

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

B. Li¹, C.H. Fan¹, Z.Q. Xiong*, Q.L. Li, M. Zhang

5 Jiangsu Key Laboratory of Low Carbon Agriculture and GHGs Mitigation, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, China

¹Contributed equally *Corresponding author. Z.Q. Xiong*

Tel: 86-25-84395148; fax: 86-25-84395210; E-mail address: zqxiong@njau.edu.cn

Abstract: An experiment was conducted to study the influences of nitrification inhibitor (NI) and
10 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
20 amendment 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
25 yield-scaled N₂O 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 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; Saggar 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).

10 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., 15 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 25

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

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 N 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, 1217 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

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.

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

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:

$$\text{Yield-scaled N}_2\text{O emissions} = \text{Cumulative N}_2\text{O emissions} / \text{vegetable yield (kg N}_2\text{O-N t}^{-1} \text{ yield)}.$$

(2)

2.5 Data processing and statistics

The values presented are given as arithmetic means \pm 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).

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.

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

5 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 $\mu\text{g N m}^{-2} \text{h}^{-1}$ were observed in all treatments across the experimental period. N₂O emissions primarily occurred with the increase in soil temperature during
10 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 and Figure 4,
15 respectively. The fertilization events considerably increased soil mineral 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. 3). In contrast to the NH₄⁺-N, a significant lower content of soil NO₃⁻-N was observed in the plots applied with nitrapyrin (Fig. 4). In addition, biochar increased the soil NH₄⁺-N content while
20 decreased the soil NO₃⁻-N contents throughout the intensive vegetable growth period, but not significantly (Fig. 3, Fig 4).

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
25 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 on cumulative N_2O emissions in both CF and CP group treatments (Table 4). However, as shown in Table 3a, 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, which also indicated that the biochar amendment rates would not result in a significant difference on the cumulative N_2O emissions. Furthermore, no significant interaction between nitrapyrin and biochar amendment was observed to affect cumulative N_2O 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 $535.4\pm 16.7\text{ t ha}^{-1}$ 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 N_2O emissions, which were related to the cumulative N_2O emissions and the fresh weight yield ranged from 0.074 ± 0.004 to $0.175\pm 0.017\text{ kg N}_2\text{O-N t}^{-1}$ yield over the entire experimental period. The lowest value of yield-scaled N_2O emissions was $0.074\pm 0.004\text{ kg N}_2\text{O-N t}^{-1}$ 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 N_2O emissions during the entire experimental period. Furthermore, significant interactions between nitrapyrin and biochar were observed to affect yield-scaled N_2O emissions throughout the experimental period (Table 4, $p<0.05$).

4 Discussion

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

N₂O emissions are directly related to the amount of mineral N available in the soil. A two-way ANOVA 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 NH₄⁺ and NO₃⁻ content of soil (Fig. 3, Fig. 4), 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. 3) 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).

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

activity of N₂O 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 N₂O emissions in vegetable field (Zhu et al., 2011) though heterotrophic nitrification is generally considered to be a minor source of N₂O (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 N₂O (Wrage et al., 2001; Huang et al., 2014). Sánchez-García et al. (2014) found that biochar increased cumulative N₂O emissions in the soil when ammonia oxidation and were the ND major processes generating N₂O emissions, whereas it decreased N₂O emissions in the soil when denitrification is the main pathway leading to N₂O 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

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, $p < 0.001$).

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

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.

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). Overall, 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.

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

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, $p < 0.05$). 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, Fig 4). 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 (Fig. 3, Fig 4), 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).

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.

Acknowledgments

We sincerely appreciate the anonymous reviewers for their critical and valuable comments to help improve this manuscript. This work was supported by the Ministry of Science and Technology (2013BAD11B01), the National Science Foundation of China (41171238, 41471192), and the Doctoral Program of Higher Education of China (20110097110001).

References

- Anderson, I. C., Poth, M., Homstead, J., and Burdige, D.: A comparison of NO and N₂O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*, *Applied and Environmental Microbiology*, 59(11), 3525-3533, 1993.
- 5 Asing, J., Saggar, S., Singh, J., and Bolan, N. S.: Assessment of nitrogen losses from urea and an organic manure with and without nitrification inhibitor, dicyandiamide, applied to lettuce under glasshouse conditions, *Soil Research*, 46, 535-541, 2008.
- Biederman, L. A., and Harpole W. F.: Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis, *Glob. Change Biol-Bioenergy*, 5, 202-214, 2012.
- 10 Boeckx, P., Xu, X., and Van Cleemput, O.: Mitigation of N₂O and CH₄ emission from rice and wheat cropping systems using dicyandiamide and hydroquinone, *Nutrient Cycling in Agroecosystems*, 72, 41-49, 2005.
- Bouwman, A. F., Beusen, A. H. W., Griffioen, J., Van Groenigen, J. W., Hefting, M. M., Oenema, O., Van Puijenbroek P. J. T. M., Seitzinger S., Slomp C. P., and Stehfest, E.: Global trends and
15 uncertainties in terrestrial denitrification and N₂O emissions, *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621), 20130112, doi:10.1098/rstb.2013.0112, 2013.
- Cayuela, M. L., Sánchez-Monedero, M. A., Roig, A., Hanley, K., Enders, A., and Lehmann, J.: Biochar and denitrification in soils: when, how much and why does biochar reduce N₂O emissions?, *Scientific reports*, 3(1732), doi:10.1038/srep01732, 2013.
- 20 Cayuela M. L., van Zwieten L., Singh B. P., Jeffery S., Roig A., and Sánchez-Monedero M. A.: Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis, *Agric. Ecosyst. Environ.*, 191, 5-16, <http://dx.doi.org/10.1016/j.agee.2013.10.009>, 2014.
- Chen, D., Suter, H. C., Islam, A., and Edis, R.: Influence of nitrification inhibitors on nitrification and nitrous oxide (N₂O) emission from a clay loam soil fertilized with urea, *Soil Biology and
25 Biochemistry*, 42, 660-664, 2010.
- Davidson, E. A.: The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860, *Nature Geoscience*, 2, 659-662, 2009.
- De Boer, W., and Kowalchuk, G. A.: Nitrification in acid soils: micro-organisms and mechanisms, *Soil Biology and Biochemistry*, 33, 853-866, 2001.

