Response of soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar amendment: a meta-analysis

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Abstract

Biochar as a carbon-rich coproduct of pyrolyzing biomass, its amendment has been advocated as a potential strategy to soil carbon (C) sequestration. Updated data derived from 50 papers with 395 paired observations were reviewed using meta-analysis procedures to examine responses of soil carbon dioxide (CO₂) fluxes, soil organic C (SOC), and soil microbial biomass C (MBC) contents to biochar amendment. When averaged across all studies, biochar amendment had no significant effect on soil CO₂ fluxes, but it significantly enhanced SOC content by 40% and MBC content by 18%. A positive response of soil CO₂ fluxes to biochar amendment was found in rice paddies, laboratory incubation studies, soils without vegetation, and unfertilized soils. Biochar amendment significantly increased soil MBC content in field studies, N-fertilized soils, and soils with vegetation. Enhancement of SOC content following biochar amendment was the greatest in rice paddies among different land-use types. Responses of soil CO₂ fluxes and MBC to biochar amendment varied with soil texture and pH. The use of biochar in combination with synthetic N fertilizer and waste compost fertilizer led to the greatest increases in soil CO₂ fluxes and MBC content, respectively. Both soil CO₂ fluxes and MBC responses to biochar amendment decreased with biochar application rate, pyrolysis temperature, or C/N ratio of biochar, while each increased SOC content enhancement. Among different biochar feedstock sources, positive responses of soil CO₂ fluxes and MBC were the highest for manure and crop residue feedstock sources, respectively. Soil CO₂ flux responses to biochar amendment decreased with pH of biochar, while biochars with pH of 8.1–9.0 had the greatest enhancement of SOC and MBC contents. Therefore, soil properties, land-use type, agricultural practice, and biochar characteristics should be taken into account to assess the practical potential of biochar for mitigating climate change.

Keywords: biochar, carbon dioxide, climate change, microbial biomass carbon, soil organic carbon

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Introduction

Atmospheric carbon dioxide (CO₂) is the most important potent greenhouse gas (GHG) with a potential radiative forcing of 1.66 W m⁻² that contributes to current global warming and impacts the earth’s climate system (Forster et al., 2007; Shindell et al., 2009). Mitigation of CO₂ release from soils has been generally achieved by enhancing CO₂ removal from the atmosphere (sequestration), reducing emissions, and avoiding (or displacing) emissions (Smith et al., 2008). Soil carbon (C) sequestration through selected soil management practice (e.g., crop residue or biochar amendment) has been proposed as a potential alternative to mitigate the rise of atmospheric CO₂ (Lal, 1999; Pan et al., 2003; Mossier et al., 2006; Smith et al., 2008; Lu et al., 2009; Shang et al., 2011).

Biochar is a carbon-rich coproduct of pyrolyzing biomass subject to high-temperature and oxygen-deprived conditions for biofuel production (Lehmann, 2007a; Laird et al., 2009) and has been advocated as a potential management strategy to improve soil quality, increase crop yield, and enhance soil carbon sequestration in fields (Marris, 2006; Lehmann, 2007b; Laird, 2008; Woolf et al., 2010; Case et al., 2014). Several recent laboratory or field studies have suggested that biochar might have the potential to mitigate climate change by increasing soil C sequestration and/or reducing GHG emissions (e.g., soil CO₂ fluxes) from soils (Lehmann, 2007b;
Chávez et al., 2012; Stewart et al., 2013; Zhang et al., 2013; Li et al., 2014). Some studies have found an increase in soil CO$_2$ fluxes following biochar amendment but with large uncertainties (Spokas & Reicosky, 2009; Smith et al., 2010; Jones et al., 2011; Rogovska et al., 2011; Zheng et al., 2012). Meanwhile, suppression of soil CO$_2$ fluxes by biochar amendment or no effect of biochar amendment has been found in other studies (e.g., Kimetu & Lehmann, 2010; Karhu et al., 2011; Lu et al., 2014).

In addition to understanding the effects on soil CO$_2$ fluxes, there is a need to understand whether biochar amendment will enhance soil organic C (SOC) sequestration (Jones et al., 2011; Luo et al., 2011). However, both positive and negative effects of biochar amendment on SOC storage have been reported in previous studies (e.g., Liang et al., 2010; Cross & Sohi, 2011). These inconsistent results may be due to differences in the nature of biochar and soil, and variation in the experimental methods adopted in individual studies (Jones et al., 2011).

Soil microbial biomass carbon (MBC) is an important soil labile C fraction that has been found to play a fundamental role in soil organic C dynamics (Grandy & Neff, 2008; Liang et al., 2011) and serves as a useful indicator of changes in soil C stabilization and nutrient dynamics following soil management practices (Sparling, 1992; Fierer et al., 2009). Recent evidence has shown that soil MBC content was higher in biochar-treated soils than in control soils (O’Neill, 2007; Liang, 2008; Biederman & Harpole, 2013). Steiner et al. (2008) found that biochar amendment to acid soils stimulated microbial activity and in turn increased soil MBC content. Thies & Rillig (2008) suggested that soil C sequestration resulting from biochar amendment cannot be attributed to a decrease in soil MBC content. Lu et al. (2014) found that biochar amendment did not alter soil microbial biomass or community structure in an incubation experiment. Together, these studies show that different results may be obtained due to variation in soil type and/or study methods.

