Reduced carbon sequestration potential of biochar in acidic soil

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HIGHLIGHTS

- CO_2 release from biochar-amended soils with different pH levels were studied.
- CO_2 release from inorganic carbon dominanted CO_2 mineralization in acid soil at early stage.
- Lower G^-/G^+ bacteria ratio associated with overall higher CO_2 emission in acid soils.
- Carbon sequestration potential of biochar was overestimated in acidic soil.

GRAPHICAL ABSTRACT
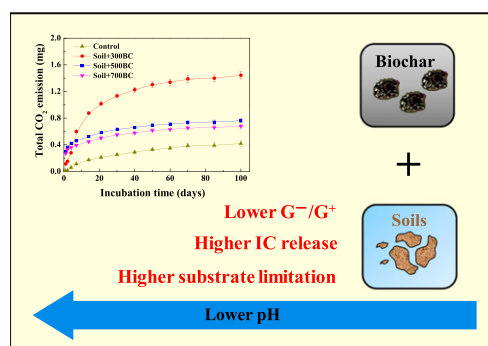
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ABSTRACT

Biochar application in soil has been proposed as a promising method for carbon sequestration. While factors affecting its carbon sequestration potential have been widely investigated, the number of studies on the effect of soil pH is limited. To investigate the carbon sequestration potential of biochar across a series of soil pH levels, the total carbon emission, CO_2 release from inorganic carbon, and phospholipid fatty acids (PLFAs) of six soils with various pH levels were compared after the addition of straw biochar produced at different pyrolysis temperatures. The results show that the acidic soils released more CO_2 (1.5–3.5 times higher than the control) after the application of biochar compared with neutral and alkaline soils. The degradation of both native soil organic carbon (SOC) and biochar were accelerated. More inorganic CO_2 release in acidic soil contributed to the increased degradation of biochar. Higher proportion of gram-positive bacteria in acidic soil (25%–36%) was responsible for the enhanced biochar degradation and simultaneously co-metabolism of SOC. In addition, lower substrate limitation for bacteria, indicated by higher C-O stretching after the biochar application in the acidic soil, also caused more CO_2 release. In addition to the soil pH, other factors such as clay contents and experimental duration also affected the phsico-chemical and biotic processes of SOC dynamics. Gram-negative/gram-positive bacteria ratio was found to be negatively related to priming effects, and suggested to serve as an indicator for priming effect. In general, the carbon sequestration potential of rice-straw biochar in soil reduced along with the decrease of soil pH especially in a short-term. Given wide spread of acidic soils in China, carbon sequestration potential of biochar may be overestimated without taking into account the impact of soil pH.

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1. Introduction

Given the increasing concerns over global warming, it is important to adopt mitigation strategies to lower the CO_2 concentration in the atmosphere. Recently, storing biochar in soil, with the potential of

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sequestrating atmospheric CO_2 (Woolf et al., 2010) and improving soil fertility (Lehmann, 2007) has attracted growing attention, which is especially appealing in developing countries. China, where the topsoil soil organic carbon (SOC) density is generally lower than the global average (Batjes, 2014; Song et al., 2005), is expected to have a higher soil carbon sequestration potential. The carbon sequestration potential of biochar is related to the properties of biochar and soil (e.g., pH, SOC, and clay content). Naturally acidic soil covers approximately 27% of the total cultivated land of China (Wilson et al., 2004) and 30% of the world's ice free land area (Vonuexkull and Mutert, 1995). Topsoil even suffers from significant acidification by a pH decline of 0.13–0.80 due to long-term overuse of nitrogen fertilizers and intensive acid deposition in China (Guo et al., 2010). Therefore, estimating the carbon sequestration potential of biochar across pH especially in acidic soils is essential for possible large-scale application of biochar.

Biochar's climate-mitigation potential primarily stems from its highly recalcitrant nature towards chemical and microbial oxidation (Keiluweit et al., 2010). The decomposition rate of biochar varies significantly with experimental durations, feedstocks, pyrolysis temperatures, soil pH and clay contents (Wang et al., 2016). Biochars produced at lower temperatures were reported to mineralize faster than those produced at higher temperature with increased aromaticity and degree of aromatic condensation (Fang et al., 2014; Zimmerman, 2010). Previous studies also indicated that more carbonates were formed in higher pyrolysis temperatures. In addition, biochars, which were made from crop residues such as tomato and corn, tend to have higher ash contents and carbonates (Murray et al., 2015; Yuan et al., 2011). Once biochar is applied into soil, acid-base neutralization will react and tend to be more intensive for acidic soil. In contrast, neutral or alkaline soil adsorbs CO_2 from the atmosphere and leads to an under-estimation of the CO_2 evolution from soil (Blagodatskaya and Kuzyakov, 2008). Despite higher CO_2 emission from carbonates in acidic soils, the abiotic process may be easily obscured by microbial mineralization (Bruun et al., 2014). More biochar C mineralized in the high pH soil was observed than in the low pH soil, largely due to the higher microbial biomass (Fang et al., 2014; Keith et al., 2011) and smaller extractable Al and Mn concentrations (Luo et al., 2011). Therefore, determining physico-chemical and biotic processes on biochar degradation across pH needs more research effort.

Generally, change in the mineralization rates of soil organic carbon (SOC) induced by additional substrates is termed as priming effect (Zimmerman et al., 2011). Both positive and negative priming effects, i.e., accelerating and retardation of SOC mineralization, respectively (Kuzyakov et al., 2000), caused by biochar have been observed (Bass et al., 2016; Zimmerman et al., 2011). After addition of biochar, various polar compounds (included lactic acid, acetic acid) contained in dissolved biochar, micronutrients and habitats favoring microbial heterotrophic activity (Castaldi et al., 2011; El-Naggar et al., 2015; Fernandez et al., 2014) through biochar amendments may result in the co-metabolism and positive priming of SOC (Mukherjee et al., 2014). Besides, soil-derived DOC sorption and encapsulation by biochar formation of organo-mineral fractions and protection from predation in porous structure (Fang et al., 2014; Joseph et al., 2010; Li et al., 2014; Lin et al., 2012) would cause suppression of native SOC decomposition (Lu et al., 2014). In recent years, meta-analyses have indicated that CO_2 fluxes with biochar amendment were negatively related to the biochar application rate, pyrolysis temperatures, and C/N ratio of the biochars (Liu et al., 2016). Biochar characterized by a low C content induced a higher positive priming effect (PE) on native soil organic carbon (Wang et al., 2016). Besides, duration of the incubation would also affect the PE, as biochar might induce a negative PE by promoting physical protection mechanisms, and C saturation of microbial communities in a long-term (Hernandez-Soriano et al., 2016; Maestrini et al., 2015).

