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Impacts of Soil Moisture and Fertilizer on N_2O Emissions from Cornfield Soil in a Karst Watershed, SW China

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Abstract: Incubation experiments using a typical cornfield soil in the Wujiang River watershed, SW China, were conducted to examine the impacts of soil moisture and fertilizer on N_2O emissions and production mechanisms. According to the local fertilizer type, we added NH_4NO_3 (N) and glucose (C) during incubation to simulate fertilizer application in the cornfield soil. The results showed that an increase in soil moisture and fertilizer significantly stimulated N_2O emissions in cornfield soil in the karst area, and it varied with soil moisture. The highest N_2O emission fluxes were observed in the treatment with nitrogen and carbon addition at 70% water-filled pore space (WFPS), reaching 6.6 mg kg^{-1} h^{-1}, which was 22,310, 124.9, and 1.4 times higher than those at 5%, 40%, and 110% WFPS, respectively. The variations of nitrogen species indicated that the production of extremely high N_2O at 70% WFPS was dominated by nitrifier denitrification and denitrification, and N_2O was the primary form of soil nitrogen loss when soil moisture was >70% WFPS. This study provides a database for estimating N_2O emissions in cropland soil in the karst area, and further helped to promote proper soil nitrogen assessment and management of agricultural land of the karst watersheds.

Keywords: N_2O emission; cornfield; karst watershed; soil moisture; nitrifier denitrification

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1. Introduction

Nitrous oxide (N_2O) has been regarded as one of the most crucial greenhouse gases, with an atmospheric concentration of 332 ppbv and global warming potential of 295–300 times that of CO_2 [1]. Hence, it is expected that N_2O will occupy a dominant position in atmospheric pollution by the 21st century since it will severely destroy the ozone layer [2]. Soil is the primary source of global N_2O emissions, accounting for about 57% of global emissions [3]. Therefore, N_2O emissions from agricultural soils have been considered a primary source of global soil [4]. Since the 1970s, N_2O emissions from agricultural soil in China and Southeast Asia have rapidly increased [3,5], mainly due to the excessive mixed application of organic fertilizers such as nitrogen fertilizer and livestock manure [4]. However, the quantification of N_2O emission fluxes and knowledge of the potential and influencing factors controlling N_2O emissions in agricultural soils remain limited [6].

Several processes have been considered responsible for N_2O production in soil, including microbial nitrification, denitrification, and nitrifier denitrification [7,8]. Nitrification usually includes two processes: ammonia oxidation and nitrite oxidation [9]. Denitrification usually refers to the process by which denitrifying bacteria gradually reduce NO_3^- NO_2^-, nitric oxide (NO), N_2O, and N_2 in a hypoxic or anaerobic environment, in which N_2O

is the intermediate product [10]. In nitrifier denitrification, ammonia-oxidizing bacteria (AOB) directly use the NO_3^- produced by the ammoxidation of nitrification as a reaction substrate [11], and the final product is mainly N_2O instead of N_2 [12], which in some cases is more substantial than traditional denitrification [13]. However, the processes of N_2O production in soil are usually jointly dominated by biogeochemical nitrogen transformations. Plenty of physicochemical parameters can affect N_2O production in soil, including soil moisture, pH, temperature, structure, and substrate supply. Among these, soil moisture has been considered a key influencing factor for N_2O production in soils, especially in agricultural soil [14].

Soil moisture is commonly expressed as a widely used indicator of water-filled pore space (WFPS) because it provides a comprehensive relationship between moisture status and total porosity in soil [15]. Soil moisture mainly affects the aeration status of soil, the redox reaction status, and then the activity of microorganisms. The response characteristics of N_2O emissions to moisture conditions in soils of different land use types are obviously different. In coniferous forest soil, nitrification may be the primary process responsible for N_2O emissions, while in broad-leaved forest soil, N_2O emissions may originate from denitrification in the same moisture treatment [16]. Moreover, optimal moisture for N_2O emissions differed significantly among different types of soils, for example, 15% WFPS in sandy Italian floodplain soil and 65% in loamy Austrian beech forest soils [17]. For agricultural soils, Banerjee et al. [18] found in western Canada that the N_2O emission flux from wheat field soil was the highest at 80% WFPS, which was similar to a study on paddy fields [19]. However, other studies have suggested that optimal moisture for N_2O emissions differed significantly among fields of different crops, ranging between 60% WFPS in rice–rapeseed rotation soil and 90% in summer maize–winter wheat crop rotation soil [20]. Well et al. [21] argued that N_2O emissions and denitrification in arable loess soil increased with moisture and that denitrification is the primary process of N_2O production in 75% WFPS and 85% WFPS treatments. In contrast, N_2O emissions in arable soils primarily resulted from denitrification at low moisture levels (<40% WFPS) [22]. Moreover, Lan et al. [23] reported that under high soil moisture conditions (>70% WFPS), nitrification was the primary process of N_2O production rather than denitrification in summer rice–winter wheat double-crop rotation soil. Commonly, these differences were regarded to be related to the soil structure, microbial community, and available substrates [24]. However, few studies have focused on N_2O emissions in agricultural soils in karst basins [25–27] and the knowledge about N_2O production and emissions in agricultural soils in a karst area is still limited.

