treatment. Band 15 belonged to Alphaproteobacteria and band 33 belonged to Deltaproteobacteria. Meanwhile, bands 14 and 18 represented Chloroflexi disappeared in BPC-PS1 treated soil. Bacterial band 22 was the sole band that vanished after BPC-PS amendment and belonged to Gammaproteobacteria.

With respect to fungal sequences, the phylogenetic analysis indicated sequences for bands 8, 9 and 12 associated with BPC-PS1 belonged to Ascomycota and sequence band 4 classified as Mucoromycotina disappeared (Fig. 5). However, band 5–1, 5–2 and 5–3 related to Basidiomycota appeared in bulk soil with the amendment of BPC-PS. Fungi band 2, which occurred in untreated salt-stressed soil belonged to Ichthyosporea was highlighted as compared to BPC-PS amended rhizosphere soil.

4. Discussion

4.1. Change in microbial growth and soil N with soil amendment

Measured C_{mic} values were lower in control plots, as compared to BPC-PS treated soils. In previous studies, salinity was decreased greatly in the first year and continued to decrease in the second year after BPC-PS treatment [10,23], this could point to the adverse impact of salt-stress on soil microbes, which in agreement with the finding of Tripathi and co-authors [35]. C_{mic} and N_{mic} contents were higher with a slightly lower microbial C/N ratio in rhizosphere than in bulk soil across treatments, suggesting a rhizosphere effect. Under BPC-PS treatments, as compared to the control, great increase (over 40%) in C_{mic} and N_{mic} both of the bulk and rhizosphere soil were observed. There was further increase in both Cmic and Nmic of bulk and rhizosphere soil under BPC-PS2 over BPC-PS1. Positive effects on microbial growth with biochar amendment

Table 2
Soil microbial biomass carbon (C_{mic}), nitrogen (N_{mic}), ammonia and nitrate nitrogen in soils from treated pots under maize cultivation.

Soil	Treatment	C_{mic} (mg kg^{-1})	N_{mic} (mg kg^{-1})	C_{mic}/N_{mic}	$NH_4^+ - N$ (μg g^{-1})	$NO_3^- - N$ (μg g^{-1})
Bulk	CK	176.62 ± 5.06c	16.41 ± 0.78c	10.78 ± 0.49b	141.13 ± 8.00c	177.90 ± 0.68a
	BPC-PS1	309.46 ± 6.76b	29.37 ± 2.25b	10.57 ± 0.58b	167.88 ± 2.17b	154.84 ± 1.01b
	BPC-PS2	475.63 ± 7.81a	34.10 ± 2.13a	13.99 ± 1.13a	190.88 ± 16.77a	138.96 ± 3.81c
Rhizosphere	CK	189.60 ± 5.06c	20.08 ± 0.78c	9.45 ± 0.36b	188.68 ± 7.25a	216.26 ± 5.65a
	BPC-PS1	322.44 ± 6.76b	33.04 ± 2.25b	9.78 ± 0.46b	195.23 ± 4.00a	106.79 ± 4.27b
	BPC-PS2	488.61 ± 7.81a	37.77 ± 2.13a	12.97 ± 0.96a	200.00 ± 5.29a	105.00 ± 4.38b

Different letters in a single column indicate significant differences ($p < 0.05$) between the treatments in bulk or rhizosphere soil.

Table 3
Improvement in soil enzyme activities (mg g^{-1} DW 24 h) in BPC-PS treatment soils under maize cultivation.

Bulk soil				Rhizosphere soil		
Treatment	Urease	Invertase	Phosphatase	Urease	Invertase	Phosphatase
CK	1.81 ± 0.10c	36.00 ± 3.00b	0.92 ± 0.03b	2.08 ± 0.32a	39.45 ± 1.89b	1.21 ± 0.02c
BPC-PS1	2.35 ± 0.06b	43.00 ± 2.00a	1.15 ± 0.08a	2.32 ± 0.47a	51.28 ± 1.76a	1.51 ± 0.07b
BPC-PS2	2.59 ± 0.02a	47.00 ± 3.61a	1.24 ± 0.02a	2.78 ± 0.52a	54.58 ± 0.97a	1.64 ± 0.01a

Different letters in a single column indicate significant differences ($p < 0.05$) between the treatments in bulk or rhizosphere soil.

had been already addressed [36]. The results here suggested remarkable improvement of microbial growth over years following biochar-manure compost amendment.