In acidic soils, the water holding capacity, nutrients, pH, and EC met significant increases after biochar addition (Molnar et al., 2016). As the stabilization of SOC can be affected by soil pH, the preferential substrate

utilization and priming effect would change after biochar application. Research indicated complexation of SOC on mineral surfaces via ligand exchange increases with decreasing pH (Gu et al., 1994). Due to the decrease in adsorption sites on the oxide surfaces, the adsorption of SOC decreased with the increase of pH (von Lutzow et al., 2006). By grouping the soils presented in the recent literatures into acid, moderately acid, neutral, and alkaline soils, increased soil microbial biomass carbon and significant positive priming effects were observed for acid soils (Liu et al., 2016). On the contrary, biochar amendments to neutral or alkaline soils caused inhibition of soil C mineralization with increased soil pH (Liu et al., 2016). Nevertheless, the biochar-induced change of substrate limitation, performed as DOC concentration and bioavailability, and simultaneous priming effects (Streit et al., 2014) across soil pH are still not available.

Changes of physicochemical properties and substrate utilization by biochar would therefore greatly affect the soil microbial communities. Bacterial PLFAs would considerably increase with rising pH (Hogberg et al., 2007), whereas fungi showed parabola trend with the highest biomass across pH of 6–7 (Pietri and Brookes, 2009; Rousk et al., 2009). Decreases ratio of fungi/bacteria (F/B) and gram-negative/gram-positive bacteria (G^-/G^+) suggested a microbial community shift in favor of gram-positive bacteria in acidic forest soil (Mitchell et al., 2015). In contrast, increased G^-/G^+ ratio were observed in neutral and alkaline soils (Ameloot et al., 2014; Gomez et al., 2014). In recent years, in light of the barcoded pyrosequencing of 16S rRNA gene amplicons, biochar-induced shift for bacteria occurred at the genus and phylum levels, for fungi at the genus level (Hu et al., 2014). Relative abundance of bacteria within the *phyla Actinobacteria*, *Gemmatimonadetes* and *Flavobacterium* in biochar-treated soils was identified (Jenkins et al., 2016; Khodadad et al., 2011; Kolton et al., 2011). In contrast, an overall loss of microbial diversity in all soils treated with oak and grass-derived biochar have also been detected (Khodadad et al., 2011), such as the abundance of *Methylophilaceae* and of *Hydrogenophilaceae* (Chen et al., 2013). Yet, soil microbial community composition related to soil properties and the relationship between greenhouse gas emissions is still ambiguous and need to be further explored.

Therefore, this study aims to investigate the effect of soil pH on the carbon sequestration potential of biochar. The biochar degradation and SOC mineralization in six typical soils were studied to reveal the effects of pH on the following processes: (1) the contribution of CO_2 release from inorganic carbonates to the total CO_2 emission; (2) the role of soil microbial community composition in the dynamic of SOC mineralization; and (3) the change in surface functional groups of soil and the consequent substrate limitation to SOC mineralization. In this study, the total CO_2 emission, $\delta^{13}C$ analysis, and related CO_2 release from inorganic carbon were investigated. Microbial community changes of soils after biochar application were revealed by phospholipid-derived fatty acids (PLFA) analysis. Change of soil carbon structure and dissolved organic carbon concentration were analyzed by the Fourier Transform infrared spectroscopy (FTIR) and total organic carbon analyzer. The results of this study are expected to calibrate the carbon sequestration potential of biochar in acidic soil on a regional or global scale.

2. Materials and methods

2.1. Soils and biochar

Six typical soils were collected from farmlands. Soil S1 was collected from Shangrao, Jiangxi province (29.2°N, 117.8°E), soil S2 was collected from Hangzhou, Zhejiang province (30.2°N, 120.1°E), soil S3 was collected from Huangshan, Anhui province (29.6°N, 117.7°E), soil S4 was collected from Hangzhou, Zhejiang province (30.3°N, 120.2°E), soil S5 was collected from Harbin, Heilongjiang province (45.7°N, 126.6°E), and soil S6 was collected from Chongqing, Sichuan province (30.3°N, 105.9°E). Soil samples were hand-picked to remove obvious plant debris and roots, air-dried, sieved through a 2 mm sieve, and analyzed

for basic properties including pH, carbon content, and particle size. For separating C mineralization sources, soils S1, S2, S4, and S5 were cultivated with typical C4 plant (corn) for years.

Rice straw was selected as the biochar feedstock given its abundance in China. It was ground, passed through a 0.154 mm sieve, and then was oven-dried overnight at 70 °C. The powder was compacted in a quartz boat and pyrolyzed for 2 h at various temperatures (300 °C, 500 °C, and 700 °C, annotated as 300BC, 500BC, and 700BC, respectively) in the tube furnace which was filled with N_2 and programmed at a heating rate of 5 °C min^{-1}. The produced biochar was then sieved to <0.154 mm for subsequent analysis.

The WHC was determined using a method adapted from Case et al. (2012). 150 g dried soil or soil-biochar mixture (2% w/w) was added to plastic cylinders (inner diameter 5 cm, height 7 cm), with the bottom end covered in a fine mesh. Each cylinder was submerged in a water tank for 8 h. The cylinders were covered with plastic film to limit evaporation. The samples were then removed, permitting drainage by gravity for 3 h, weighed after three hours, heated to 105 °C for 12 h and reweighed. The WHC of soil and soil-biochar mixture are shown in Table S1. Dissolved organic matter (DOM) was extracted from the mixture of biochar and soil with distilled water (1:1 w/v) for 30 min. The mixture was then centrifuged with a rotation speed of 3000 rpm for 15 min and the supernatant was collected and filtrated through a 0.2 mm membrane. DOC content was measured with a Shimadzu TOC-V$_{CPH}$ analyzer. Compositional changes of surface functional groups in control and biochar-amended soils were observed by Fourier Transform infrared spectroscopy (FTIR, Thermo scientific, Nicolet 6700). Chemical and physical properties of the soils are shown in Table 1. Other properties of biochar are shown in Table S1.

