

Effects of biochar amendment on greenhouse gas emissions, net ecosystem carbon budget and properties of an acidic soil under intensive vegetable production

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Abstract

Biochar addition to soils has been frequently proposed as a means to increase soil fertility and carbon (C) sequestration. However, the effect of biochar addition on greenhouse gas emissions from intensively managed soils under vegetable production at the field scale is poorly understood. The effects of wheat straw biochar amendment with mineral fertilizer or an enhanced-efficiency fertilizer (mixture of urea and nitrapyrin) on N₂O efflux and the net ecosystem C budget were investigated for an acidic soil in southeast China over a 1-yr period. Biochar addition did not affect the annual N₂O emissions (26–28 kg N/ha), but reduced seasonal N₂O emissions during the cold period. Biochar increased soil organic C and CO₂ efflux on average by 61 and 19%, respectively. Biochar addition greatly increased C gain in the acidic soil (average 11.1 Mg C/ha) compared with treatments without biochar addition (average –2.2 Mg C/ha). Biochar amendment did not increase yield-scaled N₂O emissions after application of mineral fertilizer, but it decreased yield-scaled N₂O by 15% after nitrapyrin addition. Our results suggest that biochar amendment of acidic soil under intensive vegetable cultivation contributes to soil C sequestration, but has only small effects on both plant growth and greenhouse gas emissions.

Keywords: Biochar, soil heterotrophic respiration, nitrification inhibitor, soil fertility

Introduction

The vegetable harvested area occupies 11.6% of all cultivated land in China and accounts for ca. 45% of the total world vegetable area (FAOSTAT, 2009). The annual amount of nitrogen (N) fertilizers applied in vegetable fields is three- to four-fold greater than that used for cereal production (Wang *et al.*, 2011), which leads to soil acidification (Ju *et al.*, 2007) and substantial nitrous oxide (N₂O) emissions (Xiong *et al.*, 2006; Zhu *et al.*, 2011). Any potential management strategy which could help to alleviate these issues should be critically examined.

Previous studies have demonstrated that biochar application to soils can be a ‘win-win’ solution to help meet global climatic challenges (Woolf *et al.*, 2010). A recent meta-analysis by Cayuela *et al.* (2014) suggested that biochar

reduced N₂O emissions on average by 54% across the reviewed laboratory and field studies. In contrast, weathered biochars collected from the field could negate the suppression of N₂O emissions and strongly enhance carbon dioxide (CO₂) effluxes under the subsequent laboratory incubation studies (Spokas, 2013). Although biochar addition can decrease N₂O emissions from various soils, no significant effects were observed on soils with pH <5 (Cayuela *et al.*, 2014). Furthermore, few recent studies have been carried out to address biochar effects on N₂O emissions at the field scale with a duration of at least 1 yr, and contradictory results have been emerging when compared to studies undertaken in laboratory conditions (Suddick & Six, 2013). Moreover, very few studies have investigated the effects of biochar amendment on CO₂ and non-CO₂ GHG emissions from soils at the field scale (Jeffery *et al.*, 2011; Spokas, 2013).

Recent reviews have highlighted the benefits of adding biochar to agricultural soils, such as the improvement of

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plant growth and soil properties (e.g. total carbon (C) and N content and pH) (Atkinson *et al.*, 2010; Jeffery *et al.*, 2011; Biederman & Harpole, 2012). However, information on how vegetable production, soil properties and N₂O emissions are influenced by biochar addition in acidic vegetable fields is scarce.

Considering that biochar is recalcitrant against microbial decomposition (Wang *et al.*, 2015), both positive (Fang *et al.*, 2014), negative (Zimmerman *et al.*, 2011) and no effects (Kuzyakov *et al.*, 2009) of biochar addition on mineralization of native soil organic matter (SOM) have been reported under laboratory conditions. Moreover, little information exists on the effect of adding biochar on soil respiration under field conditions (Major *et al.*, 2010). The net ecosystem C budget (NECB) can essentially provide a scientific basis for the development of C sequestration strategies (Smith *et al.*, 2010a). However, there is a paucity of data about the response of NECB for vegetable cultivation (Jia *et al.*, 2012), especially following biochar addition.

Based on the effective mitigation of N₂O emissions and agricultural benefits after biochar application (Atkinson *et al.*, 2010; Cayuela *et al.*, 2014), we hypothesized that biochar additions may contribute to decreased N₂O emissions and improved soil properties, as well as increased C sequestration in an acidic field soil growing vegetables. The objective was to investigate the effects of biochar amendment on N₂O emissions, NECB and soil properties as influenced by the application of mineral fertilizer and urea granulated with nitrapyryn.

