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The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N_2O emissions from an intensively managed vegetable field in southeastern China

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Abstract

The influences of nitrification inhibitor (NI) and biochar incorporation on yield-scaled N_2O in a vegetable field were studied using the static chamber method and gas chromatography. An experiment was conducted in an intensively managed vegetable field with 7 consecutive vegetable crops in 2012–2014 in southeastern China. With equal annual amounts of N ($1217.3\,\mathrm{kg\,N\,ha^{-1}\,yr^{-1}}$), 6 treatments under 3 biochar amendment rates, namely, $0\,\mathrm{t\,ha^{-1}}$ (C0), $20\,\mathrm{t\,ha^{-1}}$ (C1), and $40\,\mathrm{t\,ha^{-1}}$ (C2), with compound fertilizer (CF) or urea mixed with chlorinated pyridine (CP) as NI, were studied in these field experiments. The results showed that although no significant influence on soil organic carbon (SOC) content or total nitrogen (TN), CP could result in a significant increase in soil pH during the experimental period. CP significantly decreased cumulative N_2O emissions by 15.9–32.1 % while increasing vegetable yield by 9.8–41.9 %. Thus, it also decreased yield-scaled N_2O emissions significantly. In addition to the differential responses of the soil pH, biochar amendment significantly increased SOC and TN. Additionally, compared with the treatments without biochar addition, cumulative N_2O emissions showed no significant difference in the CF or the CP group treatments but increased slightly (but not significantly) by 7.9–18.3 % in the CP group treatments. Vegetable yield was enhanced by 7.1–49.5 % compared with the treatments without biochar amendment, and the yield-scaled N_2O emissions were thus decreased significantly. Furthermore, treatments applied with CP and biochar incorporation slightly increased yield-scaled N_2O emissions by 9.4 %, on average, compared with CP-C0. Therefore, the incorporation of CP could serve as an appropriate practice for increasing vegetable yield and mitigating N_2O emissions in intensively managed vegetable fields and should be further examined in various agroecosystems.

1 Introduction

Nitrous oxide (N_2O) is an important greenhouse gas and also contributes to ozone depletion in the stratosphere (IPCC, 2007; Saggar et al., 2007; Ravishankara et al., 2009). N_2O emissions increased from 10 to 12 Tg N_2O-N yr^{-1} between 1900 and 2000 and may reach 16 Tg N_2O-N yr^{-1} by 2050 (Bouwman et al., 2013). The increase in nitrogen (N) fertilizer application in agricultural ecosystems has been recognized as a major source of N_2O, representing approximately 60 % of the global anthropogenic emission rates in 2005 (Smith et al., 2007; Park et al., 2012). Emissions from soils increase markedly following the application of N fertilizers and animal manures for the purpose of increasing crop production (Mosier et al., 1998; Davidson, 2009).

Increasing grain yields is a boundary condition for "greening" Chinese agriculture in light of the increasing food demand in China. The high N fertilizer application rate contributes to high crop yields (Qin et al., 2010) but, inevitably, also to high N_2O emissions (Ding et al., 2007) and nitrate leaching (Li et al., 2007). To minimize the overall greenhouse gas (GHG) impact of agriculture while increasing crop production, the amount of N_2O emitted per unit of crop production (yield-scaled N_2O emissions) needs to be considered (Van Groenigen et al., 2010). The yield-scaled N_2O emissions approach has been suggested as a more comprehensive index to assess N_2O emissions in agricultural ecosystems (Van Groenigen et al., 2010; Grassini and Cassman, 2012). This approach is attractive in view of the need to balance crop production with the mitigation of N_2O emissions from agriculture. Only a few studies have directly addressed yield-scaled emissions in agriculture cropping systems (Halvorson et al., 2010; Wei et al., 2010; Gagnon et al., 2011), particularly intensively managed vegetable systems.

The area from which vegetables are harvested in China represents approximately 45 % of the world total (FAOSTAT, 2009). In China, the area devoted to vegetable crops has increased from 3.33 million ha in 1976 to 24.8 million ha in 2006, representing 14.5 % of the total cropland in China (FAO, 2011). Moreover, intensively managed vegetable cultivation represents a major source of N_2O emissions in the agricultural sector in China (Zheng et al., 2004; Wang et al., 2011) as a result of the practical characteristics of high N fertilization, intensive irrigation and favorable environmental conditions that are associated with this type of agriculture. Previous studies have shown that annual N fertilizer inputs are extremely high for certain intensively managed vegetable fields (Ju et al., 2006; Xiong et al., 2006; He et al., 2009). These levels are almost 3–4 times the fertilizer levels used for non-vegetable crops in fields where 2 crops are grown per year (Zheng et al., 2004). As a consequence, N_2O emissions from N fertilizer in vegetable ecosystems represent 20 % (Zheng et al., 2004) or 21.4 % (Wang et al., 2011) of the total emissions from China's farmland.

Alternative practices that could reduce N_2O emissions without necessarily reducing N inputs or crop yields have also been considered, such as nitrification inhibitor (NI) application (Zaman et al., 2009; Ji et al., 2011) and biochar amendment (Zhang et al., 2010; Jia et al., 2012; Wang et al., 2013) in agricultural soils. NIs have been used in the field to improve the efficiency of fertilizers and to reduce both nitrate leaching and denitrification by maintaining the N in the soil as NH_4^+ (Majumdar et al., 2000; Pathak and Nedwell, 2001; Malla et al., 2005; Chen et al., 2010), thus mitigating N_2O emissions and increasing the crop yield from the agricultural ecosystem. A newly developed NI, namely, chlorinated pyridine (CP), 6-chloro-2-trichlormethylpyridine, produced by chlorinating 2-methylpyridine, has been used in agricultural ecosystems to mitigate GHG emissions and simultaneously increase crop yield (Ma et al., 2013; Xiong et al., 2013). Biochar amendment of soils is currently being considered as a means of mitigating climate change by sequestering carbon (C) while concurrently improving soil properties and functions (Lehmann, 2007; Woolf et al., 2010; Zhang et al., 2012a). However, the effects of biochar amendment induced by biochar addition on N_2O emissions may be either positive or negative depending on the inherent characteristics of biochar, the addition of exogenous N and the soil water regime (Spokas and Reicosky, 2009; Xie et al., 2013; Cayuela et al., 2013, 2014). The soil pH of intensively managed vegetable fields has been found to be much lower than that of other agricultural ecosystems due to the input of large amounts of N. Most likely, this lower pH will cause a negative effect if biochar amendment is used to mitigate N_2O emissions (Cayuela et al., 2014). Overall, based on previous results, both CP application and biochar amendment could serve to decrease yield-scaled N_2O emissions in various agriculture ecosystems. However, information for investigating the combined effects of CP application and biochar amendment incorporation on yield-scaled N_2O in intensively managed vegetable agriculture is limited.

