Biochar has no effect on soil respiration across Chinese agricultural soils

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Contributions: GP designed and guided the biochar research at IREEA; XL data synthesis and drafted the manuscript; KC soil carbon and respiration analysis; JZ field measurement data checking and analysis; HZ, microbial measurement and data analysis; AF, RB, DZ, XL, BZ, YY, JQ, GW and YL respectively for monitoring in field site; SJ biochar properties and soil effects; DC soil microbial activity analysis and discussion; YK, soil respiration and C status; PS, soil carbon and emission discussion; QH, participated original data analysis and discussion, LL and JZ participated in field experiment management; XY and JW contributed to lab analysis

and assistance.

→ insert page numbers!

Abstract

Biochar soil amendment is widely accepted to enhance soil carbon sequestration through very long term storage of stable carbon in soils and to reduce N_2O emissions. However, this has been challenged with suggestions that biochar has the potential to prime decomposition of native soil organic carbon through increased microbial activities. Here we present a synthesis of data from a cross-site field study of CO_2 efflux and microbial biomass in biochar amended soils across seven sites representing rice paddies and dry croplands in China. Small changes in soil CO_2 efflux (by -1.0-10.0%) were not statistically significant, but were accompanied by significant increase in microbial biomass (by 7.9-19.0%) after biochar amendment. The increase in soil microbial biomass was not correlated with biochar rate. The small changes in soil respiration were correlated with soil pH, a potential mechanism of a liming effect on soil microbial activity. The finding of increased microbial biomass but with no increase in soil CO_2 efflux after biochar addition shows no evidence for a priming effect, and suggests that biochar could even be suppressing microbial activity under field conditions, contributing to enhanced carbon stability in biochar-amended soils.

The most important question for the paper is: have you measured microbial biomass in Biochar (without soil)??? I guess (not sure) that if you apply the Chloroform-fumigation approach to biochar (without soil), you will already have some values for "microbial biomass", despite there is no microbial biomass there. This is because Chloroform-fumigation may dissolve some lipids that are present in biochar. So, may be you measured C release from biochar after fumigation and not from the real "microbial biomass".

CHECK!!! This is very important for all your conclusions!

Keywords: biochar, carbon sequestration, greenhouse gas mitigation; croplands, microbial activity

INTRODUCTION

Biochar production and application to soils is increasingly recognized as a potential option to mitigate climate change by sequestering carbon and reduce greenhouse gases emissions in soils (Lehmann *et al.*, 2006). However, this been challenged by suggestions of a potential enhancement of soil respiration through a priming effect of biochar on native soil organic matter (Ernsting and Smolker, 2009; Verheijen et al. 2009; (Bruun and Luxhoi, 2008; Kuzyakov *et al.*, 2009; Liang *et al.*, 2010; Jones *et al.*, 2011; Luo *et al.*, 2011; Zimmerman *et al.*, 2011).

Soil respiration, regulated by the activity of soil microbes, is one of the key processes of carbon exchange between the terrestrial biosphere and the atmosphere (Schlesinger and Andrews, 2000). The microbial metabolic quotient (MQ), which is the soil respiration per unit of soil biomass carbon, has been widely used for tracing the stability of carbon under environmental change (Anderson and Domsch, 1993; Wardle and Ghani, 1995). MQ has often been observed to increase with carbon substrate input, such as crop straw or organic manure into soil, a phenomenon termed the priming effect (Kuzyakov *et al.*, 2000). Using ^{14}C labeled feedstock, Kuzyakov et al. (2009) showed a very low biochar contribution to CO_2 fluxes, despite the existence of a co-metabolic decomposition with added glucose. Meanwhile, in biochar-rich Anthrosols from the Amazon, no positive priming was found by a way of co-metabolism due to added fresh organic matter (Liang *et al.*, 2010). Again, increased release of C in laboratory studies (Kuzyakov *et al.*, 2009; Liang *et al.*, 2010; Cross and Sohi, 2011) was small relative to increased SOC storage with biochar addition, and should therefore not compromise its ability to contribute to long-term C sequestration in soil environments (Woolf and Lehmann, 2012). The persistence of organic carbon in soils could rather be regulated by site conditions of soil and climate,

than by its chemical recalcitrance alone (Schmidt *et al.*, 2011). Thus, it is still unclear whether biochar soil amendments will lead to an increase in soil respiration and CO_2 emissions that would compromise the mitigation potential and the benefits for crop N availability, before large scale projects of biochar production and use are implemented in world agriculture.