Materials and methods

Experimental site

The field experiments were conducted in July, 2012, in an intensive vegetable cultivation area (ca. 10 yr old) in Nanjing, China (32°01'N, 118°52'E). This region experiences

a subtropical monsoon climate with an annual mean precipitation of 1110 mm and a mean air temperature of 15.4 °C. The soil is classified as *Irragric Anthrosols* (WRB, 2006) with a silty clay loam texture. The physicochemical properties of soil in the Ap horizon (0–15 cm) are shown in Table 1.

Experimental design

The field management including crop species, fertilizer application rates and methods, tillage, irrigation, pesticide and weed control followed local practices. Four vegetables, namely, swamp morning glory (*Ipomoea aquatica* Forssk.), Shanghai pak choy (*Brassica chinensis* L.), coriander herb (*Coriandrum sativum* L.) and lettuce (*Lactuca sativa* L.) were grown successively over a 1-yr period (11 July 2012 to 10 July 2013). The dates of sowing (or transplanting for lettuce) and harvesting for each vegetable are shown in Figure 1. A plastic film covered the greenhouse from 14 September 2012 to 17 March 2013 to protect against cold weather following local practices. Note that the fallow periods following the

Table 1 The physicochemical properties of soil in the Ap horizon (0–15 cm) and biochar

| | Soil | Biochar |
|-----------------------------------|------|---------|
| Clay (%) | 30.1 | – |
| Silt (%) | 64.7 | – |
| Sand (%) | 5.2 | – |
| TC (g C/kg) | 15.9 | 467 |
| TN (g N/kg) | 2.1 | 5.9 |
| pH (H ₂ O) | 4.2 | 10.4 |
| CEC (cmol/kg) | 31.2 | 24.1 |
| Bulk density (g/cm ³) | 1.2 | – |
| Ash content (%) | | 20.8 |

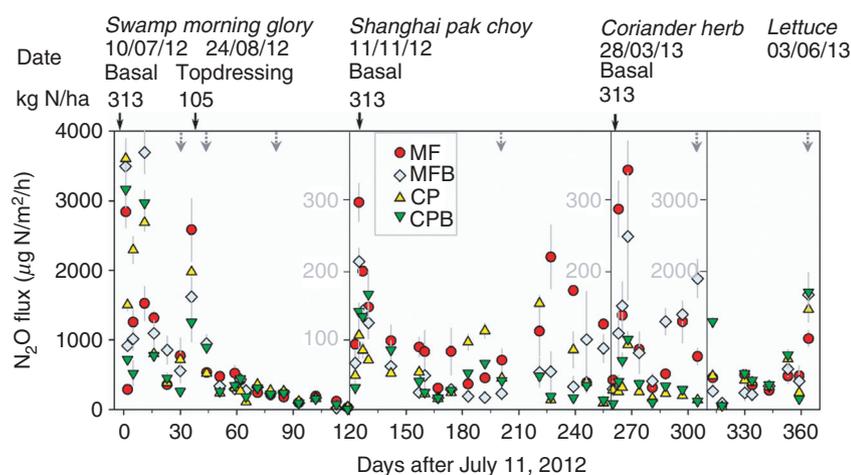


Figure 1 Seasonal dynamics of soil N₂O emissions under four treatments over four planting seasons. The solid and dashed arrows indicate basal fertilizer application and sowing/harvesting periods, respectively. Values represent means ± SE (*n* = 3). For abbreviations, see Table 2.

first two planting seasons were about one and half months, which is the local agronomic practice adopted mainly for soil aeration and tillage after crop harvest.

Four treatments were established in triplicate as follows: (i) ammonium-based mineral fertilizer applied as standard farmers' practice (MF), (ii) MF with wheat straw biochar added at the rate of 30 Mg/ha (MFB), (iii) an enhanced-efficiency fertilizer applied in the form of a mixture of urea granulated with a nitrification inhibitor, nitrapyrin [2-chloro-6-(trichloromethyl) pyridine] (CP), at a ratio of 0.24% of urea-N and (iv) CP with biochar addition at 30 Mg/ha (CPB). The plot size for each treatment was 3 m × 2 m. All treatments received the same amounts of N (1044 kg N/ha/yr), calcium superphosphate (2559 kg P₂O₅/ha/yr) and potassium chloride (1285 kg K₂O/ha/yr) based on local practices.

