1 **Changes in microbial biomass and the metabolic quotient with**
2 **biochar addition to agricultural soils: A Meta-analysis**

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19 **Abbreviations:** SR, soil respiration; SMBC and SMBN, soil microbial biomass
20 carbon and nitrogen; SOM, soil organic matter; SOC, soil organic carbon; RQ,
21 respiration quotient; MQ, microbial quotient; $q\text{CO}_2$, microbial metabolic quotient

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Abstract: Biochar has been increasingly recommended for world agriculture, but the effects on microbial activities in agricultural soils has not yet thoroughly assessed. In this study, using a meta-analysis of experiment data retrieved from literature published up to March 1, 2015, changes were examined in microbial biomass and soil respiration in agricultural soils with biochar addition. Microbial responses to biochar addition were quantified in soil respiration quotient (RQ), microbial quotient (MQ) and metabolic quotient ($q\text{CO}_2$) and their differences were evaluated between with and without biochar addition, and among groups of biochar production conditions and experiment conditions. There was an overall increase by 25% in soil microbial biomass carbon (SMBC) and nitrogen (SMBN) but a decrease by 13% in $q\text{CO}_2$, under biochar compared to the control. Whereas, microbial biomass carbon was increased by 26% but total soil CO_2 production unchanged, across all short term experiments up to 6 months following a single biochar addition. A significant reduction (by <20%) in $q\text{CO}_2$ was found under crop residue and manure biochars in term of feedstock, and biochars pyrolyzed at high temperature over 500°C in term of pyrolysis temperature. Whereas, the reduction was great (by over 30%) both in clay soils and in neutral soils but moderate (by 15%) in soil organic carbon (SOC) depleted soils, respectively in terms of soil texture, reaction and SOC level. Thus, soil conditions exerted great impacts on microbial metabolic quotient changes compared to biochar conditions. Nevertheless, microbial responses to biochar addition to agricultural soils were much uncertain with respect to both biochar and experiment conditions. Long term field experiments are still deserved to monitor soil microbial processes as long as

45 sustainable soil managements are concerned with biochar technology in agriculture.

46 **Keywords:** biochar; soil respiration; microbial biomass; carbon stability; microbial

47 activity; agricultural soil

1. Introduction

Biochar has been generally known as a black mixture of organic materials obtained via pyrolysis of waste biomass (Lehmann and Joseph, 2015). Its production and reuse in agriculture have become an emerging technology for sustainable soil management through recycling biomass waste as soil organic amendment (Cernansky, 2015). Biochar's role has been well recognized in enhancing terrestrial carbon (C) sequestration and greenhouse gas mitigation (Woelf, et al., 2010; Sohi, 2012; Smith, 2016) as well as in improving soil fertility and plant productivity (Lehmann, 2007; Jeffery et al., 2011; Liu et al., 2013). Biochar used for amendment has been also well known to improve soil aggregation and soil porosity (van Zwieten et al., 2010; Jeffery et al., 2011; Soinne et al., 2014; Omondi et al., 2016), playing a role in improving biophysical condition for microbial growth and their performance. All these could potentially affect soil health and ecosystem functions, manipulating soil organic matter decomposition and terrestrial carbon (C) cycling (Trivedi et al., 2013; Bardgett and Putten, 2014). However, biochar effects on soil microbial community and the functioning have been poorly assessed (Lehmann et al., 2011; Lehmann et al., 2015).

With special reference to C sequestration, biochar amendment with addition of exotic carbon substrates, could potentially increase soil respiration (Kuzayakov et al., 2000; Smith et al., 2010). This was often in debt as an issue of potential positive priming of decomposition of native soil organic matter, as observed in forest floor by Zimmerman et al., (2011) or with addition of fresh carbon substrates by Kuzyakov et al., (2009). There were contradictory observations in agricultural soils for soil organic carbon (SOC) storage was enhanced in black carbon rich soils from Amazon (Liang et

al., 2010) but mineralization of native SOC promoted in a black carbon added soil (Maestrini et al., 2014). Logically, the long term trajectory of SOC dynamics following biochar addition could be addressed with changes in soil microbial biomass and respiratory activity, which has been considered as indicators of SOC turnover by microbial activity (Anderson, 2003; Schloter et al., 2003; Anderson et al., 2010). While simple measures of SOC level provided the information of changes in the size of soil carbon pool (Mukherjee et al., 2016), measurements of microbial biomass and respiration rates could be used for predicting the efficiency by which microorganisms converted carbon substrates into stable organic carbon in soil (Powlson and Jenkinson, 1981; Schloter et al., 2003; Bastida et al., 2008).

Microbial carbon use efficiency has been generally considered the degree by which soil organic carbon was associated with and utilized by soil microbes. It could be taken as a key measure to understand microbial health with ecosystem succession (Bastida et al., 2008) or under human disturbance or under environmental stresses (Wardle and Ghani., 1995). Thus, the size and activity of microbial biomass and respiration could be linked to SOC dynamics across ecosystems. The earliest and simplest parameter to characterize microbial carbon use efficiency was the microbial quotient (MQ), which was the portion of microbial biomass carbon to total organic carbon pool, ranging from 1% to 5% of SOC (Sparling, 1992). The respiration quotient (RQ) was also commonly used and defined as the respiration rate per unit of SOC (Cook and Allan, 1992; Li et al., 2009). However, the metabolic quotient ($q\text{CO}_2$) considered the respiration rate $\text{CO}_2\text{-C}$ per unit microbial biomass C (Anderson and

Domsch, 1993). Conceptually based on Odum's theory of ecosystem succession, $q\text{CO}_2$ has been widely used as indicator for ecosystem succession (during which it supposedly declines) (Insam and Haselwandter, 1989) and maturity (Anderson and Domsch, 1985). Compared to SOC itself, $q\text{CO}_2$ could be more prompt to short term soil changes. A lower $q\text{CO}_2$ reflected an improved soil biophysical conditions resulted shortly from amended organic matter (Powlson and Jenkinson, 1981), but a higher $q\text{CO}_2$ indicated soil degradation under intensive land use (Masciandaro et al., 1998). Again, an increase of $q\text{CO}_2$ could be interpreted as a positive priming on decomposition of the labile SOC pool in soil, following addition of readily degradable carbon substrates to soil (Kuzyakov et al., 2000). More recently, significant increase in $q\text{CO}_2$ along with a reduction in MQ was reported in a metal polluted rice paddy, which partly explained the reduced SOC storage in the polluted soil (Bian et al., 2015). However, adding relatively inert C, particularly in the form of biochar, could also lead to a lower MQ or RQ of the treated soil. Yet, the parameter of $q\text{CO}_2$ could be considered as a promising indicator for the microbial use of carbon for their energy consumption and thus microbial health in soils (Anderson and Domsch, 1993; Anderson, 2003).

Microbial health and carbon use efficiency changes following a short term biochar addition have been not yet evaluated. The purpose of this study was to examine the biochar effects on soil microbial carbon use efficiency in relation to microbial growth in agricultural soils. With a meta-analysis, we tried to characterize the microbial changes with biochar addition using a number of microbiological

115 parameters derived from experiment data in literature. By this we aimed to address
116 bicohar's role in improving microbial growth and health, which could in turn help to
117 stabilize SOC storage in agricultural soils.