The soil microbial community and activity are the key drivers for carbon stability in field soils (Anderson *et al.*, 2011). Biochar amendment, through biotic and abiotic effects on the soil system, could lead to changes in soil microbial abundance and community structure (Lehmann *et al.*, 2011). However, changes in both microbial abundance and community composition with biochar amendments have been shown to vary with soil type, biochar feedstock and application rate. (Steinbeiss *et al.*, 2009) reported promoted fungi growth in a soil amended with yeast-derived biochar. Loss of microbial diversity was even observed in soil amended with oak and grass derived biochar (Khodadad et al. 2011). However, other studies have shown that biochar addition resulted in no changes in soil microbial biomass carbon (Zavalloni *et al.*, 2011), or with divergent impacts on microbial biomass carbon and nitrogen (Dempster *et al.*, 2012). Our previous studies in a biochar amended rice paddy from China have shown inconsistent changes both in microbial abundance (Chen *et al.*, 2013) and community structure (Chen et al., in press) between bacterial and fungal communities. With the uncertainty of the effects on the soil microbial community, the relationship between soil respiration change and changes in soil microbial activity, is not well understood when addressing carbon stability in biochar amended soils.

Here, we report an analysis of changes in soil carbon accumulation, in soil respiration and in microbial activities from a cross-site field experiment of biochar amendment across the main production regions of China. We test the hypothesis that soil

respiration could potentially be increased, as soil microbial biomass and activity is enhanced with biochar amendment in agricultural soils.

MATERIALS AND METHODS

Field experiment sites

We conducted a biochar amendment experiment in cropland at seven sites across the main crop growing areas in China (Fig.1). The seven sites are (1) Changsha (CS, rice paddy), Hunan Province, (2) Jinxian (JX, rice paddy), Jiangxi Province; (3) Guanghan (GH, rice paddy), Sichuan Province; (4) Yixing (YX, Rice paddy), Jiangsu Province; (5) Shangqiu (SQ, wheat/maize cropland), Henan Province; (6) Xinzhou (XZ, maize cropland), Shanxi Province; (7) Tai'an (TA, maize cropland), Shandong Province. As shown in Table 1, the sites vary with climate conditions and range from humid to semi-arid, with soil acidity ranging from acid to alkaline and with soil fertility ranging from very low to high organic carbon and N levels, and soil texture ranging from sandy to clay (Table 2).

Biochar used for amendment

The biochar used at the 7 experimental sites was consistently from pyrolysis of wheat straw, which is commercially available at Sanli New Energy Company, Henan, China. The carbonization for biochar production was performed at 350°C-550°C, with the residence time of about 1 hour going through the vertical kiln with a height of 5 m and a diameter of 3m. Using this technology, 0.35 tons of biochar was obtained per ton of wheat straw biomass, plus 250 kg of pyroligenous solution (wood vinegar) and 750 m^3 of syngas (Pan et al., 2011). The biochar material obtained was ground to pass a 2-mm sieve and mixed thoroughly before use in the field. The properties of biochar had been described elsewhere (Zhang et al., 2010; Liu et al. 2012).

Field experiment layout

For the field experiments, at each site, biochar was amended to soil at a rate of 0 (as the control), 20 and 40 t ha^{-1}, respectively (coded as C0, C20 and C40), after harvest of a preceding crop. Biochar was surface spread on the top soil and machine-ploughed to a depth of over 12 cm, and then thoroughly mixed and levelled with a wooden rake. No more biochar was amended in the subsequent years. The treatment was performed in triplicates and each plot was 4 m × 5 m in area, with protection rows of 0.8 m in width, and with individual irrigation and drainage outlets. The treatment plots were arranged in a randomized complete block design. The crop production was managed following the local conventional practice except for biochar use, and was consistent across the treatments in a single site.

Soil respiration field measurement

Soil respiration was measured *in situ* with a static chamber method following the procedure describe by Zou et al. (2005). In each plot, an aluminum flux collar (0.35 m×0.35 m×0.25 m) was permanently installed over the whole 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 leveled surface. The chambers were made of aluminum and wrapped with a layer of sponge and aluminum foil to minimize air temperature changes inside the chamber during the sampling period. Each chamber was equipped with a circulating fan to ensure complete gas mixing and temperature and humidity meters were installed inside the chamber.

Monitoring of gas emissions was done at weekly intervals during the whole growing season of crops. For each measurement event, gas sampling was performed during 8 to 10 a.m. following the protocol described by Zou et al. (2005). A gas sample was taken respectively at 0, 10, 20, and 30 min after chamber closure; fluxes were

determined from the slope of the mixing ratio change in these four samples. Sample sets were rejected unless they yielded a linear regression value of r^2 greater than 0.90. The mixing ratios of the above three gases were analyzed with a gas chromatograph (Agilent 7890 A) equipped with a flame ionization detector (FID). CO_2 was measured simultaneously with CH_4 and N_2O as described in Zhang et al. (2010). Seasonal amounts of CO_2 were sequentially accumulated from the emissions between every two adjacent intervals of the measurements (Zou et al., 2005).

Soil sampling and analysis

Soil samples were collected from each plot at crop harvest and placed in plastic bags before shipping to the laboratory and storing at -4℃ in a refrigerator prior to analysis.

Soil samples were air-dried at room temperature and ground to pass 2 mm sieve for basic property analysis. One portion of 2 mm samples was further ground to pass 0.25 mm and 0.1 mm for soil pH (H_2O) and organic carbon and total nutrient pool analysis. The performance for all these measurements followed the procedures described in Lu (2000).

