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Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis

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Abstract: Biochar application to soils may increase carbon (C) sequestration due to the inputs of recalcitrant organic C. However, the effects of biochar application on the soil greenhouse gases (GHGs) fluxes appear variable among many case studies; therefore the efficacy of biochar as a carbon sequestration agent for climate change mitigation remains uncertain. We performed a meta-analysis of 91 published papers with 552 paired comparisons to obtain a central tendency of three main GHG fluxes (i.e., CO_2, CH_4, and N_2O) in response to biochar application. Our results showed that biochar application significantly increased soil CO_2 fluxes by 22.14%, but decreased N_2O fluxes by 30.92% and did not affect CH_4 fluxes. As a consequence, biochar application may significantly contribute to increased global warming potential (GWP) of total soil GHG fluxes due to the large stimulation of CO_2 fluxes. However, soil CO_2 fluxes were suppressed when biochar was added to fertilized soils, indicating that

biochar application is unlikely to stimulate CO_2 fluxes in the agriculture sector, in which N fertilizer inputs are common. Responses of soil GHG fluxes mainly varied with biochar feedstock source and soil texture, and the pyrolysis temperature of biochar. Soil and biochar pH, biochar applied rate and latitude also influence soil GHG fluxes, but to a more limited extent. Our findings provide a scientific basis for developing more rational strategies towards widespread adoption of biochar as a soil amendment for climate change mitigation.

Introduction

The global average surface temperature has increased by 0.85 ^{o}C over the period 1880 to 2012 based on multiple independently produced datasets, and current projections suggest that the temperature is likely to increase by another 0.3-4.8 ^{o}C by the end of this century (IPCC, 2013). Global warming is mostly attributable to the increasing atmospheric concentrations of greenhouse gases (GHGs) due to human activities. The three main GHGs (i.e., CO_2, CH_4, and N_2O) in combination contribute to more than 90% of anthropogenic climate warming (Hansen *et al.*, 2000; IPCC, 2013).

GHG mitigation strategies include reducing and avoiding emissions as well as enhancing removal of GHGs from the atmosphere (Smith *et al.*, 2008). Soil carbon (C) sequestration through biochar amendment has been proposed as an effective countermeasure for the rising concentration of atmospheric GHGs (Lal, 1999; Pan *et al.*, 2004; Smith *et al.*, 2008). Biochar is a carbon-rich, charcoal-like product produced by burning biomass in the absence of oxygen (Laird *et al.*, 2009; Lehmann, 2007b); it contains a high proportion of recalcitrant organic C and is stable for hundreds to thousands of years after it is applied to soil (Schmidt *et al.*, 2002).

Biochar application to soils has the potential to mitigate global warming via soil C sequestration, and provide other benefits, such as improving soil fertility, retaining soil moisture, and increasing crop yields (Darby *et al.*, 2016; Bai *et al.*, 2015a,b; Hosseini Bai *et al.*, 2014; Reverchon *et al*. 2014; Laird, 2008; Lehmann, 2007a; Marris, 2006; Mukherjee *et al.*, 2014; Woolf *et al.*, 2010; Xu *et al.*, 2015a,b). However, the precise effects of biochar application on soil GHG emissions remain controversial and appear very variable among many case studies (Cayuela *et al.*, 2014; Lorenz & Lal, 2014). Soil CO_2, CH_4 and N_2O fluxes increased significantly in some studies (Jones *et al.*, 2011; van Zwieten *et al.*, 2010; Wang *et al.*, 2012; Yanai *et al.*, 2007), but substantially decreased or remained unchanged in others (Case *et al.*, 2014; Feng *et al.*, 2012; Quin *et al.*, 2015; Rogovska *et al.*, 2011; Zheng *et al.*, 2012). For example, a field trial in paddy soils amended with biochar produced from wheat straw induced a 12% increase in CO_2 emissions but a 41.8% decrease in N_2O emissions (Zhang *et al.*, 2012b). Another field experiment in pasture showed no significant effects of biochar amendment on soil CO_2 and N_2O emissions in a pasture ecosystem (Scheer *et al.*, 2011). Thus, the efficacy of biochar for climate change mitigation is largely uncertain due to these variable effects on soil GHGs emissions.

There are many hypotheses to explain why biochar may increase or decrease soil GHG fluxes. For example, increases in soil CO_2 emissions induced by biochar might be due to the labile C input and positive priming effects of biochar as well as increased belowground net primary productivity (BNPP) (Zhang *et al.*, 2012a; Zimmerman *et al.*, 2011), while the suppression of soil CO_2 emissions may be due to reduced enzymatic activity and the precipitation of CO_2 onto the biochar surface (Case *et al.*, 2014). Elevated CH_4 emissions could be attributed to the inhibitory effect of chemicals in the biochar on soil methanotrophs (Spokas, 2010). Reduced CH_4 emissions might be associated with decreased ratios of methanogenic archaea to methanotrophic proteobacteria, as the increase in oxygen supply due to biochar application supports a group of aerobic methanotrophs (Feng *et al.*, 2012).

There are also contradictory reports with respect to N_2O emissions. For example, increases in N_2O emissions may be ascribed to biochar-induced increases in soil water content, which favors denitrification, or the release of biochar embodied-N (Lorenz & Lal, 2014). In contrast, mechanisms that explain decreased N_2O emissions include: (1) improved soil aeration, (2) increased soil pH, (3) enhanced N immobilization, and (4) a toxic effect induced by biochar organic compounds (polycyclic aromatic hydrocarbons) on nitrifier and denitrifier communities (Clough *et al.*, 2013; Clough *et al.*, 2010; Hale *et al.*, 2012; Taghizadeh-Toosi *et al.*, 2011).

The contradictory reports of changes in size and even direction of soil GHG emissions when biochar is applied, and the diversity of mechanisms proposed suggest that biochar effects may depend on many factors, including soil properties, experimental methods, artificial cultivation management, biochar application rate, and biochar physic-chemical properties (Hilscher & Knicker, 2011; Lorenz & Lal, 2014). These factors may determine to what extent biochar alters soil C and N transformation processes and consequently soil GHG emissions. However, how these factors contribute to the variable responses of soil GHG emissions to biochar application across the globe still remains unclear. If these factors are not adequately addressed, the effects of biochar application on mitigating global warming cannot be fully understood.

Recently, three meta-analyses on the effects of biochar application on soil GHG fluxes have been conducted. Two of them (i.e., Cayuela *et al.*, 2015; Cayuela *et al.*, 2014) only emphasized the central tendency of soil N_2O fluxes under biochar addition, and the other by Liu *et al.* (2016) examined the response of CO_2 fluxes, soil organic C (SOC), and soil microbial biomass C (MBC) to biochar amendment. However, there is limited information on the simultaneous effects of biochar amendments on soil GHG fluxes and their global warming potential (GWP). It is necessary to compile all available data to synthesize results from individual studies to reveal the patterns of biochar-induced changes in soil GHG fluxes and to identify the major drivers for responses of GHG fluxes to biochar addition.

In this study, we compiled data from individual experimental studies that quantified the effect of soil biochar application on GHGs fluxes across various ecosystems and then quantitatively evaluated the responses of soil CO_2, CH_4 and N_2O fluxes to biochar application under different environmental and experimental conditions using meta-analysis techniques. Our objectives were to (1) quantify the effect size of biochar amendment on soil GHG fluxes across studies; (2) examine whether environmental conditions, experimental methods, and biochar characteristics would influence the responses of soil GHG fluxes to biochar application; and (3) evaluate the response of GWP of soil GHGs to biochar application.

