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Influence of Acetylene Concentration on N_2O and N_2 Emissions from an Intensive Vegetable Soil under Anoxic and Oxic Conditions

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Abstract: Acetylene (C_2H_2) is often employed to assess soil total denitrification (N_2O + N_2) due to its ease of implementation. However, this technique underestimates soil denitrification in soils with low nutrient contents, particularly those supporting grain yields. To our knowledge, there are limited studies that have specifically investigated the impact of C_2H_2 on nutrient-rich vegetable soils, especially concerning the emissions of N_2 and N_2O and the nitrogenous gas product ratio (i.e., $N_2O/(N_2O + N_2)$). In this study, we conducted both anoxic and oxic incubations at various C_2H_2 concentrations (0%, 0.01%, and 10%, v/v) and utilized a robotized sampling and analysis system to quantify soil N_2, N_2O, and CO_2 emissions. Our findings revealed that the cumulative N_2O production in soil treated with 10%C_2H_2 was significantly lower than that in soil treated with 0.01%C_2H_2 and soil without C_2H_2. Contrarily, high concentrations of C_2H_2 (10%, v/v) led to increased N_2 production. Similar trends were observed under oxic conditions, where 10%C_2H_2 concentration did not enhance N_2O production but markedly increased N_2 and CO_2 emissions. Moreover, the $N_2O/(N_2O + N_2)$ product ratio was notably higher in soils treated with 0%C_2H_2 compared to the 10%C_2H_2 treatment under anoxic conditions. These findings indicate that high concentrations of acetylene could facilitate the reduction of N_2O to N_2 and lead to underestimated soil total denitrification in vegetable soil, regardless of anoxic or oxic conditions. This discovery underscores the drawbacks when employing high concentrations of acetylene to evaluate actual total denitrification in intensive greenhouse vegetable soils, highlighting the necessity for further investigation into alternative methodologies.

Keywords: acetylene inhibition method; nitrous oxide emission; dinitrogen emission; $N_2O/(N_2O + N_2)$ product ratio; denitrification

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Citation: Cao, W.; Zhang, R.; Li, Y.; Pan, H.; Zhao, F.; Wang, C.; Xin, S.; Li, D.; Gao, Z.; Wang, Y. Influence of Acetylene Concentration on N_2O and N_2 Emissions from an Intensive Vegetable Soil under Anoxic and Oxic Conditions. *Atmosphere* **2024**, *15*, 1206. https://doi.org/10.3390/atmos15101206

Academic Editor: László Bencs

Received: 30 August 2024
Revised: 24 September 2024
Accepted: 30 September 2024
Published: 9 October 2024

1. Introduction

Nitrogen (N) is an essential nutrient that plays a crucial role in regulating ecosystem functioning and ensuring food production and security. Increased food and energy production has led to a significant increase in N fertilizer usage, including synthetic fertilizer and manure [1], causing accelerated N cycling in agricultural soils. Different agroecosystems employ various fertilization regimes, mostly depending on crop types. In intensive greenhouse vegetable production systems, the application of N fertilizer exceeds 2000 kg N ha^{-1} yr^{-1} in two cropping seasons, far beyond plant needs [2,3]. This excessive practice results in high nitrate leaching [4] and gaseous N losses from soils, partially in the form of nitrous oxide (N_2O), a greenhouse gas [5], and denitrified dinitrogen (N_2) [6,7]. These N losses are crucial in determining N availability for plants and microbes [8,9]. Measuring gaseous N losses from soils is challenging due to the high background concentration of atmospheric N_2 [10,11].

Various approaches have been developed to quantify N_2 losses from soils, such as (a) the acetylene (C_2H_2) inhibition technique [12], (b) ^{15}N traces [13], (c) the N_2/Ar technique [14], and (d) helium/O_2 atmosphere exchange [15]. The weaknesses and strengths of these methods have been reviewed [10,16]. Since its early development, the acetylene inhibition technique has become the most widely used indirect method to determine soil denitrification due to its simplicity, short incubation time, low cost, and good reproducibility [16,17]. For example, nearly half of 236 studies used the acetylene inhibition technique to measure N_2 production from the complete denitrification process [16]. Moreover, different concentrations of acetylene (C_2H_2) can be utilized to differentiate between nitrification and denitrification as sources of N_2O in soil [18–20].

Typically, low concentrations of C_2H_2 (0.01%, volume ratio, 10 Pa) are employed to evaluate the contribution of autotrophic nitrification by inhibiting soil ammonia monooxygenase (AMO) activity [21,22]. High concentrations of C_2H_2 (10%) can indirectly measure soil denitrification rates and N_2 emissions by inhibiting ammonia oxidation while preventing the reduction of N_2O to dinitrogen (N_2) via denitrification [23,24]. Nonetheless, the C_2H_2 inhibition technique also has certain limitations, leading to an underestimation of soil denitrification (i.e., $N_2O + N_2$ production) [25,26] and N_2 emissions [27]. This underestimation may be attributed to diffusion restriction of C_2H_2 to microsites, potential effects of microbial decomposition, and incomplete inhibition of N_2O reductase. These biases have mostly been observed in experiments conducted in soils with low soil nutrient and organic matter content, such as grain yield soils [26,28]. However, it remains unclear whether the C_2H_2 inhibition technique also underestimates soil denitrification in greenhouse vegetable soils with high soil nutrient levels (e.g., nitrate). Furthermore, the effects of the C_2H_2 inhibitor on soil N_2 emissions in greenhouse vegetable soils are not well understood.