Biochar was produced from wheat straw by pyrolysis at 350–550 °C (Table 1), then manually incorporated into and thoroughly mixed with the soil of the Ap horizon (0–15 cm) at 2% (w/w) on 4 July 2012.

N₂O sampling and analysis

The N₂O concentration was analysed year-round using the static opaque chamber method previously described by Wang *et al.* (2012). Briefly, three square PVC base frames were permanently fixed in each planted plot onto which were placed chambers with dimensions of 30 cm × 30 cm × 50 cm (length × width × height). Gas samples were usually measured once a week, but more frequently during the first week after fertilizer application. Four gas samples were drawn from the headspace of each chamber at 10-min intervals, and N₂O concentrations were analysed within 12 h of sampling. Air and soil temperatures were measured during gas sampling.

The N₂O concentrations were analysed using a gas chromatograph (Agilent 7890A, USA) that was equipped with an electron capture detector (ECD). The N₂O fluxes were determined using a linear function. Seasonal amounts of N₂O emissions were sequentially accumulated from the emissions between every two adjacent samplings (Wang *et al.*, 2012).

Soil heterotrophic respiration sampling and measurements

Soil heterotrophic respiration (R_h) was also analysed using the static opaque chamber method described above. Briefly, a subplot of 50 cm width without plant growth was established within the planted plots, in which three PVC ring collars as base frames with inner diameter of 16 cm were installed. A cylindrical chamber with a diameter of 17 cm and height of 24 cm was used for sampling gases. The treatments and sampling schedule was the same as for N₂O measurement.

The CO₂ concentrations were analysed using a gas chromatograph that was equipped with a hydrogen flame ionization detector (FID). The fluxes and seasonal amounts of CO₂ were determined in a way similar to that of N₂O.

To describe the relationship between soil R_h and soil temperature, the following equation was used:

$$R_h = Ae^{bT} \quad (1)$$

where R_h is the soil heterotrophic respiration rate (mg C/m²/h) at a soil temperature T (°C), A and b are the exponential fit parameters. A was considered to be a simple index of the availability and lability of biochar-C and SOC in soils.

Q₁₀ was used to describe the temperature sensitivity and calculated as follows:

$$Q_{10} = e^{10b} \quad (2)$$

where b is the exponential fit parameter.

Soil properties and vegetable biomass analyses

Soil samples were taken at each gas sampling date, at a depth of 0–10 cm, for soil water content measurement by the standard oven-drying method. Soil water-filled pore space (WFPS) was calculated using standard methods assuming a soil bulk density of 2.65 g/cm³. Soils (0–15 cm) were collected on 10 July 2013 and stored for laboratory analysis. SOC and total N concentrations were analysed by standard wet digestions.

At each vegetable harvest, the shoot parts were removed manually at the base and the roots were dug out from the soil and adhering soil particles were shaken off. Roots and shoots were washed with deionized water before drying at 65 °C for about 72 h and weighed to calculate the net primary production (NPP).

Estimation of NECB and yield-scaled N₂O emissions

We summarized the components for the NECB of short-planted croplands using the intermittent chamber measurements (Smith *et al.*, 2010a; Jia *et al.*, 2012):

$$\begin{aligned} \text{NECB (Mg C/ha)} = & \text{NPP (Mg C/ha)} - R_h \text{ (Mg C/ha)} \\ & - \text{Harvest (Mg C/ha)} + \text{Biochar (Mg C/ha)} \end{aligned} \quad (3)$$

where harvest referred to aboveground biomass removed for all vegetable crops and measured directly at harvest. The C input derived from biochar was included for the calculation of NECB.

As the total aboveground parts of leafy vegetables are usually harvested as food, yield-scaled N₂O emission was calculated as follows:

$$\begin{aligned} & \text{Yield-scaled N}_2\text{O emission (kg N/Mg yield)} \\ & = \text{N}_2\text{O emission (kg N/ha)} / \text{Aboveground biomass (Mg dry biomass/ha)} \quad (4) \end{aligned}$$

Statistical analyses

The Shapiro–Wilk test was used to check the normality of all investigated variables before analysis of variance (ANOVA) was performed. Data for some variables were natural log-transformed when the data were not normally distributed. Differences between effects of treatments and planting seasons on N_2O emissions, yield-scaled N_2O emissions, NPP and NECB were tested by two-way ANOVA. One-way ANOVA was also performed to test the effects of treatments on the annual R_h and to assess the differences of soil temperature and WFPS between vegetable growth periods. Multiple comparisons between treatments were further examined using Tukey's HSD test. All statistical analyses were performed using STATISTICA version 10 (StatSoft Inc., Tulsa, USA).

