

## Biochar has no effect on soil respiration across Chinese agricultural soils



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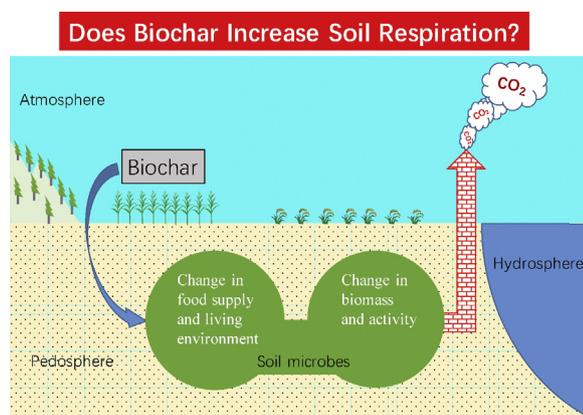
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### HIGHLIGHTS

- Biochar addition did not alter soil CO<sub>2</sub> efflux in agricultural soils.
- Biochar addition did not alter the carbon use efficiency by soil microbes.
- Biochar types and amendment rate had no effect on soil respiration.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Biochar addition to soil has been widely accepted as an option to enhance soil carbon sequestration by introducing recalcitrant organic matter. However, it remains unclear whether biochar will negate the net carbon accumulation by increasing carbon loss through CO<sub>2</sub> efflux from soil (soil respiration). The objectives of this study were to address: 1) whether biochar addition increases soil respiration; and whether biochar application rate and biochar type (feedstock and pyrolyzing system) affect soil respiration. Two series of field experiments were carried out at 8 sites representing the main crop production areas in China. In experiment 1, a single type of wheat straw biochar was amended at rates of 0, 20 and 40 t ha<sup>-1</sup> in four rice paddies and three dry croplands. In experiment 2, four types of biochar (varying in feedstock and pyrolyzing system) were amended at rates of 0 and 20 t ha<sup>-1</sup> in a rice paddy under rice-wheat rotation. Results showed that biochar addition had no effect on CO<sub>2</sub> efflux from soils

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consistently across sites, although it increased topsoil organic carbon stock by 38% on average. Meanwhile, CO<sub>2</sub> efflux from soils amended with 40 t of biochar did not significantly higher than soils amended with 20 t of biochar. While the biochars used in Experiment 2 had different carbon pools and physico-chemical properties, they had no effect on soil CO<sub>2</sub> efflux. The soil CO<sub>2</sub> efflux following biochar addition could be hardly explained by the changes in soil physico-chemical properties and in soil microbial biomass. Thus, we argue that biochar will not negate the net carbon accumulation by increasing carbon loss through CO<sub>2</sub> efflux in agricultural soils.

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## 1. Introduction

Biochar is considered as a carbon-rich organic matter with long residence time up to hundreds of years (Kuzyakov et al., 2009; Lehmann et al., 2015). Its production from waste biomass and use in agriculture has been advocated as an effective means to sequester carbon and reduce greenhouse gas emissions from soils (Lehmann et al., 2006; Atkinson et al., 2010). However, this had been frequently questioned, as several studies reported a short-term positive priming effect of biochar addition on native soil organic matter (SOM), thus negating carbon sequestration (Bruun and Luxhoi, 2008; Kuzyakov et al., 2009; Liang et al., 2010; Smith et al., 2010; Verheijen et al., 2010; Jones et al., 2011; Luo et al., 2011; Zimmerman et al., 2011; Bruun et al., 2014). Such an argument arose from an earlier study by Wardle et al. (2008) who found biochar promoted litter decomposition in soils under forest floor. Later on, Kuzyakov et al. (2009) using <sup>14</sup>C labelling technique, recognized a minor effect of biochar on soil respiration, despite of a growth-linked co-metabolic microbial decomposition of biochar after glucose addition. Similarly, Liang et al. (2010) found that the biochar-rich Amazonian Anthrosols had great capacity to resist SOM decomposition against fresh organic matter addition. A meta-analysis of 46 studies by Sagrilo et al. (2014) quantified a 28% increase in carbon dioxide (CO<sub>2</sub>) release in short-term following biochar addition to soil. This was in contrast to the finding of a similar work by Liu et al. (2015), who included longer term field studies. However, the changes in soil respiration in agricultural soils with biochar amendments are still poorly quantitatively assessed in field.

The persistence of organic matter in soils is determined by ecosystem controls related to edaphic properties, climate, plant species, litter chemistry and input rates, rather than being simply correlated with recalcitrance of the substrates alone (Schmidt et al., 2011). As a key process of carbon exchange between the biosphere and the atmosphere (Schlesinger and Andrews, 2000), carbon dioxide evolution from SOM decomposition is regulated by the size and composition of microbial communities and by the available carbon substrates under a certain vegetation. The decomposition of SOM by microbial activity could be evaluated with microbial metabolic quotient ( $qCO_2$ ), which is estimated by microbial biomass scaled soil respiration (Anderson and Domsch, 1993; Wardle and Ghani, 1995). The  $qCO_2$  is increasingly used to monitor and predict the changes in microbial decomposition of SOM under environmental disturbances (Anderson et al., 2011). Generally, fresh carbon inputs to soil from crop straw and manure selected for fast growing autochthonous microorganisms and could increase  $qCO_2$  (Leita et al., 1999). With input of carbon substrates in availability different to SOM, biochar soil amendment could lead to significant changes in microbial biomass and community structure through biotic and abiotic effects (Lehmann et al., 2011). However, these effects vary widely for different soil types, biochar feedstocks, application rates, and cropping systems. For example, Steinbeiss et al. (2009) reported an increase in fungal biomass in a soil amended with yeast-derived biochar, whereas a loss of microbial diversity was observed in a soil amended with oak and grass derived biochar (Khodadad et al., 2011). Meanwhile, with biochar addition, soil microbial biomass carbon (SMBC) or nitrogen (SMBN) could be either unchanged (Zavalloni et al., 2011) or decreased (Dempster et al., 2012). However, the change in microbial abundance and in community structure could be inconsistent between bacterial and fungal communities, as observed in some Chinese rice paddies

amended with wheat straw biochar (Chen et al., 2013, 2015). Yet, it is still unclear whether the biochar mediated changes in soil microbial activity contribute to soil respiration.