Accordingly, we estimated the effect of CP application and biochar amendment incorporation on yield-scaled N_2O emissions in intensively managed vegetable agriculture in southeastern China. The objective of the study was to find appropriate practices for increasing vegetable yield and mitigating N_2O emissions from intensively managed vegetable fields.

2 Materials and methods

2.1 Experiment site and biochar properties

A field experiment was conducted at a suburban site (31°59′ N, 118°51′ E) in Nanjing City, Jiangsu Province, China, from 2012 to 2014. This area has a subtropical monsoon climate with an annual mean rainfall of 1107 mm and an annual mean air temperature of 15.34 °C. The selected site had been conventionally continuously cultivated with vegetables for approximately 10 years and is a typical vegetable field. The studied soil was classified as Fimi-Orthic Anthrosols (RGCST, 2001), with a bulk density of 1.2 $\mathrm{g\,cm^{-3}}$, a total porosity of 51 %, a clay (< 0.002 mm diameter) fraction of 30.1 %, a silt (0.002–0.02 mm diameter) fraction of 64.7 % and a sand (0.02–2 mm diameter) fraction of 5.2 %. The main properties of this soil are as follows: pH, 5.52; total N, 1.90 $\mathrm{g\,kg^{-1}}$; organic carbon, 15.6 $\mathrm{g\,C\,kg^{-1}}$; and CEC (cation exchange capacity), 31.2 $\mathrm{cmol\,kg^{-1}}$.

For the field experiment, biochar was produced from wheat straw at the Sanli New Energy Company in Henan, China by pyrolysis and thermal decomposition at 400 °C. The biochar had a carbon content of 467 $\mathrm{g\,C\,kg^{-1}}$ and an N content of 5.9 $\mathrm{g\,N\,kg^{-1}}$.

The initial values of pH, CEC and ash content were 9.4, 24.1 cmol kg^{-1} and 20.8 %, respectively.

2.2 Treatments and vegetable management

6 treatments with the same amount of total N were applied in triplicate for 7 consecutive vegetable crops from 12 April 2012 to 12 June 2014 in Nanjing, China. Each plot had an area of 7.5 m^2 and measured 3 m × 2.5 m. Biochar was applied at rates of 0, 20 and 40 t ha^{-1} (C0, C1 and C2, respectively) with compound fertilizer (CF) or urea fertilizer mixed with CP (CP). All treatments received the same amount of N fertilizer based on the local practices during the experimental period. The total N application rate for each treatment was equal, 1217.3 kg N ha^{-1} yr^{-1} across the experimental period, of which 312.5 kg N ha^{-1} was applied for Amaranth (*Amaranthus mangostanus* L.) and Coriander (*Coriandrum sativum* L.), 600 kg N ha^{-1} was applied for Tung choy (*Ipomoea aquatica Forssk.*) and 250 kg N ha^{-1} for Baby bok choy (*Brassica chinensis* L.). Compound fertilizer with an $m(\mathrm{N}) : m(\mathrm{P_2O_5}) : m(\mathrm{K_2O})$ ratio of 15 : 15 : 15 was used for the CF group treatments, while the corresponding P and K fertilizers were broadcast in the form of calcium phosphate and potassium chloride, respectively, in addition to the CP urea for the CP group treatments. All fertilization occurred before transplanting and as the base fertilizer for each vegetable crop except for Tung choy, which had 312.6 kg N ha^{-1} as basal fertilization and 287.4 kg N ha^{-1} as top-dressing according to the local farmers' practice. Additionally, both P and K fertilizers for the CP group treatments were applied as top-dressing for the Tung choy growing period as well. Biochar was added once to the vegetable fields before sowing of the first vegetable crop (Amaranth) on 8 April 2012 and was incorporated with the soil by hand plowing at a depth of 20 cm.

7 vegetable crops were grown successively during the entire observation period (12 April 2012 to 12 June 2014). Each type of vegetable was seeded by hand and harvested at the appropriate mature stage according to the local farmers' practice. Fur-

thermore, a short fallow period was imposed after fresh biomass was harvested from each vegetable crop. Soon after harvesting each vegetable crop, the field was tilled to a depth of approximately 12–15 cm. A protective plastic film was used to cover the crops according to the growth requirements of each vegetable crop, i.e., from 12 April 2012 to 25 May 2012 for Amaranth and from 20 November 2012, to 25 May 2012 and 5 November 2013 to 15 March 2014 for Baby bok choy, as Amaranth and Baby bok choy require relatively warm weather conditions for growth. All the other management procedures, including crop species, tillage, irrigation, and pesticide and weed control, followed the local farmers' practices.

