

Response to Referees

Thank you very much for the great support and nice comments of the reviewers! We are now incorporating all the comments from two reviewers into the revised version to improve the manuscript. Please see the following point-to-point answers with the marked-up manuscript version.

Interactive comment on “The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N₂O emissions from an intensively managed vegetable field in southeastern China” by B. Li et al.

Anonymous Referee #1

Received and published: 25 November 2014

General comments

This paper presents data of a two-year experiment on the effect of the nitrification inhibitor (NI) nitrapyrin and of wheat-straw-derived biochar on yield and yield-specific N₂O emission in vegetable production in southeast China. For this purpose, experimental plots of 7.5 m², with three replicates, were established in 2012. Six different treatments, i.e., three different biochar levels (0, 20, 40 t ha⁻¹) combined with two different application forms of nitrogen fertilizer (“compound fertilizer”; and urea with nitrapyrin) were established. The amount of N fertilizer applied was the same for all treatments and amounted to approx. 1200 kg ha⁻¹ yr⁻¹. Yield and N₂O emissions were determined over seven consecutive vegetable periods within the following two years. N₂O emissions were quantified with the static chamber technique, with three replicate chambers for each treatment, and with measurements every other day for one week directly after fertilizer application, and weekly thereafter. Cumulative N₂O emissions were calculated for each cropping season, and related to the respective yield. The authors found that application of the nitrification inhibitor led to an increase in yield and to a decrease in both cumulative N₂O emissions and yield-scaled N₂O emissions, whereas the biochar also effected an increase in yield, but only a decrease in yield-scaled N₂O emissions, but not in cumulative N₂O emissions. In contrast, the combination of both factors slightly increased yield-scaled N₂O emissions. A significant difference between the two different soil amendments was observed with respect to pH: while the application of the NI led to an increase in soil pH, amendment of the soil with biochar from wheat straw was associated with a strong decrease in soil pH below 4 in the treatments without NI.

The paper presents relevant data on a timely topic, i.e., increasing agricultural nitrogen use efficiency, which is especially relevant to vegetable production, as in this special sector of agriculture

incredibly high amounts of nitrogen fertilizer are being used. The experimental design appears appropriate, the work has been conducted properly, and the paper is reasonably well written, although the language and the punctuation need some final check by a professional. The weaknesses of the paper are that:

- (i) Two different kinds of nitrogen fertilizer have been used for the two treatments with and without NI, i.e. a “compound fertilizer” for the no-NI treatment (N form was not specified), and urea + nitrapyrin for the NI treatment;

A: Thank you for your comments! In the revised version, we specified N form for the compound fertilizer on Page 5 line 27-28. We choose the compound fertilizer according to the local farmer's practice. The amount of the cumulative N₂O emissions in agriculture ecosystems were mainly depends on the amount of the N fertilizer, especially in vegetable field with such a high N fertilization application (Harrison and Webb, 2001; Qiu et al., 2010). Since a commercial product of CP for the NI treatment was in the form of urea + nitrapyrin, we can only roughly compare the effects of nitrapyrin. Besides, the corresponding P and K fertilizers were broadcast in the form of calcium phosphate and potassium chloride, respectively, in addition to the CP urea.

References

Harrison, R., and Webb, J. (2001). A review of the effect of N fertilizer type on gaseous emissions. *Advances in Agronomy*, 73, 65-108.

Qiu, W. H., Liu, J. S., Hu, C. X., Zhao, C. S., Sun, X. C., and Tan, Q. L. (2010). Effects of nitrogen application rates on nitrous oxide emission from a typical intensive vegetable cropping system. *Journal of Agro-Environment Science*, 29(11), 2238-2243.

- (ii) Beside total N, no information about the different N species in the soil were available, such as ammonium and nitrate concentrations. This would have been especially useful to interpret the effect of NI and biochar on plant-available N;

A: Thank you for your nice comments! A parallel experiment demonstrated the effects of nitrapyrin and biochar on soil ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) content was supplemented in the revised manuscript and was presented in Figure 3. Page 7 lines 14-20; Page 9 line 13-20.

- (iii) Significant pH effects, induced by the introduction of the soil amendments, superimposed the pure NI and/or biochar effects and were impossible to disentangle from them. Besides, the potential effects of these pH effects were not sufficiently discussed and taken into account for

the interpretation of the experimental results.

A: Yes, you are right! We discussed more on the effects of pH changes on Page 11 line 25-27 and Page 12 line 17-19 in our manuscript. However, it is very difficult for us to disentangle the pure NI and/or biochar effect according to the current treatments. Thank you!

However, despite the weaknesses of the study, the paper could be taken into account for publication in Biogeosciences, if the following specific comments are sufficiently addressed.

A: Thank you so much for your understanding and your great support!

Specific comments:

p. 15187, l. 22-23: Here, the literature is not fully up-to-date. There are more recent papers, e.g., Zhou et al., *Ecosystems* (2014) 17: 286–301.

A: We have revised it on Page 3 line 20. Thank you!

p. 15188, l. 18: Here and throughout the paper, you should use the common name, i.e. nitrapyrin, to facilitate literature search and comparison. The correct IUPAC name of nitrapyrin is 2-chloro-6-(trichloromethyl) pyridine.

A: We have revised CP as nitrapyrin throughout the paper. Thank you!

p. 15188, l. 29–p. 15189, l. 1: Give reason why lower pH will cause a negative effect of biochar amendment.

A: We have revised on Page 4 line 18-20. Thank you!

p. 15189, l. 7: “we estimated”: estimated or quantified?

A: We have revised it on Page 4 line 28. Thank you!

p. 15189, l. 16-17: Give source of climate information.

A: We have revised it on Page 5 line 6. Thank you!

p. 15190, l. 10: “1217.3 kg”: Here and in the following: I suggest omitting the decimals, as one decimal (i.e., 100 g N ha⁻¹) would correspond to 75 mg applied to the 7.5-m² plots. I cannot imagine that this accuracy was achievable during the experiments.

A: We have revised it on Page 5 line 24. Thank you!

p. 15190, l. 14: Specify nitrogen form.

A: We have revised it on Page 5 line 27-28. Thank you!

p. 15190, l. 21-22: How stable was the biochar over the course of the experiment (two years)?

