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Effects of Biochar Amendment on N_2O Emissions from Soils with Different pH Levels

Feng Lin [1,†], Hong Wang [2,†], Hiba Shaghaleh [3,*], Amar Ali Adam Hamad [3], Yaojun Zhang [4], Bairen Yang [5] and Yousef Alhaj Hamoud [6,*]

[1] School of Environmental Engineering, Nanjing Institute of Technology, Nanjing 211167, China
[2] College of Resource and Environment, Anhui Science and Technology University, Chuzhou 233100, China
[3] College of Environment, Hohai University, Nanjing 210098, China
[4] International Joint Research Laboratory for Global Change Ecology, School of Life Sciences, Henan University, Kaifeng 475004, China
[5] School of Environmental Science and Engineering, Yancheng Institute of Technology, Yancheng 224051, China
[6] College of Hydrology and Water Resources, Hohai University, Nanjing 210098, China
* Correspondence: hiba-shaghaleh@njfu.edu.cn (H.S.); yousef-hamoud11@hhu.edu.cn (Y.A.H.)
† These authors contributed equally to this article.

Abstract: Biochar application has the potential for mitigating N_2O emissions from agricultural soils and has been suggested as a management practice to ameliorate soil fertility and increase crop productivity. Nevertheless, the influence of biochar addition on N_2O emissions from soils with different pH levels is not yet clear, which results in a poor understanding of the mechanisms regarding biochar application to soil N_2O mitigation. A 40-day incubation experiment was carried out in the present study to investigate the impact of biochar on N_2O emissions from soils with different natural pH. Four treatments (control, nitrogen fertilizer application, biochar amendment, and N plus biochar amendment) were set up separately in soils with three different natural pH levels (acidic vegetable soil, neutral rice soil, and alkaline soil). Our results showed that adding biochar significantly decreased N_2O emissions by 20.8% and 47.6% in acidic vegetable soil for both N and no N addition treatments, respectively. For neutral and alkaline soils, the reduction of N_2O emissions by biochar amendment was only significant for N addition treatments in alkaline soil. Soil pH and NO_3^--N concentration were significantly affected by biochar amendment (soil pH increased by 1.43–1.56, 0.57–0.70, and 0.29–0.37 units for acidic vegetable soil, neutral rice soil, and alkaline soil, respectively). Thus, biochar amendment could be used as an effective management practice for mitigating N_2O emissions from acidic and alkaline soils.

Keywords: nitrous oxide emission; biochar; soil pH; acidic soil; alkaline soil

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1. Introduction

Nitrous oxide (N_2O) is an important long-lived greenhouse gas with 298 times greater global warming potential than CO_2 over a 100-year horizon [1]. Moreover, N_2O also plays a critical role in atmospheric photochemistry processes, which are involved in stratospheric ozone depletion [2]. The increasing rate of atmospheric N_2O concentration is estimated to be 0.95 ppb yr^{-1} for the last decade, and the concentration of N_2O increased to 331 ppb in 2018 [3]. Globally, agricultural soil is the major source of atmospheric N_2O emissions, which account for 80% of the increase in worldwide terrestrial N_2O emissions due to an excessive application of nitrogen fertilizer [4,5].

Biochar, produced from the low-temperature pyrolysis of biomass in a completely or partially oxygen-deficient environment, has been recommended as a means to improve soil fertility and enhance crop productivity, and, meanwhile, as an effective strategy for combating climate change [6]. To date, many studies have shown that biochar has the potential to manipulate soil nitrification, denitrification, and N immobilization [7–9]; such impact on these processes may further influence soil N_2O emissions from the agroecosystem. Additionally, all these processes are affected by various environmental factors (e.g., soil pH, soil temperature, and soil moisture) [10–14].

Soil pH is a key factor influencing soil N_2O emissions, as it regulates the production pathways of N_2O emissions [10]. Weslien et al. [15] reported that the mean annual N_2O emission was strongly correlated with soil pH, and N_2O emission increased with decreased soil pH. In contrast, He et al. [16] found that soil N_2O emissions increased in acidic soil with increased pH. As Butterbach-Bahl et al. [17] mentioned, scientific knowledge of soil N2O emissions controlled by different soil pH levels is still scarce. Furthermore, applying biochar (generally with rich carbon content and high pH) to soil may increase organic carbon contents, pH, and aeration, subsequently changing soil N_2O emissions [18,19]. Zhang et al. [20] reported that adding biochar mixed with N fertilizer decreased N_2O emissions in acidic soils by 10–23%, compared to N fertilizer alone. Nelissen et al. [21] observed that biochar amendment decreased N_2O emissions in neutral soils. In comparison, Clough et al. [22] found increased N_2O emissions in biochar treatment in alkaline soils. Conversely, Dong et al. [23] found that biochar suppressed N_2O production in alkaline soils. According to Shakoor et al. [24], applying biochar can raise the complexity of its effects on N_2O emissions under different pH levels. Therefore, different soil pH may interfere with N_2O emissions in biochar-addition soils, making adding biochar either effective or futile.

