# **Effects of Biochar Application on Soil Organic Carbon in Degraded Saline-sodic Wetlands of Songnen Plain, Northeast China**

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**Abstract:** Biochar amendment is considered as an efficient practice for improving carbon storage in soils. However, to what extent that biochar application promotes organic carbon in saline-sodic soils remains poorly understood. By comparing soil organic carbon (SOC) contents change before and after biochar addition, we deciphered the driving factors or processes that control SOC change in response to biochar application. A limited increase in SOC was observed, about by 1.16%−12.80%, even when biochar was applied at the rate of 10% of bulk soil weight. Biochar application enhanced soil dissolved organic carbon (DOC) significantly by up to 67%. It was estimated that about 50% SOC was allocated to small macroaggregates (250−2000 μm, CPOC), and SOC in silt and clay-sized particles (< 53 μm) decreased obviously after biochar addition. Microbial biomass increased with biochar amendment, of which actinomycetes (ACT), fungus (FUN), protozoon (PRO), and bacteria with straight-chain saturated fatty acids (OB) increased remarkably. Multiple linear regression models implied that DOC was governed by ACT and soil N∶P ratio, while SOC mostly depended on CPOC. The principal component analysis and the partial least square path model (PLS-PM) indicated that biochar addition aggravated nitrogen limitation in saline-sodic soils, and effects of microorganisms on regulating SOC greatly depended on nitrogen bioavailability. Biochar application had vastly changed interactions between environmental factors and SOC in saline-sodic soils. Effects of nutrients on SOC shifted to great inhibition from strong stimulation after biochar addition, meanwhile, aggregation was the only factor presenting positive effects on SOC. How to eliminate nutrient limitation and better soil aggregation process should be considered in priority when biochar was used to improve SOC in saline-sodic soils.

**Keywords:** biochar;; saline-sodic soil;; soil organic carbon;; phospholipid fatty acid;; nutrient;; Songnen Plain;; China

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# **1 Introduction**

The use of biochar for carbon sequestration and soil restoration has been proven to be an effective and cost-effective means to abate global warming [\(Joseph et al.](#page-9-0), [2020](#page-9-0)). Biochar materials are carbon-rich, resistant to degradation in soils and could better soil properties while simultaneously improve plant biomass yields([Farji-](#page-9-1)[Brener and Ghermandi, 2000](#page-9-1); [Christian, 2001](#page-9-2)). Biochar application has a large climate-change mitigation potential and could reduce about 1.8 Pg  $CO<sub>2</sub>-C$  emissions yearly, equal to 12% of current anthropogenic  $CO_2$ -C emissions [\(Christian, 2001](#page-9-2); [Zhao et al., 2015](#page-10-0)). Biocharamended soil could sequester C by physical stabilization over a long time([Novak et al., 200](#page-9-3)9; [Yin et al.](#page-10-1), [2014](#page-10-1)), and the mean residence time of biochar is estimated to about 2000 yr with a half-life of 1400 yr. Biochar application also has positive effects and in-

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hances biomass production by up to 30%, and this would yield more plant-derived biomass input into soils [\(Yin et al., 201](#page-10-1)4). Soil organic carbon mineralization was usually stimulated or suppressed by biochar through positive or negative priming effects [\(Prommer et al](#page-9-4)., [2014](#page-9-4); [Sui et al., 2016](#page-9-5)), and this might offset the benefit of soil organic carbon (SOC) improvement by biochar addition in the short-term time. However, credible data from varied field experiments and soil types are needed to assess whether soil amendment by biochar is a potentially useful option to mitigate climate change.

Saline-sodic soils cover 3.1% of the global land area, and the carbon loss (SOC) rate in saline soils is estimated to 3.47 t/ha [\(Yang et al., 2018](#page-10-2)). Improving sodic soil is a potential alternative to expand cultivated land currently to meet rising food demands from population growth. Biochar application is a prospective choice to fertilize saline-sodic soils for replantation [\(Munda et al.,](#page-9-6) [2018](#page-9-6)). Saline-sodic soils are flocculated with high soluble salts and exchangeable  $Na<sup>+</sup>$  and become disperse when pH is higher than 8.5. This results in low SOC in saline-sodic soils due to slow plant growth, low biomass, poor aeration, compaction, and low nutrient bioavailability [\(Sun et al., 2016](#page-9-7)). Since biochar is charcoal-like, high porous, and fine-grained with large surface areas, its application to soils has attracted increasing attention as an effective and economic soil amendment for improving soil quality, biomass yield and SOC pool. Biochar has been successfully used to ameliorate nutrient deficiency and salt stress [\(Sun et al., 2016](#page-9-7)), remediate degraded soils ([Sun et al., 2016](#page-9-7)), facilitate plant growth([Brodowski et al., 200](#page-9-8)5), and suppress SOC mineralization ([Lin et al., 2015\)](#page-9-9). However, the effects of biochar addition on SOC pool were usually contradictory in previous research due to differences in soil texture, biochar types, application amounts and experiment time. Biochar application can not only directly increase the SOC pool by external organic carbon input, but also improve the SOC pool indirectly by bringing more nitrogen and phosphorus that facilitate plant growth. Biochar has high adsorption capacity because of numerous inner pores, and this allows it to protect SOC from decomposition by microbes. However, there is a knowledge gap about how biochar applications affect SOC dynamics in saline-sodic soils though many works were performed on arable soils [\(Sollins et al., 1996](#page-9-10)).