Results

Soil microclimate and soil properties

No treatment differences in WFPS or soil temperature were detected during the experimental period (data not shown). The WFPS and soil temperature are therefore presented as means of all treatments (Figure 2). The values of WFPS were much higher (up to 89%) during the first week than during the remaining period of the 1-yr observation, varying between 28 and 68%. Although a plastic greenhouse was employed from 14 September 2012 to 17 March 2013, the variation in soil temperature coincided with the seasonal change of outside temperature. Predictable differences were found between planting seasons, with higher temperatures in summer and lower in the winter season ($P < 0.001$).

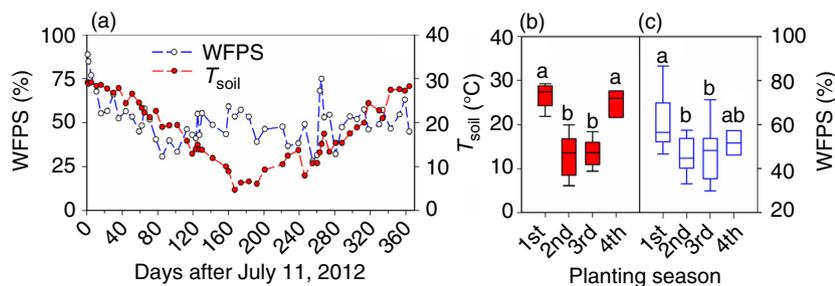


Figure 2 Temporal dynamics of soil water-filled pore space (WFPS) and soil temperature over 1 yr (a). Box plots for soil temperature (b) and WFPS (c) in different planting seasons. Data used are the means across all treatments with negligible standard errors (data not shown). Different lower case letters indicate significant differences between treatment medians ($P < 0.05$). The lines in the box represent the median of all data.

Significant differences in WFPS between different planting seasons were also detected ($P < 0.01$).

Biochar addition increased the SOC by an average of 61% compared with the treatments without biochar addition (MF and CP; $P < 0.001$, Table 2), but had no effect on total N. By the end of field measurement (day 364), contrasting results were obtained for soil pH: a significant ($P < 0.001$) decrease of 0.48 units in the MFB treatment and a 0.22 units increase for the CPB treatment compared with the treatments without biochar (Table 2).

N_2O effluxes and yield-scaled N_2O emissions

The N_2O efflux rates increased up to $4000 \mu\text{g N/m}^2/\text{h}$ after basal fertilizer application and tillage in all treatments (Figure 1). The N_2O emissions decreased subsequently with another peak of N_2O emission following fertilizer top-dressing. Peaks of N_2O emissions were also observed after basal fertilizer application and tillage during subsequent plantings. In terms of cumulative N_2O emissions, the treatment effects were significant only in the second and third planting seasons (both $P < 0.01$; Figure 3a). However, the first and fourth planting seasons accounted for 56–67% and 20–26%, of the total annual N_2O emissions, respectively.

The responses of yield-scaled N_2O emissions to biochar addition varied with different N fertilizer types and between the planting seasons ($P < 0.01$ – 0.001 ; Figure 3b). For example, biochar addition reduced the yield-scaled N_2O emissions by between 3 and 67% in the later three planting seasons following MF application, compared with a reduction of 20–40% in the first two planting seasons following CP application. The corresponding overall yield-scaled N_2O emission was either not increased by biochar in the MFB treatment or decreased by 15% in the CPB treatment ($P > 0.05$).

Soil R_h dynamics and NECB

Tillage application triggered predictable pulses of soil R_h for all the crops. Despite the similar seasonal patterns, the

Table 2 Effects of biochar addition on soil organic carbon (SOC) and total N after 1 yr and soil pH at three sampling times

| Treatment ^a | SOC (g C/kg) | Total N (g N/kg) | pH | | |
|------------------------|--------------------------|------------------|--------------|--------------|--------------|
| | | | 98 days | 221 days | 364 days |
| MF | 15.9 ± 0.2b ^b | 1.96 ± 0.04 | 4.20 ± 0.01c | 4.40 ± 0.06b | 4.82 ± 0.05b |
| MFB | 25.3 ± 0.9a | 2.02 ± 0.01 | 4.31 ± 0.07c | 4.37 ± 0.06b | 4.34 ± 0.03c |
| CP | 16.3 ± 0.7b | 1.99 ± 0.02 | 4.53 ± 0.07b | 4.92 ± 0.11a | 4.88 ± 0.05b |
| CPB | 26.5 ± 1.2a | 2.08 ± 0.05 | 4.92 ± 0.02a | 5.06 ± 0.17a | 5.10 ± 0.04a |
| <i>P</i> ^c | *** | <i>ns</i> | *** | ** | *** |

