

RESEARCH ARTICLE

# Effects of nitrogen application rate, nitrogen synergist and biochar on nitrous oxide emissions from vegetable field in south China

Qiong Yi<sup>1,2,3,4</sup> , Shuanghu Tang<sup>1,2,3</sup> \*, Xiaolin Fan<sup>4</sup> , Mu Zhang<sup>1,2,3</sup>, Yuwan Pang<sup>1,2,3</sup>, Xu Huang<sup>1,2,3</sup>, Qiaoyi Huang<sup>1,2,3</sup>

**1** Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences, Guangzhou, China, **2** Key Laboratory of Plant Nutrition and Fertilizer in South Region, Ministry of Agriculture, Guangzhou, China, **3** Guangdong Key Laboratory of Nutrient Cycling and Farmland Conservation, Guangzhou, China, **4** College of Agriculture, South China Agricultural University, Guangzhou, China

 These authors contributed equally to this work.

\* [tfstshu@aliyun.com.cn](mailto:tfstshu@aliyun.com.cn)



 OPEN ACCESS

**Citation:** Yi Q, Tang S, Fan X, Zhang M, Pang Y, Huang X, et al. (2017) Effects of nitrogen application rate, nitrogen synergist and biochar on nitrous oxide emissions from vegetable field in south China. PLoS ONE 12(4): e0175325. <https://doi.org/10.1371/journal.pone.0175325>

**Editor:** Dafeng Hui, Tennessee State University, UNITED STATES

**Received:** October 11, 2016

**Accepted:** March 23, 2017

**Published:** April 18, 2017

**Copyright:** © 2017 Yi et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** This research was financially supported by the Plan Project of Science and technology, Guangdong province (2014A020208051, 2014B090904068, 2012A020100004). Both of the funders played great roles in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

## Abstract

Globally, vegetable fields are the primary source of greenhouse gas emissions. A closed-chamber method together with gas chromatography was used to measure the fluxes of nitrous oxide (N<sub>2</sub>O) emissions in typical vegetable fields planted with four vegetables sequentially over time in the same field: endive, lettuce, cabbage and sweet corn. Results showed that N<sub>2</sub>O fluxes occurred in pulses with the N<sub>2</sub>O emission peak varying greatly among the crops. In addition, N<sub>2</sub>O emissions were linearly associated with the nitrogen (N) application rate ( $r = 0.8878$ ,  $n = 16$ ). Excessive fertilizer N application resulted in N loss through nitrous oxide gas emitted from the vegetable fields. Compared with a conventional fertilization (N<sub>2</sub>) treatment, the cumulative N<sub>2</sub>O emissions decreased significantly in the growing seasons of four plant species from an nitrogen synergist (a nitrification inhibitor, dicyandiamide and biochar treatments by 34.6% and 40.8%, respectively. However, the effects of biochar on reducing N<sub>2</sub>O emissions became more obvious than that of dicyandiamide over time. The yield-scaled N<sub>2</sub>O emissions in consecutive growing seasons for four species increased with an increase in the N fertilizer application rate, and with continuous application of N fertilizer. This was especially true for the high N fertilizer treatment that resulted in a risk of yield-scaled N<sub>2</sub>O emissions. Generally, the additions of dicyandiamide and biochar significantly decreased yield-scaled N<sub>2</sub>O-N emissions by an average of 45.9% and 45.7%, respectively, compared with N<sub>2</sub> treatment from the consecutive four vegetable seasons. The results demonstrated that the addition of dicyandiamide or biochar in combination with application of a rational amount of N could provide the best strategy for the reduction of greenhouse gas emissions in vegetable field in south China.

**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

As an important greenhouse gas, nitrous oxide ( $\text{N}_2\text{O}$ ) not only plays an important role in global warming, it also contributes greatly to ozone depletion. The observational data monitored by the World Meteorological Organization (WMO) showed that by 2012 the average concentration of  $\text{N}_2\text{O}$  increased to 325.1 ppb, which was 1.2 times higher than that in 1750 [1]. Because the warming potential of  $\text{N}_2\text{O}$  is 298 times that of  $\text{CO}_2$ ,  $\text{N}_2\text{O}$  emissions have received more attention. Agriculture contributes about 58% of total anthropogenic  $\text{N}_2\text{O}$  emissions, and soils serve as the main approach of these emissions [2]. Increase levels of atmospheric  $\text{N}_2\text{O}$  contribute about 6% of the overall global warming effect, with almost 80% of  $\text{N}_2\text{O}$  is emitted from agricultural lands; this  $\text{N}_2\text{O}$  originates from N fertilizers, soil disturbance and animal waste [3]. Over the long term, agricultural  $\text{N}_2\text{O}$  emissions are projected to increase by 35%–60% by 2030; this increase is projected to be caused by increases in application nitrogen (N) fertilizer and in animal manure production [4]. Therefore, effective mitigation measures used to mitigate  $\text{N}_2\text{O}$  emission from soil without sacrificing crop yield are urgently needed.

About 20% of the China's direct  $\text{N}_2\text{O}$  emission in the 1990s came from vegetable fields [5]. Vegetable crops cover about 1.35 million  $\text{hm}^{-2}$  in Guangdong Province ranking it fourth in the entire country, The Pearl River Delta region serves as the main vegetable production area, accounting for 37.6% of the total vegetable growing area in Guangdong Province; this region produces 32.75 million tons of vegetables per year [6–7]. Vegetable fields, a land use type with highly intensive use as well as a high rate of nitrogen application and frequent irrigation, are one of the most abundant land cover types that contribute greatly to greenhouse gas emissions in China [8]. Leaching and  $\text{N}_x\text{O}$  emission are the primary N loss pathways in vegetable fields, especially when high N application rates are used [9]. Surface soil N and environmental conditions are crucial for determining the short-term  $\text{N}_2\text{O}$  discharge during topdressing in greenhouse vegetable cultivation [10]. To reduce greenhouse gas emissions and alleviate the pressure on global warming potential (GWP), scientists have shown great interest in reducing emissions of greenhouse gases in recent years. Optimizing fertilizer N rates and applying nitrification inhibitors or changing from  $\text{NH}_4^+$  to  $\text{NO}_3^-$  based fertilizers can serve as effective measures for reducing  $\text{N}_2\text{O}$  emissions [11–12]. The addition of liming in soil with enriched fertilizer N could reduce  $\text{N}_2\text{O}$  emission, because the reduction of  $\text{N}_2\text{O}$  underground is an important process that limits  $\text{N}_2\text{O}$  emissions [13]. A markedly lower GWP, greenhouse gas intensity (GHGI) and enhance yields were observed when using the nitrification inhibitor, nitrapyrin and biological nitrification inhibitor treatments when compared to urea and a nitrification inhibitor, dicyandiamide (DCD) treatments in vegetable ecosystems [14]. Another research showed that the combination of chemical N fertilizer and manure with biochar (BC) at 30  $\text{Mg hm}^{-2}$  provided the most effective measures for reducing  $\text{N}_2\text{O}$  emissions in vegetable production [15]. The addition of BC increased soil organic carbon and total N content, vegetable yield and net ecosystem economic budget although it resulted in reduced net GWP and GHGI [16–17].

The emission of  $\text{N}_2\text{O}$  in vegetable fields is largely influenced by the cropping system used as well as by temperature, precipitation, fertilization, and vegetable species and so on. A simple short term comparison of vegetable greenhouse gas emissions among different cropping systems will provide inaccurate and unreasonable results. Although the dynamics of greenhouse gases emissions have been observed extensively in farmland, only very limited studies have been conducted related to technology that can be used to reduce greenhouse gases emissions using an evaluation index combined with N management in a vegetable field. Nevertheless, many studies have shown that DCD or BC are effective in reducing  $\text{N}_2\text{O}$  emissions, although it remained unclear which of these two materials would provide better results. More studies should be conducted that using more appropriate evaluation criterion to analyze the distinction

between DCD and BC. The seasonal dynamics of N<sub>2</sub>O fluxes were measured in a typical vegetable field planted with four vegetables grown and harvested consecutively over four growing seasons: endive, lettuce, cabbage and sweet corn. Our mainly hypotheses were that: (1) the seasonal dynamics emission fluxes and cumulative emission of N<sub>2</sub>O would be increased with the increase of nitrogen fertilizer in vegetable fields, (2) the N<sub>2</sub>O emission of DCD and BC treatment would be decreased when compared with conventional treatment, and (3) the yield-scaled N<sub>2</sub>O emission would be increased with the higher application of N fertilizer and the yield-scaled N<sub>2</sub>O emission of DCD and BC treatment would be reduced compared with conventional treatment. Yield-scaled N<sub>2</sub>O emission could be regard as an effective indicator to assess and balance the agricultural productivity with N<sub>2</sub>O emissions under this type of cultivation system.