Soil salinization has been spreading globally in over 100 countries. The Songnen Plain, Northeast China is one of the largest saline-sodic areas in the world, and alkaline land is estimated to  $3.84 \times 10^6$  ha [\(Zhao et al.](#page-10-3), [2020](#page-10-3)). Saline-sodic soils here are rich in high montmorillonite clay and sodium bicarbonate, and are poor in SOC contents. Carbon sequestration rates were restricted to < 60  $g/(m^2 \text{yr})$  [\(Sollins et al., 1996](#page-9-10)). How to improve soil texture, fertilizer saline-sodic soil for potential cultivation, and to increase soil carbon pool for mitigating rising atmospheric  $CO<sub>2</sub>$  is one of the most important environmental issue here. The objectives of the present work are: 1) to compare SOC variations under different biochar addition based on mesocosm experiments, and 2) to reveal potential factors that control SOC dynamics. Based on these two aims, it is hoped to provide manageable carbon farming solutions to the global climate and to satisfy food demand using biochar technology.

### **2 Methods and Materials**

#### **2.1 Description of soil sample sites**

Saline-sodic soils for mesocosm experiments were collected from a degraded wetland on 4 April, 2018 in the Momoge National Nature Reserve (45°54′32 ″N, 123° 45′56″E), Jilin Province, China [\(Fig. 1\)](#page-2-0). It locates in the transition zone between deserts and grasslands and supports wetlands types that are representative of the biogeographic regions, such as low plain wetlands, rivers, temperate meadow and shallow lakes. The dominant vegetation cover in the Momoge National Nature Reserve was *Leymus chinensis* with a total vegetative cover of less than 10%. The annual average temperature is 4.4℃ and the annual precipitation is 392 mm/yr (<http://www.igadc.cn/filter/d701>). The predominant vegetation specie was *Leymus chinensis* with the coverage of about 10%, and white salt spot and cracks could be observed in soil surface in the sample site. This represented the typical degraded wetlands in the Songnen Plain.

#### **2.2 Mesocosm experiments design**

Surface soils within 40 cm depth were collected, brought back to the laboratory, well mixed, and passed through a 5 mm sieve to remove stones before mesocosm experiments. To investigate the effects of biochar addition on SOC change, microcosm experiments were designed and constructed using 1 m  $\times$  1 m  $\times$  1 m polypropylene boxes. Biochar materials were prepared

<span id="page-2-0"></span>

**Fig. 1** Location and land types of the Momoge National Nature Reserve in Jilin Province, China

from rice straw at 550℃ in anaerobic conditions, and contained 422.6 g/kg of carbon, 8.4 g/kg of nitrogen, 2.2 g/kg of phosphorus, and pH of the biochar was 8.34.

Mesocosm experiments were carried out in the experiment station of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, in Changchun to avoid potential impacts from grazing in the field. Biochar was a one-off well mixed with salinesodic soils at the rate of 0.50% (T0.5), 1.0% (T1), 2.0% (T2), 5.0% (T5), and 10.0% (T10) before they were transferredinto boxes. Each treatment was performed with three replications, and the one without biochar was the control treatment (CK). The soil depth was 50 cm in mesocosmboxes. T0.5, T1, and T2 treatments were categorized into the low level of additions (LK), while T5 and T10 were the higher level (HK) [\(Yoo and Kang](#page-10-4), [2012](#page-10-4)).

Experiment boxes were placed in the field without extra water addition but natural precipitation to simulate natural soil water conditions. Experiments started in May 2018 and finished in November 2018, spanning a growing season in Northeast China. Soil samples within 40 cm depth were collected monthly for chemical analysis and three replications were collected each time.