In addition to the impact of moisture, the input of exogenous carbon and nitrogen is also crucial for the violent release of N_2O in the short term from agricultural soils, which has become one of the major topics on current global greenhouse gas emissions [28]. Exogenous nitrogen mainly consists of fertilization and nitrogen deposition [3,29]. A recent study has documented that high soil NH_4^+ concentrations would promote N_2O production via nitrifier denitrification [30]. However, the contribution of nitrifier denitrification to N_2O production has often been underestimated or ignored [7]. For example, it was reported that soil denitrification and nitrifier denitrification together contributed 61–92% of N_2O emissions [31]. However, soil organic carbon, as a carbon source and energy source for microorganisms, also impacts the production and emission of N_2O. Soils with high organic carbon content are more prone to high N_2O emissions [32]. However, several studies have suggested that in karst areas, agricultural soils have lower organic carbon stocks and effectiveness than other land uses [33,34] due to the scouring effect of surface runoff on loosened agricultural soils [35]. In incubation experiments, glucose has been widely used to simulate the influence of external carbon input because it could be efficiently utilized by microorganisms [36]. However, previous studies have extensively addressed the N_2O emission potential in agricultural soils, while lacking the analysis of nitrogen transformations in the soil accordingly.

Increasing numbers of studies have revealed the effects of soil moisture and input of exogenous nitrogen and carbon on N_2O emissions in agricultural soils, including wheat and paddy fields in flatlands [18,19,37], however, little targeted studies in lime soil in karst area have been conducted and the relevant knowledge is very limited. Due to the high steep slope, well-developed karst landform, and abundant rainfall in the karst watershed in SW China, enhanced soil erosion and surface runoff have resulted in exceedingly fragile soil layers, which are more severe than those in the European Mediterranean and Dinaric karst regions of the Balkan Peninsula [38]. Correspondingly, surface runoff has carried agricultural fertilization into rivers, changing the water chemistry [39–41], and exacerbating nitrogen pollution in water [42,43]. In addition, applying fertilizer in the growing season has promoted nitrogen runoff and leaching in agricultural soils and also simulated N_2O emissions periodically, leading to uncertainties when assessing N_2O emissions regionally and globally. Thus, the aims of this study are: (1) to clarify the response of N_2O emissions in cornfield soil to moisture and carbon/nitrogen input; (2) to assess the N_2O emission potential in cornfield soil of a typical karst watershed, its influencing factors, and transformation mechanism; and (3) to determine the key influencing factors and transformation mechanism of soil N_2O emissions.

2. Materials and Methods

2.1. Site Description and Soil Sampling

The sample site was selected in a cornfield in the Wujiang River watershed, SW China (27°15′20″ N, 106°34′28″ E), where corn is the most widely distributed crop planted one time per year and harvested in September. The sample site was located in the northern area of the Guizhou Province of China and the mid-upper reaches of the Wujiang River watershed. Corn and rice are two major crops in the study area due to the rapid growth of population and local government planning and soil management policies [44]. Therefore, the study site was named "Granary of Qianbei". Thus, due to the sizable cultivated area and typical soil types [45], we chose the sampling site as a representative of cornfields in the watershed. The region has a subtropical humid monsoon climate, with an average annual temperature of 14–16 °C and mean annual precipitation of approximately 1140 mm. The elevation and slope of the site are about 427 m and 28°, respectively. The investigated soil is typical grey-brown lime soil with low nitrogen concentration in the karst area, which is easily eroded by surface runoff.

Surface soil samples (0–10 cm) of the cornfield were collected in October 2020 by soil column cylinder (letgone wolves, Shaoxing, LN, China), and the *in situ* samples of the soil surface (0–5 cm) were collected with cutting rings. For accurately measuring the concentrations of nitrogen species in the soil samples, we applied extraction protocols in the field. Immediately after sampling, 5 g soil samples were shaken with 25 mL KCl solution (2 M) at 200 rpm for 1 h and centrifuged at 4000 rpm for 5 min, and then filtered using Whatman 0.45 μm filter papers. Extracts and soil samples were stored below 4 °C and brought back to the laboratory. Soil NH_4^+, NO_3^-, and DTN (dissolved total nitrogen) concentrations were measured using a continuous flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany) with a 10 mL aliquot of extract. Soil DOC (dissolved organic carbon) and DON (dissolved organic nitrogen) contents were measured using elemental analysis (EA2400II, PerkinElmer, Waltham, MA, USA). Soil pH was determined on a 1:5 soil/water suspension. The cutting rings were dried at 106 °C to measure the water content and bulk density. The soil samples were passed through a 2 mm sieve to remove residue after being air-dried and then stored in zip lock bags. The basic physical and chemical properties of the soil samples are shown in Table 1.

Table 1. Physical and chemical properties of cornfield soil in the study area.

pH	DTN (mg L^{-1})	DOC (mg L^{-1})	DON (mg L^{-1})	NH_4^+-N (mg L^{-1})	NO_3^--N (mg L^{-1})	Bulk Density (g cm^{-3})
7.36 ± 0.26	1.95 ± 0.07	13.92 ± 1.64	0.28 ± 0.07	0.18 ± 0.01	0.49 ± 0.04	1.2 ± 0.09

Note: All results are indicated in mean ± standard error.