## Materials and methods

### Description of the experiment

A field experiment was conducted with four crops planted and harvested independently and consecutively from Apr. 2015 to Jun. 2016 (Table 1). The experiment field was located at the test base of Guangdong Academy of Agricultural Sciences (23°8'52"N, 113°20'36"E). The region experiences a typical subtropical maritime monsoon climate with an annual mean temperature and rainfall of 22.5°C and 1517 mm, respectively. About 73.8% of all precipitation is received from March to August. The air temperature and precipitation data were obtained from nearby weather station (Fig 1, S1 Fig).

Four consecutive vegetable crops, i.e., endive (*Cichorium endivia* L.), lettuce (*Lactuca sativa* var. *ramosa* Hort.), cabbage (*Brassica oleracea* L. var. *capitata* L.) and sweet corn (*Zea mays* L.) were cultivated from 29 April 2015 to 2 June 2016. The soil properties in the top 20 cm of the latosolic red soil at the site were as follows: pH 4.88, bulk density 1.36 g cm<sup>-3</sup>, organic carbon 20.5 g kg<sup>-1</sup>, and total N 1.29 g kg<sup>-1</sup>. The experiment consisted of six treatments: (1) no fertilizer N treatment (N0), (2) low N application rate treatment with 435 kg N ha<sup>-1</sup> (N1), (3) conventional N application rate treatment with 870 kg N ha<sup>-1</sup> (N2), (4) high N application rate treatment with 1305 kg N ha<sup>-1</sup> (N3), (5) N2 plus 5% of N fertilizer synergist (N2\_DCD), (6) N2 incorporated with 10 Mg ha<sup>-1</sup> of biochar (N2\_BC). All the plots (each plot was 10 m<sup>2</sup>) were arranged in a completely randomized design with three replications. According to the local practice, urea (N 46%), superphosphate (P<sub>2</sub>O<sub>5</sub> 12%) and potassium sulfate (K<sub>2</sub>O 50%) were used to maintain soil nutrient balance and crop growth. 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 165 kg K<sub>2</sub>O ha<sup>-1</sup> were applied in the first three kinds of crops, although, 120 P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and 300 kg K<sub>2</sub>O ha<sup>-1</sup> s were applied to sweet corn during the growing season. Phosphate fertilizer was applied as basal fertilization, and potash fertilizer was applied with nitrogen fertilizer in the same proportion. DCD was applied along with fertilizer N although BC was applied along with basal

**Table 1. Cultivation time periods and N fertilization amounts and methods for four sequentially planted crops: endive, lettuce, cabbage, and sweet corn.**

Vegetation	Growth period (dd/mm/yy)	Treatment (kg N ha <sup>-1</sup> )						Topdressing	Ratio of basal N/dress N
		N0	N1	N2	N3	N2_DCD	N2_BC		
Endive	29/04/15-18/06/15	0	90	180	270	180	180	14/05/15, 01/06/15	0.3:(0.4:0.3)
Lettuce	29/09/15-16/11/15	0	75	150	225	150	150	12/10/15, 02/11/15	0.3:(0.4:0.3)
Cabbage	02/12/15-18/02/16	0	90	180	270	180	180	17/12/15, 04/01/16	0.3:(0.4:0.3)
Sweet corn	15/03/16-02/06/16	0	180	360	540	360	360	05/04/16, 11/04/16, 20/04/16	0.15:(0.3:0.15:0.4)

N0-no fertilizer N treatment; N1-low N application rate treatment (435 kg N ha<sup>-1</sup>); N2-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N3-high N application rate treatment (1305 kg N ha<sup>-1</sup>). N2\_DCD- conventional N application rate treatment plus 5% of N fertilizer DCD, N2\_BC- conventional N application rate treatment incorporated with 10 Mg ha<sup>-1</sup> of BC.

<https://doi.org/10.1371/journal.pone.0175325.t001>

fertilization. The growth period of each crop type and the dominant N fertilization practices (including N application rate, time of topdressing and ratio of basal N to dress N) are shown in Table 1. According to the local watering methods, the frequency of irrigation at the early stage, especially after transplanting was relatively high. The timing and amount of irrigation was dependent on the weather conditions.

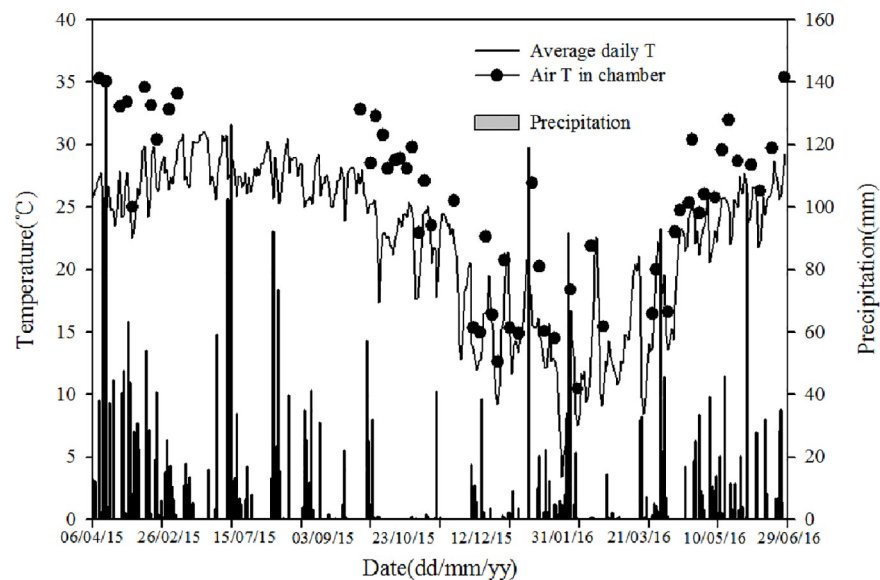
### Sampling and measurements

The closed-chamber method was used to determine the fluxes of N<sub>2</sub>O in each plot, and the concentrations of N<sub>2</sub>O were measured using an automated gas chromatograph (Agilent 7890B, USA) equipped with an electron capture detector (ECD). Gas samplings were conducted from 4 May 2015 to 28 June 2016 over 421 days. The gas collection device consisted of a chamber (0.4 m width × 0.4 m length × 0.4 m height) made of organic glass material with a stainless-steel base that was inserted into the ground. Generally, N<sub>2</sub>O flux was measured twice a week during the growing seasons. The sampling time for each chamber was 30 min in each treatment plot between 8:00 am and 12:00 am. Gas samples were collected using an injection syringe that was then taken to the laboratory as soon as possible to measure the concentration of N<sub>2</sub>O. Air temperatures outside and inside each sampling chamber were measured simultaneously with soil temperature and gravimetric moisture content at 5 cm depth for each treatment during the process of gas collection. The soil mineral nitrogen (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N) samples collected at important growing stages or after fertilization were analyzed with Continuous Flow Analysis (FUTURA II, Alliance, France). The vegetable yields were calculated from the edible part of first three crops and aboveground biomass of sweet corn.