#### **2.3 Phospholipid fatty acid (PLFA) analysis**

PLFA extraction and analysis were performed according to the method described by [Zhang et al \(2012a\)](#page-10-5). In brief, fresh soils were freeze-dried and extracted with a chloroform-methanol-citrate buffer mixture (1∶2∶0.8). The phospholipids were separated from other lipids on a silicic acid column. Phospholipid phosphate concentration was determined using the spectrometric method. Phospholipids were subjected to a mild-alkali methanolysis, and the resulting fatty acid methyl esters were separated by gas chromatography with a flame ionization detector (Agilent 6890N, Agilent, California, USA). The carrier gas was helium, and the temperature increased to 260℃ from 170℃ at a rate of 5℃/min. The inner standard, a mixture of 37 fatty acid methyl ester (FAME), was used to identify and quantify the response of individual fatty acids [\(Steinbeiss et al., 2009](#page-9-11)). The PLFA makers used for taxonomic microbial groups were shown in [Table 1](#page-3-0). PLFAs were categorized according to [Geomez et al \(2014](#page-9-12)) and [Muhammad et a](#page-9-13)l [\(2014\)](#page-9-13).

Taxonomic group	Specific PLFA markers
Bacteria (BAC)	i15 : 0, a15 : 0, i15 : 1 $\omega$ 9c; i16 : 0, i17 : 0, 17 : 0, 16 : 1 $\omega$ 9c; i16 : 1 $\omega$ 7c; cy17 : 0 $\omega$ 7c; 18 : 1 $\omega$ 5c; 18 : 1 $\omega$ 9c; $18:1\omega$ 7c; 19: $1\omega$ 6c; cy19: $0\omega$ 7c; i17: $1\omega$ 9c; 15: 0; 15: 0DMA
Actinomycetes (ACT)	$16:010$ -methyl; $17:1\omega$ 7c $10$ -methyl; $17:010$ -methyl; $18:1\omega$ 7c $10$ -methyl; $18:010$ -methyl
Fungi (Fun)	$18:1\omega$ 9c; $18:2\omega$ 6.9; $18:3\omega$ 6c
Arbuscularmycorrhizal fungi (AMF)	16:105c
Protozoon (PRO)	$20:2\omega_0$ ; $20:3\omega_0$ ; $20:4\omega_0$
Other bacteria identified with straight- chain saturated fatty acids (OB)	14:0:16:0:17:0:18:0
Gram-positive bacteria	i15 : 0; a15 : 0; i16 : 0; i17 : 0; a17 : 0
Gram-negative bacteria	$116:1\omega$ 7c; 16 : $1\omega$ 9c; 18 : $1\omega$ 5c; 18 : $1\omega$ 7c; cy17 : 0; cy19 : 0

<span id="page-3-0"></span>**Table 1** Characteristic of fatty acids of microbial functional groups

#### **2.4 Separation of different aggregation fractions**

Soil samples were air-dried for separating different aggregation fractions using a nest of four sieves having diameters of 2000 μm, 250 μm, and 53 μm ([Zhang et al.,](#page-10-6) [2012b](#page-10-6)). In brief, about 250 g dry soil samples were placed on the uppermost of the nest and had planerotary shaken mechanically for 20 min. The microsieve size ( $\leq$  250  $\mu$ m) was further sieved by hand. Mechanism sieving was done three times. Finally, three aggregation fractions were got, which were small macroaggregates part (2503−2000 μm, CPOC), macroaggregates fractions (53−250 μm, FPOC), and silt and claysized particles ( $\leq$  53  $\mu$ m, MOC). Particles larger than 2000 μm were not got in the present work. Weight and SOC contents of every aggregation fraction were determined for calculating the proportions of carbon storage.

#### **2.5 Chemical analysis**

Total soil organic carbon (SOC) and nitrogen (TN) were determined using an elemental analyzer after carbonate was removed by 1 N HCl solution (Elementar Vario Microcude, Hesse, Germany). Total phosphorus contents (TP) in soils were measured by the ammonium molybdate-ascorbic acid method. Soil samples were dried in an aluminum box to a constant weight, and soil moisture content (SWC) and bulk density (BW) were calculated by weighting mass loss before and after the soil was oven at 105℃ for 8 h.

Dissolved organic carbon (DOC) was extracted by mixing 5.00 g soil with 30.0 mL deionized water in Erlenmeyer flasks. After shaking for 30 min, the mixtures were centrifuged and filtered through a 0.45 μm filter. Filtrates were analyzed for total organic carbon using a TOC-VCPH analyzer (Gangdong, China).

#### **2.6 Statistical analysis**

All statistical analyses were performed by R software. Pearson correlation analysis was applied to explore relationships between SOC, DOC, and environmental factors, including pH, SWC, BW, nutrients, and microorganisms. Analysis of variance (ANOVA) was used to compare differences among different treatments. Principal component and multiple linear regression analyses using the stepwise regression method were carried out to decipher potential links and predominant factors that affected SOC and DOC change. Considering environmental factors were closely and inter-correlated, a partial least square path model (PLS-PM) was used to explore and visualize the effects of ecological process or components on SOC and DOC before and after biochar amendment ([Brown and Human, 1997](#page-9-14)). The PLS-PM R package can be download at [https://cran.r-project.org/](https://cran.r-project.org/src/contrib/Archive/plspm/) [src/contrib/Archive/plspm/](https://cran.r-project.org/src/contrib/Archive/plspm/).