2.3 Measurements of N_2O fluxes, soil samples and environmental factors

A static opaque chamber method was used to collect air samples from the experimental sites from 3 replicates for each treatment. Each chamber was made of PVC and consisted of a chamber body (50 cm × 50 cm × 50 cm). The outside of the chamber was coated with sponge and aluminum foil to prevent the effects of high temperatures on the chamber. The chamber was installed on a channel. The channels were inserted 0.1 m deep in the soil in each plot and filled with water to make the chamber gas-tight. Sampling was conducted between 8:30 and 10:30 LT in the morning every other day for 1 week after fertilizer application and then once per week thereafter. Gas fluxes were measured on 121 occasions over the 2 year period. On each sampling occasion, air samples were taken 0, 10, 20 and 30 min after chamber closure. The samples, collected in 20 mL syringes, were returned to the laboratory, and the N_2O was determined on the same day with a gas chromatograph (Agilent 7890A, Agilent Ltd, Shanghai, China) equipped with an electron capture detector (ECD). The carrier gas was argon-methane (5 %) at a flow rate of 40 mL min^{-1}. The column and ECD temperature were maintained at 40 and 300 °C, respectively. Concentrations of N_2O were quantified by comparing the peak area with those of reference gases (Nanjing special gas factory, Nanjing, China). N_2O fluxes were calculated by using the linear increases in gas concentration with time. The mean flux for 1 vegetable crop was calculated as the average of all measured fluxes. The measured fluxes were weighted by the interval between 2 measurements (Xiong et al., 2006). The cumulative seasonal N_2O was calculated as the product of the mean flux and the seasonal duration.

Except for the soil that was analyzed immediately before the experiment in April 2012, another batch of soil samples for each treatment was collected on 12 June 2014 and stored for laboratory analysis. In accordance with Lu (2000), the soil texture was measured using pipette analysis, the total soil organic carbon (SOC) was analyzed by wet digestion with H_2SO_4–$K_2Cr_2O_7$, and the TN was determined by semi-micro Kjeldahl digestion using Se, $CuSO_4$ and K_2SO_4 as catalysts. The soil pH was measured at a volume ratio of 1 : 2.5 (soil to water ratio) using a PHS-3C mv/pH detector (Shanghai, China). The soil temperature was measured at a depth of 15 cm beneath the collection point when the gas samples were collected. The soil water content was determined from soil samples collected at a depth of 10 cm. These samples were collected from all treatments on each gas sampling date. The samples were dried at 105 °C for 8 h and weighed. The soil moisture content obtained by oven drying was converted to water-filled pore space (WFPS) using the following equation:

$$\mathrm{WFPS} = \frac{\text{volumetric water content (cm}^3\,\text{cm}^{-3}\text{)}}{\text{total soil porosity (cm}^3\,\text{cm}^{-3}\text{)}}. \quad (1)$$

Here, total soil porosity = [1 – (soil bulk density (g cm^{-3})/2.65)] with an assumed soil particle density of 2.65 (g cm^{-3}). The total soil bulk density was determined with the cutting ring method to 0–10 cm depth according to Lu (2000).

2.4 Estimation of vegetable yields and yield-scaled N_2O emissions

The fresh vegetable yields were measured after each vegetable growth period by weighing all of the above-ground vegetable parts that were grown in each plot.

The yield-scaled N_2O emissions were related to crop yield as in Van Groenigen et al. (2010) and Grassini and Cassman (2012) and were calculated as follows:

$$\text{Yield-scaled } N_2O \text{ emissions} = \frac{\text{Cumulative } N_2O \text{ emissions}}{\text{vegetable yield } (\text{kg}\,N_2O\,\text{t}^{-1} \text{ yield})}. \quad (2)$$

2.5 Data processing and statistics

The values presented are given as arithmetic means ± standard error (SE). All figures in this study were plotted in Microsoft Excel 2003. Significant differences on soil temperature and WFPS among different vegetable crops were determined by the non-parametric Kruskal–Wallis test. A 2-way ANOVA was used to analyze the effects of CP, biochar and their interactions on vegetable yield, N_2O emissions, and yield-scaled N_2O emissions throughout the experimental period. A Tukey's multiple range tests was used to determine if significant differences occurred between the treatment means at a significance level of 0.05. All statistical analyses were performed using JMP, ver. 7.0 (SAS Institute, USA, 2007).

3 Results

3.1 Soil properties and soil microclimate

To analyze the soil properties, soil samples were collected at the end of the field experiment under the different treatments. The results of this analysis are presented in Table 1. CP had no significant influence on TN or SOC during the experimental period. However, significant increases in soil TN were observed in treatments with biochar amendments (Tables 1, $p < 0.001$). Compared with the treatments without biochar amendments, soil TN was increased by 81.5–99.3 and 44.8–63.2 % for the CF and CP group treatments, respectively. Similarly to soil TN, SOC increased significantly in the treatments with biochar amendment (Tables 1, $p < 0.001$). SOC was enhanced by

66.9–85.1 and 80.4–81.3 % for the CF and CP group treatments, respectively, compared with the treatments without biochar amendment. Moreover, CP increased soil pH significantly by 18.4–59.1 % compared with the treatments without CP ($p < 0.001$), and biochar amendment decreased soil pH significantly by 30.2–30.9 and 7.2–15.7 % for the CF and CP group treatments (Table 1, $p < 0.001$), respectively. Significant interactions between CP and biochar were observed to affect soil TN ($p < 0.05$) and soil pH ($p < 0.001$) throughout the intensive agriculture experimental period.

No statistical differences in WFPS and soil temperature were detected among all the treatments over the whole experimental period (data not shown). Dynamic variation of soil temperature was detected with seasonal change of outside temperature although plastic film was set sometimes in low-temperature seasons in the vegetable field. As shown in Fig. 1a and b, significant differences were found between different vegetable crops, with high temperature in summer and low in winter season ($p < 0.001$). Moreover, soil WFPS rates ranged from 31.93–76.04 % across all the experimental period. No significant difference on WFPS rates were detected among vegetable crops (Fig. 1c).

3.2 N_2O emissions

The dynamics of N_2O fluxes from all the treatments across the 7 vegetable growth crops are shown in Fig. 2. The pattern of these fluxes was relatively consistent and was sporadic and pulse-like. N_2O fluxes showed a similar trend during the same period in both years. Following basal fertilization, tillage and irrigation, N_2O emissions ranged from 17.2–3405.9 $\mu g\,N\,m^{-2}\,h^{-1}$ were observed in all treatments across the experimental period. N_2O emissions primarily occurred with the increase in soil temperature during the summer, from May to October. However, as shown in Fig. 2d and Fig. 2g, no significant N_2O peaks were found in certain vegetable crops to which basal or topdressing fertilization was added because of the low soil temperature after fertilization in the vegetable field.