Although a large number of experiments have examined the effect of biochar amendment on soil CO$_2$ fluxes and its potential to improve soil C sequestration under controlled (laboratory or pot) or field conditions with different soils, there is currently no systematic synthesis. Furthermore, the potential of biochar amendment to improve soil C-sink capacity (e.g., soil SOC and MBC contents) and its effect on soil CO$_2$ fluxes are still under debate, as the direction and magnitude of effects seem to depend on a variety of factors, such as soil properties, land-use type, experimental methods, vegetation presence, or biochar characteristics that vary with feedstock type and pyrolysis conditions (Hilscher & Knicker, 2011). Meta-analysis has been developed for quantitative integration of results from individual studies and is increasingly used in studies of ecological issues, greenhouse gas mitigation, and soil C sequestration (Knorr et al., 2005; Akiyama et al., 2010; Van Groenigen et al., 2010; Biederman & Harpole, 2013; Shan & Yan, 2013). Recently, Biederman & Harpole (2013) provided an evaluation of biochar effects on soil nutrient cycling (e.g., soil C fractions) in a quantitative review using meta-analysis procedures, but they only presented an overall picture and failed to distinguish different primary factors influencing biochar behavior in soils. In addition, the review did not include soil CO$_2$ fluxes, which represent an integral part of soil C cycling.

In this study, 395 individual experimental observations derived from 50 peer-reviewed publications were synthesized to examine the responses of soil CO$_2$ fluxes, SOC, and MBC to biochar amendment using meta-analysis procedures. The first objective of this study was to quantitatively examine the effect size of biochar amendment on soil CO$_2$ fluxes, SOC, and MBC contents. The second objective was to identify the key factors that influence the response of soil CO$_2$ fluxes, SOC, and MBC to biochar amendment.

Materials and methods

Data sources and compilation

We conducted a detailed review of literature published in peer-reviewed journals through the year 2014 (cutoff date on July 10, 2014). We extracted data from 50 published research papers with 395 individual observations including both control and biochar-amended treatments (Appendices S1 and S2). For each grouping category of measurements, original documented information included mean soil CO$_2$ fluxes, standard deviation (SD), and number of replicates from both biochar-amended and control treatments, as well as land-use type, soil properties (texture and pH), biochar application rate and characteristics (feedstock, pyrolysis temperature, pH, C/N ratio), vegetation cover, and experimental conditions (field/pot/incubation study, rate and source of N fertilizer, and observation length) was extracted when available. In further data compiling prior to meta-analysis, we categorized the soils into four land-use types as upland cropping system, rice paddy, grassland, and forest for soil CO$_2$ analysis based on the actual (for field-based studies) or original (for incubation or pot studies) land-use type. For SOC analysis, only three land-use types (upland cropping system, rice paddy, and grassland) were available in this analysis. However, the data available on MBC across ecosystem types were overwhelmingly derived from upland cropping systems, and thus MBC was excluded from this grouping category. Soil texture was grouped into three classes (coarse, medium, and fine) due to the inconsistent reporting of soil
texture in the literature (e.g., general qualitative description, particle size distribution, soil taxonomical unit).

Data were subjected to a standardization process to allow for comparisons. Biochar application rates were identically transformed to amount per area (expressed as t ha$^{-1}$) according to the soil layer reported in each study (or a layer of 20 cm in case of being not available) and the bulk density (BD) of soil. If BD was not directly provided in the studies, we calculated it in case of being not available.

If BD was not directly provided in the studies, we calculated it from soil texture (Saxton et al., 1986). Measurements from different biochar amendment levels from the same experiment were considered as independent observations to evaluate the overall effect of biochar on soil C dynamics. In the literature, mean soil CO2 fluxes were far more frequently reported than cumulative CO2 emissions. In the cases that seasonal or annual mean soil CO2 fluxes were not reported directly, we estimated the value by dividing total CO2 emissions into average daily fluxes over the measurement period. If only soil organic matter (SOM) was provided, we converted SOM to SOC using a Bemmelen index value of 0.58. When data were presented only in graphical form, we digitized them using the software plot digitizer ver. 2.6.2 to extract data (Cayuela et al., 2014).

**Inclusion criteria**

Studies under controlled conditions (laboratory incubation or greenhouse pot studies) were also included to better quantitatively understand the integrative effect of biochar on soil CO2 fluxes, SOC, and MBC content across soils. Studies with no replication or no reported number of replications were excluded. Moreover, grouping categories with fewer than two data pairs were excluded from the analyses. To conduct a meta-analysis, the mean, SD, and number of replicates for soil CO2 fluxes, SOC, and MBC from biochar amendment and control treatments are needed. However, 12% (47 of 395) of the observations failed to report any information on variance (SD, standard error, or variance). In these cases, efforts were made to obtain these from the corresponding authors. Otherwise, we assigned the average SD derived from other SD-reported data-sets to include as many studies as possible in the analysis (Higgins & Green, 2008).

**Data analysis**

The means of soil CO2 fluxes, SOC, and MBC contents from biochar treatment ($X_t$) and control ($X_c$) groups were used to compute effect sizes in the form of natural log-transformed response ratios (RR). The standard deviations of both biochar treatment and control were included as a measure of variance:

$$RR = \ln(X_t/X_c) = \ln(X_t) - \ln(X_c)$$

(1)

where $X_t$ and $X_c$ are means in the treatment and control groups, respectively. Its variance ($\sigma^2$) is estimated as:

$$\sigma^2 = \frac{s_t^2}{n_t} + \frac{s_c^2}{n_c}$$

(2)

where $n_t$ and $n_c$ are the sample sizes for the treatment and control groups, respectively; $s_t$ and $s_c$ are the standard deviations for the treatment and control groups, respectively.