Soil pH was measured in distilled water (soil/water ratio of 1:2.5 in mass) with a pH meter (Seven Easy Mettler Toledo, China, 2008). Organic C and N were determined with an Elementar Vario max CNS Analyser (German Elementar Company, 2003). Using fresh samples, soil microbial biomass C (MBC) and N (MBN) were determined with a chloroform fumigation–extraction protocol, with which a kEC (the portion of microbial biomass carbon extracted by K_2SO_4 solution in the procedure) of 0.45 was used to convert the measured C to MBC values (Wu *et al*., 1990). The total N in the extracts was measured by the Kjeldahl digestion–distillation procedure and calculated as MBN by using the conversion coefficient of 0.54 (Brookes *et al*., 1985).

Data processing and statistics

In order to compare between sites with different soil and crop types as well as management conditions, a meta-analysis protocol was performed following Liu et al. (2013). Data treatment and processing was performed in Microsoft Excel 2010, and calculations within the meta-analysis were conducted using the natural log of the response ratios following the procedure given by Hedges et al. (1999). However, we converted the natural log transformed ratios to relative percent changes (RC) when presenting and interpreting the results. All figures were expressed as the mean RC and 95 % confidence intervals (CIs) for each group. Means were considered significantly different from zero if the 95 % CIs did not overlap zero and were considered to be significantly different from one another if their 95 % CIs were non-overlapping.

RESULTS

Top soil organic carbon (SOC) contents were significantly higher (by 38.5% on average) in biochar amendment treatments than in the control, being basically coincident with the biochar rates, across sites (Fig. 1a). Similarly, topsoil nitrogen concentration was increased by 6.3% across sites (Fig. 1b). Meanwhile there was no statistical difference in SOC and total N increase between rice paddies and dry croplands. at the C40 treatment (40 t ha^{-1}; 8.3%) had a significantly higher soil TN concentration compared C20 (20 t ha^{-1}; 3.5%), being not parallel to biochar rates.

Soil microbial mass carbon (SMBC) and nitrogen (SMBN) tended to be higher in rice paddies than in dry maize croplands in our study. In line with the positive changes in SOC and total N, both SMBC and SMBN were found generally to increase with biochar amendment across sites (Fig. 1c), with the exception of no significant change in SMBC on some maize fields. SMBN (mean increase of 31.7%) responded more positively to biochar amendment compared to SMBC (mean increase of 13.3%). Similar to the changes in total N, the increase in both SMBC and SMBN were not parallel to biochar rates, without a direct response to increase in SOC across the treatments.

While background soil respiration and CO_2 evolution varied greatly between sites, total soil respiration over a cropping season was not significantly increased with biochar soil amendment across the seven experimental sites (Fig. 2a). The lack of a change in soil respiration was consistent between biochar application rates and between rice paddies and dry croplands. Particularly, nearly zero change in soil respiration (0.8% on average) occurred in maize soils amended with biochar, in line with their very slight change in SMBC. Furthermore, biochar decreased the soil

microbial quotient (MQ) by 18.2% on average across sites, again with no difference between rice paddies and dry maize croplands. Nevertheless, significantly lower soil MQ was observed for C40 compared to C20 (Fig. 2b). In addition, the soil microbial metabolism quotient significantly decreased with bicohar addition in rice paddies, though there was no significant change overall across sites (Fig. 2c). If all sites are considered, changes, neither changes in SMBC or in soil respiration were significantly correlated with changes in SOC after biochar amendment. Similarly, there was no significant correlation between soil respiration and SMBC across sites (Fig. 3). However, changes in SMBC (Fig. 4a) and in MQ (Fig.4b) were both positively related with changes in soil pH.. Soil pH explained 38.4% and 22.5% of the changes, respectively, in SMBC and in MQ. The percent changes in MQ were negatively correlated to soil pH change, with 42.1% of variation in MQ explained by soil pH (Fig. 4c).

DISCUSSION

The carbon sequestration potential of biochar has been questioned on the grounds of its stability in amended soils, and its potential to cause a priming effect on native SOC. Using litter bags, Wardle *et al.*, (2008) showed an ~8% increase in decomposition of native forest humus after biochar addition to the forest floor over a 10-year period.. Later studies showed no consistent increases in SOC decomposition in bicohar-amended soils (Smith *et al.*, 2010; Jones *et al.*, 2011; Luo *et al.*, 2011; Zimmerman *et al.*, 2011), leaving the the issue of biochar C stability unsettled. Our results show no significant change in soil respiration despite a great increase in soil organic carbon and a significant increase in microbial biomass with biochar amendment in croplands across sites. The findings do not support the hypothesis that soil respiration is increased with increasing soil microbial biomass. No priming effect was found even with large amendments of biochar. These findings suggest that biochar can increase carbon sequestration.

