


## Article

# Negative Pressure Irrigation System Reduces Soil Nitrogen Loss for Lettuce during Greenhouse Production

Xiang Gao , Shuxiang Zhang \*, Yanyan Song and Huaiyu Long

Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China; gxkochung@163.com (X.G.); songyy109@163.com (Y.S.); longhuaiyu@caas.cn (H.L.)

\* Correspondence: zhangshuxiang@caas.cn

**Abstract:** Negative pressure irrigation (NPI) to grow crops reduces the application of fertilizer and water while also promoting yield and quality. However, plantation vegetables usually require a large input of nitrogen (N) fertilizer in a greenhouse setting, which will lower the soil quality and accelerate the emission of greenhouse gases. Therefore, the purpose of this research was to explore planting lettuce under an NPI system that retrenches N fertilizer application and mitigates N<sub>2</sub>O emissions compared with conventional irrigation (CI). This research proved that under NPI conditions, nitrate and ammonium fluctuated slightly in the soil, stabilizing in the range of 18–28 mg kg<sup>-1</sup>, while that of CI was 20–55 mg kg<sup>-1</sup>. The NPI alleviated N<sub>2</sub>O emissions, and NPI-N150 and NPI-N105 decreased them by 18% and 32%, respectively, compared with those for CI-N150. The main explanation was that the NPI inhibited the formation of NO<sub>3</sub><sup>-</sup>-N, reduced the copies number of AOA and AOB as well as the abundance of *Nitrospira* in the soil, and weakened the soil nitrate reductase and urease activities. The results of this research provide a reliable scientific method for reducing the use of water and N fertilizer while cultivating lettuce, as well as for reducing N<sub>2</sub>O emissions from agricultural facilities.

**Keywords:** negative pressure irrigation; lettuce; NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N; AOA and AOB; N<sub>2</sub>O emission



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## 1. Introduction

Negative pressure irrigation (NPI) is a new type of subsurface irrigation method that assists crop roots in the uptake and utilization of water and fertilizers [1–3]. NPI is based on crop water consumption characteristics and soil tension. It can continuously and stably supply water and nutrition to the rhizosphere to avoid the loss of soil moisture and nutrients [1,4,5]. Since the NPI system supplies nitrogen (N) fertilizer and water in the rhizosphere, it improves the N utilization efficiency (NUE) of crops [3]. Its energy-saving technology has led to it being used in greenhouse and field trials [3,6,7]. In previous research on NPI, the yield of cucumbers, spinach, tomato, cabbage, and other crops was increased by 17~51%, while water consumption was reduced by 14~53% compared with traditional irrigation [7–9]. However, although plantation vegetables require less water and fertilizer under an NPI system, the application of N fertilizer is also related to a series of greenhouse gas (GHG) emissions [10–12]. N<sub>2</sub>O is an important greenhouse gas, and nearly 70% of its emissions are derived from the production and utilization of N fertilizer in agriculture [13–15]. Whether NPI could alleviate N<sub>2</sub>O emissions has not yet been reported.

Greenhouse vegetable production is usually associated with excessive application of N fertilizer, which causes soil consolidation and N leaching into the water, which pollutes rivers and the groundwater [11,14,16,17] and leads to high levels of greenhouse gas emissions [18,19]. Nearly 70% of the N will be lost and returned to the atmosphere or leached due to the catalysis of soil-nitrifying microbes during nitrification and denitrification processes [13,20–22]. The soil nitrification is dominantly driven by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) [23–25]. The subsequent product NO<sub>3</sub><sup>-</sup>-N is a high-mobility N source in the soil, affecting eutrophication and groundwater pollution, and it also causes N<sub>2</sub>O-driven climate change through denitrification [18,26,27].

Therefore, reducing the application of N fertilizer and slowing down N-driven nitrification and denitrification are changes capable of significantly alleviating the emission of  $N_2O$ .