^aMF, mineral fertilizer; MFB, wheat biochar plus MF; CP, a mixture of urea and nitrapyrin; CPB, wheat biochar plus CP. ^bValues represent means ± SE (*n* = 3). Different lower case letters in the same column indicate statistically significant differences between treatments at the *P* < 0.05 level, letters not shown when differences not significant. ^c****P* < 0.01; ****P* < 0.001; *ns*, not significant.

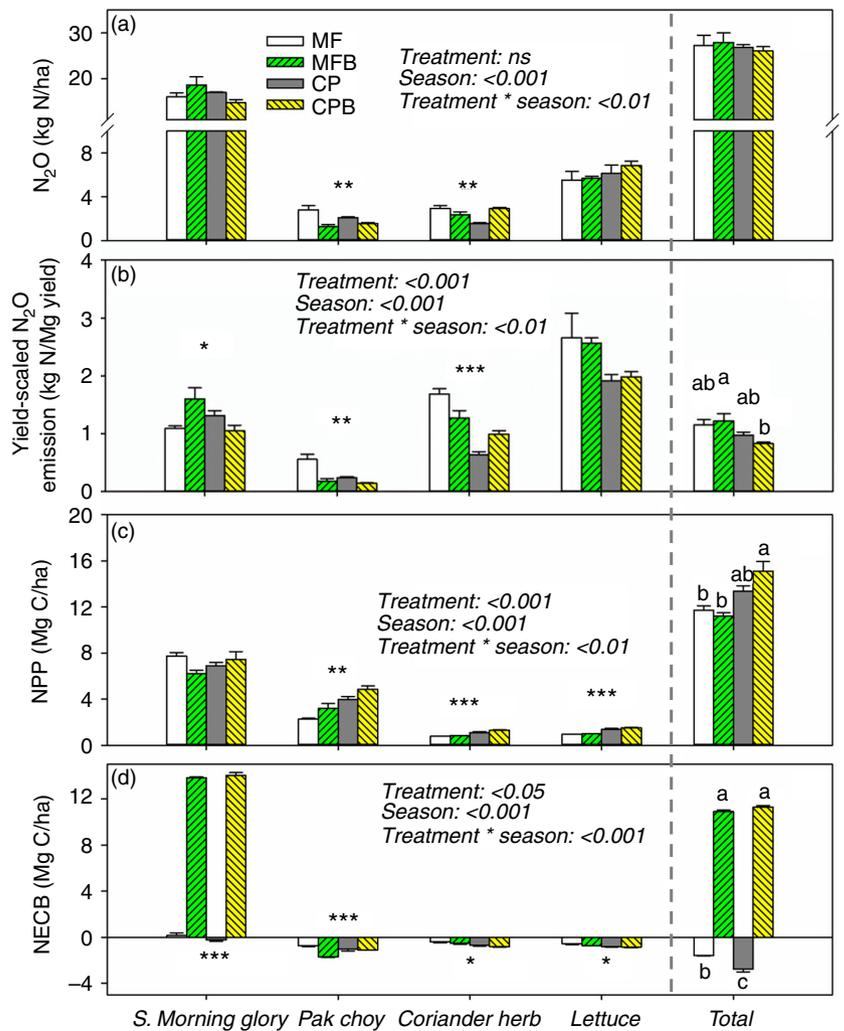


Figure 3 Soil cumulative N₂O emissions (a), yield-scaled N₂O emission (b), NPP (c) and NECB (d) under four treatments for each planting season. ANOVA results for each variable are inserted in each subfigure. Within each planting season, ANOVA results are indicated by asterisks (**P* < 0.05; ***P* < 0.01; ****P* < 0.001; *ns*, not significant). Values represent means ± SE (*n* = 3). Different lower case letters indicate significant differences between treatment means (*P* < 0.05), letters not shown when differences not significant. For abbreviations see Table 2.