2.2. Experimental Design

The experimental designs and protocols of the incubation experiment in this study were conducted according to Qin et al. [19]. To assess the impacts of nitrogen/carbon on N_2O emissions in soil, NH_4NO_3 and glucose were used to simulate the excessive application of nitrogen fertilizer and atmospheric deposition [18]. Two groups of incubations were conducted as follows: (1) in the treatment group (NC for short, followed by moisture content, such as NC40%), the solutions of NH_4NO_3 (200 μg N g^{-1} soil) and glucose (300 μg C g^{-1} soil) were uniformly distributed on the soil surface by head sprayer, and moisture content was maintained by deionized water; (2) in the control check group (CK, followed by moisture content, such as CK40%, was used to indicate treatments without the addition of nitrogen and carbon), deionized water was dripped to make the soil moisture content reach 5%, 40%, 70%, and 110% WFPS, respectively. For better analysis, in the NC groups, each incubation (NC5%, NC40%, NC70%, and NC110%) had 11 replicates, with 5 subgroups for the N_2O concentration measurements and 6 subgroups for soil sample collection; in the CK groups, each incubation (CK5%, CK40%, CK70%, and CK110%) had 3 replicates for the N_2O concentration measurements. Thus, there were a total of 56 incubations (4 × 11 + 4 × 3) conducted in this study, and detailed information is shown in Table 2.

Table 2. The parameters and nutrient protocols in each incubation experiment.

Experimental Condition	Soil Moisture (WFPS)	C and N Addition	Temperature (°C)	Number of Replicates	Soil Sample Collection
CK5%	5%	×	25	3	×
CK40%	40%	×	25	3	×
CK70%	70%	×	25	3	×
CK110%	110%	×	25	3	×
NC5%	5%	√	25	11	√
NC40%	40%	√	25	11	√
NC70%	70%	√	25	11	√
NC110%	110%	√	25	11	√

Note: "×" means no treatment; "√" means treatment conducted.

Specifically, the detailed protocols and procedures were performed according to Cheng et al. [16] and Banerjee et al. [18]. Firstly, the soil samples were pre-incubated for 7 days. For that, 30 g of air-dried soil was weighed and filled into a wild-mouth bottle airtightly covered with a two-hole rubber stopper, which was inserted with two glass tubes for inflowing and outflowing gas. On the one hand, several studies have emphasized that suitable soil moisture (40–70% WFPS) and temperature (20 °C–25 °C) could enrich nitrifying bacteria and enhance nitrification-denitrification during the pre-incubation experiment in soil [46,47]; on the other hand, the *in situ* temperature of the cornfield soil samples employed in this study reaches 21 °C–26 °C in the daytime during summer. Therefore, we set the incubation temperature to that of the *in situ* temperature of the topsoil, ~25 °C [18]. Accordingly, in the pre-incubation stage, the moisture and temperature of the incubated soil samples were kept at 50% WFPS and 25 °C, respectively, in a dark incubator for 7 days. Soil moisture was maintained using the weighing method by deionized water. Because the rewetting effect of soil would lead to the enhancement of soil microorganism activity and acceleration of the mineralization rate [48], after pre-incubation, bottles were opened and placed in the incubator where the moisture and temperature were 0% and

25 °C, respectively, for 1 day to provide proper moisture for the next stage. Then, the soil moisture in each of the treatments was adjusted to 0%, 35%, 65%, and 105% WFPS, respectively, and subsequent experiments were carried out after 36 h of pre-incubation to reduce the influence of soil rewetting and stabilize the flora.

For N_2O concentration analysis, each incubation bottle was opened and placed in a ventilated place to aerate for 10 min adequately, then covered and sealed with a rubber stopper. After airtight incubation for 1 h, the silicone tube connected to a three-way stopcock was unscrewed (the lower height of the glass tube in the bottle) and connected to a G2308 gas concentration analyzer (Picarro, Santa Clara, CA, USA) to determine the N_2O concentrations. After the N_2O concentrations of all the samples were measured, each bottle was fully aerated for another 10 min in chronological order and then covered with a parafilm membrane to prevent water evaporation. Finally, all bottles were placed in the incubator at 25 °C and measured for N_2O concentrations every 24 h for 13 days. The soil samples (5 g dry soil) were collected on days 1, 2, 4, 6, 8, 10, and 13 (destructive sampling), respectively, for analysis of the nitrogen species.

2.3. Analysis and Calculation

After being collected from the incubation bottles, the soil samples were immediately measured (5 g), filled in a 50 mL centrifuge tube, and extracted with 25 mL KCl solution (2 M). The mixed solutions were shaken at 200 rpm for 1 h, centrifuged at 4000 rpm for 5 min, and then filtered using a Whatman 0.45 μm filter membrane. After that, the concentrations of NH_4^+ and NO_3^- were determined by a continuous flow analyzer (AA3, SEAL Analytical, Norderstedt, Germany) at Tianjin Normal University. During the whole incubation experiment, a gas concentration analyzer (G2308, Picarro, Santa Clara, CA, USA) was used to measure the daily N_2O emission fluxes. The relative standard deviations were below 5%, and the detected concentrations of the soil properties and N_2O fluxes were within the range of certified values. In addition, the results showed that the standard deviations of all the replicates were within ± 5%.