To our knowledge, few studies have examined the influence of biochar addition on N_2O emissions in soils with various natural pH levels. For this study, we conducted a laboratory incubation by incorporating biochar and N fertilizer into soils with different natural pH. This incubation study aimed to investigate the influence of biochar amendment on N_2O emissions from three soils with different natural pH levels (acidic vegetable soil, neutral rice soil, and alkaline soil).

2. Materials and Methods

2.1. Soil and Biochar

All samples were collected from three typical arable soils in the upper 15 cm layer. The three kinds of soil samples were collected from a vegetable field (Suoshi Village, 32°04′ N, 118°58′ E) and a rice field (Jiangsu Academy of Agricultural Sciences, 32°03′ N, 118°87′ E) located in Nanjing, Jiangsu province, China, and a coastal saline field (field station of Nanjing Agricultural University, 33°19′ N, 120°45′ E) located in Dafeng, Jiangsu province, China, respectively. Visible plant detritus and fragments were removed from soil samples after being air-dried. Then, soil samples were passed through a 2 mm stainless steel sieve. The biochar used in our incubation was produced from wheat straw, which was pyrolyzed at 500 °C. Similar to soil samples, the biochar was ground and passed through a 2 mm sieve before incubation. The physicochemical properties of the selected biochar and soil samples are shown in Table 1.

Table 1. The physicochemical properties of soil and biochar.

Soil Type	Total C (g kg^{-1})	Total N (g kg^{-1})	Soil pH	Sand (%)	Silt (%)	Clay (%)	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Bulk Density (g cm^{-3})
V	15.9	2.1	5.40	54.4	30.36	15.18	22.95	172.63	1.11
R	12.8	1.1	7.56	69.5	10.16	20.32	11.79	7.10	1.36
A	11.2	0.8	8.80	69.7	20.15	10.08	13.46	1.37	1.56
Biochar	467.2	6.0	10.90	-	-	-	<1	<1.2	0.41

Abbreviations: V, acidic vegetable soil; R, neutral rice soil; A, alkaline soil.

2.2. Incubation Experiment

A 40-day incubation experiment was carried out to examine the impact of biochar amendment on N_2O emission. Four treatments were performed: control, only N application (N), only biochar amendment (B), and N plus biochar amendment (NB). Four replicates were conducted for each treatment. Biochar at a rate of 4% (*w*/*w*, dry weight) and 80 g of soil (oven-dry weight) was added to an Erlenmeyer flask (250 mL) and mixed thoroughly. Soil moisture was regulated to 60% water-holding capacity (WHC), and samples were pre-incubated at 25 °C in the dark for one week to stabilize the microbial activity and avoid an undesired microbial peak. For N and NB treatments, 34.8 mg of urea containing 16 mg of N was dissolved and added to each flask. The flasks were covered with parafilm to prevent water evaporation and maintain soil humidity. In addition, the flasks were weighed and distilled water was added to compensate water losses when needed. Meanwhile, four other replicates for each treatment were set up and sampled to determine the changes in soil mineral N (NO_3^--N, NH_4^+-N) concentrations on days 3, 5, 10, 20, and 40.

2.3. N_2O Measurements

Gas samples from the headspace of the flasks were collected using a gas-tight syringe on days 1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 15, 18, 20, 22, 25, 30, 35, and 40 of the incubation. The headspace air in the flasks was mixed with ambient air for 5 min at a rate of 200 mL min^{-1} before taking a sample for each measurement. Then, the flasks were sealed with rubber stoppers and fitted with butyl rubber for 2 h for gas sampling. The ambient air sample was the initial concentration to calculate the N_2O emission rate. The headspace air in the flasks was collected to measure the increase in gas concentration. After gas sampling, flasks were flushed with ambient air and kept open. The N_2O concentration was determined by a gas chromatograph (GC) (7890A GC System, Agilent Technologies, Santa Clara, CA, USA) equipped with an electron capture detector (ECD). The ECD and column were set at 300 °C and 40 °C, respectively. A mixture of argon–CH_4 (5%) was used as the carrier gas, and the flow rate was set at 40 mL min^{-1}. The N_2O flux was calculated using a nonlinear fitting approach as follows:

$$F = h\frac{dC}{dt}\bigg|_{t=0}$$

where h is the height of the chamber in m, C is the concentration in ppb, and t is the time in s. The slope dC/dt is the slope of the gas concentration curve, which can be estimated using the exponential regression method [25].