### **3 Results**

# **3.1 Soil physical-chemical property changes with biochar amendment**

On the whole, BW, SWC, and TN decreased with biochar addition, while TP, SOC, and DOC contents showed noticeable or small increase tendencies. TP increased about 46%, from 0.24 g/kg in CK to 0.35 g/kg in T10. TN contents decreased by 10% with biochar addition. SOC increased from 12.06 g/kg in CK to 13.60 g/kg in T10. DOC increased significantly with biochar addition, about by 25% and 67% in T5 and T10, though the difference between CK and LK was not at a significant level. As expected, soil pH was increasing with biochar addition but withina [small r](#page-4-0)ange, from 8.22 in CK to 8.44 in T10 treatment ([Table 2\)](#page-4-0).

	Treatment	pH	$SWC / \%$	BW / (g/cm <sup>3</sup> )	DOC/(g/kg)	SOC / (g/kg)	TN/(g/kg)	TP/(g/kg)
<b>CK</b>		$8.22 \pm 0.33^{\text{a}}$	$21 \pm 10^a$	$0.67 \pm 0.10^a$	$0.09 \pm 0.02^b$	$12.06\pm3.08^a$	$0.90 \pm 0.07$ <sup>a</sup>	$0.24 \pm 0.02^b$
LK	T <sub>0.5</sub>	$8.43 \pm 0.34$ <sup>a</sup>	$17 \pm 12^a$	$0.64 \pm 0.07$ <sup>a</sup>	$0.13 \pm 0.04^{ab}$	$12.20 \pm 3.13^a$	$0.88 \pm 0.10^a$	$0.27 \pm 0.02^b$
	T <sub>1</sub>	$8.18 \pm 0.28$ <sup>a</sup>	$15 \pm 10^a$	$0.62 \pm 0.09^a$	$0.09 \pm 0.02^b$	$13.55 \pm 3.01^a$	$0.84 \pm 0.12^a$	$0.28 \pm 0.06^b$
	T <sub>2</sub>	$8.13 \pm 0.21$ <sup>a</sup>	$14\pm10^a$	$0.59 \pm 0.07$ <sup>a</sup>	$0.09 \pm 0.03^{b}$	$11.62 \pm 4.45^a$	$0.87 \pm 0.10^a$	$0.26 \pm 0.03^b$
<b>HK</b>	T <sub>5</sub>	$8.31 \pm 0.19^a$	$16 \pm 12^a$	$0.61 \pm 0.06^a$	$0.12\pm0.05^{ab}$	$12.33 \pm 2.75^a$	$0.81 \pm 0.08^a$	$0.33 \pm 0.03^a$
	T <sub>10</sub>	$8.44 \pm 0.19^a$	$15 \pm 9^a$	$0.59 \pm 0.09^a$	$0.15 \pm 0.05^a$	$13.60 \pm 3.68^a$	$0.81 \pm 0.09^a$	$0.35 \pm 0.05^a$

<span id="page-4-0"></span>**Table 2** Soil properties change with different biochar addition treatments.

Notes: Different letters meant significant differences; SWC, soil moisture contents; BW, bulk density; DOC, dissolved organic carbon; SOC, soil organic carbon; TN, total content; TP, total phosphorus; T0.5, T1, T2, T5 and T10 meant that biochar was added at the mass rate of 0.5%, 1.0%, 2.0%, 5.0% and 10.0% to soil amounts; CK was the control treatment without biochar application, LK represented low biochar addition amounts, and HK represented high biochar addition amounts

#### **3.2 SOC contents in different aggregate fractions**

Over the whole study, FPOC stored about 41.6%−49.7% of total SOC in soils. CPOC irregularly but slowly raised from 27.9% in CK to 36.7% in T10 with biochar addition [\(Fig. 2](#page-4-1)). MOC showed a noticeable decrease trend with biochar addition.

# **3.3 Soil microorganism community change with biochar amendment**

ANOVA analysis indicated that total PLFAs did not vary significantly among CK, LK, and HK treatments. PLFA contents in CK were close to those in LK and increased to 320 nmol/g in HK treatments.