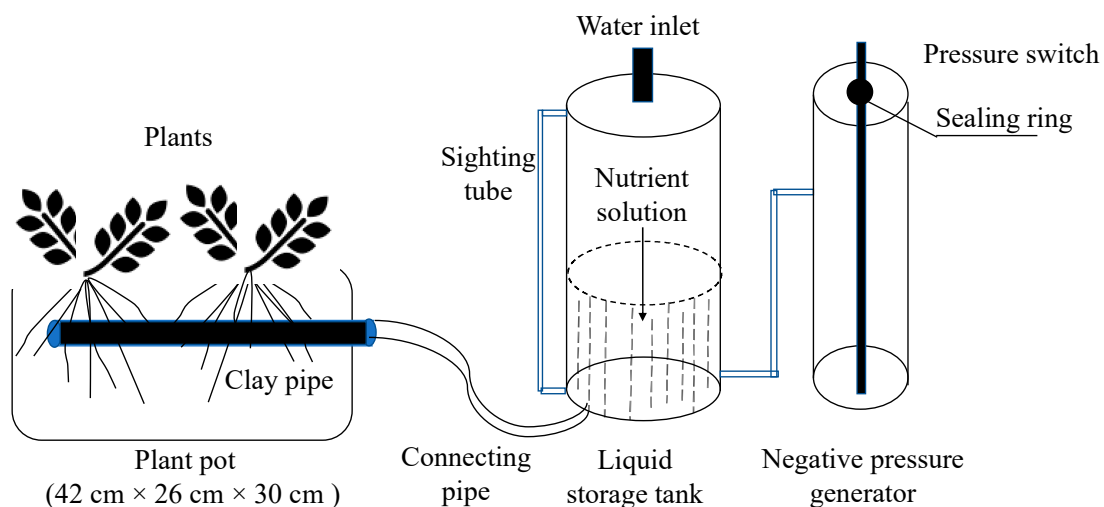
Although China's vegetable production accounts for half of the world's production, it accounts for only 7.8% of the global use of crop chemical fertilizers and 6.6% of the greenhouse gas emissions [21]. Alleviation of the environmental pollution and GHG emissions caused by N application could be achieved by using traditional ways of growing vegetables to reduce the loss of N fertilizer in greenhouses, but alternatively, a feasible irrigation strategy using NPI is capable of reducing the use of N fertilizer [3,6,28,29]. Relatedly, the current drip irrigation (DI) technology principally introduces water and fertilizer into the topsoil, which is likely to result in transpiration leading to a decrease in water and nutrition use efficiency [30–32]. Although using synthetic nitrification inhibitors (SNIs) can effectively suppress the loss of N and reduce greenhouse gas emissions, they have many drawbacks, including cost, environmental pollution, and entry into the food system [13,18]. Consequently, it is necessary to formulate strategies to mitigate N loss processes, such as the NPI system. It seems to be an excellent solution, with restraints on nitrification and the leaching of N to ensure nitrogen utilization efficiency and environmental health and safety.

Our previous studies demonstrated that the NPI system improved the yield and quality of a variety of vegetable crops compared with CI and DI while maintaining a relatively stable water and fertilizer supply and microbial diversity in the rhizosphere in greenhouse and field trials [2,6–8]. Currently, few studies have evaluated whether NPI is able to reduce N loss and mitigate GHG emissions, especially  $N_2O$  emissions, and there is no theoretical support. Therefore, this research aimed to employ irrigation regimes (CI and NPI) with a gradient of N availability to determine whether the growth and quality of lettuce are significantly different, to determine whether NPI can reduce the formation of  $NO_3^-$ -N and the abundance of nitrifying activity in the soil, and to develop an understanding of the mechanism that is suppressing  $N_2O$  emissions.

## 2. Materials and Methods

### 2.1. Materials

The NPI system was designed by the Chinese Academy of Agricultural Sciences (USA patent, 4235561A, and China patent, ZL201610329413.3) (Figure 1). It provides a constant water and nutrition supply to the rhizosphere zone for plant growth [2–5]. The CI treatment simply directly sprinkles water and fertilizer into the topsoil.