2.4. Chemical Analysis of Soil and Biochar

Soil mineral N (NH_4^+-N and NO_3^--N) was extracted by 2 M KCl at a soil/water ratio of 1:5 and shaken (200 rev min^{-1}) at 25 °C for 1 h. Concentrations of soil NH_4^+-N and NO_3^--N were determined by an ultraviolet spectrophotometer (HITACHI U-2900, Tokyo, Japan) following two-wavelength ultraviolet spectrometry [26]. The total N content of soil and biochar was determined by Nessler′s colorimetric method, and the total C content of soil and biochar was determined by the combustion method [27]. Soil and biochar pH were analyzed by a PHS-3 C mv/pH detector (Shanghai, China) in a volume ratio of 1:2.5 (soil or biochar/water).

2.5. Data Analysis

A statistical analysis was conducted using SPSS software version 21 (IBM, Armonk, NY, USA). The data were presented as means ± SE (n = 4). A one-way analysis of variance (ANOVA) was performed to evaluate the significant differences in cumulative N_2O emissions among all the treatments for each soil. A two-way ANOVA was performed to examine the differences in chemical characteristics and cumulative N_2O emissions affected by biochar, N fertilizer, and their interactions. A linear regression analysis was performed to test the correlation between soil pH and N_2O emissions.

3. Results

3.1. Soil pH

Soil pH varied from 4.78 to 6.97 in acidic vegetable soil, from 7.05 to 8.00 in neutral rice soil, and from 8.18 to 8.76 in alkaline soil across all treatments (Figure 1). For all soils, biochar amendment increased soil pH compared to no biochar treatments (Figure 1). On the last day of the incubation, biochar amendment increased soil pH by 1.43 and 1.56 units, 0.70 and 0.57 units, and 0.37 and 0.29 units in the N addition and no N addition treatments for acidic vegetable soil, neutral rice soil and alkaline soil, respectively. The increase was more pronounced in acidic vegetable soil. In contrast, applying N fertilizer reduced soil pH compared to the control across the entire incubation period. Compared to the control, N plus biochar amendment showed an increase in soil pH for all soils. Meanwhile, soil pH for the NB treatments was lower than that for the B treatments. The two-way ANOVA showed that soil pH was significantly influenced by biochar and N fertilizer for each soil type ($p \leq 0.001$, Table 2). In contrast, their interactions were insignificant for all soils after the 40-day incubation.