The changes in C_{mic} and N_{mic}, known as general indicators of microbial growth, could indicate the resilience of a salt-stressed soil as influenced by BPC-PS treatment, to which soil microbes appeared very sensitive. The change in copy numbers followed this trend for microbial biomass, although increase in fungal gene abundance under BPC-PS2 was lower than BPC-PS1 for both bulk and rhizosphere soils (Fig. 1). Yan and Marschner [37] suggested that the low microbial biomass in soils could be mainly due to low quantities of substrate inputs in soils with poor plant growth and only secondarily to the effects of high salinity. Salinity affected soil macro- and microorganisms primarily by decreasing osmotic potential and mostly sensitive cells could be killed at low osmotic potential. This, in turn, could select for microorganisms that adapt

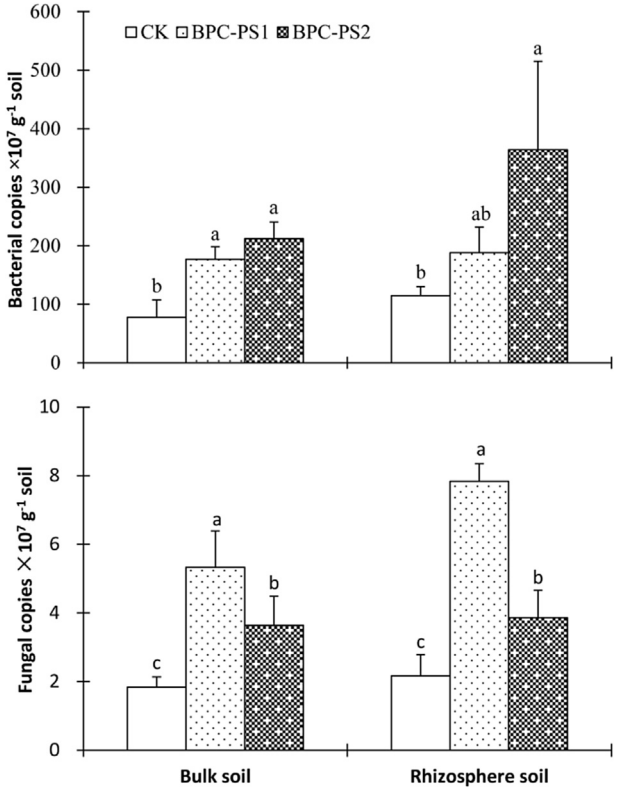

Fig. 1. Abundance of total bacteria and fungi based on the qPCR in bulk and rhizosphere soil of maize plant under BPC-PS treatments. Different letters indicates significant differences between treatments at P < 0.05 (n = 3; error bars of ±SD).