A: Biochar is relatively stable in agriculture soil for it due to its inert recalcitrant C component, so it can contribute long-term C sequestration in soil (Kuzyakov et al., 2014). Thank you!

Reference

Kuzyakov, Y., Bogomolova, I., and Glaser, B.: Biochar stability in soil: Decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis, *Soil Biol. Biochem.* 70, 229-236, 2014.

p. 15190, l. 25: Do not start a sentence with a number, i.e.: Seven...

A: Thank you for your comments! We have revised it on Page 5 line 18 and Page 6 line 7.

p. 15191, l. 7-9: All management procedures should be summarized in a table.

A: We have summarized them in Table 1 on Page 26. Thank you!

p. 15191, l. 15: frame(s), not channel(s)

A: We have revised it on Page 6 line 21. Thank you!

p. 15191, l. 27-28: Give detection limit of your N_2O flux measurements, i.e., minimum detectable N_2O flux.

A: Thank you for your comments! The concentration of the standard gas for measuring N_2O flux is 320 ppb. With the characteristic of high N fertilization and frequent irrigation, the N_2O fluxes in vegetable field are relatively higher than other upland ecosystems, so the N_2O fluxes measured in our experiment were all detectable.

p. 15192, l. 1-2: In which way were the fluxes weighted? Was only the period preceding the measurement used for weighting, or half of the preceding and half of the succeeding period?

A: We just use the period preceding the measurement used for weighting. Thank you!

p. 15192, l. 6: Stored for how long? At which temperature? Well-aerated or in closed bags?

A: We have revised it on Page 7 line 7. Thank you!

p. 15193, l. 3: $\text{kg N}_2\text{O t}^{-1}$ yield is the unit of the fraction, not of the denominator (and it should be $\text{kg N}_2\text{O-N t}^{-1}$ yield).

A: Thank you for your comments! We have revised the unit of yield-scaled N_2O emissions as $\text{kg N}_2\text{O-N t}^{-1}$ yield throughout our manuscript.

p. 15193, l. 16-18: The first two sentences are dispensable.

A: We have deleted the first two sentences on Page 8 line 14. Thank you!

p. 15193, l. 22: "Similar to..."

[A: We have revised it on Page 8 line 17. Thank you!](#)

p. 15194, l. 3-5: Give increase/decrease of pH in absolute terms. To specify a relative change for a logarithmic number is problematic.

[A: We have revised it on Page 8 line 21-22. Thank you!](#)

p. 15194, l. 14: One digit is sufficient.

[A: We have revised it on Page 9 line 2. Thank you!](#)

p. 15194, l. 22: Probably the decimals are dispensable, depending on your detection limit.

[A: We have revised it on Page 9 line 8. Thank you!](#)

p. 15195, l. 9: Replace “In addition” with “In contrast”.

[A: We have revised it on Page 9 line 29. Thank you!](#)

p. 15196, l. 1: Split (too long) sentence between “period, and”.

[A: We have revised it on Page 10 line 20. Thank you!](#)

p. 15196, l. 19: “related to mitigating”

[A: We have revised it on Page 11 line 7. Thank you!](#)

p. 15198, l. 9: “primarily due to“

[A: We have revised it on Page 12 line 25. Thank you!](#)

p. 15198, l. 15: “result in adversely affecting”

[A: We have revised it on Page 12 line 29. Thank you!](#)

p. 15198, l. 20-23: Change sentence slightly to: “. . . biochar increased cumulative N₂O emissions in the soil when ammonia oxidation and nitrifier-denitrification (ND) were the major processes generating N₂O emissions, whereas it decreased N₂O emissions in the soil when denitrification was the main pathway. . .”

[A: We have revised it on Page 13 line 6-9. Thank you!](#)

p. 15199, l. 9: Replace “in a rice paddy” with “in paddy rice”.

[A: We have revised it on Page 13 line 22. Thank you!](#)

p. 15200, l. 6-9: Here, you mix up yield-scaled with grain N yield-scaled N₂O emissions. Venterea et al. (2011) found grain yield-scaled N₂O emissions in the range of 0.046-0.073 kg N₂O-N t⁻¹ yield in conventional tillage, and 0.067-0.1 kg N₂O-N t⁻¹ yield in no-till systems, i.e. comparable with your findings.

[A: Thank you for your comments! We have deleted the incomparable result on Page 14 line 13.](#)

p. 15201, l. 11: “interactions”: Need to be specified between which factors.

A: We have revised it on Page 15 line 4. Thank you!

Table 1: Unclear, where P and *** refer to (last line of table).

A: We have some supplement in Table 2 on Page 27. Thank you!

Figure 2: Font size in Fig. 2 should be increased.

A: We have changed the font size in Figure 2. Thank you!

Figure caption 2: Replace “dashed line apart the vegetable growing and fallow periods” with “dashed line separates the vegetable growing and fallow periods.”

A: We have revised it in Figure caption 2 on Page 31 line 9. Thank you!

Interactive comment on “The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N₂O emissions from an intensively managed vegetable field in southeastern China” by B. Li et al.

Anonymous Referee #3

The paper tries to assess the combined effects of nitrification and biochar application on vegetable yield and N₂O. This is a two-year field experiment, with useful information collected. It is well within the scope of Biogeoscience. However, the manuscript suffers from some major and minor problems.

A: Thank you very much for your great support and nice comments! We are now incorporating all of your comments into the revised version to improve the manuscript. Since we have modified the manuscript according to the interactive comments of anonymous Referee #1 on 3 Dec., 2014, the modifications here will be on the latest version according to your comments. Please see the following point-to-point answers with the marked-up version of the manuscript.

1. This experiment set up six treatments to study the effects of NI and biochar on vegetable and N₂O emission, including CF-C0, CF-C1, CF-C2 and CP-C0, CP-C1, CP-C2. But two types of chemical N fertilizer species, compound fertilizer and urea, are also included. How the authors can assure N species exert no difference on N₂O emission and vegetable yield? Replacing the compound fertilizer with urea can make the results more persuasive. Two rates of biochar are included in the experiment, but few words are used to analyze the biochar application amount induced impacts on yield or N₂O.