Biochar addition changed microbial community structures. It was obvious that BAC and AMF biomass changed little and even slightly reduced under LK treatments, but increased greatly by 21% and 24% com-

<span id="page-4-1"></span>

**Fig. 2** Carbon storage proportions in aggregation fractions. CPOC, small macroaggregates part (2503−2000 μm); FPOC, macroaggregates fractions (53−250 μm); MOC, silt and claysized particles ( $\leq$  53  $\mu$ m); T0.5, T1, T2, T5 and T10 meant that biochar was added at the mass rate of 0.5%, 1.0%, 2.0%, 5.0% and 10.0% to soil amounts; CK was the control treatment without biochar application

pared with those in CK treatments. The increase trends were more obvious for ACT, FUN, PRO, and OB, which increased by 11.5%, 44.1%, 24.0%, and 108.0% in HK treatments than those in CK treatments, respectively. The difference of FUN between HK and CK treatments was to a statistically significant level ([Table 3](#page-5-0)).

### **3.4 Correlations between soil organic carbon and environmental factors**

Significant positive correlations were observed for DOC vs. pH, TP, MOC, PLFA, BAC, ACT, PRO, and OB, while negative correlations were found for DOC vs. BW, C∶N, C∶P, N∶P, and COPC. Positive correlations were found between SOC and BW, C∶N, C∶P, and CPOC, while negative correlations were observed between SOC and SWC, FOPC, MOC, PLFA, PRO, and OB at a significant level ([Fig. 3](#page-5-1)).

### **4 Discussion**

# **4.1 Effects of biochar amendment on SOC and DOC change**

Incorporating biochar into the soil could reduce organic carbon loss by suppressing  $CO<sub>2</sub>$  emissions, enhancing plant productivity, and protecting SOC degradation. However, the impacts of biochar on soil carbon dynamics on longevity and [magnitude ranged widely from](#page-9-15) weeks to several years [\(MacKenzie and Quideau, 2010](#page-9-15)). Biochar application has [positive, neutral](#page-9-16), or negative effects on SOC and DOC [\(Liu et al., 2016](#page-9-16)). SOC contents are usually altered by biochar amendment within a great change range, from a few percent to several folds. However, DOC conte[nts varie](#page-6-0)d in different [researches](#page-9-17) with biochar addition ([Table 4](#page-6-0)). For instance, [Smebye's](#page-9-17)

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Treatment	PLFAs / (nmol/g)	Microorganism species $/$ (nmol/g)						
		<b>BAC</b>	ACT	<b>FUN</b>	AMF	PRO	<b>OB</b>	
СK	$265.37\pm21.50^a$	$171.07\pm12.99^a$	$36.98\pm4.04^{\circ}$	$25.56 \pm 1.77^{\circ}$	$8.48\pm0.65^{\circ}$	$4.46 \pm 0.70^a$	$4.98 \pm 2.31$ <sup>a</sup>	
LK	$264.83 \pm 11.10^a$	$163.90\pm6.38^{\circ}$	$37.23 \pm 2.10^a$	$30.32 \pm 1.17^{ab}$	$8.14 \pm 0.36$ <sup>a</sup>	$4.60 \pm 0.34$ <sup>a</sup>	$7.49 \pm 2.70$ <sup>a</sup>	
НK	$320.52 \pm 20.12^a$	$198.76 \pm 11.54^a$	41.23 $\pm$ 2.81 <sup>a</sup>	$36.84 \pm 2.43^b$	$10.09 \pm 0.63$ <sup>a</sup>	$5.53 \pm 0.53^a$	$10.38 \pm 1.55^{\circ}$	

<span id="page-5-0"></span>**Table 3** Change of soil microbe's biomass under biochar addition

<span id="page-5-1"></span>Notes: Different letters meant a significant difference at 0.05 level. PLFAs, total phospholipid fatty acids; BAC, the bacteria groups indicative by chain or branch PLFAs; ACT, actinomycetes; FUN, fugun; AMF, arbuscularmycorrhizal fungus; PRO, protozoon; OB, other bacteria identified with straight-chain saturated fatty acids.CK was the control treatment without biochar application, LK represented low biochar addition amounts, and HK represented high biochar addition amounts



**Fig. 3** Correlations analysis of soil organic carbon (SOC), dissolved organic carbon (DOC) and environmental factors. CN, CP and NP were C∶N, C∶P and N∶P ratios; SWC, soil moisture contents; BW, bulk density; TN, total content; TP, total phosphorus; PLFA, total phospholipid fatty acids; BAC, the bacteria groups indicative by chain or branch PLFAs; ACT, actinomycetes; FUN, fugun; AMF, arbuscularmycorrhizal fungus; PRO, protozoon; OB, other bacteria identified with straight-chain saturated fatty acids.; CPOC, small macroaggregates part (2503−2000 μm); FPOC, macroaggregates fractions (53−250 μm); MOC, silt and clay-sized particles (< 53 μm)