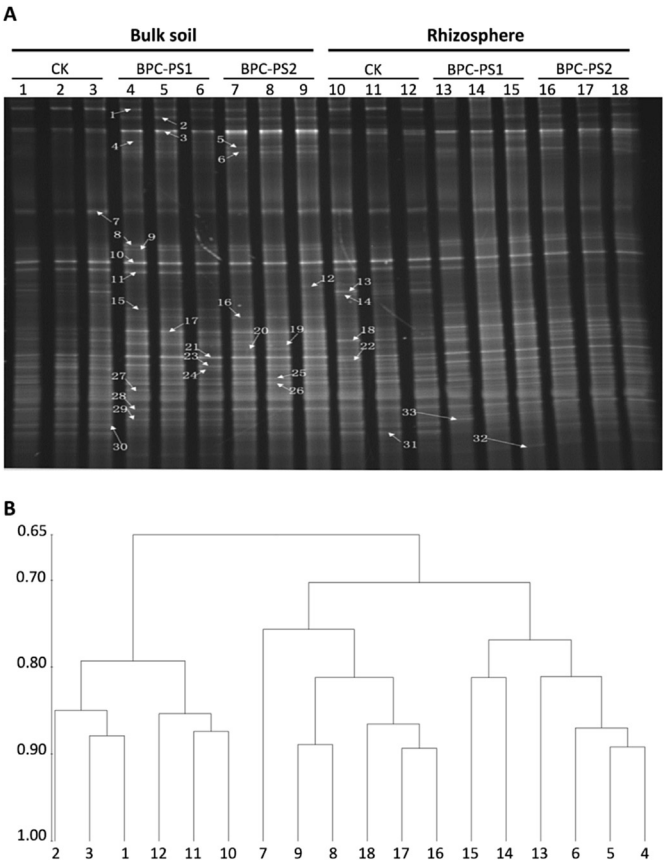

Fig. 2. DGGE profiles (A) and cluster analysis (B) of amplified 16S rRNA gene fragments of bacterial communities in BPC-PS amended salt-affected soil during the vegetative growth stage of maize. Arrows indicate the excised bands (B1–B33) those were sequenced.

Table 4
Effect of BPC-PS amendment on diversity of bacteria and fungi under salt-stressed soil using DGGE bands pattern.

Soil	Treatment	Shannon index		Richness	
		Bacteria	Fungi	Bacteria	Fungi
Bulk	CK	3.04 ± 0.00b	2.24 ± 0.06a	25 ± 1b	10 ± 1a
	BPC-PS1	3.23 ± 0.02a	2.39 ± 0.19a	28 ± 0a	11 ± 2a
	BPC-PS2	3.28 ± 0.07a	2.28 ± 0.10a	29 ± 2a	10 ± 1a
Rhizosphere	CK	3.12 ± 0.09b	2.43 ± 0.14a	27 ± 3a	12 ± 2a
	BPC-PS1	3.22 ± 0.02 ab	2.39 ± 0.09a	29 ± 1a	11 ± 1a
	BPC-PS2	3.31 ± 0.05a	1.94 ± 0.00b	31 ± 2a	7 ± 0b

Different letters in a single column indicate significant differences ($p < 0.05$) between the treatments in bulk or rhizosphere soil.

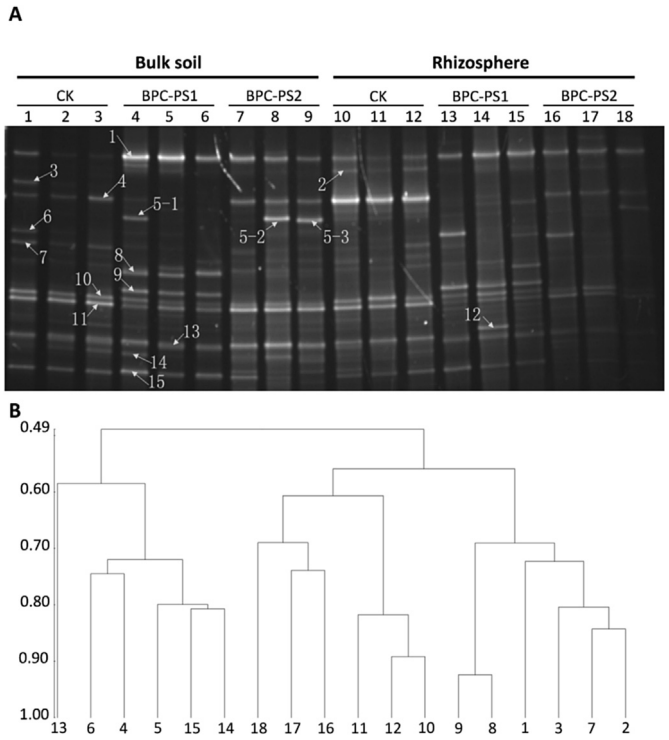

Fig. 3. DGGE profiles (A) and cluster analysis (B) of amplified 18S rRNA gene fragments of fungal communities with BPC-PS amendments in salt-affected soil of (bulk and maize rhizosphere) at the vegetative growth stage of maize crop. Arrows indicate the excised bands (B1–B15) used for sequencing.

to saline conditions by accumulating osmolytes [4], including amino acids in bacteria [38].