Materials and Methods

Data sources

Publications were searched using Web of Science (1900-2015) with the following search terms: (biochar or black carbon or charcoal) and (soil greenhouse gases (GHGs) or CO_2 or CH_4 or N_2O or global warming potential (GWP)). The selection criteria were as follows: (i) experiments had at least one pair of data (control and treatment) and measured soil CO_2, CH_4, or N_2O fluxes; (ii) the method of biochar application was clearly described, including experimental duration, amount of biochar application, physico-chemical characteristics of biochar, and soil properties such as pH and C/N ratio; (iii) the means, standard deviations/errors, and sample sizes of variables in the control and treatment groups could be extracted directly from tables, graphs or contexts. In total, 91 research papers on biochar application were selected from more than 2000 published papers. The geographic distribution of the selected studies over the world is presented in Fig. 1. The studies contained multiple biochar application levels (Case *et al.*, 2012; Stewart *et al.*, 2013), biochar types (Ameloot *et al.*, 2013; Spokas & Reicosky, 2009), soil types (Gomez *et al.*, 2014; Wang *et al.*, 2011) or N fertilization levels (Barbosa de Sousa *et al.*, 2014; Sun *et al.*, 2014) were treated as multiple independent studies.

Four categories of data were extracted from the literature of biochar application experiments: (1) soil GHG fluxes, including CO_2, CH_4 and N_2O fluxes; (2) soil properties, including pH, total C, total N, and C/N ratio; (3) biochar properties, including biochar feedstock types, pyrolysis temperature, rate of biochar applied, pH, total C, total N, and C/N ratio; and (4) other auxiliary variables, including latitude, longitude, experiment types (field, pot and incubation), experimental duration, and N fertilization (whether or not). The variables listed in category (2), (3) and (4) were used as explanatory factors (either categorical or continuous) of the variation in GHG fluxes in response to biochar application.

Analysis

We followed the methods used by Hedges *et al.* (1999) and Luo *et al.* (2006) to evaluate the responses of soil CO_2, CH_4 and N_2O fluxes to biochar application. A response ratio (*RR*, natural log of the ratio of the mean value of a variable in biochar treatment plots to that in control) was used to calculate effect sizes as below:

$$RR = \ln\frac{X_t}{X_c} = \ln(X_t) - \ln(X_c) \quad (1)$$

where X_t and X_c are means in the treatment and control groups, respectively. The variance (v) of each individual *RR* is estimated as:

$$v = \frac{S_t^2}{n_t X_t^2} + \frac{S_c^2}{n_c X_c^2} \quad (2)$$

where n_t and n_c are the sample sizes of the variable in treatment and control groups, respectively; S_t and S_c are the standard deviations for the treatment and control groups.

The mean response ratio (RR_{++}) was calculated from *RR* of individual pairwise comparisons between treatment and control as below,

$$RR_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} W_{ij} RR_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} W_{ij}} \quad (3)$$

where m is the number of groups, and k is the number of comparisons in the i^{th} group. The reciprocal of its variance () was considered as the weight (*W*) of each *RR*.

We used a bootstrapping method to obtain the 2.5% and 97.5% percentiles as the lower and upper limits of our 95% bootstrap confidence interval (CI) based on 5000 iterations (Adams *et al.*, 1997; Zhou *et al.*, 2014). When the 95% CI of RR_{++} for soil GHG emissions overlapped with zero, biochar application had no significant impact on the variable. Otherwise, the biochar-induced response was considered as significance (Luo *et al.*, 2006). The percentage change of variables was calculated on the basis of [exp(RR_{++})-1]100%. The frequency distribution of the individual response ratio (*RR*) was tested by a Normal-test and fitted by a Gaussian function using Equation (5) in SigmaPlot software (Systat Software Inc., CA, USA).

$$y = \alpha \exp[-\frac{(x-\mu)^2}{2\sigma^2}] \quad (4)$$

where *x* is *RR* of a variable; *y* is the frequency (i.e., number of *RR* values); α is a coefficient showing the expected number of *RR* values at *x*=μ; and μ and α are the mean and variance of the frequency distributions of *RR*, respectively.

In addition, global warming potential (GWP) was calculated when three soil GHG (i.e., CO_2, CH_4, and N_2O) fluxes were extracted simultaneously from one study (IPCC, 2007). It should be noted that the units of soil CO_2, CH_4, and N_2O fluxes were unified before the calculation of the GWP. The GWP (t CO_2 equivalent ha^{-1}) was then determined as follows:

$$CO_2 \times 1 + CH_4 \times 25 + N_2O \times 298 \quad (5)$$

The between-group heterogeneity (Q_b) across all data for a given response variable was calculated to further analyze the biochar effect among different subgrouping categories. A random effect model was used to explore the soil and biochar properties and other auxiliary variables that may explain the response of soil GHG fluxes to biochar application. We also conducted meta-regression to examine the relationships between *RR* (GHGs) and continuous forcing factors. The correlations of *RR* (GHGs) among different variables were examined by correlation analysis applied in R (R Core Team, 2015).

The publication bias was tested by funnel plot method and assessed using Kendell's Tau (Moller & Jennions, 2001). If the mean effect had significant difference from zero (i.e., indicating the existence of publication bias), Rosenthal's fail-safe number was calculated (MetaWin 2.1) to estimate whether our conclusion is likely to be affected by the non-published studies (Rosenberg, 2005).

Results

Effects of biochar application on soil greenhouse gas (GHG) fluxes

The individual response ratios (*RRs*) of soil GHG fluxes (i.e., CO_2, CH_4, and N_2O) all displayed normal/Gaussian distributions (Fig. S1). On average, biochar application significantly increased soil CO_2 fluxes by 22.14% with a mean weighted RR_{++} of 0.20 (CI = [0.12, 0.31]), but decreased soil N_2O fluxes by 30.92% with a RR_{++} of 0.37 (CI = [-0.48, -0.28]). Soil CH_4 fluxes were not significantly affected by biochar application (RR_{++} = -0.03, CI = [-0.35, 0.23]) (Fig. 2, Table S2). Publication bias for this analysis was not suggested by Rosenthal's method (Table S3).

The response of soil CO_2 flux to biochar application depended significantly on biochar properties, experimental method, nitrogen (N) fertilization, and latitude. Soil texture and biochar pH were the two most critical parameters affecting the response of soil CH_4 flux to biochar addition. Biochar-induced changes in soil N_2O fluxes were significantly associated with soil and biochar properties, i.e., biochar feedstock type and applied rate, soil texture and pH (Table 1).