Cumulative N_2O emissions for each treatment across the entire experimental period are shown in Table 3. These emissions varied widely among different crops during the individual vegetable crop-growing season. Additionally, the cumulative N_2O emissions showed significant differences among all the treatments. The greatest N_2O-N flux was observed in the CF-C0 treatment (54.6 ± 1.5 kg N ha^{-1}), whereas the lowest flux was in the CP-C0 treatment (37.1 ± 4.4 kg N ha^{-1}). As shown in Tables 2 and 3a, significant decreases in cumulative N_2O emissions were detected in the treatments with NI. The decreases ranged from 15.9–32.1 % compared with the treatments without NI application ($p < 0.001$). In addition, biochar amendment had no significant influence on cumulative N_2O emissions in both CF and CP group treatments. However, as shown in Table 3, slight increases in cumulative N_2O emissions were observed. These increases were 7.9 and 18.3 % for CP-C1 and CP-C2, respectively, but were not significant compared with CP-C0. Furthermore, no significant interaction between CP and biochar amendment was observed to affect cumulative N_2O emissions throughout the intensive vegetable experimental period (Table 2).

3.3 Vegetable yield and yield-scaled N_2O emissions

Table 3 shows details of the fresh weight for each vegetable crop. The highest fresh weight yield for the 7 consecutive vegetable crops was $535.4 \pm 16.7 \times 10^3$ kg ha^{-1} for CP-C1, an increase of 71.7 % compared with CF-C0. Both CP application ($p < 0.001$) and biochar amendment ($p < 0.05$) significantly increased the yield in the intensively managed vegetable system over the experimental period. As shown in Table 3, NI significantly increased vegetable yield by 9.8–41.9 % while biochar amendment also resulted in significant increases of 7.1–49.5 % in vegetable yield, compared with the treatments without CP nor biochar, respectively. Moreover, significant interactions between CP and biochar amendment were observed to affect vegetable yield throughout the intensive vegetable experimental period (Table 2, $p < 0.05$).

Table 3 shows the yield-scaled N_2O emissions, which were related to the cumulative N_2O emissions and the fresh weight yield ranged from 0.074 ± 0.004 to

$0.175 \pm 0.017\,\mathrm{kg\,N_2O\,t^{-1}}$ yield over the entire experimental period, and the lowest value of yield-scaled N_2O emissions was $0.074 \pm 0.004\,\mathrm{kg\,N_2O\,t^{-1}}$ yield for CP-C0, namely, the treatment with CP application and without biochar amendment. Both CP application ($p < 0.001$) and biochar amendment ($p < 0.05$) significantly decreased the yield-scaled N_2O emissions during the entire experimental period. Furthermore, significant interactions between CP and biochar were observed to affect yield-scaled N_2O emissions throughout the experimental period (Table 2, $p < 0.05$).

4 Discussion

4.1 Effects of CP as nitrification inhibitor on N_2O emissions and vegetable yield

N_2O emissions are directly related to the amount of mineral N available in the soil. A two-way ANOVA indicated that seasonal N_2O emissions during the vegetable-growing periods were significantly affected by CP application (Table 2, $p < 0.001$), in agreement with previous results (Ma et al., 2013; Xiong et al., 2013). Different types of NI that were effective in reducing N_2O have been reported by previous studies (Xu et al., 2000; Boeckx et al., 2005; Zaman et al., 2008, 2009; Zaman and Blennerhassett, 2010). CP could efficiently inhibit the activity of the ammonia oxidase and reducing the abundance of *nirK* gene in vegetable soil, and thus inhibit the nitrification process while regulating the NO_3^- and NH_4^+ content of soil (Di et al., 2009, 2014), which is highly related to mitigate N_2O emissions. Moreover, CP application in vegetable soil resulted in a significant increase in soil pH though insignificant in soil TN and SOC across the 7 consecutive vegetable growing periods (Table 1, $p < 0.001$). The pH increase effect may have been a result of the production of hydroxyl (OH^-) ions during urea hydrolysis (De Boer and Kowalchuk, 2011). Similarly, Zaman et al. (2008) have reported a low rise in soil pH after applying Agrotain-treated urea to pasture soil. A laboratory incubation of DCD decomposition also showed a soil pH increase with the addition of urine with or without DCD (Singh et al., 2008). Zhu et al. (2011) reported a negative correlation between soil pH and N_2O emission rate in vegetable soils, which may indicate that the increasing of soil pH is another factor mitigating N_2O emissions.

A previous study has shown that N fertilizers combined with NI such as DCD and DMPP may improve the yield and quality of agricultural and horticultural crops (Pasda et al., 2001). Similarly, CP produced a significant increase in vegetable yield in our study across the experimental period (Tables 2 and 3b, $p < 0.001$). Possible explanations for higher crop yields obtained with NH_4^+-containing fertilizers supplemented with CP include the reduction of N losses by leaching and volatilization and the improved bio-availability of N in vegetable soil resulting from the added CP. Ma et al. (2013) reported that 2 types of NIs (CP and DCD) increased average wheat yield by 9.7 % under conventional and no-till practices during the winter wheat-growing season.