2. Materials and Methods

2.1. Data source

We searched literature published since 2001 and up to March 1, 2015 via electronic databases including Wiley-Blackwell, Springer Link, Web of Science, and the Chinese Magazine Network (CNKI). A bulk data base of 550 papers was first created by searching using the key words “biochar” and “soil”, after which the database was further filtered using the individual key words “respiration, mineralization and microbial biomass”. Subsequently, the collected literature was carefully checked to exclude studies conducted with non-agricultural soils. The used biochar could not specified as pure or mixed biochar as detailed information of the applied biochar was not always available in some of the reported experiments.

Among the final literature archive of 97 publications, 69 studies reported soil respiration (SR) (including 30 ones reporting also SOC), 54 reported soil microbial biomass C and/or N (SMBC/N) (including 23 ones also reporting SOC) and 26 reported both SMBC and SR. In these studies, SR was estimated either as the reported total CO₂ evolved or as the sum of individual single measurements CO₂ efflux weighted by time intervals, over a defined period. Totally, 1073 individual data pairs of single biochar treatments with and without biochar, were retrieved and used in this analysis. Some of the reported experiments in the collected literature included also treatments with biochar mixed with other material, which were not included as data pairs used for this study. The established data archive is given in Supplementary Information (Table. S1).

Used in the meta-analysis were the means and standard deviation (SD) of the measurements of SOC, SR and SMBC/SMBN, and the number of replicates of a data pair with and without biochar in a retrieved study. In case with no numerical data

shown, the software program Grafula 3 was used to extract numerical data from figures in a publication. For analyzing factors influencing microbial response to biochar, information of biochar production and soil condition as well as experiment condition were also retrieved and categorized. Soil properties were taken into account including soil pH, texture and SOC level while biochar production conditions including the feedstock and pyrolysis temperature as well as the application rate of biochar used. In addition, information of experiment types (field, pot trial or laboratory incubation) and the time since the biochar had been applied to the soil were also recorded.

2.2. Calculation and data processing

Specific quotients were calculated as follows:

$$RQ = SR/SOC \quad (1)$$

$$MQ = SMBC/SOC \quad (2)$$

$$qCO_2 = SR/SMBC \quad (3)$$

Where, SR is soil respiration in g CO₂-C g⁻¹ soil, SOC is the concentration of total soil organic carbon in g C kg⁻¹ soil, SMBC is soil microbial biomass C in mg kg⁻¹ soil as measured using fumigation-extraction protocols. Values for SOC and SR included those from biochar persisted since an addition to the soil.

A meta-analysis was conducted to characterize microbial abundances and respiration parameters for treatments with and without biochar addition. Following Hedges et al. (1999), an effect size was calculated as the natural log value of the response ratio:

$$R = X_t/X_c \quad (4)$$

Where, X_t and X_c were the mean of a tested parameter under a biochar amendment treatment and the control (without biochar amendment) respectively of a single

experiment. A standard error for a tested parameter was calculated from the standard deviation and the number of replicates reported in the experiment data. The weight values for each measured effect were considered as the inverse of the variance.

For analyzing the responses to biochar addition, the reported studies were grouped according to the defined categories of biochar, soil and experiment conditions. In detail, experiment types were categorized into incubation, pot or field studies as claimed in the reported studies, while experiment duration into classes of <6, 6-12 and >12 months. Studies were grouped according to feedstock of biochar from crop residue, wood residue and manure, and to pyrolysis temperature at 350, 350-500 and >500 centigrade and to application rate of <10, 10-20, 20-40 and >40 tons per hectare, respectively. Meanwhile, soil conditions before biochar addition were respectively categorized into classes of acid (pH <6.5), neutral (pH 6.5-7.5) and alkaline (pH >7.5) soils in term of soil reaction, of sand, loam and clay in term of soil texture and of <10, 10-20 and >20 g kg⁻¹ of SOC in term of the size of carbon pool. In addition, soil condition was also concerned with or without N fertilizer in a reported experiment. Data groups with fewer than three studies within a defined category were excluded from analysis.

Data treatment and processing were performed with Microsoft Excel 2010 and calculations with meta-analysis were conducted in natural log of response ratios following the procedure given by Hedges et al., (1999). When presenting and interpreting the biochar effect, we converted the natural log transformed ratios to the relative percent changes (RC) by biochar addition over the control of no biochar addition. All changes were graphed based on 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 significantly different from one another

193 if their 95 % CIs were non-overlapping.

3. Results

3.1. Microbial biomass and respiration responses as influenced with experimental types

Across the reported studies, biochar provided an overall increase in SMBC (by 26% on average; CI: 22% to 30%). The increase was on average by 22%, 34% and 29% with a CI of 16% to 27%, 21% to 49% and 21% to 37% respectively in lab incubation, pot and field experiments (Fig. 1a), showing no difference between experiment types. In contrast, the changes with biochar in SMBN varied in a wide range with experiment types. In detail, SMBN was significantly increased only in incubation studies (mean: 42%; CI: 24% to 62%) but not significantly different from the control in pot or field studies.

No significant change in SR was found across the reported studies of laboratory incubation and field study despite of a large uncertainty in the fewer pot studies (Fig. 1b). While no consistent change in the field experiments, a significant reduction in $q\text{CO}_2$ was observed indeed in both pot and lab incubation studies, giving an overall decrease by 16% on average (CI: 11% to 21%).

Short term experiments with a duration less than 6 months were the majority (79%) of the studies used and exerted a significant reduction in $q\text{CO}_2$ by 17% on average (CI: 12% to 21%). Of course, large uncertainty about the $q\text{CO}_2$ changes existed in the much fewer studies with a duration longer than 6 months, with a 8% (CI: 0% to 16%) decrease in those having a duration over 12 months compared to those having a duration in-between 6-12 months (Fig. 2).

216 With an exception of the few pot studies, a reduction in both RQ and MQ was
217 found across experiment types, being greater in lab incubation than in pot and field
218 experiments (Fig. S1). Moreover, the reduction in RQ was not significantly different
219 across the groups of experiment duration, though reduction in RQ seemed higher in
220 experiments having a duration in-between 6-12 months (Fig. S2).

221 Changes in SMBC and SR were further analyzed with experiment durations (Fig.
222 3 and 4). SMBC was significantly but moderately increased by 26% (CI: 20% to 32%)
223 in incubation experiments with durations shorter than 6 months but unchanged in
224 those sustained over 6 months, following biochar addition. Compared to no significant
225 change in the field studies having durations longer than 12 months, SMBC was
226 significantly but largely increased by 34% (CI: 21% to 49%) and 35% (CI: 26% to
227 45%) respectively in the pot and field studies having durations less than 12 months.
228 Meanwhile, bulk soil respiration in incubation studies was not significantly changed
229 in short term experiments (less than 6 month) while significantly but slightly
230 increased (mean: 10%; CI: 6% to 15%) in those longer term (over 6 months)
231 experiments (Fig. 4). Finally, biochar addition had no visible effect on SR in pot
232 studies conducted for less than 12 months while a negative (mean: -7%; CI: -11% to
233 -3%) change in SR was observed in field studies sustained 6 -12 months since biochar
234 addition.

