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Impacts of High-Frequency Chicken Manure Biochar Application on N₂O and CH₄ Emissions from Vegetable Field in Subtropical China

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Abstract: Vegetable production in Subtropical China is distinguished by excessive nitrogen (N) fertilization, frequent irrigation, and multiple crop rotations in a single year. The aforementioned variables are closely related to soil nitrous oxide (N₂O) and methane (CH₄) emissions. Hence, we conducted a field trial to measure N₂O and CH₄ emissions using static chamber–gas chromatograph. Four treatments were used: control (CK) with no fertilizer, 100% chemical N fertilization (CN), the conventional 30% chicken manure N plus 70%CN (CMN + CN), and 30% chicken manure biochar N plus 70%CN (CMBN + CN). The annual cumulative N₂O emissions reached 12.4, 63.5, 111.8, and 44.1 kg N₂O-N ha⁻¹ for the CK, CN, CMN + CN, and CMBN + CN treatments, respectively. Compared to the CN and CMN + CN treatments, the CMBN + CN treatment reduced N₂O emissions by 35.9%–65.7%, while it simultaneously increased the total vegetable yield by 16.1% compared to the CN treatment. Seven seasons mean N₂O emission factors are 1.3% for CN, 3.8% for CMN + CN, and 0.9% for CMBN + CN. The CH₄ emission was negligible, ranging from 0.07 kg CH₄-C ha⁻¹ for the CK treatment to 0.8 kg CH₄-C ha⁻¹ for the CN treatment. N₂O emissions peaked under the conditions of an interior chamber temperature of around 31.9 °C and the water-filled pore space (WFPS) of the soil being approximately 60%. Future climate change will intensify, triggering higher N₂O emissions from subtropical vegetable fields. CMB can be one of the best substitutes for direct chicken manure application as a soil supplement because it has a beneficial effect on improving vegetable yield and reducing N₂O emissions in Subtropical China.

Keywords: cumulative emission; emission factor; substitute fertilizer; subtropical climate; chicken manure biochar



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1. Introduction

The 21st century poses a serious threat to humanity due to global warming, caused by greenhouse gas (GHG) emissions [1]. On a 100-year time scale, the global warming potential of nitrous oxide (N₂O) and methane (CH₄), two significant GHGs, is 265 and 28 times more than that of carbon dioxide (CO₂) [2]. Through the rest of this century, the main anthropogenic cause of the stratospheric ozone's depletion will be N₂O, a powerful warming gas. N₂O and CH₄ concentrations in the atmosphere are rising at rates of 0.4% and 0.26% per year, respectively [3]. The largest anthropogenic source of N₂O emissions, substantial nitrogen (N) fertilization, is being used by cropland systems, especially economic crops to obtain relatively higher target yields [4]. Generally, N₂O is mainly generated

through the processes of nitrification, which occurs under aerobic conditions, followed by denitrification under anoxic conditions [5]. The aforementioned process is mainly dominated by the competition between soil water and air content.

Almost 45% of all vegetable production in the world is done in China [6]. Hence, vegetable cultivation results in 19.2% of GHG emissions from Chinese croplands [7]. And the net GHG emissions of open-field and greenhouse vegetable system are about 3.6–23.1 and 2.2–14.1 times that of the main staple cropping systems for producing wheat and maize in China, respectively [8]. N fertilizer was identified as the largest contributor, accounting for 78.2% of the net GHGs in the form of N₂O from vegetable production [7]. Very high N₂O emissions, 35.9–238.0 kg N₂O-N ha⁻¹, for four vegetable seasons were reported from Nanjing Province, Southeast China, where vegetable field totally received 1492 kg chemical N fertilizer plus 810 kg pig manure N [9]. Subtropical vegetable fields also experience excessive N fertilizer (375 kg N ha⁻¹ for each leafy vegetable season), frequent irrigation (once a day for leafy vegetables if no rainfall), more rainfall and a relatively higher temperature, and several crop rotations (around 8 seasons per year) in a single year [10]. A 50 m distance near our location and 137-day field study found a quite high N₂O emission factor (EF, 8.9%) with urea fertilization in swamp cabbage cultivation during the relatively high-temperature and rainy season [11]. However, the former result might overestimate the N₂O EF of leafy vegetable production in Subtropical China, because N₂O emissions from croplands were significantly impacted by fertilizer management, soil temperature, and moisture [3]. A new meta-analysis result showed that the EFs of N₂O in the vegetable production systems of China have an obvious regional difference: notably, the EF in South China was 6.7 times higher than that in Northwest China [12]. However, we found that the measurements of N₂O emissions are relatively lower in South China than in other regions, and the duration of some studies was not a whole year, which might increase the uncertainty of the N₂O EF for this region in previous study. Hence, the N₂O emissions, especially the annual cumulative emissions and their mitigation measures, urgently need to be assessed in subtropical vegetable fields in China to help minimize the uncertainty of the N₂O EF for this region in future analyses.

Antibiotic resistance genes (ARGs), as one of the emerging pollutants, is receiving more and more attention. About 58.0% of antibiotics used in livestock breeding in China are discharged into feces [13], and the concentration of antibiotics in animal manure in China is generally chicken manure > pig manure > cow manure [14]. To address ARGs, more intensive farms are implementing pyrolysis technology and producing biochar from animal manure [15]. Pyrolysis is a method in which biochar is created by thermally carbonizing biomass in an oxygen-restricted environment inside of an airtight chamber. Growing interest has been shown in the last 10 years in the biochar application to cropland as a sustainable method to combat climate change by capturing carbon from the atmosphere and reducing soil GHG emissions [16]. The use of biochar has been shown to significantly improve soil organic carbon (SOC), water holding capacity, and soil aeration, thus decreasing the need for chemical fertilizers and nutrient leaching, improving crop growth and yield, and lowering anthropogenic GHGs emissions [9]. Because biochar is a carbonaceous substance with a significant surface area, a porous structure, and an abundance of functional groups that is produced as a result of the thermochemical conversion [17]. Due to its special characteristics and associated effects, when applied, biochar will have an impact on soil physiochemical properties, and these changes will affect the composition of the soil microbial communities which are responsible for soil nitrification and denitrification, as well as further affecting soil N₂O production [18]. Biochar based on feedstocks has a different quality. For instance, compared to biochar made from wood and plant matter, animal manure biochar has a smaller specific surface area and a significantly higher quantity of nutrients [19]. However, the potential impact of the partial application of chicken manure biochar (CMB) instead of direct chicken manure application on N₂O and CH₄ emissions and crop output of the continuous planting leafy vegetables in subtropical China is not well studied.

This study specifically focuses on (i) the emission characteristics of N₂O and CH₄ from the continuous leafy vegetables rotations in subtropical regions, (ii) the impact of repeated CMB application on CH₄ and N₂O emissions and vegetable yield, and (iii) understanding the factors influencing N₂O and CH₄ emissions from vegetable fields in Subtropical China. Therefore, we hypothesize that the subtropical vegetable field emits a high amount of N₂O due to the intensive vegetable cropping system and high N fertilizer application and that CMB would mitigate N₂O emissions. This study provides better and more environmentally friendly agricultural techniques for vegetable production in this area.

2. Materials and Methods

2.1. Experiment Site and Design

The field experiment started in April 2021 and finished in May 2022 on a vegetable field in Houxi Town, Xiamen City, with the coordinates 118°02′01.31″ E, 24°38′16.93″ N. With a temperate and wet climate, Xiamen is a part of the subtropical maritime monsoon climate region. The average annual temperature is around 20.8 °C, the average annual sunshine duration is approximately 2233.6 h per year, the average annual sunshine rate is about 51%, and the average annual rainfall is approximately 1200 mm [10]. Vegetables and rice are the two main crops grown in Xiamen all year round because of the city's moderate temperature, ample sunshine, and plentiful rainfall. Typically, crops are grown all year long, with several cycles, intense planting, and a high fertilizer need. Brown sandy loam soil makes up the soil at the experiment location, and the fundamental physical and chemical characteristics of the topsoil (0–20 cm) before our cultivation experiment are a bulk density of 1.43 g cm⁻³; pH of 4.56; maximum field water holding capacity of 28.37%; soluble salt content of 0.08%, ammonium N content of 11.11 mg kg⁻¹; nitrate N content of 45.46 mg kg⁻¹; organic matter content of 13.6 g kg⁻¹; total N of 1.0 g kg⁻¹; total potassium of 1.8 g kg⁻¹; total phosphorus of 1.5 g kg⁻¹; available phosphorus of 110.2 mg kg⁻¹; available potassium of 287.5 mg kg⁻¹; and soil particle composition of 0.04% clay, 21.95% powder, and 78.01% sand.