4.2 Effects of biochar on N_2O emissions and vegetable yield

As shown in Tables 2 and 3a, biochar had no significant influence on cumulative N_2O during the experimental period. Decreases in the net emissions of N_2O from certain agricultural ecosystems as a result of soil amendment with biochar have been well documented by previous studies (Cayuela et al., 2014; Felber et al., 2014). Additionally, a meta-analysis of 30 papers (published from 2007 to 2013) by Cayuela et al. (2014) found that soil N_2O emissions, which were affected by biochar characteristics, soil characteristics and N fertilizer type, were reduced by 54 % in both laboratory and field studies. However, biochar amendment had no significant influence on cumulative N_2O emissions during the experimental period and even slightly increased cumulative N_2O by 7.9–18.3 % in the CP group treatments (Tables 2 and 3a). Thus, the mitigating effect of biochar amendment on N_2O emissions did not work in the intensively managed vegetable field in this study, which is in consistent with previous short-term laboratory incubation results in acidic soils (Yuan and Xu, 2011; Wang et al., 2014). This finding may firstly be due to the decrease in soil pH in the treatments amended with biochar (Table 1). In contrast with the significant increase in soil pH due to the liming effect of biochar generally reported in previous studies (Biederman and Harpole, 2012; Zhang et al., 2010), soil pH decreased significantly in the plots amended with biochar (Table 1, $p < 0.001$). Biochar brings extra carbon source to provide the energy for heterotrophic nitrification process though biochar was considered to be stable in soil, which may also cause an increase in the H^+ content due to nitrification processes in the soil (Schmidt, 1982; De Boer and Kowalchuk, 2001; Wrage et al., 2001) and thus decrease the soil pH significantly. In addition, the decrease in soil pH may, most likely, be attributed to the weathering effect of biochar after 2 years of incorporation into fields (Jones et al., 2012; Spokas, 2013), particularly in intensively managed vegetable fields. Yao et al. (2010) reported that the pH of biochar samples decreased from 8.4 to 7.5, due primarily to the loss of base cations through leaching and probable carbonation during the weathering process, and biochar offers the practical benefits of high N fertilization input and may also be weathered more easily in vegetable soils. Cayuela et al. (2014) also reported that the effectiveness of biochar application on mitigating N_2O emissions was significant in neutral and alkaline soils but not in acidic soils with $pH < 5$. Low soil pH may result in adversely affect the activity of nitrous oxide reductase in vegetable field (Liu et al., 2010). The decrease in soil pH may also increase the heterotrophic nitrification rate, which may cause the increase of N_2O emissions in vegetable field (Zhu et al., 2011) though heterotrophic nitrification is generally considered to be a minor source of N_2O (Anderson et al., 1993). Moreover, Sánchez-García et al. (2014) found that biochar increased cumulative N_2O emissions in the soil where ammonia oxidation and nitrifier-denitrification (ND) being the major processes generating N_2O emissions, and whereas decreased N_2O emissions in the soil where denitrification is the main pathway leading to N_2O emissions under the same experimental conditions. Vegetable field had a high amount of N input and may expect ammonia oxidation and linked ND being important processes generating N_2O (Wrage et al., 2001; Huang et al., 2014).

Biochar amendment significantly increased SOC and soil TN (Table 1, $p < 0.001$) and thus significantly improved vegetable yield compared with the treatments that received no biochar addition (Table 2, $p < 0.05$), which agree well with previous reported benefit of adding biochar to soils (Major et al., 2010; Jia et al., 2012). In our study, SOC increased significantly by 66.9–85.1 % in the treatments amended with biochar over the experimental period (Tables 1, $p < 0.001$). This result is in consistent with the finding that average SOC increased by 61 % due to biochar addition, reported in a meta-analysis by Biederman and Harpole (2012). Biochar amendment significantly increased SOC, most likely due to its inert recalcitrant C component, which can contribute to soil carbon sequestration, at least over periods of decades to millions of years (Kuzyakov et al., 2009, 2014; Lehmann et al., 2011). Additionally, biochar amendment also significantly increased soil TN (Table 1, $p < 0.001$), which is in consistent with previous study in a rice paddy (Zhang et al., 2012b). Most likely, this difference in soil TN is probably due to the release of N in soil from the biochar (Singh et al., 2010; Schouten et al., 2012). The N content of the biochar used in our experiment was 5.9 $\mathrm{g\,N\,kg^{-1}}$, and this potential N source may also have increased the soil TN. Additionally, the amendment of biochar would offer a further opportunity to achieve N fertilizer savings in vegetable soil, which may have resulted in an increase of soil TN due to the higher inorganic N absorption effects, as seen in comparison with treatments without biochar amendment in an intensively managed vegetable field (Ding et al., 2010). Furthermore, vegetable yield enhancement effect of biochar may also be associated with increases in root exudation in the plots amended with biochar (Gregory, 2006). Biochar amendment in agricultural soil may stimulate microbial activity, resulting in nutrient release (Steinbeiss et al., 2009), reducing nutrient leaching (Laird et al., 2010), and improving crop nutrient availability and plant N uptake (Saarnio et al., 2013) in the intensively managed vegetable field. Moreover, as shown in Table 3, a significant difference in vegetable yield among the treatments was found under different biochar application rates with compound N fertilization (CF-C0, CF-C1 and CF-C2), in agreement with the results reported by Jeffery et al. (2011) although the plots amended with biochar showed a significantly lower soil pH (Table 1, $p < 0.001$).

4.3 The combined effects of CP and biochar incorporation on yield-scaled N_2O emissions

Analyzing N_2O emissions on a yield basis provides interesting information for estimating the environmental impacts of intensive agricultural production systems. As shown in Table 3, yield-scaled N_2O emissions ranged from 0.074 ± 0.004–0.175 ± 0.017 kg N_2O-N t^{-1} yield, much lower than previously reported values (Van Groenigen et al., 2010; Ma et al., 2013). Venterea et al. (2011) observed that grain N yield-scaled N_2O emissions ranged between 4 and 8 kg N_2O-N t^{-1} N and that aboveground N yield-scaled N_2O emissions ranged between 2 and 7 kg N_2O-N t^{-1} N in a corn-soybean rotation in Rosemount, USA, with a fertilizer N input of 146 kg N ha^{-1} yr^{-1}. Ordinarily, vegetable crops require higher N application rates than staple food crops such as rice, wheat and maize (Li and Wang, 2007). Moreover, the leafy vegetables in this study differed from other crops and all aboveground portions of the vegetable plants were considered as the yield, resulting in low values of yield-scaled N_2O in our vegetable field.