**Figure 1.** Schematic of lettuce growth using a negative pressure irrigation system.

The greenhouse experiment was conducted at the Chinese Academy of Agricultural Sciences (116.3 E, 39.9 N). The tested soil was sandy loam with the following soil physical

and chemical characteristics: bulk density,  $1.36 \text{ g cm}^{-3}$ ; pH 6.08; organic matter, 1.62%; total N,  $1.52 \text{ g kg}^{-1}$ ; available N,  $18.57 \text{ mg kg}^{-1}$ ; Olsen P,  $16.26 \text{ mg kg}^{-1}$ ; and available potassium,  $82.37 \text{ mg kg}^{-1}$ . The greenhouse conditions were a day/night temperature regime of  $30/25 \text{ }^\circ\text{C}$ , humidity maintained at 70%, the photosynthetic photon following the natural sunlight, and photoperiod in the season. The lettuce was supplied by the Chinese Academy of Agricultural Sciences.

## 2.2. Experimental Design

### 2.2.1. Experiment 1

The trial pots ( $40 \text{ cm} \times 25 \text{ cm} \times 25 \text{ cm}$ ) were filled with 30 kg of sandy loam. There was a total of 8 treatments, including the irrigation and N treatments, where the irrigation system was CI or NPI, and N consisted of 4 fertilizer concentrations: the different urea-N rates of 150, 120, 105, and  $90 \text{ N kg ha}^{-1}$ , respectively. Four lettuce plants in each pot and the treatments were randomly arranged and repeated 3 times, and the standard concentration of a Hoagland solution formula for plant with a slight modification was supplied [2]. The fertilizer and water were mixed and supplied to the crop for absorption. According to the previous results of NPI experiments, the negative pressure value was set to  $-5 \text{ kPa}$ , and the clay pipe for supplying water was buried 15 cm depth in the pot [2,5]. The water consumption of the NPI was the difference between the amount of water added to and released from the water storage tank (around 10 L). The water consumption of CI was approximately 13 L, and fertilization was carried out 0, 15, and 30 days after sowing. The CI treatment directly sprinkled water into the topsoil, and irrigation was performed 6 times (first 3 L, others time 2 L, for a total of 13 L water). When the NPI system is running, the irrigation emitter supplies water to the plant, and the amount of liquid at the surface of the storage tank will decline. The amount of water consumption can be obtained by recording the amount of water in the storage tank [2,5]. Harvest was conducted 42 days after sowing lettuce in a greenhouse in April 2017.

### 2.2.2. Experiment 2

Based to the results of Experiment 1, further research in Experiment 2 was performed with same equipment. This included the irrigation and N treatments, where the irrigation system was CI or NPI, and N consisted of 2 fertilizer concentrations for CI: a traditional urea-N rate of  $150 \text{ N kg ha}^{-1}$  (CI-N150) or CI-N150 with dicyandiamide (DCD, Sigma, MO, USA) at 10% of the applied N (CI-N150 + DCD). For NPI, the N was NPI-N150 ( $150 \text{ N kg ha}^{-1}$ ) or NPI-N105 ( $105 \text{ N kg ha}^{-1}$ ), based on the yield results of Experiment 1. According to the soil weight of 2.25 million kg per ha, the dosage of nitrogen fertilizer is  $150 \text{ kg/ha}$ . In this study, 2 g of N was requested for 30 kg of soil by calculating, and the urea-N contained an N amount of 46%, with  $150 \text{ kg}$  of N dosage for  $4.35 \text{ g/pot}$ . A total of  $105 \text{ kg N/ha}$  was requested for  $3.05 \text{ g/pot}$ . Harvest was conducted 42 days after sowing lettuce in a greenhouse in June 2017. In addition, 10 g soil samples were collected at 0, 4, 8, and 12 days after the third fertilization (30 days after sowing) and core soil sampled (0–25 cm) for determination of their nitrate and ammonium contents, respectively, to minimize damage to plant roots. The agronomic management and pest control of vegetables followed local greenhouse practices [2].

## 2.3. Analyses

The yield and quality of the plants, as well as their N contents, were measured after harvest. The fresh weight of the lettuce in each pot was measured as the yield, the nitrate content was determined by salicylic acid colorimetry, the soluble sugar by anthrone colorimetry, and vitamin C (VC) by 2,6-chlorophenol spectrophotometry [2]. In addition, the plants were dried at  $75 \text{ }^\circ\text{C}$  until completely dry and then crushed to test their concentrations of N (Kjeldahl 2300; FOSS, Hoganas, Sweden). The N content of the plants ( $\text{mg pot}^{-1}$ ) = biomass (g)  $\times$  N concentration ( $\text{mg g}^{-1}$ ) [2].