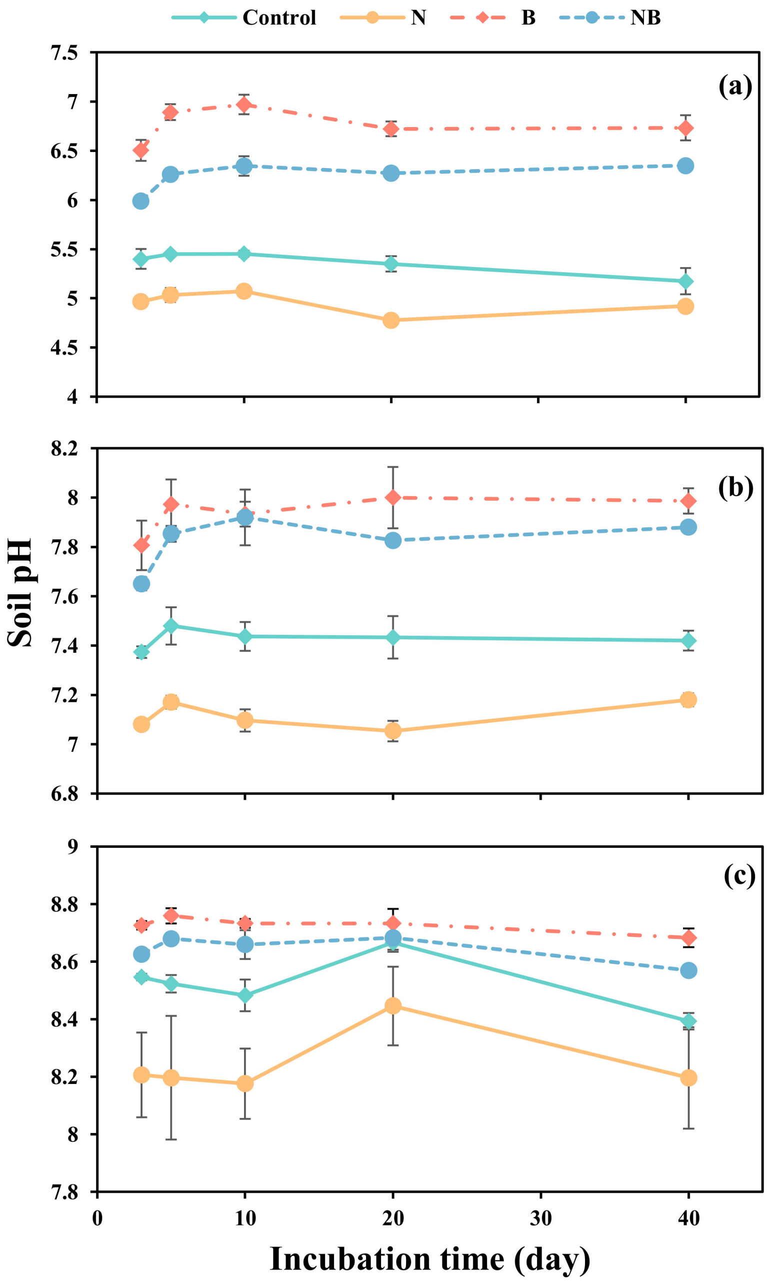

Figure 1. Changes in soil pH from (**a**) acidic vegetable soil, (**b**) neutral rice soil, and (**c**) alkaline soil during the 40-day incubation. The error bars indicate the standard errors of means (n = 3).

Table 2. Two-way ANOVA (F and *p* values) for the effects of biochar and N fertilizer on nitrous oxide emissions and soil properties.

Soil Type	Variable	Biochar (B)		N Fertilizer (N)		N × B	
		F	*p*	F	*p*	F	*p*
V	Cum. N_2O	216.227	<0.001	1871.412	<0.001	47.065	<0.001
	NH_4^+-N	0.114	0.741	0.265	0.616	11.325	0.006
	NO_3^--N	6.995	0.021	51.986	<0.001	0.299	0.594
	pH	1423.772	<0.001	64.216	<0.001	2.691	0.127
R	Cum. N_2O	0.937	0.352	459.391	<0.001	0.13	0.725
	NH_4^+-N	0.039	0.846	3.414	0.089	3.728	0.077
	NO_3^--N	314.596	<0.001	700.507	<0.001	2.135	0.17
	pH	1952.271	<0.001	146.724	<0.001	21.334	0.001
A	Cum. N_2O	10.132	0.008	575.44	<0.001	8.656	0.012
	NH_4^+-N	4.851	0.048	12.466	0.004	3.86	0.073
	NO_3^--N	43.787	<0.001	987.621	<0.001	3.526	0.085
	pH	17.186	0.001	79.771	<0.001	1.237	0.288

Abbreviations: V, acidic vegetable soil; R, neutral rice soil; A, alkaline soil; Cum. N_2O, cumulative nitrous oxide emissions.

3.2. Soil NH_4^+-N and NO_3^--N

At the beginning of the incubation, soil NH_4^+-N concentrations increased after N addition for all soils (Figure 2a,c,e). NH_4^+-N decreased sharply on day 5 for N addition treatments in neutral rice soil. In acidic vegetable soil, NH_4^+-N for N addition treatments decreased to the level of non-nitrogen treatments in the middle of the incubation on day 20. In comparison, NH_4^+-N increased on day 5 and decreased gradually with incubation time in alkaline soil. Soil NH_4^+-N concentration for NB treatment was lower than that for N treatment. For no N addition treatments, soil NH_4^+-N concentrations remained low and rarely changed in all soils. The two-way ANOVA showed that adding biochar and N did not significantly affect NH_4^+-N concentrations in acidic vegetable and neutral rice soil after 40-day incubation (Table 2). Nevertheless, their interactions significantly influenced NH_4^+-N concentration in acidic vegetable soil. Converse to acidic vegetable soil, biochar and N fertilizer significantly influenced the NH_4^+-N concentration in alkaline soil ($p < 0.05$) but were not significantly affected by their interactions.

Relative to no N addition treatments, soil NO_3^--N concentrations remained high in acidic vegetable and neutral rice soil throughout the entire incubation period for the N addition treatments (Figure 2b,d), while in alkaline soil, soil NO_3^--N concentration was relatively low for the N addition treatments at the beginning of the incubation and increased with incubation time (Figure 2f). The concentration of NO_3^--N was higher in acidic vegetable soil (118.8–174.8 mg kg^{-1}) than in neutral rice (48.9–103.0 mg kg^{-1}) and alkaline (16.4–83.8 mg kg^{-1}) soils. The NO_3^--N concentrations for the control and biochar-only (B) treatments remained low during the entire incubation period in all soils. Across all the treatments, soil NO_3^--N concentrations were highest in the nitrogen-only treatment and lowest in the biochar-only (B) treatments. The two-way ANOVA showed soil NO_3^--N was significantly affected by biochar and N fertilizer ($p < 0.05$) but not significantly affected by their interactions in all soils (Table 2).