235 In addition, there was no significant correlation between soil respiration and
236 SBMC, despite of a weak correlation detected in lab incubation experiments (Fig. 5).
237 Of course, the data was abnormally distributed though 85% of total pairs that showed

changes in a range of $\pm 50\%$.

3.2. Microbial respiration response as influenced by biochar conditions

Data of microbial changes in MQ, RQ and $q\text{CO}_2$ with biochar application rates were shown in Fig. 6 and Fig. S3. Overall, responses of MQ to biochar addition decreased with application rate, with the largest (mean: 57%; CI: 49% to 63%) reduced by an addition over 40 t ha^{-1} . Similarly, the negative changes in RQ under a biochar addition were larger between $10\text{-}40 \text{ t ha}^{-1}$ (mean: -27%; CI: -29% to -24%) than $<10 \text{ t ha}^{-1}$ (mean: -14%; CI: -17% to -10%), although few data available at rates over 40 t ha^{-1} . There was a wide negative response of $q\text{CO}_2$ (mean: -15%; CI: -22% to -6%) across the studies, without significant differences between the application rate groups.

As shown in Fig. 7 and Fig. S4a, the significant reduction in both RQ and $q\text{CO}_2$ was not different among feedstock groups of biochars, though a reduction in MQ was lower with manure biochar than crop residue biochar. The biochars produced under pyrolysis temperatures over 500 centigrade induced a significant reduction in $q\text{CO}_2$ by 15% on average (CI: 10% to 20%) and a greater reduction (by 37% on average, CI: 33% to 42%) in RQ, compared to 12% on average (CI: 2% to 27%) at pyrolysis temperatures <350 and in-between 350-500 centigrade (Fig. S4a). Most (81% of total) of biochars used in the reported studies were alkaline ($\text{pH} > 9$). With an exception of non-alkaline biochar having a pH value under 8, both MQ and RQ were significantly decreased with alkaline biochars having a pH value over 8. These reductions, by a mean of 25% and 49% and a CI range of 22% to 28% and of 43% to 55% respectively

for RQ and MQ, were higher with biochars having a pH value over 10 than those having a pH value in a range of 9-10 (Fig. S4). Differently, a reduction in $q\text{CO}_2$ was significant by 14% (CI: 10% to 19%) with biochars having a pH value over 9 though insignificant with biochars having a pH value under 9.

3.3. Microbial respiration response as influenced by soil conditions

As shown in Fig. S5, negative responses of RQ were observed regardless of N fertilization. Whereas, MQ had a larger reduction following a biochar addition in unfertilized soils (mean: 43%; CI: 37% to 48%) than in N-fertilized soils (mean: 21%; CI: 12% to 28%). However, the reduction with biochar in $q\text{CO}_2$ by 20% on average was significant (CI: 14% to 25%) in unfertilized soils but not in fertilized soils (Fig. 8).

Despite of no change in RQ with soil texture, the reduction in MQ was greater in clay soils than in sandy soils (Fig. S5). Likewise, a reduction in $q\text{CO}_2$ was greater in clay soils (mean: 32%; CI: 17% to 43%) than in loamy soils (mean: 8%; CI: 2% to 13%). With regard to soil reaction, the reduction in RQ was greater in alkaline soils (pH >7.5) (by 35% with a CI range of 32% to 38%) than in acidic (pH <6.5) (by 14% with a CI range of 8% to 20%) and in neutral (pH 6.6-7.5) (by 19% with a CI range of 10% to 27%) soils. Whereas, the reductions in MQ were significant both in acid (by 39% with a CI range of 33% to 44%) soils and alkaline soils (by 32% with a CI range of 22% to 40%) but insignificant in neutral soils. In contrast, the reduction in $q\text{CO}_2$ was higher in neutral soils (by 32% with a CI range of 23% to 39%)) than in acid soils (by 11% with a CI range of 6% to 15%) though $q\text{CO}_2$ was increased by 7 % on

average (CI: 1% to 14%) in a few studies with alkaline soils (Fig. 8).

When examined with SOC concentration before biochar addition, biochar exerted a large decrease both in RQ (mean:30%; CI: 27% to 33%) and MQ (mean: 42%; CI: 37% to 45%) in soils having a SOC concentration under 20g kg⁻¹ though no change in soils having a SOC value over 20g kg⁻¹ (Fig. S5). In contrast, biochar addition moderately decreased $q\text{CO}_2$ by 6% to 23% in soils having SOC less than 20 g kg⁻¹ but significantly increased by 11% (CI: 0% to 24%) in the soils with a SOC concentration over 20 g kg⁻¹ (Fig. 8).

4. Discussions

4.1 Biochar induced microbial change: biomass size versus respiration

The size of microbial biomass was generally accepted as a key indicator of soil quality (Lehmann et al., 2011) and soil respiration was considered the main process of carbon transport from terrestrial ecosystems to the atmosphere and thus a major player in the increasing global atmospheric CO₂ concentration (Schlesinger and Andrews, 2000). Variations in soil microbial biomass and respiratory activity posed one of the greatest uncertainties in current models of global carbon cycling (Hartman and Richardson 2013). SOC sequestration in agricultural soils was understood with good management practices through increasing organic carbon pool but stabilizing soil respiration (Smith et al. 2008; Paustian et al. 2016), which was sensitive to warming (Lloyd and Taylor, 1994). There has been many studies showing overall reductions in greenhouse gases including CO₂ with greatly enhanced C storage with biochar in agricultural soils (Lehmann and Rondon., 2006; Sohi et al., 2010; Sohi, 2012; Zhang et al., 2012; Liu et al., 2013). Also, there has been emerging evidence that stabilized SOC was of microbial rather than of plant input (Grandy and Neff, 2008; Cotrufo et al., 2013). So far, how microbial biomass and soil respiration coevolved with biochar addition has still not been yet clearly understood.

In our meta-analysis, an overall moderate increase both in SMBC by 26% and SMBN by 21% was visible respectively for the 413 and 106 pairs of data reported (Fig. 1a). More interestingly, an increase in SMBC was more or less consistent (CI in a range of 22%-26%) across all the experiments. Whereas, the divergent change in

SMBN across the experiment types could be attributed to the N competition by plants in pot and field trials (Lehmann *et al.*, 2003). Here, increase in SMBC tended to decrease with increasing duration, especially in experiments prolonged up to 12 months (Fig. 3). The increase in microbial biomass could be accounted for with the small labile or extractable carbon pool (Lou *et al.*, 2015) in biochar, which could be exhausted or aged in soils in a range of days to months (Kuzayakov, *et al.* 2009; Hale *et al.*, 2012). Of course, biochar could act as a suitable habitat for microbial growth and protection from predators (Pietikäinen *et al.*, 2000). Therefore, soil microbial growth could be potentially promoted following short term biochar addition, though the size of microbial biomass was smaller relative to the total carbon pool for the nature of largely recalcitrant carbon in biochar (Lehmann *et al.*, 2015).