In this study, two types of experiment design were used to investigate the effect of biochar amendment on soil respiration. Three questions will be addressed: 1) How does soil respiration respond to biochar application? 2) Does biochar application rate and soil types affect the response of soil respiration to biochar amendment? 3) Does biochar types (in terms of feedstock and pyrolyzing condition) affect the response of soil respiration to biochar amendment?

## 2. Materials and methods

### 2.1. Field experiment

Two types of experiment design were used to characterize the effect of biochar amendment on soil respiration. Experiment 1 (Exp. 1) was carried out at seven sites in a range of agricultural soils across the main crop production areas of China (Supplementary Fig. S1). The objective of Exp. 1 was to investigate the effect of a single biochar (wheat straw biochar) at three application rates on soil CO<sub>2</sub> fluxes. The seven sites cover various soil types, climatic conditions and land uses. The sites included four rice paddies of CS (Changsha, Hunan Province), JX (Jinxian, Jiangxi Province), GH (Guanghan, Sichuan Province) and YX (Yixing, Jiangsu Province) and three dry croplands of SQ (Shangqiu, Henan Province), XZ (Xinzhou, Shanxi Province) and TA (Tai'an, Shandong Province).

Experiment 2 (Exp. 2) was carried out to compare the effects of biochars from different feedstocks at two pyrolyzing system on soil CO<sub>2</sub> fluxes at a single site. The experiment was conducted at Changshu (CSU), Jiangsu Province in a rice paddy in the Tai Lake plain, Southeast China. In this area, summer rice is rotated with winter wheat.

The experimental sites represent a wide range of climatic conditions from humid to semi-arid, with mean annual temperature between 10.5 and 17.7 °C and mean annual precipitation between 400 and 1500 mm (Table 1). Soil pH varied from 4.9 to 8.4 (Table 2). The concentrations of soil organic carbon (SOC) and total nitrogen (TN) and textures vary also across sites.

### 2.2. Biochars

In Experiment 1, only wheat straw biochar (WSBC) was tested across sites, which is commercially available at Sanli New Energy Company, Henan, China. The biochar was produced *via* pyrolysis with a residence time of 1 h at temperature in a range of 350–550 °C. The system is a vertical kiln with 5 m in height and 1.5 m in diameter. The biochar was ground to pass a 2-mm sieve and homogenized before amending to soils. The properties of this biochar have been described in detail earlier (Zhang et al., 2010; Liu et al., 2012).

In Experiment 2, the four types of biochars were produced from three feedstocks at two pyrolysis systems. Both wheat straw biochar (WSBC) and maize stalk biochar (MSBC1) were obtained from the Sanli New Energy Company, produced under same pyrolysis conditions outlined above. The other two biochars were produced from rice straw (RSBC) and maize stalk biochar (MSBC2) by Nanjing Qinfeng Crop Straw Technology Company. Both RSBC and MSBC2 were produced *via* pyrolysis from rice straw and maize stalk biomass respectively at

**Table 1**

Air temperature, precipitation, crop rotations, crop cultivars and fertilizer regimes in the experimental sites.

Experiment	Soil management	Location	MAT <sup>a</sup> (°C)	MAP <sup>b</sup> (mm)	Crop rotation	Cultivar	Fertilizer (kg ha <sup>-1</sup> Season <sup>-1</sup> )		
							N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Exp. 1	Rice paddy	Changsha (CS)	17.1	1500	Rice-rice	Zhongjiazao17	150	90	90
		Jinxian (JX)	17.7	1400	Rice-rice	Yougong98	300	220	150
		Guanghan (GH)	16.3	890	Rice-wheat	DYou202	240	150	75
	Dray cropland	Yixing (YX)	15.7	1177	Rice-wheat	Wuyunjing7	300	125	125
		Shangqiu (SQ)	13.9	780	Wheat-maize	Zhengdan 958	300	75	90
		Xinzhou (XZ)	10.5	400	Maize	Xianyu335	220	90	180
		Tai'an (TA)	12.8	727	Maize	Zhengdan 958	430	75	0
Exp. 2	Rice paddy	Changshu (CSU)	15.4	1054	Rice-wheat	Changyou 5	165	35	95

<sup>a</sup> MAT: mean annual temperature.<sup>b</sup> MAP: mean annual precipitation.

temperatures of 550–650 °C with residence time of about 1 h. The system is a 12 m long rotatory kiln with 0.9 m in diameter. There were five treatments in total, including the control with no biochar amended (CK), WSBC, MSBC1, MSBC2 and RSBC. As shown in Supplementary Table S1, the total carbon pool varied in a range of 233–572 g C kg<sup>-1</sup>, dissolved organic carbon (DOC) in a range of 216–824 mg kg<sup>-1</sup> and pH in a range of 9.4–10.4, across the 4 types of biochars used.