Water use efficiency (WUE,  $\text{g kg}^{-1}$ ) = yield/water consumption  $\times$  100%, where yield was the fresh weight ( $\text{g pot}^{-1}$ ), and the water consumption of NPI and CI was approximately 10 L and 13 L, respectively. Partial factor productivity (PFP) of the applied N = yield/applied N amount ( $\text{kg kg}^{-1}$ ).

The soil samples were used to determine the  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N contents with a FIAstar 5000 continuous flow injection analyzer (FOSS, Swiss). The soil apparent nitrification rate (ANR) =  $\text{NO}_3^-$ -N/( $\text{NH}_4^+$ -N +  $\text{NO}_3^-$ -N)  $\times$  100%, and the data for nitrate and ammonium were derived from the soil measured at harvest. Soil urease and nitrate reductase activity were determined by colorimetric technique methods [33]. Soil DNA was extracted from the rhizosphere soil using a DNA Spin Kit for soil (OMEGA, GA, USA) according to the manufacturer's instructions. Quantitative PCR (qPCR) was conducted to assess the abundance of the amoA genes of both ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). amoA-AOA, GenF: ATAGAGCCTCAAGTAGGAAAGTTCTA, GenR: CCAAGCGGCCATCCAGCTGTATGTCC. amoA-AOB, Fomd: CTGGGGTTCTACTGGTGGTC, GenR: GCAGTGATCATCCAGTTGCG [34].

In Experiment 2, the rhizosphere soil attached to the plant roots was collected to determine the composition of the bacterial communities. High-throughput sequencing was conducted by Beijing Allwegene Technology Co. Ltd. The QIIME (v1.8.0) software was applied to quantify the abundance of *Nitrospira* at the genus level.

The  $\text{N}_2\text{O}$  emissions experiment used 100 mL glass bottles containing 10 g rhizosphere soil (the oven dry equivalent) and 200 mg  $\text{N kg}^{-1}$   $(\text{NH}_4)_2\text{SO}_4$ . The microcosms were incubated in 70% water-filled pore space in the dark at 20 °C with 80% RH for 18 days. After 24 h of incubation, a 20 mL gas sample was taken from the headspace with a syringe at different sampling intervals on days 1, 3, 5, 7, 9, 11, 13, 15, and 18 and injected into a 10 mL prevacuum vial. All bottles were ventilated thoroughly for 2 min by an aeration pump after sampling.  $\text{N}_2\text{O}$  was measured with a gas chromatograph fitted with an electron capture detector (HP7820A, Agilent, CA, USA). The cumulative  $\text{N}_2\text{O}$  emissions were summed at the different sampling days. The  $\text{N}_2\text{O}$  incubation method followed Hink (2018) [35].

#### 2.4. Statistical Analysis

All data from the experiments were statistically analyzed by ANOVA using Excel 2010 and SAS 9.1 software (SAS Inc., Cary, NC, USA).

### 3. Results

#### 3.1. Performance of the Lettuce in Experiment 1

NPI improved the performance of lettuce (Table 1). Compared with CI, NPI increased the yield and total N content by an average of 25% and 38%, respectively. Meanwhile, NPI reduced plant nitrate content by an average of 20%. NPI increased WUE and PFP by an average of 49% and 26%, respectively. In addition, there was not significant difference between the yields of N150 and N105 treatments under NPI, but N105 treatment reduced the nitrate content by 14% and also increased PFP by 37% compared to N150 treatment.

#### 3.2. Yield and Quality of Lettuce in Experiment 2

NPI can promote the yield and quality of lettuce in greenhouse production (Table 2). N150 under the NPI treatment significantly increased the yield of lettuce by 18% and 10% compared to the same N levels and DCD addition under CI, respectively. Although the NPI-N105 treatment reduced the N application by 30%, the lettuce yield was still 10% higher than that of CI-N150, but there was no noteworthy difference between it and the CI-N150 + DCD treatment. The N content of the plants also had a similar trend: NPI-N150 had the highest N content, and CI-150 had the lowest N content.