The primary objective of this study is to evaluate the effects of C_2H_2 on soil denitrification and N_2 production in a greenhouse vegetable cropping system. To attain this, soil samples were collected from a long-term fertilization site, established in 2004, located in the Shouguang area. We conducted laboratory incubation experiments under both anoxic and oxic conditions with different acetylene concentrations. The concentrations of N_2O and N_2 were quantified utilizing a robotic sampling and analysis system, ensuring accuracy and precision in our measurements.

2. Materials and Methods

2.1. Sampling Site

Soil samples were collected from a site in Luozhuang Village, Gucheng Street, Shouguang City, Shandong Province (36°55′ N, 118°45′ E) that was established by China Agricultural University in 2004. The greenhouse vegetable soil there is characterized by a sandy loam texture. The study area exhibits a mean annual air temperature of 12.4 °C and mean annual precipitation of 558 mm. More details on the layout of the experiment are provided in the previous literature [29]. In brief, there were two growing seasons per year with continuous cropping of tomato: the autumn–winter (AW) and winter–spring (WS) seasons. The AW season began in early August and ended the following January, while the WS season started in early February and ended in the middle of June. A brief summer fallow period lasted less than two months. The tomato vines were removed from the greenhouse at the final harvest according to conventional practice. Conventional fertilization treatment with three replicates was selected in the vegetable greenhouse. Prior to tomato planting, chicken manure was utilized as a basal fertilizer at rates of 270 and 190 kg N ha^{-1} in the AW and WS seasons, respectively. The annual application rate of chemical nitrogen fertilizer was 1200 kg N ha^{-1}. Phosphorus and potassium fertilizers were applied at 150 kg P_2O_5 ha^{-1} and 520 kg K_2O ha^{-1}, respectively, in each growing season. Furrow irrigation and conventional fertilization followed, in which the fertilizers were dissolved and applied with the furrow irrigation water. Irrigation time and amount of irrigation were determined by farmers based on local weather, soil moisture contents, and crop growth. In each growing season, 10 to 11 irrigation events took place, and 540 mm to 560 mm water was supplied.

2.2. Anoxic and Oxic Incubations and Gas Monitoring

To investigate the effects of varying acetylene concentrations on N_2O, N_2, and CO_2 emissions, soil samples (0–20 cm) were collected at the end of the AW season from conventional fertilization treatment. Fresh soil samples were sieved through a 2 mm mesh to remove roots and other debris and were thoroughly mixed before being stored at 4 °C for use. The laboratory incubation comprised six treatments, considering the oxygen levels (anoxic and oxic conditions) and acetylene concentrations (0%, 0.01%, and 10%, v/v). For anoxic incubations, there were three groups: a control without inhibitor (0%C_2H_2, anoxic environment with 99.999% helium gas), a low acetylene concentration (0.01%C_2H_2, anoxic environment with 0.01% C_2H_2 (v/v) in the headspace), and a high acetylene concentration (10%C_2H_2, anoxic environment with 10% C_2H_2 in the headspace). Under oxic conditions, the design included a control without inhibitor (0%C_2H_2, oxic environment with 18% O_2 in the headspace), a combination of a low concentration of C_2H_2 and O_2 (0.01%C_2H_2, oxic environment with 18% O_2 and 0.01% C_2H_2 in the headspace), and a combination of a high concentration of C_2H_2 and O_2 (10%C_2H_2, oxic environment with 18% O_2 and 10% C_2H_2 in the headspace). Each treatment was replicated 5 times to minimize errors. Soil N_2O, N_2, and CO_2 emissions were measured with a robotic incubation system at a constant temperature of 20 °C. All soils were analyzed for ammonium, nitrite, and nitrate after incubation.

The robotic incubation system consisted of an automatic sampling module and a gas analysis module (Agilent 7890A gas chromatograph, Santa Clara, CA, USA). The automatic sampling module consisted of a headspace autosampler (CTC GC-Pal) and a bidirectional helical peristaltic pump (Gilson Model 222, Gilson, Corbonod, France). The gas analysis module included an electron capture detector (ECD), a thermal conductivity detector (TCD), and a flame ionization detector (FID) the gas chromatograph, which could monitor changes in N_2O (ECD, TCD), N_2 (TCD), and CO_2 (TCD) gas concentrations. Specifically, ECD data were used for N_2O concentrations below 10 ppmv, and TCD data were used for N_2O concentrations above 10 ppmv. Further details on the robotic incubation system, such as the periodic sampling and analysis of the headspace gas, are described by Molstad et al. [30]. The specific experimental procedure was as follows: Approximately 12.0 g of dried soil was weighed and added to 120 mL serum flasks. A certain amount of deionized water was added to the serum flasks using a syringe to adjust the soil moisture content to 20%. The serum flasks were sealed with rubber stoppers and aluminum caps. A vacuum-inflation system (Beijing Ferren Science & Technology Co., Ltd., Beijing, China) was used to flush the serum flasks with high-purity helium (99.999%); a mixture of 0.01% C_2H_2 and helium; a mixture of 10% C_2H_2 and helium; a mixture of 18% O_2 and helium; a mixture of 18% O_2, 0.01% C_2H_2, and helium; and a mixture of 18% O_2, 10% C_2H_2, and helium. Each serum flask was flushed five times and then filled with the corresponding gas and kept under overpressure for 3 min. The pressure in the headspace of the serum flasks was equilibrated with atmospheric pressure using a syringe filled with deionized water. All the flushed serum flasks were placed in a robotized incubation/monitoring system to conduct a 40 h incubation period. During this period, the concentrations of N_2O, N_2, and CO_2 were monitored at intervals of 8 h. The production rates of these gasses were calculated based on their concentrations, as recorded by the robotic incubation system, compared with known standard gas concentrations. The AIT-bias was calculated from the following equation:

$$\text{AIT-bias} = \frac{(N_2O)_{+C_2H_2} - (N_2O + N_2)_{-C_2H_2}}{(N_2O + N_2)_{-C_2H_2}} \times 100\%$$

where the parameters $(N_2O)_{+C_2H_2}$ and $(N_2O + N_2)_{-C_2H_2}$ represent the N_2O production rate (in nmol g^{-1} h^{-1}) and the total N_2O and N_2 production rate (in nmol g^{-1} h^{-1}) in the presence and absence of acetylene, respectively. The N_2O product ratio $(N_2O/(N_2O + N_2))$ was calculated as the ratio of the N_2O production rate to the total N_2O and N_2 production rate [28].

2.3. Analysis of Soil Physical and Chemical Properties

Soil total nitrogen (TN) was determined using a Kjeldahl nitrogen analyzer. Soil pH was measured potentiometrically in deionized water (1:2.5 *w*/*v*) using a combination pH electrode (PHS-3E, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China). Soil organic carbon (SOC) was determined using the potassium dichromate–sulfuric acid oxidation titration method. Available phosphorus was determined using the molybdenum–antimony anti-absorption spectrophotometry method after extraction with 0.5 mol L^{-1} $NaHCO_3$. Soil moisture was determined by drying the soil in an oven at 105 °C for 24 h. Soil ammonium (NH_4^+) and nitrate (NO_3^-) were determined by extraction with 1 mol L^{-1} KCl followed by measurement using a continuous flow analyzer (TRAACS 2000, Bran Luebbe, Norderstedt, Germany). Soil NO_2^- was measured using the sulfanilamide method [31]. In this study, all the reported calculations were conducted based on dry soil mass. The major properties of the greenhouse vegetable soil used are summarized in Table 1.

Table 1. Basic soil characteristics prior to incubation.

pH	Soil Organic Carbon (g kg^{-1})	Total Nitrogen (g kg^{-1})	Olsen-P (mg kg^{-1})	Exchangeable Potassium (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_2^--N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
6.54 ± 0.02	9.56 ± 0.38	1.55 ± 0.036	232 ± 0.75	240 ± 2.7	21.2 ± 0.24	0.066 ± 0.002	297 ± 44

2.4. Statistical Analysis

SPSS software (version 20.0) was used to perform one-way analysis of variance (ANOVA) with least significant difference (LSD). The experimental data were plotted using Sigmaplot 12.5; dashed lines represent mean values, solid lines represent medians, and bar graphs represent mean values ± standard deviations.

3. Results

3.1. Cumulative N_2O Production

As depicted in Figure 1, a markedly higher cumulative N_2O production was observed during anoxic incubation than during oxic incubation, indicating that the anoxic conditions greatly enhanced N_2O emissions. The cumulative N_2O production under anoxic conditions was 110.0, 107.6, and 0.37 nmol N g^{-1} in the 0%C_2H_2, 0.01%C_2H_2, and 10%C_2H_2 treatments, respectively. Notably, a high concentration of acetylene (i.e., 10%C_2H_2) did not result in increased N_2O production compared to the 0.01%C_2H_2 treatment. Under oxic conditions, the cumulative N_2O production was 0.046, 0.16, and 0.044 nmol N g^{-1} for the 0%C_2H_2, 0.01%C_2H_2, and 10%C_2H_2 treatments, respectively. The cumulative N_2O production in the 0.01%C_2H_2 treatment was significantly higher than those in the other treatments ($p < 0.05$). Notably, similar to anoxic incubation, a high acetylene concentration did not elevate N_2O production during oxic incubation.