### 2.3. Field experiment layout

In Exp. 1, biochar was amended at rates of 0, 20 and 40 t ha<sup>-1</sup> at each of the 7 sites. In Exp. 2, each biochar type was added to the rice paddy at rate of 0 and 20 t ha<sup>-1</sup>. After the harvest of a summer crop, biochar was spread on to the soil surface and incorporated into the topsoil to a 15 cm depth by plowing and levelling with a wooden rake. The field experiment at each site was laid out in a randomized block design with three replications. All the plots were 4 m × 5 m in area with individual irrigation and drainage outlets. To minimize mutual interference between the treatment plots and to reduce soil disturbance during gas sampling, boardwalks were fixed at 0.8 m away from the chamber. The field experiment following a similar procedure was initiated in 2009 at YX, in 2010 at CS, JX, GH and SQ, in 2011 at XZ and TA, and in 2014 at CSU (Supplementary Table S2). No more biochar was added subsequently following a single biochar amendment when the experiment initiated, across the sites. Crop production was managed following the local conventional practice, except for biochar use, and was consistent across the treatments.

### 2.4. CO<sub>2</sub> efflux measurement

Soil CO<sub>2</sub> efflux was measured with a static chamber following a procedure described by Zou et al. (2005). In each plot, an aluminum flux collar (0.35 m × 0.35 m × 0.25 m) was permanently installed in the ground and kept in place over the entire crop growing season. The top edge of each collar had a groove (5 cm in depth) for filling with water to seal the rim of the chamber with a levelled surface. The chambers were wrapped with a layer of foam insulation and covered with

aluminum foil to minimize air temperature changes inside the chamber during the gas sampling. No plants were included in the chambers. Soil CO<sub>2</sub> efflux was measured at one-week intervals during the entire growing season for each crop. For each measurement event, gas sampling was performed in the morning from 8 to 10 a.m. (Zou et al., 2005). A gas sample was taken at 0, 10, 20, and 30 min after a chamber closure. CO<sub>2</sub> efflux rate was determined from the slope of the CO<sub>2</sub> concentration in these four sequential samples. Sample sets were rejected unless they yielded a linear regression value of r<sup>2</sup> < 0.90. The CO<sub>2</sub> concentration was analyzed with a gas chromatograph (Agilent 7890 A) equipped with a flame ionization detector. A mean daily CO<sub>2</sub> flux over a crop growing season was estimated by dividing the accumulated CO<sub>2</sub> fluxes by the number of crop days (Zou et al., 2005).

### 2.5. Soil sampling and analysis

Topsoil sampling was conducted after the harvest of the second crop (Supplementary Table S2). Topsoil (0–15 cm depth) samples were collected from each plot and placed in plastic bags before shipping to the laboratory and storing at -4 °C prior to analysis. A portion of a fresh moist sample was ground to pass a 2 mm sieve for analyzing SMBC and SMBN. Soil samples were air-dried at room temperature and ground to pass a 2 mm sieve for soil pH analysis. A portion of the 2 mm sieved sample was further ground to pass 0.15 mm sieves for SOC and TN analysis (Elementar Vario max CNS Analyser, Elementar Company, 2003). Microbial biomass was determined with a chloroform fumigation-extraction protocol, with which a k<sub>EC</sub> (the portion of microbial biomass carbon extracted by 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> solution in the procedure) of 0.45 (Wu et al., 1990). The total N in the extracts was measured by the Kjeldahl digestion-distillation procedure and calculated to microbial biomass N by using the conversion coefficient of 0.54 (Brookes et al., 1985). The SMBC in biochar amended soils could be overestimated by using fumigation-extraction method, for chloroform-fumigation could dissolve some lipids present in biochar (Kuzakov et al., 2014). To test this, SMBC in biochar was determined using the same fumigation-extraction protocol with soils. Three randomly selected fresh wheat straw biochar samples were fumigated

**Table 2**

Selected soil properties across experimental sites.

Experiment	Soil management	Location	Soil type	pH (H <sub>2</sub> O)	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )	Clay (%)	Silt (%)	Sand (%)
Exp. 1	Rice paddy	Changsha (CS)	Hydroagric stagnic anthrosol	6.2 ± 0.1	18.8 ± 0.5	1.8 ± 0.1	18	28	54
		Jinxian (JX)	Hydroagric stagnic anthrosol	4.9 ± 0.2	17.7 ± 0.4	1.6 ± 0.1	20	38	42
		Guanghan (GH)	Hydroagric stagnic anthrosol	6.0 ± 0.1	20.1 ± 0.6	1.8 ± 0.1	16	32	52
	Dray cropland	Yixing (YX)	Hydroagric stagnic anthrosol	6.1 ± 0.1	23.5 ± 1.6	1.8 ± 0.2	17	37	46
		Shangqiu (SQ)	Calcaric entic fluvent	8.4 ± 0.1	9.9 ± 0.7	0.9 ± 0.1	\	\	\
		Xinzhou (XZ)	Typic orchrept (cinnamon soils)	8.4 ± 0.1	4.4 ± 0.3	0.4 ± 0.1	15	36	49
Exp. 2	Rice paddy	Tai'an (TA)	Typic hapludalf (brown soils)	5.9 ± 0.1	8.4 ± 0.2	0.8 ± 0.1	6	32	62
		Changshu (CSU)	Hydroagric stagnic anthrosol	7.2 ± 0.2	26.5 ± 1.4	2.7 ± 0.3	\	\	\

"\" indicates not determined.

and other three were not. Results showed that there were no significant differences in the extractable carbon content of chloroform fumigated and non-fumigated biochar.

## 2.6. Data processing and statistics

The microbial quotient (MQ) was calculated by dividing SMBC content with SOC content. Here,  $q\text{CO}_2$  was estimated by dividing seasonal total  $\text{CO}_2$  efflux with SMBC content and expressed as  $\text{g CO}_2\text{-C g}^{-1} \text{SMBC Season}^{-1}$ .