Both biochar and manure input could increase microbial biomass and provide additional benefits for soil fauna and flora by increasing soil organic carbon stocks and thus improving soil habitats. Soil microbial diversity could also reflect the resilience of soils and influence their biochemical transformation for plant nutrient availability through enhanced microbially biochemical activity [39]. The findings of Azam and Ifzal [40] suggested that salinity with sodium chloride had higher impact on $NO_3^- - N$ production than on $NH_4^+ - N$ through mineralization of organic matter. BPC-PS amendment here preserved high levels of ammonium ($NH_4^+ - N$) while reduced nitrate ($NO_3^- - N$) contents in both bulk and rhizosphere soils. This could be also attributed to increased N supply from manure composted with biochar and to enhanced adsorption of ammonia on biochar particles to be less accessible to nitrifiers. In fact, retention of ammonium onto biochar had been considered as a mechanism for the role of biochar in depressing N_2O emission through reduced nitrification and the subsequent denitrification [41]. Apparently, BPC-PS amendments helped preserve added nitrogen in soil. In fact, the increased rate of nitrification in soil affected by soil salinity could lead to increase in loss of applied nitrogen and reduction in availability for crop growth [42]. In this study, BPC-PS amendment was highly important for soil health and transformation of applied nitrogen for the enhanced microbial growth in the alleviated saline soil as small size of microbial biomass but low metabolic activity were mostly found in salt-affected soils [43].

4.2. Change in microbial community structure in BPC-PS treated salt soil

Here, microbial community composition was shown to be affected by salinity. There was also some evidence that fungi could be more sensitive to salt-stress as compared to bacteria [44]. Results of the present research indicated a significant increase in bacterial diversity in bulk soil under BPC-PS but a decrease in fungal diversity in rhizosphere soil under the BPC-PS2 treatment. Thus, bacterial resilience with the biochar-based amendment was more consistent than fungal for the latter could require more fresh carbon input for soil amendment. Sun and co-authors [45] reported changes in soil bacterial community structure but not in fungi in a long term cropping system in conjunction with manure application. And a recent research observed no effect of biochar on bacterial genetic diversity in a wheat crop [46]. Meanwhile, this study showed great and consistent effects in bacterial community structure with biochar-manure compost amendment over two years while increase in fungi abundance weakened in the second year, following amendment in the salt-stressed soil. In agreement with this finding, Chen and co-authors [47] reported significant increase in bacterial 16S rRNA gene abundance but a decrease in fungal 18S rRNA gene abundance following biochar amendment to a rice paddy soil.

Soil salinization could impact on microbial community structure due to adverse effects of toxic salts [5,48], as well as to lose of soil aggregates and soil structure that could lead to poor habitat for microbial growth. Biochar had been known to improve soil aggregate formation when incorporated into soil, and could become more effective on improving the growth and development of soil organisms, when added with manure compost [36]. In general, biochar amendment increased the relative abundance of members of the Actinobacteria and Bacteriodetes phyla [11,16]. Biochar improved certain genera of microorganisms, especially those belonging to *Pseudomonas*, *Mesorhizobium*, *Brevibacillus*, *Bacillus and Trichoderma*, which in the rhizosphere could improve crop growth [17,49]. Amendment of BPC-PS has been shown previously to be highly effective for improvement of salt-stressed soil and crop yield mainly with improvement of plant nutrition on saline soils [10], which had been known mostly affected by sodium. Promotion of beneficial microbial taxa (Acidobacteria, Proteobacteria and Gammaproteobacteria) and the emergence of two new single bands representing Alphaproteobacteria and Deltaproteobacteria was observed following BPC-PS amendment in the salt-stressed soil. Fungal 18S rRNA gene abundance also was observed significantly increased with the BPC-PS amendment in both bulk and rhizosphere soils. The phylogenetic sequence analysis of DGGE bands in the present study indicated some new bands of bacterial phyla with BPC-PS amendment both in bulk and maize rhizosphere soil. Improvement of microbial community structure with certain specific strains is visible in the present study, suggesting improvements in soil health as previously observed by Lashari and co-authors [10,23].