In other field studies with biochar amendment, soil respiration was not affected in a Mediterranean wheat cropland (Castaldi *et al.*, 2011), or even decreased in a Miscanthus bioenergy crop (Case *et al.*, 2014; Schimmelpfennig *et al.*, 2014), and ecosystem respiration was not changed in a temperate grassland (Schimmelpfennig *et al.*, 2014). These all controdict the findings from lab incubation studies where increases in soil respiration, as a result of a potential priming effect, were observed (Jones *et al.*, 2011; Luo *et al.*, 2011; Troy *et al.*, 2013).

However, significant increases in soil microbial biomass were consistently observed across the seven sites, though not in parallel with biochar application rates, suggesting

promoted microbial growth in biochar amended soils. Such promotion could be also seen in a work by (Paz-Ferreiro *et al.,* 2012) who found significant increase in microbial abundance in biochar-amended soil. In some sites with rice paddies, microbial biomass/gene abundance, and particularly of bacterial, was significantly enhanced with biochar amendment though community structure change was variable between sites (Chen *et al.*, 2013). Moreover, here we found a general decline in soil microbial MQ with biochar amendment across sites. The fact that microbial biomass increased, but MQ decreased, suggests that biochar could suppress the activity of the soil microbial community in the amended soils, regardless of soil types of rice paddy or maize fields. This could suggest again an enhanced biological stability of soil carbon, instead of a potential priming effect, after biochar amendment. Some early studies by (Rogovska *et al.*, 2011; Zavalloni *et al.*, 2011) had already showed no promotion of soil respiration with addition of biologically available new substrates such as crop straw and manure to biochar amended soil.

However, the protection of soil carbon against decomposition by increasing soil microbial growth is still not thoroughly understood. Of course, the added biochar is physically associated with soil particles and this prevents it from decomposing (Brodowski *et al.*, 2005b). Improved carbon resource efficiency could be expected with biochar due to co-location of various resources in and around biochar particles, while soil biotic and abiotic conditions were improved (Pietikainen *et al.*, 2000; Lehmann *et al.*, 2011), including improvements in soil aggregation and moisture conditions (Brodowski *et al.*, 2005a; Karhu *et al.*, 2011; Zhang *et al.*, 2012; Liu *et al.*, 2014). While soil pH has been recognized as a primarily driver for changes in soil microbial activity (Anderson and Domsch, 1993), pH change affected the changes in SMBC (Fig. 4a) and in MQ (Fig.4b) in our study. Slightly positive changes in soil pH,

mostly in acid and slightly acid soils, with the added alkaline biochar (pH around 10), contributed to a decreased soil microbial MQ. This suggests prompt microbial adaptation to alleviated soil acidity in the biochar amended soils. However, the changes in microbial community structure and composition with regard to carbon utilization efficiency is still an urgent need in field studies.

In summary, biochar amendment did not increase CO_2 efflux from soil but promoted soil microbial biomass across sites. We suggest that biochar application to agricultural soils could even protect soil carbon, instead of inducing a priming effect. However, the concurrent changes in chemical recalcitrance, soil microbial community structure and their biochemical activity need further study.

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AUTHORSHIP STATEMENT

JQ, BZ, AZ, RB, XL, GW, DZ, XL, YL, YY, LC, XY did the field works and/or lab analysis; KC, XL, LL, JZ,XZ, JZ, JS, DC, PS, YK, QH analyzed data with discussions;

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Figure captions

Fig. 1 Change in soil organic carbon storage (a), total nitrogen (b), soil microbial biomass carbon (c) and nitrogen (d) with biochar amendment across sites.

Fig. 2 Change in soil respiration (a), soil microbial quotient (b) and metabolic quotient (c) with biochar soil amendment across sites