A: Thank you for your comments! In the revised version, we specified N form for the compound

fertilizer on Page 5 line 27-28. We choose the compound fertilizer according to the local farmer's practice. The amounts of the cumulative N₂O emissions in agriculture ecosystems were mainly depended on the amount of the N fertilizer, especially in vegetable field with such a high N fertilization application (Harrison and Webb, 2001; Qiu et al., 2010). Since a commercial product of CP for the NI treatment was in the form of urea + nitrapyrin, we can roughly compare the effects of nitrapyrin. The corresponding P and K fertilizers were broadcast in the form of calcium phosphate and potassium chloride, respectively, in addition to the CP urea. We added some results and discussion to analyze the biochar application amount induced impacts on yield and N₂O emissions on Page 10 line 3-5, Page 10 line 13-14, Page 13 line 13-15, and Page 15 line 26-29.

References

Harrison, R., and Webb, J. (2001). A review of the effect of N fertilizer type on gaseous emissions. *Advances in Agronomy*, 73, 65-108.

Qiu, W. H., Liu, J. S., Hu, C. X., Zhao, C. S., Sun, X. C., and Tan, Q. L. (2010). Effects of nitrogen application rates on nitrous oxide emission from a typical intensive vegetable cropping system. *Journal of Agro-Environment Science*, 29(11), 2238-2243.

2. Many previous studies focus on only single factor of biochar and NI. These authors studied the combined effect of both. The rationale to study the combined effect has to be explored. Say, in some previous studies, biochar improves the soil pH, while NI can result in more NH₄⁺ in soil. These two soil amendments applied together may promote NH₃ volatilization significantly?

A: Thank you for your precious comments! We have added some rationale to study the combined effects of nitrapyrin and biochar in the introduction part on Page 4 line 21-25.

3. Many results confused me in the paper. Line 18-19 in the abstract "Vegetable yield was enhanced by 7.1-49.5% compared with the treatments without biochar amendment". This result is not consistent with that in Table 3. Table 3 tells us in CP treatment, applying biochar at the rate of 40t/ha reduced yield, compared to CP-C0. Page 15196, line 3 "Both CP application and biochar amendment significantly decreased the yield-scaled N₂O emissions during the entire experimental period". According to data in Table 3, compared to CP-C0, CP-C1 and CP-C2 could not decrease the yield-scaled N₂O emission significantly, how can such conclusion be got?

A: We have revised it on Page 1 line 23-24 and Page 10 line 22. Thank you! We have discussed the overall significance of nitrapyrin and biochar on N₂O emissions and vegetable yield with a

two-way ANOVA analysis according to Table 4 and the comparison of different treatments with a multiple comparisons according to Table 3. Since biochar had different effects on soil properties, vegetable yield and cumulative N₂O emissions when combined with compound fertilizer (CF) or nitrapyrin (CP), we separately described the results and discussion about them as CF and CP group treatments in the manuscript sometimes for further understanding. Thank you for your comments!

4. “1217.3 kg N ha⁻¹ yr⁻¹ across the experimental period”. The experiment lasted about two years. Is 1217.3 kg N applied every year or total two years period?

A: Thank you for your comments! We now specified the unit in the Abstract and the manuscript as 1217 kg N ha⁻¹ yr⁻¹. The average N application amount per year is calculated as the total N application (2637.5 kg N) amount divided by the total experimental growth period (26 months) in this intensively managed vegetable field, and then multiplies it by 12 months. That is to say, the 1217.3 kg N was applied every year in the vegetable field.

5. The paper wants to explore the coupled effects of biochar and NI on yield and N₂O emission, but most results and discussions concerned the single impacts of NI or biochar application. Only several lines in the fourth paragraph of section 4.3 discussed the coupled effects of biochar and NI. Authors should pay emphasis on exploring this combined impact rather than analyzing their single effect lengthily. There are some interesting results, say, as compared to CP-C0, CP-C1 increased total yield of vegetable, while further improving the biochar application rate to 40t/ha (CP-C2), the yield was decreased. But in the three CF treatments, while increasing the biochar application amount from 0 to 40t/ha, the yield is enhanced from 311 to 446t/ha. Authors said no words about the phenomenon.

A: Thank you for your comments! Since nitrapyrin application and biochar amendment could both have effects on N transformation processes in vegetable field, we have firstly discussed the main effect of nitrapyrin or biochar on N₂O emissions and vegetable yield to gain a better understanding of the combined use of them. Additionally, we have revised the discussion part on Page 15 line 6-29 about their combined effects on yield-scaled N₂O emissions.

Biochar significantly increased the vegetable yield in CF group treatments while had no significant effects in CP group treatments, even an insignificant decrease in CP-C2 treatment. This may mainly attribute to the fact that the vegetable yields in CP group treatments were relatively high

compared with other ecosystems although both nitrapyrin and biochar separately can significantly increase vegetable yield. We have revised it on Page 15 line 26-27.

6. In consistent with most early studies, biochar addition significantly decreased soil pH in this study. Authors thought weathering process of biochar resulted in this effect. You said biochar may be weathered more easily in vegetable soils. Powerful data or published papers should be provided to support this opinion. I think although weathering process of biochar can lower the soil pH, a significant level is hard to achieve. Can you provide the values of soil pH after each seasonal vegetable is harvested? The changes of pH during the seven seasons may provide hints to explain this phenomenon.

A: Thank you for your comments! As described on Page 12 line 16-21 in the manuscript, biochar significantly decreased the soil pH because biochar amendment stimulated the heterotrophic nitrification process in acid vegetable field, which may also cause an increase in the H⁺ content due to nitrification processes in the vegetable soil. The weathering process of biochar was not the main reason for the pH decreasing effect of biochar. However, the soil pH after each seasonal vegetable shows no meaningful regular pattern to explain this phenomenon, which may due to the heterogeneity of the vegetable soil in this field work.

7. Biochar amendment significantly increased SOC and TN. Please calculate how much SOC and TN was increased in the whole soil horizon, and please considers where these increased carbon and nitrogen stem from did.

A: Thank you for your comments! We have revised it in Table 2 on Page 27. Increases of SOC and TN were stem from the biochar amended in the vegetable soil, and we have illustrated where these increases may stem from on Page 13 line 18-30.

The following are some minor problems:

1. Effects of the first and second sentences of Abstract overlap. The first sentence should state the purpose of the study.