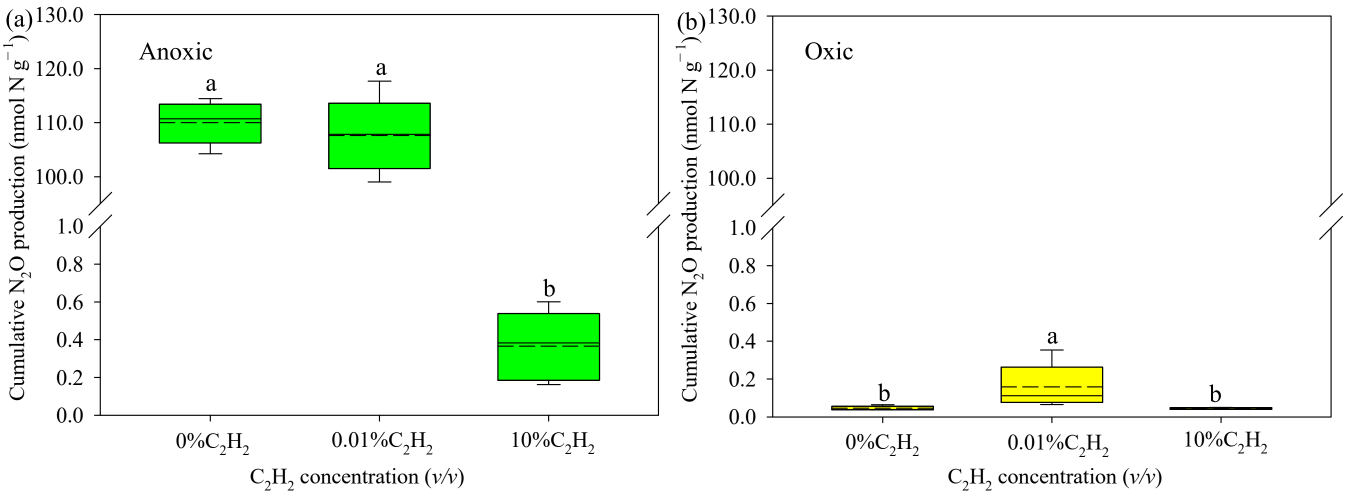

Figure 1. Soil cumulative N_2O production under anoxic (**a**) and oxic (**b**) conditions. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.2. Cumulative N_2 Production

As illustrated in Figure 2, the cumulative N_2 production was 44.0, 88.2, and 297.2 nmol N g^{-1} for the $0\%C_2H_2$, $0.01\%C_2H_2$, and $10\%C_2H_2$ treatments, respectively. The addition of acetylene concentrations of 0.01% and 10% significantly increased N_2 production compared to the $0\%C_2H_2$ treatment ($p < 0.05$), indicating that acetylene stimulated nitrate reduction. Moreover, under oxic conditions, the $10\%C_2H_2$ treatment resulted in significantly higher cumulative N_2 production than the $0\%C_2H_2$ and $0.01\%C_2H_2$ treatments ($p < 0.05$), which further supports the notion that high concentrations of acetylene can promote N_2 production.

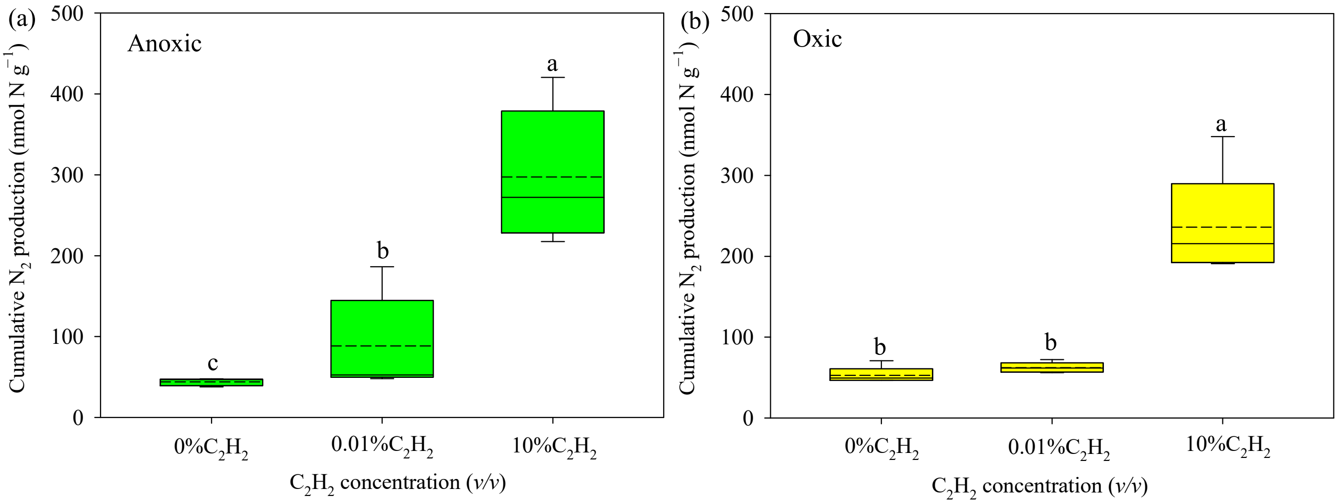

Figure 2. Soil cumulative N_2 production under anoxic (**a**) and oxic (**b**) conditions. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.3. Nitrogenous Gas Production

The production of nitrogenous gasses, namely N_2O and N_2, was calculated as the sum of the production of the individual gas at the end of incubation (Figure 3). Under anoxic conditions, the treatment with a $10\%C_2H_2$ concentration exhibited significantly greater gas production compared to the treatments without C_2H_2 and with $0.01\%C_2H_2$ ($p < 0.05$). These findings suggest that a high C_2H_2 concentration markedly enhances nitrogenous gas production under anoxic conditions. Likewise, during oxic incubation, the $10\%C_2H_2$

treatment demonstrated significantly higher nitrogenous gas production compared to the other treatments ($p < 0.05$).