In Exp. 1, a three-way ANOVA was performed for  $\text{CO}_2$  efflux from soil based on three factors: biochar application rate, site and crop season, and their interactions as fixed effects. A two-way ANOVA was used to test the effect of biochar addition and experimental site, and their interaction on SOC, TN, SMBC, SMBN, MQ and  $q\text{CO}_2$ . In Exp. 2, a two-way ANOVA was conducted to test the effect of biochar type and measurement season, and their interaction on  $\text{CO}_2$  efflux from soil. A difference between biochar treatments was considered significant at  $p < 0.05$ . All data was presented as means  $\pm$  standard deviation of the three replicates.

## 3. Results

### 3.1. $\text{CO}_2$ efflux from biochar amended soils

Exp. 1, mean daily  $\text{CO}_2$  flux for a specific crop season varied greatly between sites ( $p < 0.01$  for site effect, overall ANOVA; Table 3; Fig. 1). Generally, dry cropland soils respired much more  $\text{CO}_2$  than rice paddies. The soil for maize cultivation at SQ site respired  $29.81 \pm 1.3 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$ , which was 6.2 times as large as from the rice paddy at CS site ( $4.78 \pm 0.37 \text{ kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$ ) in the second season. Mean daily soil  $\text{CO}_2$  flux varied also between crop seasons in a single site, but the changes in direction were inconsistent across sites ( $p < 0.01$  for crop season, Fig. 1). For dry cropland soils, daily soil  $\text{CO}_2$  flux was significantly higher in the first season than in the second season at XZ and TA, while much higher in the second than in the first season at SQ. Overall, biochar amendment had no effect mean daily soil  $\text{CO}_2$  flux across sites ( $p = 0.16$  for BC effect; Table 3; Fig. 1). Moreover, there were no significant interactions between biochar and experimental site or crop season ( $p = 0.91$  for  $\text{BC} \times \text{Site}$  and  $p = 0.38$  for  $\text{BC} \times \text{Season}$ ).

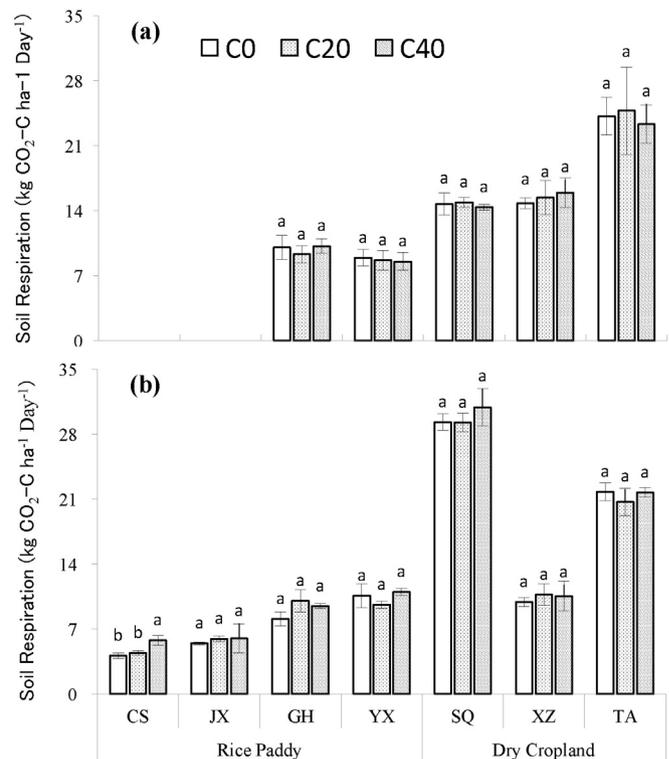
In Exp. 2, mean daily soil  $\text{CO}_2$  flux varied in a range of 12.7–15.2  $\text{kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$  for rice season and of 8.5–10.1  $\text{kg CO}_2\text{-C ha}^{-1} \text{ d}^{-1}$  for the subsequent wheat season. Although the four biochars used had different carbon pools and properties (Supplementary Table S1), the  $\text{CO}_2$  efflux from these biochars amended soils did not vary ( $p = 0.76$  for treatment; Supplementary Table S3; Fig. 2).

### 3.2. Soil carbon and nitrogen

Soil organic carbon increased significantly with biochar amendment across application rates and sites in Exp. 1. Hereby, the degree of change corresponded to biochar amendment rates (Fig. 3a). Likewise, total N increased in soils amended with biochar (Fig. 3b). Soil microbial mass

**Table 3**  
Overall ANOVA statistics for factors affecting soil respiration in Exp. 1.

Source	Sum of squares	df	Mean square	F	Sig.
Model	6051	35	173	94.93	<0.01
Biochar (BC)	7	2	3	1.85	0.16
Site	4151	6	692	379.91	<0.01
Season	108	1	108	59.46	<0.01
BC $\times$ site	11	12	1	0.49	0.91
BC $\times$ season	4	2	2	0.98	0.38
Site $\times$ season	1222	4	305	167.74	<0.01
BC $\times$ site $\times$ season	13	8	2	0.90	0.52
Error	131.128	72	2		

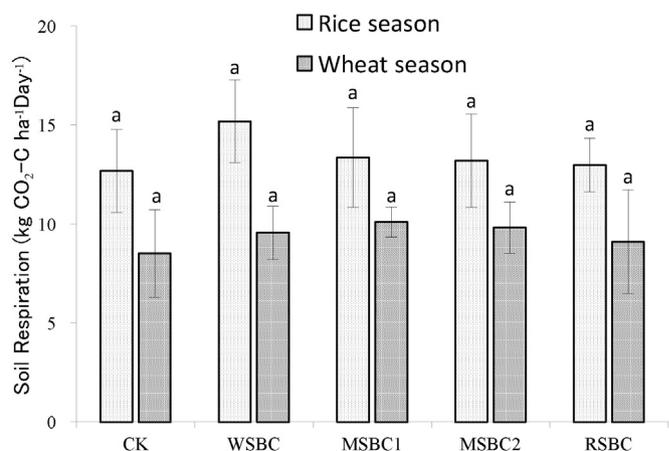


**Fig. 1.** Soil  $\text{CO}_2$  efflux over time (a, first rice/maize season; b, second rice/maize season) in response to biochar amendment across the seven sites in Exp. 1. Soil  $\text{CO}_2$  efflux in CS and JX was not measured in the first season after biochar amendment.