A: We have revised it on Page 1 line 9-12. Thank you!

2. The NI used in this study is chlorinated pyridine, or 6-chloro-2-trichlormethyl pyridine, or “chlorinated pyridine, a mixture of urea and nitrapyrin” (Table 1)? Please be consistent. Likely nitrapyrin is used more common.

A: We have unified CP as nitrapyrin throughout the manuscript. Thank you!

3. Page 15187, line 4, global N₂O emission.

A: We have revised on Page 3 line 3. Thank you!

4. Page 15187, line 24-28, redundant.

A: We have deleted the redundant part on Page 3 line 22. Thank you!

5. Unit of Equation 2 is incorrect.

A: We have revised the unit as kg N₂O-N t⁻¹ yield throughout the manuscript. Thank you!

6. Page 15197, line 5-10, the effects of CP on yield is an important conclusion in this paper, more persuasive reasons should be explored. The available N in soil is not likely a limiting factor for yield.

A: We have revised it on Page 11 line 22-23 and Page 11 line 27-29. Thank you!

7. Page 15201, line 13-15, you need reference or data to support your opinion.

A: We have revised it on Page 15 line 5-8. Thank you!

8. Language needs improvement greatly.

A: We have modified the language use throughout the manuscript. Thank you!

Thank you very much once again for your helpful comments!

Best Regards!

Zhengqin

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The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N₂O emissions from an intensively managed vegetable field in southeastern China

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10 **Abstract:** An experiment was conducted to study the influences of nitrification inhibitor (NI) and biochar incorporation on yield-scaled N₂O using the static chamber method and gas chromatography in an intensively managed vegetable field with 7 consecutive vegetable crops from 2012 to 2014 in southeastern China. With equal annual amounts of nitrogen (N) application rate (1217 kg N ha⁻¹ yr⁻¹), 6

treatments under 3 biochar amendment rates, namely, 0 t ha⁻¹ (C0), 20 t ha⁻¹ (C1), and 40 t ha⁻¹ (C2), with compound fertilizer (CF) or urea mixed with NI of nitrapyrin as chlorinated pyridine (CP), were

15 studied in these field experiments. The results showed that although no significant influence on soil organic carbon (SOC) content or total nitrogen (TN), nitrapyrin could result in a significant increase in soil pH during the experimental period. Nitrapyrin significantly decreased cumulative N₂O emissions by 15.9%–32.1% while increasing vegetable yield by 9.8%–41.9%. Thus, it also decreased yield-scaled N₂O emissions significantly. In addition to the differential responses of the soil pH, biochar amendment

20 significantly increased SOC and TN. Compared with the treatments without biochar addition, the cumulative N₂O emissions showed no significant difference in the CF or the CP group treatments but increased slightly (not significantly) by 7.9%–18.3% in the CP group treatments. Vegetable yield was enhanced by 7.1%–49.5% in CF group treatments compared with the treatments without biochar amendment while had no significant difference in CP group treatments, and the yield-scaled N₂O

25 emissions were thus decreased significantly. Furthermore, treatments applied with nitrapyrin and biochar incorporation slightly increased yield-scaled N₂O emissions by 9.4%, on average, compared with CP-C0. Therefore, the application of nitrapyrin could serve as an appropriate practice for increasing vegetable yield and mitigating N₂O emissions in intensively managed vegetable fields and

删除的内容: Abstract: The influences of nitrification inhibitor (NI) and biochar incorporation on yield-scaled N₂O 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

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should be further examined in various agroecosystems.

1 Introduction

Nitrous oxide (N₂O) is an important greenhouse gas and also contributes to ozone depletion in the stratosphere (IPCC, 2007; Saggarr et al., 2007; Ravishankara et al., 2009). [Global](#) N₂O emissions increased from 10 Tg to 12 Tg N₂O-N yr⁻¹ between 1900 and 2000 and may reach 16 Tg N₂O-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 N₂O, 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 N₂O 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 N₂O emitted per unit of crop production (yield-scaled N₂O emissions) needs to be considered (Van Groenigen et al., 2010). The yield-scaled N₂O emissions approach has been suggested as a more comprehensive index to assess N₂O 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 N₂O 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; [Zhou et al., 2014](#)), particularly intensively managed vegetable systems.

~~Intensively managed vegetable cultivation represents a major source of N₂O 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, N₂O emissions from N fertilizer in vegetable ecosystems represent 20% (Zheng et al., 2004) or 21.4% (Wang et al., 2011) of~~

删除的内容: 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, i

the total emissions from China's farmland.

Alternative practices that could reduce N₂O 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 NH₄⁺ (Majumdar et al., 2000; Pathak and Nedwell, 2001; Malla et al., 2005; Chen et al., 2010), thus mitigating N₂O emissions

and increasing the crop yield from the agricultural ecosystem. A newly developed urea mixed with NI of nitrapyrin as chlorinated pyridine (CP), has been used in agricultural ecosystems to mitigate GHG emissions and simultaneously increase crop yield (Ma et al., 2013; Xiong et al., 2013; Zhang et al., 2015). 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 N₂O 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 soil pH will cause a negative effect if biochar amendment is used to mitigate N₂O emissions because it would affect activity of N₂O reductase in soil (Cayuela et al., 2014). Overall, based on previous results, both nitrapyrin application and biochar amendment could serve to decrease yield-scaled N₂O emissions in various agricultural ecosystems. As nitrapyrin application and biochar amendment could both significantly affected the soil pH, the combined use of the two practices may have different effects on N transformation processes and NH₃ volatilization, and thus affect the N₂O emissions and crop yield in intensively managed vegetable field (Zhu et al., 2011; Soares et al., 2012; Gong et al., 2013). However, information for investigating the combined effects of nitrapyrin application and biochar amendment incorporation on yield-scaled N₂O in intensively managed vegetable agriculture is limited.