[work \(2016\)](#page-9-17) indicated that DOC contents increased by 2775% when biochar was added into arable soils at the rate of 10%. DOC was also changed by −5.59%−26.67% when biochar was applied into the crop field([Yang et](#page-10-2) [al., 2018](#page-10-2)). In contrast to results from arable soils, the magnitude of SOC contents change in the present work were relatively smaller, from 1.16% to 12.80% even biochar was added at the rate of 10%, than the remarkable increase in sugarcane, paddy field, and other agriculture fields [\(Table 4](#page-6-0)). DOC contents increased greatly from no effect to 66.7%, and this amplification was higher than those in arable soils. It indicated that driving factors affecting SOC dynamics in saline-sodic soils differed greatly from those in crop soils ([Zimmerman et](#page-10-7) [al., 2011\)](#page-10-7).

Biochar addition had little improvement effects on

Soils/land	Biochar amendment	SOC change / %	DOC / $\%$	References
Acidic acrisol	$10\%$ (wt/wt)		2775.00	Smebye et al., 2016
Arable soil	$30\%$ (wt/wt)	$100 - 127$		Steinbeiss et al., 2009
Paddy field	$10-40 / (t/ha)$	$10.8 - 55.6$		Zhang A et al., $2012$
Sugarcane field	$0.68\% - 1.04\%$ (wt/wt)	54	$-11.80$	Yin et al., 2014
Arable field	$24 - 72 / (t/ha)$	153	$-9.75$	Prommer et al., 2014
Rice field	$1.78 - 29.60 / (t/ha)$	$63 - 65$		Sui et al., 2016
Soil without types	$2\% - 5\%$ (wt/wt)		$-1.31-31.10$	Zhao et al., 2015
Coastal saline soil	$0.5\% - 2.0\%$ (wt/wt)	$5.2 - 68.0$		Novak et al., 2009
Agriculture field	$15.75 - 47.25 / (t/ha)$	$27.08 - 92.61$	$5.59 - 26.67$	Yang et al., 2018
Rice field	$1-10/(t/ha)$	$43.8 - 169.0$		Munda et al., 2018
Coastal saline soil	$0.5\% - 2.0\%$	$-3.83 - 87.00$		Sun et al., 2016
Costal saline soil	$3.2 - 32 / (t/ha)$	$31.0 - 298.0$		Lin et al., 2015
Saline soil	$0.5\% - 10.0\%$ (wt/wt)	$1.16 - 12.80$	66.70	The present work

<span id="page-6-0"></span>**Table 4** Comparison of soil organic carbon (SOC), dissolved organic carbon (DOC) change with biochar addition in the present work and previous studies

Note: wt, weight

SOC in saline-sodic soils than those in agricultural and coastal saline soils. SOC contents in CK treatments had no difference with LK treatments but differed significantly with HK treatments though the increase was only 12.80%. Limited SOC improvement by biochar might be ascribed to serious nutrient limitations and less microbial activities in saline-sodic soils. Besides as a direct carbon source into soil carbon pool, biochar addition improved SOC pools by perfecting soil texture, facilitating plant growth, and producing more litter biomass. Principal component analysis indicated that SOC and TN had positive loadings on the first principal component (PC1, 38.71% of total variance), and PLFA had positive loadings on the second principal component (PC2, 17.03% of total variances)([Fig. 4](#page-6-1)). This meant that nutrient limitation and microbial activities exerted strong control on SOC and DOC contents. The dependence of CPOC on SOC was presented by the neighboring location of SOC and CPOC in [Fig. 3](#page-5-1). The increasing contribution of CPOC to SOC might be caused by direct biochar addition.

Proportions of MOC to SOC decreased with biochar addition, implied that degraded SOC mainly stemed from MOC. MOC was closely related to OB in the present work. The OB were indicators of physiological or nutritional stress in bacterial co[mmunities, and low](#page-9-18)er proportions meant lower stress [\(Bossio et al., 199](#page-9-18)8). Proportions of OB to PLFA increased to 3.64% from

<span id="page-6-1"></span>

**Fig. 4** The PC1 × PC2 loadings with soil organic carbon (SOC), dissolved organic carbon (DOC) and environmental factors used in the principal component analysis. TN, total content; TP, total phosphorus; PLFA, total phospholipid fatty acids; BAC, the bacteria groups indicative by chain or branch PLFAs; ACT, actinomycetes; FUN, fugun; AMF, arbuscularmycorrhizal fungus; PRO, protozoon; OB, other bacteria identified with straight-chain saturated fatty acids; CPOC, small macroaggregates part (2503−2000 μm); FPOC, macroaggregates fractions (53−250 μm); MOC, silt and clay-sized particles ( $\leq$  53  $\mu$ m)