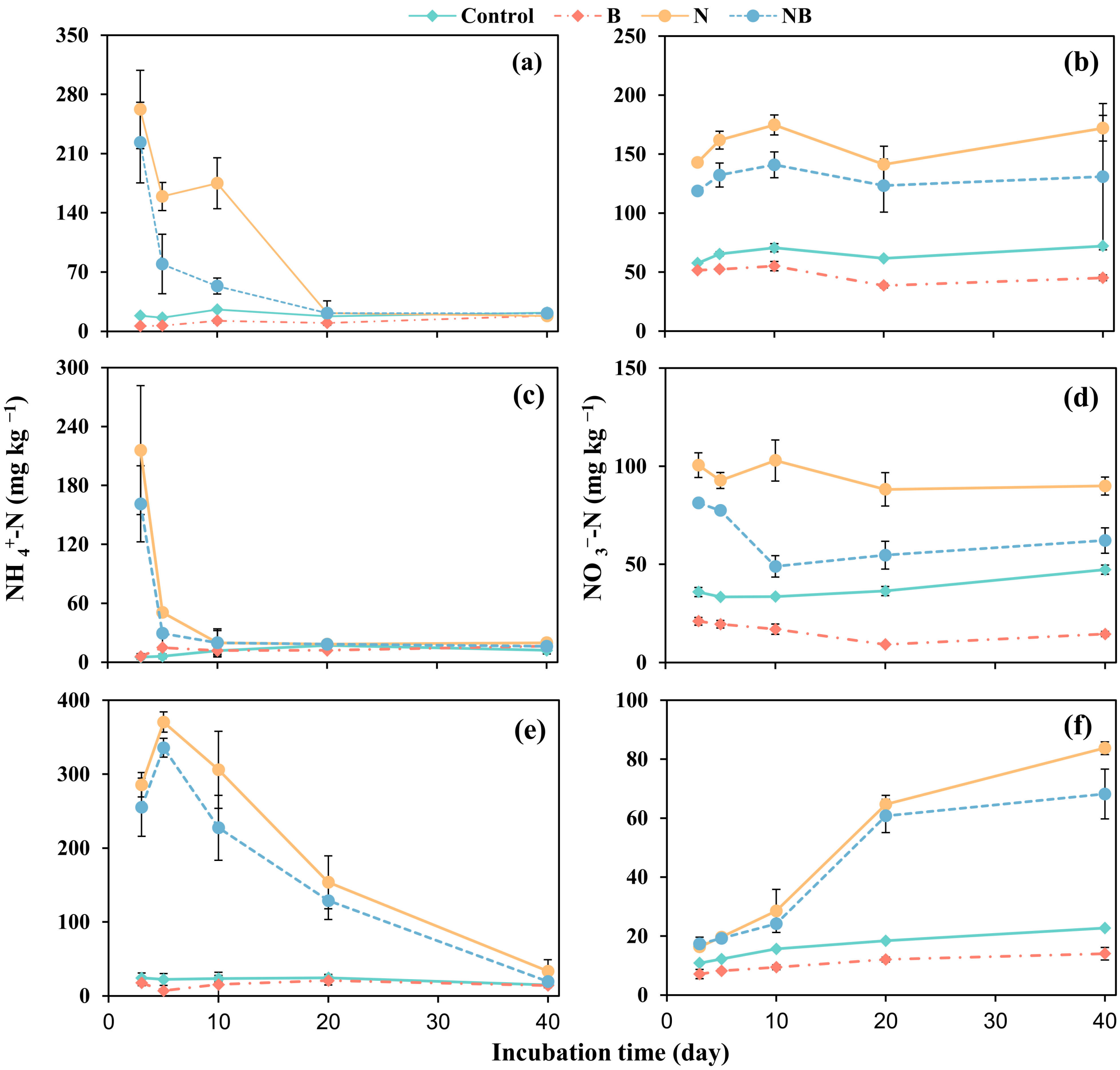

Figure 2. Dynamics of NH_4^+-N and NO_3^--N from (**a**,**b**) acidic vegetable soil, (**c**,**d**) neutral rice soil, and (**e**,**f**) alkaline soil. The error bars indicate the standard errors of means (*n* = 3).