Chicken manure from the market was used in this experiment because it is a common organic fertilizer that can be found in commercial organic fertilizer marketplaces in Xiamen, and around 60% of the farmers like to use chicken manure in leafy vegetable cultivation, according to their planting experiences [10]. In order to simulate the production process of making biochar from chicken manure in intensive livestock and poultry farms, the same chicken manure bought from marketplaces as a feedstock was converted into biochar through a pyrolysis process that we performed ourselves. We pyrolyzed chicken manure for 1 h at 500 °C in an oxygen-limited environment to produce biochar. Table 1 displays the physical and chemical characteristics.

Table 1. Physical and chemical properties of chicken manure and CMB.

Organic Fertilizer	Water Content ¹ (%)	pH ²	TOC ² (%)	TN ² (%)	C/N ² (%)	TP ² (%)	TK ² (%)
Chicken manure	11.9 ± 1.5	7.6 ± 0.6	28.7 ± 6.2	3.0 ± 1.5	11.0 ± 5.3	1.3 ± 0.2	2.0 ± 1.4
CMB	1.7 ± 1.1	12.2 ± 0.2	249.0 ± 42.7	2.2 ± 0.3	11.5 ± 0.4	3.1 ± 1.0	6.1 ± 1.3

Notes: ¹ Measured in wet matter. ² Measured in dry matter. TOC (total organic carbon); TN (total nitrogen); TP (total phosphorous); TK (total potassium).

A field survey was conducted on traditional crop cultivation and management (including NPK fertilizer types and application rate, organic and inorganic application ratio, and substrate and chasing ratio) in four vegetable-growing areas outside Xiamen Island, from November 2020 to January 2021. The results show that the application rates of N, P₂O₅, and K₂O fertilizers for conventional leafy vegetables in Xiamen were 375, 225, and 263 kg ha⁻¹, respectively; the ratio of substrate to chase fertilizer was 6:4 for N fertilizer; and the average ratio of organic and inorganic N fertilizer application was about 3:7 [10].

Based on aforesaid survey result, four treatments were set up in this field experiment: N-free control (CK), conventional 100% chemical fertilizer N (CN), conventional 30% chicken manure N (4.2 t ha^{-1}) plus 70%CN (CMN + CN), and 30% chicken manure biochar N (5.2 t ha^{-1}) plus 70% chemical fertilizer N (CMBN + CN). Three replications, 12 plots, plot size $2.5 \text{ m} \times 8.0 \text{ m}$ (20 m^2), containing a planting size of $1.7 \text{ m} \times 8.0 \text{ m}$ (13.6 m^2), were arranged in randomized group. Before the experiment began, a 50 cm wide by 0.8 mm thick plastic film was pre-buried between adjacent plots and replicates to stop inter-plot water and fertilizer interactions.

Two common leafy vegetables (pak choi and swamp cabbage) were cultivated during this study. From April 2021 to May 2022, a total of 13 months, we carried out field-cultivating experiments for seven different seasons: (S1) pak choi (9 April to 8 May 2021); (S2) swamp cabbage (15 June to 30 August 2021); (S3) pak choi (2 September to 28 September 2021); (S4) pak choi (13 October to 10 November 2021); (S5) pak choi (2 December 2021 to 11 January 2022); (S6) pak choi (29 January to 14 March 2022); and (S7) pak choi (10 April to 8 May 2022). Pak choi grows for only a short time: about 28 days during the warm months of March through November and 40 days during the cold months of December through February of the following year. The growing cycle of swamp cabbage takes about 75–80 days. Pak choi seeds were spread out across each plot at a rate of 42 g in the first season and 33 g in the next seasons. An experienced farmer uniformly distributes pak choi seeds into each plot before incorporating them into the earth, using a plow. With a density of 12 plants per square meter, swamp cabbages are transplanted with seedlings from the nearby farmer's fields.

For N, the basal-dressing-to-top-dressing ratio is 6:4. Based on 30% of the N application quantity (or $112.5 \text{ kg N ha}^{-1}$), the precise amount of various organic compounds was computed and applied. Urea (N 46.0%), super phosphate (P_2O_5 12.0%), and potassium sulfate (K_2O 50.0%) make up the chemical fertilizers used. In order to meet the required P/K dosage, more single super phosphate or potassium sulfate should be added to organic fertilizer if its P or K content falls below 98 kg P ha^{-1} or 218 kg K ha^{-1} . All treatments received the same amount of P and K fertilizer applied to leafy vegetables in Xiamen, and P and K were used as basal fertilizer before planting. Irrigation, weeding, and other cultivating management techniques were carried out in accordance with the regional customary norms and remained constant across various treatments. The irrigation equipment employed was a vertical rotating sprinkler system, and each treatment received the same amount of irrigation. According to farmers' experience, we irrigated once after topdressing the soil surface with urea; and then we irrigated once a day during the rainless period, when the soil surface appears to be dry, and irrigated twice if the soil surface temperature was too high for vegetables in the summer season. Typically, pesticides were applied once or twice during the growing season for vegetables.

At each harvest season, the biomass of the plants' aboveground parts in a $1 \text{ m} \times 1 \text{ m}$ (1 m^2) region from each plot was harvested and weighed. The fresh weight of the aboveground biomass is referred to as the crop yield. Due to the swamp cabbage's lengthy growth cycle, the aboveground stems and leaves were harvested three times in 2021, on 18 July, 2 August, and 30 August; and the yield was calculated as the total of the three harvests.

2.2. Sample Collection and Measurement Method of Greenhouse Gases

GHGs emissions were measured from April 2021 to May 2022, in all seven vegetable growth seasons. Emissions were measured manually using a closed static chamber method which was explained with details in another study [3]. Each chamber was composed of a stainless-steel frame with a water-filled groove on top which sealed the upper chamber with a diameter of 40 cm and a height of 30 cm. To reduce the rise in air temperature, an insulating material was placed over the upper chamber. The frames remained in place for the whole of the crop-growing season after being placed 10 cm into the soil. A total of four gas samples were collected during chamber closure at intervals of 10 min, using 60 mL plastic syringes, through a three-way stopcock and a Teflon tubing attached to the

chamber. The initial sample was collected right after enclosure. After each basal-dressing and top-dressing fertilization, the emissions of N₂O and CH₄ were measured once every two days for 10 days and once every 4 days for regular days. Between 9:00 and 11:30 am, measurements were performed.

Gas chromatograph (GC, Agilent 7890, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with an electron capture detector (ECD) operating at 330 °C for N₂O concentration and a flame ionization detector (FID) operating at 250 °C for CH₄ concentration was used to examine the concentrations of N₂O and CH₄; the process was explained with details in another study [20].

The N₂O and CH₄ fluxes were calculated using the following equation:

$$F = k_1 \times P_0/P \times 273/(273 + T) \times M/V \times H \times dc/dt$$

where F (μg N m⁻² h⁻¹ or μg C m⁻² h⁻¹) is N₂O fluxes; k₁ is a coefficient (0.001) for dimensional conversion; P₀ (hPa) is the atmospheric pressure in the chamber which was assumed to not differ substantially from P (1013 hPa), which is the standard atmospheric pressure at the experimental site; T (°C) is the average air temperature in the chamber during the enclosure; M (28 g N₂O-N mol⁻¹) is the molecular weight of N₂ in the N₂O molecule; V (L mol⁻¹) is the molecular volume at 1013 hPa and 273 K; H (m) is the chamber height; c (μL L⁻¹) is the concentration of N₂O; t (h) is the chamber enclosure time; and dc/dt (μL L⁻¹ h⁻¹) is the rate of increase in N₂O concentration in the chamber [3]. The fluxes of three replications for each measurement period were averaged to obtain the mean N₂O and CH₄ emissions. The arithmetic means of the two days that were closest to the days without measurements were used to estimate the N₂O and CH₄ fluxes for those days. The total N₂O and CH₄ emissions for each vegetable growing season were then calculated by adding the daily estimations. To find the N₂O emission factor (EF) for our vegetable field, the amount of emitted N₂O-N per kg N fertilizer was measured.

2.3. Soil Sampling and Measurements

Soil samples were taken during each vegetable crop season from each plot once every two days for ten days after each fertilization, and once every eight days on regular days, to determine soil moisture and soil mineral N (N_{min}, NH₄⁺-N + NO₃⁻-N). A 3 cm in diameter gage auger was used to collect soil samples at a depth of 20 cm. Soil samples were transported to laboratory in an ice box and immediately frozen at -18 °C before extracting the soil N_{min}. A 20 g soil sample was oven-dried to a constant weight at 105 °C to determine the soil water content and to calculate water filled pore space (WFPS) using the equation described in [3]. Then, 12 g of fresh soil samples was weighed in a 200 mL plastic bottle, 100 mL of a 1 mol L⁻¹ potassium chloride (KCl) solution was added, and the mixture was shaken at 180 r for 1 h before filtering. The extracts were stored at -18 °C prior for analysis of NH₄⁺-N and NO₃⁻-N, using a SEAL Analytical Auto Analyzer 3 (AA3, SEAL Analytical GmbH, Hamburg, Germany).