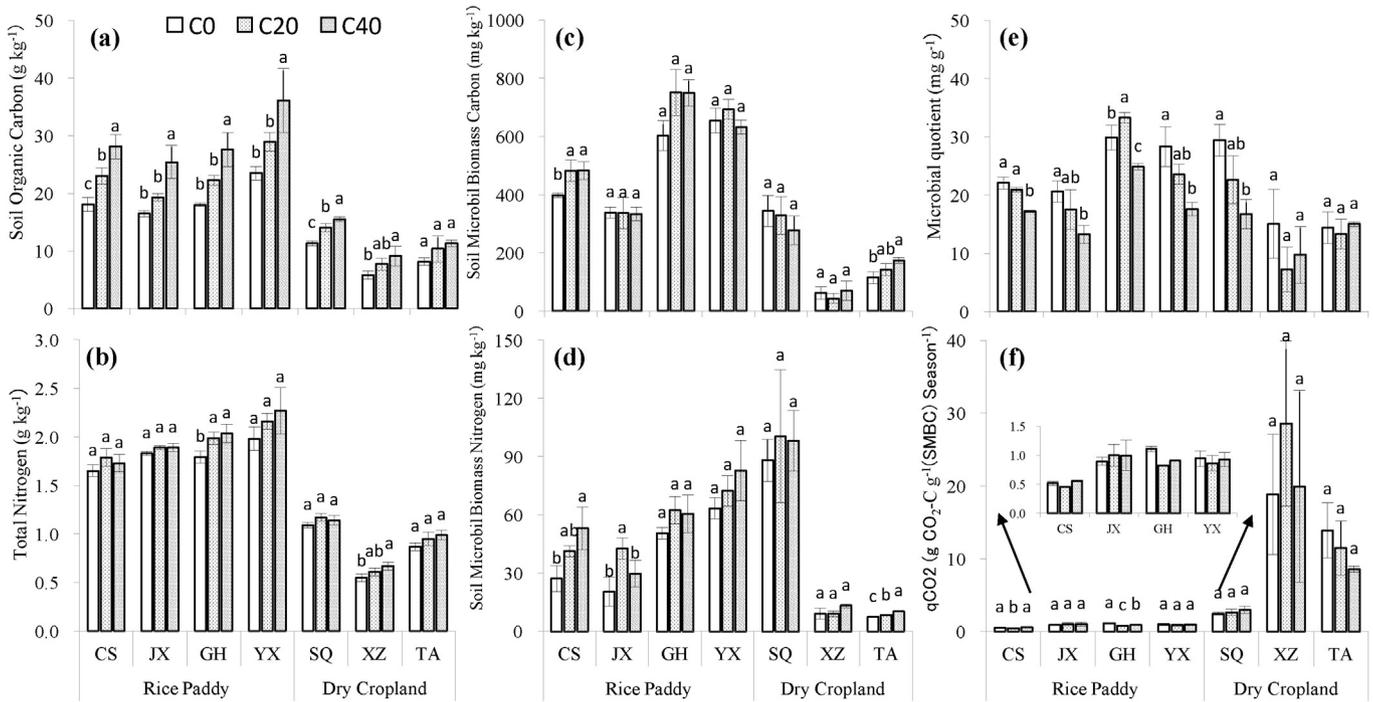
carbon was generally higher in rice paddies than in maize croplands (Fig. 3c) but the effect of biochar on SMBC was site specific ( $p < 0.01$  for site effect; Supplementary Table S4). Overall, SMBN increased with biochar by 32% on average of all the sites.

Biochar addition had a negative effect on soil MQ ( $p < 0.01$  for BC effect; Fig. 3e). The MQ decreased by 13.4% and 28.4% under biochar amendment rate of 20 and 40  $\text{t ha}^{-1}$ . There was a significant interactive effect between biochar and site on MQ ( $p < 0.01$  for  $\text{BC} \times \text{Site}$  effect). In detail, the MQ with biochar amendment decreased at site CS, JX, YX and SQ but unchanged at XZ and TA. Unlike MQ, the  $q\text{CO}_2$  was not affected by biochar rates across sites ( $p = 0.51$  for BC effect;  $p = 0.63$  for  $\text{BC} \times \text{Site}$  effect; Supplementary Table S3; Fig. 3f).

The correlations between soil  $\text{CO}_2$  efflux and SOC, SMBC and pH were evaluated in Exp. 1. Soil  $\text{CO}_2$  flux was correlated neither to SOC



**Fig. 2.** Soil  $\text{CO}_2$  efflux over time in response to different biochars at CSU in Exp. 2. Soil  $\text{CO}_2$  efflux was measured during rice and wheat growing season.



**Fig. 3.** Soil organic carbon (SOC, a), total nitrogen (TN, b), soil microbial biomass carbon (SMBC, c), microbial biomass nitrogen (SMBN, d), microbial quotient (MQ, e) and microbial metabolic quotient ( $qCO_2$ , f) in response to biochar amendment across the seven sites in Exp. 1. Soil samples were collected after the second crop harvest.

nor to SMBC (Supplementary Fig. S2). Furthermore, there was no significant correlation between SOC and SMBC (Supplementary Fig. S2). However, SMBC was weakly correlated to soil pH (Supplementary Fig. S3), explaining 28% of the overall change in SMBC. Again, the percent changes in  $qCO_2$  followed a weak decreasing trend with increasing soil pH ( $p = 0.07$ ).