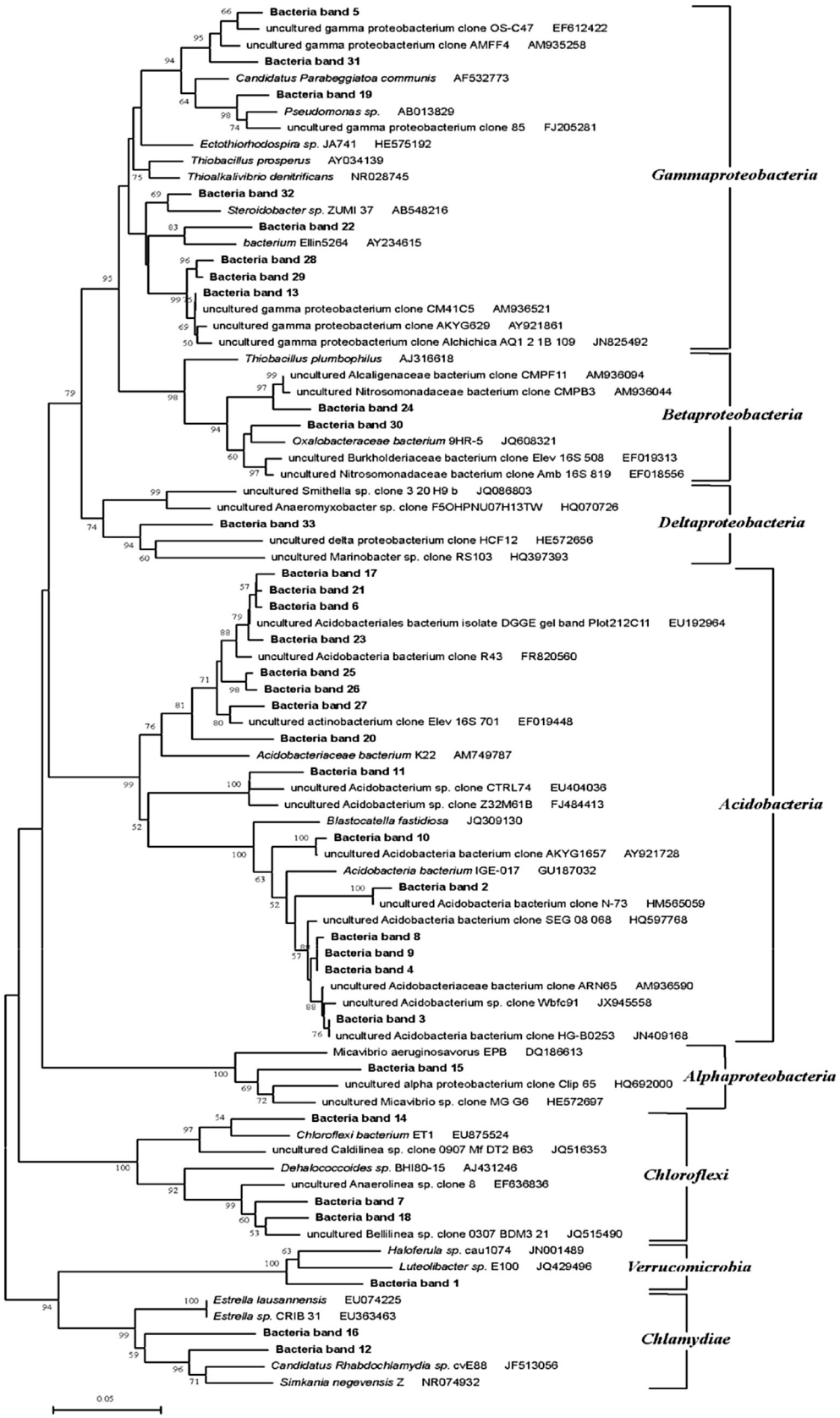

Fig. 4. Phylogenetic tree of bacterial 16S rRNA gene clone sequences from salt-affected soil of Central Great Plan China with the amendments of BPC-PS in bulk and rhizosphere soil of maize plants at the vegetative growth stage of maize crop. Bootstrap values greater than 50 are shown (1000 replications). The scale bar represents 0.05 substitutions per nucleotide.