### Statistical analysis

N<sub>2</sub>O flux was calculated by using a temporal increase in N<sub>2</sub>O concentration in the chamber over time, and using Eq (1):

$$N_2O \text{ flux } (\mu g \cdot m^{-2} \cdot h^{-1}) = \rho \times \frac{V}{A} \times \frac{dc}{dt} \times \frac{273}{273 + T} \tag{1}$$



**Fig 1. Air temperature inside and outside chamber and precipitation during consecutive four vegetable seasons.**

<https://doi.org/10.1371/journal.pone.0175325.g001>

where  $\rho$  is the density of  $N_2O$  under standard state,  $\frac{dc}{dt}$  is the change rate of  $N_2O$  concentration along with time,  $V$  is the volume of the chamber,  $A$  is the cover area of the chamber and  $T$  is the air temperature in chamber during sampling.

The cumulative  $N_2O$  emissions were calculated as the sum of daily estimates of  $N_2O$  flux obtained by linear interpolation between two adjacent sampling dates, with an assumption that  $N_2O$  flux measured on a sampling date was a representative of the average daily  $N_2O$  emissions.

The water-filled pore space (WFPS) was calculated using Eq (2):

$$\text{WFPS}(\%) = (\text{volumetric water content}/\text{total soil porosity}) \times 100 \quad (2)$$

where total soil porosity =  $1 - (\text{soil bulk density}/2.65)$ , with  $2.65 \text{ (g cm}^{-3}\text{)}$  being the assumed particle density of the soil.

Yield-scaled  $N_2O$  emissions ( $\text{g } N_2O\text{-N kg}^{-1}$  aboveground N uptake) were calculated using Eq (3) [18]:

$$\text{Yield-scaled } N_2O \text{ emission} = (\text{Cumulative } N_2O \text{ emission}/\text{aboveground N uptake}) \quad (3)$$

where aboveground N uptake denotes the total amount of N in aboveground biomass ( $\text{kg N ha}^{-1}$ ).

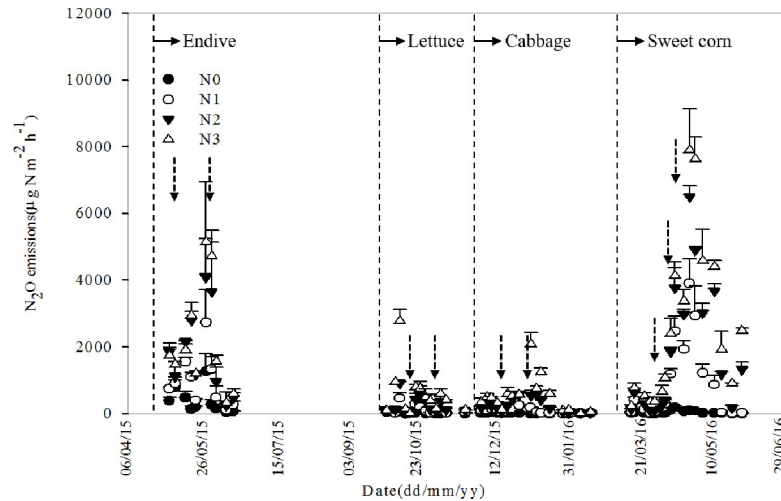
A general linear model (GLM) procedure was used for analysis of experimental data. Analysis of variance using Duncan's new multiple at a 5% confidence level was performed on the  $N_2O$  fluxes, the cumulative  $N_2O$  emissions and yield-scaled  $N_2O$  emissions. The correlation between  $N_2O$  fluxes and the N application rate, mineral N content and N application rate was analyzed by a linear model procedure. All data were analyzed using the SAS software package for Windows (SAS 9.0).

## Results

### Dynamic changes of $N_2O$ fluxes and accumulation of $N_2O$ emissions under different N application rates

Dynamics changes were observed in  $N_2O$  emissions fluxes during the growing seasons of the four crops (Fig 2, S2 Fig). The results showed that increases in  $N_2O$  fluxes were closely related to the rate of N application. The  $N_2O$  emissions occurred in pulses and the peak of  $N_2O$  emissions varied greatly with crop. In addition, the peak value of  $N_2O$  emissions increased with an increase in the N application rate. During the growth periods for endive, lettuce and sweet corn,  $N_2O$  emissions peaked at 30 days after transplanting (DAT) for all treatments, 10 DAT and 41–45 days after sowing (DAS), respectively. However, in cabbage, the  $N_2O$  emissions peaked at inconsistent times, a finding that may have been caused by the relatively low emission peak and low  $N_2O$  concentration.

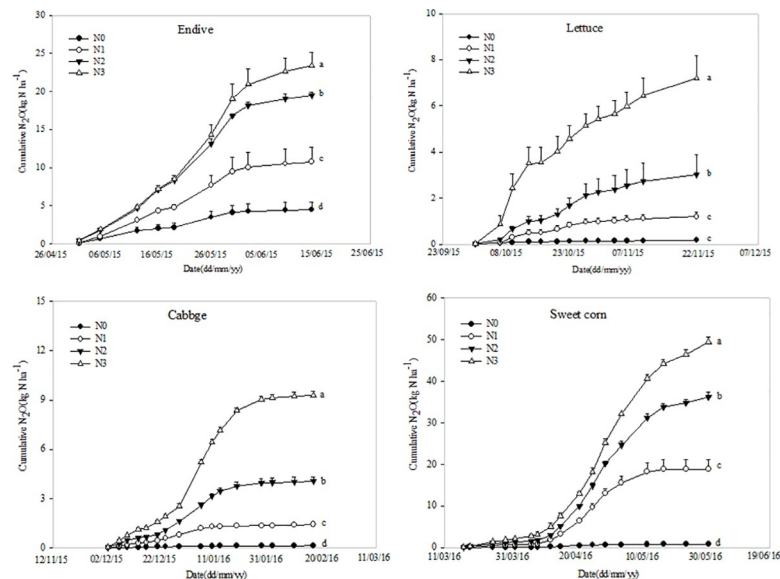
Cumulative  $N_2O$  fluxes of treatments with different N application rates for each crop growing season fluctuated greatly from 5.6 to 89.7  $\text{kg N ha}^{-1}$  (Fig 3, S3 Fig). When the different crops were compared, the lettuce growing season showed the lowest cumulative  $N_2O$  emissions among all N application treatments (except for the N0 treatment), accounting for less than 8.0% of total emissions from the observation periods. In contrast, the highest cumulative  $N_2O$  emission occurred during the sweet corn growing season, accounted for more than 46.1% of the total emissions (except for N0 treatment) during all four cropping seasons. This result was not only caused by the high level of N fertilization, but could also be partially attributed to the change in fertilization method to furrow application of the base fertilizer in sweet corn. Clearly, the trends cumulative  $N_2O$  emissions among different treatments during the growing seasons of four species were almost similar. A significant



**Fig 2. Temporal changes in N<sub>2</sub>O fluxes under different N application rates during four consecutive cropping seasons.** The dotted lines in figure indicate transplanting/sowing of each crop, while the dashed arrows indicate fertilization time. N0-no fertilizer N treatment; N1-low N application rate treatment (435 kg N ha<sup>-1</sup>); N2-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N3-high N application rate treatment (1305 kg N ha<sup>-1</sup>). The bars represent the standard error of the means (*n* = 3).

<https://doi.org/10.1371/journal.pone.0175325.g002>

difference in cumulative N<sub>2</sub>O emissions was observed between different N application levels in each growing season (N3 > N2 > N1 > N0), which indicated that N<sub>2</sub>O emissions in vegetable fields are strongly affected by fertilizer N input. Great N application input resulted in more N<sub>2</sub>O emission.



**Fig 3. Cumulative N<sub>2</sub>O emissions from different N application rates in four consecutive cropping seasons.** Different letters within each growing season indicated difference among treatments at *P* < 0.05 level by Duncan's new multiple range test. N0-no fertilizer N treatment; N1-low N application rate treatment (435 kg N ha<sup>-1</sup>); N2-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N3-high N application rate treatment (1305 kg N ha<sup>-1</sup>). The bars represent the standard error of the means (*n* = 3).