## 4. Discussion

### 4.1. Biochar effects on soil respiration: experimental conditions

The carbon sequestration potential of biochar has been questioned based on observations of short term priming effect on native SOC decomposition, leading to temporary increases in soil respiration. This issue was first addressed by Wardle et al. (2008) who found an 8% decrease in forest SOM decline when amended with biochar in a forest floor, which was evidenced by the substantially increased soil respiration in the first year following the amendment. Sagrilo et al. (2014) highlighted a much higher increase in soil respiration following biochar addition, being by 28% on average of the observations from 46 lab and short-term field studies. However, the results from this study did not support their conclusion from relatively lab-biased studies. On the contrary, soil respiration in terms of measured  $CO_2$  effluxes did not show a significant response to biochar amendment, which was consistent across biochar types differing in carbon pools and experiment sites differing in climatic and crop production conditions (Figs. 1 & 2).

Different protocols used for soil respiration measurement or estimation could contribute to the distinct findings reported in different studies. Most of the studies included in meta-analysis of Sagrilo et al. (2014) estimate soil respiration by incubating soils in laboratory. The soils and biochars used were normally ground into very fine particles, instead of soil aggregates in field condition. Thereby, the carbon substrates were not physically protected and they became highly accessible to soil microorganisms. This resulted in a much higher soil respiration rate following biochar addition (Lu et al., 2007; Jones et al., 2011; Luo et al., 2011; Troy et al., 2013). In field condition, however, the organic substances could be physically protected in macro aggregates and/or well

bound to soil mineral particles, limiting their access by soil microbes (Brodowski et al., 2005; Liang et al., 2010). In addition, the favorable environment with consistent temperature and soil moisture in incubation could also promote soil microbes' growth and active response to exotic carbon accessible. Similar to our finding here, Castaldi et al. (2011) and Schimmelpfennig et al. (2014) reported no significant changes in soil respiration following biochar amendment in field condition. In some instances, soil respiration even depressed in *Miscanthus* bioenergy croplands with biochar addition (Case et al., 2014; Schimmelpfennig et al., 2014). Recently, Herath et al. (2015) argued that the added biochar could exert a negative instead of a positive priming effect on native soil organic matter decomposition, at least in some soil types.

Application rate, the amount of biochar used in a single test, could be another factor that regulating the response of soil respiration to biochar addition. The biochar application rates (20 and 40  $t\ ha^{-1}$ ) used in this study were relatively low compared to the studies reported higher soil respiration rate following biochar amendment. In the meta-analysis by Sagrilo et al. (2014), the data of high soil respiration ( $CO_2$  effluxes) were associated with extremely high biochar application rates (up to 480  $t\ ha^{-1}$ ), with added biochar as double as native SOC. As reported in a study by Stewart et al. (2012), soil respiration was increased by over 35% when biochar amended at (around 100  $t\ ha^{-1}$  (>5% of soil)) but only by 8% at rate of 1% (around 25  $t\ ha^{-1}$ ). Nevertheless, use of biochar at very high dosages could be impractical for the sake of cost, which already constrained farmers' adoption of biochar directly in their production (Clare et al., 2014).

### 4.2. Biochar effects on soil respiration: biochar conditions

Pyrolysis temperature had been known as a key factor for biochar's property and its performance in soils. The biochars pyrolyzed at higher pyrolysis temperatures are generally more recalcitrant than those pyrolyzed at low temperatures (Al-Wabel et al., 2013). Sagrilo et al. (2014) also highlighted that the pyrolyzing temperature is another key factor contributing to the positive response of soil respiration in addition to application rates. In their study, biochars pyrolyzed at temperatures below 350 °C significantly increased soil respiration, while those over

350 °C had no effect. In this study, all the biochars used in Exp. 1 and Exp. 2 were produced at temperature of 350–650 °C, which is within the range reported by Sagrilo et al. (2014). A recent analysis on long-term studies with <sup>13</sup>C and <sup>14</sup>C contrasting biochars revealed that biochar slightly decreased SOM decomposition, but the effect was dependent on soil and biochar properties (Wang et al., 2015). However, for a moderate biochar application rate, a significant increase in soil respiration could only occur in soil amended with low temperature biochar, especially for the biochars produced at temperature lower than 300 °C. At this temperature, it is likely that the un-charred raw materials (feedstocks) contribute more to soil respiration compared to the charred biochar. Therefore, we argue that the production condition of biochar (for completely charred process) seemed no influence on biochar's effect on soil respiration when amended to agricultural soils in this study. The assumption that biochars containing more labile carbon could increase soil respiration by providing more food for microbial decomposition (Knoblauch et al., 2011) was not supported by this study. The biochars used in Exp. 2 contained different levels of labile carbon (Supplementary Table S1). However, soil respiration did not vary across the treatments with these different biochars amended. This suggested that potential short term pulse from labile carbon decomposition could not account for a measureable difference when examined for a whole crop production cycle. In biochar amended soil, labile organic molecules could be protected or bound to biochar particles mostly of hydrophobic nature, decreasing their bioavailability to soil microbial degradation (Pignatello et al., 2006). In addition, in biochar amended soils, the evolved CO<sub>2</sub> could be partially absorbed by biochar particles (Cornelissen et al., 2013), reducing direct release to the atmosphere. But it is still poorly known whether these processes could negate the potential priming by use of existing labile carbon pool in amended biochar; and the long-term influence of biochar on SOM decomposition need to be investigated in future studies (Singh and Cowie, 2014).