2.2. Incubation experiment

In this experiment 24 treatments were set with five replicates, consisting of 6 controls with soils only and 18 treatments with permutations of 6 soils and 3 kinds of biochar. For controls, 5 g soil was incubated in 40 ml sample vials. For the 18 treatments, 5 g soil and 100 mg biochar were thoroughly mixed in 40 ml sample vials. As increased moisture content reduces oxygen availability to soil microorganisms, the change of water holding capacity would directly affect the generation of CO_2 (Baronti et al., 2014; Case et al., 2012; Zong et al., 2016). To ensure the same soil moisture and aeration for biochar-treated soils, deionized water of 80% soil or soil-biochar mixture water holding capacity was added to the vials, which were sealed and incubated in the dark at 25 °C. Three empty vials were set to measure the concentration of CO_2 of surrounding atmosphere.

To determine the CO_2 release, 100 μl gas samples were withdrawn from the vials and measured with a gas-tight glass syringe (Agilent) through injecting the headspace CO_2 into the gas chromatograph (Fuli 9790). Flame Ionization Detector (FID) was used to detect CO_2 at 250 °C. Porapak Q column was used to separate CO_2 with a column temperature of 80 °C. Limits of detection (LOD) of CO_2 measurement was 2 ppm, and the relative standard deviation (RSD) were lower than 5%.

Table 1
Selected soil properties.

Soil	pH	SOC[a] (mg/g)	SIC[b] (mg/g)	WHC[c] (%)	Sand (%)	Silt (%)	Clay (%)	Soil type
S1	4.34	2.15	0.0120	35.3	19.2	23.6	57.2	Ferralsols
S2	5.19	12.7	0.0361	41.8	18.5	21.1	60.4	Ferralsols
S3	5.88	26.2	0.0739	37.5	59.7	29.3	11.0	Anthrosols
S4	7.74	12.3	0.127	32.3	68.1	22.6	9.3	Luvisols
S5	7.81	35.7	2.32	40.5	25.2	47.1	27.7	Phaeozems
S6	7.91	14.4	1.37	41.2	36.2	40.2	23.6	Cambisols

[a] SOC: Soil organic carbon.
[b] SIC: Soil inorganic carbon.
[c] WHC: Water holding capacity.

After the measurement, the vials caps were kept open for 20 min, leaving the vials refilled with the atmosphere air for re-incubation. The incubation experiments was carried over a period of 100 days, and CO_2 evolution was measured periodically at 1, 2, 4, 7, 14, 21, 30, 40, 50, 60, 70, 85, 100 days of incubation. For treatments of soils S1, S2, S4, and S5, another 100 μl samples from the vials were carried out simultaneously for $\delta^{13}C$ analysis to determine the carbon mineralization source.

2.3. CO2 release from mineralization of organic carbon and inorganic carbonates

In this experiment 24 treatments were set with three replicates, consisting of 6 controls with soils only and 18 treatments with permutations of 6 soils and 3 biochars. For the controls, 5 g soil was incubated in 40 ml sample vials. For the treatments, 5 g soil and 100 mg biochar were thoroughly mixed in 40 ml sample vials. These treatments were heat-treated at 160 °C for 2 h. Deionized water of 80% water holding capacity with excessive concentration of NaN_3 or $HgCl_2$ in a mass ratio of 0.5% was added to maintain the sterile state. For soils S1, S2, and S3 (pH < 7.0), $HgCl_2$ was added to sterilize. For the other soils (pH > 7.0), NaN_3 was used to sterilize. The headspace CO_2 was measured as cumulative CO_2 release by using the method described in Section 2.3.

The CO_2 release from mineralization of organic carbon was calculated by subtracting abiotic from the total CO_2 emission. The effect of biochar application on the total CO_2 emission and CO_2 release from mineralization of organic carbon was calculated as: $(C_T - C_S)/C_S \times 100\%$, where C_T is the total C mineralized from the biochar-amended soil, C_S is the amount of CO_2-C evolved from the control soil. The effect was calculated as percentage (%) of non-biochar treatment. Furthermore, CO_2 release from inorganic carbon/total CO_2 emission (IC/TC) ratio was used to calculate the contribution of abiotic process for soils.

2.4. Carbon mineralization source and priming effects

100 μl gas samples were withdrawn from the incubated soils and soil-biochar mixture vials to the sample bridge for $\delta^{13}C$ analysis using Precon-IRMS (Delta V Thermo Finnigan). The amount of biochar mineralized (C_B) from biochar-amended soil was calculated as:

$$C_B = \frac{C_T \left(\delta_T^{13}CO_2 - \delta_S^{13}CO_2 \right)}{\left(\delta_B^{13}CO_2 - \delta_S^{13}CO_2 \right)} \quad (1)$$

where C_T is the total C mineralized from the biochar-amended soil, $\delta_T^{13}CO_2$ is the $\delta_T^{13}C$ value of the CO_2-C evolved from the biochar-amended soils, $\delta_S^{13}CO_2$ is the $\delta_T^{13}C$ value of CO_2-C evolved from the control soil, and $\delta_S^{13}CO_2$ is the initial $\delta_T^{13}C$ value of the biochar. The amount of the soil-C mineralized ($C_{S,B}$) from the biochar-amended soil and the priming effects (PE) induced by the biochar on mineralization of the native organic carbon in the soils were calculated as:

$$C_{S,B} = C_T - C_B \quad (2)$$

$$PE = C_{S,B} - C_S \quad (3)$$

where C_S is the amount of CO_2-C evolved from the control soil, C_T is the total C mineralized from the biochar-amended soil, C_B is the amount of C mineralized from biochar. The positive priming effect means accelerating of SOC mineralization by biochar. In contrast, negative priming effect means the suppression of SOC mineralization by biochar.