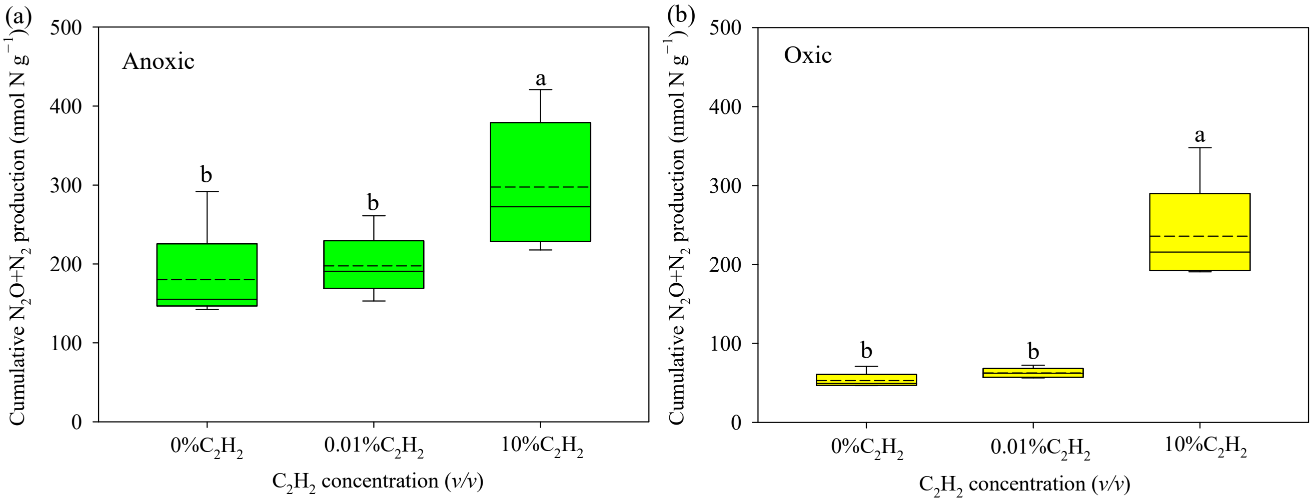

Figure 3. Nitrogenous gas ($N_2O + N_2$) production under anoxic (**a**) and oxic (**b**) conditions. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.4. N_2O and N_2 Production Rates and $N_2O/(N_2O + N_2)$ Product Ratio

As shown in Table 2, under anoxic conditions, the treatment with a high concentration of C_2H_2 (i.e., 10%C_2H_2) exhibited a significantly lower N_2O production rate than that with 0.01%C_2H_2 and that without C_2H_2. Clearly, the high concentration of C_2H_2 corresponded to a significant increase in the N_2 production rate ($p < 0.05$), consequently lowering the $N_2O/(N_2O + N_2)$ product ratio and increasing the AIT-bias. This finding suggests a substantial underestimation of nitrogen gaseous emissions from the soil. Under oxic conditions, a similar effect was observed in that the high concentration of acetylene (10%, v/v) also led to underestimated nitrogen gaseous loss ($N_2O + N_2$) in greenhouse vegetable soil.

Table 2. N_2O and N_2 production rates, $N_2O/(N_2O + N_2)$, and bias in greenhouse vegetable soil under anoxic and oxic conditions with varying acetylene levels.

Production Rate	**Anoxic Conditions**			**Oxic Conditions**		
	0%C_2H_2	**0.01%C_2H_2**	**10%C_2H_2**	**0%C_2H_2**	**0.01%C_2H_2**	**10%C_2H_2**
N_2O-N (nmol g^{-1} h^{-1})	2.75 ± 0.10 a	2.69 ± 0.17 a	0.01 ± 0.005 b	0.0011 ± 0.0003 b	0.004 ± 0.0029 a	0.0011 ± 0.0001 b
N_2-N (nmol g^{-1} h^{-1})	1.10 ± 0.11 b	2.21 ± 1.49 b	7.44 ± 2.07 a	1.32 ± 0.25 b	1.56 ± 0.16 b	5.91 ± 1.61 a
$(N_2O + N_2)$-N (nmol g^{-1} h^{-1})	3.85 ± 0.20 b	4.90 ± 1.47 b	7.44 ± 2.07 a	1.32 ± 0.25 b	1.56 ± 0.16 b	5.91 ± 1.61 a
$N_2O/(N_2O + N_2)$ (%)	71.47 ± 1.66 a	58.25 ± 14.6 b	0.13 ± 0.08 c	0.09 ± 0.03 b	0.26 ± 0.21 a	0.02 ± 0.005 b
AIT-bias (%)		−30.1 ± 4.50 b	−99.8 ± 0.12 a		−99.7 ± 0.22 a	−99.9 ± 0.01 a

Note: Different letters (e.g., ‘a’, ‘b’, ‘c’) represent significant differences ($p < 0.05$) at various acetylene levels under the same oxygen condition.