3.3. Nitrous Oxide Emissions

The N_2O fluxes showed different temporal variations in the three soils across the incubation (Figure 3). The flux peak was highest in neutral rice soil (122.12 µg N kg^{-1} h^{-1}) and lowest in acidic vegetable soil (10.59 µg N kg^{-1} h^{-1}). N_2O flux peaks occurred on day 4 and day 5 for NB and N treatment for acidic vegetable soil. The N_2O fluxes for NB treatment declined more quickly than for N treatment and reached a steadily low level 6 days earlier than the N treatment (Figure 3a). The pattern of N_2O fluxes in neutral rice soil showed a typical pulse release followed by the N addition. The N_2O fluxes climbed rapidly to the top on days 2 and 3 for N and NB treatment. Afterward, N_2O fluxes declined sharply during the next 2 or 3 days and remained relatively low till the end of incubation (Figure 3b). For alkaline soil, after N addition, the fluxes of N_2O increased and reached their peaks on days 12 and 15 for N and NB treatment, respectively, then declined until day 25 of the incubation, and then kept relatively stable for the rest of the period (Figure 3c). The flux peak was lowest in acidic vegetable soil (10.59 µg N kg^{-1} h^{-1}) and highest in neutral rice soil (122.12 μg N kg^{-1} h^{-1}). Across the entire incubation period, N_2O fluxes were relatively low and steady for the control and B treatments in all soils (Figure 3).

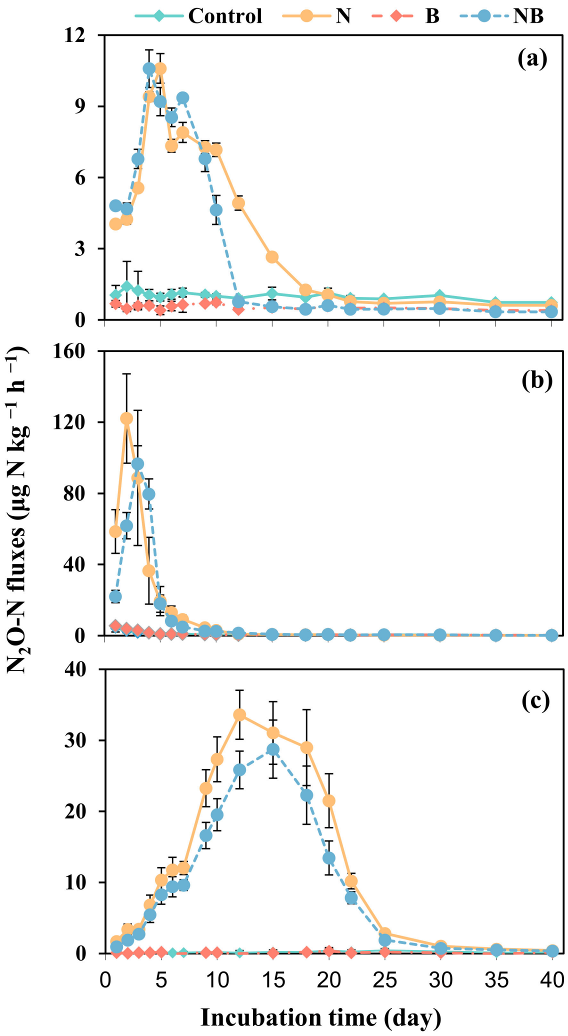

Figure 3. Temporal dynamics of N_2O fluxes from (**a**) acidic vegetable soil, (**b**) neutral rice soil, and (**c**) alkaline soil during the 40-day incubation. The error bars indicate the standard errors of means ($n = 3$).

The highest cumulative N_2O emissions were measured in N treatment, averaging 2.72, 9.03, and 11.28 mg N kg^{-1} for acidic vegetable, neutral rice, and alkaline soil, respectively (Figure 4). In acidic vegetable soil, the cumulative N_2O emissions were significantly depressed by biochar addition for B (47.6%) and NB (20.8%) treatments relative to the control and N treatments, respectively. Although biochar addition decreased cumulative N_2O emissions by 10.6% and 15.4% for B and NB treatments in neutral rice soil, the differences were insignificant. In alkaline soil, NB treatment significantly reduced cumulative N_2O emissions by 22.3% relative to N treatment. Meanwhile, the difference between the control and B treatment was not significant. The two-way ANOVA showed that cumulative N_2O emission was significantly affected by biochar, N fertilizer, and their interactions in acidic vegetable and alkaline soils ($p < 0.05$). Nevertheless, cumulative N_2O emission was only significantly influenced by N fertilizer in neutral rice soil (Table 2).