2.4. Determination of Temperature and Precipitation

A digital thermometer was used to measure the ambient temperature and the soil temperature at a depth of 10 cm during this study. The recorded ambient temperature and soil temperature are shown in Figure 1. The air temperature in the headspace of the chambers was measured at the start and end of gas sampling, using digital thermometers. The mean value represents the temperature during gas sampling, and it was further used to calculate the GHG fluxes. And precipitation data were recorded by an automatic weather station at the experimental site, as shown in Figure 2.

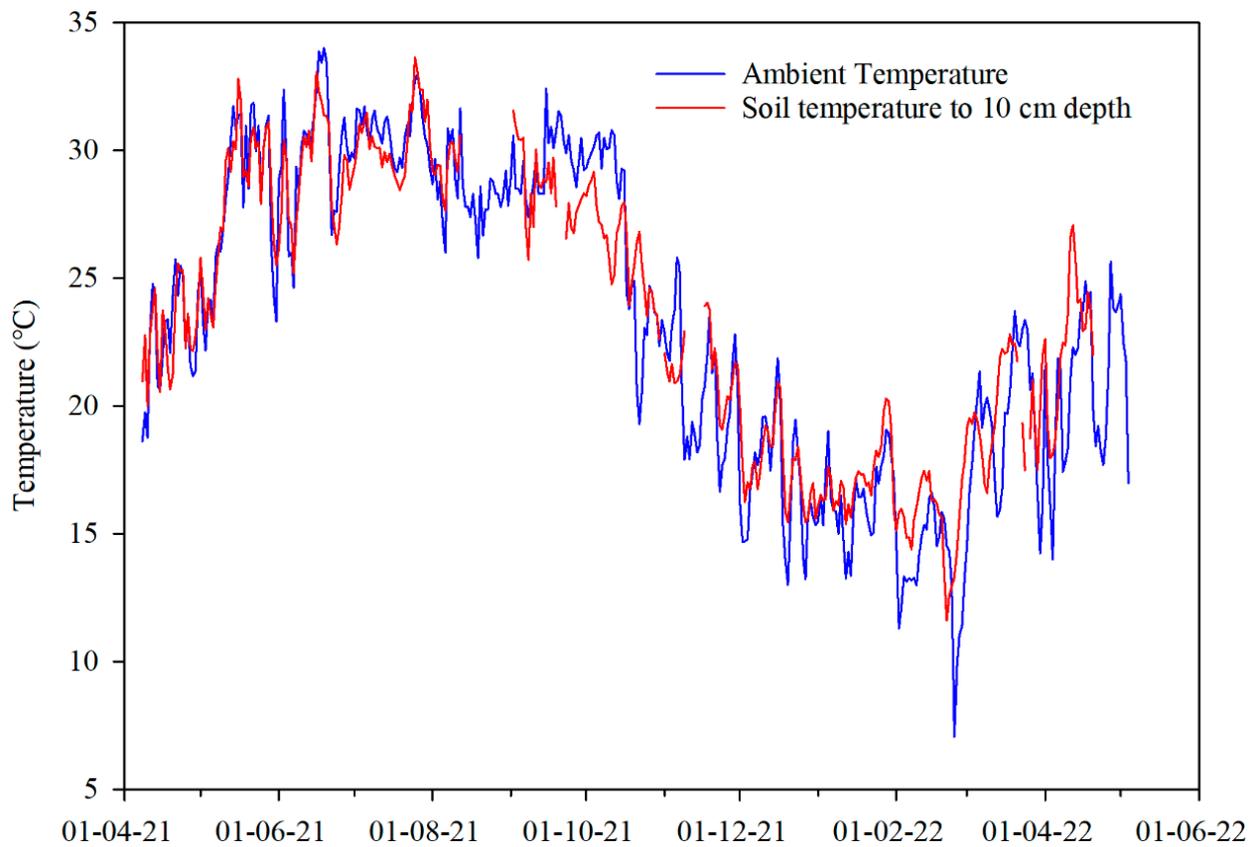


Figure 1. The ambient temperature and the soil temperature at a depth of 10 cm during the whole study duration.

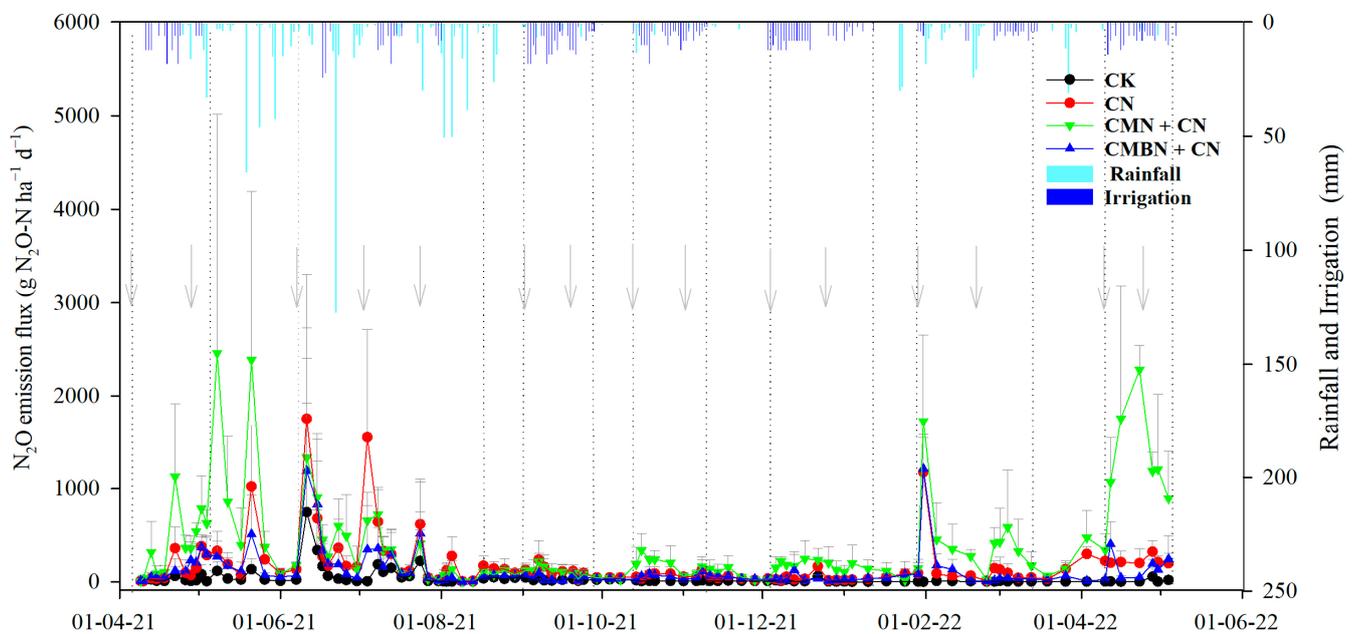


Figure 2. Mean N_2O emissions from four treatments, and rainfall and irrigation over the duration of the field experiment. Error bars represent standard deviation of the mean N_2O emissions, and arrows indicate fertilization. CK (nitrogen-free or control), CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% chicken manure biochar N + 70% CN).

2.5. Analysis of the Relationship between N₂O Emissions and Temperature and Soil Moisture

The links between N₂O emission, temperature, and soil moisture were analyzed using the group mean value and the boundary-line method. Refer to [3] for more information about this approach. When analyzing relationships among various parameters, the usage of group mean values has multiple advantages, including data conclusion, outlier reduction, statistical power increase, result comparison, and improved communication of study findings [21].

2.6. Statistical Analysis

To evaluate the statistically significant differences in indicators among crop yields of various treatments at the 5% level ($p < 0.05$), SPSS 25.0 statistical software (IBM, Armonk, NY, USA) was used for data analysis. To create the necessary statistical analysis charts, SigmaPlot version 14.0 software (Inpixon, Palo Alto, CA, USA) was used.