- Di, H. J., Cameron, K. C., Podolyan, A., and Robinson, A.: Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil, *Soil Biology and Biochemistry*, 73, 59-68, 2014.
- Di H. J., Cameron K. C., Shen J. P., Winefield C. S., Callaghan M. O., Bowatte S., and He J. Z.:
5 Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils, *Nat. Lett.*, 2, 621-624, 2009.
- Ding, W., Cai, Y., Cai, Z., Yagi, K., and Zheng, X.: Nitrous oxide emissions from an intensively cultivated maize-wheat rotation soil in the North China Plain, *Science of the total environment*, 373, 501-511, 2007.
- 10 Ding, Y., Liu, Y. X., Wu, W. X., Shi, D. Z., Yang, M., and Zhong, Z. K.: Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns, *Water, Air, & Soil Pollution*, 213(1-4), 47-55, 2010.
- Felber, R., Leifeld, J., Horák, J., and Nefel, A.: Nitrous oxide emission reduction with greenwaste biochar: comparison of laboratory and field experiments, *European Journal of Soil Science*, 65,
15 128-138, 2014.
- Gagnon, B., Ziadi, N., Rochette, P., Chantigny, M. H., and Angers, D. A.: Fertilizer source influenced nitrous oxide emissions from a clay soil under corn, *Soil Science Society of America Journal*, 75, 595-604, 2011.
- Gong, W., Zhang, Y., Huang, X., and Luan, S.: High-resolution measurement of ammonia emissions
20 from fertilization of vegetable and rice crops in the Pearl River Delta Region, China, *Atmospheric Environment*, 65, 1-10, 2013.
- Grassini, P., and Cassman, K. G.: High-yield maize with large net energy yield and small global warming intensity, *Proc. Natl. Acad. Sci.*, 109, 1074-1079, 2012.
- Gregory, P. J.: Roots, rhizosphere and soil: the route to a better understanding of soil science?,
25 *European Journal of Soil Science*, 57, 2-12, 2006.
- Halvorson, A. D., Del Grosso, S. J., and Alluvione, F.: Tillage and inorganic nitrogen source effects on nitrous oxide emissions from irrigated cropping systems, *Soil Science Society of America Journal*, 74, 436-445, 2010.
- He, F. F., Jiang, R. F., Chen, Q., Zhang, F. S., and Su, F.: Nitrous oxide emissions from an intensively
30 managed greenhouse vegetable cropping system in Northern China, *Environ. Pollut.*, 157,

1666-1672, 2009.

Huang, T., Gao, B., Hu, X. K., Lu, X., Well, R., Christie, P., Bakken, L., and Ju, X. T.: Ammonia-oxidation as an engine to generate nitrous oxide in an intensively managed calcareous Fluvo-aquic soil, *Scientific reports*, 4(3950), doi: 10.1038/srep03950, 2014.

5 IPCC-Intergovernmental Panel on Climate Change: *Climate Change 2007: The Physical Science Basis*, Cambridge University Press, Cambridge, UK and New York, NY, USA, 2007.

Jampeetong, A., Konnerup, D., Piwpuan, N., and Brix, H.: Interactive effects of nitrogen form and pH on growth, morphology, N uptake and mineral contents of *Coix lacryma-jobi* L, *Aquatic Botany*, 111, 144-149, 2013.

10 Jeffery, S., Verheijen, F. G. A., Van Der Velde, M., and Bastos, A. C.: A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis, *Agriculture, Ecosystems & Environment*, 144, 175-187, 2011.

Ji, Y., Yu, J., Ma, J., Li, X. P., Xu, H., and Cai Z. C.: Effect of timing of DCD application on nitrous oxide emission during wheat growing period. *Acta. Ecol. Sin.*, 31, 7151-7160, 2011. (In Chinese)

15 Jia, J., Li, B., Chen, Z., Xie, Z., and Xiong, Z. Q.: Effects of biochar application on vegetable production and emissions of N₂O and CH₄, *Soil Science and Plant Nutrition*, 58, 503-509, 2012.

Jones, D. L., Rousk, J., Edwards-Jones, G., Deluca, T. H., and Murphy, D. V.: Biochar mediated changes in soil quality and plant growth in a three year field trial, *Soil Biol. Biochem.*, 45, 113-124, 2012.

20 Ju, X. T., Kou, C. L., Zhang, F. S., and Christie, P.: Nitrogen balance and groundwater nitrate contamination: Comparison among three intensive cropping systems on the North China Plain, *Environ. Pollut.*, 143, 117-125, 2006.

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

25 Kuzyakov, Y., Subbotina, I., Chen, H. Q., Bogomolova, I., and Xu, X. L.: Black carbon decomposition and incorporation into microbial biomass estimated by ¹⁴C labeling, *Soil Biol. Biochem.*, 41, 210-219, 2009.

Lehmann, J.: A handful of carbon, *Nature*, 447, 143-144, 2007.

30 Laird, D., Fleming, P., Wang, B., Horton, R., and Karlen, D.: Biochar impact on nutrient leaching from

- a Midwestern agricultural soil, *Geoderma*, 158, 436-442, 2010.
- Lehmann, J., Rillig, M., Thies, J., Masiello, C. A., Hockaday, W. C., and Crowley, D.: Biochar effects on soil biota—a review, *Soil. Biol. Biochem.*, 43, 1812-1836, 2011.
- Li, D. J., and Wang, X. M.: Nitric oxide emission from a typical vegetable field in the Pearl River Delta, China, *Atmos. Environ.*, 41, 9498-9505, 2007.
- Li, X., Hu, C., Delgado, J. A., Zhang, Y., and Ouyang, Z.: Increased nitrogen use efficiencies as a key mitigation alternative to reduce nitrate leaching in north china plain, *Agricultural Water Management*, 89, 137-147, 2007.
- Liu, B., Mørkved, P. T., Frostegard, A., and Bakken, L. R.: Denitrification gene pools, transcription and kinetics of NO, N₂O and N₂ production as affected by soil pH, *FEMS microbiology ecology*, 72, 407-417, 2010.
- Liu, C., Wang, K., and Zheng, X.: Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat–maize cropping system, *Biogeosciences*, 10(4), 2427-2437, 2013.
- Lu, R. K.: *Soil Agro-chemical Analyses*, Agricultural Technical Press of China, Beijing, 2000. (In Chinese)
- Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., and Brookes, P. C.: Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH, *Soil Biology and Biochemistry*, 43(11), 2304-2314, 2011.
- Ma, Y., Sun, L., Zhang, X., Yang, B., Wang, J., Yin, B., Yan, X., and Xiong, Z.: Mitigation of nitrous oxide emissions from paddy soil under conventional and no-till practices using nitrification inhibitors during the winter wheat-growing season, *Biology and fertility of soils*, 49, 627-635, 2013.
- Major, J., Rondon, M., Molina, D., Riha, S. J., and Lehmann, J.: Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol, *Plant Soil*, 333, 117-128, 2010.
- Majumdar, D., Kumar, S., Pathak, H., Jain, M.C., and Kumar, U.: Reducing nitrous oxide emission from an irrigated rice field of North India with nitrification inhibitors. *Agriculture, ecosystems & environment*, 81, 163-169, 2000.
- Malla, G., Bhatia, A., Pathak, H., Prasad, S., Jain, N., and Singh, J.: Mitigating nitrous oxide and methane emissions from soil under rice-wheat system with nitrification inhibitors, *Chemosphere*

58, 141-147, 2005.