Overall, CP significantly decreased yield-scaled N_2O emissions across the experimental period (Tables 2 and 3c, $p < 0.001$), attributing to the N_2O reducing and vegetable increasing effects of CP in intensively managed vegetable field. Our results indicate that the yield-scaled N_2O emissions were minimal in the CP-C0 treatment (0.074 ± 0.004 kg N_2O-N t^{-1} yield). This treatment showed the lowest cumulative N_2O emissions (37.1 ± 4.4 kg N ha^{-1}) and the second highest vegetable yield (500.3 ± 34.9 t ha^{-1}). Under the application of equal amounts of N, CP application was a more efficient way to reduce the yield-scaled N_2O emissions in our case. This approach may significantly improve vegetable yield while causing a decrease in N_2O emissions and, thus, improved agronomic N use efficiency (NUE) in intensively managed vegetable agriculture (Li et al., 2009; Asing et al., 2008). Thus, CP application without biochar amendment (CP-C0) can serve as an appropriate way of mitigating N_2O emissions while increasing vegetable yield in intensively managed vegetable agriculture.

Biochar amendment significantly decreased the yield-scaled N_2O emissions across the experimental period (Tables 2 and 3c, $p < 0.05$), mainly due to the increasing effect of vegetable yield with biochar. Obvious interactions in yield-scaled N_2O emissions were observed between CP and biochar addition (Table 2, $p < 0.05$). Significant interactions in yield were observed though no significant differences in vegetable yield were found among different combinations of CP and biochar as CP-C0, CP-C1 and CP-C2 (Table 3), indicating biochar incorporation rate did not increase vegetable yield when combined with CP application. Most likely, the vegetable yields were relatively high compared with other ecosystems although both CP and biochar separately can significantly increase vegetable yield.

However, no significant interactions in N_2O emissions were observed in the current study. Treatments with CP and biochar incorporation slightly increased the cumulative N_2O by 7.9–18.3 %, but not significantly (Table 3), which may probably due to the reason that biochar be able to diminish the mitigating effect of CP by affecting the N transformation processes in vegetable soil. Di et al. (2014) reported that DCD was highly effective in inhibiting the growth of AOB communities, and reducing N_2O emissions under high soil moisture, whereas Yanai et al. (2007) found that when the soils were rewetted at 83 % WFPS, the suppressive effects of charcoal addition on N_2O emissions were not observed. Thus, biochar addition diminished the mitigation effect of CP on cumulative N_2O emissions in vegetable soil under the high WFPS condition due to frequent irrigations (Fig. 1). Additionally, biochar would increase the heterotrophic nitrification rate in the treatments applied with both CP and biochar (CP-C1 and CP-C2) compared with CP-C0 treatment, and thus increase the N_2O emissions in vegetable field. Since we did not measure the individual N transformation process responding to N_2O emissions, the combined effects of NI and biochar incorporation on N_2O emissions in intensively managed vegetable fields need further study.

5 Conclusions

Yield-scaled N_2O emissions were significantly affected by both CP application and biochar amendment in intensively managed vegetable agriculture. Throughout the experimental period, although significant influences on soil TN and SOC were not found, CP application significantly increased soil pH and vegetable yield while significantly decreasing cumulative N_2O emissions in the intensively managed vegetable field, therefore causing a significant decrease in yield-scaled N_2O emissions over the experimental period. Moreover, biochar amendment significantly increased soil TN, SOC and vegetable yield but had no significant influence on cumulative N_2O emissions, whereas this amendment significantly decreased soil pH and yield-scaled N_2O emissions. CP and biochar incorporation into vegetable soil slightly increased yield-scaled N_2O emissions during the experimental period. Yield gains were the most important factor for lower yield-scale N_2O emissions in our case compared with previous studies. Overall, taking environmental and economic benefits into consideration, CP application in the vegetable field was the best procedure for reducing the yield-scaled N_2O emissions. The long-term combined effects of NI applications and biochar amendment and their underlying mechanisms on N transformation processes in intensively managed vegetable agriculture should be further studied.

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Table 1. Influence of biochar amendment on soil total nitrogen (TN), soil organic carbon (SOC), and soil pH in 6 different treatments over the entire experimental period. Values represent means ± SD ($n = 3$).

Treatments	TN ($\mathrm{g\,N\,kg^{-1}}$)	SOC ($\mathrm{g\,C\,kg^{-1}}$)	pH
T0	1.90	15.6	5.52
CF-C0	1.46 ± 0.03 d	12.7 ± 0.6 c	5.27 ± 0.08 c
CP-C0	1.74 ± 0.12 c	12.8 ± 0.8 c	6.24 ± 0.08 a
CF-C1	2.65 ± 0.02 b	21.8 ± 0.2 b	3.64 ± 0.04 d
CP-C1	2.52 ± 0.16 b	23.2 ± 0.3 a	5.79 ± 0.05 b
CF-C2	2.91 ± 0.05 a	23.5 ± 0.3 a	3.68 ± 0.06 d
CP-C2	2.84 ± 0.12 a	23.1 ± 0.9 a	5.26 ± 0.08 c
P	***	***	***

T0 provides the initial soil condition prior to the experiments;
CF, compound fertilizer;
CP, chlorinated pyridine, a mixture of urea and nitrapyrin;
C0, Biochar 0 $\mathrm{t\,ha^{-1}}$;
C1, Biochar 20 $\mathrm{t\,ha^{-1}}$;
C2, Biochar 40 $\mathrm{t\,ha^{-1}}$.
Means ± SD with different letters in the same column indicate significant differences between treatments according to Tukey's multiple range test ($p < 0.05$).
*** $p < 0.001$.

Table 2. Two-way ANOVA for the effects of biochar (Bc) and chlorinated pyridine (CP) on the cumulative N_2O emissions, vegetable yield and yield-scaled N_2O over the entire experimental period.