2.95% with biochar addition, and this implied that bacteria suffered growing resource stress and nutrient limitation after biochar addition, which was also confirmed by ne[gative](#page-4-1) correlations between OB and C∶N and C∶P [\(Fig. 2](#page-4-1)). Biochar addition brought exogenous nutrient loading into soils, specifically total phosphorus, and this effectively facilitated microbial growth as FUN and AFM. These relations were in good agreement with

previous results ([Liu et al., 2018](#page-9-19)). Correlations analysis confirmed that PLFA, BAC, FUN, and AMF were significantly and positively related to TP. FUN and AMF increased obviously with biochar which would compete with bacteria for space and resources. Biochar is the solid remains of biomass produced from the thermochemical conversion under oxygen limitation and is dominantly composed of multiple aromatic C which is not bioavailable for bacteria [\(Liu et al., 2018](#page-9-19)). Particles less than 53 μm contain an abundance of polysaccharides, proteins, and lipids, which composed 41.7%, 4.2%, and 11.1% of MOC, respectively [\(Grandy and Neff, 2008](#page-9-20)). These easily-consumed compounds could be as alternative carbon and nutrient sources for bacteria.

### **4.2 Predominant factor affecting SOC and DOC**

SOC and DOC contents were co-controlled by multiple environmental factors, and the multiple linear regression analyses could identify the most important one. ACT biomass and N∶P ratios were the common factors that were used for predicting DOC contents and the proportions of DOC to SOC (RC) ([Table 5](#page-7-0)). ACT species, as pioneers in nitrogen-deficient environments, have a predilection for barren soils and can acclimate to stressful conditions such as drought, high salinity, and extreme pH. Biochar addition caused increasing soil pores (low BW), drought (low SWC), and high pH, and assisted ACT growth when simultaneously restricted other microorganisms. As effective decomposer of C compounds, actinomycetes are booming when N limitation occurs in soil [\(MacKenzie and Quideau, 201](#page-9-15)0). DOC was expected to be derived from ACT excretion or products of refractory SOC decomposed by ACT, and this deduction was further supported by the positive Beta coefficients of ACT in regression models, which was 0.635.

N∶P ratios were the common negative factor controlling DOC and RC in soils, implying nitrogen limitation occurrence in saline-sodic soils. Soil N∶P ratios increased with biochar addition, which confirmed the rising limitation of microbial growth by nitrogen after biochar addition. In regression models, the Beta coefficients of N∶P to DOC and RC were both negative, this matched well with the diagonal location of DOC, PLFA, and TN and N∶P in [Fig. 3.](#page-5-1) Globally, there is a Redfieldlike atomic C∶N∶P ratio, about 60∶7∶1, for the soil microbial community ([Lehmann et al., 2011](#page-9-21)). The wellconstrained N∶P ratios reduced to 5.3 in HK, to 7.1 in LK from 8.3 in CK treatments, suggested aggravating limitation to microorganisms by nitrogen in saline-sodic soils after biochar addition.

Only the CPOC was introduced into the regression model for predicting SOC contents, and this was in good agreement with the positive loading of CPOC on SOC in [Fig. 3](#page-5-1). CPOC could explain 73% of SOC variation as the Beta coefficient shown in [Table 5](#page-7-0). Considering no aggregate larger than 2000 μm was separated and biochar was fine powders, it was guessed that increasing CPOC might directly come from biochar materials. Biochar could form the organic-inorganic complex with soil minerals, and this interaction could enclose biochar carbon effectively and protect them against further microbial decomposition [\(Brodowski et](#page-9-8) [al., 2005](#page-9-8)). Biochar application caused SWC and BW to decrease and soil pores to increase, and it facilitated  $Fe<sup>3+</sup>$  and  $Al<sup>3+</sup>$  deposition in biochar surface, reduced the

<span id="page-7-0"></span>**Table 5** Summary of multiple linear regression models for predicting soil organic carbon (SOC), dissolved organic carbon (DOC) and RC using environmental factors

Dependent	Factor	Coefficient	Beta	$R^2_{\text{adj}}$	F	$\boldsymbol{P}$
<b>DOC</b>	<b>ACT</b>	0.002	0.635	0.571	27.648	${}_{0.000}$
	N : P	$-0.015$	$-0.233$			
	pH	0.026	0.181			
RC	ACT	0.018	0.516	0.704	72.393	${}_{0.000}$
	NP	$-0.020$	$-0.568$			
<b>SOC</b>	<b>CPOC</b>	0.156	0.730	0.525	67.314	${}_{0.000}$

Notes: Factors were the independent factors for regression analysis; Coefficients were the coefficient values for independent factors; Beta was the corresponding standardard regression coefficients that reflect the contributions of independent factors on dependent factor variations;  $R^2_{\text{adj}}$  was the adjusted coefficient value representing the goodness of fit. F was the values of variance analysis and *P* showed the statistically significant level when it was lower than 0.05; ACT, actinomycetes; NP, N∶P ratios; CPOC, small macroaggregates part (2503−2000 μm)

microbial accessibility to biochar, and finally protected biochar-derived C from decomposition([Sollins et al](#page-9-10)., [1996](#page-9-10)). However, more evidences or parameters, such as black carbon biomarker, are needed to confirm links between carbon in CPOC aggregates and biochar materials.