The N_2O emission fluxes were calculated using the following formula [46]:

$$F = \frac{\left(C_{sample} - C_{air}\right) \times \left(V_{bottle} - V_{soil}\right) \times 44 \times P}{R \times \left(T + 273.15\right) \times \Delta t \times m_{soil}} \quad (1)$$

where F is the emission flux of N_2O (mg N g^{-1} h^{-1}), C_{sample} is the N_2O concentration after incubation in the bottle (nmol L^{-1}), C_{air} is the N_2O concentration before incubation in the bottle (nmol L^{-1}), V_{bottle} is the volume of the bottle (m^3), V_{soil} is the volume of incubated soil in the bottle (m^3), 44 is the molecular weight of N_2O, P is one standard atmospheric pressure (101.325 kPa), R is the gas constant (8.314), T is the actual temperature in the bottle, 273.15 is the thermodynamic temperature, Δt is the incubation time (1 h), and m_{soil} is the soil weight (30 g).

$$N_2O \text{ cumulative emissions} = \sum F_n \times 24\ h \quad (2)$$

where F_n is the N_2O emission flux measured daily.

3. Results

3.1. N_2O Emission Varied with Soil Moisture

The N_2O emissions from incubated soil samples varied significantly with different moisture and carbon/nitrogen (Figure 1). Except for NC5%, the soil N_2O emission fluxes of NC40%, NC70%, and NC110% reached their maximums on the first two days and steadily decreased afterwards. Specifically, the N_2O emission fluxes in NC40% and NC70% treatments reached their maximum fluxes on the second day, while those in the NC110% treatment reached the maximum on the first day. In contrast, the soil N_2O emission fluxes in NC40% and NC 70% reached equilibrium on the 7th and 11th days, respectively, while those in NC110% decreased rapidly to a steady state after the third day.

Among the four treatments, the maximum N_2O emission flux occurred in NC70%, reaching 6.56 mg kg^{-1} h^{-1}, which was 124.9 (0.05 mg kg^{-1} h^{-1}) and 1.4 (4.67 mg kg^{-1} h^{-1}) times higher than those in NC40% and NC110%, respectively. Conversely, in NC5%, the N_2O emission flux was relatively stable and low, fluctuating within the small range of 0.26–0.32 μg kg^{-1} h^{-1}.

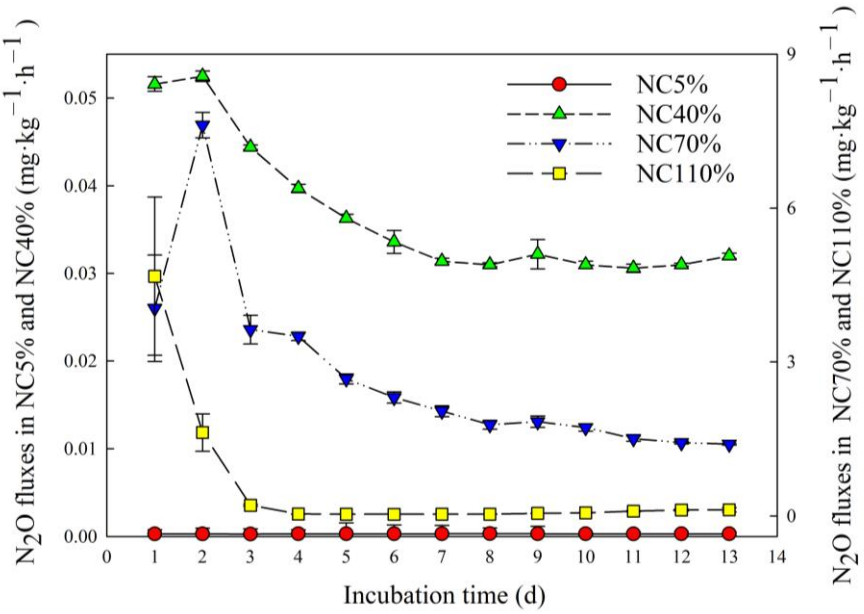

Figure 1. Soil N_2O emission fluxes as affected by exogenous NH_4NO_3 (200 μg N g^{-1} soil) and glucose (300 μg C g^{-1} soil) addition at different soil moistures (5%, 40%, 70%, and 110% WFPS) during the incubation period (d = 13). Data are presented as mean ± standard error (n = 5).