Factors	DF	Cumulative N_2O emissions			Vegetable yield			Yield-scaled N_2O emissions		
		SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Bc	2	66.28	1.21	0.3577	12 977.7	9.58	< 0.05	0.0029	6.63	< 0.05
CP	1	570.09	19.29	< 0.001	76 098.8	111.36	< 0.001	0.0171	78.04	< 0.001
Bc × CP	2	86.83	1.47	0.2687	17 542.7	12.95	< 0.05	0.0044	10.13	< 0.05
Model	5	723.21	4.89		106 619.4	31.48		0.0244	22.31	
Error	12	354.67			8126.9			0.0026		

SS: sum of squares;
F value: ratio of mean squares of 2 independent samples;
p value: index of differences between the control group and the experimental group. If $p < 0.05$ and $p < 0.001$, significant differences exists between the control group and the experimental group.

Table 3. Cumulative N_2O emissions, vegetable yield and yield-scaled N_2O emissions under different treatments over the entire experimental period.

Treatments	Amaranth 12 Apr 2012– 10 Jul 2012 (41d/90d)	Tung choy 11 Jul 2012– 19 Nov 2012 (100d/131d)	Baby bok choy 20 Nov 2012– 27 Mar 2013 (98d/127d)	Coriander herb 28 Mar 2013– 30 Jun 2013 (62d/94d)	Tung choy 1 Jul 2013– 4 Nov 2013 (106d/126d)	Baby bok choy 5 Nov 2013– 15 Mar 2014 (67d/129d)	Amaranth 15 Mar 2014– 12 Jun 2014 (58d/89d)	Total rotation (791d)
Cumulative N_2O emissions ($kg\,N\,ha^{-1}$)								
CF-C0	9 ± 0.8 a	17.7 ± 0.9 ab	1.1 ± 0.7 a	4.1 ± 1.2 a	16.5 ± 2.8 a	0.8 ± 0.2 ab	5.3 ± 0.3 a	54.6 ± 1.5 a
CP-C0	6.7 ± 1.2 d	15.6 ± 1.2 b	0.8 ± 0.1 b	2.7 ± 0.5 a	6.5 ± 1.4 c	0.6 ± 0.1 c	3.8 ± 1.6 a	37.1 ± 4.4 d
CF-C1	9.3 ± 0.5 ab	21.3 ± 4.0 a	1.1 ± 0.1 ab	4.4 ± 0.7 a	11.4 ± 3.8 b	0.9 ± 0.2 a	4.8 ± 1.6 a	52.6 ± 10.2 ab
CP-C1	8.3 ± 0.6 c	16.1 ± 0.4 b	0.9 ± 0.2 ab	3.9 ± 1.9 a	9.6 ± 2.3 bc	0.7 ± 0.1 abc	4.7 ± 1.6 a	43.9 ± 5.8 bcd
CF-C2	9.4 ± 0.7 bc	20.7 ± 1.7 a	0.9 ± 0.2 ab	3.6 ± 0.7 a	8.8 ± 2.2 bc	0.7 ± 0.1 abc	4.2 ± 0.6 a	47.3 ± 2.4 abc
CP-C2	8.6 ± 0.9 b	15.7 ± 2.2 b	0.9 ± 0.1 ab	3.1 ± 0.4 a	7.6 ± 0.6 bc	0.6 ± 0.1 bc	3.9 ± 0.8 a	39.8 ± 3.5 cd
Vegetable yield ($t\,ha^{-1}$)								
CF-C0	11.1 ± 1.8 c	83.1 ± 12.4 c	10.2 ± 3.6 d	18.6 ± 1 bc	132.5 ± 6 b	44.9 ± 4.6 a	11.5 ± 4.4 c	311.8 ± 23.8 d
CP-C0	19.8 ± 0.4 a	140.5 ± 11.9 a	58.9 ± 5.7 b	24.7 ± 4.1 b	163.6 ± 16.3 a	47.2 ± 9.3 a	45.5 ± 2 a	500.3 ± 34.9 a
CF-C1	19.3 ± 1.4 ab	95.2 ± 3.9 c	39 ± 8.1 c	25.8 ± 5.2 b	131.9 ± 14.1 b	49.9 ± 6.1 a	16.1 ± 4.4 c	377.2 ± 23.4 c
CP-C1	23.3 ± 0.9 a	123.7 ± 12.6 ab	81 ± 6.5 a	24.9 ± 3.2 b	177.2 ± 14.5 a	61.6 ± 13.2 a	43.7 ± 6.8 ab	535.4 ± 16.7 a
CF-C2	14.6 ± 5.7 bc	128.9 ± 6.8 ab	57.8 ± 11.4 b	12.6 ± 2.6 c	172.2 ± 4.9 a	43.1 ± 17.3 a	17 ± 9.3 c	446.2 ± 36.1 b
CP-C2	19.5 ± 2.9 ab	120.7 ± 7.2 b	62.1 ± 13.4 b	35.3 ± 8.5 a	161.4 ± 9.7 a	56.7 ± 9.4 a	33.9 ± 6.5 b	489.7 ± 12.7 ab
Yield-scaled N_2O emissions ($kg\,N_2O\,t^{-1}$ yield)								
CF-C0	0.814 ± 0.082 a	0.216 ± 0.031 a	0.135 ± 0.105 a	0.218 ± 0.062 ab	0.125 ± 0.026 a	0.019 ± 0.005 a	0.516 ± 0.207 a	0.175 ± 0.017 a
CP-C0	0.338 ± 0.051 c	0.112 ± 0.005 c	0.014 ± 0.002 b	0.131 ± 0.101 b	0.041 ± 0.011 c	0.012 ± 0.004 a	0.084 ± 0.032 c	0.074 ± 0.004 d
CF-C1	0.475 ± 0.027 bc	0.225 ± 0.048 a	0.029 ± 0.01 b	0.176 ± 0.057 ab	0.085 ± 0.035 b	0.017 ± 0.005 a	0.302 ± 0.07 b	0.139 ± 0.027 b
CP-C1	0.337 ± 0.022 c	0.131 ± 0.011 bc	0.029 ± 0.002 b	0.167 ± 0.089 ab	0.054 ± 0.011 bc	0.012 ± 0.027 a	0.114 ± 0.051 bc	0.081 ± 0.009 cd
CF-C2	0.659 ± 0.317 ab	0.161 ± 0.009 b	0.016 ± 0.001 b	0.293 ± 0.083 a	0.051 ± 0.013 bc	0.019 ± 0.011 a	0.302 ± 0.154 b	0.106 ± 0.009 c
CP-C2	0.417 ± 0.065 bc	0.131 ± 0.025 bc	0.015 ± 0.006 b	0.089 ± 0.019 b	0.047 ± 0.005 c	0.011 ± 0.004 a	0.019 ± 0.019 bc	0.081 ± 0.008 cd

See Table 1 for treatment codes. The total range of observation dates includes sampling that occurred during vegetable planting or not during vegetable planting. The values indicate (mean ± SD). Data in the brackets indicate the vegetable growing days/total days including the following fallow period for each vegetable crop in rotation. Different letters indicate a significant difference between treatments ($p < 0.05$).