Cumulative biochar degradation was calculated. Furthermore, to eliminate the impact of soil SOC content on biochar degradation, the mineralization rate of biochar as per unit native SOC were used to describe the degradation of biochar in different soils.

2.5. Phospholipid-derived fatty acids (PLFA) analysis

The PLFA analysis was used as a rapid and sensitive method to detect the change in the soil microbial community. The main microbial taxa, including bacteria, fungi, and actinomycetes were indicated by specific PLFA biomarkers. Soil PLFA extraction and purification was performed according to a previous study (He et al., 2013). The fatty acids were extracted from 3 g freeze-dried soil samples by the extraction mixture containing phosphate buffer, chloroform and methanol after 100 days' incubation. The lipid extractions were then purified by a solid phase extraction column (500 mg; 3 ml; Agilent Technologies Inc., UK). The neutral lipids, glycolipids, and phospholipids were eluted with chloroform, acetone and methanol, respectively, after being dried with N_2. PLFAs were subsequently derivatized through mild alkaline methanolysis reaction. The following PLFA analyses were performed by using the Agilent 6890 N gas chromatograph (Agilent, Wilmington, DE) fitted with a MIDI Sherlock microbial identification system (Version 4.5, MIDI, Newark, NJ).

The PLFAs used as markers of each of the specific groups were described in Table S3. The microbial community composition was also assessed by the ratios of gram-negative bacteria/gram-positive bacteria (G^-/G^+) and fungal/bacterial (F/B), thus to better analyze the microbial community change and dominated population.

2.6. Statistical analyses

Statistical evaluation of the data sets was performed on SPSS version 20.0 (SPSS Inc., Chicago, USA). Significant differences between treatments at different times of incubation were tested using the one-way analysis of variance (ANOVA). The principle component analysis (PCA) was performed to evaluate the difference in microbial community change of soil PLFAs. The statistically significant level was set to 0.05.

3. Results and discussion

3.1. Effect of soil pH on the CO_2 release from carbonates and mineralization of organic carbon

Biochar significantly increased the total CO_2 emission in acidic soils ($p < 0.05$). In contrast, the total CO_2 emission was suppressed for the 500BC and 700BC treatments in neural and alkaline soils (Fig. 1a). 300BC-amended soils released much more CO_2 than 500BC and 700BC-amended soils (Fig. 1a, Fig. S1). Since lower-temperature-pyrolyzed biochar have higher amount of aliphatic and volatile components (Zimmerman, 2010), more labile fraction would be oxidized during incubation.

The CO_2 release from the carbonates of biochar was responsible for the short-term increase of the observed CO_2 emission in acidic soils. A

negative relationship between the IC/TC and the soil pH values was observed (Fig. 1b). All biochar-amended soils showed higher IC/TC ratios compared to the control in the acidic soils (with the highest IC/TC ratio 61.5%, 32.5%, and 11.9% for S1, S2, and S3, respectively at early stage of the incubation). In addition, the highest mineralization rate of biochar appeared on the first day for soils S1 and S2, following an order of 700BC > 500BC > 300BC. Moreover, the mineralization of 700BC on the first day in soils S1 and S2 were almost the same, occupying 65% and 59% of the total CO_2 emission. The transient peak of degradation rate from biochar in soils S1 and S2 on the first day with 500BC and 700BC followed by a sharp decline indicated that a faster abiotic release of carbonates from biochar with lower pH (Fig. S2). The effect of abiotic release of carbonates in biochar for acid soils only occurs sharply in the early stage of incubation, based on the content of carbonates in biochar. Although more carbonates in the biochar generated at higher temperature, CO_2 release from inorganic carbon was observed to be higher in 500BC than 700BC in acidic soils, mainly due to the higher soil pH induced by 700BC.

Higher-temperature-pyrolyzed biochar-amended neutral or alkaline soils adsorbed much more CO_2 from the atmosphere. In S4-S6 (pH > 7.0), the highest IC/TC ratio was observed in 300BC-amended soil, CO_2 release from inorganic carbon significantly decreased for 500BC and 700BC treatments ($p < 0.05$). Since H_2CO_3 was the unique form that dissociated immediately into CO_2 and H_2O at pH < 4. When the pH was higher, the equilibrium between CO_2 and carbonates promoted the formation of carbonates (Maestrini et al., 2015), thus to adsorb more CO_2 from the atmosphere and might lead to an under-estimation of the CO_2 evolution (Blagodatskaya and Kuzyakov, 2008).

In general, the promotion or suppression of abiotic CO_2 release was a short-term effect and determined by the amount of carbonates of biochar, where pH took effect through interaction between biochar and soil. Murray et al. (2015) compared the stability of low- and high-ash biochars, and found more CO_2 released from Ferralsol (acidic soil) in the first day after applying tomato-biochar and blue-mallee-biochar. The results suggested a higher abiotic release and less stable structure of low-ash tomato-biochar in acidic soils (Murray et al., 2015). In this study, biochar was made rice straw and had a relative lower ash content and less stable carbon structure, which tended to promote more CO_2 than biochar made from other feedstocks. The IC/TC ratio decreased during the incubation, accompanied with the equilibrium of pH in the soil-biochar mixture. Furthermore, after subtracting the abiotic CO_2 release, the CO_2 release from organic carbon mineralization still showed the same trend as the total carbon release. Therefore, dissociation of carbonates was not the only way responsible for the increase of the observed CO_2 emission. Other mechanism such as organic carbon mineralization by microbial communities also contributed to the phenomenon.
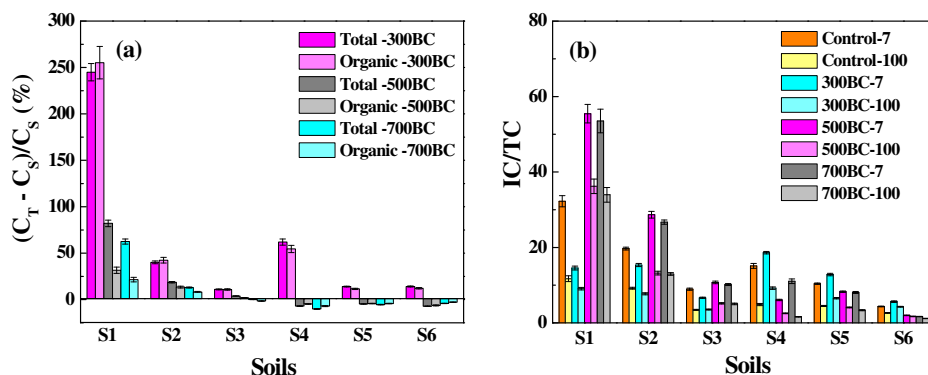