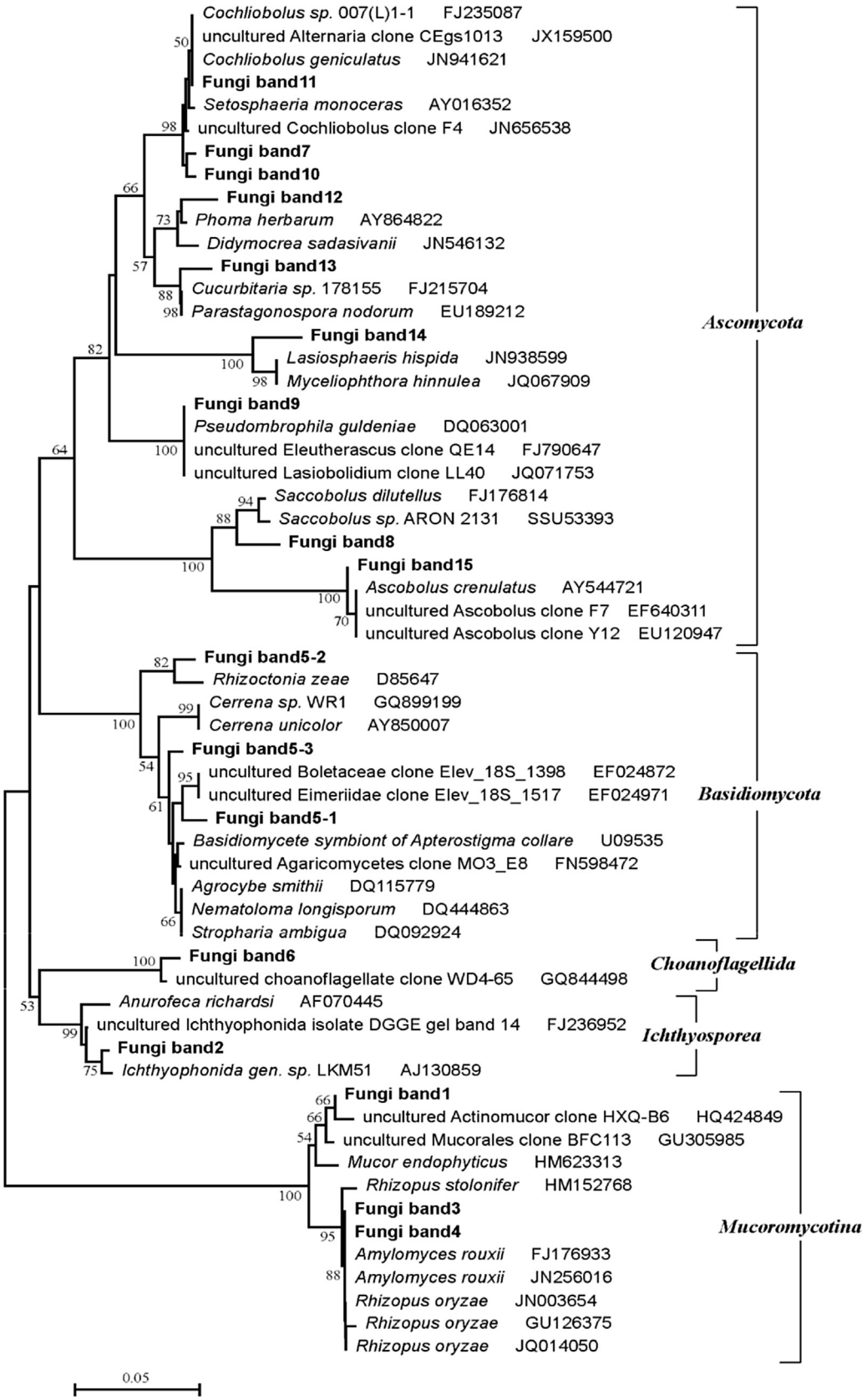

Fig. 5. Phylogenetic tree of fungal 18S rRNA gene clone sequences from salt-affected soil of Central Great Plan China with the amendments of BPC-PS in bulk and rhizosphere soil of maize plants at the vegetative growth stage of maize crop. Bootstrap values greater than 50 are shown (1000 replications). The scale bar represents 0.05 substitutions per nucleotide.

4.3. Changes in soil enzyme activity in BPC-PS treated soil

Soil enzyme activity had been accepted as a key indicator for microbial function for nutrient conservation and nutrient transformations related to soil fertility and quality [35]. In particular, urease and protease were known to hydrolyse nitrogen compound protein [50]. Soil salinity could play considerable adverse effects on plant root exudation, exudates quality and quantity [51]. The urease activity had been accepted as a highly effective soil enzymatic indicator for N mineralization of soil organic matter and of applied fertilizer. Generally, soil urease activity had been shown to be negatively correlated with soil salinity and/or alkalinity [52]. In this study, with BPC-PS amendment, significant positive correlations existed between activities of soil enzymes and microbial biomass in both bulk and rhizosphere soils except urease in the rhizosphere soils. Incorporations of manure into salinized soil, enhanced microbial and soil enzyme activities, that function for nutrient cycling [53]. Reduction in salt-stress could lead to increases in soil organic carbon, microbial biomass and activity that refer to soil fertility [54]. Tripathi and co-authors [35] reported significant negative correlation between soil salinity and urease, with additions of organic material increased the enzyme activities. Saline soil had generally lower enzymatic activity, and the major effects of salt-stress depended on salt content of the soil [48]. Reduction in soil enzyme activities could result from lower microbial biomass under salt-stress [55]. Salinity inflicts extremely a harmful influence on biochemical processes essential for maintenance of soil quality [7]. Likewise, soil biochemical activity (urease and alkaline phosphatase activity as well as the respiration rates) in soil and plant