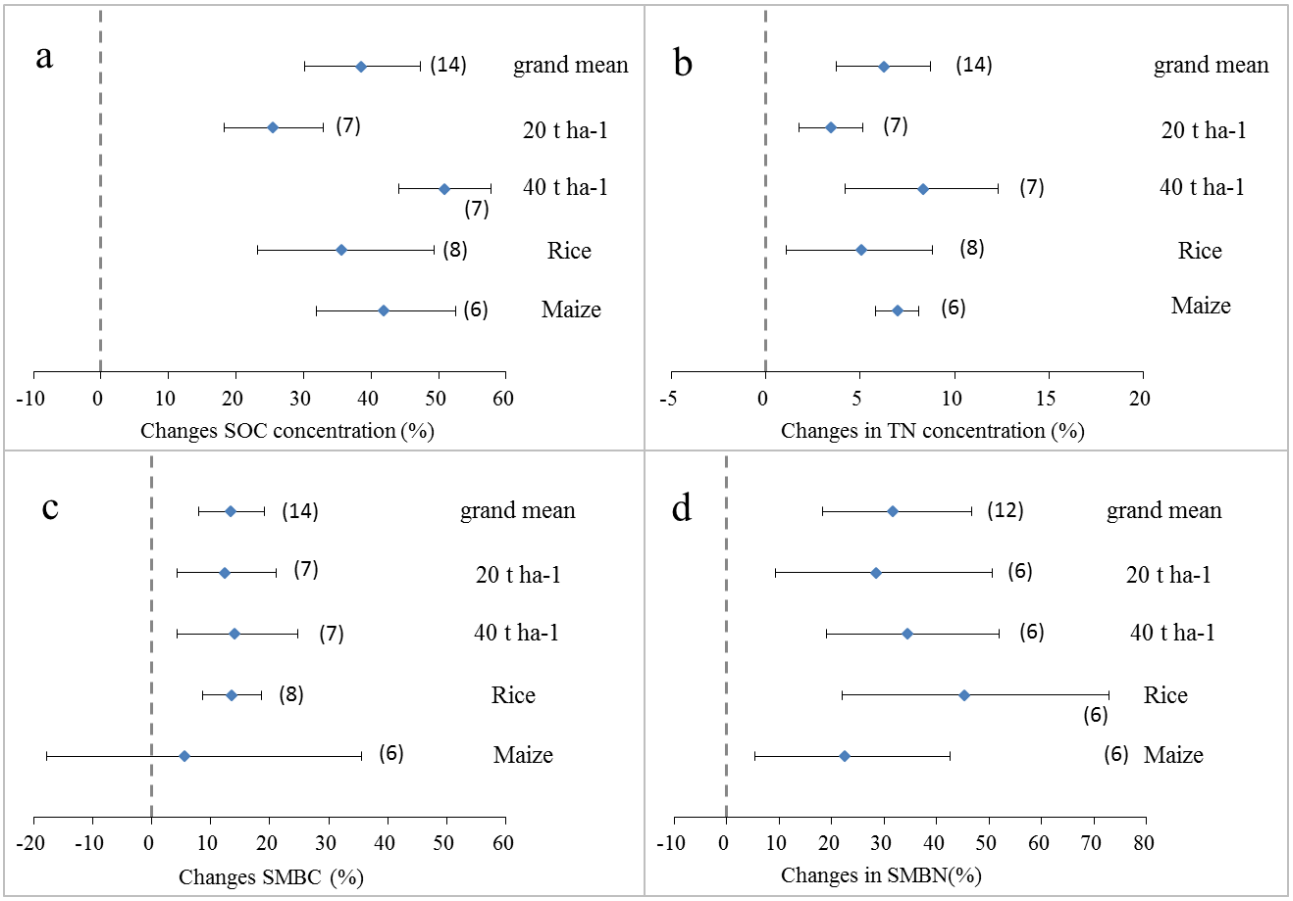
Fig. 3 Relationships between soil organic carbon and microbial biomass carbon (a) and soil respiration (b) and soil respiration with soil microbial biomass carbon (c)

Fig. 4 Relationships between soil pH and soil microbial biomass carbon (a) and soil microbial quotient (b) and metabolic quotient (c) across the experimental sites

Table 1 Site conditions of the field experiments with biochar amended soils

NEVER USE P2O5, K2O!!! This is a writing style of 50 (!) years ago!

Crop	Site	MAT(oC)	MAP(mm)	Crop rotation	Cultivar	Fertilizer (kg ha^{-1} $season^{-1}$)		
						N	P_2O_5	K_2O
Rice	CS	17.1	1500	Rice-Rice	Zhongjiazao17	150	90	90
	JX	17.7	1400	Rice-Rice	Yougong98	300	220	150
	GH	16.3	890	Rice-Wheat	DYou202	240	150	75
	YX	15.7	1177	Rice-Wheat	Wuyunjing7	300	125	125
Maize	SQ	13.9	780	Wheat-Maize	Zhengdan 958	300	75	90
	XZ	10.5	400	Maize	Xianyu335	220	90	180
	TA	12.8	727	Maize	Zhengdan 958	430	75	0



→ this is good! Make central point larger and in Black, or – better: make different colors (center and the lines!) for the 4 treatments, but the mean in black!
Write "mean" instead of "grand mean"!!!
Fig. 1