<https://doi.org/10.1371/journal.pone.0175325.g003>

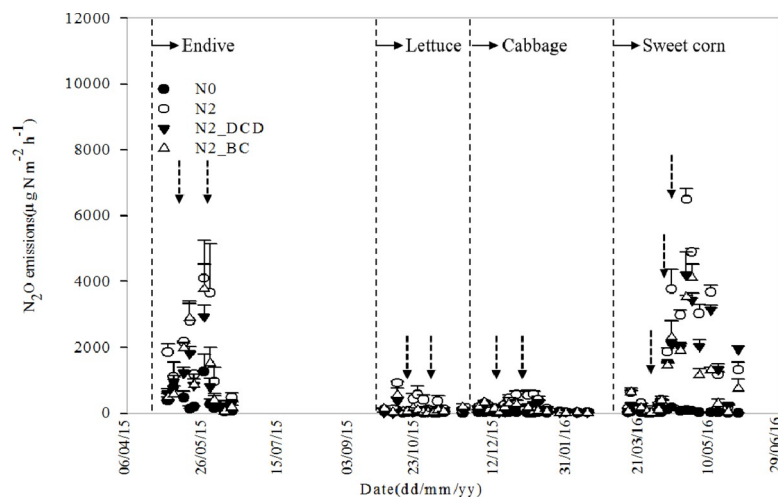
## Dynamic seasonal emission fluxes and cumulative emission characteristics of N<sub>2</sub>O between DCD and BC

The addition of DCD and BC could effectively reduce seasonal N<sub>2</sub>O emission fluxes when compared with the N<sub>2</sub> treatment (Fig 4, S4 Fig). N<sub>2</sub>O emission peaks of N<sub>2</sub>\_DCD and N<sub>2</sub>\_BC from four crop growing seasons were lower than that of N<sub>2</sub> treatment.

Overall, when compared with the treatment using the same amount of N fertilizer, the N<sub>2</sub>\_DCD and N<sub>2</sub>\_BC treatments significantly decreased cumulative N<sub>2</sub>O emissions in the observation periods by 34.6% and 40.8%, respectively. The cumulative data showed that compared with the N<sub>2</sub> treatment, cumulative N<sub>2</sub>O emissions from the N<sub>2</sub>\_DCD treatment decreased by 42.8%, 61.8%, 54.0% and 25.7% in the endive, lettuce, cabbage and sweet corn growing seasons, respectively. Meanwhile, the N<sub>2</sub>\_BC treatment resulted in decreased N<sub>2</sub>O emissions by 28.4%, 53.6%, 56.9% and 44.5% in comparison with the N<sub>2</sub> treatment for the same four crop growing seasons. Interestingly, the effects of N<sub>2</sub>\_DCD and N<sub>2</sub>\_BC treatments on cumulative N<sub>2</sub>O were quite different during the crop seasons of four crops grown consecutively (Fig 5, S5 Fig). In the endive season, the N<sub>2</sub>\_DCD treatment resulted in significantly reduced cumulative N<sub>2</sub>O emissions when compared with the N<sub>2</sub>\_BC treatment. In the lettuce and cabbage seasons, no significant differences were observed between N<sub>2</sub>\_DCD and N<sub>2</sub>\_BC on the reduction of the cumulative N<sub>2</sub>O emissions. Until sweet corn season, the BC treatment resulted in significantly reduced cumulative N<sub>2</sub>O emissions when compared with the N<sub>2</sub>\_DCD treatment. The results indicated that the effects of BC on N<sub>2</sub>O emission reduction became more and more obvious over time.

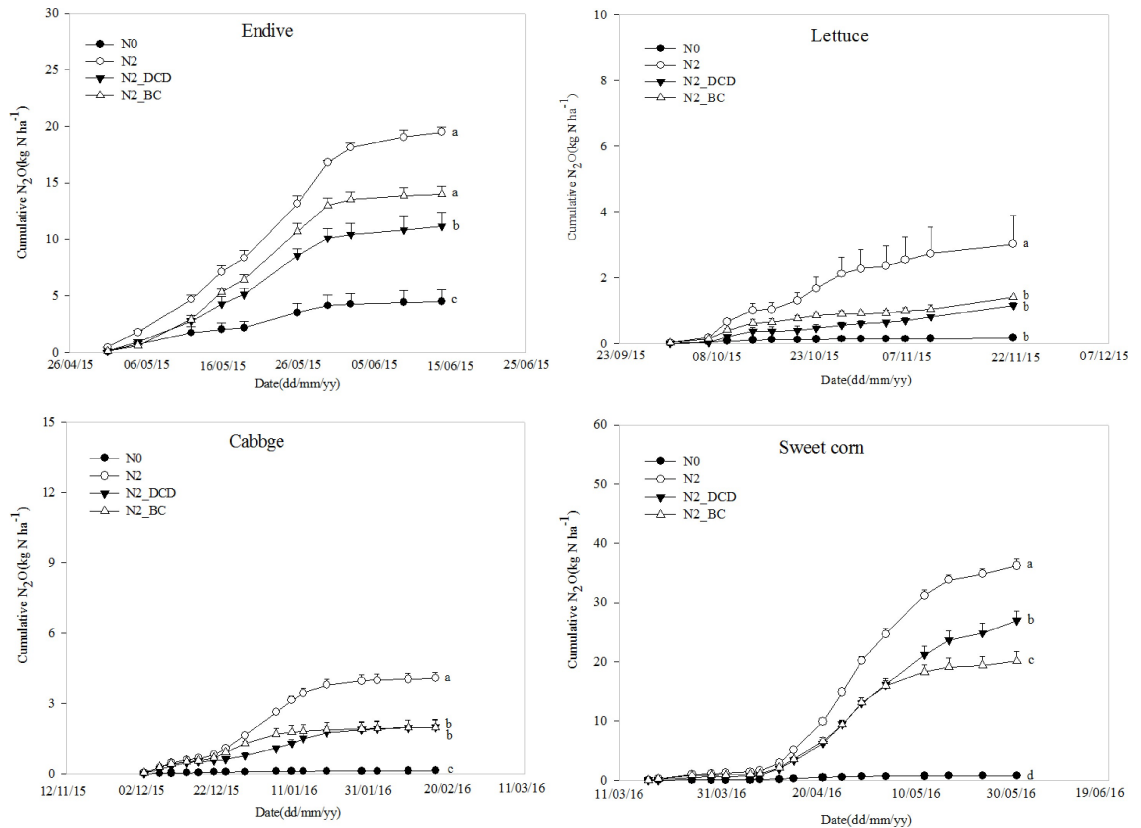
## Effects of N application rate on yield-scaled N<sub>2</sub>O emissions

The yield-scaled N<sub>2</sub>O emissions from different N application rates varied greatly with crops and N applications ranging from 8.2 to 593.6 g N<sub>2</sub>O-N kg<sup>-1</sup> N (Fig 6, S6 Fig). The yield-scaled N<sub>2</sub>O emissions in endive and sweet corn seasons were relatively higher than those of lettuce and cabbage seasons, which had the same trend of cumulative N<sub>2</sub>O emissions. This may have occurred because of the variations of climate in different seasons and the differences of the



**Fig 4. Temporal changes in N<sub>2</sub>O fluxes between dicyandiamide (DCD) and biochar (BC) during four consecutive cropping seasons.** The dotted lines in figure mean transplanting/sowing of each crop, although the dashed arrows mean fertilization incident. N<sub>0</sub>-no fertilizer N treatment; N<sub>2</sub>-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N<sub>2</sub>\_DCD-conventional N application rate treatment plus 5% of DCD, N<sub>2</sub>\_BC-conventional N application rate plus incorporated with 10 Mg ha<sup>-1</sup> of BC.

<https://doi.org/10.1371/journal.pone.0175325.g004>



**Fig 5. Cumulative N<sub>2</sub>O emission characteristics between dicyandiamide (DCD) and biochar(BC) treatment in four consecutive cropping seasons.** N<sub>0</sub>-no fertilizer N treatment; N<sub>2</sub>- conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N<sub>2</sub>\_DCD-conventional N application rate treatment plus 5% of DCD, N<sub>2</sub>\_BC-conventional N application rate plus incorporated with 10 Mg ha<sup>-1</sup> of BC.