**Table 1.** Performance of lettuce under different irrigation and N treatments in Experiment 1.

Irrigation	N Treatments	Yield g pot <sup>-1</sup>	Total N Content g pot <sup>-1</sup>	Nitrate mg kg <sup>-1</sup>	WUE kg m <sup>-3</sup>	PPF Kg Kg <sup>-1</sup>
CI	N150	361 ± 16 b	17.7 ± 0.9 b	508 ± 12 a	22.6 ± 1.0 b	89 ± 4 d
	N120	338 ± 20 bc	14.5 ± 0.3 d	485 ± 30 a	21.1 ± 1.3 b	104 ± 6 c
	N105	288 ± 20 de	12.0 ± 0.8 e	417 ± 24 b	18.3 ± 1.2 c	101 ± 7 c
	N90	263 ± 19 e	10.3 ± 0.4 f	391 ± 25 b	16.4 ± 1.2 c	108 ± 8 c
NPI	N150	421 ± 12 a	20.5 ± 0.8 a	403 ± 17 b	29.9 ± 1.2 a	104 ± 3 c
	N120	410 ± 18 a	19.6 ± 0.7 a	381 ± 15 b	30.3 ± 1.8 a	126 ± 6 b
	N105	403 ± 14 a	18.9 ± 0.9 ab	346 ± 14 c	29.6 ± 0.6 a	142 ± 5 a
	N90	327 ± 25 bcd	16.4 ± 0.4 c	319 ± 17 c	26.9 ± 2.3 a	135 ± 10 ab

CI is conventional irrigation, and NPI is negative pressure irrigation. N150~N90: 150 kg N ha<sup>-1</sup>, ~N90: 90 kg N ha<sup>-1</sup>, respectively. All of the data are the means of three replicates ± SE, and the different letters after numbers in the same column for the same trait indicate a significant difference at 0.05 ( $p < 0.05$ ) by Duncan's tests.

**Table 2.** Yield and quality of lettuce under different irrigation and N treatments in experiment 2.

Irrigation	N Treatments	Yield g pot <sup>-1</sup>	Plant N g pot <sup>-1</sup>	Nitrate mg kg <sup>-1</sup>	Soluble Sugar mg g <sup>-1</sup>	VC mg kg <sup>-1</sup>
CI	N150	373 ± 21 c	18.8 ± 1.1 c	493 ± 21 a	25.8 ± 1.2 b	217 ± 9 b
	N150 + D	399 ± 20 bc	20.6 ± 1.1 b	387 ± 14 b	24.9 ± 0.9 b	214 ± 6 b
NPI	N150	439 ± 16 a	23.4 ± 1.1 a	394 ± 36 b	27.2 ± 1.0 a	233 ± 5 a
	N105	412 ± 15 ab	21.3 ± 1.0 b	352 ± 20 c	27.9 ± 0.3 a	238 ± 8 a

CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>; D is DCD addition. All of the data are the means of three replicates ± SE, and the different letters after numbers in the same column for the same trait indicate a significant difference at 0.05 ( $p < 0.05$ ) by Duncan's tests.