Fig. 1. (a) Effect of biochar on the total CO_2 emission and the CO_2 release from mineralization of organic carbon at the end of the incubation: $(C_T - C_S)/C_S \times 100\%$, where C_T–CO_2 release of biochar-amended soil; C_S–CO_2 release of control soil. Positive $(C_T - C_S)/C_S$ means increased CO_2 release after biochar application, and negative $(C_T - C_S)/C_S$ suggests suppression of CO_2 release. (b) Effect of biochar on the inorganic carbon/total carbon (IC/TC) mineralized (%) for six soils on the early stage (7th day) and the end (100th day) of the incubation.

3.2. Biochar degradation and induced priming effect of SOC across different soil pH

The degradation rate of biochar was negatively correlated with the soil pH (Fig. 2). Despite of lower abiotic release for biochar pyrolysis at lower temperature (300 °C), faster degradation during the incubation was shown (Fig. S3), which was in accordance with a previous study (Singh et al., 2012). Biochar pyrolyzed at higher temperature has greater chemical recalcitrance and stability. Furthermore, surface covering by silicates derived from ashed tissues could also protect biochar pyrolyzed at higher temperature from decomposition (Bertrand et al., 2007; Xiao et al., 2014). Despite the rapid dissolution of carbonates in acidic soil on the first day, more biochar C was mineralized in the high-pH soil than in the low-pH soil (Fig. S2). Higher microbial biomass was attributed to higher biochar mineralization in the high-pH soil, which was related to the content of SOC by offering more available substrates for microbial growth. Nevertheless when expressed as the mineralization of biochar per unit native SOC in different soils, the revised degradation of biochar had a negative relationship with the soil pH.

Apart from the soil pH, other factors might also affect the biochar stability, such as feedstocks, pyrolysis temperatures, SOC and clay contents. The long-term stability of biochars was in the order of manure biochar < leaf biochar < wood biochar. Besides, a greater degree of mineralization was found for low-temperature biochars from the same feedstock (Singh and Cowie, 2014). It was resulted from a more aromatic structure with higher aryl C content (Luo et al., 2011), which is consistent with the results of this study. The large amount of native SOC also support greater microbial activity and biomass for biotic mineralization of biochar-C (Fang et al., 2014; Keith et al., 2011); (Kimetu and Lehmann, 2010). The stability of biochar in soils might also attribute to its reduced accessibility via organo-mineral associations (Fang et al., 2014). The soil with abundant Fe and Al oxides provided greater stabilization to biochar C compared to the soil with abundant phyllosilicates dominated soil (Murray et al., 2015). The organo-mineral associations could also explain the slightly decreased biochar degradation rate in soils S1 and S2 at the end of the incubation. Further studies on the mechanism and contribution on those factors influencing biochar stability should be conducted.

In acidic soils, biochar addition initially (first 7 days) accelerated the SOC mineralization. The application of the low-temperature-pyrolyzed biochar induced more drastically positive priming effect than the high-temperature-pyrolyzed biochar (Fig. 3a). Co-metabolism and liming effect were considered to be the main reasons for the priming effects in acidic soils. Since biochar C pyrolyzed at lower temperature contained higher volatile matter, which was more easily mineralized and resulted in increased microbial biomass and the simultaneous increase in enzyme production (Blagodatskaya and Kuzyakov, 2008; Keith et al., 2011; Kuzyakov et al., 2009). The lag phase and lower priming effect of 300BC amended soils within 7 days introduced another

mechanism such as liming effect. As soils amended with higher-temperature-pyrolyzed biochar had higher pH and higher respiratory quotient (Bruun et al., 2014), the mineralization of SOC would be more drastically stimulated. However, the positive priming effect of 500BC-amended soil S2 shifted from positive (11.9 μg C on the 4th day) to negative (13.07 μg C on the 100th day) across time. As the labile C components of biochar were depleted quickly, the effect of co-metabolism would decrease, accompanied with other mechanisms such as microbial adaption though the incubation, the stabilization of biochar adsorption and more stable organo-mineral interactions (Gu et al., 1994; Yang et al., 2016).

Compared with the acidic soils, the neutral and alkaline soils showed negative priming effect in the early stage of incubation. Biochar initially exhibited a suppression of SOC mineralization (30.92, 44.86, 68.65 μg C of 300BC, 500BC, 700BC amended soil S5 on the 7th day of the incubation, respectively). The neural and alkaline soils amended with higher-temperature-pyrolyzed biochar resulted in a higher negative priming effect. Adsorption of DOC to biochar was introduced to explain this negative priming effect. The concentration of DOC in biochar-amended soil significantly decreased (Fig. S6), suggesting the protection SOC from microbial degradation and offset the excess CO_2 release by co-metabolism. Besides, as the smaller adsorption capacity and less stable structure of 300BC, negative priming effect switched to positive priming effect over time (Fig. 3b).

The results showed a negative relationship between the priming effect and the soil pH. Similarly, other studies suggested a more pronounced positive priming effect caused by liming effect of biochar in low-pH soil (Luo et al., 2011). On the contrary, a meta-analysis on pyrogenic organic matter induced priming effect of previous researches did not observe a significant relationship between the priming effect and the soil/biochar pH (Maestrini et al., 2015). The meta-analysis was taken by a time scale of over a year and the priming effect would switch from positive to negative priming or in the opposite across time, which would obscure the effect of pH. Other factors such as SOC, biochar application rates, temperatures, experimental duration and moisture contents might also influence the results. Therefore, more factors should be taken into account for evaluating the carbon sequestration potential of biochar.