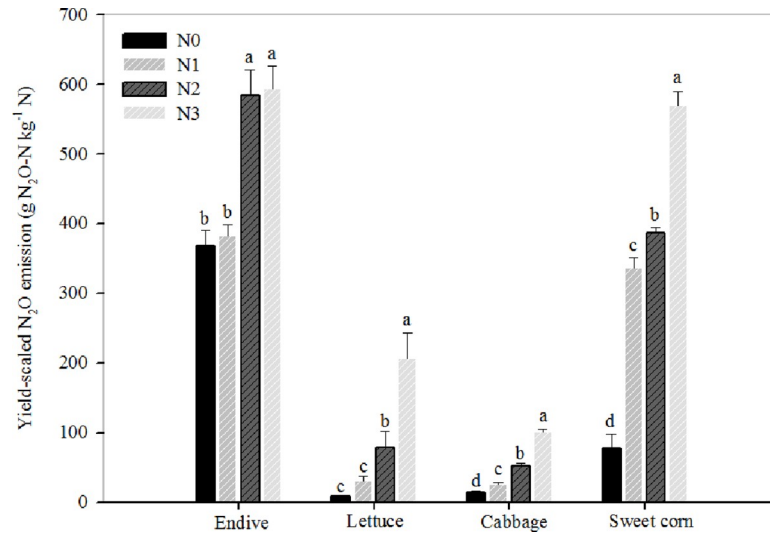
<https://doi.org/10.1371/journal.pone.0175325.g005>

production index. The yield-scaled N<sub>2</sub>O emissions in four consecutive growing seasons increased with an increase in the N application rate (Fig 6, S6 Fig). No significant difference in yield-scaled N<sub>2</sub>O emissions was observed between the N<sub>0</sub> and N<sub>1</sub> treatments in the first two vegetable growing seasons. However, a significant difference was observed in yield-scaled N<sub>2</sub>O emissions between N<sub>0</sub> and N<sub>1</sub> in the last two vegetable growing seasons. The N<sub>3</sub> treatment resulted in significantly increased yield-scaled N<sub>2</sub>O emissions by 1.7%, 163.6%, 93.5% and 47.4% when compared with the N<sub>2</sub> treatments. The results indicated that the continuous application of N fertilizer, especially for the high N fertilizer treatment, resulted in the risk of yield-scaled N<sub>2</sub>O emissions.

### Effects of DCD and BC on yield-scaled N<sub>2</sub>O emissions

The yield-scaled N<sub>2</sub>O emissions from DCD and BC also varied greatly among crops ranging from 8.7 to 583.9 g N<sub>2</sub>O-N kg<sup>-1</sup> N (Fig 7, S7 Fig). Compared with the N<sub>2</sub> treatment, the N<sub>2</sub>\_DCD treatment resulted in significantly decreased the yield-scaled N<sub>2</sub>O emissions by 48.1%, 61.4%, 56.5% and 17.6% in endive, lettuce, cabbage and sweet corn, respectively. Similarly, the application of the BC treatment also resulted in significantly reduced the yield-scaled N<sub>2</sub>O emissions by 42.2%, 56.8%, 55.0% and 28.7% in the same four crops, respectively. On average, the DCD and BC treatments resulted in decreased yield-scaled N<sub>2</sub>O-N emissions by 45.9% and 45.7% when compared with the N<sub>2</sub> treatment, which indicated that the effects of

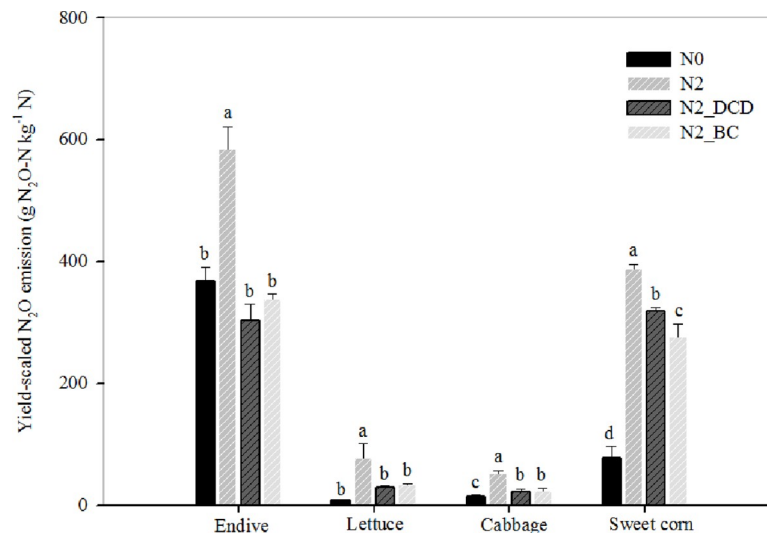




**Fig 6. Effects of different N application rate on yield-scaled N<sub>2</sub>O emissions from four consecutive cropping seasons.** N0-no fertilizer N treatment; N1, low N application rate treatment (435 kg N ha<sup>-1</sup>); N2-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N3-high N application rate treatment (1305 kg N ha<sup>-1</sup>). The yield here refers to edible part of a vegetable in first three crops and aboveground biomass of sweet corn. Different letters indicate significantly difference between treatments at *P*<0.05 by Duncan's new multiple range test.

<https://doi.org/10.1371/journal.pone.0175325.g006>

the nitrification inhibitor (DCD) and BC on N<sub>2</sub>O emission reduction under the conditions of this experiment were quite remarkable, and the effects of BC on N<sub>2</sub>O emission reduction was better than DCD to some extent, particularly in the late crops of the test.



**Fig 7. Effects of dicyandiamide (DCD) and biochar (BC) on yield-scaled N<sub>2</sub>O emissions from four consecutive cropping seasons.** N0-no fertilizer N treatment; N2-conventional N application rate treatment (870 kg N ha<sup>-1</sup>); N2\_DCD-conventional N application rate treatment plus 5% of DCD, N2\_BC-conventional N application rate plus incorporated with 10 Mg ha<sup>-1</sup> of BC. The yield here refers to edible part of a vegetable in first three crops and aboveground biomass of sweet corn. Different letters indicate significantly difference between treatments at *P*<0.05 by Duncan's new multiple range test.