We conducted weighted meta-analyses using RRs, where mean effect size for each category was calculated using a categorical random effects model. Groups with fewer than two treatments were excluded from the analyses. The overall mean effect size and 95% confidential interval (CI) of each grouping category generated by bootstrapping (9999 iterations) were calculated with MetaWin version 2.0 statistical software (Rosenberg et al., 2000). Mean effect sizes were considered significant if the 95% CI did not overlap with zero, and $X_t$ and $X_c$ were considered significantly different from one another if their 95% CIs did not overlap. In addition, to further analyze biochar effect among different subgrouping categories, between-group heterogeneity ($Q_b$) was examined across all data for a given response variable.

Publication bias is the selective publication of articles showing certain types of results over others, which is the tendency for journals to publish studies with statistically significant results. Such publication bias will lead to an overestimation of effect size in meta-analysis. To avoid this problem, we examined publication bias by both Rosenthal’s method ($z = 0.05$) and Orwin’s method (negligible effect = 0.2) (Rosenberg et al., 2000).

In addition to the meta-analysis procedure, fitting of data to linear and Gaussian distribution functions was carried out using the SigmaPlot version 12.0 software, and the frequency distribution of RR was plotted to reflect variability among individual studies with the following Gaussian function (i.e., normal distribution) (Luo & Zhou, 2006):

$$y = a \exp\left(\frac{(x - \mu)^2}{2\sigma^2}\right)$$

(3)

where $y$ is the frequency of RR values within an interval, $x$ is the mean of RR for the given interval, $\mu$ and $\sigma^2$ are the mean and variance across all RR values, respectively, and $a$ is a coefficient indicating the expected number of RR at $x = \mu$.

**Sensitivity analyses**

We performed sensitivity analyses to test the robustness of our meta-analyses. For the first sensitivity analysis, we excluded outlier studies, repeated the meta-analysis, and compared the results to those of the original meta-analysis. For the second sensitivity analysis, we excluded datasets that did not report variances, repeated the meta-analysis, and then compared the results to those of the original meta-analysis.

**Results**

**Biochar effects on soil CO2 fluxes, SOC, and MBC**

Across all observations, the datasets were homogenous for soil CO2 fluxes with a normal distribution pattern of effect sizes (Fig. 1). A significant positive linear relationship was observed between soil CO2 fluxes in biochar-amended and control treatments (Fig. 1a). However, the slope of a linear regression was not significantly greater than one ($P = 0.08$), suggesting that the response of soil...
CO₂ fluxes to biochar amendment was not significant when averaged across all studies. Similarly, the distribution of effect sizes across all observations was also normal for SOC and MBC (Fig. 1). The slopes of linear regressions for SOC and MBC in biochar-amended against those in the control treatments were significantly greater than one (SOC, \( P < 0.01 \); MBC, \( P = 0.02 \)), suggesting that biochar amendment significantly increased SOC and MBC contents (Table 1; Fig. 1b, c). Biochar-induced changes in SOC content were significantly and positively correlated with those in MBC content across all observations (Fig. 2a). However, no such correlation of biochar-induced changes in soil CO₂ fluxes with SOC (Fig. 2b, \( P = 0.65 \)) or MBC was found (data not shown).

The response of soil CO₂ fluxes to biochar amendment depended significantly on soil properties, vegetation presence, fertilizer, and biochar feedstock source and characteristics (Table 2). Of these, soil texture and pH, vegetation presence, feedstock source, and C/N ratio of biochar were the key factors mediating the response of soil CO₂ fluxes to biochar amendment. Land-use type and biochar C/N ratio were the two most critical parameters affecting the response of SOC
Table 1 Percent change and fitted Gaussian (normal) distributions of soil CO₂ fluxes, SOC, and MBC responses to biochar amendment

| Target variables | Percent change ($e^{R_{xx}-1} \times 100\% \pm SD$) | Sampling size ($n$) | Gaussian distribution |
|------------------|-------------------------------------------------|---------------------|----------------------|
| Soil CO₂ fluxes  | 3.05 ± 0.24                                     | 167                 | $a = 32.69, \sigma = 0.12, \mu = 3.78, \sigma^2 = 0.98^{***}$ |
| SOC              | 52.20 ± 1.78                                    | 148                 | $a = 20.45, \sigma = 0.18, \mu = 0.42, \sigma^2 = 0.81^*$          |
| MBC              | 36.34 ± 0.93                                    | 80                  | $a = 8.72, \sigma = 0.24, \mu = 18.58, \sigma^2 = 0.92^{**}$       |

* $P < 0.05; \** P < 0.01; \**

Fig. 2 Correlations of biochar-induced response ratios of SOC with those of soil MBC content (a) and CO₂ fluxes (b).