3.5. Cumulative CO_2 Production

As shown in Figure 4, under anoxic conditions, the results of cumulative CO_2 production were 337.3, 353.3, and 403.2 nmol C g^{-1} in the 0%C_2H_2, 0.01%C_2H_2, and 10%C_2H_2 treatments, respectively. When compared with the 0%C_2H_2 and 0.01%C_2H_2 treatments, a high C_2H_2 concentration (i.e., 10%C_2H_2) significantly increased CO_2 production ($p < 0.05$). A similar trend was observed under oxic conditions, with a high concentration of C_2H_2 (10%) treatment exhibiting the highest cumulative CO_2 production (445.5 nmol C g^{-1}) compared to the other treatments.

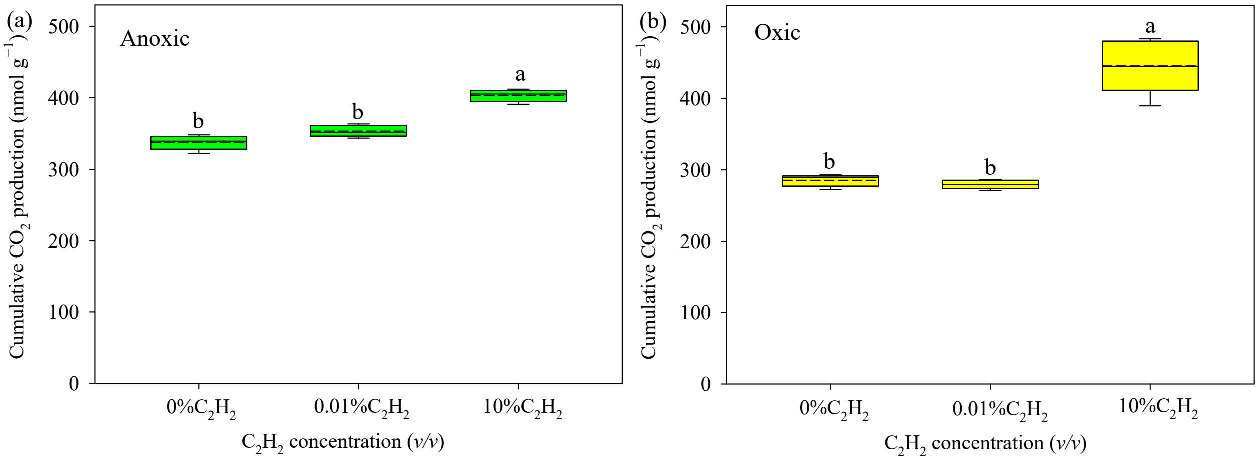

Figure 4. Soil cumulative CO_2 production under anoxic (**a**) and oxic (**b**) conditions. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

3.6. Nitrite and Ammonium Concentration

Under anoxic conditions, the nitrite (NO_2^-) accumulated concentration was 2.36, 2.18, and 0.095 mg N kg^{-1} in the $0\%C_2H_2$, $0.01\%C_2H_2$, and $10\%C_2H_2$ treatments, respectively (Figure 5a). The accumulations of NO_2^- in the $0\%C_2H_2$ and $0.01\%C_2H_2$ treatments were significantly ($p < 0.05$) higher than that in the $10\%C_2H_2$ treatment, showing that higher acetylene concentration could slow down NO_2^- accumulation, possibly by enhancing NO_2^- reduction during anoxic incubation. Conversely, NO_2^- concentration was significantly higher in the treatment with a high acetylene concentration (i.e., $10\%C_2H_2$) than in that without acetylene (i.e., $0\%C_2H_2$) during oxic incubation (Figure 5b, $p < 0.05$).