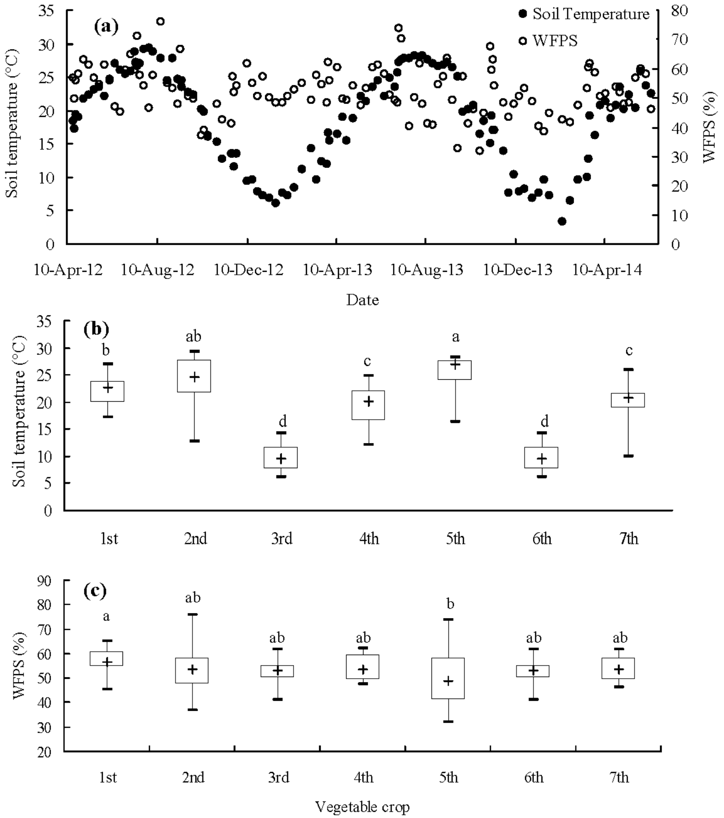

Figure 1. Dynamics of soil temperature (T) and WFPS across the experimental period **(a)**. Box plots for soil temperature **(b)** and WFPS **(c)** in different vegetable crops.
The 1st to 7th mean different vegetable crops in rotation across the experimental period. Different letters indicate significant difference among all treatment medians ($p < 0.05$). The plus mark in the box represents the medians of all data.

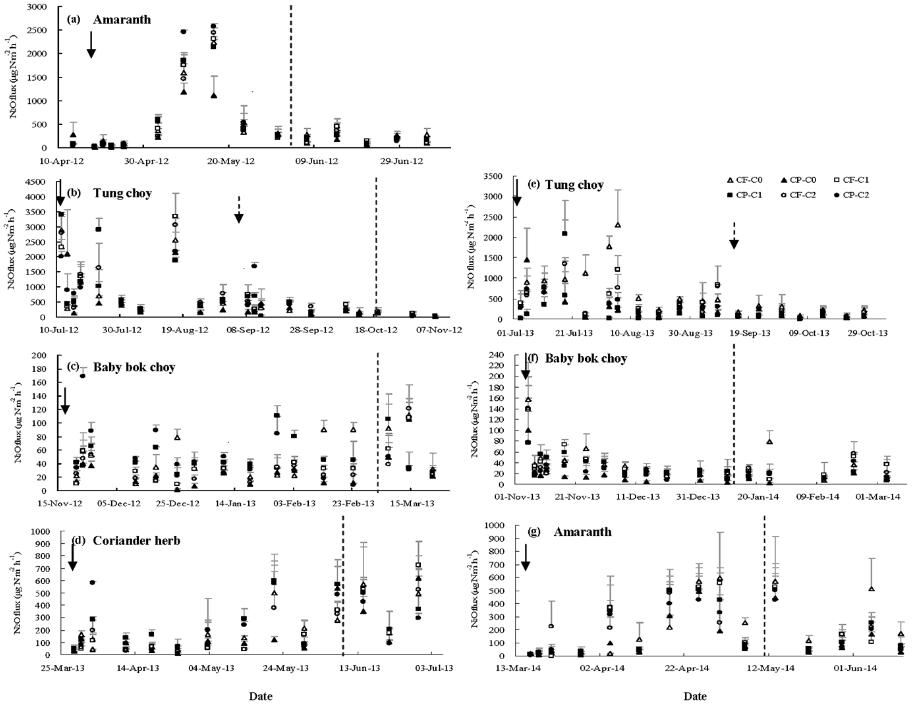

Figure 2. Dynamics of soil N_2O emissions fluxes under different treatments in vegetable fields with 7 consecutive vegetable crops from 2012 to 2014 in southeastern China.
The solid and dashed arrows indicate basal fertilization and top-dressing, respectively. The dashed line apart the vegetable growing and fallow periods. The bars indicate the standard error of the mean (+ SE) for the 3 replicates of each treatment. See Table 1 for treatment codes.

BGD
11, 15185–15214, 2014
The combined effects of nitrification inhibitor and biochar incorporation
B. Li et al.

Title Page
Abstract | Introduction
Conclusions | References
Tables | Figures
◀| |▶
◀ ▶
Back | Close
Full Screen / Esc
Printer-friendly Version
Interactive Discussion