3. Results and Analysis

3.1. N₂O Emission

The peaks of the N₂O emissions were typically recorded after N fertilization followed by irrigation or rainfall events (Figure 2). All treatments, except CK, received the same amount of N fertilizer; however, the N₂O emissions showed significant variations under different N sources. The CMN + CN treatment showed that the direct use of chicken manure combined with chemical N fertilizer promotes greater N₂O emissions than the single chemical N fertilizer application treatment, CN. Two large peaks with an amount of (2467.3 and 2365.6 g N₂O-N ha⁻¹ d⁻¹) occurred after a relatively heavy rainfall in the CMN + CN treatment in fallow after the first season on 8 May and 20 May 2021, respectively. However, N₂O emission fluxes were relatively small when the rainfall was high, like 130 mm on 22 June 2021. Compared to the CN and CMN + CN treatments, the CMBN + CN treatment significantly reduced the N₂O emission to the background level by processing chicken manure into biochar and applying it in combination with chemical N fertilizer. In addition, large seasonal variations existed during our study's duration. Large peaks for N₂O emissions were mainly observed from February to July, and relatively small N₂O emissions occurred during August and next-year January.

3.2. CH₄ Fluxes

During the course of our field experiment, no significant variations in CH₄ fluxes were noted in the different N fertilizer treatments. The CH₄ fluxes were mainly around 0 during most of the study's duration. There were some small peaks of CH₄ emissions and uptakes. The CH₄ emissions were mainly caused by the high soil WFPS conditions after enormous or continuous precipitation (Figure 3). We noticed a peak of CH₄ uptake after soil plowing at the beginning of the second season in all treatments; however, it disappeared immediately after an enormous rainfall of 127 mm on 22 June 2021 (Figure 3). All the peaks of CH₄ uptake were short-term, and they were mainly following the events of soil plowing at the beginning of each season.

3.3. Cumulative N₂O and CH₄ Emissions

Cumulative N₂O emissions displayed a fascinating pattern of alterations over time: they were notably high and showed significant differences in various treatments (Figure 4a). When viewed from the perspective of cumulative dynamics, except for the CK treatment, all treatments exhibited an exponential increase in N₂O emissions at the beginning. Then, between August 2021 and February 2022, N₂O emissions partially stabilized. From February to April 2022, we noticed an upward trend in emissions again. After 9 April 2022, a new cumulative cycle occurred.

Although there was no N application, the cumulative emission from 9 April 2021, to 9 April 2022 reached 12.4 ± 10.3 kg N₂O-N ha⁻¹ yr⁻¹ for the CK treatment. The cumulative N₂O emissions of the conventional CN and CMN + CN treatments reached 63.5 ± 20.0

and $111.8 \pm 25.0 \text{ kg N}_2\text{O-N ha}^{-1} \text{ yr}^{-1}$, respectively. Compared to the CN and CMN + CN treatments, the cumulative N_2O emissions significantly decreased by 35.9% and 65.7% in the CMBN + CN treatment, which applied 30% chicken manure biochar N together with 70% chemical N fertilizer.

Although there were some waves, the cumulative CH_4 emissions showed a whole increase trend since the field experiment's beginning to the middle of December 2021 (Figure 4b). Then, it stepped down until the end of the last vegetable growing season. Relative to that of N_2O , the net cumulative CH_4 emission was negligible, only maintained at a small level, ranging from $0.08 \text{ kg CH}_4\text{-C ha}^{-1} \text{ yr}^{-1}$ for the CK treatment to $0.55 \text{ kg CH}_4\text{-C ha}^{-1} \text{ yr}^{-1}$ for the CN treatment.

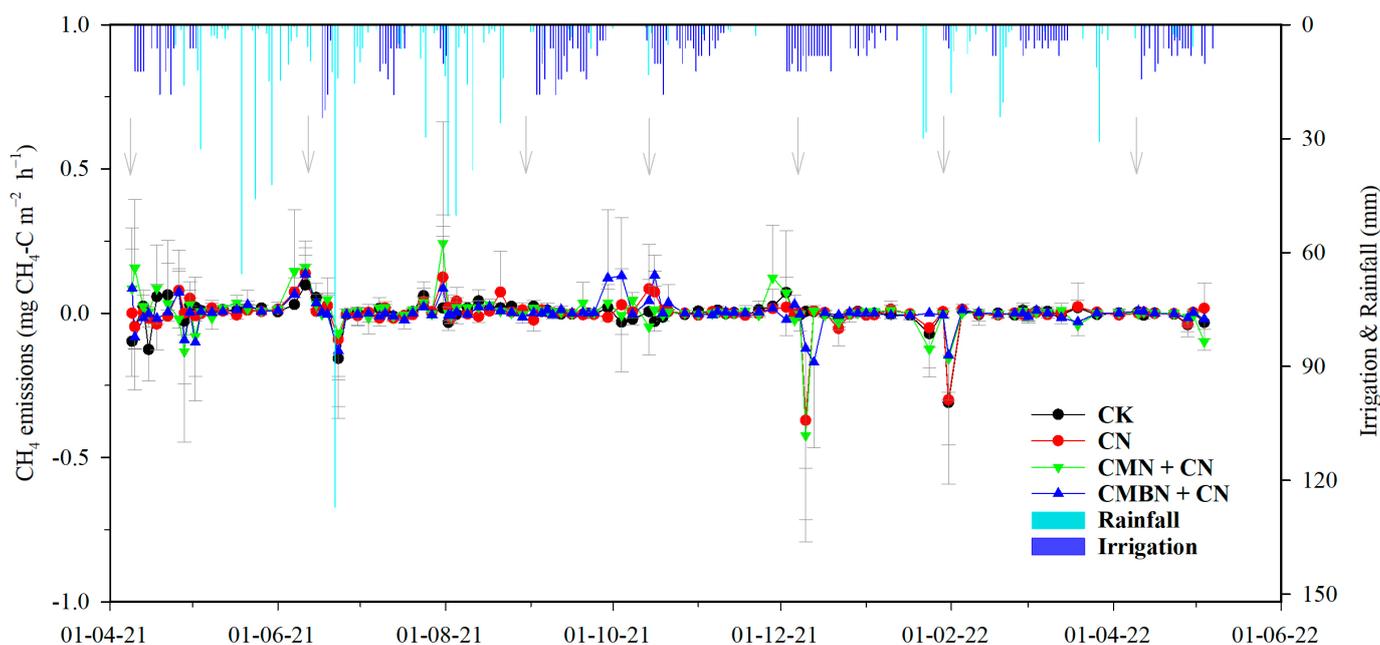


Figure 3. Mean CH_4 fluxes in four treatments. Error bars represent standard deviation, and arrows indicate soil tillage at the beginning of each vegetable season. CK (nitrogen-free or control), CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% chicken manure biochar N + 70% CN).

3.4. N_2O Emission Factor (EF)

The mean N_2O EFs of three fertilized treatments differed significantly from one another (Figure 5): 1.3%, 3.8%, and 0.9% for the CN, CMN + CN, and CMBN + CN treatments, respectively. A relatively high EF appeared in three treatments in S2 from the middle of June to the end of August in 2021 and in the conventional treatment with CMN + CN in S6 and S7 in 2022. The mean N_2O EFs from seven seasons in CMBN + CN decreased by 30.7% and 76.3% when compared to that of the two conventional treatments, CN and CMN + CN.

3.5. Leafy Vegetable Yield Comparison

The yield of different treatments (Figure 6) showed that the soil basic fertility could maintain the vegetable yield at a relatively high level, even though there was no N fertilizer application in treatment CK when the field trail started. However, as time passed, the vegetable yield remained at a low level, significantly lower than that of the CN, CMN + CN, and CMBN + CN treatments. There were no discernible output differences in the three different N fertilizer treatments, with the exception that the pak choi yield in the CN treatment was lower than that of the CMN + CN and CMBN + CN treatments in S5 and S6. The seven seasons' overall yield output of the treatments showed that the conventional single chemical N fertilizer application (treatment CN) obtained 219.3 Mg ($1 \text{ Mg} = 10^6 \text{ g}$) ha^{-1} .

Compared with the CN treatment, the vegetable yield showed an increasing trend by 12.4% and 16.1% in the CMN + CN and CMBN + CN treatments, respectively.