<https://doi.org/10.1371/journal.pone.0175325.g007>

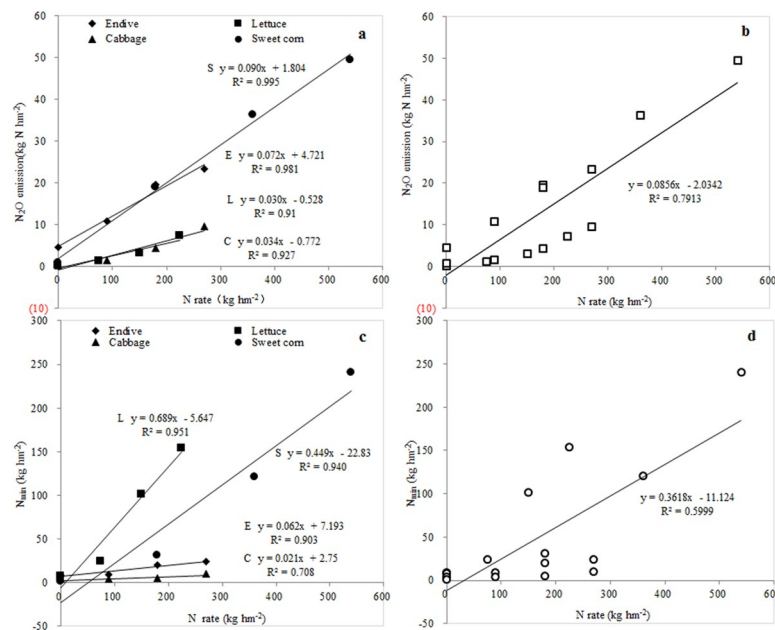
## Discussions

### The influence of different N fertilizer levels on N<sub>2</sub>O emissions

Nitrogen fertilization markedly influenced the soil N<sub>2</sub>O emission, although the effects of N fertilization were quite different in terms of nitrogen applications rates and types, crops, and seasons [19]. Both fertilization and plant types significantly altered N<sub>2</sub>O emission [20]. Small changes in N fertilizer can have a substantial environmental impact. A change from 75 to 50 kg N hm<sup>-2</sup> reduced the GWP per hm<sup>-2</sup> by 18% [21]. It is usually assumed that N<sub>2</sub>O emissions will increase with an increase in the N application rate [22]. Conversely, some studies have reported that there was a nonlinear response of N<sub>2</sub>O emission to incremental additions of N fertilizer [23], and the N<sub>2</sub>O emissions exhibited the same seasonal pattern whatever the treatment and the type of crop had little impact on the level of N<sub>2</sub>O emission [24]. In this study, N<sub>2</sub>O emissions were linearly associated with the N application rate. When considering the four consecutive crops studied here, seasonal N<sub>2</sub>O emissions had strong positive correlations with N application rates for each growing season (Fig 8A, S8 Fig) and the four cropping seasons ( $r = 0.8878^{**}$ ,  $p < 0.0001$ ,  $n = 16$ ) (Fig 8B, S8 Fig). Besides, significant difference in cumulative N<sub>2</sub>O emissions was found among crop types, which mainly attributed to the influence of soil mineral N content and temperature factor. What's more, the residual mineral nitrogen in the vegetable fields was also closely associated with the N application rate for each growing season (Fig 8C, S8 Fig) and four cropping seasons ( $r = 0.7745^{**}$ ,  $p = 0.0004$ ,  $n = 16$ ) (Fig 8D, S8 Fig).

### The influence of DCD and BC on N<sub>2</sub>O emissions

The application of nitrification inhibitors in agricultural soils is considered to be a promising approach for increasing N use efficiency and reducing N<sub>2</sub>O emissions to the environment



**Fig 8. The correlation between N<sub>2</sub>O emissions, mineral N content and N application rate from vegetable fields.** E-Endive, L-Lettuce, C-Cabbage and S-Sweet corn. N<sub>min</sub>-soil mineral nitrogen, the sum of NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N. a and b indicate a significant relationship exists between N application rate and N<sub>2</sub>O emissions in each growing season and in four consecutive seasons, respectively, c and d mean a significant correlation exists between N<sub>2</sub>O emissions and N<sub>min</sub> in each growing season and in four consecutive seasons, respectively.

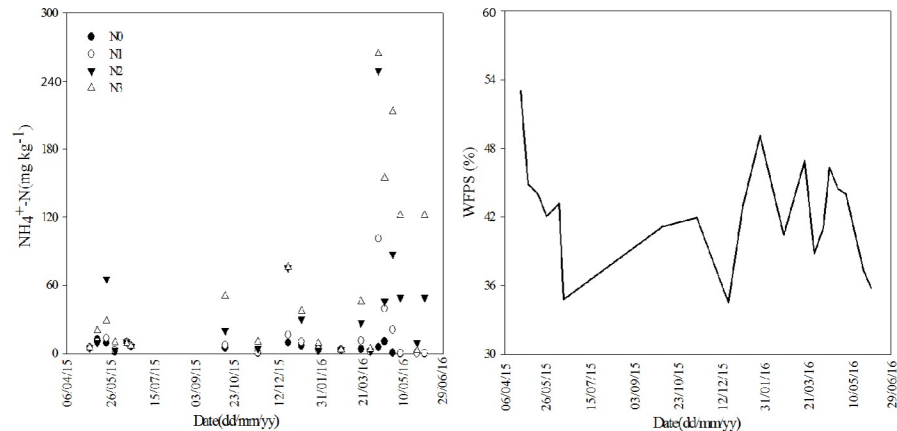
<https://doi.org/10.1371/journal.pone.0175325.g008>

[25]. The structure of DCD contains similar amino and imino functional groups in the  $\text{NH}_3$  structure, and this structure results in DCD in the form of substrate competition to disturb the use of ammonia oxidation on the substrate, thereby inhibiting the nitrification [26]. DCD had the most significant effect in reducing  $\text{N}_2\text{O}$  emissions under the highest nitrogen application rate, and a higher rate of DCD will be more effective in reducing  $\text{N}_2\text{O}$  emission [22, 27]. BC is widely used in soil improvement and allows for a reduction in carbon emissions because of its special functions and characteristics. The addition of BC into agricultural soils significantly increased soil total N, soil organic C and vegetable yield [28]. In this study, the results showed that both DCD and BC materials could effectively mitigate  $\text{N}_2\text{O}$  emission fluxes and cumulative  $\text{N}_2\text{O}$  emissions although the reduction mechanisms might be different for these two materials. The effects of DCD on reducing  $\text{N}_2\text{O}$  emissions may attribute to the significant reduced the AOB *amoA* gene copy numbers especially with high nitrogen application rates [22]. However, the mechanism for  $\text{N}_2\text{O}$  emission reduction of BC lies in how it affects many of the soil biogeochemical processes involved with the changes in organic carbon, nitrogen and enzymatic activities [29]. Unlike the DCD treatment, BC did not limit the availability of inorganic nitrogen to nitrifying and denitrifying bacteria; thus, the supply of ammonium and nitrate ions in the soil could not reveal inhibition of  $\text{N}_2\text{O}$  emissions [30]. We also found no significant difference between the  $\text{N}_2\text{-BC}$  treatment and conventional treatment in vegetable yields (data not shown). BC treatment significantly reduced accumulation of  $\text{N}_2\text{O}$  emissions and yield-scaled  $\text{N}_2\text{O-N}$  emissions, and this was beneficial for enhancing nitrogen use efficiency and reducing N loss caused by  $\text{N}_2\text{O}$  release.

### The driving factors and evaluation indicator of $\text{N}_2\text{O}$ emissions

Soil moisture, air temperature and N application significantly affected  $\text{N}_2\text{O}$  emissions [31–32]. In addition, the  $\text{N}_2\text{O}$  emissions increase when soil pH decreases, and the addition of DCD resulted in a significant decrease in total  $\text{N}_2\text{O}$  emissions in the acid condition and decreased peak  $\text{N}_2\text{O}$  emissions in all pH treatments [33]. High content of soil available nitrogen, especially for ammonium nitrogen, caused higher  $\text{N}_2\text{O}$  emissions of vegetables when compared with winter wheat fields [34–35]. The  $\text{N}_2\text{O}$  emissions from soil with ammonium nitrogen fertilizer application were relatively higher than soil with nitrate nitrogen fertilizer application [36]. The present study also found an obvious correlation between peak  $\text{N}_2\text{O}$  emissions and ammonium nitrogen content. The peak  $\text{N}_2\text{O}$  emissions usually occurred within two weeks after the highest content of soil ammonium nitrogen (Fig 9, S9 Fig). The result indicated that an abundant accumulation of ammonium ions more likely resulted in an increase in loss of nitrous oxide gas from vegetable fields. Soil  $\text{N}_2\text{O}$  emission flux and its source was closely related with the dynamic change of ammonium nitrogen and nitrate content in soil [37]. However, the WFPS in this study showed no significant correlation with  $\text{N}_2\text{O}$  emissions.