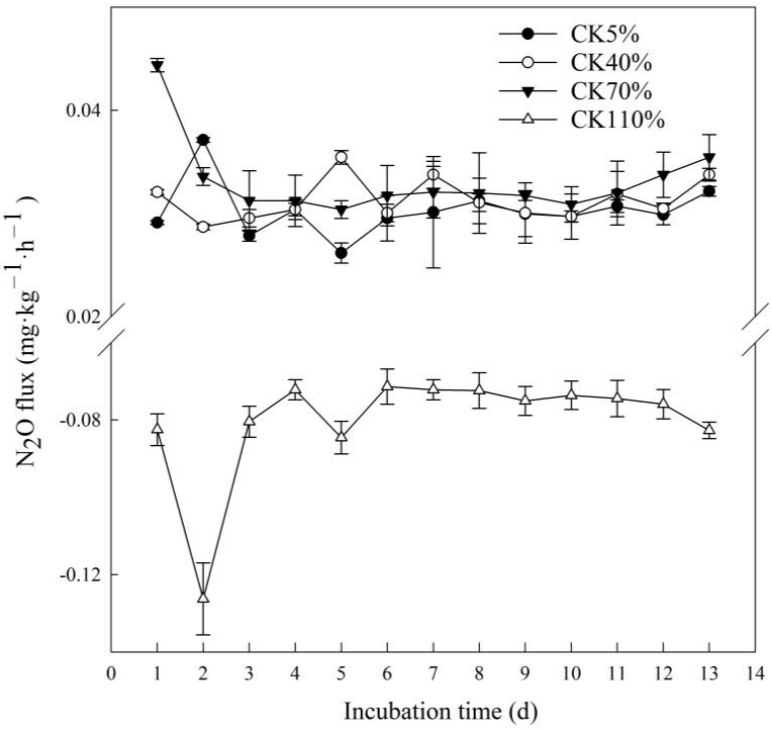

Figure 2. N_2O emission fluxes under different moisture treatments without carbon/nitrogen addition (5%, 40%, 70%, and 110% WFPS) during the laboratory incubation period (d = 13). Data are presented as mean ± standard error (n = 3).

In total, N_2O emission fluxes in the CK treatments were two to five orders of magnitude lower than those in the NC treatment. No differences in N_2O emission fluxes were observed among the treatments of CK5%, CK40%, and CK70% ($p < 0.05$) (Figure 2), and nearly no fluctuation was observed during the incubation period. Additionally, apparent N_2O sinks in CK110% were observed during the incubation, and N_2O was consumed with the highest rate of -0.13 μg kg^{-1} h^{-1} on the second day, three times higher than the maximum emission flux value of 0.04 μg kg^{-1} h^{-1} in other CK treatments.

The cumulative N_2O emission of NC70% was the highest of all the treatments (Figure 3), reaching 456.1 mg kg^{-1}, and was 2.7 times that of NC110% (170.7 mg kg^{-1}) and 39.8 times that of NC40% (11.5 mg kg^{-1}). The cumulative N_2O emissions in NC110% almost reached the maximum amount on the second day and remained stable thereafter. Cumulative emissions in NC70% presented logarithmic growth, while those in NC5% and NC40% showed linear growth. The cumulative emissions of NC5%, NC40%, NC70%, and NC110% are significantly different ($p < 0.01$).

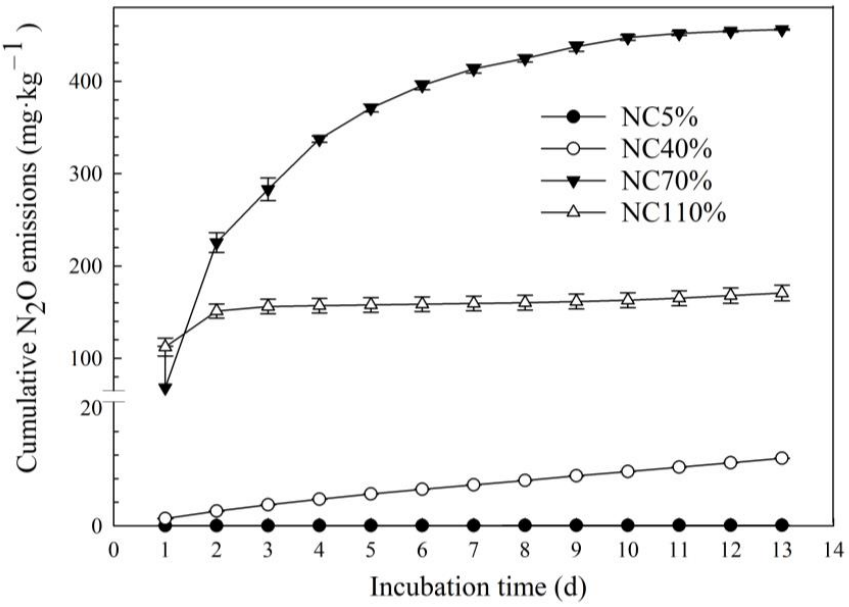

Figure 3. Cumulative N_2O emissions as affected by exogenous NH_4NO_3 (200 μg N g^{-1} soil) and glucose (300 μg C g^{-1} soil) addition at different water moistures (5%, 40%, 70%, and 110% WFPS) during the laboratory incubation period (d = 13). Data are presented as mean ± standard error ($n = 5$).

3.2. Nitrogen Forms

The decreasing rates of NH_4^+ concentrations in NC40% (9.6 mg L^{-1} d^{-1}) and NC70% (10 mg L^{-1} d^{-1}) were higher than that of NC110% (3.6 mg L^{-1} d^{-1}) in the first two days (Figure 4a) and decreased to 0 in the later stage. The N_2O emission fluxes were positively correlated with the NH_4^+ concentrations of NC40% ($p < 0.05$) and NC70% ($p < 0.01$), respectively (Figure 5). In the first two days of incubation, NO_3^- concentrations in the NC treatments were consumed rapidly, from 88.4 to 0 mg/L in NC110% with a consumption rate of -44.2 mg L^{-1} d^{-1}, and from 88.4 to 28.0 mg/L in NC 70% with a consumption rate of -30.2 mg L^{-1} d^{-1}.