Combined effect of biochar with N fertilization on soil GHG fluxes

The combination of biochar with N fertilizer application significantly decreased soil CO_2 and increased CH_4 fluxes whereas it did not change soil N_2O fluxes (Fig. 2 and S2). In unfertilized soils, biochar application significantly increased soil CO_2 fluxes by 43.3% with a RR_{++} of 0.36 (CI = [0.24, 0.50]), but decreased soil CO_2 fluxes by 8.6% in N-fertilized soils (RR_{++} = 0.09, CI = [-0.17, -0.02]) (Table S2). Meanwhile, biochar application significantly increased soil CH_4 fluxes by 11.6% with a RR_{++} of 0.11 in N-fertilized soils (CI = [0.01, 0.25]), but had no significant effect in unfertilized soils (CI = [-0.62, 0.27]). Biochar application significantly reduced soil N_2O fluxes by 33.0% and 28.8% in both fertilized and unfertilized soils with RR_{++} of 0.4 (CI = [-0.53, -0.28]) and 0.34 (CI = [-0.51, -0.18]), respectively (Fig. 2).

Effects of biochar applying methods on GHG fluxes

Experimental methods (i.e., field studies, laboratory incubations, and pot experiments) had a significant effect on the response of soil CO_2 and CH_4 fluxes to biochar application, while it was not pronounced for N_2O fluxes (Table 1, Fig. S3). On average, biochar application significantly increased soil CO_2 fluxes by 30.34% in laboratory incubations, but had no changes under field studies and pot experiments. Biochar application significantly increased soil CH_4 fluxes by 25.4% in field studies, but did not change in laboratory incubations and pot experiments. In addition, experimental duration showed no significant effect on responses of soil GHG fluxes to biochar application (Fig. S4).

Interestingly, the effect of fertilization on GHG fluxes in biochar amended soil appears closely related to experiment methodology. Only laboratory incubations showed significant increase of CO_2 fluxes to biochar application in unfertilized soils compared to those in field and pot experiments, while there were no responses in fertilized soils. For CH_4 fluxes, only field studies showed significant positive responses to biochar application in fertilized soils, and other treatments did not exhibit any significant effects (Fig. 2).

Effects of soil and biochar properties on soil GHG emissions

The response of soil GHG fluxes to biochar application differed for biochar feedstock source (i.e., wood, herb, and biowaste, Table 1, Fig. 3a-c). Among all biochar feedstock sources, wood source had the smallest positive effect for CO_2 fluxes and negative effect for N_2O fluxes. Meanwhile, biowaste source induced the largest positive effect and negative effect for CO_2 and N_2O fluxes, respectively. The effects of biochar application on soil CH_4 fluxes were not significant among different feedstock sources.

The response of soil GHG fluxes to biochar application also varied with soil texture (Table 1, Fig. 3d-f). For CO_2 fluxes, positive effects of biochar application occurred in soils with coarse and medium texture, while no significant effects were found in fine texture. CH_4 fluxes showed a significant negative response to biochar amendment only in coarse soils. N_2O fluxes significantly decreased by biochar application in all soil types, but the smallest negative response occurred in medium soils.

Response ratios of soil GHG fluxes across all the studies were significantly correlated with biochar pyrolysis temperature (Tem), biochar pH (BpH), soil pH, and biochar application rate (App), and latitude (Lat) (Table 1, fig. 4). The response of soil CO_2 and CH_4 fluxes to biochar amendment slightly decreased with pyrolysis temperature and biochar pH (*P*<0.001), but increased with application rate and latitude of the study for soil CO_2 fluxes (*P*<0.001). In addition, the responses of soil N_2O fluxes to biochar application revealed negative trends with soil pH (*P*=0.001) and application rate (*P*<0.001). Although these correlations were statistically significant, their contributions in explaining the variation of GHGs fluxes responses were low ($0.04<R^2<0.11$, Fig. 4).

Effects of biochar application on global warming potential (GWP)

With those data measured simultaneously for soil CO_2, CH_4, and N_2O fluxes, biochar application positively affected GWP (RR_{++} = 0.44, CI = [0.22, 0.69]). Meanwhile, biochar application significantly increased GWP by a mean response ratio of 0.69 (CI = [0.39, 0.99]) in unfertilized soils compared to a minor negative effect in N-fertilized soils (RR_{++} = -0.08, CI = [-0.15, -0.03], Figs. 5a-c). Interestingly, laboratory incubations showed significant positive responses of GWP to biochar application, while field and pot experiments exhibited no effects (Fig. 5d). The different responses between laboratory incubations, field and pot experiments for all data were the same as those in unfertilized soils (Fig. $5d_1$), while, in fertilized soils, there were no significant effects of biochar application on GWP (Fig. $5d_2$). This pattern generally matched the effect of biochar on soil CO_2 fluxes.

Discussion

Responses of CO_2 CH_4, and N_2O fluxes to biochar application

On average, our meta-analysis showed that biochar application significantly increased soil CO_2 fluxes by 22.14%. Among individual studies, biochar application affected soil CO_2 fluxes with diverse magnitudes and even directions (Augustenborg *et al.*, 2012; Scheer *et al.*, 2011; Zhang *et al.*, 2012a). The stimulating effects of biochar application on soil CO_2 fluxes were usually ascribed to higher labile-C mineralization and/or inorganic C release from biochar (Fig. 6; e.g. Jones *et al.*, 2011; Smith *et al.*, 2010; Zimmerman *et al.*, 2011). Furthermore, as suggested by Liu *et al.* (2016), biochar application enhanced soil organic C (SOC) by 40% and soil microbial biomass C (MBC) content by 18%. This indicates that the stimulation of soil CO_2 fluxes might be associated with the higher SOC status and the more active soil microbial activities (Fig. 6).

Soil CO_2 fluxes declined with biochar pyrolysis temperature. Low pyrolysis temperature results in more microbial-available C and nutrients in biochar than a high pyrolysis temperature, which promotes high soil microbial activities to decompose soil organic matter (SOM) and release more CO_2 from soil (Chan *et al.*, 2008; Hale *et al.*, 2012; Novak *et al.*, 2010). This results in the negative relationship between *RR* (CO_2) and biochar pyrolysis temperature and a positive relationship between *RR* (CO_2) and application rate (Figs. 4a, d). Meanwhile, high-temperature biochars may contain higher relative concentrations of toxic compounds (i.e., polycyclic aromatic hydrocarbons) (Nakajima *et al.*, 2007), which can affect soil microbial biomass and activity. In addition, the *RR* (CO_2) exhibited a negative correlation with biochar pH probably because biochar with pH < 7 had a relatively high input of labile-C fractions and triggered a higher priming effect on soil C mineralization (Crombie *et al.*, 2015). Our results indicated that CO_2 fluxes did vary over time after biochar application. However, mechanisms involved in soil CO_2 stimulation after biochar application may differ in short term compared to long term. In short term, soil CO_2 stimulation may have been originated from the breakdown of organic C and the release of inorganic C contained in the biochar (Jones *et al.*, 2011). In the long-term, biochar can promote rapid loss of humus and belowground C (Wardle *et al.*, 2008). Meanwhile, increased belowground NPP induced by biochar amendment maybe also causing the stimulation of CO_2 emissions during the long-time experiments (Major *et al*., 2010).

In addition, biochar-induced changes in soil CO_2 fluxes significantly increased with latitude, which may be related to increase soil temperature after biochar application (Bozzi *et al.*, 2015). The increasing temperature may induce the larger stimulation on soil microbes and thereby CO_2 fluxes, in the high latitude soils, where microbial activities and soil respiration are strongly limited by temperature (Mikan *et al.*, 2002).