- Mosier, A.R., Kroeze, C., Nevison, C., Oenema, O., Seitzinger, S. P., and Van Cleemput, O.: Closing the global N₂O budget: nitrous oxide emissions through the agricultural nitrogen cycle, OECD/IPCC/IEA phase II development of IPCC guidelines for national greenhouse gas inventory methodology, *Nutrient Cycling in Agroecosystems*, 52, 225-248, 1998.
- 5 Pasda, G., Hähndel, R., and Zerulla, W.: Effect of fertilizers with the new nitrification inhibitor DMPP (3, 4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops, *Biology and Fertility of Soils*, 34, 85-97, 2001.
- Park, S., Croteau, P., Boering, K. A., Etheridge, D. M., Ferretti, D., Fraser, P. J., Kim, K-R., Krummel, P. B., Langenfelds, R. L., van Ommen, T. D., Steele L. P., and Trudinger, C. M.: Trends and seasonal cycles in the isotopic composition of nitrous oxide since 1940, *Nature Geoscience*, 5, 261-265, 2012.
- 10 Pathak, H., and Nedwell, D. B.: Strategies to reduce nitrous oxide emission from soil with fertilizer selection and nitrification inhibitor, *Water Air Soil Poll.*, 129, 217-228, 2001.
- 15 Qin, S., He, X., Hu, C., Zhang, Y., and Dong, W.: Responses of soil chemical and microbial indicators to conservational tillage versus traditional tillage in the North China Plain, *European Journal of Soil Biology*, 46, 243-247, 2010.
- Ravishankara, A. R., Daniel, J. S., and Portmann, R. W.: Nitrous oxide (N₂O): the dominant ozone-depleting substance emitted in the 21st century, *Science*, 326, 123-125, 2009.
- 20 O'Connor P. J., Hennessy, D., Brophy, C., O'Donovana, M., and Lynch M. B.: The effect of the nitrification inhibitor dicyandiamide (DCD) on herbage production when applied at different times and rates in the autumn and winter, *Agric Ecosyst Environ* 152, 79-89, 2012.
- CRGCST (Cooperative Research Group on Chinese Soil Taxonomy): *Chinese Soil Taxonomy* (3rd Edition), Science Press, Beijing, New York, 2001
- 25 Saarnio, S., Heimonen, K., and Kettunen, R.: Biochar addition indirectly affects N₂O emissions via soil moisture and plant N uptake, *Soil Biology and Biochemistry*, 58, 99-106, 2013.
- Saggar, S., Giltrap, D. L., Li, C., and Tate, K. R.: Modelling nitrous oxide emissions from grazed grasslands in New Zealand, *Agriculture, ecosystems & environment*, 119, 205-216, 2007.
- Sánchez-García, M., Roig, A., Sanchez-Monedero, M. A., and Cayuela, M. L.: Biochar increases soil N₂O emissions produced by nitrification-mediated pathways, *Soil Processes*, 2(25), doi:
- 30

10.3389/fenvs.2014.00025, 2014.

Schmidt, E. L.: Nitrification in soil, *Nitrogen in agricultural soils, (nitrogeninagrics)*, 22, 253-288, 1982.

Schouten, S., van Groenigen, J. W., Oenema, O., and Cayuela, M. L.: Bioenergy from cattle manure? Implications of anaerobic digestion and subsequent pyrolysis for carbon and nitrogen dynamics in soil, *GCB Bioenergy*, 4, 751-760, 2012.

Singh, J., Saggar, S., Giltrap, D. L., and Bolan, N. S.: Decomposition of dicyandiamide (DCD) in three contrasting soils and its effect on nitrous oxide emission, soil respiratory activity, and microbial biomass-an incubation study, *Soil Research*, 46, 517-525, 2008.

Singh, B. P., Hatton, B. J., Singh, B., Cowie, A. L., and Kathuria, A.: Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils, *J. Environ. Qual.*, 39, 1224-1235, 2010.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogleh, S., O'Marai, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenko, V., Schneider, U., and Towprayoon, S.: Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture, *Agriculture, Ecosystems & Environment*, 118, 6-28, 2007.

Soares, J. R., Cantarella, H., and de Campos Menegale, M. L.: Ammonia volatilization losses from surface-applied urea with urease and nitrification inhibitors. *Soil biology and biochemistry*, 52, 82-89, (2012).

Spokas, K. A.: Impact of biochar field aging on laboratory greenhouse gas production potentials, *GCB Bioenergy*, 5, 165-176, 2013.

Spokas, K.A., and Reicosky, D.C.: Impacts of sixteen different biochars on soil greenhouse gas production, *Annals of Environmental Science*, 3(1), 4, 2009.

Steinbeiss, S., Gleixner, G., and Antonietti, M.: Effect of biochar amendment on soil carbon balance and soil microbial activity, *Soil Biol. Biochem.*, 41, 1301-1310, 2009.

Van Groenigen, J. W., Velthof, G. L., Oenema, O., Van Groenigen, K. J., and Van Kessel, C.: Towards an agronomic assessment of N₂O emissions: a case study for arable crops, *Euro. J. Soil. Sci.*, 61, 903-913, 2010.