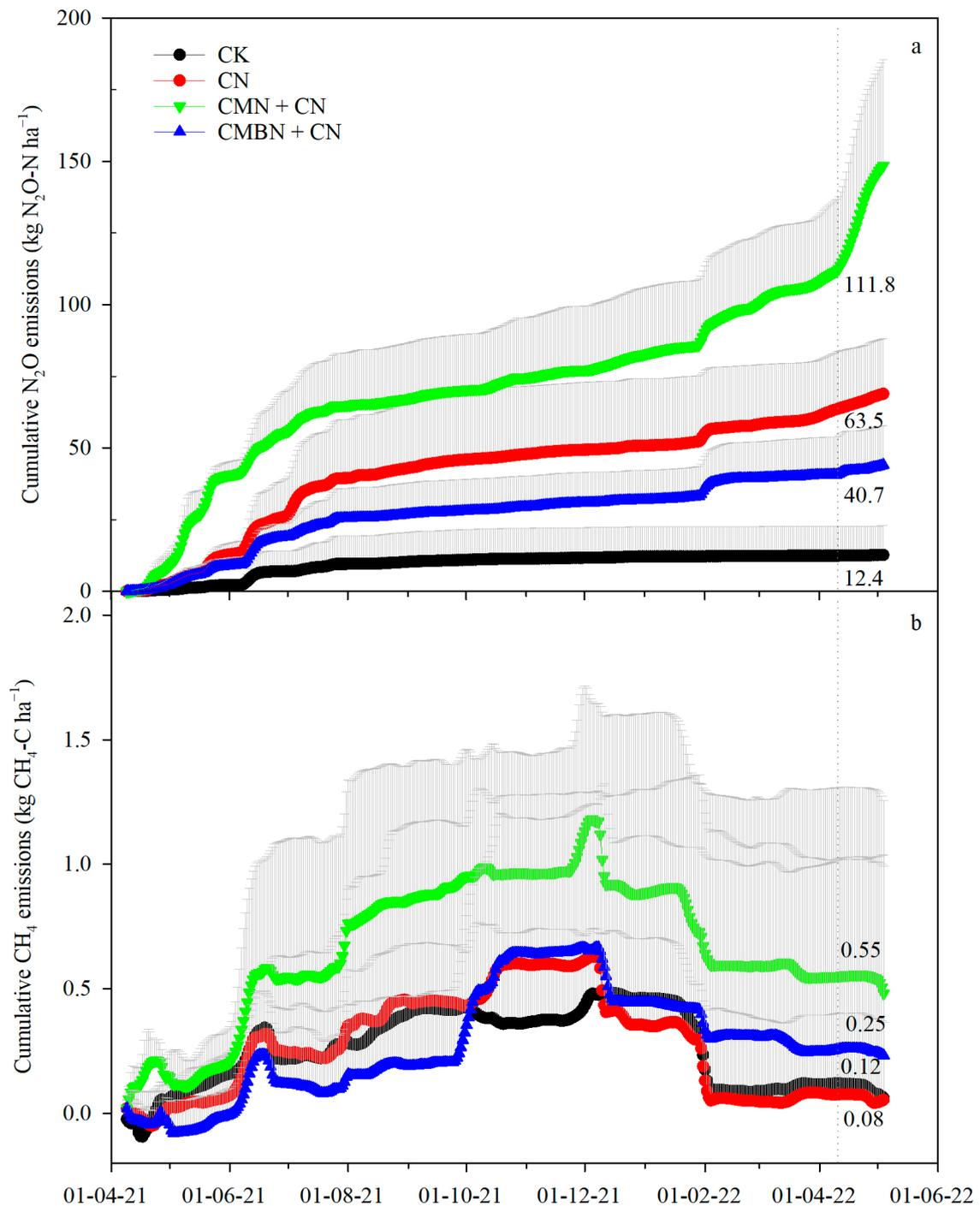


Figure 4. Daily cumulative N₂O (a) and CH₄ (b) emissions over the duration of the field experiment. Error bars represent standard deviation. CK (nitrogen-free or control), CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% chicken manure biochar N + 70% CN).

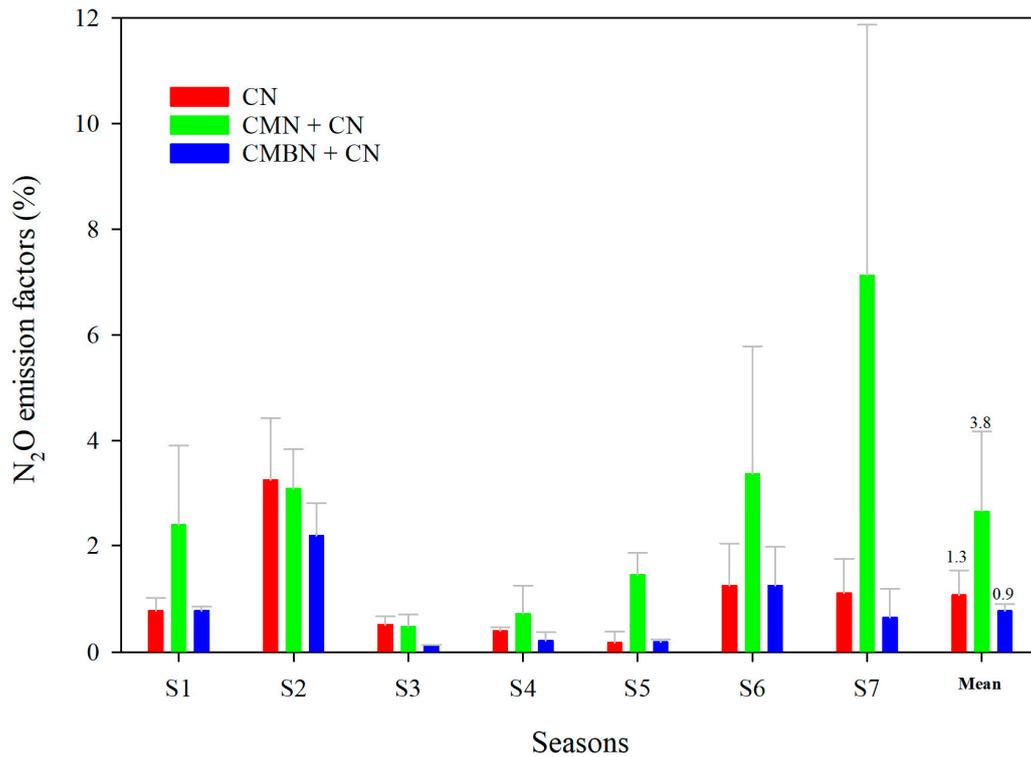


Figure 5. Seasonal N₂O EFs and mean N₂O EFs from all vegetable growth seasons. Error bars represent standard deviation. CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% chicken manure biochar N + 70% CN).

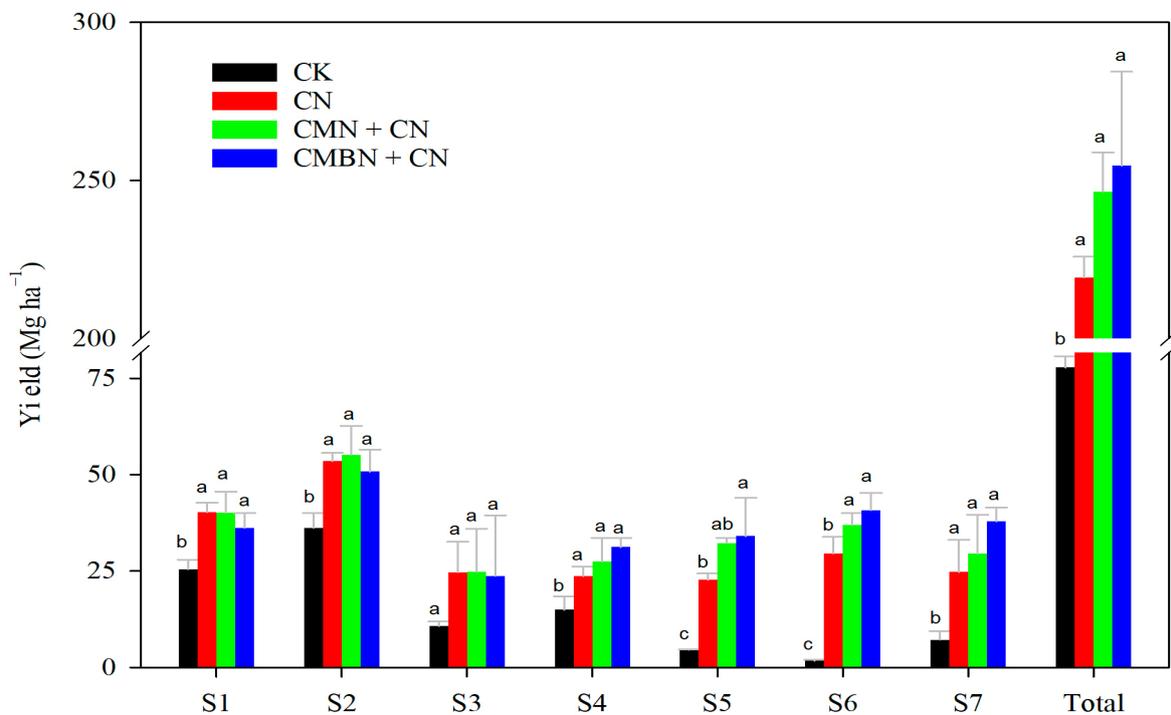


Figure 6. Seven seasons' leafy vegetable yield from different fertilizer treatments. S1–S7, the first to the seventh cultivating season. Error bars represent standard deviation. Identical letters above the error bars indicate that no statistically significant difference occurred among different treatments of each crop, while different letters indicate significant differences occurred ($p < 0.05$). CK (nitrogen-free or control), CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% chicken manure biochar N + 70% CN).