To note, the observed increase in microbial biomass was not followed by the changes in soil respiration across the experiments. Data in Fig. 1b showed no positive change in SR, with the narrow CI ranges similar to that of SMBC. Variation of changes in SR was relatively wide in pot experiments for soil respiration rates, measured mostly as CO₂ effluxes could be subject to physical disturbance, as discussed for tillage effects (Fiedler *et al.*, 2015). The changes in SR with prolonged experiment durations for more than 6 months (Fig. 4) were different between lab incubation and field trials. On contrary, when aged in field conditions, biochar could improve soil aggregation and consequently the organic-mineral-microbial interactions (Major *et al.*, 2010; Jones *et al.*, 2012; Liu *et al.*, 2016), and thus tended to harness soil respiration in the long run (Smith *et al.*, 2010). Indeed, Ameloot *et al.* (2014) observed

a lowered soil microbial respiration two years following a single biochar addition at 49 t ha⁻¹.

Whether the observed increases in microbial growth contributed to soil respiration in individual studies could again help to understand the carbon stabilization in biochar added soils. Using the data of the studies reporting both microbial and respiration measurements, changes in soil respiration are plotted against those in SMBC (Fig. 5). In fact, there was not a significant correlation between SR and SMBC, regardless of experiment types. The finding that changes in SR were not in parallel to increases in microbial biomass, especially in pot and field studies, could suggest some microbial community shifts. This has been evidenced with some taxa-specific community changes in the works by Farrell et al., (2013), by Gomez et al., (2014), by Khodadad et al., (2011), by Ameloot et al. (2014) and by Chen et al. (2013; 2015a) as well as potential changes in fungal community such as mycorrhizae (Vanek and Lehmann 2015). Particularly in a recent work by Chen et al., (2015b), a potential shift in bacterial groups using less labile carbon pools such as aromatic compounds could exist a few years following a biochar addition to a rice soil. Thus, lower turnover of SOC indicated by greatly enhanced C pool but unchanged respiration could be linked to potential microbial community changes in biochar added soils. This provided an insight of a new bio-physical mechanism for stabilizing SOC in agricultural soils in short term following biochar amendment (Ameloot et al. 2013; Zheng et al. 2016).

Changes in $q\text{CO}_2$ could further support the above finding of enhanced microbial

growth but no net increase in soil respiration following biochar addition to agricultural soils. The parameter $q\text{CO}_2$ was accepted as a sensitive indicator of environmental stresses on soil microbial community microbial stress (Wardle and Ghani., 1995). And lower $q\text{CO}_2$ generally indicated lower stresses in less disturbed ecosystems or with best management practices (Thirukkumaran and Parkinson, 2000; Merrington et al., 2002; Anderson and Domsch, 2010). Across the studies (Fig. 1b), there was an overall decrease by 13% in $q\text{CO}_2$, though no net change in the few field experiments. The negative change was very significant and more or less consistent in lab incubation studies (Fig. 1b) and in experiments with duration shorter than 6 months (Fig. 2). This was in contrast to the finding of a potential positive priming effect on native SOM decomposition (Zimmerman et al 2011; Maestrini et al. 2014; Weng et al., 2015). Thus, we argued that biochar addition could exert short term negative priming on decomposition of soil organic carbon in agricultural soils. Biochar added in soil could help manipulate microbial health and thus reduce carbon exhaustion for their energy use for biochar could act as a suitable habitat for microbial growth and also provide protection from predators (Pietikäinen et al., 2000). Of course, the changes in $q\text{CO}_2$ could be related to biochar's physical properties and the amount added to soil.

4.2. Microbial metabolic quotient response to biochar: biochar condition versus soil condition

While a significant reduction in $q\text{CO}_2$ was observed but not different across groups of feedstock, a decrease in $q\text{CO}_2$ was significant with biochars only produced

at a pyrolyzing temperature over 500 centigrade or having a pH value over 9 (Fig. 7). Biochar pyrolyzed at high temperatures (and thus high pH) has been known rich in aromatic carbon, higher in porosity and surface area than biochar produced at lower temperatures (Novak et al., 2010; Zimmermann 2010; Harvey et al., 2012), thus better promoting soil aggregation and aeration. In a recent meta-analysis, soil aggregation and moisture retention were better enhanced with higher temperature biochars than lower temperature ones (Omondi et al., 2016). Improved aeration with enhanced aggregation provided better aerobic metabolism, which was more efficient than anaerobic metabolism and allowed a greater efficiency in converting labile carbon to microbial biomass (Picek et al., 2000). Biochar addition at higher doses provided larger amounts of stable biochar carbon to soils, thus enhancing SOC storage while improving soil structure (Sohi et al., 2010; Sohi, 2012). All these biophysical changes could partly explain the decrease in $q\text{CO}_2$ weakly correlated to biochar application rates (Fig. 6). However, changes in $q\text{CO}_2$ were not significantly different between groups of biochar property and between groups of application rates, in this study (Fig. 6 and 7). Thus, the biophysical improvement could play a major role in the bulk reduction in $q\text{CO}_2$ following biochar addition, which could not depend much on feedstock types of biochars. In other words, the extent of such effect could be subject to soil conditions that could be potentially improved with biochar addition.

The changes in $q\text{CO}_2$ were smaller within a group of soil conditions compared to those of biochar property. However, there were wider but significant changes between the groups respectively in term of N fertilization, soil texture, soil reaction and soil

organic carbon pool. As expected, biochar addition led to a lower $q\text{CO}_2$ in soils relatively low in SOC concentration (Fig. 8). Comparatively, greater (by 32%) reduction in $q\text{CO}_2$ was observed in clay soils. Physical protection has known as a primary mechanism for sequestration of labile C (Hassink 1996; Six et al., 2000) and carbon sources from added biochar was less accessible to microorganisms in clayey soils than in sandy or loam soils (Gul et al., 2015). As reported by Omandi et al., (2016), biochar promoted soil aggregation in clay soils rather than in loam soil. Findings above could be explained with a much better improvement of soil aggregation in clay soils than in loam soils, and in carbon depleted soil than in carbon rich soils. In a recent study, microbial growth and activity were promoted via enhanced soil aggregation with the accumulation of physically stabilized OC in rice paddy (Wang et al., 2015). This study highlighted the soil impacts on microbial metabolic quotient responses to biochar addition, depending on the potential that soil biophysical condition could be improved.

Furthermore, changes in $q\text{CO}_2$ were also significantly different between the experiment groups of soil reaction (Fig. 8). In detail, $q\text{CO}_2$ was moderately and greatly decreased in acid (by 11%) and neutral soils (by 32%) but slightly increased in alkaline soils. Addition of alkaline biochar to low pH soils has been shown to improve microbial growth and activity, and increase the carbon use efficiency (Bruun et al., 2008; Steiner et al., 2008; Jin, 2010; Biederman and Harpole, 2013), owing to elevated soil pH values. In acid soils, compared to neutral or alkaline soils, greater plant productivity was generally observed (Liu et al., 2013) and resultantly more fresh

carbon sources could be provided, reducing the energy need for nutrient exhaustion. Chen et al., (2016) and Zheng et al., (2016) both reported a significant reduction in $q\text{CO}_2$ along with a slight but significant change in microbial community composition in a slightly acid rice paddy 4 years following a single biochar amendment. Whereas, in alkaline soils with salinity constraints, biochar addition could potentially increase the salt stress on soil microbes, potentially increasing their energy need for their relief (Wichern et al., 2006; Song et al., 2014). Therefore, soil reaction played a strong role in $q\text{CO}_2$ changes under biochar, through the direct impact on microbial growth and health.