Wang, L., Butterly, C. R., Wang, Y., Herath, H. M. S. K., Xi, Y. G., and Xiao, X. J.: Effect of crop

- residue biochar on soil acidity amelioration in strongly acidic tea garden soils, *Soil Use and Management*, 30(1), 119-128, 2014.
- Wang, J. Y., Xiong, Z. Q., and Yan, X. Y.: Fertilizer-induced emission factors and background emissions of N₂O from vegetable fields in China, *Atmos. Environ.*, 45, 6923-6929, 2011.
- 5 Wang, Z., Zheng, H., Luo, Y., Deng, X., Herbert, S., and Xing, B.: Characterization and influence of biochars on nitrous oxide emission from agricultural soil, *Environmental Pollution*, 174, 289-296, 2013.
- Wei, X. R., Hao, M. D., Xue, X. H., Shi, P., Horton, R., Wang, A., and Zang, Y. F.: Nitrous oxide emission from highland winter wheat field after long-term fertilization, *Biogeosciences*, 7, 10 3301-3310, 2010.
- Woolf, D., Amonette, J. E., Street-Perrott, F. A., Lehmann, J., and Joseph, S.: Sustainable biochar to mitigate global climate change, *Nature communications*, 1, 56, doi: 10.1038/ncomms1053, 2010.
- Wrage, N., Velthof, G. L., Van Beusichem, M. L., and Oenema, O.: Role of nitrifier denitrification in the production of nitrous oxide, *Soil Biology and Biochemistry*, 33(12), 1723-1732, 2001.
- 15 Xie, Z., Xu, Y., Liu, G., Liu, Q., Zhu, J., Tu, C., Amonette, J., Cadisch, G., Yong, J., and Hu, S.: Impact of biochar application on nitrogen nutrition of rice, greenhouse-gas emissions and soil organic carbon dynamics in two paddy soils of China, *Plant and soil*, 370, 527-540, 2013.
- Xiong, W., Xia, Y. Q., Zhou, W., and Yan, X. Y.: Relationship between nitrogen application rate and nitrous oxide emission and effect of nitrification inhibitor in vegetable farming system, *Acta Pedologica Sinica*, 50, 743-751, 2013. (In Chinese)
- 20 Xiong, Z. Q., Xie, Y. X., Xing, G. X., Zhu, Z. L., and Butenhoff, C.: Measurements of nitrous oxide emissions from vegetable production in China, *Atmos. Environ.*, 40, 2225-2234, 2006.
- Xu, X., Zhou, L., Van Cleemput, O., and Wang, Z.: Fate of urea-¹⁵N in a soil-wheat system as influenced by urease inhibitor hydroquinone and nitrification inhibitor dicyandiamide, *Plant and Soil*, 220, 261-270, 2000.
- 25 Yanai, Y., Toyota, K., and Okazaki, M.: Effects of charcoal addition on N₂O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments, *Soil Science and Plant Nutrition*, 53, 181-188, 2007.
- Yao, F. X., Arbestain, M. C., Virgel, S., Blanco, F., Arostegui, J., Maciá-Agulló, J. A., and Macías, F.: 30 Simulated geochemical weathering of a mineral ash-rich biochar in a modified Soxhlet reactor,

Chemosphere, 80, 724-732, 2010.

Yuan, J. H., and Xu, R. K.: The amelioration effects of low temperature biochar generated from nine crop residues on an acidic Ultisol, *Soil Use and Management*, 27(1), 110-115, 2011.

5 Zaman, M., and Blennerhassett, J. D.: Effects of the different rates of urease and nitrification inhibitors on gaseous emissions of ammonia and nitrous oxide, nitrate leaching and pasture production from urine patches in an intensive grazed pasture system, *Agric. Ecosyst. Environ.*, 136, 236-246, 2010.

Zaman, M., Nguyen, M. L., and Saggar, S.: N₂O and N₂ emissions from pasture and wetland soils with and without amendments of nitrate, lime and zeolite under laboratory condition, *Soil Research*, 46, 526-534, 2008.

10 Zaman, M., Saggar, S., Blennerhassett, J. D., and Singh, J.: Effect of urease and nitrification inhibitors on N transformation, gaseous emissions of ammonia and nitrous oxide, pasture yield and N uptake in grazed pasture system, *Soil Biology and Biochemistry*, 41, 1270-1280, 2009.

Zhang, A., Bian, R., Pan, G., Cui, L., Hussain, Q., Li, L., Zheng, J., Zheng, J., Zhang, X., Han, X., and Yu, X.: Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles, *Field Crops Research*, 127, 153-160, 2012b.

Zhang, A., Cui, L., Pan, G., Li, L., Hussain, Q., Zhang, X., Zheng, J., and Crowley, D.: Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China, *Agriculture, ecosystems & environment*, 139, 469-475, 2010.

20 Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J., and Zhang, X.: Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain, *Plant and soil*, 351, 263-275, 2012a.

Zhang, M., Fan, C. H., Li, Q. L., Li, B., Zhu, Y. Y., and Xiong, Z. Q.: A 2-yr field assessment of the effects of chemical and biological nitrification inhibitors on nitrous oxide emissions and nitrogen use efficiency in an intensively managed vegetable cropping system, *Agriculture, Ecosystems & Environment*, 201, 43-50, 2015.

Zheng, X. H., Han, S. H., Huang, Y., Wang, Y. S., and Wang, M. X.: Re-quantifying the emission factors based on field measurements and estimating the direct N₂O emission from Chinese croplands, *Global Biogeochemical Cycles*, 18(2), doi: 10.1029/2003GB002167, 2004.

30 Zhou, M., Zhu, B., Brüggemann, N., Bergmann, J., Wang, Y., and Butterbach-Bahl, K.: N₂O and CH₄

- emissions, and NO_3^- leaching on a crop-yield basis from a subtropical rain-fed wheat–maize rotation in response to different types of nitrogen fertilizer, *Ecosystems*, 17(2), 286-301, 2014.
- Zhu, T. B., Zhang, J. B., and Cai, Z. C.: The contribution of nitrogen transformation processes to total N_2O emissions from soils used for intensive vegetable cultivation, *Plant Soil*, 343, 313-327, 2011.
- 5 Zhu, X., Burger, M., Doane, T. A., and Horwath, W. R.: Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability, *Proceedings of the National Academy of Sciences*, 110(16), 6328-6333, 2013.

Table 1 Vegetable species and management procedure over the entire experimental period.