3.6. Dynamics of Soil Moisture

Soil moisture ranged from 28.3% to 88.4% WFPS and showed significant seasonal variations (Figure 7). Four treatments maintained the same dynamics of WFPS during the whole field experiment period. The highest soil WFPS (>60.0%) occurred from the middle of May to August 2021, and February 2022, which were mainly dominated by rainfall events (Figure 2). In the rest of the period, the WFPS mainly fell into the range from 40.0% to 60.0%.

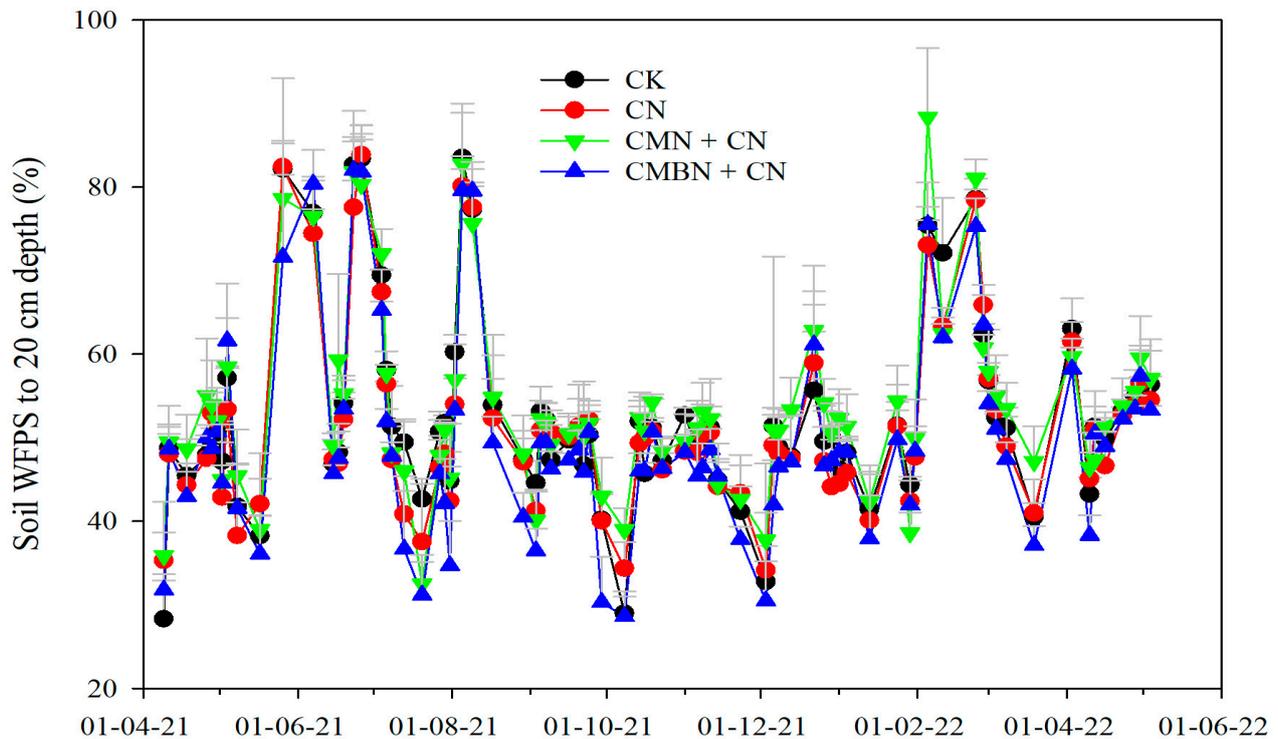


Figure 7. Dynamics of WFPS to 20 cm depth during whole field experiment period. Error bars represent standard deviation. CK (nitrogen-free or control), CN (100% chemical nitrogen), CMN + CN (30% chicken manure N + 70% CN), and CMBN + CN (30% Chicken manure biochar N + 70% CN).

3.7. Relationship between Soil Moisture and Temperature, and N_2O Emissions

The boundary-line approach was used to study the relationships between N_2O emissions and air temperature in the chamber and the WFPS in the topsoil (Figure 8). N_2O emissions were relatively low when the air temperature inside the chamber was around 14.3 °C. Maximum emissions of 2431 g N_2O -N $ha^{-1} d^{-1}$ on average occurred at 31.9 °C; at higher temperatures, that exceeded around 35.0 °C, N_2O emissions tended to decrease again. The emissions of N_2O were also relatively low when the soil moisture in the top 20 cm of soil was below 30% WFPS. The highest emissions were measured when the WFPS was around 60%; at a higher soil moisture level, when the WFPS exceeded 65%, N_2O emissions also tended to decrease again.

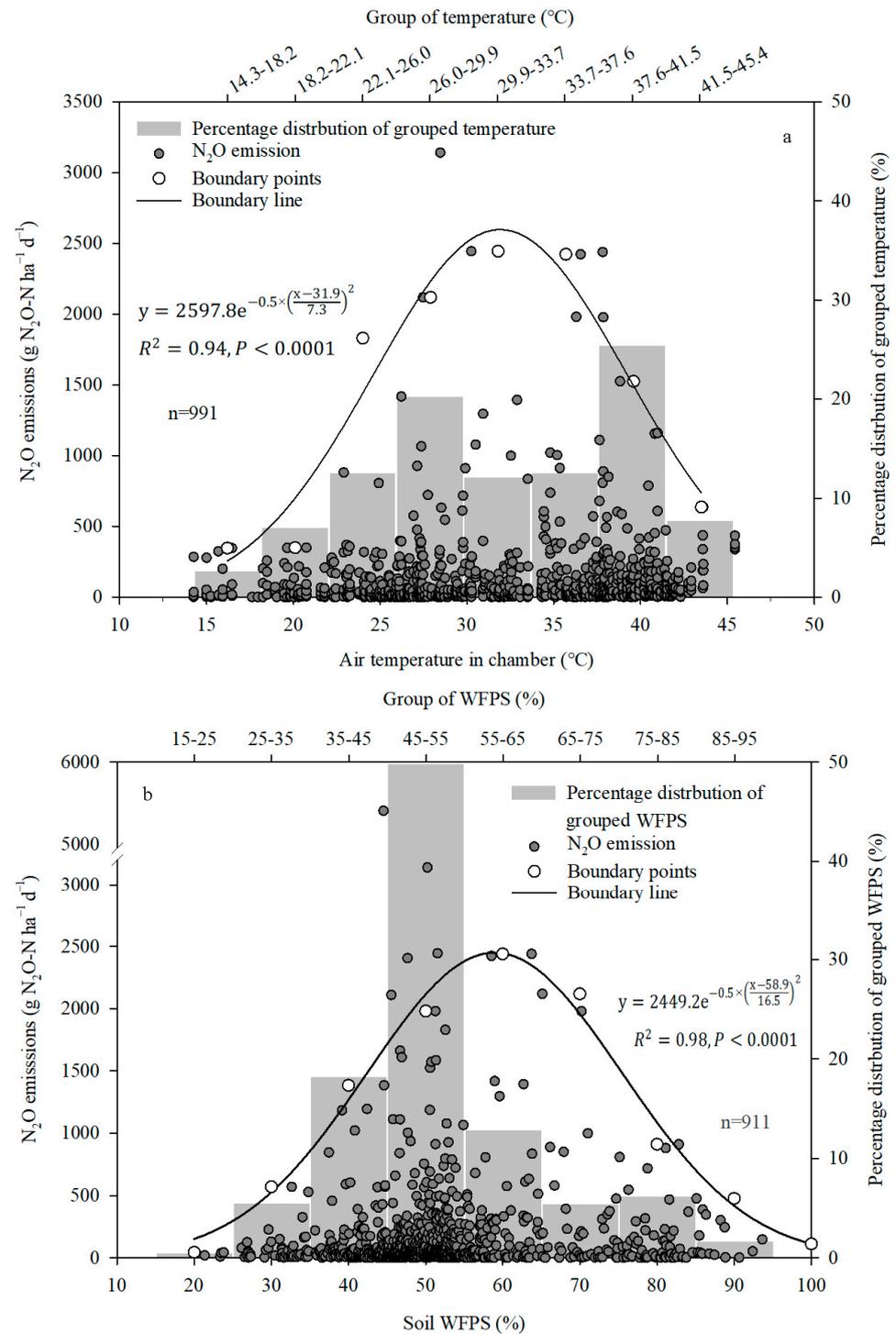


Figure 8. The relationships between (a) N₂O emissions and air temperature in the chamber and (b) water field pore space (WFPS, %) in the top 20 cm soil depth and N₂O emissions.