annual total *R_h* rates showed significant differences between the MFB, CPB, CP and MF treatments as indicated on Figure 4. Seasonal dynamics of soil *R_h* rates were similar

among all treatments and strongly correlated with the temporal variations in soil temperature (Figures 2a and 5). Biochar application increased the availability and lability of

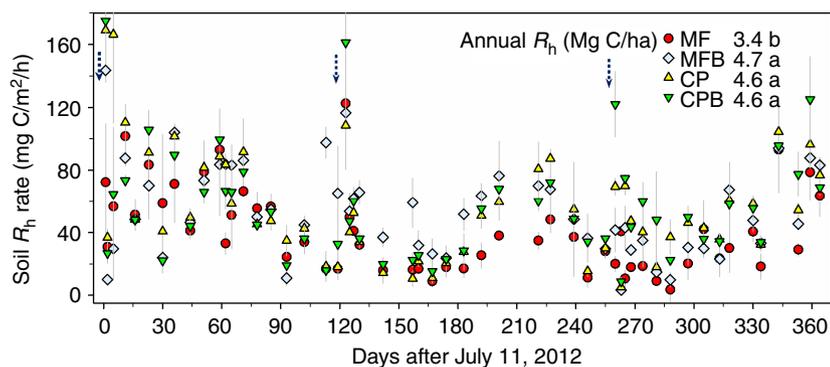


Figure 4 Temporal dynamics of soil R_h rates under four treatments over 1 yr. The annual soil R_h amounts are inserted next to the legend. Values represent means \pm SE ($n = 3$). The dotted arrows represent tillage application. Different lower case letters indicate significant differences between treatment means ($P < 0.05$). For abbreviations, see Table 2.

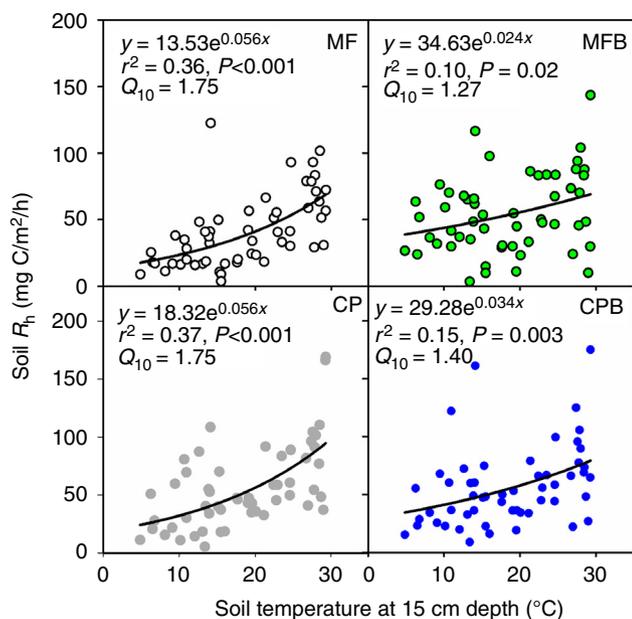


Figure 5 Relationships between soil R_h rates and soil temperature at a depth of 15 cm in the MF, MFB, CP and CPB treatments, respectively. Values represent means of three replications of soil R_h . For abbreviations, see Table 2.

the soil organic substrate for both types of N fertilizer, although the Q_{10} was decreased.

About 49–66% of the annual total NPP was derived from the first planting season across all treatments (Figure 3c). Despite similar NPP values for all treatments in the first planting season, higher NPP values were generally observed on the CP (by 55%) and CPB (by 81%) treatments compared with the MF treatment across the remaining three planting seasons.

The NECB ranged from -2.8 Mg C/ha in the CP treatment to 11.3 Mg C/ha in the CPB treatment over the annual observation (Figure 3d). The greatest positive values of NECB were observed from the treatments with biochar addition in the first planting season. For the non-biochar treatments, significant but small C losses were observed over a 1-yr period.

Discussion

Dynamics of N_2O emissions from soil under vegetable cultivation

The measured annual N_2O fluxes of 26–28 kg N/ha (Figure 3a) are comparable to previous observations in Chinese vegetable fields (Mei *et al.*, 2011). These fluxes are generally about six times higher than that from the rice–wheat rotation system in the same region (Ma *et al.*, 2013). The enhanced N_2O production ratios of both the nitrification and denitrification processes in an acidic vegetable soil (pH = 4.3) have been suggested to contribute to greater N_2O effluxes (Zhu *et al.*, 2011). Temperature is another major factor influencing N_2O emissions from soils, which could be reflected in the differences observed between planting seasons (Figures 2 and 3a). Mei *et al.* (2011) also identified that soil temperature was a significant regulator of seasonal variations in N_2O emissions from field vegetables. Within a 4-yr period, occurrences of higher N_2O emissions due to fertilizer application coincided with high soil temperature conditions (mean: 21 °C), whereas N_2O emissions were not measurable at lower temperatures (mean: 7.2 °C) (Mei *et al.*, 2011).