Vegetable Species	Growth Period	Fertilization Time	Tillage Time	Irrigation Time	Pesticide	Greenhouse Setting
Amaranth	2012/4/12- 2012/7/10	2012/4/18	2012/4/16	2012/4/17 2012/5/4 2012/5/8 2012/5/14	2012/4/16	2012/4/12- 2012/5/25
Tung choy	2012/7/11- 2012/11/19	2012/7/10 2012/9/7	2012/7/9	2012/7/10 2012/7/25 2012/8/15 2012/9/18 2012/9/25 2012/10/8	2012/7/9	
Baby bok choy	2012/11/2- 2013/3/27			2012/12/9 2012/12/29 2013/1/15 2013/2/1		2012/11/20- 2013/2/24
Coriander herb	2013/3/28- 2013/6/30	2013/3/27	2013/3/26	2013/3/27 2013/4/13	2013/3/26	
Tung choy	2013/7/1- 2013/11/4	2013/7/2 2013/9/8	2013/7/1	2013/7/2 2013/8/6 2013/8/15 2013/9/14	2013/7/1	
Baby bok choy	2013/11/5- 2014/3/14	2013/11/4	2013/11/3	2013/11/4 2013/12/1 2013/12/18		2013/11/5- 2014/3/14
Amaranth	2014/3/15- 2014/6/12	2014/3/14	2014/3/13	2014/3/14 2014/4/7 2014/4/23	2014/3/13	2014/3/15- 2014/5/12

Table 2

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	1.9	4.56	15.6	37.44	5.52
CF-C0	1.46±0.03 d	3.52±0.08	12.7±0.6 c	30.4±0.81	5.27±0.08 c
CP-C0	1.74±0.12 c	4.2±0.29	12.8±0.8 c	30.79±1.96	6.24±0.08 a
CF-C1	2.65±0.02 b	6.38±0.03	21.8±0.2 b	50.74±0.47	3.64±0.04 d
CP-C1	2.52±0.16 b	6.05±0.4	23.2±0.3 a	55.7±0.33	5.79±0.05 b
CF-C2	2.91±0.05 a	6.96±0.11	23.5±0.3 a	56.3±0.62	3.68±0.06 d
CP-C2	2.84±0.12 a	6.83±0.29	23.1±0.9 a	55.52±2.13	5.26±0.08 c
<i>P</i>		***		***	***

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.

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

*** $p < 0.001$

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

Table 3Cumulative N₂O emissions, vegetable yield and yield-scaled N₂O emissions under different treatments over the entire experimental period.

Treatments	Amaranth	Tung choy	Baby bok choy	Coriander herb	Tung choy	Baby bok choy	Amaranth	Total rotation
	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)	2012/4/18- 2014/6/12 (779d)
(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±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
(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±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
(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±0.017 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 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 ($p < 0.05$).

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	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>	SS	<i>F</i>	<i>P</i>
CP	1	570.09	19.29	<0.001	76098.8	111.36	<0.001	0.0171	78.04	<0.001
Bc	2	66.28	1.21	0.3577	12977.7	9.58	<0.05	0.0029	6.63	<0.05
CP×Bc	2	86.83	1.47	0.2687	17542.7	12.95	<0.05	0.0044	10.13	<0.05
Model	5	723.21	4.89		106619.4	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 exist between the control group and the experimental group

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 vertical line in each sub figure 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 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.

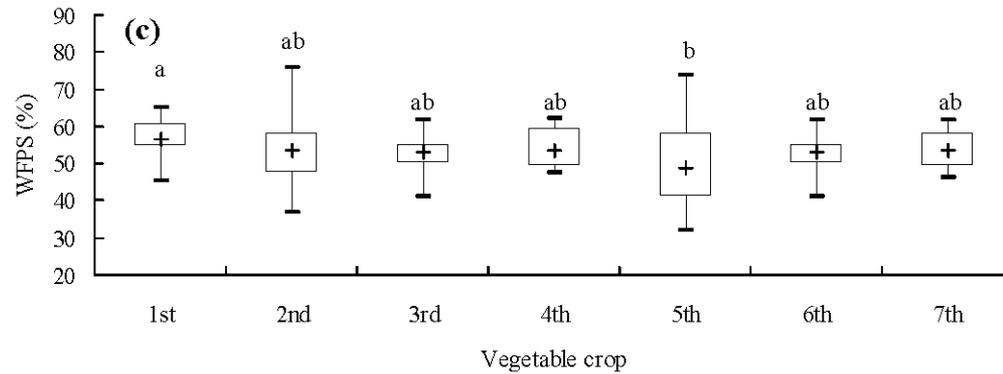
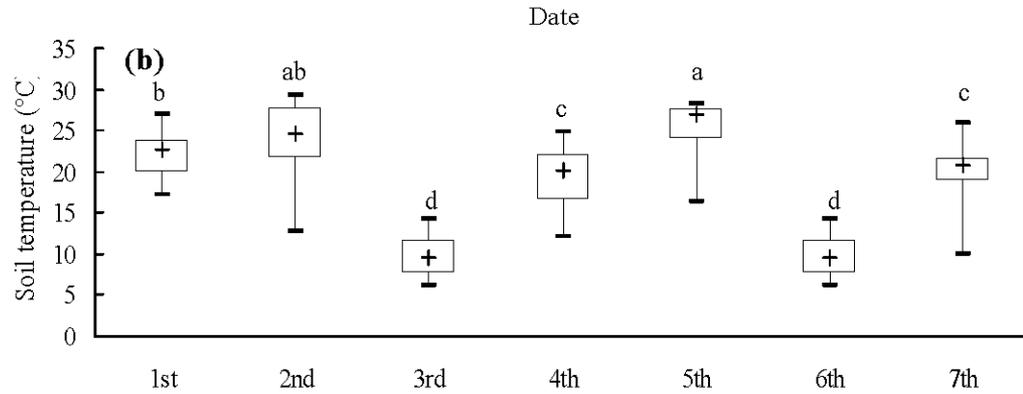
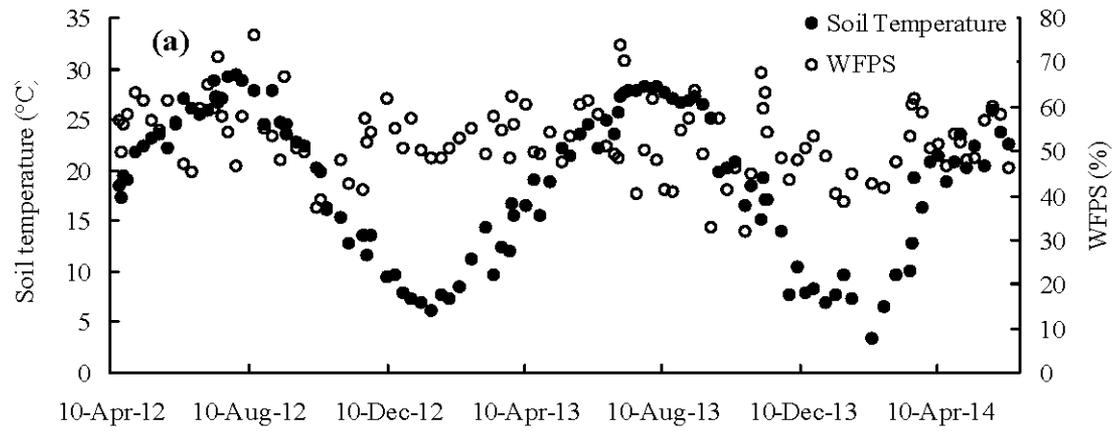
The solid and dashed arrows indicate basal fertilization and top-dressing, respectively. The dashed vertical line in each sub figure separates different vegetable growth periods. The bars indicate the standard error of the mean (+SE) for the 3 replicates of each treatment. See Table 2 for treatment codes.