Accordingly, we quantified the effect of nitrapyrin application and biochar amendment incorporation on yield-scaled N₂O emissions in intensively managed vegetable agriculture in southeastern China. The objective of the study was to find appropriate practices for increasing

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vegetable yield and mitigating N₂O 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 1,107 mm and an annual mean air temperature of 15.34°C ([Nanjing Meteorology](#)). 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 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 g kg⁻¹; organic carbon, 15.6 g C kg⁻¹; and CEC (cation exchange capacity), 31.2 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 g C kg⁻¹ and an N content of 5.9 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

~~There were~~ 6 treatments with the same amount of total N₀ in triplicate for 7 consecutive vegetable crops from Apr. 12, 2012 to Jun. 12, 2014 in Nanjing, China. Each plot had an area of 7.5 m² 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 [nitrapyrin](#) (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, 121.7 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(N):m(P₂O₅):m(K₂O) ratio of 15:15:15 was used for the CF group treatments and the N form of the compound fertilizer is ammonium fertilizer, while the corresponding P and K fertilizers were broadcast in the form of calcium phosphate and potassium chloride, respectively, in addition to the [nitrapyrin](#) urea

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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.

5 Biochar was added once to the vegetable fields before sowing of the first vegetable crop (Amaranth) on Apr. 8, 2012 and was incorporated with the soil by hand plowing at a depth of 20 cm.

There were 7 vegetable crops grown successively during the entire observation period (Apr. 12, 2012 to Jun. 12, 2014). Each type of vegetable was seeded by hand and harvested at the appropriate mature stage according to the local farmers' practice. Furthermore, a short fallow period was imposed

10 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 Apr. 12, 2012 to May 25, 2012 and Mar. 15, 2014 to May 12, 2014 for Amaranth and from Nov. 20, 2012, to Feb. 24, 2013 and Nov. 5, 2013 to Mar. 14, 2014 for Baby bok choy, as Amaranth and Baby bok choy require

15 relatively warm weather conditions for growth. All the other management procedures, including crop species, tillage, irrigation, and pesticide, followed the local farmers' practices are presented in Table 1.

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2.3 Measurements of N₂O 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

20 (50×50×50 cm³). 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 frame. The frames 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 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

25 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 N₂O 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

30 at 40°C and 300°C, respectively. Concentrations of N₂O were quantified by comparing the peak area

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with those of reference gases (Nanjing special gas factory, Nanjing, China). N₂O 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 N₂O 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 Jun. 12, 2014 and stored at -20 °C 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 H₂SO₄-K₂Cr₂O₇, and the TN was determined by semi-micro Kjeldahl digestion using Se, CuSO₄ and K₂SO₄ 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.

Simultaneously with the determination of the trace gas fluxes, soil sampling at 0–15 cm depth was conducted for the determination of soil mineral N and soil water content. Soil mineral N was determined at approximately 7–15 days interval. The soil NH₄⁺-N and NO₃⁻-N were extracted by shaking for 1 h on a rotary shaker with 2 mol L⁻¹ KCl solution. According to Lu (2000), soil NH₄⁺-N and NO₃⁻-N contents were measured following the two wavelength ultraviolet spectrometry and indophenol blue methods, respectively, using an ultraviolet spectrophotometer (HITACHI, U-2900, Japan). The soil moisture content obtained by oven drying was converted to water-filled pore space (WFPS) using the following equation:

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

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 N₂O 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 N₂O emissions were related to crop yield as in Van Groenigen et al. (2010) and Grassini and Cassman (2012) and were calculated as follows:

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Yield-scaled N₂O emissions = Cumulative N₂O emissions / vegetable yield (kg N₂O-N t⁻¹ yield).

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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 nonparametric Kruskal-Wallis test. A 2-way ANOVA was used to analyze the effects of [nitrapyrin](#), biochar and their interactions on [soil TN, SOC, soil pH, soil mineral N](#), vegetable yield, N₂O emissions, and yield-scaled N₂O 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).

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3 Results

3.1 Soil properties and soil microclimate

[Nitrapyrin](#) had no significant influence on [soil TN](#) or SOC during the experimental period. However, significant increases in soil TN were observed in treatments with biochar amendments (Table 2, $p < 0.001$). 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. Similar to soil TN, SOC increased significantly in the treatments with biochar amendment (Table 2, $p < 0.001$). 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, [nitrapyrin](#) increased soil pH significantly by [0.97–2.15 units](#) compared with the treatments without [nitrapyrin](#) ($p < 0.001$), and biochar amendment [significantly](#) decreased soil pH by [1.59–1.63 units](#) and [0.45–0.98 units](#) for the CF and CP group treatments (Table 2, $p < 0.001$), respectively. Significant interactions between [nitrapyrin](#) and biochar were observed to affect soil TN ($p < 0.05$) and soil pH ($p < 0.001$) throughout the intensive [vegetable](#) experimental period.

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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 Figure 1a and 1b, 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 31.9–76.0% across all the experimental period. No significant difference on WFPS rates were detected among vegetable crops (Fig. 1c).

3.2 Dynamics of N₂O fluxes and soil NH₄⁺-N and NO₃⁻-N content

The dynamics of N₂O fluxes from all the treatments across the 7 vegetable growth crops are shown in Figure 2. The pattern of these fluxes was relatively consistent and was sporadic and pulse-like. N₂O fluxes showed a similar trend during the same period in both years. Following basal fertilization, tillage and irrigation, N₂O emissions ranged from 17–3406 μg N m⁻² h⁻¹ were observed in all treatments across the experimental period. N₂O emissions primarily occurred with the increase in soil temperature during the summer, from May to October. However, as shown in Figure 2d and Figure 2g, no significant N₂O peaks were found in certain vegetable crops to which basal or top-dressing fertilization was added because of the low soil temperature after fertilization in the vegetable field.

Soil NH₄⁺-N and NO₃⁻-N contents ranged from 58.0–413.9 mg N kg⁻¹ and 36.4–279.1 mg N kg⁻¹ across the 7 vegetable crops growth period, respectively as shown in Figure 3. The fertilization events considerably increased soil inorganic N (NO₃⁻ + NH₄⁺) content. As the nitrapyrin limited the nitrification process, significant higher soil NH₄⁺-N contents were observed for the treatments with nitrapyrin compared to the treatments with compound fertilizer at the same N rate (Fig. 3a). In contrast to the NH₄⁺-N, a significant lower content of soil NO₃⁻-N was observed in the plots applied with nitrapyrin (Fig. 3b). In addition, biochar increased the soil NH₄⁺-N content while decreased the soil NO₃⁻-N contents throughout the intensive vegetable growth period, but not significantly (Fig. 3).