Land-use change and experimental method

When averaged across all studies, biochar amendment did not significantly increase soil CO₂ fluxes (mean: 5%; CI: −3% to 12%). Specifically, the response of soil CO₂ fluxes to biochar amendment was not pronounced in upland cropping systems, grasslands, and forests, while biochar amendment significantly increased soil CO₂ fluxes in rice paddies by 18% (CI: 10% to 22%) (Fig. 3a). Biochar amendment significantly increased SOC content by 40% across all ecosystem types (CI: 32% to 51%). The largest increases in SOC content in biochar-amended treatments were found in rice paddies (mean: 68%; CI: 75% to 128%).

Responses of soil C to biochar amendment in terms of soil CO₂ fluxes, SOC, and MBC depended on experimental method (Table 2, Fig. 3b). Biochar amendment significantly decreased soil CO₂ fluxes in pot experiments by 18% (CI: −46% to −3%), while positive effects on soil CO₂ fluxes were observed in laboratory incubations (mean: 28%; CI: 10% to 26%) and field studies (mean: 8%; CI: 1% to 10%). Among the laboratory incubation, greenhouse pot, and field studies, pot studies showed the greatest increases in SOC response to biochar amendment. Field studies showed significant positive responses of MBC to biochar amendment, while soil MBC content was decreased by biochar amendment in incubation and pot studies (Fig. 3b).

Soil texture and pH

Soil texture and pH had a significant effect on the response to biochar amendment for soil CO₂ fluxes and MBC (Table 2, Fig. 4). Among soils with coarse, medium, and fine texture, significant positive effects of biochar amendment on soil CO₂ fluxes occurred in soils with coarse texture (mean: 24%; CI: 18% to 30%), while positive effects on soil CO₂ fluxes were observed in laboratory incubations (mean: 28%; CI: 10% to 26%) and field studies (mean: 8%; CI: 1% to 10%). Among the laboratory incubation, greenhouse pot, and field studies, pot studies showed the greatest increases in SOC response to biochar amendment. Field studies showed significant positive responses of MBC to biochar amendment, while soil MBC content was decreased by biochar amendment in incubation and pot studies (Fig. 3b).

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Table 2 Between-group variability ($Q_b$) among observations ($k$) suggesting their potential as predictor variables influencing soil CO$_2$ fluxes, SOC, and MBC responses to biochar amendment

| Categorical variables          | Soil CO$_2$ fluxes | SOC   | MBC   |
|-------------------------------|--------------------|-------|-------|
|                               | $k$ | $Q_b$ | $k$  | $Q_b$ | $k$  | $Q_b$ |
| All studies                   | 167 | –     | 148  | –     | 80   | –     |
| Land-use type                 | 167 | 0.32* | 148  | 0.75**| 80   | –     |
| Experimental method           | 167 | 0.14* | 148  | 0.56* | 80   | 1.98**|
| Soil texture                  | 167 | 1.67**| 146  | 0.45  | 78   | 2.18**|
| Soil pH                       | 167 | 1.51**| 146  | 0.73  | 78   | 2.03**|
| Fertilizer source (kg N ha$^{-1}$) | 167 | 0.28* | 145  | 0.12  | 80   | 3.49***|
| Role of vegetation presence   | 167 | 1.20**| 148  | 0.21  | 80   | 2.41**|
| Biochar applied rate (t ha$^{-1}$) | 167 | 1.05* | 146  | 1.17* | 78   | 1.83**|
| Feedstock source              | 167 | 1.31***| 148  | 0.56* | 80   | 1.26**|
| Pyrolysis temperature (°C)    | 167 | 0.78* | 148  | 0.69* | 80   | 2.69**|
| Biochar pH                     | 167 | 0.25* | 146  | 0.62* | 74   | 3.12**|
| Biochar C/N ratio              | 165 | 1.02**| 148  | 1.15**| 78   | 1.32* |

A larger $Q_b$ is a better predictor of variation than a variable with a small $Q_b$.

Statistical significance of $Q_b$: *P < 0.05; **P < 0.01; ***P < 0.001.

acid soils (mean: 30%; CI: 16% to 69%) and no significant effect in acid and alkaline soils (Fig. 4b). The smallest and greatest effects of biochar on SOC were found in acid soils (mean: 25%; CI: 18% to 23%) and alkaline soils (mean: 53%; CI: 32% to 96%), respectively. Biochar amendment had no effect on MBC in neutral or alkaline soils, while it significantly increased soil MBC content by 49% in acid soils (CI: 42% to 85%). The existence of publication bias was suggested only by Rosenthal’s method on SOC among the three investigated variables, but no bias was indicated by Orwin’s method.

Combined effect of biochar with N fertilizer

The combined effect of biochar amendment with N fertilizer was significant for soil CO$_2$ fluxes and MBC, while it was not pronounced for SOC (Table 2, Fig. 5). Biochar amendment to unfertilized soils significantly increased soil CO$_2$ fluxes by 21% (CI: 15% to 43%), in contrast to a minor negative effect of biochar amendment on soil CO$_2$ fluxes in N-fertilized soils (mean: −5%; CI: −11% to −1%). Among different N fertilizer types, the combined use of biochar with synthetic N fertilizer led to a 45% increase in soil CO$_2$ fluxes (CI: 34% to 68%), while the response of soil CO$_2$ fluxes to biochar amendment was not pronounced when organic N fertilizer or other waste organic N sources were used. In contrast to soil CO$_2$ fluxes, the response of MBC to biochar amendment was not pronounced in N-unfertilized soils, while biochar amendment significantly increased MBC by 42% (CI: 26% to 58%) when N fertilizer was applied. The greatest positive combined effects on MBC were found when biochar amendment was coupled with waste compost N fertilizer (Fig. 5).