It had been argued that N might be a limiting factor in biochar amended soils (Lehmann and Rondon, 2006). In this study of a meta-analysis, biochar induced $q\text{CO}_2$ reduction was greater in unfertilized soils than in fertilized ones, though $q\text{CO}_2$ unchanged in fertilized soils over control without biochar (Fig. 8). Many field studies showed higher N use efficiency in fertilized agricultural soils with biochar than without biochar (Chan et al., 2008; Zhang et al., 2012; Liu et al., 2014; Qian et al., 2014; Zhang et al., 2016a). Yet, Jin (2010) also reported that soils amended with higher rates of biochar exerted smaller effects on $q\text{CO}_2$, compared to lower rates. Here, the trend of decreasing $q\text{CO}_2$ with increasing biochar addition could support the prevailing role of physical condition improvement over the N limitation impact, resulting in promoting microbial activity. Therefore, the findings without N fertilization could confound the understanding of microbial metabolic activity changes with biochar in fertilized agricultural soils.

4.3. Carbon dynamics with biochar related to experiment type and the time frame

Changes of microbial activity and biomass varied largely with the types and duration of the experiments examined in this study. There were wide differences in $q\text{CO}_2$ among the experiment types but moderate differences between the duration length groups though few experiments with duration longer than 6 months (Fig. 2). Unlike the findings from lab incubation and pot studies, there was no overall change in $q\text{CO}_2$ with biochar in field conditions (Fig. 1). This could be attributed to the highly dynamic conditions in the field where soils underwent wetting and drying cycles, which could result in carbon pulses when soils rewetted (Liu et al., 2016). Laboratory incubations were generally conducted with controlled temperature and moisture regime but without plant growth, pot and field trials could provide robust but temporally and spatially heterogeneous conditions with dynamic soil-plant-microbial interactions (Lehmann et al., 2015). Indeed, a weak but significant correlation of SR to SBMC was found only in lab incubation experiments ($y=0.48x+4.67$, $R^2=0.24$, $p < 0.01$) but not in pot and field experiments (Fig. 5). When considering the data of SR and of SMBC independently, biochar induced no changes (Fig. 1b) in SR but significant increase in SMBC (Fig. 1a), across all the experiment types. This could translate to an overall decrease in $q\text{CO}_2$ for the bulk of the total experiments examined in this study. This provided information for better understanding the changes in microbial health and carbon dynamics following biochar addition to agricultural soils.

A significant negative change in $q\text{CO}_2$ occurred in experiments having durations shorter than 6 months (by -16% on average) and longer than 12 months (by -8% on

average), compared to a slight but insignificant increase in those experiments sustained in-between 6-12 months (0.5% on average) (Fig. 2). Comparatively, no change was observed for SR (Fig. 4) but increases for SMBC (Fig. 3) in both pot and field studies that were carried out for longer than 6 months. This again could correspond to a net decrease in $q\text{CO}_2$. Thus, the time frame that the microbial changes had been monitored since biochar was added was also a key issue for assessing biochar induced changes (Lehman et al., 2015).

The above variation with experiment duration could be concerned with potential temporal change in carbon substrates available to microbial utilization. Most of carbon in the added biochar could persist in soils on a centennial scale, with a very small labile pool (Lehmann et al., 2015; Wang et al., 2016). Such labile pool could contain some amount of bioactive molecules promoting biological activity in plants (Lou et al., 2015) and in rhizosphere (Sun et al., 2017). In relevance to this small labile carbon pool, potential positive responses of microbial respiration could persist less than 6 months (Wang et al., 2016) or up to years (Kuzyakov et al., 2009; Smith et al., 2010), since biochar was added to soil. Owing to the predominant recalcitrant carbon pool (Woelf et al., 2010), biochar tended to provide a persistent habitat with alleviated environmental stress for microbial growth. While biochar's effect on soil fertility and C sequestration/GHGs mitigation sustained over years (Liu et al. 2014), increased methane production from accessible labile carbon from added biochar became invisible in the next rice season (Zhang et al., 2012), following a single amendment. Instead, a negative priming effect by biochar could potentially appear

over the long-term following a single addition (Maestrini et al. 2014).

Short term lab or pot experiments may provide biased information for interpreting biochar's effect on soil and agricultural production (Zhang et al. 2016b). And field experiments has been increasingly urged to conduct over years for better understanding biochar's role in agriculture, beyond carbon sequestration (Liu et al 2013; Liu et al., 2016; Zhang et al., 2016b). The meta-analysis here demonstrated the large uncertainty about the microbial response to biochar across the experiments with different lengths of duration. Yet, information from the existing studies mainly with short term lab incubations had limited our understanding of soil microbial response to biochar and the potential impact on carbon dynamics in agricultural soils. As commented by Zhang et al., (2016b), more long term studies should be conducted with crop residue biochar to poor soils as long as biomass waste recycling as sustainable soil management were concerned with biochar technology in agriculture of the world.

5. Conclusions

By a meta-analysis of literature data, this study demonstrated an overall short term increase in microbial biomass but a decrease in $q\text{CO}_2$ together with an unchanged soil respiration in agricultural soils following a biochar addition. This finding confirmed a promising role by biochar in lowering C turnover while promoting microbial health in soils in short term. While a significant reduction in $q\text{CO}_2$ was consistent across feedstock groups, only high temperatures ($>500\text{ }^{\circ}\text{C}$) biochar exerted a significant reduction (by 16%) in $q\text{CO}_2$ in pyrolyzing temperature groups. Whereas, this reduction was great (by over 30%) both in clay soils and in neutral soils but

moderate (by 15%) in SOC depleted soils. These effects were likely due to improved microbial habitats and alleviated environmental stresses, with which soil conditions exerted great impacts rather than biochar conditions. However, the large uncertainty across the experiments with different lengths of duration constrained robust characterization of microbial changes with biochar. Thus, well designed long term field experiments are urgent to conduct to gain sound understanding of microbial and C dynamics with biochar in agricultural soils.

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References:

- Ameloot, N., De Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y. N., Bouckaert, L., Sleutel, S., 2013. Short-term CO₂ and N₂O emissions and microbial properties of biochar amended sandy loam soils. *Soil Biol. Biochem.* 57, 401-410.
- Ameloot, N., Sleutel, S., Case, S.D.C., Alberti, G., McNamara, N.P., Zavalloni, C., Vervisch, B., Vedove, G., Neve, S.D., 2014. C mineralization and microbial activity in four biochar field experiments several years after incorporation. *Soil Biol. Biochem.* 78, 195–203.
- Anderson, T.H., Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. *Biol.*

536 Fertil. Soils. 1(2), 81-89.

537 Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO₂ ($q\text{CO}_2$) as a
538 specific activity parameter to assess the effects of environmental conditions, such
539 as pH, on the microbial biomass of forest soils. Soil Bio. Biochem. 25, 393-395.