salt tolerance were improved by incorporation of organic amendment in a rice-barley rotation system [53]. Landgraf and Klose [56] reported that the ß-glucosidase activity was highly associated with the amount of easily mineralizable organic carbon. Such increase could be related to the microbial availability of a high quantity substrates added to the affected soil [48]. In this study, significantly improved urease and invertase activities in bulk and rhizosphere soil with BPC-PS amendment could be attributed to the exogenous addition of enzymes from the compost in addition to the improvement of microbial growth. Soil microbes could produce and release a large amount of extracellular phosphatase due to their large combined biomass, high metabolic activity and short life cycles. In addition, phosphatase activity was directly linked to soil organic carbon and available phosphorus [57]. In our findings, phosphatase activity was significantly improved in bulk and rhizosphere of maize after BPC-PS amendment, as in agreement with previous studies showing changes in soil phosphorus [23]. Therefore, changes in soil enzyme activities contributed to the improvement of soil nutrient availability, and in turn, improved plant growth and maize yield.

5. Conclusions

Using two single field experiments on a saline soil, the present study demonstrated significant improvement of microbial abundance and enzyme activities over years following amendment of biochar-manure compost. This was in line with the alleviation of soil salinity described in a previous study. However, with highly positive effects on soil bacterial community, fungal gene abundance was relatively weakly increased but the diversity reduced on years following the amendment. The great increase in soil enzyme activities of urease and phosphatase and higher ammonium retention probably contributing to the improved maize productivity in the saline soil under the amendment. Thus, combined use of biochar poultry manure compost and pyroligneous solution could be an effective low-cost method to alleviate microbial stress and to improve crop productivity in saline soils.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2015.07.005.

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