Vegetation present effect

Whether or not vegetation was present, biochar amendment significantly increased SOC content. However, vegetation presence altered the effect of biochar amendment on soil CO$_2$ fluxes and MBC (Fig. 6a). Response of soil CO$_2$ fluxes to biochar amendment shifted from a 10% decrease (CI: −15% to −5%) in soils with vegetation to an 18% (CI: 10% to 28%) increase in the soils without vegetation present. In contrast, MBC response to biochar amendment differed from a 10% decrease (CI: −21% to −6%) in soils without vegetation to a 45% increase (CI: 24% to 76%) in soils with vegetation present. Publication bias for this analysis was not suggested by either of Rosenthal’s or Orwin’s methods.

Biochar applied rate

Effects of biochar amendment on soil CO$_2$ fluxes, SOC, and MBC varied with its application rate (Table 2, Fig. 6b). Response of soil CO$_2$ fluxes to biochar amendment decreased with biochar application rate, with the highest positive response (mean: 25%; CI: 8% to 22%) when biochar was amended at the rate of <20 t ha$^{-1}$ and the lowest negative response (mean: −12%; CI: −9% to −3%) at the rate of 40–60 t ha$^{-1}$ (Fig. 6b). In contrast, the significant positive response of SOC increased with biochar application rate, ranging from 23% (CI: 17% to 30%) to 59% (CI: 37% to 80%). Soil MBC content was significantly increased by 50% (CI: 34% to 65%) when biochar was applied at lower rates (<20 t ha$^{-1}$), while biochar amended at relatively higher rate (20–40 t ha$^{-1}$) led to an 8% decrease in MBC content (CI: −16% to −1%). The existence of publication
bias was suggested only by Orwin’s method for soil CO₂ emissions, while not for SOC and MBC analyses.

**Biochar leading characteristics**

Most studies included in this meta-analysis have been undertaken using wood (149 datasets, 38% of total) and crop residue (164 datasets, 42% of total) as biochar source materials, while only a small number of studies used manure (26 datasets) and biowaste (24 datasets) as biochar feedstock sources. Responses of soil CO₂ fluxes, SOC, and MBC to biochar amendment depended on biochar characteristics such as feedstock source, pyrolysis temperature, pH, and C/N ratio (Table 2, Fig. 7). The response of soil CO₂ fluxes to biochar amendment depended on feedstock sources (Fig. 7a), with the highest positive (mean: 46%; CI: 23% to 69%) and smallest negative responses (mean: −12%; CI: −20% to −3%) for manure and biowaste sources, respectively. Among all biochar feedstock sources, wood source had the largest enhancement of SOC content (mean: 48%; CI: 32% to 51%). MBC was significantly increased by biochar with a crop residue source (mean: 48%; CI: 32% to 51%), while, in contrast, biochar with a wood source led to a decrease in MBC content (mean: −8%; CI: −13% to −3%).
Responses of soil CO₂ fluxes and MBC to biochar amendment decreased with pyrolysis temperature (Fig. 7b), shifting from a significant positive effect at pyrolysis temperature <400 °C (soil CO₂ fluxes, mean: 24%, CI: 21% to 36%; MBC, mean: 64%, CI: 43% to 84%) to a negative effect at >600 °C (soil CO₂ fluxes, mean: 15%, CI: 4% to 25%; MBC, mean: 11%, CI: 12% to 2%). In contrast, the extent of SOC enhancement due to biochar amendment increased with pyrolysis temperature, achieving the highest response (mean: 56%, CI: 16% to 95%) at pyrolysis temperature >600 °C.

Responses of soil C to biochar amendment also varied with pH and C/N ratio of biochar for soil CO₂ fluxes, SOC, and MBC (Table 2, Fig. 7c, d). In general, responses of soil CO₂ fluxes decreased with pH of biochar, with the largest positive effect occurred for acid biochar (Fig. 7c; mean: 18%, CI: 5% to 23%). In contrast, slight alkaline biochar (pH 8.1–9.0) had the largest increase in effect on both SOC (mean: 75%; CI: 46% to 104%) and MBC (mean: 68%; CI: 50% to 88%). Response of soil CO₂ fluxes to biochar amendment decreased with C/N ratio of biochar (Fig. 7d). The largest positive effect was observed for biochar with the C/N ratios <50 (mean: 68%; CI: 50% to 88%), while a significant negative effect occurred with the C/N ratios falling within the range of 201–300 (mean: −97%; CI: −148% to −65%). The extent of SOC enhancement due to biochar amendment tended to increase with C/N ratio of biochar, with the highest positive response to biochar with C/N ratio of 201–300, although limited data were available in this meta-analysis. Among biochars with different C/N ratios, only the biochar falling within relatively lower C/N ratios (50–100) showed a significant positive effect on MBC (mean: 62%; CI: 43% to 80%). Among the above grouping categories, publication bias was suggested only for biochar C/N ratio by Rosenthal’s method.
Robustness of meta-analysis

Robustness of our meta-analysis was confirmed by repeating the same meta-analysis procedure after removing the outlier datasets or data without original variances, and then comparing the results with those of the original meta-analysis. In this meta-analysis, removal of outlier datasets did not change the general results. After removing the outliers, the mean effect sizes of biochar treatments was 5% (CI: −2% to 12%) for CO2, 40% (CI: 30% to 58%) for SOC, and 19% (CI: 12% to 24%) for MBC, comparable to 5% (CI: −2% to 12%), 40% (CI: 30% to 58%), and 19% (CI: 12% to 24%) for CO2, SOC, and MBC when all datasets included, respectively.