Biochar application had no significant effect on soil CH_4 fluxes in our meta-analysis, although individual studies showed diverse effects. In experimental studies, multiple factors (e.g., soil aeration and porosity, methanogens, and methanotrophs) have been proposed to explain the different effects of biochar application on soil CH_4 fluxes (Karhu *et al.*, 2011; Lehmann & Rondon, 2006), but the underlying mechanisms are still poorly understood (Lorenz & Lal, 2014). Soil CH_4 fluxes are largely determined by methanogens and methanotrophs at a microbial scale (Bodelier & Laanbroek, 2004). Therefore, decreased soil CH_4 fluxes under biochar application might be due to the higher ratios of methanogenic to methanotrophic bacteria observed in some studies (Fig. 6; Feng *et al.*, 2012), and others suggested that improved soil aeration and CH_4 oxidation after biochar application suppressed soil CH_4 fluxes (Fig. 6; Karhu *et al.*, 2011). In contrast, the increased soil CH_4 fluxes under biochar application could be attributed to biochar compounds that inhibit the activity of methanotrophs (Spokas, 2013).

Biochar application decreased CH_4 fluxes in coarse soils, whereas it increased CH_4 fluxes in fine soils. Biochar application to the coarse soils is likely to improve soil aeration, thus making the soils more favorable for the aerobic methanotrophs communities and increases CH_4 oxidation (van Zwieten *et al.*, 2009). However, in the fine textured soils, the porous structure of biochar may be filled with a clay and fine silt fraction, which could offset the aeration effect. A weak stimulation of CH_4 fluxes induced by biochar amendment may be due to enhancing soil methanogenic archaea (Feng *et al.*, 2012). In addition, the biochar-induced effects on soil CH_4 fluxes decreased with biochar pH, probably resulting from altered soil microbial community structure, especially the ratio of soil methanogenic to methanotrophic abundance (Anders *et al.*, 2013).

Our meta-analysis showed that biochar application decreased soil N_2O fluxes by 30.92%, consistent with another meta-analysis reported by Cayuela *et al.* (2014). This response was probably driven by changes in the activity of the nitrifiers and denitrifiers that produce N_2O. Biochar application enhances soil aeration (absorbing/holding an excess of soil moisture) and reduces N leaching as a result of NH_4^+ and NO_3^- adsorption by biochar (Fig. 6; Bai *et al.*, 2015a; Hosseini Bai *et al.*, 2014; Reverchon *et al.*, 2014; Rogovska *et al.*, 2011; Steiner *et al.*, 2008; Yanai *et al.*, 2007). The enhanced soil aeration and reduced compaction may inhibit denitrification due to more oxygen being present, and the diminished N leaching may decrease the inorganic N pool available for soil nitrifiers and denitrifiers (Fig. 6). Moreover, biochar amendment stimulates the *nosZ* transcription (i.e., denitrifying bacteria gene markers), which suggests that biochar mitigates N_2O fluxes by further reducing it to N_2 (Xu *et al.*, 2014). In addition, biochar facilitates the transfer of electrons to soil denitrifying microorganisms, which promotes the reduction of N_2O to N_2 (Fig. 6; Cayuela *et al.*, 2013).

Furthermore, our study found that biochar-induced decreases in N_2O fluxes were enhanced with increasing biochar application rate. Larger amounts of microbial-available and active nutrients due to high biochar application rates may promote complete denitrification to N_2 (Lorenz & Lal, 2014), which may largely contribute to the suppression of soil N_2O fluxes as well as high molar H: C_{org} ratio (Cayuela *et al.*, 2015).

Regulation of Nitrogen (N) fertilization on biochar impacts

Our results showed that biochar application increased soil CO_2 fluxes by 43.33% in unfertilized soils, but decreased by 8.61% in N-fertilized soils, consistent with the meta-analysis of Liu *et al.* (2016). More available inorganic N source for soil microbes and/or plant roots could stimulate soil microbial C mineralization after N is added (Lu *et al.*, 2011; Zhou *et al.*, 2014), but the absorption of NH_4^+ and NO_3^- by biochar would decrease the soil inorganic-N pool after N-fertilizers were applied (Clough *et al.*, 2013; Steiner *et al.*, 2008). Therefore, immobilization of soil inorganic-N induced by biochar application may be the main reason for the slight suppression of soil CO_2 fluxes in N-fertilized soils. In unfertilized soils, the significant stimulation of soil CO_2 fluxes was mainly explained by the relatively higher nutrient availability for soil microbes and/or the priming effect on native soil C decomposition after biochar application (Smith *et al.*, 2010; Wardle *et al.*, 2008).

Biochar application increased soil CH_4 fluxes by 11.67% in N-fertilized soils, but had no significant effect on unfertilized soils. Soil CH_4 fluxes increased weakly under corn and strongly under rice cultivation with N fertilization, respectively, during the entire growing season (Zhang *et al.*, 2010; Zhang *et al.*, 2012b). Biochar input under N addition is likely to alleviate C limitation to microbes. Therefore, the activities of soil methanogenic archaea are enhanced and more CH_4 is produced. Alternatively, some studies showed that decreasing of soil CH_4 fluxes could be partly explained by the facilitated CH_4 oxidation after biochar application (Karhu *et al.*, 2011; Yu *et al.*, 2012), and a more stimulatory effect of biochar on methanotrophic proteobacteria than on methanogenic archaea in unfertilized soils (Feng *et al.*, 2012).

The biochar-induced decrease in soil N_2O fluxes was not significantly different in unfertilized (28.82%, CI = [39.95%, 16.47%]) soils from those of N-fertilized soils (32.97%, CI = [41.14%, 24.42%]). As N addition increased N_2O fluxes by 216% on average across the globe (Liu & Greaver, 2009), the quantity of soil N_2O fluxes mitigated by biochar application in N-fertilized soils is much larger than that in unfertilized soils. As mentioned above, this might be due to more soil NH_4^+ and/or NO_3^- absorbed by biochar after N-fertilizer application, likely causing denitrification to decline (Russow *et al.*, 2008) and/or a facilitation of N_2O reduction to N_2 (Dalal *et al.*, 2003).

Biochar effects on soil GHG fluxes varying with experimental types

The effects of biochar application on soil CO_2 fluxes differed with experimental types. Our study found a significant positive response in unfertilized soils mainly in laboratory incubations, but not in field and pot experiments. The positive response of soil CO_2 fluxes in laboratory incubation is most likely due to the mineralization of the labile C fractions existed in biochar (Zimmerman *et al.*, 2011), as well as increased soil surface area due to pore structures which promotes microbial activity (Chia *et al.*, 2014). In field experiments, the non-significant difference of CO_2 fluxes between control and biochar treatments largely resulted from low application rates and/or high biochar labile C leaching due to rainfall (Kuzyakov *et al.*, 2009; Spokas & Reicosky, 2009). In N-fertilized soils, there were no significant differences in biochar-induced changes of soil CO_2 fluxes among field studies, pot experiments, and laboratory incubations. The positive effects of biochar application on soil CO_2 fluxes as mentioned above may be offset by absorption of soil inorganic N (NH_4^+ and NO_3^-) when biochar is applied (Smith *et al.*, 2010; Steiner *et al.*, 2008; Wardle *et al.*, 2008). Therefore, no changes were observed in soil CO_2 fluxes.