3.3. Biochar induced microbial structure change across different soil pH

The predominant group of microorganisms was gram-positive bacteria which comprised 25.1% and 38.6% of the total microbial PLFAs in soils S1 and S2, respectively. The PLFA concentrations suggested a preferential community for gram-negative bacteria in the other four soils which comprised 37.8% to 53.2% of the total microbial biomass (Table S4). As stronger ligand exchange existed between carboxyl/hydroxyl functional groups in natural organic matter and iron oxide surfaces, especially under acidic or slightly acidic pH conditions (Gu et al., 1994). The distribution of soil microbial communities by pH was attributed to more stable organo-mineral structure, as well as the lower nutrient content in acidic soils. Gram-positive bacteria which had the ability to form spores (Moche et al., 2015), dominated in severe environmental conditions as presented here in acidic soils. Since gram-positive bacteria was known for their importance in the decomposition of aromatic C (Santos et al., 2012), the presented higher gram-positive bacteria in acidic soils would favor the degradation of biochar which was rich in aromatic C, in consistence with higher degradation for biochar. On the contrary, a more gram-negative bacteria-dominated community in neural and alkaline soils favored easily degradable carbon substrates (Feng et al., 2010) and was weak in degradation of biochar.

The addition of biochar significantly increased total PLFA-C abundance ($p < 0.05$), especially at the early stage of the incubation (Table S4, Fig. S3). The increased amount of PLFAs in the 300BC-amended soil was observed to be significantly higher than that of 500BC and 700BC. In accord with the other study using PCR-DGGE, significantly and greatly increased bacterial gene abundance, as well as the small increase in
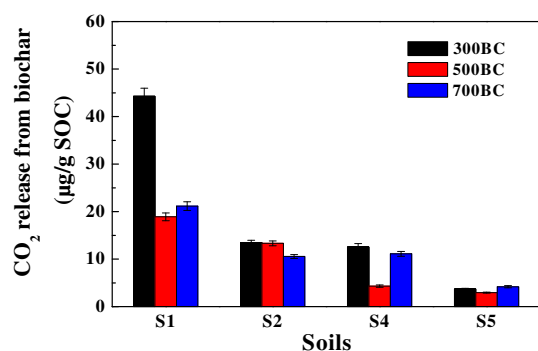

Fig. 2. CO_2 release from biochar per unit of soil organic carbon (μg/g SOC) under different pH values over 100 days of incubation.
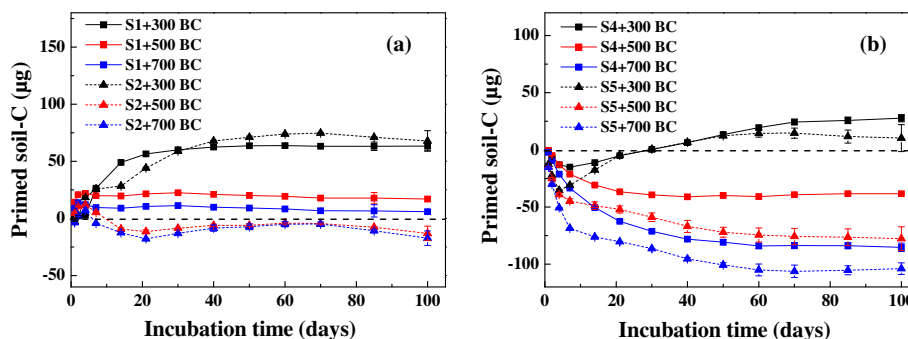
Fig. 3. Primed soil organic carbon (μg) of (a) the acidic soils S1 and S2; and (b) the alkaline soils S4 and S5. Positive primed soil organic carbon (SOC) indicates increased carbon mineralization of SOC in biochar-amended soils, and negative soil organic carbon indicates decreased SOC mineralization.

fungal gene abundance were observed under biochar application (Lu et al., 2015). Despite the increase of PLFAs, total CO_2 emission decreased for neural and alkaline soils in 500BC and 700BC, suggesting other mechanisms such as the distribution of microbial communities (G^-/G^+ ratio and F/B ratio) would also affect the mineralization of soil carbon. As indicated in Fig. S4, the PLFA change was mainly related to soil types rather than the kinds and application of biochar, and the effects of soil types became more drastic as time goes on. The results also suggest that the site effects are greater than biochar treatment, emphasizing the importance of soil properties in the carbon sequestration by biochar.

The G^-/G^+ ratio exhibited a positive correlation with the soil pH ($p < 0.05$). Increased soil pH and change of substrates bioavailability by biochar induce the higher G^-/G^+ ratio. Higher G^-/G^+ ratio were observed in higher-temperature-pyrolyzed biochar amended acidic soils except soil S1 (Fig.4a). The easy bioavailable materials in biochar such as hemicelluloses might be responsible for the increase G^-/G^+ ratio especially at the early stage of incubation. The increased G^-/G^+ ratio was supported by previous study which also showed the decline of gram-positive *Acidbacteria* and abundance of gram-negative Alphaproteobacteria and *Bacteroidetes* (Kolton et al., 2011; Xu et al., 2016). Other suggested a shift from acid to alkaline phosphomonoesterase activity may suggest an ecological and functional shift towards a more copiotrophic ecology (Jenkins et al., 2016). As the gram-negative bacteria had not been detected in control for soil S1 at the end of the incubation, higher aromatic structure in 500BC and 700BC stimulated the growth of gram-positive bacteria.

The G^-/G^+ ratio showed a higher positive relationship with soil pH in the end of the incubation ($p < 0.05$, $R^2 = 0.52$) than in the early stage of the incubation ($p < 0.05$, $R^2 = 0.39$). This was largely due to the depletion of labile C in biochar. At the end of the incubation, due to stronger adsorption capacity for DOC and enzyme in higher-temperature- pyrolyzed biochar, lower G^-/G^+ ratio was observed in neural and alkaline soils. Furthermore, decreasing ratios of G^-/G^+ bacterial PLFAs in the

end of the incubation suggest a possible microbial community shift towards Gram-positive bacteria over time, resulting from the exhausting of labile organic carbon in the treatment.