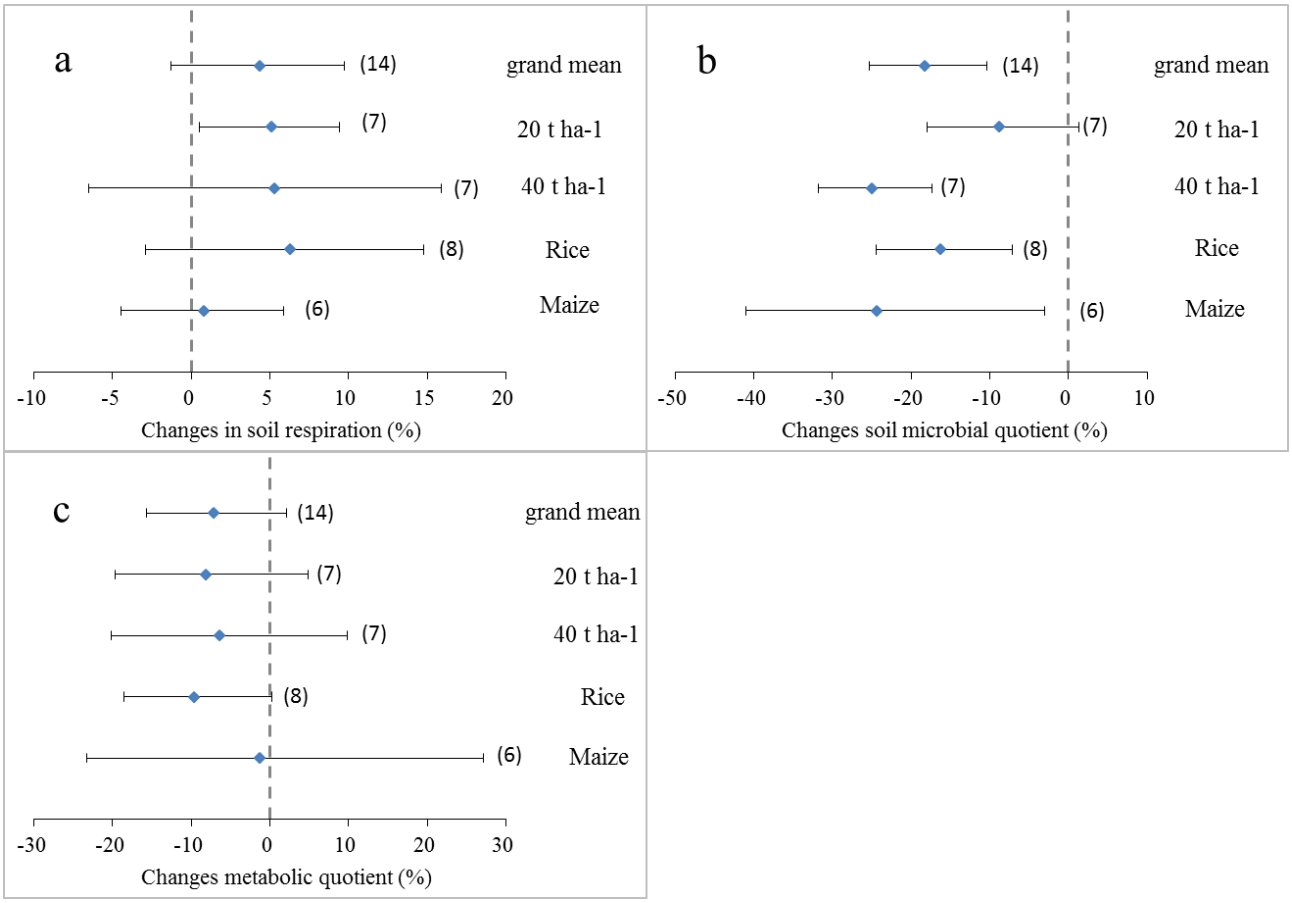
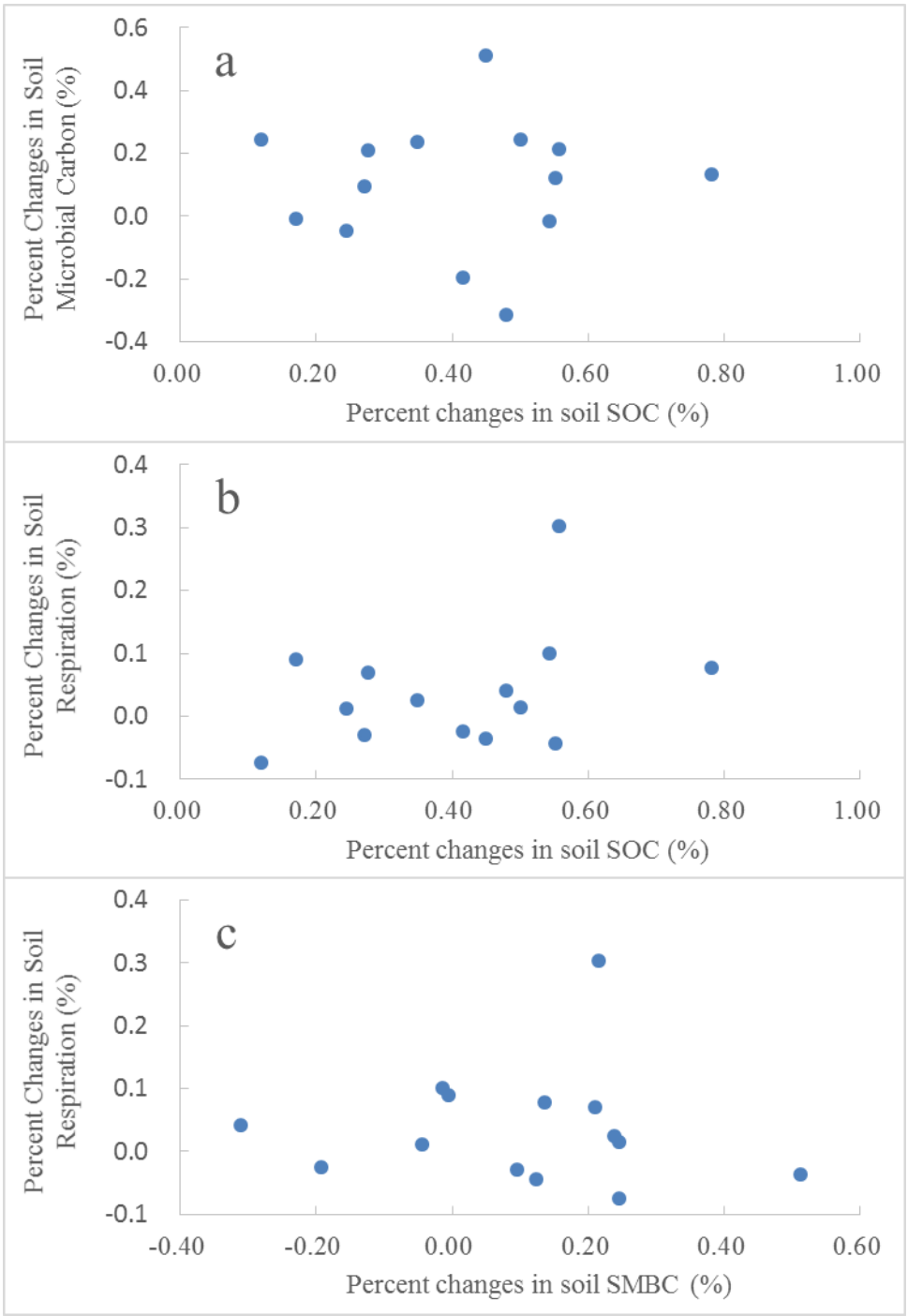