540 Anderson, T.H., 2003. Microbial eco-physiological indicators to asses soil quality.
541 Agr. Ecosys. Environ. 98(1), 285-293.

542 Anderson, T.H., Domsch, K.H., 2010. Soil microbial biomass: the eco-physiological
543 approach. Soil Bio. Biochem. 42(12), 2039-2043.

544 Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem
545 functioning. Nature 515(7528), 505-511.

546 Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of
547 soil quality indices: a biological perspective. Geoderma 147(3), 159-171.

548 Bian, R., Cheng, K., Zheng, J., Liu, X., Liu, Y., Li, Z., Li, L., Pete, S., G, Pan., David,
549 C., Zheng, J., Zhang, X., Zhang, L., Qaiser, H., 2015. Does metal pollution matter
550 with C retention by rice soil? Scientific Reports 5, 13233.

551 Biederman, L.A., Harpole, W.S., 2013. Biochar and its effects on plant productivity
552 and nutrient cycling: a meta-analysis. Glob. Change Biol. Bioenergy 5(2), 202-214.

553 Bruun, S., Jensen, E.S., Jensen, L.S., 2008. Microbial mineralization and assimilation
554 of black carbon: dependency on degree of thermal alteration. Org. Geochem. 39,
555 839–845.

556 Cernansky, R., 2015. State-of-the-art soil. Nature 517(7534), 258-260.

557 Chan, K. Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Agronomic

558 values of greenwaste biochar as a soil amendment. *Soil Res.* 45(8), 629-634.

559 Chen, J., Liu, X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J.,
560 Zhang, X., Wang, J., Yu, X., 2013. Biochar soil amendment increased bacterial but
561 decreased fungal gene abundance with shifts in community structure in a slightly
562 acid rice paddy from Southwest China. *Appl. Soil Ecol.* 71, 33-44.

563 Chen, J., Liu, X., Li, L., Zheng, J., Qu, J., Zheng, J., Zhang, X., Pan, G., 2015a.
564 Consistent increase in abundance and diversity but variable change in community
565 composition of bacteria in topsoil of rice paddy under short term biochar treatment
566 across three sites from South China. *Appl. Soil Ecol.* 91, 68-79.

567 Chen, J., Liu, X., Li, L., Zheng, J., Qu, J., Zheng, J., Pan, G., 2015b. Consistent
568 increase in abundance and diversity but variable change in community composition
569 of bacteria in topsoil of rice paddy under short term biochar treatment across three
570 sites from South China. *Appl. Soil Ecol.* 91, 68-79.

571 Cook, B.D., Allan, D.L., 1992. Dissolved organic carbon in old field soils: total
572 amounts as a measure of available resources for soil mineralization. *Soil Bio.*
573 *Biochem.* 24(6), 585-594.

574 Cotrufo, M.F., Wallenstein, M., Boot, C.M., Denef, K. Paul, E.A., 2013. The
575 Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant
576 litter decomposition with soil organic matter stabilization: do labile plant inputs
577 form stable soil organic matter? *Glob. Change Biol.* 19, 988 – 995.

578 Farrell, M., Kuhn, T.K., Macdonald, L.M., Maddern, T.M., Murphy, D.V., Hall, P.A.,
579 Singh, B.P., Baumann, K., Krull, E.S., Baldock, J.A., 2013. Microbial utilization of

580 biochar-derived carbon. *Sci. Total Environ.* 465, 288–297

581 Fiedler, S.R., Buczko, U., Jurasinski, G., Glatzel, S., 2015. Soil respiration after
582 tillage under different fertiliser treatments—implications for modelling and
583 balancing. *Soil. Till. Res.* 150, 30–42.

584 Gomez, J.D., Denef, K., Stewart, C.E., Zheng, J., Cotrufo, M.F., 2014. Biochar
585 addition rate influences soil microbial abundance and activity in temperate soils.
586 *Eur. J. Soil Sci.* 65, 28–39.

587 Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical
588 decomposition sequence and its impact on soil organic matter structure and
589 function. *Sci. Tot. Environ.* 404(2), 297–307.

590 Gul, S., Whalen, J.K., Thomas, B.W., 2015. Physico-chemical properties and
591 microbial responses in biochar-amended soils: Mechanisms and future directions.
592 *Agr. Ecosyst. Environ.* 206, 46–59.

593 Hartman, W. H., Richardson, C. J., 2013. Differential nutrient limitation of soil
594 microbial biomass and metabolic quotients ($q\text{CO}_2$): Is there a biological
595 stoichiometry of soil microbes? *PLoS ONE* 8, e57127.

596 Harvey, O., Kuo, L., Zimmerman, A., 2012. An index-based approach to assessing
597 recalcitrance and soil carbon sequestration potential of engineered black carbons
598 (biochars). *Environ. Sci. Technol.* 46, 1415–1421.

599 Hassink, J., 1996. Preservation of plant residues in soils differing in unsaturated
600 protective capacity. *Soil Sci. Soc. Am. J.* 60(2), 487–491.

601 Hale, S. E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T.,

602 Shitumbanuma, V., O'Toole, A., Sundqvist, K.L., Arp, H.P.H., Cornelissen, G.,
 603 2012. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and
 604 dioxins in biochars. *Environ. Sci. Technol.* 46, 2830–2838.

605 Hedges, L. V., Gurevitch, J., Crutis, P.S., 1999. The meta-analysis of response rations
 606 in experimental ecology. *Ecology* 80, 1150–1156.

607 Insam, H., Haselwandter, K., 1989. Metabolic quotient of the soil microflora in
 608 relation to plant succession. *Oecologia* 79(2), 174-178.

609 Jeffery, S., Verheijen, F.G.A., Van Der Velde, M., Bastos, A.C., 2011. A quantitative
 610 review of the effects of biochar application to soils on crop productivity using
 611 meta-analysis. *Agr. Ecosyst. Environ.* 144(1), 175-187.

612 Jin, H., 2010. Characterization of microbial life colonizing biochar and
 613 biochar-amended soils. PhD Dissertation, Cornell University, Ithaca, NY.

614 Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012.
 615 Biochar-mediated changes in soil quality and plant growth in a three year field
 616 trial. *Soil Bio. Biochem.* 45, 113-124.

617 Khodadad, C. L., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011.
 618 Taxa-specific changes in soil microbial community composition induced by
 619 pyrogenic carbon amendments. *Soil Bio. Biochem.* 43(2), 385-392.

620 Kuzyakov, Y., Friedel, J. K., Stahr, K., 2000. Review of mechanisms and
 621 quantification of priming effects. *Soil Bio. Biochem.* 32, 1485-1498.

622 Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon
 623 decomposition and incorporation into soil microbial biomass estimated by ^{14}C

624 labeling. *Soil Bio. Biochem.* 41, 210–219.

625 Lehmann, J., da Silva Jr, J. P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003.

626 Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of

627 the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant*

628 *soil* 249(2), 343-357.