4. Discussion

4.1. N₂O and CH₄ Emissions in Subtropical Vegetable Field in China and Their Controlling Factors

Our study specifically looked at the impact of temperature, soil moisture, and N fertilization on N₂O and CH₄ emission rates in a subtropical vegetable farm in China. We found that N₂O emissions in our experiment were noticeably high. One of the main reasons can be frequent N fertilization to increase crop output. N₂O, a strong GHG, can be released into the atmosphere, when soil microorganisms' access to more inorganic

N sources, such as NO_3^- -N and NH_4^+ -N, in the soil is subjected to nitrification and denitrification processes [22]. In subtropical vegetable fields, there are indications that N input can reach up to 375 kg N ha^{-1} during each vegetable season [10], which is about 4.2–4.6 times that of the aboveground N uptake by leaf vegetables [23]. It has been proved that the N input amount has a positive correlation with N_2O emissions, especially surplus N rates [24]. Throughout the course of our field experiment, we noticed a rise in the N_2O emission graph following each N fertilization, followed by irrigation or precipitation (Figure 2). It demonstrates a strong and positive correlation between N_2O emissions and N fertilization. So, it is crucial to optimize the fertilization rate in subtropical vegetable fields.

The conventional chemical fertilization treatment (CN) in our study produced relatively high cumulative N_2O emissions ($63.5 \text{ kg N ha}^{-1}$) (Figure 4a). This can be explained by comparing the multiple vegetable seasons (seven or eight) in a year and applying a high quantity of N input to meet vegetable needs and obtain a higher yield in response to market demands. Furthermore, without timely irrigation water cooling, the soil surface temperature in the area of our field experiment is too high for vegetable crops and the soil becomes easily dry in the absence of rainfall or irrigation. From May to September, we usually need to irrigate twice in a day during the high-temperature days and once in a day during the regular days. Wet–dry cycling, which is produced by this process, is quite frequent and has been shown to increase N_2O emissions in upland areas [24]. An additional factor contributing to elevated N_2O emissions in this area can be the soil acidity, which is one of the main characteristics of subtropical soil. The application of chemical N fertilizer lowers soil pH, which alters the microbial composition of the soil and accelerates denitrification and finally increases the N_2O emissions [25]. Biochar application can be one of the best solutions to enhance soil pH. Lastly, this region has a relatively high temperature; even during the wintertime, the mean air and soil temperatures are still above 15°C (Figures 1 and 8). There are some indications that N_2O emissions will be promoted when the soil temperature exceeds the threshold value of 10 to 15°C [3]. We also found that the N_2O emission is low when the chamber's temperature is below 15°C . However, in our field experiment region, only a few sample days have temperatures below 15°C . The largest emissions occurred at around 30°C . When the temperature rose above about 35°C , N_2O emissions started to decline again. On the basis of a boundary-line analysis, another study conducted in Northwestern China reported that N_2O emissions were low when the soil temperature was below 11°C . In the ten days following fertilization and/or irrigation, higher N_2O fluxes were recorded at soil temperatures between 11°C and 22°C [26]. Results from another study conducted on the North China Plain can be used to validate our findings. Even after applying N, they claimed that N_2O emissions were still noticeably low when the soil temperature was below 10°C at a depth of 5 cm. Average maximum emissions were recorded at 23.8°C ; however, when temperature increased to 25°C , N_2O emissions started to decline again [3]. But our result showed a relatively larger temperature range suitable to N_2O emissions than that of the North China Plain.

The traditional organic fertilizer treatment, which entailed applying 70% chemical N and 30% chicken manure N (CMN + CN), was reported to be significantly high in N_2O emissions, and this can be attributable to a number of reasons. The application of chicken manure, which is a well-known rich source of N [5], can be one of the main factors for the increasing N_2O emissions. The high N concentration and available organic carbon in chicken manure may have encouraged an increase in the denitrification rate. When the soil moisture is at a high level, denitrification plays a crucial role in N_2O production [27]. This might be directly illustrated by several peaks of N_2O in the treatment CMN + CN, following the heavy rainfall (Figure 2). This study showed that soil moisture also played a significant role in N_2O emissions, since we noted diverse emission patterns at various soil moisture levels. N_2O emissions were noticeably low when the WFPS in the top 20 cm of the soil was less than 30% (Figure 8), which is comparable to the outcome of a field experiment conducted on cropland in North China Plain [3]. Almost identical results were observed from a cropland in Northwestern China, where it was reported that N_2O

emissions were consistently less than $200 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ when soil moisture was below 40% WFPS. When WFPS was around 70%, the highest emissions were recorded, and emissions decreased again above this point [26]. Reduced denitrification and microbial activity can result in lower N_2O emissions when soil moisture levels are insufficient [28]. Our findings can also be supported by the outcomes of an incubation study conducted in the Wujiang River basin, Southwest China, using normal cornfield soil. According to this study, the treatments with N and carbon addition at 70% WFPS had the largest N_2O emission, reaching $6.6 \text{ mg kg}^{-1} \text{h}^{-1}$, which was 22, 310, 124.9, and 1.4 times greater than those at 5%, 40%, and 110% WFPS, respectively [29]. Moreover, we saw a considerable increase in N_2O emissions as the WFPS reached about 60% (Figure 8). This result is consistent with previous research that showed the availability of water-stimulated microbial activities and increased N_2O generation [3]. For N_2O emissions, a soil moisture range of about 60% seems to be ideal. Fascinatingly, N_2O emissions began to decline again when the WFPS was over 65% (Figure 8). According to another study, the most favorable soil moisture levels for the highest N_2O production from nitrification and denitrification are 60% and 70% WFPS, respectively [30]. This observation can have the following plausible explanation: with higher soil moisture, denitrifiers can be more active, but when the soil moisture approaches a higher-than-specified range, their activities are disturbed. When soil moisture levels approach water-logging conditions, they change the microbial populations so that some microbial communities are more efficient at denitrification and N_2O generation than others, and there are water logging effects, which hamper the diffusion of gases, such as oxygen (O_2), across the soil–air interface; as a result, less N_2O will be emitted [28].

The results of our investigation into various treatments indicated variances in mean N_2O EFs, with the CN, CMN + CN, and CMBN + CN treatments showing percentages of 1.3%, 3.8%, and 0.9%, respectively (Figure 5). The mean N_2O EF for the tropics and subtropics was 1.2% overall, falling within the IPCC-EF's uncertainty range. Mean N_2O EFs by region were 1.4% for Africa, 1.1% for Asia, 0.9% for Australia, and 1.3% for Central and South America [31]. It is evident from comparing our results with worldwide EF estimates for subtropical regions that the EFs from our experiment fields, particularly those from our two conventional treatments, especially CMN + CN are greater. High N_2O emissions from open-field pepper (*Capsicum annuum* L.) were also reported in another study, which was conducted in China under similar climatic conditions. They stated that the cumulative N_2O emission and EF reached $4.05 \text{ kg N ha}^{-1} \text{ season}^{-1}$ and 0.96%, respectively, from the conventional treatment consisting of only chemical N fertilization ($400 \text{ kg ha}^{-1} \text{ season}^{-1}$) [32]. Based on a meta-analysis, the intensive vegetable systems in China exhibited an average N_2O emission in one vegetable growth season of $3.91 \text{ kg N}_2\text{O-N ha}^{-1}$ and an EF of 0.69% [33]. It is important to remember that, because of different environmental factors in different regions, the outcomes of N_2O emissions could be regionally unique. In our field area, the primary causes for high emissions could be the intensive cropping rotation, high N fertilization, and suitable environmental conditions.

In contrast, very low CH_4 fluxes were identified in this study. The four treatments' overall mean cumulative CH_4 emissions throughout the course of our experiment ranged from $0.08 \text{ kg CH}_4\text{-C ha}^{-1}$ to $0.55 \text{ kg CH}_4\text{-C ha}^{-1}$ (Figure 4b). These small emissions can be attributed to the high soil moisture after some heavy rainfall. Subtropical areas frequently have greater temperatures and moisture, thus enhancing the development and activity of methanogens. The anaerobic conditions brought on by the water-logged soils in these places encourage the production of CH_4 [34]. On the other hand, upland soil acted as a small CH_4 sink in North China Plain, with significant seasonal variation [3]. Upland soil in North China is often well-drained and has relatively low temperatures. It can be caused by several circumstances, which restrict the activity of methanogens, the bacteria that generate CH_4 . In addition, the soil's ability to drain well encourages aeration, which aids methane-oxidizing bacteria in oxidizing CH_4 [35]. These elements can support the limited atmospheric CH_4 sink function of upland soil in North China.