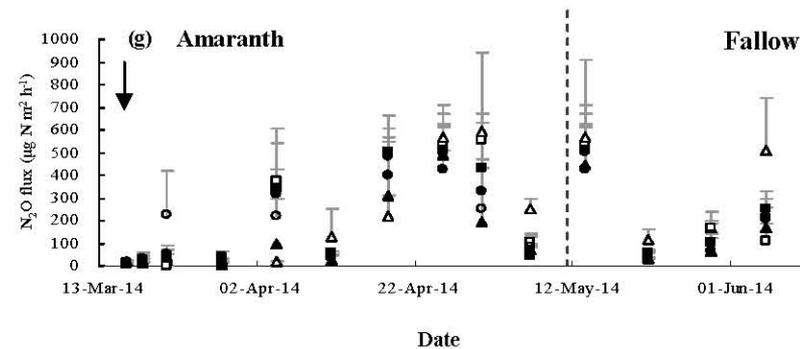
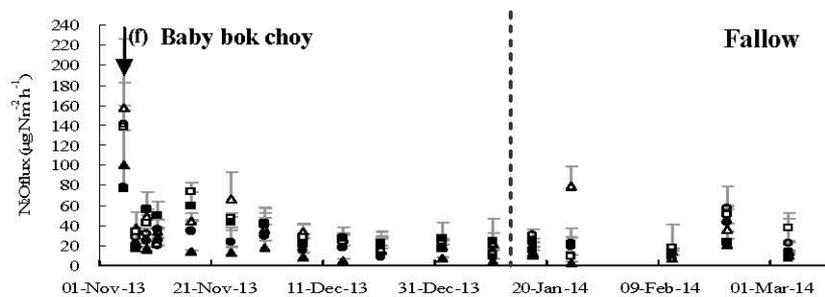
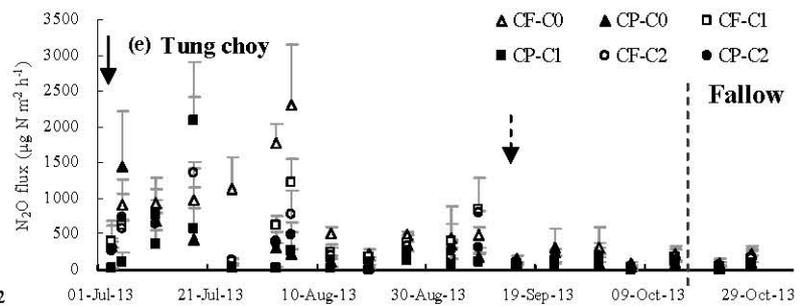
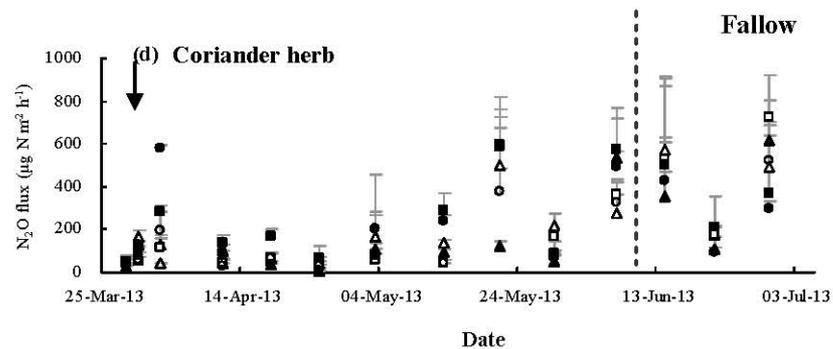
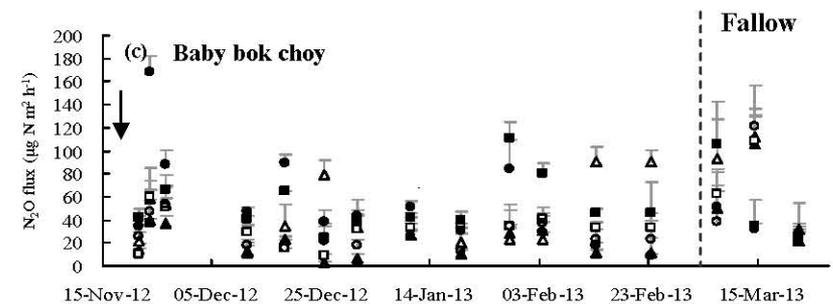
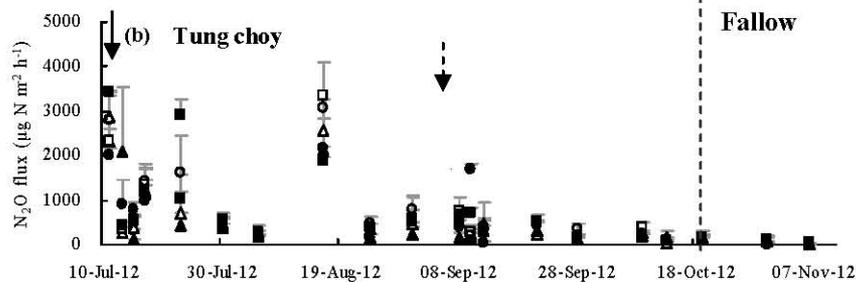
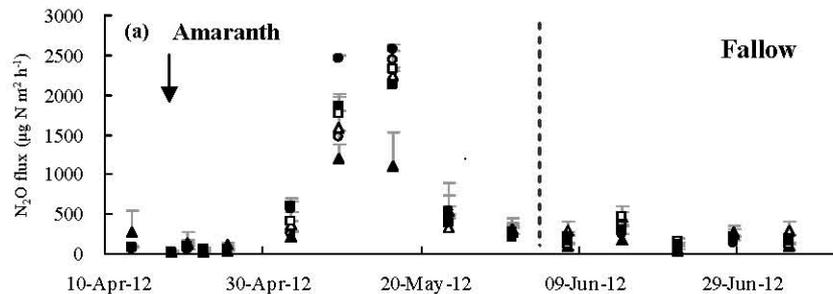
*** $p < 0.001$; n.s. not significant.