Removing the datasets that did not report any measure of variance did not change the general results. However, the analyses of factors were impacted as the number of datasets was considerably reduced from 167 to 112 for CO2 and some category analyses failed to perform for lack of or too few datasets included in these categories. After removing the datasets without any variance available, the mean effect size of biochar for CO2 was 6% (CI: −4% to 13%), while that including all datasets was 5% (CI: −3% to 12%). Only five datasets for SOC and three datasets for MBC failed to report any measure of variance, and the results were not altered after removing them in this meta-analysis.

Discussion

Biochar effects on soil CO2 fluxes and SOC varying with land use

Across the entire set of studies, biochar amendment had no significant effect on soil CO2 fluxes. A lack of a significant response of soil CO2 fluxes to biochar amendment in upland cropping systems, grasslands, and forests matches other reports in the literature (Kuzynkov et al., 2009; Spokas & Reicosky, 2009; Van Zwieten et al., 2010; Singh & Cowie, 2014). However, this meta-analysis showed a significant positive response of soil CO2 fluxes to biochar amendment in rice paddies (Fig. 3a). The positive response of soil CO2 fluxes to biochar amendment is typically attributed to the mineralization of the labile biochar C fractions or its priming.
effect on native soil C decomposition through biotic or abiotic means (Kolb et al., 2009; Smith et al., 2010; Zimmerman et al., 2011). Kimetu & Lehmann (2010) found that the biochar effect on soil CO2 fluxes depended on SOC status, suggesting that enhanced soil CO2 fluxes might also be associated with the relatively high SOC content in rice paddies. In addition, high inputs of synthetic N fertilizer are typically carried out in rice paddies, which could be responsible for the significant positive response of soil CO2 fluxes to biochar amendment. Indeed, this meta-analysis showed that biochar amendment coupled with synthetic N fertilizer significantly increased soil CO2 fluxes (Fig. 5). Nevertheless, the small short-term C release in biochar-treated soils should not overshadow its potential for long-term C sequestration (Jones et al., 2011).

A consistent significant positive effect on SOC was observed following biochar amendment across land-use types. Biochar amendment had the largest positive effect on SOC in rice paddies as compared with upland cropping systems or natural grasslands, which might be associated with the relatively lower rate of soil organic C decomposition due to waterlogging in rice paddies (Biederman & Harpole, 2013; Xie et al., 2013). The response of SOC to biochar amendment was lower in upland cropping systems than in natural grasslands. It is likely that agricultural practice (e.g., soil tillage or harvest) involved in upland cropping systems accelerates the SOC turnover rate (Wang et al., 2005; Liu et al., 2006).

**Biochar effect on soil CO2, SOC, and MBC altered by experimental methods**

The effect of biochar amendment on soil CO2 fluxes differed with experimental methods, and a significant positive response occurred in laboratory incubation or field experiments, contrary to a significant negative response in greenhouse pot studies (Fig. 3b). The negative response of soil CO2 fluxes to biochar amendment in pot studies is most likely due to the relatively poor SOC status for the datasets enclosed in this category analysis as compared to other experimental methods. On the other hand, biochar amendment exerted a consistent and significant positive effect on SOC across the experimental methods, with the greatest positive response of SOC to biochar amendment in pot experiments. Indeed, significant higher relative rates of biochar were used in pot experiments than in laboratory or field studies based on the present database (Fisher’s exact test, two-way: \( P = 0.01 \)), which could directly lead to the significant positive response of SOC to biochar amendment (Fig. 6b). In controlled experiments, furthermore, biochar amendment to limited amounts of sampled soils tended to have a higher potential to enhance SOC content as compared to field studies. However, a quite opposite result was obtained in terms of biochar effect on MBC across the study methods, where biochar amendment had a significant positive effect on MBC in field studies, while negative effect was observed in controlled studies. It is most likely that field-based relative to controlled experimental conditions can improve the availability of microbial habitats and easy access of microbial food resources especially following biochar amendment and thus increasing microbe population size in soils (Zackrisson et al., 1996; Pietikäinen et al., 2000).

**Biochar effect on soil CO2, SOC, and MBC depending on soil properties**

Biochar amendment in coarse soils exerted a significant positive effect on soil CO2 fluxes, while significant negative effects were observed in fine-textured soils (Fig. 4a). Presumably, the easier adequate mixture and larger contact area of biochar with soil particles in coarse soils may greatly improve soil aeration and thus accelerating the soil organic C decomposition rate with sufficient oxygen supply (Wardle et al., 2008; Rogovska et al., 2011; Stewart et al., 2013). Similarly, MBC were significantly increased following biochar amendment in coarse soils (Fig. 4a). This is supported by the evidence that biochar amendment to sandy-textured soils greatly increased crop or biomass productivity and thus enhancing soil C accumulation due to its promotion of soil aggregation and retention of both soil nutrients and moisture (Oguntunde et al., 2004; Gaskin et al., 2010; Van Zwieten et al., 2010; Jeffery et al., 2011). Besides, biochar amendment to sandy soils relative to loam or silt soils would more benefit for improving soil fertility due to its original low soil fertility as well as easy loss of soil nutrients through leaching and runoff and thereby promote soil microbial activities (e.g., Lehmann, 2007b; Woolf et al., 2010).