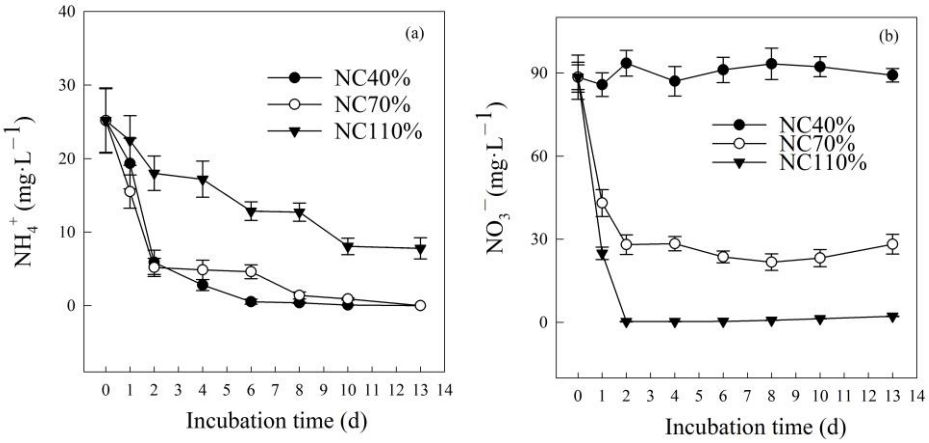

Figure 4. Nitrogen form concentrations as affected by exogenous NH_4NO_3 (200 μg N g^{-1} soil) and glucose (300 μg C g^{-1} soil) addition at different soil moistures (40%, 70%, and 110% WFPS) during the incubation period (d = 13): (**a**) NH_4^+ concentrations; (**b**) NO_3^- concentrations. Data are presented as mean ± standard error (n = 3).

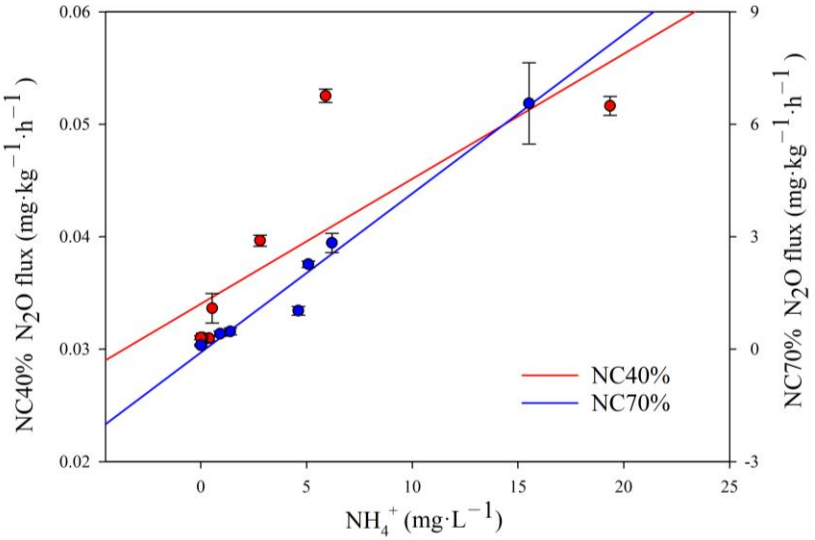

Figure 5. Relationship between N_2O flux and NH_4^+ content over the incubation period. Data are presented as mean ± standard error (n = 5).

4. Discussion

4.1. Responses of N_2O Emissions and Production Mechanisms to Soil Moisture

As a key driving factor of N_2O emissions in soil, soil moisture determines the aeration status of the soil and controls the oxygen supply of soil microorganisms, thereby affecting the activity of nitrifying–denitrifying bacteria [49]. Figure 1 shows that the N_2O emission fluxes were quite different among the NC groups. The maximum emission flux and cumulative emissions both occurred in 70% WFPS, which were consistent with previous findings on N_2O emissions in cornfield soils [50,51]. Basically, soil moisture affects the mobility of dissolved inorganic nitrogen [52]. In detail, soil moisture stimulates the utilization rate of nitrogen species by microorganisms [16]. Additionally, when soil moisture increases, the addition of organic carbon promotes the respiration of heterotrophic microorganisms in the soil, resulting in a decrease in soil oxygen partial pressure and an enhancement of denitrification [53]. Moreover, 70% WFPS moisture provides both aerobic and anaerobic microsites within the soil, which can enhance nitrification and denitrification simultaneously [51,54].

In the NC110%, the maximum emission flux and cumulative emission of soil N_2O were 0.77 and 0.47 times that of NC70%, respectively, and were much higher than those in NC40% and NC5% (Figures 1 and 3). Many researches have suggested that oversaturated

moisture content obstructs oxygen and N_2O diffusion [9,55], and high soil moisture can create a hypoxic or anaerobic environment that facilitates denitrification [17], which also promotes the complete denitrification and thus reduces N_2O to N_2 [23]. In addition, aerobic nitrifying bacteria are inhibited in anaerobic or low-oxygen environments, and N_2O is mainly produced by denitrification [49,56]. Unlike the lower moisture treatment, the N_2O emission peak appeared on the first day and dropped rapidly after the second day, which was because the denitrification rate in the soil is faster than other nitrogen conversion processes [7].