3.3 Cumulative N₂O emissions, vegetable yield and yield-scaled N₂O emissions

The cumulative N₂O emissions for each treatment across the entire experimental period are shown in Table 3a. These emissions varied widely among different crops during the individual vegetable crop-growing season. Additionally, the cumulative N₂O emissions showed significant differences among all the treatments. The greatest N₂O-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 Table 3a and Table 4 significant decreases in the cumulative N₂O emissions were detected in the treatments with nitrapyrin ($p<0.001$). The decreases ranged from 15.9%–32.1% compared with the treatments without nitrapyrin application ($p<0.001$). In contrast, biochar amendment had no significant influence

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on cumulative N₂O emissions in both CF and CP group treatments (Table 4). However, as shown in Table 3a, slight increases in cumulative N₂O 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, which also indicated that the biochar amendment rates would not result in a significant difference on the cumulative N₂O emissions. Furthermore, no significant interaction between nitrapyrin and biochar amendment was observed to affect cumulative N₂O emissions throughout the intensive vegetable experimental period (Table 4).

Table 3b shows details of the fresh weight for each vegetable crop. The highest fresh weight yield for the 7 consecutive vegetable crops was 535.4±16.7 t ha⁻¹ for CP-C1, an increase of 71.7% compared with CF-C0. Both nitrapyrin application ($p<0.001$) and biochar amendment ($p<0.05$) significantly increased the yield in the intensively managed vegetable system across the experimental period (Table 4). As shown in Table 3b, nitrapyrin significantly increased vegetable yield compared with the treatments without nitrapyrin. In addition, the vegetable yield was significantly enhanced by 9.8%–41.9% with the increase of the biochar amendment rates in CF group treatments (Table 3b). However, a decrease of vegetable yield was observed in CP-C2 treatment compared with CP-C0. Moreover, significant interactions between nitrapyrin and biochar amendment were observed to affect vegetable yield throughout the intensive vegetable experimental period (Table 4, $p<0.05$).

Table 3c shows the yield-scaled N₂O emissions, which were related to the cumulative N₂O emissions and the fresh weight yield ranged from 0.074±0.004 to 0.175±0.017 kg N₂O-N t⁻¹ yield over the entire experimental period. The lowest value of yield-scaled N₂O emissions was 0.074±0.004 kg N₂O-N t⁻¹ yield for CP-C0, namely, the treatment with nitrapyrin application and without biochar amendment. As shown in Table 4, both nitrapyrin application ($p<0.001$) and biochar amendment ($p<0.05$) significantly decreased the yield-scaled N₂O emissions during the entire experimental period. Furthermore, significant interactions between nitrapyrin and biochar were observed to affect yield-scaled N₂O emissions throughout the experimental period (Table 4, $p<0.05$).

4 Discussion

4.1 Effects of nitrapyrin as nitrification inhibitor on N₂O emissions and vegetable yield

N₂O emissions are directly related to the amount of mineral N available in the soil. A two-way ANOVA

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indicated that seasonal N₂O emissions during the vegetable-growing periods were significantly affected by [nitrapyrin](#) application (Table 4, $p < 0.001$), in agreement with previous results (Ma et al., 2013; Xiong et al., 2013; Zhang et al., 2015). Different types of NI that were effective in reducing N₂O have been reported by previous studies (Xu et al., 2000; Boeckx et al., 2005; Zaman et al., 2008, 2009; Zaman and Blennerhassett, 2010). [Nitrapyrin](#) 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 NO₃⁻ and NH₄⁺ content of soil (Fig. 3), which is highly related to [mitigating](#) N₂O emissions (Di et al. 2009, 2014). Moreover, [nitrapyrin](#) 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 2, $p < 0.001$). 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 N₂O emission rate in vegetable soils, which may indicate that the increasing of soil pH is another factor mitigating N₂O 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, [nitrapyrin](#) produced a significant increase in vegetable yield in our study across the experimental period (Table 3b, Table 4, $p < 0.001$). Possible explanations for higher crop yields obtained with NH₄⁺-containing fertilizers supplemented with [nitrapyrin](#) (Fig. 3a) include the reduction of N losses by leaching and volatilization, and improved bio-availability of N and N uptake of the crops (O'Connor et al. 2012) in the vegetable soil. Ma et al. (2013) reported that 2 types of NIs ([nitrapyrin](#) and DCD) increased average wheat yield by 9.7% under conventional and no-till practices during the winter wheat-growing season. In addition, since [nitrapyrin](#) could significantly increase soil pH, the uptake rates of the inorganic N would increase due to the increasing effect of [nitrapyrin](#) on soil pH in vegetable field (Jampeetong et al., 2013). Moreover, Zhang et al. (2015) find CP significantly increased vegetable yield by 12.6% in an intensively managed vegetable field which may due to CP was beneficial for the growth and N assimilation of the crops (Liu et al., 2013).

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4.2 Effects of biochar on N₂O emissions and vegetable yield

As shown in Table 3a and Table 4, biochar had no significant influence on cumulative N₂O during the experimental period. Decreases in the net emissions of N₂O 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 N₂O 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 N₂O emissions during the experimental period and even slightly increased cumulative N₂O by 7.9%–18.3% in the CP group treatments (Table 3a, Table 4). Thus, the mitigating effect of biochar amendment on N₂O 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 2). 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 2, $p < 0.001$). With such a high amount of N fertilization application, biochar may lose the buffering effects of soil pH changes. Luo et al (2011) reported that biochar with low pyrolysis temperature had more water extractable organic carbon. Although considered to be stable in soil, biochar brings extra carbon source for heterotrophic nitrification process, 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, primarily due 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 N₂O emissions was significant in neutral and alkaline soils but not in acidic soils with pH < 5, which probably due to that low soil pH may result in adversely affecting the

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activity of N_2O 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, vegetable field had a high amount of N input and may expect ammonia oxidation and linked nitrifier-denitrification (ND) being important processes generating N_2O (Wrage et al., 2001; Huang et al., 2014). Sánchez-García et al. (2014) found that biochar increased cumulative N_2O emissions in the soil when ammonia oxidation and were the ND major processes generating N_2O emissions, whereas it decreased N_2O emissions in the soil when denitrification is the main pathway leading to N_2O emissions under the same experimental conditions.