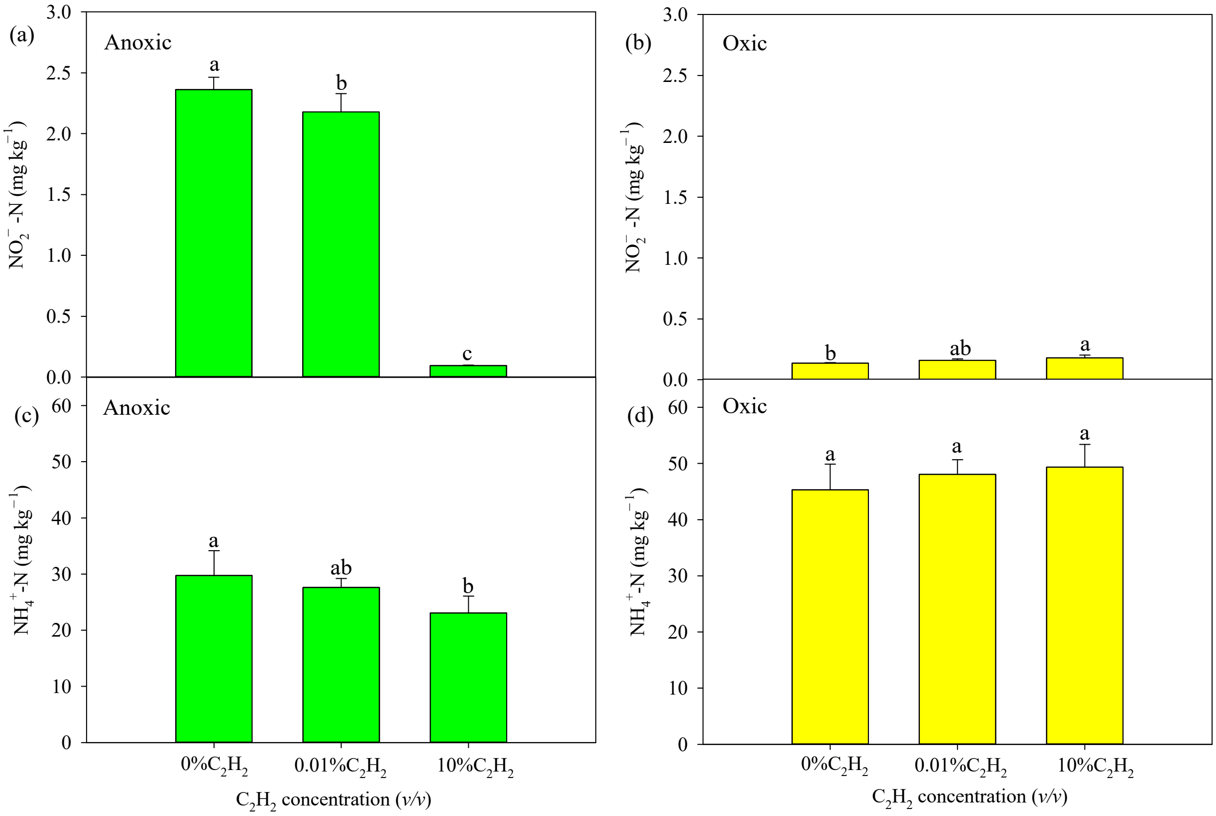

Figure 5. Content of soil NO_2^- (**a**,**b**) and NH_4^+ (**c**,**d**) after incubation under anoxic (**a**,**c**) and oxic (**b**,**d**) conditions. Different lowercase letters indicate significant differences among treatments ($p < 0.05$).

As shown in Figure 5c, ammonium (NH_4^+) accumulation during anoxic incubation ranged from 23.1 to 29.7 mg N kg^{-1}. These NH_4^+ accumulations were increased at the end of incubation compared with the initial NH_4^+ concentration (21.2 mg N kg^{-1}, Table 1). Under oxic conditions, no significantly higher NH_4^+ accumulations were observed among the 0%C_2H_2, 0.01%C_2H_2, and 10%C_2H_2 treatments (Figure 5d). Additionally, there were no significant differences in nitrate (NO_3^-) accumulations among all treatments, regardless of anaerobic or aerobic conditions, indicating that acetylene did not markedly affect soil nitrate concentration at the end of incubation.

4. Discussion

4.1. Effect of Acetylene Inhibitor on Soil N_2O and N_2 Production

Under anoxic conditions, our results showed that a high concentration of C_2H_2 (i.e., 10%) had a significant impact on reducing the cumulative N_2O production in a greenhouse vegetable soil with high nitrate concentration compared with a 0%C_2H_2 treatment. This finding diverges from earlier studies [28,32], which reported an increase in N_2O concentration with C_2H_2 treatment over time. Smith et al. [28] noted that the effectiveness of C_2H_2 in inhibiting the reduction of N_2O to N_2 is enhanced at high NO_3^- concentrations. The prevailing view is that 10% (v/v) C_2H_2 leads to high N_2O concentrations by inhibiting the N_2O reductase enzyme in denitrifying microbes, as established by Knowles [27] and by N_2 production assessed by measuring the difference in N_2O produced between C_2H_2-treated and untreated flasks [33,34]. However, our data suggest that high C_2H_2 concentrations actually promote the reduction of N_2O to N_2 under anoxic conditions (Figure 2), challenging the findings of Qin et al. [28], which indicated lower N_2 increases with C_2H_2 treatment. There are several reasons for the increased N_2 production at high C_2H_2 levels. First, some denitrifiers are not sensitive to C_2H_2, weakening its inhibitory effect [25,35]. Second, some products (e.g., acetate, ethanol) of the degradation of acetylene via soil microorganisms can be utilized by denitrifying bacteria [36,37], promoting the reduction of N_2O. And the availability of carbon is likely to be a major limiting factor in greenhouse vegetable soil during denitrification [6]. In oxic conditions, elevated C_2H_2 levels did not escalate N_2O production but significantly boosted N_2 yields, as indicated in Figure 2. This could be ascribed to the micro-anaerobic conditions induced by C_2H_2 degradation among soil microorganisms [38], which in turn may have enhanced the reduction of N_2O to N_2.