Figure 4 Dynamics of the soil 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 vertical line in each sub figure separates different vegetable growth periods. The bars indicate the standard error of the mean (+SE) for the 3 replicates of each treatment. See Table 2 for treatment codes.

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



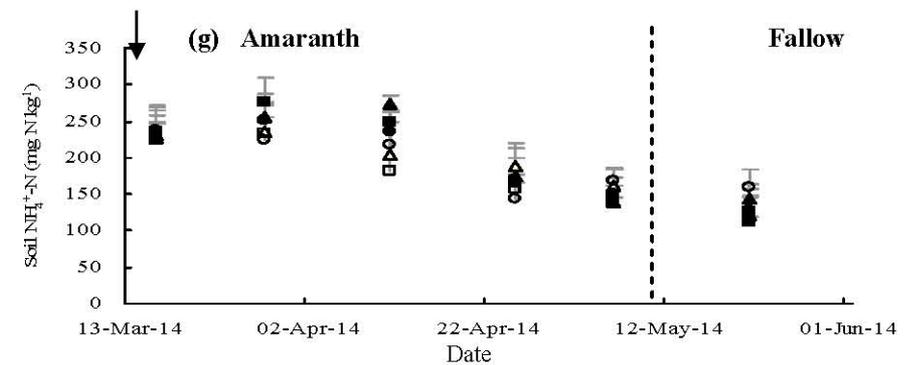
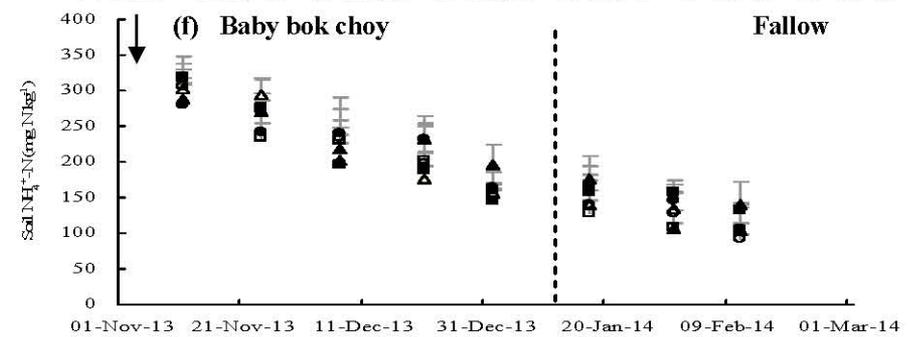
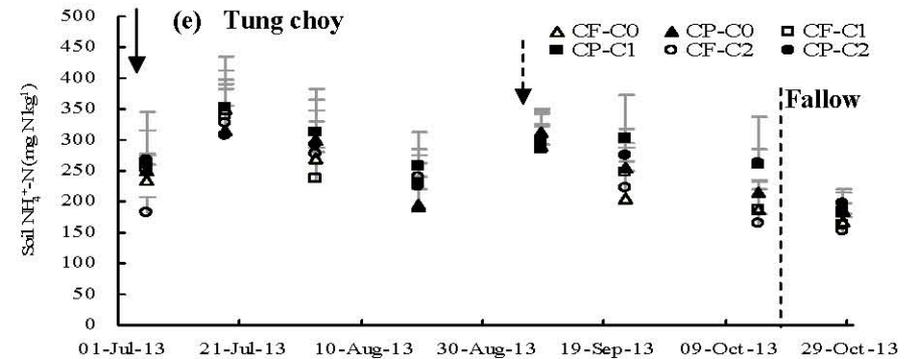
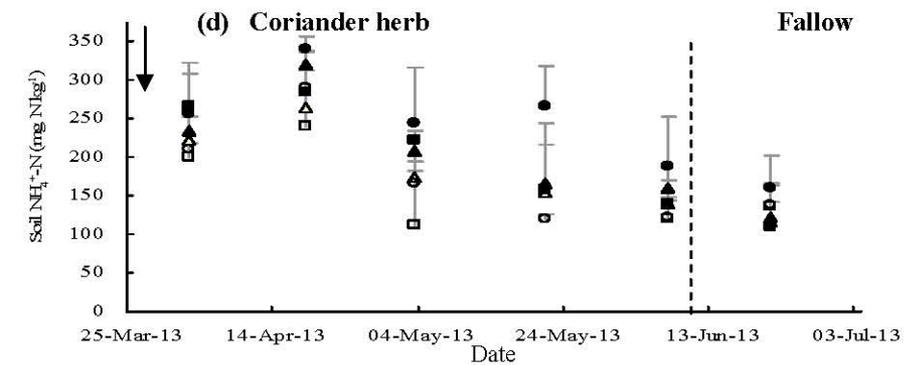
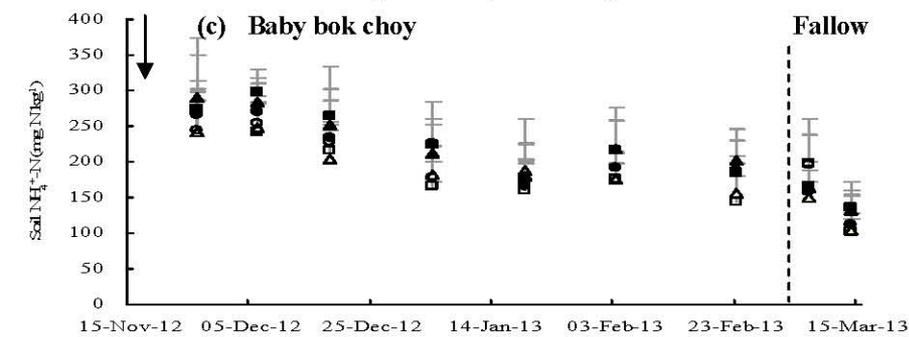
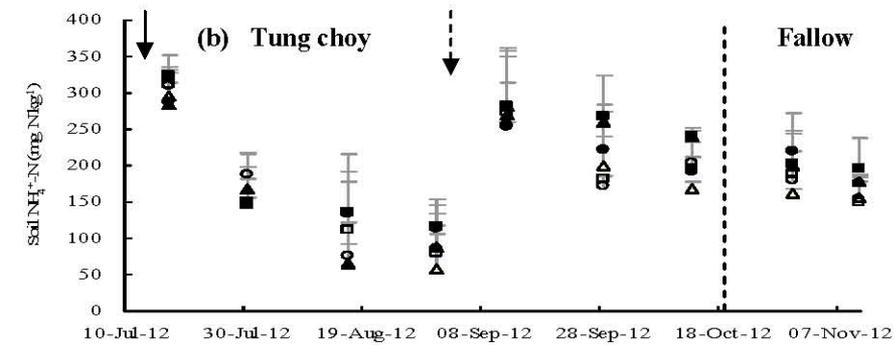
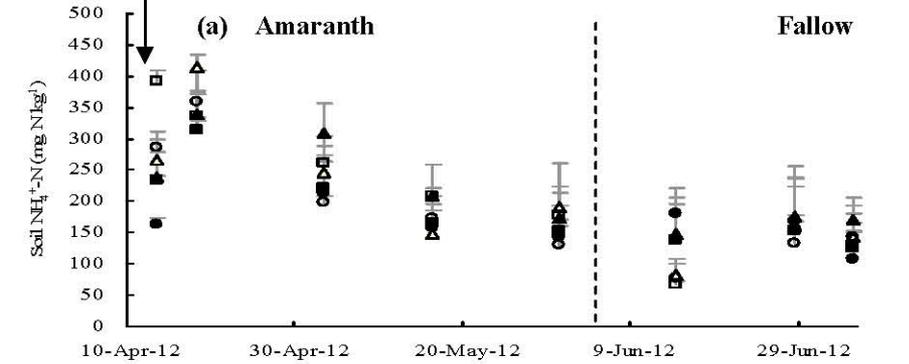