Biochar effects on N_2O emissions and yield-scaled N_2O emissions

Despite numerous laboratory studies and greenhouse experiments which have showed depressed N_2O emissions due to adding biochar (Cayuela *et al.*, 2014), we found that biochar did not reduce total annual N_2O emissions. This finding agrees with the results of a small-scale vegetable rotation system (Suddick & Six, 2013) and several other field experiments (Xie *et al.*, 2013).

There are a number of possible reasons for the absence of biochar effects on N_2O mitigation. Firstly, the availability of N and soil temperature are far more influential in controlling N_2O emission rates (Mei *et al.*, 2011) than the presence of biochar. Surplus N inputs probably mask the beneficial effect of biochar addition on N transformation, such as

inorganic N absorption and microbial NH_4^+ immobilization (Clough *et al.*, 2010). Significantly reduced N_2O emissions following biochar addition were observed in the second planting season under the cold conditions probably corresponding to low N availability (Figures 1 and 2), although available N was not measured in this study. Others have also found stimulated N_2O emissions in the presence of biochar containing high N (Singh *et al.*, 2010; van Zwieten *et al.*, 2010) or combined with high N addition (Wang *et al.*, 2012). Secondly, the absence of biochar effects on mitigating N_2O emissions could be attributed to the small change in soil pH following biochar addition (Table 2), although soil pH increased after adding biochar at the first measurement which agrees with results of short-term laboratory biochar incubations using acidic soils (Yuan & Xu, 2011). The pH of biochar has been found to significantly decrease by one to four units as a consequence of weathering 3 yr after field incorporation (Jones *et al.*, 2012). The effectiveness of biochar application on mitigating N_2O emissions in our studied soil is consistent with other results for acidic soil with pH <5, but not in neutral and alkaline soils (Cayuela *et al.*, 2014). Thirdly, the enhanced CEC (Cation exchange capacity) of biochar-amended soils can contribute to decreased N_2O emissions (Singh *et al.*, 2010); however, in this study, the soil and the biochar had similar CEC values (31.2 and 24.1 cmol/kg, respectively) (Table 1), and hence, the soil buffering capacity would not have changed greatly.

Biochar amendment did not reduce yield-scaled N_2O emission for either N fertilizer over the year, because of the absence of biochar effects on vegetable yield (Figure 3b). This conclusion is supported by Suddick & Six (2013) who found that biochar additions had neither a positive or negative effect on crop yield nor cumulative annual N_2O emissions in a small-scale vegetable rotation system in northern California. Jones *et al.* (2012) concluded from a 3-yr field experiment that biochar addition to highly productive agricultural land may not produce the benefits to crop yield and biomass seen in other studies (e.g. Singh *et al.*, 2010; Biederman & Harpole, 2012). This is consistent with our current finding. Nitrapyrin application in the CP applied treatment lowered the yield-scaled N_2O emissions, which was because the nitrification inhibitor allowed more N to be taken up by the growing crop and hence increasing the NPP (Figure 3b,c). This result agrees with a previous study on yield-scaled N_2O emissions in winter wheat (Ma *et al.*, 2013), where CP application led to more effective N_2O emission mitigation than dicyandiamide application.

As demonstrated in a recent global meta-analysis of biochar studies, biochar's positive impacts on crop yields are most often reported in China (Crane-Droesch *et al.*, 2013). Based on limited field results, however, Clare *et al.* (2014) estimated that the absolute contribution of biochar-induced soil N_2O emission reductions in China was relatively small. Consequently, before biochar can be commonly used in

China and elsewhere, more attention should be paid to assessing the potential for crop yield improvement and N_2O mitigation.