Biochar was effective at decreasing soil CO2 fluxes but did not benefit SOC enhancement in neutral or alkaline soils, and significant positive responses of soil CO2 fluxes were observed in moderately acid soils, contrary to the negative responses in neutral soils (Fig. 4b). The increased soil CO2 fluxes and less enhancement of SOC content in biochar-amended acid soils may be explained by several reasons: (i) biochar addition to acid soils greatly enhanced its consumption through microorganisms for balanced soil pH conditions (Bruun et al., 2008); (ii) biochar in acid soils may trigger a more vigorous priming effect on native SOC mineralization (Cross & Sohi, 2011; Foe Reid et al., 2011; Jones et al., 2011); and (iii) greater crop or biomass productivity was observed in acid soils following biochar amendment and thus indicating more organic C substrate for biochar-induced SOC mineralization (Liu et al., 2013). Instead, biochar amendment to neutral or alkaline soils would incur an inhibition of soil C mineralization with enhanced soil pH. Soil MBC content was significantly increased by
biochar amendment in acid soils relative to in neutral or alkaline soil conditions. It is possible that biochar, in most cases as an alkaline buffer when applied into acid soils, can maximize the microbial activities and in turn increase microbe populations (Steiner et al., 2008).

**Combined effects of biochar with fertilizer on soil CO₂, SOC, and MBC**

Biochar amendment significantly increased soil CO₂ fluxes when synthetic N fertilizer was applied. It might be attributed to the fact that balanced soil C/N ratio resulting from the combination of biochar with synthetic N fertilizer would incur a motivation of soil microbial C mineralization due to more soil available C and N sources (Zou et al., 2004). Besides, synthetic N fertilizer relative to other N fertilizer sources in biochar-treated soils may more readily facilitate a priming effect on soil C mineralization through direct and rapid nutrients release.

Responses of SOC to biochar amendment did not significantly differ in soils with or without N fertilizer application (Fig. 5). Among the different N fertilizer sources, biochar co-applied with organic N fertilizer had the largest increment potential for SOC, which was mainly due to the fact that organic N fertilizer input greatly enriched the soil organic C pools (Halvorson et al., 1999; Lu et al., 2009). In contrast, biochar addition to N-fertilized soils resulted in a significant positive effect on MBC, mainly contributed by biochar combined with waste compost N fertilizer or synthetic N fertilizer. The biochar-induced increase in MBC in synthetic N-fertilized soils may be explained by the greatly improved direct soil nutrients supply for microbial activities due to synthetic N fertilizer input, in addition to biochar benefits itself (Yamato et al., 2006; Steiner et al., 2007; Asai et al., 2009). In addition, the waste compost N fertilizer source (with a low C/N ratio of 12) may offer the best needs for microbe populations and their associated activities through increased N mineralization and its variety of microbial nutrient sources (Ding et al., 2013).

**Biochar effect on soil CO₂, SOC, and MBC differing with biochar applied rate**

Responses of soil CO₂ fluxes decreased with biochar applied rates, corresponding to SOC content increasing with biochar applied rates (Fig. 6b). As proposed by Lehmann & Rondon (2006), biochar amended at higher rates may not only increase the C credit benefit, but simultaneously suppress soil C mineralization as a high C/N ratio leading to low microbial N availability. Furthermore, the decrease in soil CO₂ fluxes at higher applied rates of biochar may be associated with specific biochar characteristics and their impacts on soil properties. It is obvious that biochar amendment at higher rates was more effective at enhancing SOC content, as more stable biochar C input to the soils. In contrast, biochar amendment at relatively higher rates had a negative effect on soil MBC, which is given by the evidence that larger applied amounts of biochar with high C/N ratio tended to induce an immobilization of soil microbial N and thus led to low microbe-associated activities (Case et al., 2012; Ameloot et al., 2013). In addition, the biochar amendment method was not clearly reported in most of the literatures; therefore, we were unable to fully address the difference in effect size among the current grouping categories. The same challenge was also encountered in some other quantitative review study of biochar impacts on environmental concerns or agricultural productivity (e.g., Jeffery et al., 2011; Cayuela et al., 2013).
2014). As pointed out in previous studies (e.g., Spokas & Reicosky, 2009), nevertheless, the response of soil CO₂ fluxes and topsoil C fractions to biochar amendment depended more on its own characteristics, soil properties, and land-use types, rather than its application rates.