In general, yield-scaled greenhouse gas (GHG) emissions provide a valuable measure for assessing the ability of management to mitigate emission without affecting by interaction management on crop productivity compared to area basis emission [38]. Therefore, the yield-scaled  $\text{N}_2\text{O}$  emission can serve as an indicator to express  $\text{N}_2\text{O}$  emissions in relation to crop productivity by calculating the  $\text{N}_2\text{O}$  emissions per unit aboveground N uptake [18]. Yield-scaled  $\text{N}_2\text{O}$  emissions changed greatly with the plantation crop species and different fertilization treatments. Besides, yield-scaled  $\text{N}_2\text{O}$  emissions varied widely in agricultural soils, a level of variation that is caused by many factors, such as N source, climate, cropping system and sites [39–40]. Burzaco et al. [41] showed that yield-scaled  $\text{N}_2\text{O-N}$  emissions increased with N application rates. In this study, the yield-scaled  $\text{N}_2\text{O}$  emissions from the intensively fertilized vegetable fields were 8.2%–593.6%. These percentages were extremely higher than other



**Fig 9. Dynamic changes of the content of soil  $\text{NH}_4^+\text{-N}$  and water-filled pore space (WFPS) during four vegetable growing periods.** N0-no fertilizer N treatment; N1-low N application rate treatment ( $435 \text{ kg N ha}^{-1}$ ); N2-conventional N application rate treatment ( $870 \text{ kg N ha}^{-1}$ ); N3-high N application rate treatment ( $1305 \text{ kg N ha}^{-1}$ ).

<https://doi.org/10.1371/journal.pone.0175325.g009>

reports and may have been partially caused by the high rates of precipitation, high temperatures, concentrated cultivated pattern and so on. The total  $\text{N}_2\text{O}$  emissions from treatments in this study ranged from  $32.3$  to  $89.7 \text{ kg N hm}^{-2}$ , accounting for  $4.8\%$ – $7.4\%$  of the total nitrogen input. The results of the present study indicated that emissions from vegetable fields are important potential sources of China's  $\text{N}_2\text{O}$  inventory. However, large uncertainties existed in the estimation of direct  $\text{N}_2\text{O}$  emissions and background emissions of  $\text{N}_2\text{O}$  from vegetable fields because different cropping systems have different emission characteristics, especially for these intensively managed vegetable fields. It also showed that yield-scaled  $\text{N}_2\text{O}$  emissions were  $22\%$  lower with nitrapyrin than without the inhibitor at the same level of N fertilizer, but these did not interact with N rate or timing [41]. The result of this study also showed a significant reduction in yield-scaled  $\text{N}_2\text{O}$ -N emissions with nitrification inhibitor and biochar treatment by  $45.9\%$  and  $45.7\%$  respectively compared with N2 treatment. The response of yield-scaled  $\text{N}_2\text{O}$ -N emissions to fertilizer N addition was positive while to the addition of DCD and BC was negative, which mainly caused by the rate of increase in  $\text{N}_2\text{O}$  emission comparison to aboveground N uptake. Therefore, minimizing yield-scaled  $\text{N}_2\text{O}$ -N emissions could be realized by optimizing N application rates with high yields.

## Conclusions

In the present study,  $\text{N}_2\text{O}$  emissions were linearly associated with the N application rate in vegetable fields. The  $\text{N}_2\text{O}$  emissions occurred in pulses and the peak of  $\text{N}_2\text{O}$  emissions varied greatly with crops and treatments. The peak value of  $\text{N}_2\text{O}$  emissions increased with an increase in the N application rate. The total  $\text{N}_2\text{O}$  emissions from treatments in this study ranged from  $32.3$  to  $89.7 \text{ kg N hm}^{-2}$ , accounting for  $4.8\%$ – $7.4\%$  of the total nitrogen input. This finding indicated that emissions from vegetable fields are important potential sources of  $\text{N}_2\text{O}$  emissions in China. Compared with the same amount of N fertilizer treatment, N2\_DCD and N2\_BC treatment significantly decreased cumulative  $\text{N}_2\text{O}$  emissions by  $34.6\%$  and  $40.8\%$ , respectively. These results indicated that BC was better at reducing  $\text{N}_2\text{O}$  emissions than DCD, particularly in the late growth stage of the four crops tested here. Yield-scaled  $\text{N}_2\text{O}$  emissions varied greatly with crops under different N level treatments. Overall, this study provides insights for the effective technical measure related to inhibiting  $\text{N}_2\text{O}$  emissions under field

conditions in southern China. In addition, the yield-scaled N<sub>2</sub>O emissions also could be regarded as an environment parameter that can be used to evaluate N<sub>2</sub>O emission potential or calculate the N<sub>2</sub>O inventory. Furthermore, N management strategies also should be adjusted to enhance the efficiency of fertilizer use and provide for vegetable production without sacrificing yield and without the increasing N<sub>2</sub>O emissions. However, further study should be considered on the economic effects of controlling N<sub>2</sub>O emissions with the goal of providing environment friendly sustainable development.

## Supporting information

**S1 Fig. Original data for Fig 1.**

(XLSX)

**S2 Fig. Original data for Fig 2.**

(XLSX)

**S3 Fig. Original data for Fig 3.**

(XLSX)

**S4 Fig. Original data for Fig 4.**

(XLSX)

**S5 Fig. Original data for Fig 5.**

(XLSX)

**S6 Fig. Original data for Fig 6.**

(XLSX)

**S7 Fig. Original data for Fig 7.**

(XLSX)

**S8 Fig. Original data for Fig 8.**

(XLSX)

**S9 Fig. Original data for Fig 9.**

(XLSX)

## Acknowledgments

We sincerely appreciate the editors and anonymous reviews for their critical and valuable comments to help improve this manuscript. Thanks come to my colleagues and field staff for their efforts.

## Author Contributions

**Conceptualization:** QY SHT.

**Data curation:** QY SHT MZ.

**Funding acquisition:** QY SHT.

**Investigation:** QY XH QYH.

**Methodology:** QY SHT XLF.

**Project administration:** SHT QY YWP.

**Writing – original draft:** QY.

Writing – review & editing: SHT XLF.