In this study, very low N_2O emissions were observed when the soil moisture in CK treatments was below 70% WFPS. Generally, at low soil moisture, both the soil mineralization rates and the diffusion rates of NH_4^+, NO_3^-, and DOC were found to be relatively low. Correspondingly, the nutrient utilization rate of nitrifying–denitrifying bacteria was also kept at a relatively low level [57]. However, the activity of anaerobic denitrifying bacteria was inhibited because of favourable ventilation in the soil. Correspondingly, nitrification could be enhanced [9]. The low N_2O flux at low soil moisture, as Cheng et al. [16] reported, mainly resulted from the negative correlation between the ratio of NO/N_2O emitting from soil and soil moisture. Similarly, previous studies also suggested that as soil moisture decreases, the emission flux of NO will increase, which partly explains the low N_2O emission fluxes in low-moisture soils [58]. Moreover, the N_2O emission flux will decrease with the increase in total nitrification rate at low moisture levels (<50% WHC), which is also an important reason for low fluxes.

4.2. Estimation of the Contribution of Nitrogen Transformation to N_2O Production

As noted previously, N_2O is produced from three primary nitrogen transformation processes, including nitrification, denitrification, and nitrifier denitrification, which is closely related to the variation in N_2O emissions [9]. In order to quantify the contribution of the three processes to N_2O production in this study, the results of previous studies and this study were jointly employed. In this study, the rapid change in nitrogen forms occurred mainly in the first two days during the incubation period, and N_2O emissions also manifested a similar characteristic, which indicated that the nitrogen conversion rate in the soil was occurring fast with the input of exogenous nitrogen and carbon. The cumulative N_2O emission of NC70% in the first two days was about 224.9 mg kg^{-1} (Figure 3), and the consumption of NH_4^+ and NO_3^- was about 23.8 mg L^{-1} and 12.1 mg L^{-1}, respectively (Figure 4). This is because the contribution of nitrification to N_2O production is relatively low, usually less than 1% of the total NH_4^+ oxidation [59]. In addition, nitrifier denitrification will be enhanced in a hypoxic environment [11] and may account for up to 100% of N_2O emissions reduced from NH_4^+ under the right conditions in soils [13]. Based on the above findings in the literature, to quantify the contribution of nitrogen transformation to N_2O production, it was assumed that the final product of nitrifier denitrification in the first two days was only N_2O, and the N_2O produced by nitrification accounted for 1% of the total NH_4^+ oxidation in NC70%.

It was generally assumed that N_2 could be produced by complete denitrification only at high soil moisture levels (>90% WFPS), but the production of N_2 was usually significantly less than that of N_2O [60]. Moreover, about one-third of the denitrifying bacteria lack the *nosZ* gene encoding N_2O reductase (N_2OR), and the final product is usually N_2O [61]. Krause et al. [62] found that when the moisture level was under 90% WFPS, the $N_2O/(N_2O + N_2)$ emission value was between 0.4 and 0.88 in incubation in the cornfield soil. In addition, plenty of influencing factors could affect the ratio of $N_2O/(N_2O + N_2)$; for example, in addition to oxygen levels, NO_3^- content also significantly affects the ratio [63]. Therefore, in this study, we assumed that 80–90% of the final product of denitrification was N_2O, and then we constructed a polynomial calculation using existing data and hypotheses. Afterwards, the result indicated that 58.3–21.1% of the N_2O emissions came from nitrifier denitrification, and 37.3–75.6% came from denitrification. A study using the isotope tracing method suggested that at 70% WFPS, about 49.9% of total N_2O emissions were from nitrifier denitrification, and 16.1% were from

denitrification [7]. This study reported that nitrifier denitrification might be the main reason for the high N_2O flux in the soil, and the contribution of nitrification was pretty low. However, Congreves et al. [64], using the $^{15}N_2O$ labelling method, argued that the production of N_2O was contributed by both nitrification and denitrification at 70% WFPS. This may be due to differences in the soil structure, pH, and C/N ratio [65].

After two days of incubation, the soil NO_3^- in NC110% was almost completely consumed. From the third day to the end of the incubation, the cumulative N_2O emission was about 19.5 mg kg^{-1}, and the cumulative consumption of NH_4^+ was about 9.5 mg L^{-1}. Previous studies reported that the production of N_2O hardly comes from nitrification when the soil moisture level is high [51,60]. Kool et al. [7]. reported that 92.1% of N_2O production came from denitrification, and the contribution of nitrification was between 0 and 3.4% at 90% WFPS. Thus, in this study, we assumed that nitrification did not occur from the third day to the end at the 110% WFPS because NO_3^- was consumed significantly, and denitrification did not occur. Thus, only nitrifier denitrification could be responsible for N_2O production. The results showed that nitrifier denitrification contributed approximately 34% of the final product as N_2O after the second day. On the first day of the incubation, NO_3^- was rapidly consumed as a denitrification substrate (-63 mg L^{-1} d^{-1}), the cumulative emission of N_2O was about 112.1 mg kg^{-1}, and the consumption of NH_4^+ was 3.3 mg L^{-1}. Thus, we assumed that the production of N_2O-N accounted for 34% of the total NH_4^+-N oxidation. The results indicated that NO_3^- was used as the reaction substrate of denitrification, of which about 94% was reduced to N_2O and 6% reduced to N_2. On the second day, with the same assumptions, 0.013 mmol of N_2O-N was produced by nitrifier denitrification, 0.04 mmol of N_2O-N was produced by denitrification, and about 67% and 33% were reduced to N_2O and N_2, respectively. Generally, in addition to oxygen levels, NO_3^- content also significantly affects the ratio in soil [63]. Moreover, the ratio is not invariable after denitrification occurs, and its variation may be related to the induction kinetics of the denitrification enzyme [66]. An incubation experiment revealed that $N_2O/(N_2O+N_2)$ value decreased from 0.8 to 0.05 within 80 h after applying nitrogen fertilizer [67]. Wang et al. [63] used a combination of field experiments and incubation in cornfields and found that the values varied greatly in the range of <0.01 to 0.67, which was closely related to soil moisture, and the highest values were observed for fertilized soils at high soil moisture (>60% WFPS). Finally, nitrifier denitrification was weak because ammonia-oxidizing bacteria mainly produce NO under a completely anaerobic condition, and N_2O could only be produced when aerobic conditions are restored [68].