# **4.3 How biochar amendment changed the soil carbon pool?**

Soil carbon pool variation usually results from multiple combined factors as soil physical conditions, nutrient availability, microorganism activity, and aggregation processes. To differentiate impacts of environmental effects on SOC is vital to evaluate the benefit of biochar application on soil properties and fertility. The partial least squares path model (PLS-PM) could provide a visual block diagram that revealed complex multivariate relationships among observed and latent variables. In the present work, five blocks were used to reveal the importance of environmental factors on carbon change in saline-sodic soils before and after biochar addition.

The Phy block consisted of SWC, BW, and pH variables, the Nut block contained TN, TP, C∶N, C∶P and N∶P variables, the Agg block contained CPOC, FPOC, and MOC variables, the Carbon block contained DOC and SOC variables, and the Mic block contained PLFA, BAC, ACT, FUN, AMF, PRO and OB variables. The Phy, Nut, Agg, Carbon, and Mic blocks represented information of soil basic physical-chemical properties, nutrient availability, aggregation process, carbon dynamics, and microorganism communities, respectively.

The PLS-PM model indicated that biochar application had greatly changed interactions among these five blocks. In CK treatments, Phy, Nut, Mic, and Agg all had positive effects on Carbon, of which Nut had the largest effect on Carbon while mic had the smallest effect. In LK and HK treatments, Agg was the only one that had positive effects while other blocks had negative effects on Carbon, and Nut still had the largest importance [\(Fig. 5\)](#page-8-0).

The effects of microorganisms on carbon dynamic shifted to weakly negative in  $LK + HK$  (-0.0768) from weakly positive in CK treatments (0.0043). It was concluded that biochar addition triggered the negative priming effects of microorganisms on the SOC pool [\(Zim](#page-10-7)[merman et al., 2011](#page-10-7)). However, no significant effects of Mic on SOC were observed as expected in other work

<span id="page-8-0"></span>

**Fig. 5** Effects of physical-chemical properties (Phy), nutrient availability (Nut), microorganisms (Mic), aggregation process (Agg) on carbon before and after biochar amendment. The gof is a pseudo goodness of fit measure that accounts for the model quality at both the measurement and the structural models; CK was the control treatment without biochar application, LK represented low biochar addition amounts, and HK represented high biochar addition amounts

([Prayogo et al., 201](#page-9-22)4). It confirmed that roles of microorganisms in regulating carbon cycles greatly depended on nutrient limitation in saline-sodic soils, especially nitrogen bioavailability. Biochar initially promoted microorganism biomass via bringing more nutrients by biochar, which was proved by rising  $CO<sub>2</sub>$  production over the short term in arable soils [\(Prommer et](#page-9-4) [al., 2014\)](#page-9-4). However, microorganisms would utilize SOC associated with clay minerals as nitrogen or carbon sources when extra nitrogen from biochar was depleted. This was confirmed by the effects change of nut on carbon, which divered to −0.6284 in LK + HK from 0.9684 in CK treatments. Nutrients were the primary driver affecting SOC pool in saline-sodic soils,

Meanwhile, the Agg block was the only one that had positive effects on SOC, from 0.0820 in CK to 0.3478 in  $LK + HK$ . Aggregation improvement caused by biochar addition could better the SOC pool, and this was in good agreement with PCA and the regression analysis results. However, as mentioned above, SOC increase after biochar addition might come from biochar materials but not from the native soil organic matter, and effects of biochar on native SOC p[reserve should b](#page-9-19)e studied over the long-term timescale ([Liu et al., 2018\)](#page-9-19).

# **5 Conclusions**

Our study demonstrated that rice straw-derived biochar addition had limited improvement effects, by 1.16%− 12.80%, on SOC in saline-sodic soils, However, DOC

increased significantly by up to 67% with biochar application. Biochar amendment could facilitate FUN and ACT biomass but aggravated nitrogen limitation on microorganisms. ACT and N∶P were the predominant factors controlling DOC contents, while CPOC accounted for most SOC changes. The PLS-PM models implied that mitigating nutrient limitation and improving the soil aggregation process should be considered in priority when biochar was used to remediation saline-sodic soils.

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