629 Lehmann, J., Rondon, M., 2006. Biochar soil management on highly weathered soils

630 in the humid tropics. In: *Biological Approaches to Sustainable Soil Systems*

631 517–530. CRC Press, Boca Raton, FL.

632 Lehmann, J., 2007. A handful of carbon. *Nature* 447,143–144.

633 Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D.,

634 2011. Biochar effects on soil biota - A review. *Soil Bio. Biochem.* 43, 1812-1836.

635 Lehmann, J., Joseph, S., 2015. Biochar for environmental management: science,

636 technology and implementation. Routledge, New York.

637 Lehmann, J., Kuzyakov, Y., Pan, G., Ok, Y.S., 2015. Biochars and the plant-soil

638 interface. *Plant Soil* 395, 1-5.

639 Li, Y.T., Rouland, C., Benedetti, M., Li, F.B., Pando, A., Lavelle, P., Dai, J., 2009.

640 Microbial biomass, enzyme and mineralization activity in relation to soil organic C,

641 N and P turnover influenced by acid metal stress. *Soil Bio. Biochem.* 41(5),

642 969-977.

643 Liang, B., Lehmann, J., Sohi, S.P., Theis, J.E., O'Neill, B., Trujillo, L., Gaunt, J.,

644 Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects

645 the cycling of non-black carbon in soil. *Org. Geochem.* 41(2), 206-213.

646 Liu, X., Zhang, A., Ji, C., Joseph, S., Bian, R., Li, L., Pan, G., Paz-Ferreiro, J., 2013.
647 Biochar's effect on crop productivity and the dependence on experimental
648 conditions—a meta-analysis of literature data. *Plant Soil* 373(1-2), 583-594.

649 Liu, X., Ye, Y., Liu, Y., Zhang, A., Zhang, X., Li, L., Pan, G., Zheng, J., 2014.
650 Sustainable biochar effects for low carbon crop production: A 5-crop season field
651 experiment on a low fertility soil from Central China. *Agr. Syst.* 129, 22-29.

652 Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Zou, J., 2016. Response of soil
653 carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar
654 amendment: a meta-analysis. *Glob. Change Biol. Bioenergy* 8(2), 392-406.

655 Lloyd, J., Taylor, J.A., 1994. On the temperature dependence of soil
656 respiration. *Functional ecology* 315-323.

657 Lou, Y., Joseph, S., Li, L., Graber, E.R., Liu, X., Pan, G., 2015. Water Extract from
658 Straw Biochar Used for Plant Growth Promotion: An Initial Test.
659 *BioResources* 11(1), 249-266.

660 Maestrini, B., Herrmann, A.M., Nannipieri, P., Schmidt, M.W., Abiven, S., 2014.
661 Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen
662 mineralization in a temperate forest soil. *Soil Bio. Biochem.* 69, 291-301.

663 Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil - applied black
664 carbon: downward migration, leaching and soil respiration. *Glob. Change*
665 *Biol.* 16(4), 1366-1379.

666 Masciandaro, G., Ceccanti, B., Gallardo-Lancho, J.F., 1998. Organic matter properties
667 in cultivated versus set-aside arable soils. *Agr. Ecosyst. Environ.* 67, 267-274.

668 Maestrini, B., Herrmann, A.M., Nannipieri, P., Schmidt, M.W., Abiven, S., 2014.
669 Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen
670 mineralization in a temperate forest soil. *Soil Bio. Biochem.* 69, 291-301.

671 Merrington, G., Nfa, L.W., Parkinson, R., Redman, M., Winder, L., 2002.
672 Agricultural pollution: environmental problems and practical solutions. Spon Press,
673 London.

674 Mukherjee, S., Weihermueller, L., Tappe, W., Vereecken, H., Burauel, P., 2016.
675 Microbial respiration of biochar-and digestate-based mixtures. *Biol. Fertil. Soils* 52,
676 151-164.

677 Novak, J.M., Busscher, W.J., Watts, D.W., 2010. Short-term CO₂ mineralization after
678 additions of biochar and switchgrass to a Typic Kandiudult. *Geoderma* 154,
679 281–288.

680 Omondi, M.O., Xia, X., Nahayo, A., Liu, X., Korai, P.K., Pan, G., 2016.
681 Quantification of biochar effects on soil hydrological properties using
682 meta-analysis of literature data. *Geoderma* 274, 28-34.

683 Paustian, L., Babcock, B., Hatfield, J.L., Lal, R., McCarl, B.A., McLaughlin, S.,
684 Rosenzweig, C., 2016, April. Agricultural mitigation of greenhouse gases: science
685 and policy options. In 2001 Conference Proceedings, First National Conference on
686 Carbon Sequestration. Washington, DC: Conference on Carbon Sequestration.

687 Picek, T., Šimek, M., Šantrůčková, H., 2000. Microbial responses to fluctuation of
688 soil aeration status and redox conditions. *Biol. Fertil. Soils* 31(3-4), 315-322.

689 Pietikäinen, J., Kiikkilä, O., Fritze, H., 2000. Charcoal as a habitat for microbes and

its effect on the microbial community of the underlying humus. *Oikos* 89(2),
231-242.

Powlson, D.S., Jenkinson, D.S., 1981. A comparison of the organic matter, biomass,
adenosine triphosphate, and mineralizable nitrogen contents of ploughed and direct
drilled soils. *The J. Agric. Sci.* 97, 713-721.

Qian, L., Chen, L., Joseph, S., Pan, G., Li, L., Zheng, J., Zhang, X., Zheng, J., Wang,
J., Yu, X., Wang, J., 2014. Biochar compound fertilizer as an option to reach high
productivity but low carbon intensity in rice agriculture of China. *Carbon Manag.*
5(2), 145-154.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon
cycle. *Biogeochemistry*, 48(1), 7-20.

Schlöter, M., Dilly, O., Munch, J.C., 2003. Indicators for evaluating soil quality. *Agr.*
Ecosyst. Environ. 98(1), 255-262.

Six, J.A.E.T., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and
microaggregate formation: a mechanism for C sequestration under no-tillage
agriculture. *Soil Bio. Biochem.* 32(14), 2099-2103.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H.H., Kumar, P., McCarl, B., Ogle,
S., O'Mara, F., Rice, C., Scholes, R.J., Sirotenko, O., Howden, M., McAllister, T.,
Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenbach, M., Smith,
J.U., 2008. Greenhouse gas mitigation in agriculture. *Philos. Trans. R. Soc. B*
363, 789-813.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil

712 respiration. *Soil Bio. Biochem.* 42, 2345–2347.

713 Smith, P., 2016. Soil carbon sequestration and biochar as negative emission
714 technologies. *Glob. Change Biol.* 22(3), 1315-1324.

715 Sohi, S. P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use
716 and function in soil. *Advances in Agronomy* 105, 47-82.

717 Sohi, S. P., 2012. Carbon storage with benefits. *Science* 338(6110), 1034-1035.

718 Song, Y., Zhang, X., Ma, B., Chang, S.X., Gong, J., 2014. Biochar addition affected
719 the dynamics of ammonia oxidizers and nitrification in microcosms of a coastal
720 alkaline soil. *Biol. Fertil. Soils* 50(2), 321-332.