Fig. 2 → see comments above!



%%% of what? Make everywhere the “0” lines!
Use normal black and good blue color (not slightly blue!)

Fig. 3

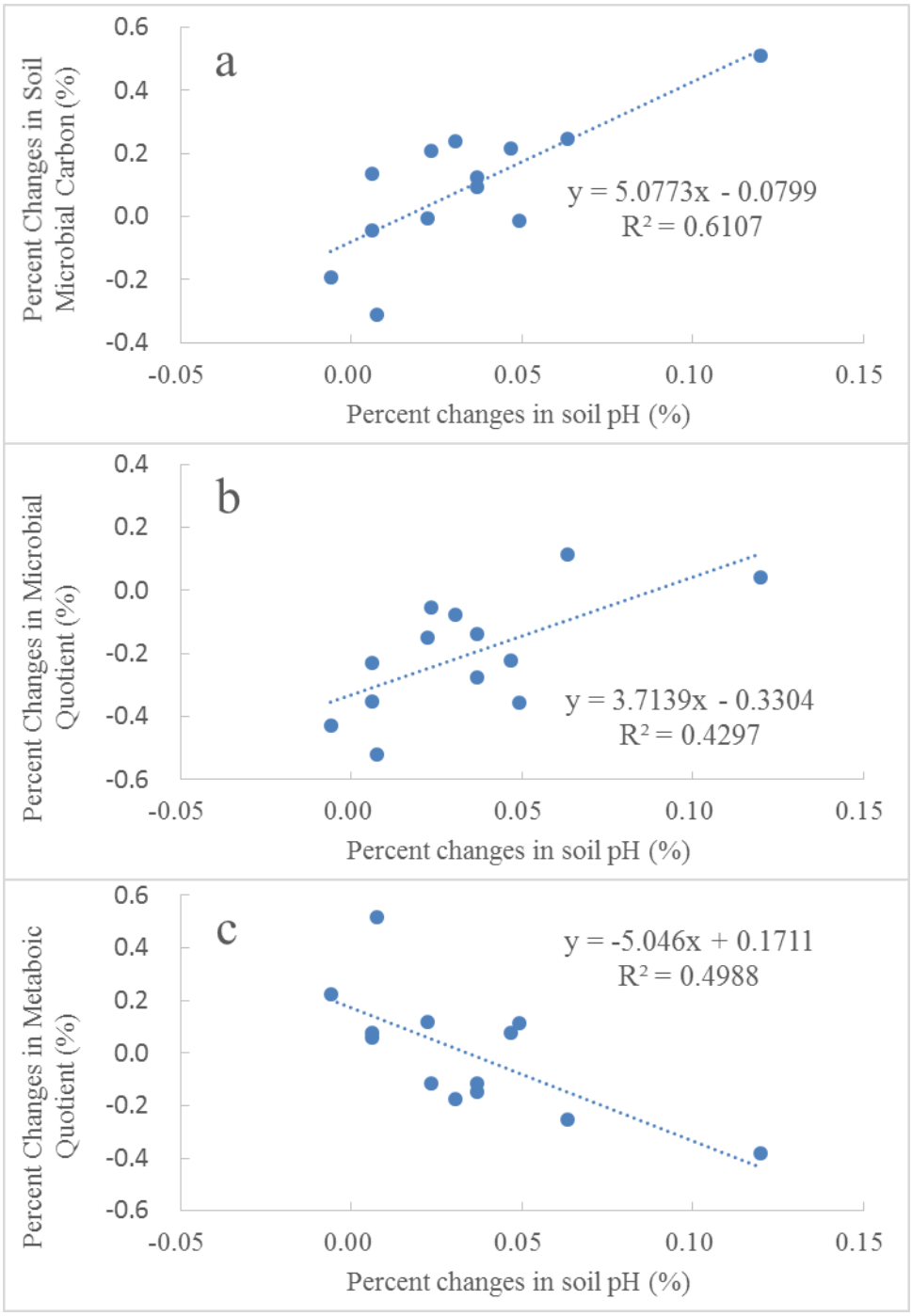


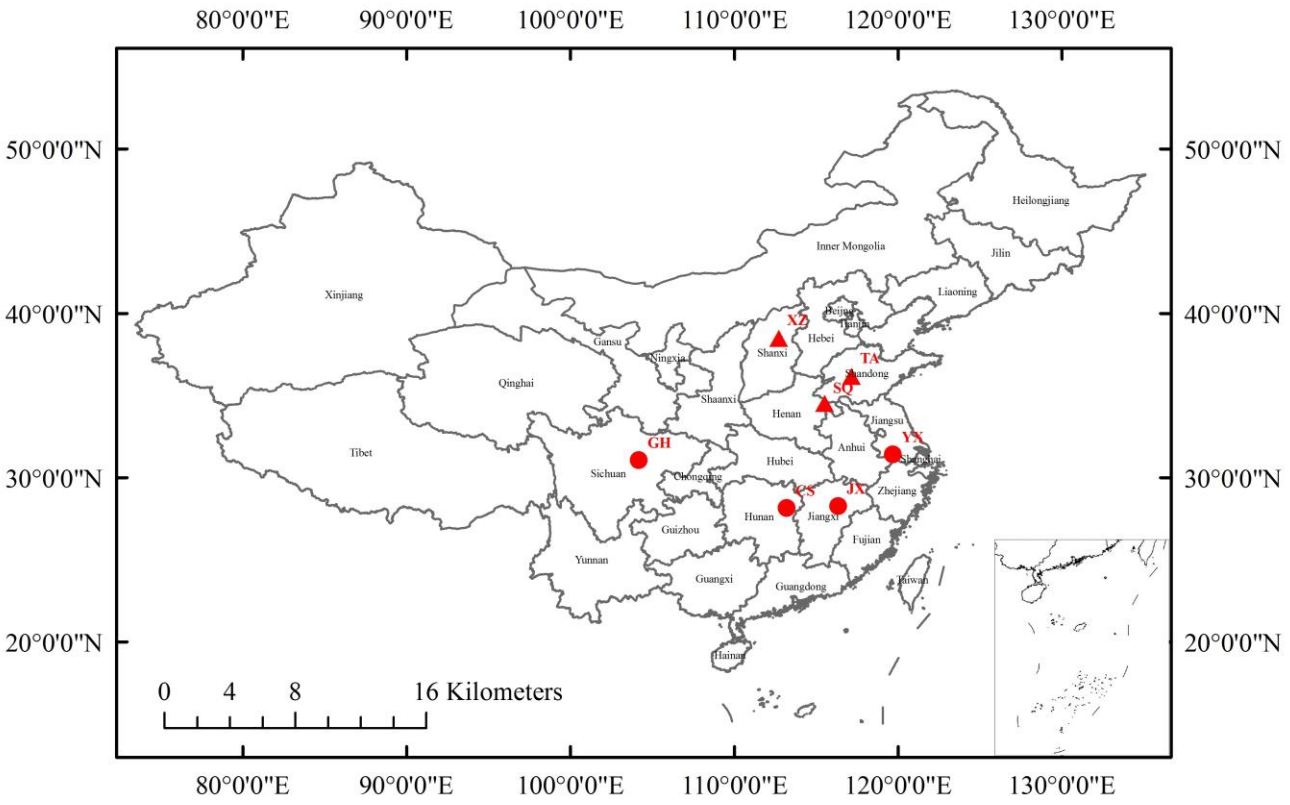
Fig. 4 → the number of digits is completely irrelevant!

It is not clear from these Figs – are the lines significant or not

See comments to Fig 3

Supplement Table 1 Biochar and soil basic properties across sites

Site	pH (H_2O)	SOC (g kg-1)	TN (g kg-1)	Clay (%)	Silt (%)	Sand (%)
CS	6.2	18.8	1.8	18	28	54
JX	4.9	17.7	1.6	20	38	42
GH	6.0	20.1	1.8	16	32	52
YX	6.1	23.5	1.8	17	37	46
SQ	8.4	9.9	0.9	\	\	\
XZ	8.4	4.4	0.4	15	36	49
TA	5.9	8.4	0.8	6	32	62
Biochar	10.4	467	5.9	-	-	-



Supplement Fig. 1