Across all studies, soil CH_4 fluxes showed a positive response to biochar application in field studies, but no significant changes in laboratory incubations and pot experiments. The positive effects in field studies mainly reported from the treatments with N fertilization. The increase in soil CH_4 fluxes under N addition probably resulted from stimulation of soil microbial activities, especially the methanogenic archaea and methanotropic bacteria (Bodelier & Laanbroek, 2004). As reported by Liu *et al.* (2016), biochar amendment significantly increased soil microbial biomass C (MBC) in the field experiments, whereas MBC decreased in controlled studies. This likely resulted from improving the availability of microbial habitats and accessibility of microbial food resources in the field-based experiments compare to the controlled conditions especially under biochar amendment (Pietikainen *et al.*, 2000).

In contrast, the responses of soil N_2O fluxes to biochar application showed a consistent trend across all treatments (Figs. 4a-c). However, laboratory incubations showed greater N_2O

flux decreases than field studies with respect to biochar application in unfertilized soils (Fig. 4b), likely due to the difference in mixing of biochar with soil in controlled and field studies. Biochar is mixed thoroughly with soils in most controlled studies, which enhances soil aeration, but in field studies biochar is applied to the soil surface (e.g., Bamminger *et al.*, 2014; Case *et al.*, 2014; Scheer *et al.*, 2011; Wang *et al.*, 2012).

Responses of GWP of soil GHGs to biochar application

Global warming potential (GWP) is a simplified index to estimate the potential future impacts of GHGs on the global climate system based on their radiative forcing and lifetimes (IPCC, 2013). Overall, biochar application significantly increased GWP by 46.22% (CI = [19.72%, 82.20%]). The fluxes are governed by different mechanisms (Fig. 6), but largely resulting from the significant stimulation of soil CO_2 fluxes. The increased amount of soil CO_2 fluxes induced by biochar application was nearly a one thousand times the size of CH_4 or N_2O fluxes in most studies (e.g., Scheer *et al.*, 2011; Wang *et al.*, 2012; Zhang *et al.*, 2012a). In addition, biochar increased the GWP of soil GHGs in unfertilized soils, but decreased it in N-fertilized soils due to the suppression of soil CO_2 and N_2O fluxes under N addition.

Significant amounts of CO_2, CH_4, and N_2O were released to the atmosphere from agriculture, which accounted for nearly one-fifth of the annual increase in radiative forcing of climate change (Cole *et al.*, 1997). Soil GHG fluxes would increase substantially after N fertilizers were applied, especially in croplands (Hall & Matson, 1999; Liu & Greaver, 2009; McSwiney & Robertson, 2005; Zhou *et al.*, 2014). Agricultural GHG emissions from crop and livestock production was 5.3 Pg of carbon dioxide equivalents (CO_2 eq) in 2011 (FAO 2014). Tian et al. (2016) estimated CH_4 and N_2O emissions in the agricultural ecosystems were 169

±26 Tg C yr^{-1} and 4.9±0.3 Tg N yr^{-1}, respectively. According to our estimates with a decrease of 7.69% for GWP under N fertilization, 0.41 Pg CO_2 eq yr^{-1} could potentially be mitigated by biochar applied to agricultural soils in combination with N fertilizers. Moreover, biochar application would increase average yield of 10% and nearly 14% in acidic soils (Jeffery *et al.*, 2011). Given that our study elicits that biochar application reduces CO_2 fluxes and GWP in N-fertilized soil, biochar therefore appears to be a good strategy to mitigate global warming in fertilized agro-ecosystems.

Implications for future experiments and land surface models

The compiled database in our meta-analysis mainly obtained from laboratory incubations, and the results were different for the responses of soil GHG fluxes to biochar application compared to those from field studies (Scheer *et al.*, 2011; Spokas *et al.*, 2009; Fig 4). The lack of field-scale studies, especially those lasting at least two successive seasons (Lorenz & Lal, 2014), may hamper our evaluation of soil GHG fluxes in response to biochar application in the longer term. In addition, most biochar application experiments had been conducted in North America, Europe, and China. There remains a dearth of field studies in other regions, including Africa, Southeast Asia, and South America. Thus, long-term field experiments with biochar amendments are especially needed in these regions.

Nitrogen fertilization mediated the responses of soil GHG fluxes and their GWP to biochar application. Since N deposition increased from ~34 Tg N yr^{-1} in 1860 to 100 Tg N yr^{-1} in 1995 and is predicted to reach 200 Tg N yr^{-1} in 2050 (Galloway *et al.*, 2008; IPCC, 2013), the interactive effects between biochar and N addition may dramatically influence soil microbial community structure and ecosystem functioning as well as soil GHG fluxes in the future (Liu *et al.*, 2016). To address this issue, biochar experiments with diverse types of N fertilization (e.g. fertilizer type and level) are needed to examine potential nonlinear responses to biochar application.

In the nature, biochar is often produced by wildfire, and currently, industrially produced-biochar application becomes more common, especially in agriculture. Our meta-analysis results from laboratory, pot, and field studies found significant effects of biochar application on soil GHG fluxes and their GWPs. These results may provide some insights into how the fire-generated biochar affects net climate forcing from soil GHG fluxes and offers recommendations for development and improvement of land surface models. Tempo-spatial variability of soil GHG fluxes is mostly attributed to soil temperature, soil moisture, fire severity, aspect and time since fire in wildfire models (Gathany & Burke, 2011). However, wildfire-produced and industrial biochar may play critical roles in shaping terrestrial ecosystem processes and affecting soil GHG fluxes. Thus, future land surface models may need to incorporate biochar-induced effects to natural ecosystem processes, especially soil GHG fluxes and their GWPs for better forecasting the feedback of terrestrial ecosystems to climate change. Additionally, the combined or interactive effects of N fertilization with biochar amendments can be incorporated into future land surface models to improve the predictions about N-mediated feedback of ecosystem C cycles to climate systems from soil GHG fluxes.

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Supporting information

Text S1 A list of 91 papers from which the data were extracted for this meta-analysis.

Table S1 Response ratio (RR) and number of paired observations extracted from each of the papers.

Table S2 Percentage changes of soil greenhouse gas (GHG) emissions in response to biochar application.

Table S3 The Kendall's Tau for RR(CO_2), RR(CH_4), RR(N_2O) and RR(GWP) in different treatments.

Fig S1 Frequency distributions of response ratios (RR) of soil CO2, CH4, and N2O emissions to biochar application.

Fig S2 Frequency distributions of response ratios (*RR*) of soil CO_2, CH_4, and N_2O emissions to biochar application on unfertilized soils and N-fertilized soils.

Fig S3 The effect of biochar application on soil CO_2, CH_4, and N_2O emissions differed with experimental method.

Fig S4 Effects of experimental duration on response ratios of GHG emissions to biochar application.

Table 1 Between-group variability (Q_b) among observations (n) suggesting their potential as predictive variables influencing soil GHG emissions responses to biochar application.