Biochar effects on R_h , NECB and soil C content

The annual R_h was 3.4–4.7 Mg C/ha (Figure 4) and is comparable to the 3.5–5.1 Mg C/ha from the rice–wheat rotation system in this region (Zheng *et al.*, 2008). The stimulatory effects of biochar application on soil R_h were detectable, although we were not able to partition biochar-derived and SOM-derived CO_2 (Figure 4). The increased soil R_h may have resulted from the increased availability and lability of organic substrates for microbes in the presence of biochar (as shown by the higher A index values in Figure 5) (Fang *et al.*, 2014). On the other hand, Xie *et al.* (2013) demonstrated, in an Ultisol soil, a pronounced C loss of 15.8% following application of wheat straw biochar calculated from changes in SOC stocks over a rice-growing season. Biochar-derived CO_2 emissions have also been observed 2 yr after biochar application (Major *et al.*, 2010) not just initially (Smith *et al.*, 2010b). In addition, in agreement with the findings of Fang *et al.* (2014), the presence of biochar decreased the Q_{10} values of SOC mineralization (Figure 5), suggesting a high C sequestration potential of biochar application in soil under future warming conditions. Nonetheless, no consensus has been reached on how much biochar decomposes and for how long it can reside in soil under field conditions. The greater soil R_h in the CP treatment could be partly ascribed to the increased C quality compared with the MF treatment (A index values of 18.32 vs 13.53; Figures 4 and 5), and urea hydrolysis in the CP treatments also releases extra CO_2 as evidenced by van Zwieten *et al.* (2010). Hence, long-term field observations are warranted, particularly with isotopic techniques.

The positive value of NECB represents ecosystem C gain after harvest on a crop season scale (Smith *et al.*, 2010a). In the four vegetable rotations in this study, the C loss could be mainly attributed to soil R_h and the harvest of above-ground biomass from the vegetable field (Figures 3 and 4). Carbon entering into the field was due mainly to biochar application and the residue of below-ground biomass. The negative values of NECB indicate that our experimental field exhibited C loss without biochar addition (Figure 3d), which is consistent with the observations of Jia *et al.* (2012) from an intensive vegetable field in southeast China. The positive values of NECB observed in the biochar-added treatments (Figure 3d) suggest that biochar application contributes greatly to soil C sequestration, which agrees well with our recent meta-analysis of biochar (Wang *et al.*, 2015). Over the 1-yr period, the NECBs were significantly affected by biochar addition (Figure 3d). Despite greater R_h observed from biochar-added treatments, biochar addition clearly

contributed to the greatest C gain compared with the corresponding treatments without biochar addition. This finding supports the results of Woolf *et al.* (2010), who suggested that the direct input of biochar-C in soils is one of the largest beneficial feedbacks for mitigating climate change in agriculture.

The increase of SOC averaged by 61% after biochar addition (Table 2) agrees with the finding of Biederman & Harpole (2012). Biochar increased SOC due mainly to its recalcitrant C, thus contributing to soil C sequestration on a scale of tens to thousands years (Kuzyakov *et al.*, 2009; Wang *et al.*, 2015). More importantly, biochar can be easily obtained from the slow pyrolysis of many kinds of biomass (Woolf *et al.*, 2010; Lehmann *et al.*, 2011). The large increase of soil C following biochar incorporation (mean: 14.7 Mg C/ha) may substantially mitigate the net global warming potential (Woolf *et al.*, 2010), although biochar addition failed to depress both N₂O emissions and soil R_h from the acidic vegetable field in our study (Figures 3a and 4). No difference in soil total N was observed (Table 2), which is consistent with previous reports on biochar application to soils cultivated with maize and grass (Jones *et al.*, 2012) but in contrast to the conclusion of Biederman & Harpole (2012). The detailed and specific characteristics of the biochar and soil, as well as the application amount of biochar, should be taken into consideration when evaluating both soil C sequestration and GHGs mitigation.

Conclusions

In conclusion, application of wheat biochar did not reduce total annual N₂O emissions regardless of N fertilizer type, whereas both biochar and CP additions reduced N₂O emissions under cold conditions. Relative to the MF treatment, biochar and CP applications stimulated soil R_h via additional C sources. Nevertheless, biochar addition significantly contributed to C gain in the vegetable field compared with the non-biochar-added treatments. The effect of nitrapyrin on N₂O mitigation should be further investigated in vegetable ecosystems. The differential responses of yield-scaled N₂O emissions in the MFB and CPB treatments indicate that further studies on the combined effects of biochar and nitrification inhibitors are required. Long-term field experiments are necessary to prove the negligible effects of biochar addition on N₂O emissions and crop biomass, as well as the stimulatory effects on both soil R_h and NECB observed under vegetable cultivation.

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