#### 4.3. Biochar effects on soil respiration: change in soil microbial activity

While soil respiration could be generally mediated by soil microorganisms, the insignificant change in soil CO<sub>2</sub> release following a single biochar amendment was explained by the changes neither in soil microbial biomass nor in SOC in this study (Supplementary Fig. S2). Microbial abundance and their activity could be promoted with biochar, even with sludge biochar containing high level of toxic heavy metals (Paz-Ferreiro et al., 2012). For some rice paddy soils included in this study, microbial biomass/gene abundance, particularly for bacteria, was significantly enhanced following a single biochar amendment, although the specific changes in community structure could vary between sites (Chen et al., 2013). Improvement of soil microbial growth following biochar amendment had been often reported in the literature (Anderson and Domsch, 1993; Tian et al., 2008; Aciego Pietri and Brookes, 2009; Chen et al., 2015; Lu et al., 2015). In this study, however, the increase in microbial abundance did not lead to a promotion of soil respiration. This could suggest improved carbon use efficiency in biochar amended soils while the biotic and abiotic conditions are improved greatly (Pietikainen et al., 2000; Lehmann et al., 2011).

## 5. Conclusions

The present study, using one single biochar in different soil-crop systems and different biochars in a single rice soil, demonstrated no change in soil respiration but a general increase in microbial abundance in agricultural soils following biochar amendment at 20–40 t ha<sup>-1</sup>. As the unchanged soil respiration and CO<sub>2</sub> effluxes was consistent across environmental and biochar conditions, carbon turnover could be likely slowed in biochar amended soil, thus promising the biochars' role in soil carbon sequestration. Continued monitoring studies should be warranted to explore long-term changes in carbon cycling, especially with the dynamics of soil aeration, water regime and pH we well as carbon

pools in these biochar amended soils. Particularly, future studies should examine the relationships between changes in microbial community structure and composition and the extent to which soil respiration could be affected.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.02.179>.

## References

- Aciego Pietri, J.C., Brookes, P.C., 2009. Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. *Soil Biol. Biochem.* 41, 1396–1405.
- Al-Wabel, M.I., Al-Omran, A., Ei-Naggar, A.H., Nadeem, M., Usman, A.R.A., 2013. Pyrolysis temperature induced changes in characteristics and chemical composition of biochar produced from conocarpus wastes. *Bioresour. Technol.* 131, 374–379.
- Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO<sub>2</sub> (qCO<sub>2</sub>) as a specific activity parameter to assess the effects of environmental-conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* 25, 393–395.
- Anderson, C.R., Condon, L.M., Clough, T.J., et al., 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* 54, 309–320.
- Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and Soil* 337, 1–18.
- Brodowski, S., Amelung, W., Haumaier, L., Abetz, C., Zech, W., 2005. Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. *Geoderma* 128, 116–129.
- Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil-nitrogen – a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842.
- Bruun, S., Luxhoi, J., 2008. Is biochar production really carbon-negative? *Environ. Sci. Technol.* 42, 1388.
- Bruun, S., Clauson-Kaas, S., Bobulska, L., Thomsen, I.K., 2014. Carbon dioxide emissions from biochar in soil: role of clay, microorganisms and carbonates. *Eur. J. Soil Sci.* 65, 52–59.
- Case, S.D.C., McNamara, N.P., Reay, D.S., Whitaker, J., 2014. Can biochar reduce soil greenhouse gas emissions from a *Miscanthus* bioenergy crop? *Global Change Biology Bioenergy* 6, 76–89.
- Castaldi, S., Rioldino, M., Baronti, S., et al., 2011. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere* 85, 1464–1471.
- Chen, J., Liu, X., Zheng, J., et al., 2013. Biochar soil amendment increased bacterial but decreased fungal gene abundance with shifts in community structure in a slightly acid rice paddy from Southwest China. *Appl. Soil Ecol.* 71, 33–44.
- Chen, J., Liu, X., Li, L., et al., 2015. Consistent increase in abundance and diversity but variable change in community composition of bacteria in topsoil of rice paddy under short term biochar treatment across three sites from South China. *Appl. Soil Ecol.* 91, 68–79.
- Clare, A., Barnes, A., McDonagh, J., Shackley, S., 2014. From rhetoric to reality: farmer perspectives on the economic potential of biochar in China. *Int. J. Agric. Sustain.* 12, 440–458.
- Cornelissen, G., Rutherford, D.W., Arp, H.P.H., et al., 2013. Sorption of pure N<sub>2</sub>O to biochars and other organic and inorganic materials under anhydrous conditions. *Environmental Science & Technology* 47 (14), 7704–7712.

- Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., Murphy, D.V., 2012. Decreased soil microbial biomass and nitrogen mineralisation with *Eucalyptus* biochar addition to a coarse textured soil. *Plant and Soil* 354, 311–324.
- Herath, H.M.S.K., Camps-Arbestain, M., et al., 2015. Experimental evidence for sequestering C with biochar by avoidance of CO<sub>2</sub> emissions from original feedstock and protection of native soil organic matter. *Global Change Biology Bioenergy* 7, 512–526.
- Jones, D.L., Murphy, D.V., Khalid, M., et al., 2011. Short-term biochar-induced increase in soil CO<sub>2</sub> release is both biotically and abiotically mediated. *Soil Biol. Biochem.* 43, 1723–1731.
- Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biol. Biochem.* 43, 385–392.
- Knoblauch, C., Maarifat, A.-A., Pfeiffer, E.-M., Haefele, S.M., 2011. Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. *Soil Biol. Biochem.* 43, 1768–1778.
- Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biol. Biochem.* 41, 210–219.
- Kuzyakov, Y., Glaser, B., 2014. Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific <sup>14</sup>C analysis. *Soil Biol. Biochem.* 70, 229–236.
- Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems – a review. *Mitig. Adapt. Strat. Glob. Chang.* 11, 395–419.
- Lehmann, J., Rillig, M.C., Thies, J., et al., 2011. Biochar effects on soil biota - A review. *Soil Biol. Biochem.* 43, 1812–1836.
- Lehmann, J., Abiven, S., Kleber, M. et al. (2015) Persistence of biochar in soil. in: Lehmann J, Joseph S, (editors). Biochar for Environmental Management, Chapter 10, Earthscan, USA. pp235–281.
- Leita, L., De Nobili, M., Mondini, C., et al., 1999. Influence of inorganic and organic fertilization on soil microbial biomass, metabolic quotient and heavy metal bioavailability. *Biol. Fertil. Soils* 28, 371–376.
- Liang, B., Lehmann, J., Sohi, S.P., et al., 2010. Black carbon affects the cycling of non-black carbon in soil. *Org. Geochem.* 41, 206–213.
- Liu, X., Qu, J., Li, L., et al., 2012. Can biochar amendment be an ecological engineering technology to depress N<sub>2</sub>O emission in rice paddies?—a cross site field experiment from South China. *Ecol. Eng.* 42, 168–173.
- Liu, S., Zhang, Y., Zong, Y., et al., 2015. Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis. *GCB Bioenergy* 8, 392–406.
- Lu, Z., Pan, G., Zheng, J., et al., 2007. Change in CO<sub>2</sub> production potential by soil respiration from a paddy soil under aerobic incubation by using differently disturbed samples. *Ecol. Environ.* 16 (3), 987–993 (In Chinese with English abstract).
- Lu, H., Lashari, M.S., Liu, X., et al., 2015. Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroigneous solution in a saline soil from central China. *Eur. J. Soil Biol.* 70, 67–76.
- Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biol. Biochem.* 43, 2304–2314.
- Paz-Ferreiro, J., Gasco, G., Gutierrez, B., Mendez, A., 2012. Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. *Biol. Fertil. Soils* 48, 511–517.
- Pietikainen, J., Kiikkila, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos* 89, 231–242.
- Pignatello, J.J., Kwon, S., Lu, Y., 2006. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): attenuation of surface activity by humic and fulvic acids. *Environ. Sci. Technol.* 40, 7757–7763.
- Sagrilo, E., Jeffery, S., Hoffland, E., Kuyper, T.W., 2014. Emission of CO<sub>2</sub> from biochar-amended soils and implications for soil organic carbon. *GCB Bioenergy* 7, 1294–1304.
- Schimmelpennig, S., Mueller, C., Gruenhagen, L., Koch, C., Kammann, C., 2014. Biochar, hydrochar and uncarbonized feedstock application to permanent grassland—effects on greenhouse gas emissions and plant growth. *Agr. Ecosyst. Environ.* 191, 39–52.
- Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20.
- Schmidt, M.W.I., Torn, M.S., Abiven, S., et al., 2011. Persistence of soil organic matter as an ecosystem property. *Nature* 478, 49–56.
- Singh, B.P., Cowie, A.L., 2014. Long-term influence of biochar on native organic carbon mineralisation in a low-carbon clayey soil. *Sci. Rep.* 4.
- Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. *Soil Biol. Biochem.* 42, 2345–2347.
- Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol. Biochem.* 41, 1301–1310.
- Stewart, C.E., Zheng, J., Botte, J., Cotrufo, M.F., 2012. Co-generated fast pyrolysis biochar mitigation greenhouse gas emissions and increases carbon sequestration in temperate soils. *Global Change Biology Biochemistry* 5, 153–164.
- Tian, Y., Haibara, K., Toda, H., et al., 2008. Microbial biomass and activity along a natural pH gradient in forest soils in a karst region of the upper Yangtze River, China. *J. For. Res.* 13, 205–214.
- Troy, S.M., Lawlor, P.G., O'Flynn, C.J., Healy, M.G., 2013. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. *Soil Biol. Biochem.* 60, 173–181.
- Verheijen, F.G.A., Jeffery, S., Bastos, A.C., van de Velde, M., Diafas, I., 2010. Biochar application to soils – a critical scientific review of effects on soil properties, processes and functions, EUR24099EN. Office for the Official Publications of the European Communities, Luxembourg.
- Wang, J., Xiong, Z., Kuzyakov, Y., 2015. Biochar stability in soil: meta-analysis of decomposition and priming effects. *Global Change Biology Biochemistry*. <http://dx.doi.org/10.1111/gcbb.12266>.
- Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO<sub>2</sub>) as a bioindicator of disturbance and ecosystem development. *Soil Biol. Biochem.* 27, 1601–1610.
- Wardle, D.A., Nilsson, M.-C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. *Science* 320, 629.
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass c by fumigation extraction – an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169.
- Zavalloni, C., Alberti, G., Biasiol, S., et al., 2011. Microbial mineralization of biochar and wheat straw mixture in soil: a short-term study. *Appl. Soil Ecol.* 50, 45–51.
- Zhang, A., Cui, L., Pan, G., et al., 2010. Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agr. Ecosyst. Environ.* 139, 469–475.
- Zimmerman, A.R., Gao, B., Ahn, M.-Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol. Biochem.* 43, 1169–1179.
- Zou, J.W., Huang, Y., Jiang, J.Y., Zheng, X.H., Sass, R.L., 2005. A 3-year field measurement of methane and nitrous oxide emissions from rice paddies in China: effects of water regime, crop residue, and fertilizer application. *Global Biogeochem. Cycles* 19. <http://dx.doi.org/10.1029/2004GB002401>.