	CO_2		CH_4		N_2O	
Variables	n	Q_b	n	Q_b	n	Q_b
All studies	402	--	121	--	371	--
Role of N fertilization	402	13.43***	121	7.70**	371	0.37
Experimental method	402	19.52***	121	9.33**	371	2.34
Feedstock source	402	4.28	121	10.60**	371	19.37***
Soil texture	277	9.95*	86	115.98***	256	14.34**
Pyrolysis temperature (°C)	385	37.27***	110	6.85**	354	1.94
Biochar pH	327	25.08***	103	14.22***	317	3.05
Soil pH	390	0.55	117	1.62	351	10.19**
Applied rate (Lg (t ha^{-1}))	400	15.65***	120	4.53*	371	39.05***
Latitude (^{o})	401	50.44***	121	0.00	371	2.50
Soil C/N ratio	212	0.25	58	0.02	183	2.35
Duration (day)	402	0.02	121	0.51	371	1.62
Biochar C/N ratio	387	0.06	120	0.64	363	0.53

A variable with larger Q_b is a better predictor than a variable with smaller Q_b.

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

Figure captions

Figure 1 Global distribution of 137 study sites selected in this meta-analysis. Letters C, M and N represent the sites with CO_2, CH_4 and N_2O measurements, respectively.

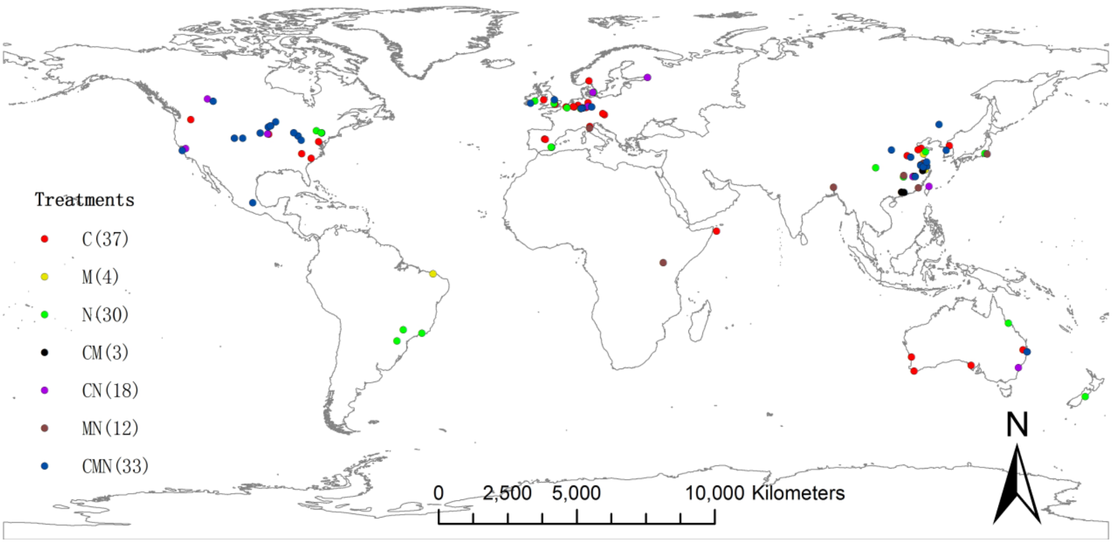
Figure 2 The effect of biochar application on soil CO_2 (a), CH_4 (b), and N_2O (c) emissions differed with experimental method (including field studies (F), laboratory incubation (I), and pot experiments (P)) in unfertilized soils and N-fertilized soils, shown as weighted response ratio (RR_{++}). Mean effect and 95% CIs are shown. If the CI did not overlap with zero, the response was considered significant ('*'). Numerals indicate number of observations. 'Overall' indicates the integrated biochar effect across N fertilization as compared with controls.

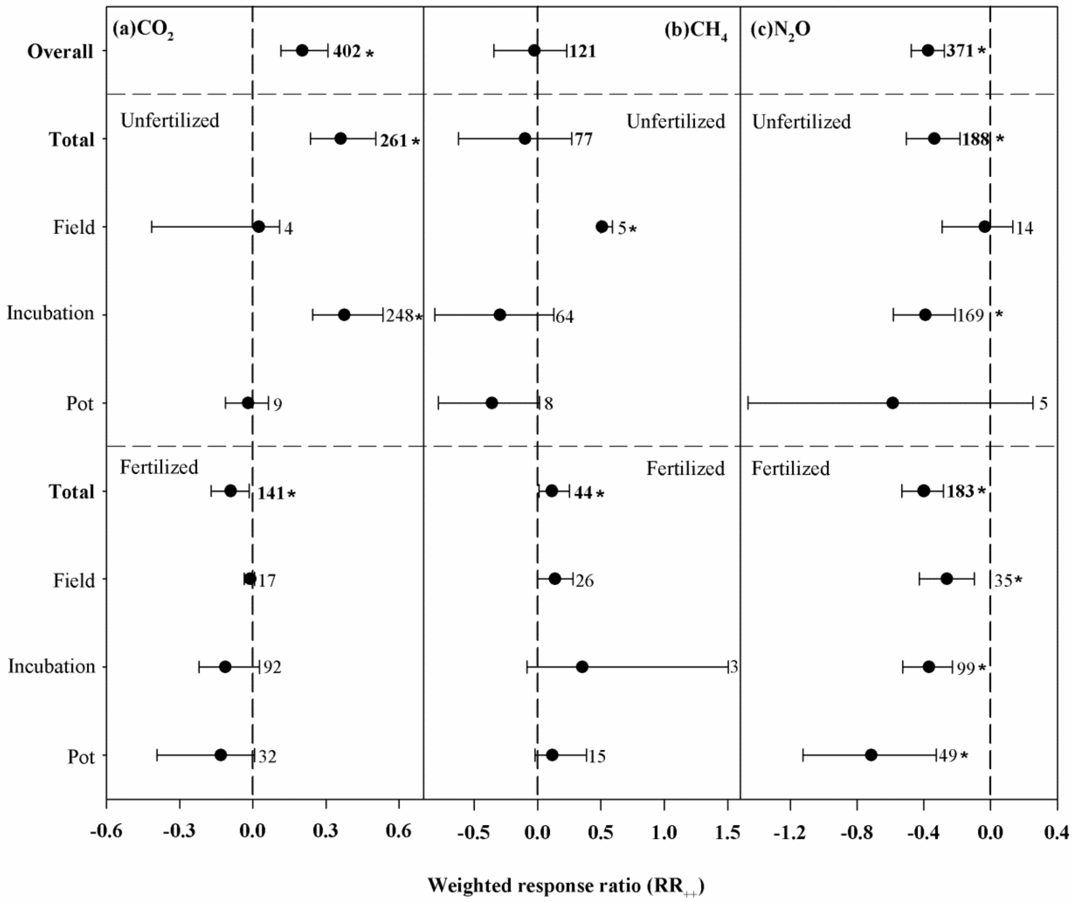
Figure 3 The effect of biochar application on soil CO_2 (a and d), CH_4 (b and e), and N_2O (c and f) emissions depended on biochar feedstock source and soil texture, shown as weighted response ratio (RR_{++}). Mean effect and 95% CIs are shown. If the CI did not overlap with zero, the response was considered significant ('*'). Numerals indicate number of observations.