Biochar amendment significantly increased SOC and soil TN (Table 2, $p < 0.001$) and thus significantly improved vegetable yield compared with the treatments that received no biochar addition (Table 4, $p < 0.05$), which agree well with previous reported benefit of adding biochar to soils (Major et al. 2010; Jia et al., 2012). In addition, vegetable yield was significantly increased with the increase of biochar amendment rates in the CF group treatments, which is in agree with previous results (Zhang et al., 2010). In our study, SOC increased significantly by 66.9%–85.1% in the treatments amended with biochar over the experimental period (Table 2, $p < 0.001$). 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 2, $p < 0.001$), which is in consistent with previous study in paddy rice (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 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

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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 3b, 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 2, $p<0.001$).

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4.3 The combined effects of nitrapyrin and biochar incorporation on yield-scaled N₂O emissions

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Analyzing N₂O emissions on a yield basis provides interesting information for estimating the environmental impacts of intensive agricultural production systems. As shown in Table 3c, yield-scaled N₂O emissions ranged from 0.074±0.004–0.175±0.017 kg N₂O–N t⁻¹ yield, much lower than previously reported values (Van Groenigen et al., 2010; Ma et al., 2013). 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 N₂O in our vegetable field.

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Overall, nitrapyrin significantly decreased yield-scaled N₂O emissions across the experimental period (Table 3c, Table 4, $p<0.001$), attributing to the N₂O reducing and vegetable increasing effects of nitrapyrin in intensively managed vegetable field. Our results indicate that the yield-scaled N₂O emissions were minimal in the CP-C0 treatment (0.074±0.004 kg N₂O–N t⁻¹ yield). This treatment showed the lowest cumulative N₂O 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, nitrapyrin application was a more efficient way to reduce the yield-scaled N₂O emissions in our case. This approach may significantly improve vegetable yield while causing a decrease in N₂O emissions and, thus, improved agronomic N use efficiency (NUE) in intensively managed vegetable agriculture (Li et al., 2007; Asing et al., 2008). Thus, nitrapyrin application without biochar amendment (CP-C0) can serve as an appropriate way of mitigating N₂O emissions while increasing vegetable yield in intensively managed vegetable agriculture.

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Although biochar amendment did not significantly decreased the cumulative N₂O emissions, it

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significantly decreased the yield-scaled N₂O emissions across the experimental period (Table 3c, Table 4, $p < 0.05$), mainly due to the increasing effect of vegetable yield with biochar. Obvious interactions in yield-scaled N₂O emissions were observed between nitrapyrin and biochar addition (Table 4, $p < 0.05$).

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5 However, no significant interactions between nitrapyrin and biochar in N₂O emissions were observed in the current study. Treatments with nitrapyrin and biochar incorporation slightly increased the cumulative N₂O by 7.9%–18.3%, but not significantly (Table 3a, Table 4), which indicated that biochar could be able to diminish the mitigating effect of nitrapyrin, namely, the effect of inhibiting the nitrification process in vegetable soil (Fig. 3). Vegetable soil in our study is acid due to large N application in which biochar would increase the heterotrophic nitrification rate in the treatments applied with both nitrapyrin and biochar (CP-C1 and CP-C2) compared with CP-C0 treatment, and thus increase the N₂O emissions in vegetable field. Additionally, with such low soil pH, the denitrification process and the ND process may also become the major contributors to the N₂O pool (Zhu et al., 2011; Zhu et al., 2013). Although biochar had no significant effect on soil inorganic N content, it decreased the soil NH₄⁺-N content and increased the soil NO₃⁻-N content in the treatments amended with biochar in both CF and CP group, which may simultaneously increase the NO₂⁻ content in the vegetable soil, and thus increase the cumulative N₂O emissions linked with other N₂O generating processes in vegetable field across the experimental period. Moreover, Di et al. (2014) reported that DCD was highly effective in inhibiting the growth of AOB communities, and reducing N₂O 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 N₂O emissions were not observed. Thus, biochar addition diminished the mitigation effect of nitrapyrin on the cumulative N₂O emissions in vegetable soil under the high WFPS condition due to frequent irrigations (Fig. 1).

15 Significant interactions in yield were observed though no significant differences in vegetable yield were found among different combinations of nitrapyrin and biochar as CP-C0, CP-C1 and CP-C2 (Table 3b), indicating biochar incorporation rate did not increase vegetable yield when combined with nitrapyrin application. Most likely, the vegetable yields were relatively high compared with other ecosystems although both nitrapyrin and biochar separately can significantly increase vegetable yield, which can also explain the fact that increasing biochar amendment rates did not resulted in significant increase in vegetable yield across the experimental period (Table 3b). Since we did not measure the individual N transformation process and the microbe in vegetable soil responding to N₂O emissions and

vegetable yield, the combined effects of nitrapyrin and biochar incorporation on N₂O emissions in intensively managed vegetable fields need further study.

5 Conclusions

Yield-scaled N₂O emissions were significantly affected by both nitrapyrin application and biochar amendment in intensively managed vegetable agriculture. Throughout the experimental period, although significant influences on soil TN and SOC were not found, nitrapyrin application significantly increased soil pH and vegetable yield while significantly decreasing cumulative N₂O emissions in the intensively managed vegetable field, therefore causing a significant decrease in yield-scaled N₂O emissions over the experimental period. Moreover, biochar amendment significantly increased soil TN, SOC and vegetable yield but had no significant influence on the cumulative N₂O emissions, whereas this amendment significantly decreased soil pH and yield-scaled N₂O emissions. Nitrapyrin and biochar incorporation into vegetable soil slightly increased yield-scaled N₂O emissions during the experimental period. Yield gains were the most important factor for lower yield-scale N₂O emissions in our case compared with previous studies. Overall, taking environmental and economic benefits into consideration, nitrapyrin application in the vegetable field was the best procedure for reducing the yield-scaled N₂O emissions. The long-term combined effects of nitrapyrin application 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 2