In NC40%, the N_2O emission flux decreased rapidly after reaching the peak on the second day (Figure 1); thus, we assumed that the denitrification of denitrifying bacteria did not occur at this time, and the cumulative amount of NO_3^- was derived from the oxidation of NO_2^-. The cumulative N_2O emission was approximately 2.5 mg kg^{-1} (Figure 3), the consumption of NH_4^+ was about 19.3 mg L^{-1}, and the cumulative amount of NO_3^- was about 5.5 mg L^{-1} (Figure 4) during the first two days. The results showed that only 8.3% of NH_4^+-N was oxidized to NO_3^--N, and the gaseous nitrogen loss of N_2O-N only accounted for 1.1% of NH_4^+ consumption. Most NH_4^+ consumption was still unclear, considering that the sample soil was weakly alkaline; the volatilization of NH_4^+ might be a possible reason for its significant consumption at low soil moisture [69].

4.3. Impacts of Exogenous Carbon and Nitrogen on N_2O Production and Emission from Soils with Different Moisture

Soil microbial available nitrogen is another essential factor that affects soil nitrogen conversion. The input of exogenous nitrogen could provide substrates for nitrifying–denitrifying bacteria and further affect N_2O emissions in soil [46,70]. Soil organic carbon (SOC) have been regarded as the primary energy source and carbon source of soil microorganisms, affecting microorganism activity and the microflora distribution characteristics. Thus, in general, SOC content has been found to be positively correlated with N_2O emissions [71], while the present studies indicate that it also depends on the soil moisture

and soil types. In this study, the investigated soil was weakly alkaline and suitable for nitrifying and denitrifying microbial activities [9,72]. However, firstly, the concentrations of NH_4^+, NO_3^-, and TN in the filed were low, reaching only 0.18 mg L^{-1}, 0.49 mg L^{-1}, and 1.95 mg L^{-1}, respectively (Table 1); secondly, no significant differences of N_2O emissions have been observed among CK5%, CK40%, and CK70%, indicating that all of them were in a stable state during the incubation period (Figure 2), which revealed that low available nitrogen content might have limited the nitrification and denitrification of the original soil. However, after adding exogenous carbon and nitrogen, N_2O emission flux from NC5% was still much lower than that in NC treatments, indicating that the available nitrogen could not be effectively transformed and won't produce N_2O at low soil moisture [18]. N_2O emission fluxes in CK40% and CK70% were significantly lower than those in NC40% and NC70%, respectively, indicating that sufficient soil available nitrogen and organic carbon could promote the N_2O production by increasing the richness and diversity of nitrifying–denitrifying microflora at appropriate soil moisture [36,48]. Differently, N_2O was absorbed in CK110% and reached the peak consumption rate on the second day. The main reasons are: firstly, soil water has a certain solubility of N_2O, i.e., at 25 °C and 101.3 kPa, the solubility in water is 5.9 mL L^{-1} [73]; secondly, the extremely low effective nitrogen content in the soil allows denitrifying bacteria to use N_2O as an electron acceptor instead of NO_3^- to be reduced to N_2 [74]; thirdly, in flooded or anaerobic environments, N_2O produced in the soil would be further reduced to N_2 by denitrifying bacteria during diffusion processes. Schlesinger [75] found an average soil N_2O uptake potential of 4 mg m^{-2} h^{-1} during field monitoring and a maximum in soils with high water content, such as wetlands. Audet et al. [76] found that N_2O emissions from riverine wetlands in agricultural watersheds were −44–122 mg m^{-2} h^{-1}, showing a more significant absorption potential.

5. Conclusions

N_2O emission fluxes from cornfield soil in Guizhou Province, SW China, were low in the natural state due to low nitrogen content but obviously stimulated by the inputs of exogenous carbon/nitrogen (e.g., fertilization, nitrogen deposition) at the proper soil moisture (>40% WFPS). Moreover, the experimental soil was more sensitive to moisture than soils from other areas, in which the microbial processes of N_2O production at different moisture levels were significantly different. Nitrifier denitrification was a crucial process dominating N_2O production at a high soil moisture level (70% WFPS), which has often been overlooked or underestimated in previous studies. In the NC110% treatment, N_2O was mainly produced by denitrification, and the emission peaked earlier than other moisture treatments, suggesting that there will be a large N_2O emission from soils in a short time when rainfall occurs, which was very difficult to capture in previous field monitoring work. We speculated that the reaction rate of denitrification at 110% WFPS is faster than that in other treatments. In the current estimates of global soil N_2O emissions, cornfields in karst regions are indispensable due to the high potential of N_2O emissions. More studies on N_2O production mechanisms are needed to provide a more rigorous explanation for the N_2O emissions from lime soils in karst areas.

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