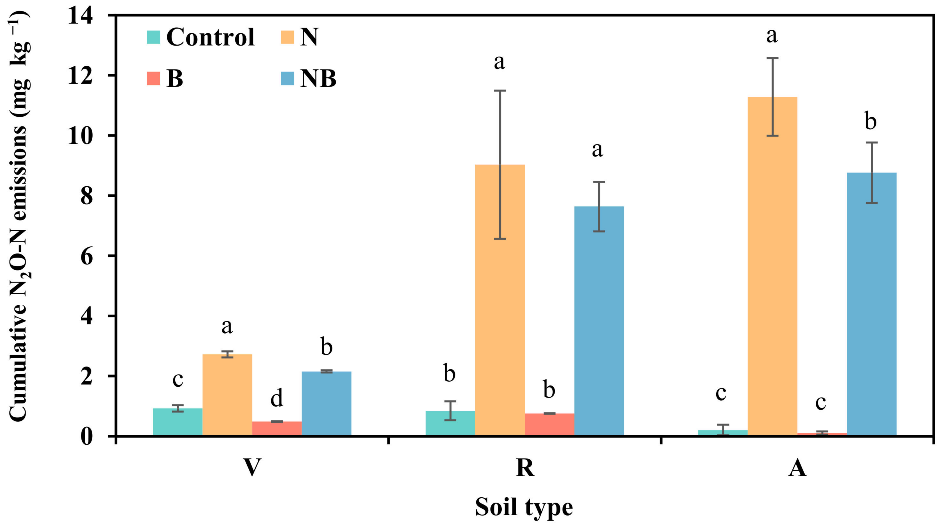

Figure 4. Cumulative N_2O emissions for soils with different pH. The error bars indicate the standard errors of means (n = 3). Different letters among the treatments indicate significant differences at $p < 0.05$ level.

4. Discussion

Nitrification, denitrification, and nitrifier denitrification are the three primary microbial processes for N_2O production in soil [24]. Previous studies have indicated that the activity of the nitrifier peaked at approximately 60% WHC (water holding capacity) and decreased with a drop in oxygen, while the activity of the denitrifier increased above 70% WHC [10]. In our study, WHC was maintained at 60% across the entire incubation period, and the coupling of NH_4^+-N decrease and NO_3^--N increase was observed clearly in alkaline soil (Figure 2e,f), indicating a strong nitrification process in alkaline soil. However, the coupling of NH_4^+-N and NO_3^--N did not occur in acidic and neutral soils (Figure 2a–d). Chen et al. [28] also demonstrated that the nitrification process is generally higher in alkaline soil than in acidic soil because the oxidization process regulated by autotrophic nitrifier was stronger under slightly alkaline conditions. The availability of mineral nitrogen (NH_4^+-N, NO_3^--N) is a major factor influencing soil nitrous oxide release. Generally, applying N fertilizer increased soil N_2O emissions in agricultural systems [29,30]. Consistent with this, all three soils showed large N_2O emission peaks after the addition of exogenous nitrogen in our study (Figure 3). The addition of exogenous nitrogen increased NH_4^+ concentration, and high NH_4^+ contents suppressed NO_2^- transformed into NO_3^- and stimulated NO_2^- accumulation, which contributed to an increase in N_2O emissions [31]. Thus, adding biochar may alter the availability of mineral nitrogen substrates (NH_4^+-N, NO_3^--N) and further affect N_2O production. In our incubation, biochar amendment accelerated the NH_4^+-N consumption when compared with the N fertilization-only treatments, especially for the acidic soil (Figure 2a). This might be attributed to the fact that biochar amendments accelerated nitrification and inhibited the influence of high NH_4^+ content on NO_2^- transforming to NO_3^-, then suppressed N_2O formation [32]. In addition, NH_4^+ could have been directly sorbed by biochar, which reduced the N availability for N_2O formation by nitrification [33]. The reduction in mineral N was also attributed to enhancing NH_3 volatilization due to increased soil pH with biochar amendment [34]. Throughout the incubation, soil NO_3^--N concentrations were much lower for biochar-amended treatments than those for unamended treatments (Figure 2b,d,f), and biochar amendment significantly influenced soil NO_3^--N contents in all soils (Table 2). This might be because biochar with a high C: N ratio suppresses the activity of denitrifiers via microbial N immobilization [35]. Alternatively, the surface absorption of NO_3^- by biochar decreases NO_3^- availability [36]. The depressed amount of available NO_3^- could result in a decrease in N_2O emissions.