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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		SOC		pH
	(g N kg ⁻¹)	(t N ha ⁻¹)	(g C kg ⁻¹)	(t C ha ⁻¹)	
T0	<u>1.9</u>		<u>15.6</u>		<u>5.52</u>
CF-C0	<u>1.46±0.03 d</u>	<u>3.52±0.08</u>	<u>12.7±0.6 c</u>	<u>30.4±0.81</u>	<u>5.27±0.08 c</u>
CP-C0	<u>1.74±0.12 c</u>	<u>4.2±0.29</u>	<u>12.8±0.8 c</u>	<u>30.79±1.96</u>	<u>6.24±0.08 a</u>
CF-C1	<u>2.65±0.02 b</u>	<u>6.38±0.03</u>	<u>21.8±0.2 b</u>	<u>50.74±0.47</u>	<u>3.64±0.04 d</u>
CP-C1	<u>2.52±0.16 b</u>	<u>6.05±0.4</u>	<u>23.2±0.3 a</u>	<u>55.7±0.33</u>	<u>5.79±0.05 b</u>
CF-C2	<u>2.91±0.05 a</u>	<u>6.96±0.11</u>	<u>23.5±0.3 a</u>	<u>56.3±0.62</u>	<u>3.68±0.06 d</u>
CP-C2	<u>2.84±0.12 a</u>	<u>6.83±0.29</u>	<u>23.1±0.9 a</u>	<u>55.52±2.13</u>	<u>5.26±0.08 c</u>
<i>P</i>	<i>***</i>		<i>***</i>		<i>***</i>

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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 t·ha⁻¹; C1, Biochar 20 t·ha⁻¹; C2, Biochar 40 t·ha⁻¹.

The increased SOC and TN in the whole soil horizon were calculated according to a depth of 20cm topsoil.

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Means ± SD with different letters in the same column indicate significant differences between treatments according to Tukey's multiple range test ($p<0.05$).

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*** $p<0.001$

P value: the index of differences between the control group and the experimental group. If $p<0.05$ and

$p<0.01$, significant differences exists between the control group and the experimental group

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Table 3

Cumulative N₂O emissions, vegetable yield and yield-scaled N₂O emissions under different treatments over the entire experimental period.

	Amaranth	Tung choy	Baby bok choy	Coriander herb	Tung choy	Baby bok choy	Amaranth	To rota
Treatments	2012/4/18- 2012/7/10 (41d/83d)	2012/7/11- 2012/11/19 (100d/131d)	2012/11/20- 2013/3/27 (98d/127d)	2013/3/28- 2013/6/30 (62d/94d)	2013/7/1- 2013/11/4 (106d/126d)	2013/11/5- 2014/3/14 (67d/129d)	2014/3/15- 2014/6/12 (58d/89d)	(77)
(a) Cumulative N₂O 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±
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±
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±1(
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.
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.
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
(b) 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±
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±
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±
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±
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±
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±
(c) Yield-scaled N₂O emissions (kg N₂O-N 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
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

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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
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.
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
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.

See Table 2 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 ($p < 0.05$).

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Table 4

Two-way ANOVA for the effects of nitrapyrin (CP) and biochar (Bc) on the cumulative N₂O emissions, vegetable yield and yield-scaled N₂O emissions over the entire experimental period.

Factors	DF	Cumulative N ₂ O emissions			Vegetable yield			Yield-scaled N ₂ O emissions		
		SS	F	P	SS	F	P	SS	F	P
<u>CP</u>	<u>1</u>	<u>570.09</u>	<u>19.29</u>	<u><0.001</u>	<u>76098.8</u>	<u>111.36</u>	<u><0.001</u>	<u>0.0171</u>	<u>78.04</u>	<u><0.001</u>
<u>Bc</u>	<u>2</u>	<u>66.28</u>	<u>1.21</u>	<u>0.3577</u>	<u>12977.7</u>	<u>9.58</u>	<u><0.05</u>	<u>0.0029</u>	<u>6.63</u>	<u><0.05</u>
<u>CP×Bc</u>	<u>2</u>	<u>86.83</u>	<u>1.47</u>	<u>0.2687</u>	<u>17542.7</u>	<u>12.95</u>	<u><0.05</u>	<u>0.0044</u>	<u>10.13</u>	<u><0.05</u>
<u>Model</u>	<u>5</u>	<u>723.21</u>	<u>4.89</u>		<u>106619.4</u>	<u>31.48</u>		<u>0.0244</u>	<u>22.31</u>	
<u>Error</u>	<u>12</u>	<u>354.67</u>	<u>—</u>	<u>—</u>	<u>8126.9</u>	<u>—</u>	<u>—</u>	<u>0.0026</u>	<u>—</u>	<u>—</u>

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

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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 separates 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 2 for treatment codes.

Figure 3 Dynamics of the soil NH_4^+-N and NO_3^-N concentrations within the 0–15 cm soils 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 separates different vegetable growth periods. See Table 2 for treatment codes.

*** $p < 0.001$; n.s. not significant.

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Temporal variations of the soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ content from all the treatments across the 7 vegetable growth crops are shown in Figure 3.

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between nitrapyrin and

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ere observed to affect soil NH_4^+ -N and NO_3^- -N contents throughout the intensive vegetable

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growth period (Fig. 3).

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C

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NI

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application. Most likely, the vegetable yields were relatively high compared with other ecosystems although both nitrapyrin

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and biochar separately can significantly increase vegetable yield.

However, no significant interactions between nitrapyrin and biochar in N_2O emissions were observed in the current study. Treatments with nitrapyrin

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and biochar incorporation slightly increased the cumulative N₂O by 7.9%–18.3%, but not significantly (Table 3a, Table 4), which may probably due to the reason that biochar be able to diminish the mitigating effect of nitrapyrin

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by affecting the N transformation processes in vegetable soil (Fig. 3). Di et al. (2014) reported that DCD was highly effective in inhibiting the growth of AOB communities, and reducing N₂O 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 N₂O emissions were not observed. Thus, biochar addition diminished the mitigation effect of nitrapyrin

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on cumulative N₂O 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 nitrapyrin

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and biochar (CP-C1 and CP-C2) compared with CP-C0 treatment, and thus increase the N₂O emissions in vegetable field. Since we did not measure the individual N transformation process responding to N₂O emissions, the combined effects of NI and biochar incorporation on N₂O emissions in intensively managed vegetable fields need further study.

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