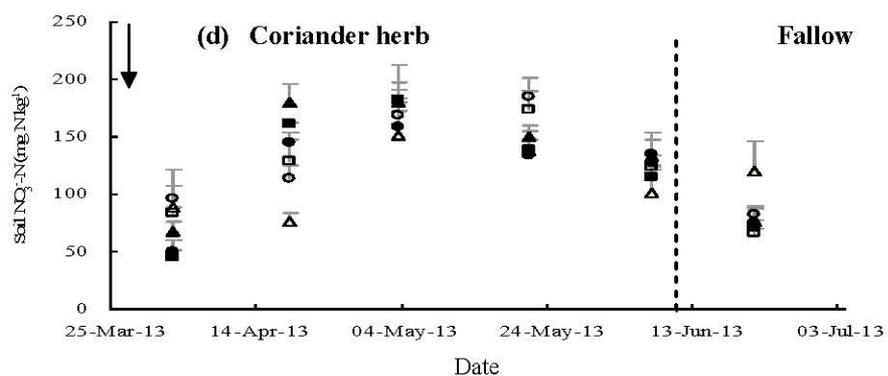
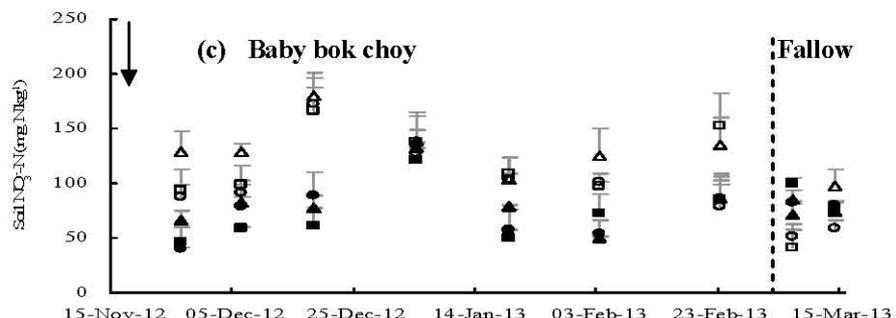
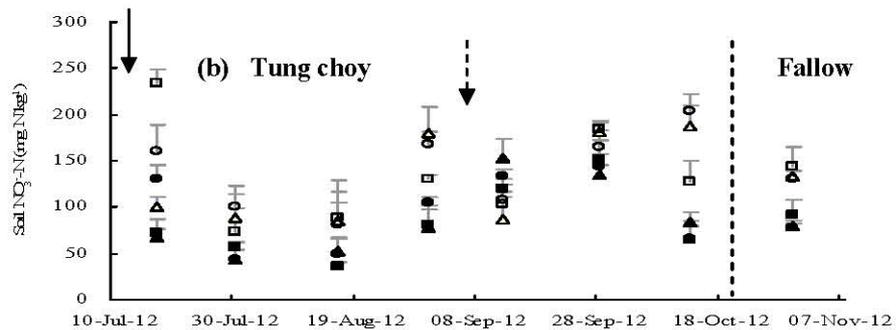
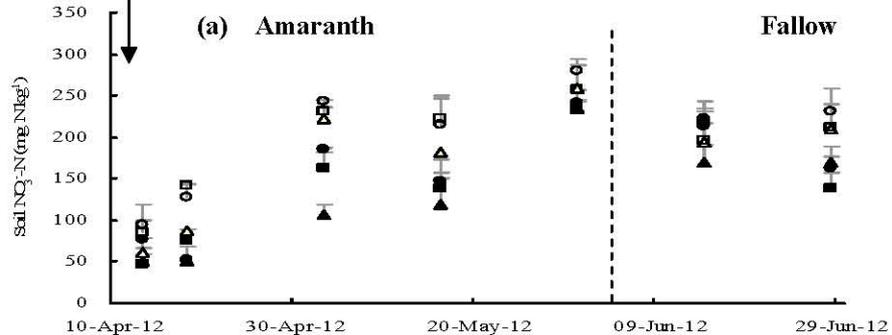
Total experimental period:

CP:***

Bc: n.s.

CP × Bc: n.s.





Total experimental period:

CP:***

Bc: n.s.

CP × Bc: n.s.