4.2. Impacts of CMB on Crop Yield and GHGs Emissions of Vegetable Field in Subtropical Regions

The results of this study support our hypothesis that, in comparison to conventional fertilization management, CMB application can successfully reduce GHG emissions and maintain high vegetable output in subtropical region of China.

CMB boosted the vegetable output when compared to the traditional single chemical fertilizer-application treatment (Figure 6). The overall mean output of the CMBN + CN, during all seven seasons, was 13.9% higher than that of the CN treatment. A similar investigation in a vegetable field had comparable results to ours, as this study showed that the growth and yield characteristics of cabbage were greatly enhanced by the combined application of CMB and NPK fertilizers. In comparison to the control, the application of 5 t ha⁻¹ CMB + 50% NPK raised the soil pH, organic carbon, available phosphorus, and cation exchange capacity (CEC) by 26.6%, 41.4%, 296%, and 78.7%, respectively. This integrated application increased cabbage output by 73% and 27% in comparison to the control and 50% NPK treatments, respectively [36]. There are a number of reasons why the application of biochar can be responsible for increasing crop output. First of all, biochar is a rich source of organic matter, which can improve the soils fertility and nutrient availability and promote the growth and development of plants. Additionally, biochar can enhance the soil structure by enhancing the soil's water holding capacity and decreasing soil compaction [37], thus favorably influencing root development and nutrient uptake. Therefore, the partial application of CMB as an optimized approach is highly advised for farmers in order to preserve the environment, particularly decreasing GHGs emission like N₂O from cropland without sacrificing crop yield. Based on the results of our field study, it is advised to apply CMB over a longer duration of time. We did not see any appreciable differences in the vegetable production in optimized treatment CMBN + CN for the first three vegetable seasons of our study. The biochar may not have been sufficiently broken down or integrated into the soil during the shorter growing seasons to affect the vegetable yield. After the third season, the vegetable yield in treatment CMBN + CN experienced positive changes. One of the primary causes can be the fact that biochar needs time to stabilize and develop to its full capacity. The biochar may have gone through additional mineralization and decomposition over time, enabling it to release nutrients gradually and enhance soil quality [38].

Furthermore, CMB impacts N₂O emission from cropland. Our investigation showed that the application of CMB significantly reduced N₂O emissions. The cumulative N₂O emissions significantly decreased in the CMBN + CN treatment, which applied 30% chicken manure biochar N, along with 70% chemical N fertilizer, compared to CN and CMN + CN treatments, by 35.9% and 63.6%, respectively (Figure 4a). When compared to two conventional treatments, CN and CMN + CN, the mean N₂O emission factors from all seasons in CMBN + CN is 0.9%, reduced by 30.7% and 76.3%, respectively (Figure 5). The consistency of our findings is supported by a study from Guangdong Province in South China; the study reported that adding rice-straw biochar to vegetable cropping systems that had received N fertilizer considerably reduced annual N₂O emissions by 34% to 67% [39]. The adsorption abilities of biochar, which can successfully collect and retain N within the soil, make soil N less favorable to nitrification and denitrification processes and thereby result in lower N₂O emissions. Biochar application minimizes the amount of N that is available for transformation into N₂O by decreasing N losses through volatilization and leaching, leading to fewer emissions [40]. These results highlight the potential of biochar for reducing N₂O.

In addition, the EF factor of the optimal treatment, CMBN + CN, was comparable with that of other regions in China. Furthermore, the average annual N₂O emission factor reported in the vegetable production in Jiangdu City, Jiangsu Province, on the east coast of China, ranged from 1.1% to 1.9% [41], which is greater than the EF of the CMBN + CN treatment. The EF of chemical N for the vegetable field throughout the course of the entire year, according to an earlier study from the North China Plain, was 1.1–1.8%, which is also higher than our outcome. Our findings demonstrated the effectiveness and applicability of

this treatment approach by correlating with EF seen in different geographic areas and other parts of China.

In conclusion, our investigation revealed the potential of CMB for improving vegetable yield and mitigating climate change. Although our optimal treatment's 0.9% annual EF factor still appears to indicate higher N₂O emissions in this area, this can be explained by comparing the multiple vegetable seasons (seven or eight) in a year, as discussed above. This study did not cover the application of biochar with different rates and feedstocks. Hence, further studies are needed to comprehensively understand the potential of biochar regarding sustainable agriculture and mitigating climate change.

4.3. The Potential Impacts of CMB on Contaminated Agricultural Soil

Many studies have reported that heavy metals from a variety of sources, such as feed additives and environmental toxins, are frequently found in chicken wastes. These metals can accumulate in soil and pose threats to the environment, as well as to public health [42]. In a similar way, the antibiotics residue which is present in chicken manure may help to promote the growing problem of ARGs [43]. Studies have looked into how well biochar can immobilize heavy metals and antibiotics in soil. Inhibiting the transport and bioavailability of heavy metals, biochar's porous structure acts as a physical barrier, lowering the likelihood that they can accumulate in crops or leach into groundwater. Soil heavy-metal concentrations and crop uptake have been shown to significantly decrease as a result of biochar application [27]. Certain research indicates that biochar can help lower ARGs by changing the composition of the soil microbial community, boosting microbial diversity, and affecting the physicochemical characteristics of the soil [44]. According to a prior study, after the bamboo biochar was added to the soil–lettuce system, the relative abundances of ARGs decreased by 51.8%, 43.4%, and 44.1% in the lettuce leaves, roots, and soil. This is primarily due to the fact that biochar can lower the abundance of pathogenic and host bacteria for ARGs in the soil [45]. By growing the world's population, the food demands also will be increased. To meet the markets' needs for meat, more poultry farms will be established and produce much more chicken waste. To deal with this challenge associated with the direct application of chicken manure as an organic fertilizer, pyrolysis can be one of the promising pathways.

Moreover, based on a study conducted on leafy vegetable fields, applying CMB to Pb-contaminated soils increased the amount of Pb that was accessible in the soil but decreased the concentration of Pb in plants. Even in the presence of elevated Pb mobilization in the soil, the application of amendments at increasing doses leads to a decrease in Pb concentration in plants [46]. Another study reported that the availability of Zn, Cu, Pb, Cd, and Ni was decreased by 13.3, 8.3, 13.8, 9.1, and 3.6%, respectively, when cow manure biochar was applied at a rate of 4 t ha⁻¹ in comparison to the control treatment, and the amount of Zn, Cu, Pb, Cd, and Ni in the edible parts of zucchini plants was reduced by 10, 17, 66, 20, and 26%, respectively, when 8 t ha⁻¹ of cow manure biochar was applied, compared to the untreated soil [47]. Some researchers emphasized integrating biochar and bacteria. They reported that heavy metal remediation and gradual soil condition improvements are possible with the integration of biochar and bacteria.

Additionally, when biochar is used to aid phytoremediation processes, bacteria highly encourage plant development [48]. We can infer from a comparison of research findings that various quality and feedstock biochar have distinct effects on heavy metal levels in soil. Furthermore, biochar can be one of the strategies to combat salt-affected soil. It is confirmed by a study that reported that adding biochar to salt-affected soils improves their chemical, biological, and physical characteristics [49]. These results suggest that the application of chicken manure biochar can support sustainable agricultural practices by reducing climate change, boosting productivity, and reducing potential environmental and health problems related to the application of traditional chicken manure to vegetable fields.

5. Conclusions

We conducted a field experiment in Subtropical China to assess the impact of the continuous application of CMB on N₂O and CH₄ emissions, as well as vegetable yield under field conditions. Our findings indicated that the Chinese subtropical vegetable field emits significantly more N₂O than previously reported. This resulted from intensive crop rotation, high fertilizer N levels, and suitable environmental conditions, as previously discussed. The subtropical vegetable field faces significant pressure to reduce N₂O emissions due to future climate change, including increased heat, drought, and storms. CMB improves vegetable yield and reduces N₂O emissions. However, it has no significant impact on the exchange of CH₄ between the soil and atmosphere. To address emerging pollutants such as antibiotic resistance genes, more intensive farms are implementing pyrolysis technology and producing biochar from animal manure. Our research suggests that using chicken manure biochar as a soil supplement, along with chemical N fertilizer, can improve vegetable yield and reduce N₂O emissions in subtropical vegetable fields. Further research is needed to investigate the application of different biochar with different rates in this context.

Author Contributions: M.J.A. designed the study, performed the experiments, analyzed data, and wrote the paper; X.F. and D.Z. performed the experiments, provided original data, and revised the paper; W.Z. provided essential assistance for this study; W.H. revised and proofread the paper. B.G. and S.C. conceptualized, performed Data curation, and supervised the project. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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