Fungi accounted for a higher proportion in lower-pH soils (Table S5), and F/B ratio increased with the decrease of the soil pH (Fig. 4). While the F/B ratio exhibited a significant increase for soils S1 and S2 at the early stage of incubation ($p < 0.05$) after the biochar application, no significant change of the F/B ratio was observed for the neural and alkaline soils. The F/B ratio showed a higher negative relationship with soil pH in the end of the incubation ($p < 0.05$, $R^2 = 0.47$) than in the early stage of the incubation ($p < 0.05$, $R^2 = 0.24$). Fungi could be the consumer of aromatic biochar and lignin, accelerating the degradation of biochar (Watzinger et al., 2014). However, despite the higher F/B ratio in acidic soils, it did not show obvious relationship between the biochar degradation and the priming effect of SOC. It was because fungal flora was more efficient in assimilating substrate C than bacterial flora. Generally, fungal flora assimilated 30–40% of C into its new mycelium while bacterial flora only assimilated 5–10% of substrate C into its new cells (Sakamoto and Oba, 1994). As a result, the metabolic quotient decreased with an increase in the F/B ratio (Sakamoto and Oba, 1994), thus to dissimilate less C substrate and caused less CO_2 release (Fig. 5).

Soils with lower G^-/G^+ ratios tended to have positive priming effect, and the extent depended on the pyrolysis temperature of biochar (Fig. 5). Stronger biochar degradation by gram-positive bacteria might be accompanied by co-metabolism of SOC, which would therefore cause the positive priming effect for SOC in acidic soils. Rapid degradation rate of biochar at lower G^-/G^+ ratios also indicated the change of preferential substrate utilization. It was suggested that soil with low biodegradability SOC and nutrient-poor soils were more likely affected by priming effects (Blagodatskaya and Kuzyakov, 2008). Since theses soils were more limited by the availability of energy substrates, the added substrates would act as an important energy source for microbial metabolism. Other studies also suggested that the mineralizability of the SOC determined whether pyrogenic organic matter carbon was a
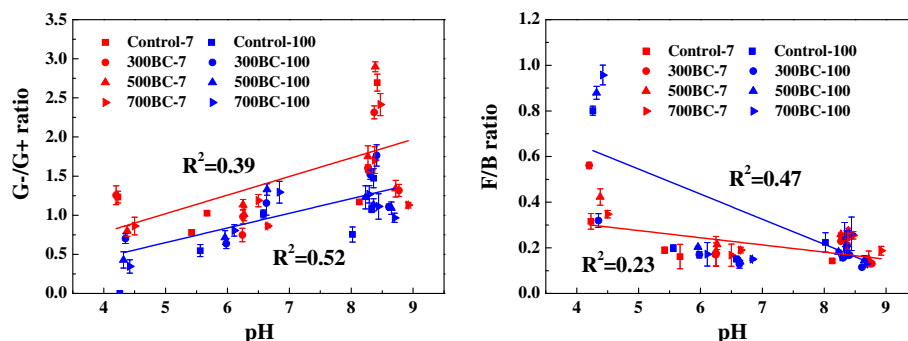

Fig. 4. Relationship of the pH with (a) the gram-negative/gram-positive bacteria (G^-/G^+) ratio and (b) the fungal/bacteria (F/B) ratio.
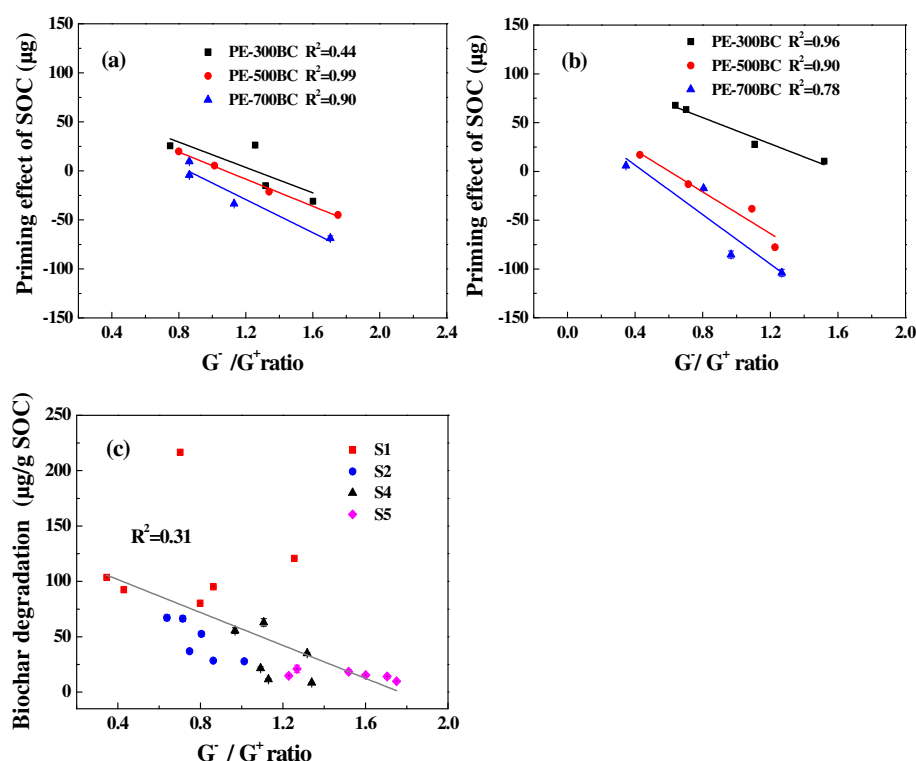
Fig. 5. Relationship between the gram-negative/gram-positive bacteria (G^-/G^+) ratio and the priming effect of the four soils (S1, S2, S4, and S5) in (a) the early stage (7th day) and (b) the end of the incubation (100th day); (c) The relationship between G^-/G^+ ratio and biochar degradation per unit of soil organic carbon (SOC).

more attractive substrate (Whitman et al., 2014). As the heterogeneous structure of carbon in SOC, biochar and the resulting diversity of microbial communities, the G^-/G^+ ratio was suggested be a promising indicator for the prediction of priming effect. Further researches on the relationship between G^-/G^+ ratio and other factors (SOC, water contents, and incubation duration) is needed, as well as for the changes at taxonomic level determined by 16 s rRNA.