## References

1. World Meteorological Organization Greenhouse Gas Bulletin, The State of Greenhouse Gases in the Atmosphere Based on Global Observations through. 2013; 9.
2. US-EPA, 2006: Global Anthropogenic Non-CO<sub>2</sub> Greenhouse Gas Emissions: 1990–2020. United States Environmental Protection Agency, EPA 430-R-06-003, June 2006. Washington, D.C., <http://www.epa.gov/nonco2/econ-inv/downloads/GlobalAnthroEmissionsReport.pdf>.
3. Dalal RC, Wang WJ, Robertson GP and Parton WJ. Nitrogen oxide emission from Australian agricultural lands and mitigation options: a review[J]. *Aust. J. Soil Res.* 2003; 41: 165–195.
4. FAO, 2003: World Agriculture: Towards 2015/2030. An FAO Perspective. FAO, Rome, pp. 97.
5. Zheng XH, Han SH, Huang Y, Wang YS, Wang MX. Re-quantifying the emission factors based on field measurements and estimating the direct N<sub>2</sub>O emission from Chinese croplands [J]. *Global Biogeochem. Cy.* 2004; 18 (2), GB2018.
6. Guangdong rural statistical yearbook editing committee. Guangdong rural statistical yearbook [M]. Beijing: China Statistics Press, China statistical, 2015.
7. National bureau of statistics of the People's Republic of China. Statistical yearbook of Guangdong [M]. Beijing: China Statistics Press, China statistical, 2015.
8. Yan HL, Xie LY, Guo LP, Fan JW, Diao TT, Lin M, et al. Characteristics of nitrous oxide emissions and the affecting factors from vegetable fields on the North China Plain [J]. *J. Environ. Manage.* 2014; 144: 316–321 <https://doi.org/10.1016/j.jenvman.2014.06.004> PMID: 24991790
9. Min J, Zhao X, Shi WM, Xing GX, Zhu ZL. Nitrogen balance and loss in a greenhouse vegetable system in southeastern China [J]. *Pedosphere.* 2011; 21 (4): 464–472.
10. Riya S, Min J, Zhou S, Shi WM, Hosomi M. Short-term responses of nitrous oxide emissions and concentration profiles to fertilization and irrigation in greenhouse vegetable cultivation [J]. *Pedosphere.* 2012; 22 (6): 764–775.
11. Ju XT, Lu X, Gao ZL, Chen XP, Su F, Kogge M, et al. Processes and factors controlling N<sub>2</sub>O production in an intensively managed low carbon calcareous soil under sub-humid monsoon conditions [J]. *Environ. Pollut.* 2011; 159:1007–1016. <https://doi.org/10.1016/j.envpol.2010.10.040> PMID: 21251741
12. Gilsanz C, Báez D, Misselbrook TH, Dhanoa MS, Cárdenas LM. Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP [J]. *Agr. Ecosyst. Environ.* 2016; 216: 1–8.
13. Hansen M, Clough TJ, Elberling B. Flooding-induced N<sub>2</sub>O emission bursts controlled by pH and nitrate in agricultural soils [J]. *Soil Biol. Biochem.* 2014; 69: 17–24.
14. Zhang M, Fan CH, Li QL, Li B, Zhu YY, Xiong ZQ. 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 [J]. *Agr. Ecosyst. Environ.* 2015; 201: 43–50.
15. Jia JX, Li B, Chen ZZ, Xie ZB, Xiong ZQ. Effects of biochar application on vegetable production and emissions of N<sub>2</sub>O and CH<sub>4</sub> [J]. *Soil Sci. Plant Nutr.* 2012; 58: 503–509.
16. Wang XB, Zhou W, Liang GQ, Song DL, Zhang XY. Characteristics of maize biochar with different pyrolysis temperatures and its effects on organic carbon, nitrogen and enzymatic activities after addition to fluvo-aquic soil [J]. *Sci. Total Environ.* 2015; 538: 137–144. <https://doi.org/10.1016/j.scitotenv.2015.08.026> PMID: 26298256
17. Li B, Fan CH, Zhang H, Chen ZZ, Sun LY, Xiong ZQ. Combined effects of nitrogen fertilization and biochar on the net global warming potential, greenhouse gas intensity and net ecosystem economic budget in intensive vegetable agriculture in southeastern China [J]. *Atmos. Environ.* 2015; 100:10–19.
18. Van Groenigen JW, Velthof GL, Oenema O, Van Groenigen KJ, Van Kessel C. Towards an agronomic assessment of N<sub>2</sub>O emissions: a case study of arable crops [J]. *Europ J Soil Sci.* 2010; 61(6): 903–913.
19. Cao B, He FY, Xu QM, Yin B, Cai GX. Denitrification losses and N<sub>2</sub>O emissions from nitrogen fertilizer applied to a vegetable field [J]. *Pedosphere.* 2006; 16(3): 390–397.
20. Kavdir Y, Hellebrand HJ, Kern J. Seasonal variations of nitrous oxide emission in relation to nitrogen fertilization and energy crop types in sandy soil. *Soil & Tillage Research*, 2008, 98: 175–186.
21. Goglio P, Grant BB, Smith WN, Desjardins RL, Worth DE, Zentner R, et al. Impact of management strategies on the global warming potential at the cropping system level [J]. *Sci. Total Environ.* 2014; 490: 921–933. <https://doi.org/10.1016/j.scitotenv.2014.05.070> PMID: 24911772
22. Dai Y, Di HJ, Cameron KC, He JZ. Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N<sub>2</sub>O emissions in a grazed pasture soil [J]. *Sci. Total Environ.* 2013; 465: 125–135. <https://doi.org/10.1016/j.scitotenv.2012.08.091> PMID: 23021462

23. Mscwiney CP, Robertson GP. Nonlinear response of N<sub>2</sub>O flux to incremental fertilizer addition in a continuous maize (*Zea mays* L.) cropping system[J]. *Global Change Biol.* 2005; 11, 1712–1719.
24. Hénault C, Devis X, Lucas JL, Germon JC. Influence of different agricultural practices (type of crop, form of N-fertilizer) on soil nitrous oxide emissions. *Biol Fertil Soils*, 1998, 27: 299–306
25. Liu Y, Yang Y, Qin HL, Zhu YJ, Wei WX. Differential responses of nitrifier and denitrifier to dicyandiamide in short- and long-term intensive vegetable cultivation soils [J]. *J.Integr.Agr.* 2014; 13(5): 1090–1098.
26. Zacherl B, Amberger A. Effect of the nitrification inhibitors dicyandiamide, nitrapyrin and thiourea on *Nitrosomonas-europaea*[J]. *Fertilizer Res.* 1990; 22: 37–44.
27. Luo J, Ledgard S, Wise B, Welten B, Lindsey S. Effect of dicyandiamide (DCD) delivery method, application rate, and season on pasture urine patch nitrous oxide emissions[J]. *Biol. Fertility Soils*, 2015; 51 (4):453–464.
28. Li B, Fan CH, Xiong ZQ, Li QL, Zhang M. The combined effects of nitrification inhibitor and biochar incorporation on yield-scaled N<sub>2</sub>O emissions from an intensively managed vegetable field in southeastern China[J]. *Biogeosci. Discuss*, 2014; 11, 15185–15214.
29. Nelissen V, Saha BK, Ruyschaert G, Boeckx P. Effect of different biochar and fertilizer types on N<sub>2</sub>O and NO emissions [J]. *Soil Biol. Biochem.* 2014; 70:244–255.
30. Case SDC, McNamara NP, Reay DS, Stott AW, Grant HK, Whitaker J. Biochar suppresses N<sub>2</sub>O emissions while maintaining N availability in a sandy loam soil [J]. *Soil Biol. Biochem.* 2015; 81: 178–185.
31. Qiu W H, Liu J S, Hu C X, Sun X C, Tan Q L. Effects of nitrogen and soil moisture on nitrous oxide emission from an alfisol in Wuhan, China[J]. *J. Food Agri. Environ.* 2010; 8(3–4):592–596.
32. Rashti MR, Wang WJ, Moody P, Chen CR, Ghadiri H. Fertiliser-induced nitrous oxide emissions from vegetable production in the world and the regulating factors: A review [J]. *Atmos. Environ.* 2015; 112: 225–233.
33. Robinson A, Di HJ, Cameron KC, Podolyan A, He JZ. The effect of soil pH and dicyandiamide (DCD) on N<sub>2</sub>O emissions and ammonia oxidizer abundance in a stimulated grazed pasture soil[J]. *J. Soil Sediment.* 2014; 14(8): 1434–1444.
34. Yao ZS, Zheng XH, Zhou ZX, Xie B, Meo B, Gu J, et al. Nitrous oxide emission from winter wheat and vegetable fields in the Taihu region: A comparison case study [J]. *Climatic Environ. Res.* 2006; 11(6): 691–701.
35. Li DJ, Wang XM. Nitric oxide emission from a typical vegetable field in the Pearl River Delta, China [J]. *Atmos. Environ.* 2007; 41: 9498–9505.
36. Xu J, Shi W, Tong Y'A. Effect of different N fertilizer forms on N<sub>2</sub>O emission in Anthrosol [J]. *Chinese J. Soil Sci.* 2009; 40(2): 325–330 (in Chinese).
37. Yan HL, Zhang X, Xie LY, He CC, Ren HY, Zhang H, et al. Study on the pathway and dynamics of N<sub>2</sub>O emissions from the vegetable soil fertilized with ammonium nitrogen [J]. *Chinese J. Agrometeorology.* 2014; 35(2): 141–148 (in Chinese).
38. Johnson JMF, Weyers SL, Archer DW, Barbour NW. Nitrous oxide, methane emission, and yield-scaled emission from organically and conventionally managed systems. *Soil Sci. Soc. Am. J.*, 2012, 76:1347–1357
39. Venterea R T, Maharjan B, Dolan M S. Fertilizer source and tillage effects on yield-scaled nitrous oxide emissions in a corn cropping system[J]. *J. Environ. Qual.* 2011; 40:1521–1531. <https://doi.org/10.2134/jeq2011.0039> PMID: 21869514
40. Chris K, Rodney V, Johan S, Arlene AM, Bruce L, Keesjan VG. Climate, duration, and N placement determine N<sub>2</sub>O emissions in reduced tillage systems: a meta-analysis[J]. *Global Change Biol.* 2013; 19, 33–44.
41. Burzaco JP, Smith DR, Vyn TJ. Nitrous oxide emissions in Midwest US maize production vary widely with band-injected N fertilizer rates, timing and nitrapyrin presence[J]. *Environ. res. letters*, 2013; 8(3): 0.35031.