721 Soinne, H., Hovi, J., Tammeorg, P., Turtola, E., 2014. Effect of biochar on
722 phosphorus sorption and clay soil aggregate stability. *Geoderma* 219–220,
723 162–167.

724 Sparling, G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a
725 sensitive indicator of changes in soil organic matter. *Aust. J. Soil Res.* 30, 195–207.

726 Steiner, C., Glaser, B., Geraldte Teixeira, W., Lehmann, J., Blum, W.E., Zech, W.,
727 2008. Nitrogen retention and plant uptake on a highly weathered central
728 Amazonian Ferralsol amended with compost and charcoal. *J. Plant Nutr. Soil Sci.*
729 171(6), 893-899.

730 Sun, J., Drosos, M., Mazzei, P., Savy, D., Todisco, D., Vinci, G., Pan, G., Piccolo, A.,
731 2017. The molecular properties of biochar carbon released in dilute acidic solution
732 and its effects on maize seed germination. *Sci. Tot. Environ.* 576, 858–867.

733 Thirukkumaran, C.M., Parkinson, D., 2000. Microbial respiration, biomass, metabolic

734 quotient and litter decomposition in a lodgepole pine forest floor amended with
 735 nitrogen and phosphorous fertilizers. *Soil Bio. Biochem.* 32(1), 59-66.

736 Trivedi, P., Anderson, I.C., Singh, B.K., 2013. Microbial modulators of soil carbon
 737 storage: integrating genomic and metabolic knowledge for global prediction.
 738 *Trends Microbiol.* 21(12), 641-651.

739 Van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S.,
 740 Cowie, A., 2010. Effects of biochar from slow pyrolysis of papermill waste on
 741 agronomic performance and soil fertility. *Plant Soil* 327, 235–246.

742 Vanek, S.J., Lehmann, J., 2015. Phosphorus availability to beans via interactions
 743 between mycorrhizas and biochar. *Plant Soil* 395(1-2), 105-123.

744 Wang, P., Liu, Y., Li, L., Cheng, K., Zheng, J., Zhang, X., Zheng, J., Stephen, J., Pan,
 745 G., 2015. Long-term rice cultivation stabilizes soil organic carbon and promotes
 746 soil microbial activity in a salt marsh derived soil chronosequence. *Scientific*
 747 *Reports* 5, 15704..

748 Wang, J., Xiong, Z., Kuzyakov, Y., 2016. Biochar stability in soil: meta - analysis of
 749 decomposition and priming effects. *Glob. Change Biol. Bioenergy* 8(3), 512-523.

750 Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO₂)
 751 as a bioindicator of disturbance and ecosystem development. *Soil Bio. Biochem.*
 752 27(12), 1601-1610.

753 Weng, Z, Van. Zwieten, L, Singh, B.P, Kimber, S, Morris, S, Cowie, A., Macdonald,
 754 L., 2015. Assessing biochar stability and native soil carbon stabilisation in pasture.
 755 *Soil Bio. Biochem.* 90, 111-121.

756 Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial
 757 communities and the decomposition of maize in acidic soils. *Geoderma* 137(1),
 758 100-108.

759 Woolf, D., Amonette, J., Street-Perrott, F., Lehmann, J., Joseph, S., 2010. Sustainable
 760 biochar to mitigate global climate change. *Nat. commun.* 1, 56.
 761 doi:10.1038/ncomms1053.

762 Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J., Zhang, X., 2012. Effect of
 763 biochar amendment on maize yield and greenhouse gas emissions from a soil
 764 organic carbon poor calcareous loamy soil from Central China Plain. *Plant Soil*
 765 351(1-2), 263-275.

766 Zhang, D., Pan, G., Wu, G., Kibue, G.W., Li, L., Zhang, X., Zheng, J., Zheng, J.,
 767 Cheng, K., Stephen, J., Liu, X., 2016a. Biochar helps enhance maize productivity
 768 and reduce greenhouse gas emissions under balanced fertilization in a rainfed low
 769 fertility inceptisol. *Chemosphere* 142, 106-113.

770 Zhang, D., Yan, M., Niu, Y., Liu, X., van Zwieten, L., Chen, D., Bian, R., Cheng, K.,
 771 Li, L., Stephen, J., Zhang X., Crowley, D., Filley, T., Pan, G., Zheng, J., 2016b. Is
 772 current biochar research addressing global soil constraints for sustainable
 773 agriculture? *Agr. Ecosyst. Environ.* 226, 25-32.

774 Zheng, J., Chen, J., Pan, G., Liu, X., Zhang, X., Li, L., Bian, R., Cheng, K., Jinwei, Z.,
 775 2016. Biochar decreased microbial metabolic quotient and shifted community
 776 composition four years after a single incorporation in a slightly acid rice paddy
 777 from southwest China. *Sci. Total Environ.* 571, 206-217.

778 Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced
779 black carbon (biochar). *Environ. Sci. Technol.* 44, 1295–1301.

780 Zimmerman, A.R., Gao, B., Ahn, M.Y., 2011. Positive and negative carbon
781 mineralization priming effects among a variety of biochar-amended soils. *Soil Bio.*
782 *Biochem.* 43, 1169-1179.

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Figure captions

Fig. 1. Response of SMBC and SMBN (a), SR and $q\text{CO}_2$ (b) to biochar addition expressed as the average percentage changes from control with 95% confidence intervals across experiment types. The number of pairs is shown in parenthesis.

Fig. 2. Changes following biochar addition in $q\text{CO}_2$ with experiment duration. The central point and the bar represent the grand mean and the 95% confidence interval. The number of pairs is shown in parenthesis.

Fig. 3. Changes following biochar addition in SMBC with experiment duration. The central point and the bar represent the grand mean and the 95% confidence interval. The number of pairs is shown in parenthesis.

Fig. 4. Changes following biochar addition in SR with experiment duration. The central point and the bar represent the grand mean and the 95% confidence interval, respectively. The number of pairs is shown in parenthesis.

Fig. 5. Changes in SR against changes in SMBC for the overall 140 data pairs retrieved and for 118 data pairs (dashed block) with changes in $\pm 50\%$ changes over the control. The latter boosted in the embedded figure and correlation equation, the explanatory (R^2) and probability level shown on the top left for lab incubation, pot and field experiments respectively of 81, 9 and 28 data points.

Fig. 6. Response of $q\text{CO}_2$ to biochar addition expressed as the average percentage changes from control with 95% confidence intervals for biochar application rate (in tons per hectare). The number of pairs is shown in parenthesis.

Fig. 7. Response of $q\text{CO}_2$ to biochar addition expressed as the average percentage changes from control with 95 % confidence intervals for feedstock, pyrolyzing temperature, and pH of biochars. The number of pairs is shown in parenthesis.

Fig. 8. Response of $q\text{CO}_2$ to biochar addition expressed as the average percentage

811 changes from control with 95% confidence intervals for nitrogen fertilizer (N0,
812 no nitrogen; N1, with nitrogen), soil texture, soil pH, SOC. The number of pairs
813 is shown in parenthesis.