4.2. Effect of Acetylene Inhibitor on Actual Soil Denitrification and Gaseous Product Ratio

Our findings demonstrated that a high concentration of C_2H_2 significantly influenced the production of nitrogenous gas (i.e., the sum of N_2O and N_2 production) in greenhouse vegetable soils, irrespective of anoxic or oxic conditions. Specifically, the presence of high C_2H_2 (10%) led to enhanced actual soil denitrification (Figure 3). This raises questions about the efficacy of using high C_2H_2 concentrations as an inhibitor method to evaluate actual soil denitrification potential. Our data showed that relying on high C_2H_2 concentrations may unpredictably underestimate actual soil denitrification, as C_2H_2 promotes the reduction of N_2O to N_2. This is corroborated by previous studies that have noted incomplete inhibition of N_2O reduction to N_2 when employing the C_2H_2 inhibition technique as opposed to the ^{15}N-nitrate tracer or alternative approaches [24,26,28,38,39]. In our study, under anoxic conditions, there was a significant reduction in the $N_2O/(N_2O + N_2)$ product ratio in greenhouse vegetable soil due to the presence of high C_2H_2 concentrations, as detailed in Table 2. This result aligns with previous findings from grain field soils, which also noted a decrease in the $N_2O/(N_2O + N_2)$ product ratio in response to C_2H_2 [28]. This alignment might be due to high concentrations of NO_3^- inhibiting the reduction of N_2O to N_2 during denitrification, as NO_3^- is favored over N_2O as an electron acceptor [40–43]. The accumulation of NO_3^- in greenhouse vegetable soil, as shown in Table 1, may thus have impeded N_2O consumption, resulting in a higher $N_2O/(N_2O + N_2)$ product ratio in the absence of C_2H_2 treatment. On the other hand, denitrifying microorganisms can metabolize certain byproducts of acetylene degradation [27], potentially accelerating the reduction of NO_3^- and N_2O in the soil [44]. This mechanism could contribute to a significant underestimation of total denitrification in vegetable soil, as evidenced by the data presented in Table 2.

4.3. Effect of Acetylene Inhibitor on Soil Inorganic Nitrogen

Denitrification processes are characterized by a sequential reduction pathway, typically proceeding from NO_3^- to NO_2^-, then to nitric oxide (NO), N_2O, and finally to N_2, with NO_3^- reduction occurring first under completely anoxic conditions [43]. Under scenarios where organic carbon is scarce, competition for electrons among the key denitrification reductases (e.g., Nar, Nir, Nor, and Nos) becomes intense. Our study showed that the concentration of nitrite (NO_2^-) was considerably lower in the treatment with 10%C_2H_2 compared with the treatment without C_2H_2 under anoxic conditions (Figure 5). This difference can be attributed to certain byproducts of acetylene degradation serving as an alternative carbon source that enhanced the reduction of NO_2^-. Conversely, under oxic conditions, an elevated concentration of NO_2^- was detected in the 10%C_2H_2 treatment, which can be primarily ascribed to the increased anoxic microsites resulting from C_2H_2 degradation promoting denitrification and nitrate reduction. Additionally, the accumulation of ammonium in the greenhouse vegetable soil at the conclusion of anoxic incubation suggests that dissimilatory nitrate reduction to ammonium may also have occurred, a process supported by findings from Blackmer and Bremner [40] and further studies [45,46]. Furthermore, no significant differences in nitrate production were observed between the treatments with and without C_2H_2, which could be attributed to the substantial background concentration of NO_3^- in the soil. Lastly, the short incubation period resulted in minimal nitrogenous gas production, having a negligible effect on the overall NO_3^- dynamics.

5. Conclusions

This study revealed that an elevated concentration of C_2H_2 (10%, v/v) substantially boosted the generation of N_2 and the overall yield of nitrogenous gasses (i.e., $N_2O + N_2$) in greenhouse vegetable soils under anoxic conditions. Consequently, there was a significant reduction in the $N_2O/(N_2O + N_2)$ product ratio. Under oxic conditions, a similar trend was observed; high C_2H_2 concentrations did not elevate N_2O production but markedly increased N_2 production. These results suggest that high levels of acetylene can expedite the conversion of N_2O to N_2, potentially leading to an underestimation of the actual total denitrification occurring in greenhouse vegetable soil, irrespective of anoxic or oxic conditions. Given these findings, it is imperative to carefully reconsider the use of C_2H_2 as an inhibitor when assessing the denitrification potential of soils within this specific cropping system. The capacity of high C_2H_2 concentrations to promote the reduction of N_2O to N_2 is a significant factor that merits further investigation and contemplation.

Author Contributions: Conceptualization, W.C., R.Z. and Y.W.; methodology, W.C., R.Z. and Y.W.; software, W.C., R.Z., F.Z., C.W., D.L., Z.G. and Y.W.; validation, R.Z., H.P. and Y.W.; formal analysis, Y.L., S.X., D.L. and Z.G.; investigation, W.C.; resources, W.C. and Y.W.; data curation, W.C., C.W. and Y.W.; writing—original draft preparation, W.C. and R.Z.; writing—review and editing, W.C., R.Z. and Y.L.; visualization, W.C. and Y.W.; Supervision, Y.W.; Project administration, W.C. and Y.W.; funding acquisition, W.C. and Y.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (grant no. 42107345, 41230856) and was also supported by the Scientific Talent Fund Project of Weifang University of Science & Technology of China (grant no. KJRC2022003), the Special Project of Discipline Construction of Weifang University of Science and Technology (grant no. 2021XKJS23), the Weifang Science and Technology Development Plan Project (grant no. 2022GX037), and the National key research and development program (grant no. 2023YFE0104700).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors. The data are not publicly available due to privacy.

Acknowledgments: We would like thank Lars Molstad (UMB Nitrogen group, Norwegian University of Life Sciences) for designing and programming our robotized incubation system for analyzing gas kinetics.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Wenchao Cao is an employee of Shouguang Vegetable Industry Holding Group Company Limited. The paper reflects the views of the scientists, and not the company.

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