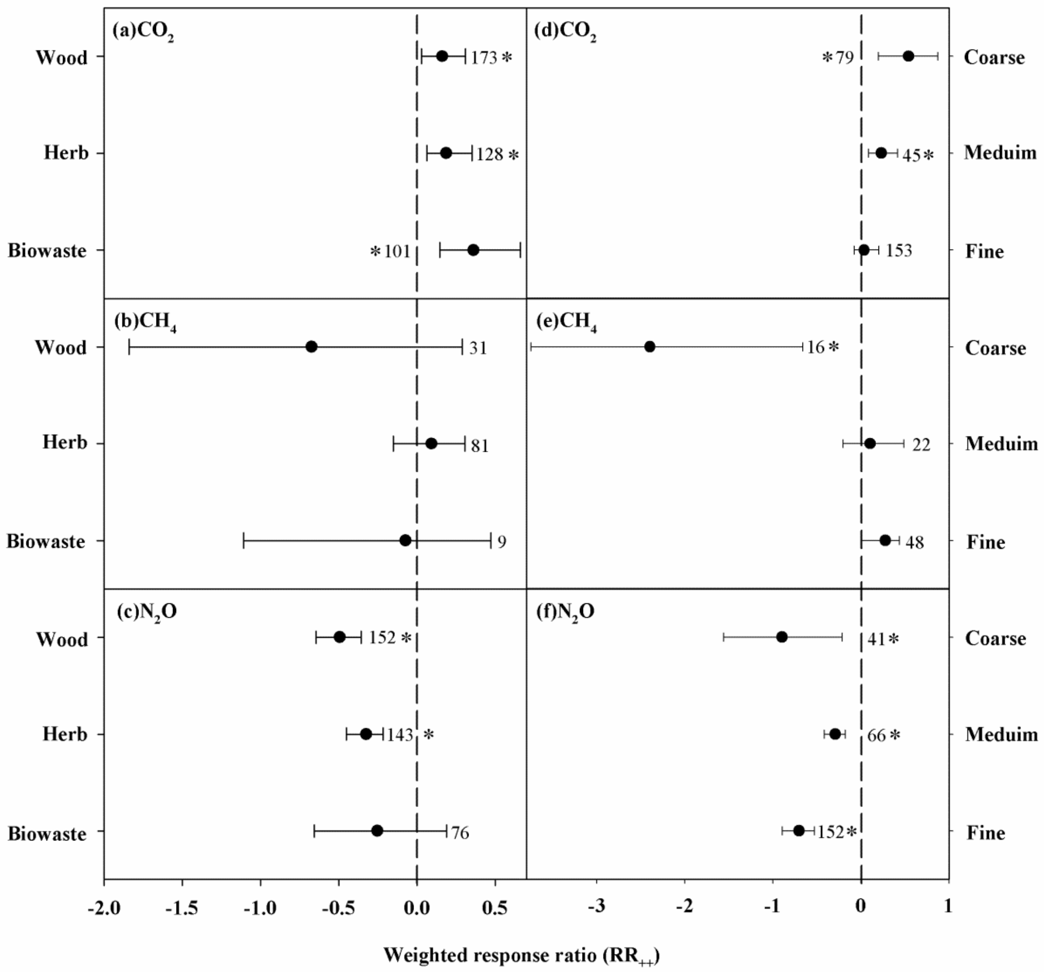
Figure 4 Effects of biochar pyrolysis temperature, biochar pH, soil pH, applied rate, and latitude on response ratios of soil CO_2 emissions (a, b, c, d, and e), CH_4 emissions (f, g, h, i, and j), and N_2O emissions (k, l, m, n, and o) to biochar application.

Figure 5 Frequency distributions of response ratio (RR) of global warming potential (GWP, panel a) to biochar application, GWP in unfertilized soils (b) and N-fertilized soils (c). The sample size (n), weighted response ratio (RR_{++}) and 95% CIs are shown. The effect of biochar application on GWP differed with experimental method (d), and GWP differed with experimental method in unfertilized soils (d_1) and N-fertilized soils (d_2). Mean effect and 95% CIs are shown. If the CI did not overlap with zero, the response was considered significant ('*'). Numerals indicate number of observations.

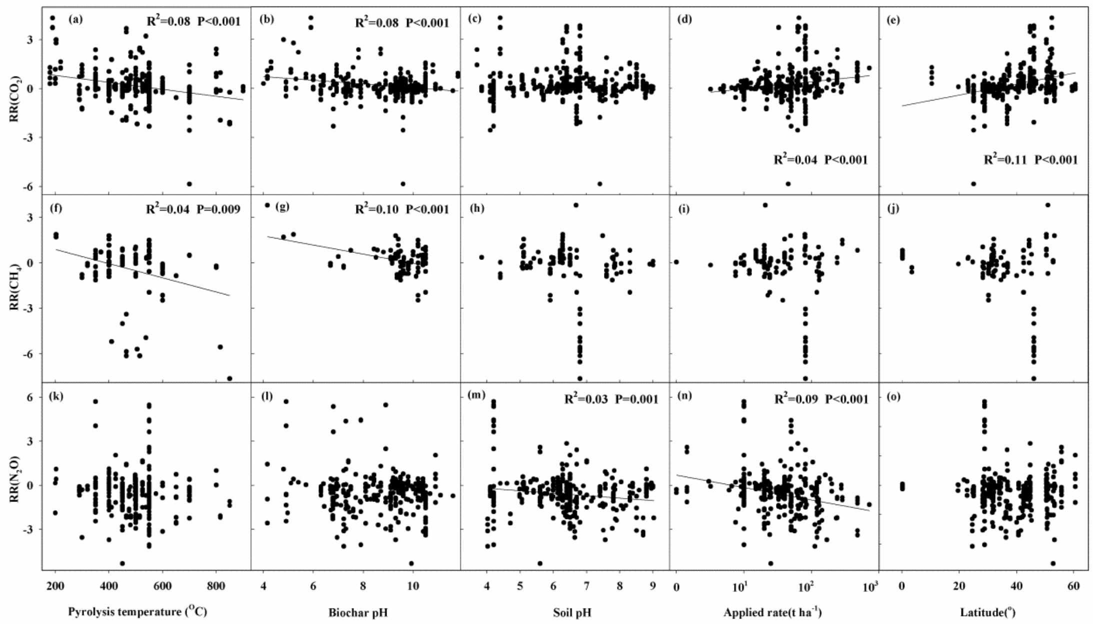
Figure 6 Potential mechanisms of soil GHG fluxes in response to biochar amendment. The red line and blue line represent the positive and negative regulations, respectively.