**Biochar effect on soil CO₂, SOC, and MBC response dependent on its characteristics**

Responses of soil CO₂ fluxes to biochar amendment depended on biochar feedstock materials, with the highest positive response for manure-derived biochar and the significant negative response for biowaste sources (Fig. 7a). Biochar generated from manure with relatively lower C/N ratios (grand mean of C/N ratio: 13) enhanced the soil C mineralization rate for their quick decomposition due to facilitated microbial N availability in soils (Huang et al., 2004; Zou et al., 2004). In contrast, the negative response of soil CO₂ fluxes to biochar derived from biowaste (e.g., municipal solid waste, sewage sludge) might be attributed to the adverse effect of heavy metals or other unknown contaminants in bio-waste sources on soil C mineralization activities (Hospido et al., 2005; Chan & Xu, 2009). For all feedstock sources, biochar amendment consistently enhanced SOC content, which was primarily ascribed to the combined effect of biochar C input and the improved productivity or higher biomass gain and consequently larger amounts of plant residue retained in soils due to biochar amendment (Jeffery et al., 2011; Liu et al., 2013). In contrast with negative response of soil MBC to wood-derived biochar amendment, crop residue source had a significant positive effect on soil MBC. As proposed by individual studies (e.g., Graham, 1981; Rondon et al., 2007; Lehmann et al., 2011), biochar materials with lower C/N ratios can greatly improve soil microbial activities for facilitated C and N availability and in turn increased the microbe population size in soils.

Pyrolysis temperature plays an important role in the response of soil CO₂ fluxes and SOC to biochar amendment. In this meta-analysis, the responses of soil CO₂ fluxes and SOC to biochar amendment decreased and increased with pyrolysis temperature, respectively (Fig. 7b). In general, biochar created at high temperatures is more resistant to decomposition and thereby would serve as better candidates for soil C sequestration (Novak et al., 2010; Harvey et al., 2012). As suggested by Day et al. (2005), low pyrolysis temperature of biochar would be more suitable for improving soil nutrition balance and in turn promote soil microbial C mineralization activities, while high-temperature biochar would generally lead to a material analogous to activated C (Ogawa et al., 2006). Some studies showed that biochar generated at high temperature tended to be alkaline and thus had a greater positive effect on aboveground productivity, leading to a higher rate of plant residue retain (Bagreev et al., 2001; Novak et al., 2009). In addition, biochar generated at high temperature contains less bioactive volatile compounds (Gundale & DeLuca, 2006; Hale et al., 2012) that can otherwise limit plant growth. A significant positive response of MBC was mainly associated with the relatively higher level of microbial-available and active nutrients introduced into soil with biochar pyrolyzed at low temperatures (Chan et al., 2008). Therefore, the pyrolysis temperature of biochar during generation processes should be given more attention in terms of its potential effect on soil CO₂ fluxes and soil C storage.

Responses of soil C to biochar amendment varied with pH of biochar, with the significant positive response of soil CO₂ fluxes to biochar with pH <7, and the highest positive responses of SOC and MBC to biochar with pH of 8.1–9.0 (Fig. 7c). A recent study showed that biochar with pH <7 tended to trigger a higher priming effect on soil C mineralization in addition to its relatively higher labile C fractions input (Crombie et al., 2014), although conflicting results have been obtained in previous reports (Bell & Worrall, 2011; Jones et al., 2011; Zimmerman et al., 2011). As previously mentioned, the influence of biochar pH on soil processes depended greatly on its actual buffer capacity under a certain soil condition (Yuan et al., 2011). The biochar with moderate alkalinity would benefit lower SOC mineralization but higher overall microbial activities depending on soil properties, while biochar with extreme high or low pH values would incur macronutrient deficiencies and finally lead to low soil microbial activities, in line with the results of this meta-analysis (Chan & Xu, 2009).

Biochar C/N ratio was another important parameter influencing soil CO₂ fluxes, SOC, and MBC (Fig. 7d). This meta-analysis showed a shift from a significant positive effect on soil CO₂ fluxes for biochar with the lowest C/N ratios (<50) to a pronounced negative response for those with the highest ones (201–300). Biochar C/N ratio effects on soil CO₂ fluxes were correlated negatively with its effects on SOC, where opposite patterns were observed (Fig. 7d). Besides influencing C substrate input, biochars with high C/N ratios often result in microbial N immobilization in soils (Baggs et al., 2000; Cayuela et al., 2010). Thus, less soil N is available for microbial processes such as soil C mineralization, although greatly decreased bioavailability of C in biochar may limit the induction of N immobilization (Major et al., 2010; Singh & Cowie, 2014). Similarly, decreased soil microbial activities as a consequence of N deficiency with biochar with high C/N ratios would lead to a decline in microbe population size. On the other hand, biochars with low C/N ratios were
generally produced under lower pyrolysis temperatures and thus accompanied by higher labile C fractions that contribute to soil CO2 fluxes following incorporation into soils (Crombie et al., 2014).

Limitations and future challenges

Variance of variables was generally heterogeneous among the grouping categories, which was expected for the meta-analysis enclosing data from a range of soil types, land-use changes, and climatic regions. In some instances, however, the high level of variance may be ascribed to the limited number of studies included within some certain categories. Moreover, we did not take into consideration the data on environmental and management conditions or the auxiliary data on other soil properties (e.g., soil inorganic C and N) due to lack of relevant information in studies included. However, these factors may have interactive effects with biochar on soil C dynamics. Indeed, further work is expected to investigate whether the large variability reflects high underlying variability in outcomes associated within certain categories or the limited number of datasets currently available in the given category.

The limited range of study durations did not allow us to examine the effect of biochar aging on soil CO2 fluxes, SOC, and MBC in this meta-analysis. No studies ran more than 4 years, and only 21% of the observations included in this analysis showed results over a whole growing season with the presence of vegetation cover. To evaluate the effect of biochar on soil CO2 fluxes and its effectiveness at enhancing soil C sequestration potential, therefore, field experiments with longer durations across a wider range of spatial and temporal scales are required.

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