Soil pH is a crucial factor influencing N_2O production and release [37]. In the current study, biochar amendment significantly influenced soil pH in all soils (Table 2; Figure 1). It has been reported that biochar can be used as a conditioner to ameliorate acid soils [38] due to its high concentrations of carbonates and organic anions. In our study, biochar addition significantly inhibited cumulative N_2O emission by 20.8% and 47.6%, and increased soil pH by 1.43 and 1.56 units in acidic vegetable soil for both N addition and no N addition treatments, respectively (Figures 1 and 4). The decrease in N_2O might be related to the large gap between the pH of biochar and acidic vegetable soil, which increased the soil pH. Previous studies analogously reported that biochar amendment significantly reduced N_2O emissions in acidic soil, which used an equally high pH (around 10.9) biochar as in this study [39–41]. Similar to acidic vegetable soil, biochar amendment significantly reduced cumulative N_2O emissions for N addition treatments in alkaline soil. This could be ascribed to the incorporation of biochar with high C: N ratios increasing soil microbial N immobilization, which results in a decrease in N_2O emission [42]. Wu et al. [43] also found that biochar amendment significantly decreased cumulative N_2O emission in alkaline soil. The authors attributed this to the liming effect of biochar, which reduced the N_2O/N_2 ratio and resulted in lower N_2O emissions [44]. Although nitrification was identified as the predominant process in alkaline soil in our incubation, importing organic C and available nitrogen might promote microbial growth and activity, consequently accelerating oxygen consumption and creating temporary anaerobic microsites favorable for denitrification [45]. Therefore, the reduction of N_2O in alkaline soil in our incubation might be partially associated with biochar's liming effect, which favored the N_2 formation [35].

Previous studies have shown that adding biochar could increase soil porosity and aeration, and suppress N_2O emissions [10,43]. Nevertheless, biochar amendment did not significantly decrease N_2O emissions for neutral rice soil, despite the soil aeration of neutral soil being the same as the other two soils (the same soil texture for all soils and the same WHC for all treatments). Thus, the present study may indicate that the influence of enhanced soil aeration with biochar is minimal. Among all three soils, cumulative N_2O emissions were highest in alkaline soil and lowest in acidic soil for N addition treatments (Figure 4). Furthermore, for N addition treatments across all the soils, cumulative N_2O emissions were, to a significant degree, positively correlated with soil pH (Figure 5). Generally, the nitrification process in alkaline soils is higher than that in acidic soils [24,46], hence increasing N_2O emissions.

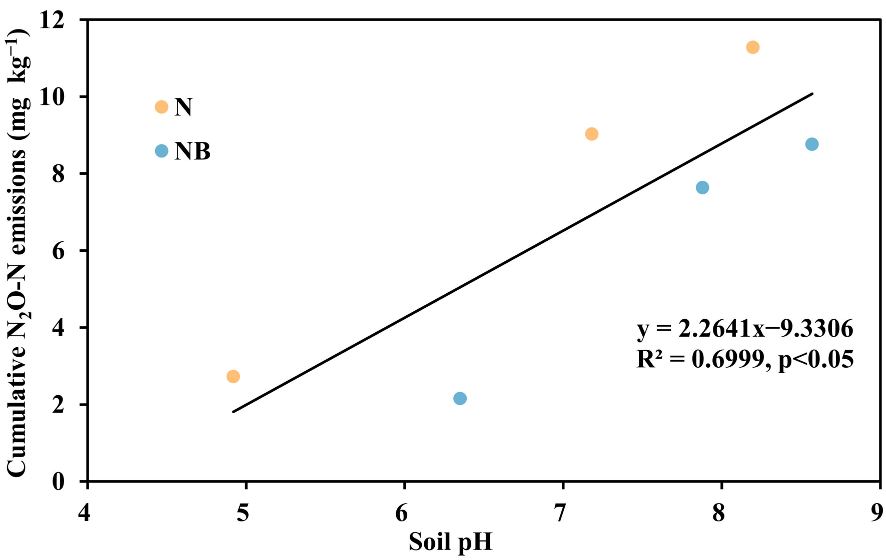

Figure 5. Relationship between cumulative N_2O emissions and soil pH for N addition treatments across all the soils.

5. Conclusions

Our study showed that (1) biochar addition significantly decreased cumulative N_2O emission for both N and no N addition treatments and can buffer soil acidity in acidic vegetable soil; (2) biochar addition also significantly decreased N_2O emissions in alkaline soil for N addition treatment. In all the soils, biochar amendment reduced available nitrogen contents. A positive relationship was observed between cumulative N_2O emissions and soil pH across N addition treatments.

These results suggest that the incorporation of biochar into soils could be an effective management strategy for mitigating soil N_2O emissions, but its effectiveness varies with changing soil pH. Since our study was conducted as a short-term incubation without growing plants, additional long-term studies are required to evaluate the effect of biochar amendment on N_2O emissions across different soil types under field conditions.

Author Contributions: Conceptualization, H.S. and Y.A.H.; data curation, H.S. and A.A.A.H.; formal analysis, A.A.A.H. and B.Y.; investigation, Y.Z. and B.Y.; methodology, F.L., H.W. and Y.Z.; supervision, Y.A.H.; validation, Y.A.H.; writing—original draft, F.L., H.W., H.S. and Y.A.H.; writing—review and editing, H.S. and Y.A.H. All authors have read and agreed to the published version of the manuscript.

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

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