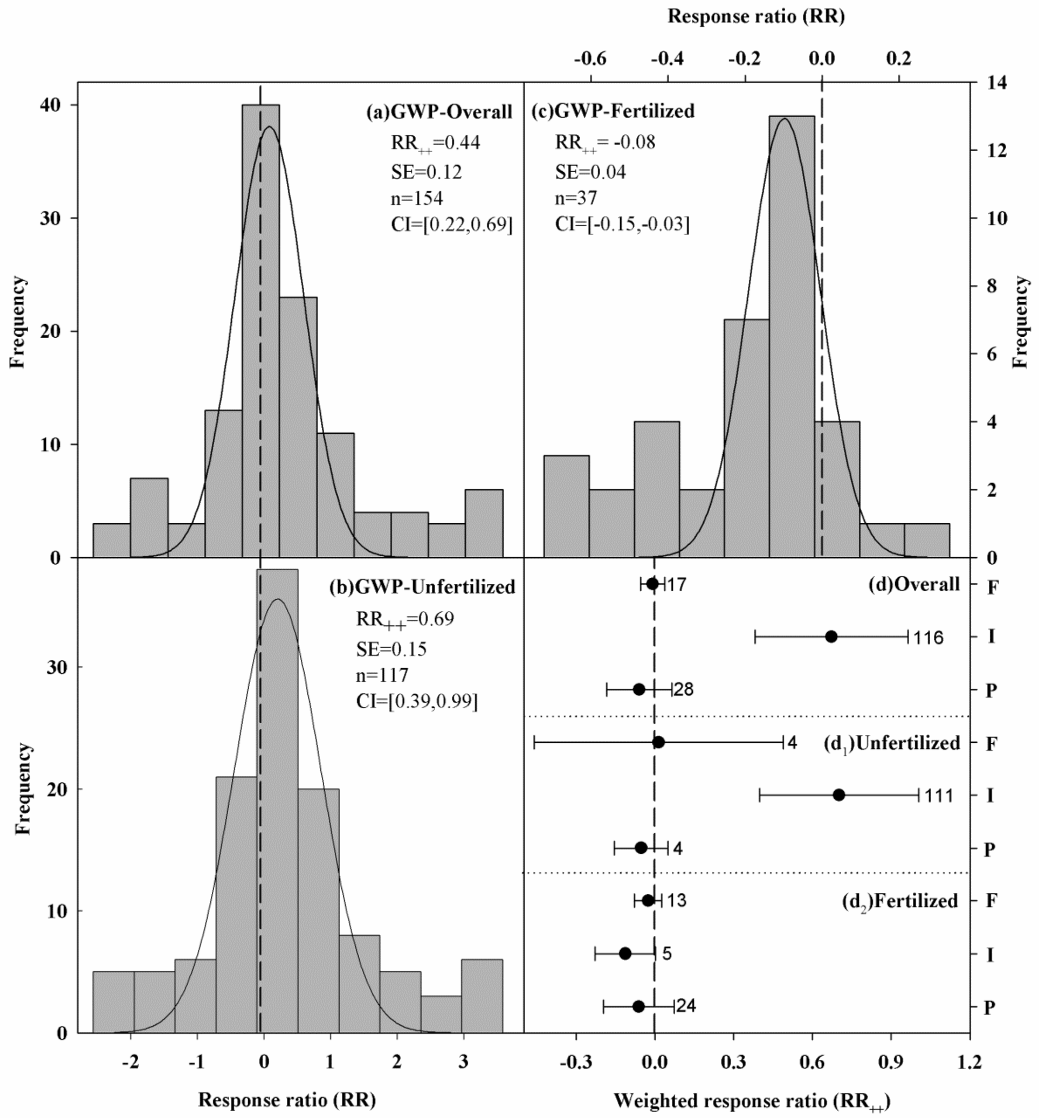

(a)CO2
(b)CH4
(c)N2O
Overall
Unfertilized
Total
Field
Incubation
Pot
Fertilized
402 *
121
371 *
261 *
77
188 *
4
5 *
14
248 *
64
169 *
9
8
5
141 *
44 *
183 *
17
26
35 *
92
3
99 *
32
15
49 *
-0.6 -0.3 0.0 0.3 0.6
-0.5 0.0 0.5 1.0 1.5
-1.2 -0.8 -0.4 0.0 0.4
Weighted response ratio (RR++)



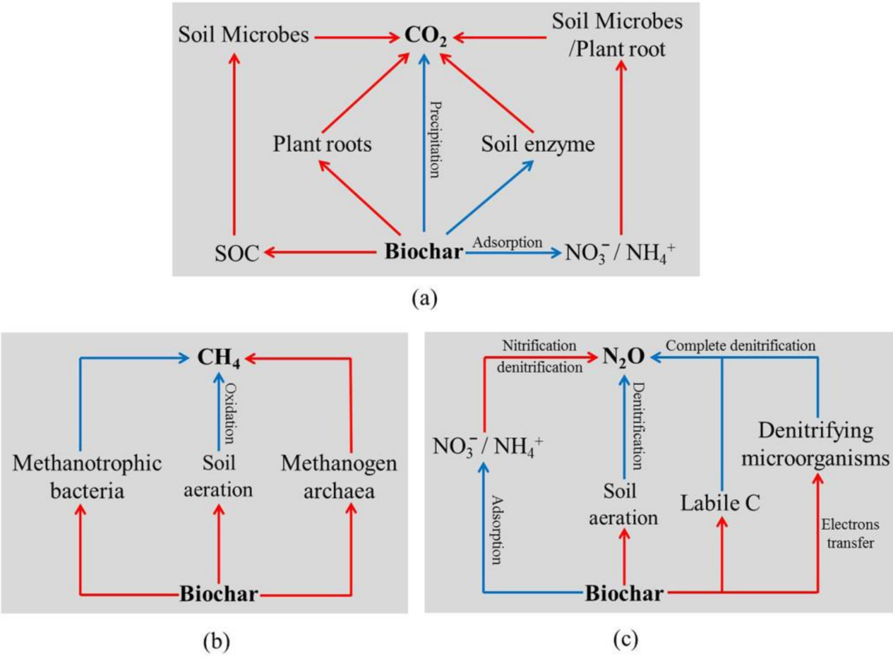
(a)CO2
Wood
Herb
Biowaste
173 *
128 *
* 101
(b)CH4
31
81
9
(c)N2O
152 *
143 *
76
(d)CO2
Coarse
Meduim
Fine
* 79
45 *
153
(e)CH4
16 *
22
48
(f)N2O
41 *
66 *
152 *
-2.0
-1.5
-1.0
-0.5
0.0
0.5
-3
-2
-1
0
1
Weighted response ratio (RR++)



(a) R^2=0.08 P<0.001
(b) R^2=0.08 P<0.001
(c)
(d) R^2=0.04 P<0.001
(e) R^2=0.11 P<0.001
(f) R^2=0.04 P=0.009
(g) R^2=0.10 P<0.001
(h)
(i)
(j)
(k)
(l)
(m) R^2=0.03 P=0.001
(n) R^2=0.09 P<0.001
(o)
RR(CO_2)
RR(CH_4)
RR(N_2O)
Pyrolysis temperature (°C)
Biochar pH
Soil pH
Applied rate(t ha^{-1})
Latitude(°)



Response ratio (RR)
(a)GWP-Overall
RR++=0.44
SE=0.12
n=154
CI=[0.22,0.69]
(c)GWP-Fertilized
RR++= -0.08
SE=0.04
n=37
CI=[-0.15,-0.03]
(b)GWP-Unfertilized
RR++=0.69
SE=0.15
n=117
CI=[0.39,0.99]
(d)Overall
(d1)Unfertilized
(d2)Fertilized
Frequency
Response ratio (RR)
Weighted response ratio (RR++)


Soil Microbes
CO2
Soil Microbes
/Plant root
Precipitation
Plant roots
Soil enzyme
SOC
Biochar
Adsorption
NO3- / NH4+
(a)
CH4
Oxidation
Methanotrophic
bacteria
Soil
aeration
Methanogen
archaea
Biochar
(b)
Nitrification
denitrification
N2O
Complete denitrification
Denitrification
NO3- / NH4+
Denitrifying
microorganisms
Adsorption
Soil
aeration
Labile C
Electrons
transfer
Biochar
(c)