3.4. Carbon structure change of biochar-amended soil and consequent substrates limitation

More stable organ-mineral structure and lower nutrients in acidic soils resulted in higher substrate limitation. As indicated by FTIR, the peak at 913 cm^{-1} attributed to the deformation of Al-Al-OH stretching vibration, and higher proportion of aromatic C=C groups (1637 cm^{-1}), confirmed the existing of organo-mineral interaction (Fig. 6a). Previous research also showed the presence of significant char like aryl and O-aryl C of SOC in the aridic arenosol (Farrell et al., 2013). As the more stable nature of SOC in acid soils, the energy sustaining active

microbial populations and production of enzymes was limited, thus contributed to higher substrate limitation and G^-/G^+ ratio. On the contrary, higher aliphatic C—H stretching vibration (1406 cm^{-1}) and "amide-II" N—H (1550 cm^{-1}) were detected in alkaline soils, indicating more easily available substrates and micronutrients, contributed to higher proportion of gram-negative bacteria.

The change of surface functional groups induced by biochar resulted in increased substrate bioavailability in acid soils. After biochar amendment, aromatic C=C and C=O groups strongly increased in DOC, indicating the existing of dissolved aromatic compound (Qian and Chen, 2014) by biochar. Furthermore, Due to labile fraction as carbon source in dissolved biochar, stronger resolved band of C—O at 1168 cm^{-1} and peaks centered around 1040 cm^{-1} as indicative of cellulosic material (Li et al., 2014) were also observed after biochar amendment in acidic soils (Fig. 6b, Fig. S6a).

Consistent with surface functional groups change, a negative relationship between the bacteria substrate limitation and the pH ($R^2 = 0.44$, $p < 0.05$) was observed. In addition, a relative weaker negative correlation between bacteria substrates limitation and the DOC
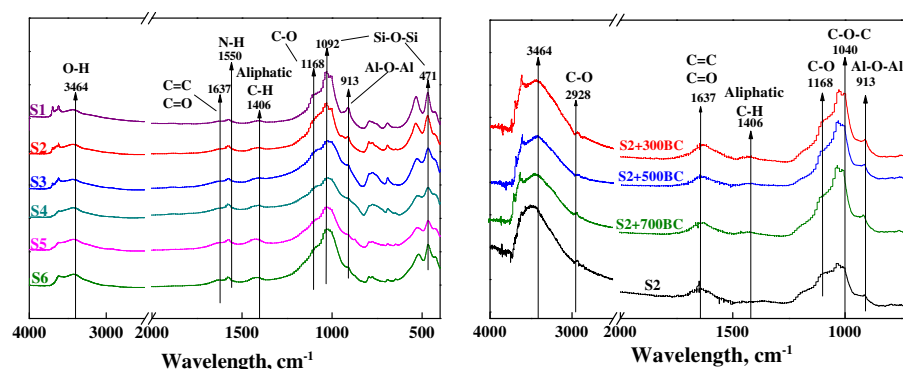

Fig. 6. Fourier Transform infrared spectroscopy (FTIR) profiles of (a) the six original soils and (b) DOC in biochar-amended S2 after 100 days incubation.
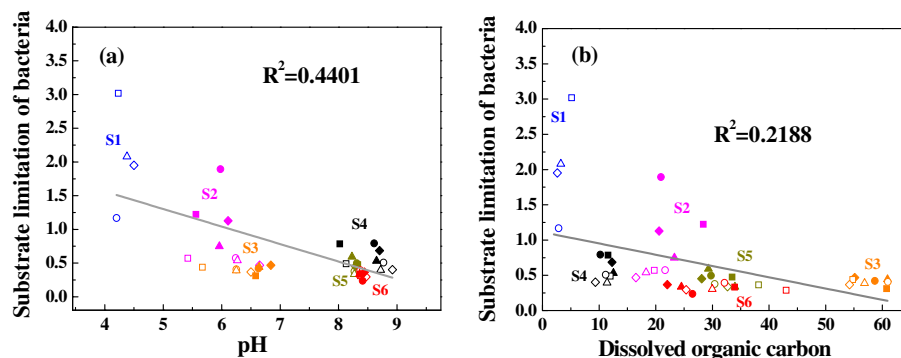
Fig. 7. Relationship of the substrate limitation of bacteria with (a) the pH and (b) the dissolved organic carbon (DOC). Each color represents a soil. Hollows represent soils in the 7th day of incubation, and solids represent soils at the end of the 100th day of incubation. Squares, circles, triangle and diamond mean the control soil, soil amended with 300BC, 500BC, and 700BC, respectively. Data of substrates limitation was unavailable at the end of the incubation for all treatments of soil S1 as the PLFA 16:1w7 has not been detected.

content was also observed ($R^2 = 0.22$, $p < 0.05$). Despite of the decreased DOC (Fig. 7b), biochar addition increased polysaccharides (increase in intensity of infrared absorption at 1050–1000 cm^{-1}) and resulted in drastically decreased substrate limitation in acidic soils. On the contrary, substrate limitation only met a slightly increase for alkaline soils, largely due to adsorption of DOC by biochar. The DOC content of soil S6 amended with 700BC just accounted for 60.47% of the DOC of the control soil, indicating strong sorption of SOC by biochar. The higher stretching vibration of aromatic material also contributed to the substrate limitation, thus to explain why higher microbial biomass existed in neural soils after biochar addition generated lower carbon mineralization.

4. Conclusions

Biochar carbon sequestration potential in acidic soil was limited where the degradation of both SOC and biochar was accelerated. In acidic soil, more carbonates in biochar were released due to the more acidic environment. Higher proportion of gram-positive bacteria and fungi in acidic soils favored the degradation of biochar and stimulated the SOC mineralization. Biochar amendment could relieve the substrate limitation in acidic soil, thus promoted the degradation of SOC and biochar. Although the priming effect of SOC differ in various soils and changed over time, the G$^-$/G$^+$ ratio was introduced as an promising indicative for prediction of carbon sequestration, as lower G$^-$/G$^+$ ratios tended to have positive priming effect. Given a considerable percent of agriculture soil in China is red soil, the carbonates and priming effect of SOC should be taken into account in evaluating the carbon sequestration potential of biochar. As the higher ash content and low carbon content in rice-straw biochar, further research is required to explore the micromechanism, especially the biological process and long-time-scale experiments of soil pH on carbon sequestration potential of various biochar.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.07.140.

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