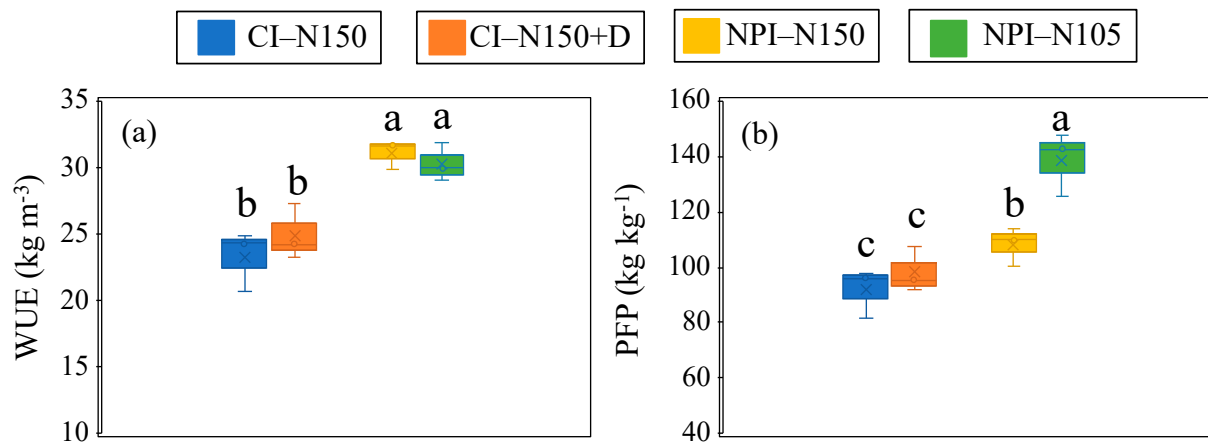
Compared with the CI treatment, the NPI treatment increased the soluble sugar and VC contents and decreased the nitrate content of the lettuce (Table 2). For example, the soluble sugar content of NPI-N150 was significantly increased by 5.4% and 9.2% compared with the two CI treatments, respectively. NPI-N105 had the lowest nitrate content at 352 mg kg<sup>-1</sup>, which was 29% lower than that of the CI-N150 treatment.

### 3.3. Water Use Efficiency and Partial Factor Productivity of Applied N

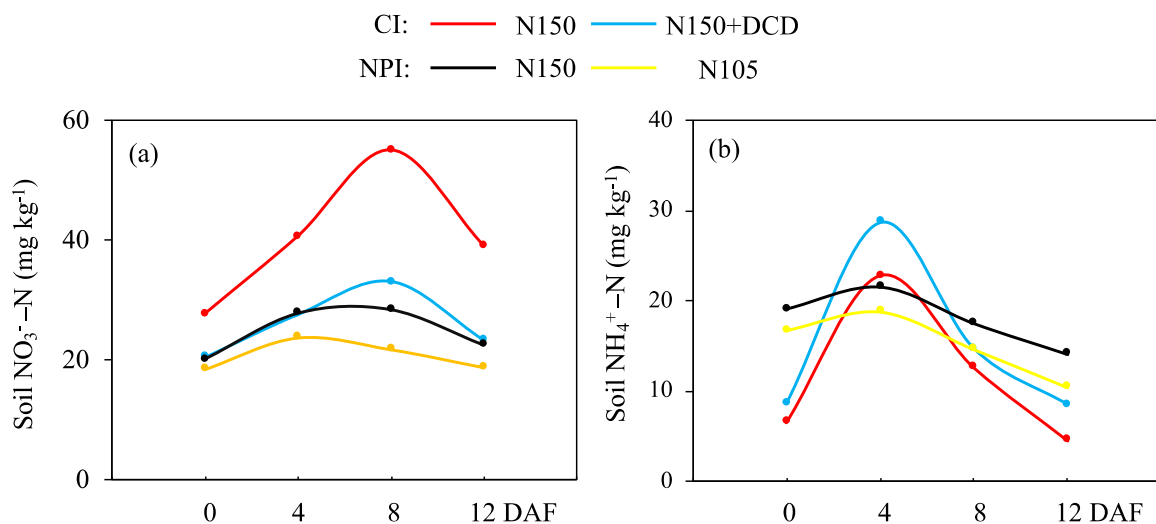
According to the results shown in Figure 2, the WUE and PFP of the NPI treatments were remarkably higher than those of CI, but there was no significant difference in WUE at the two N levels under NPI, and the DCD addition did not notably improve WUE in the CI treatment. Compared with CI-N150, NPI-N150's WUE and PFP were conspicuously promoted by 33% and 17%, respectively. These results show that NPI could reduce water and nutrient applications.

### 3.4. Dynamic Change of Soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N Content

The contents of soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N were influenced by irrigation patterns and N treatments, which fluctuated slightly under the NPI treatment, resulting in inhibition of the formation of NO<sub>3</sub><sup>-</sup>-N (Figure 3a). CI-N150 maintained the highest soil NO<sub>3</sub><sup>-</sup>-N level, with the highest peak appearing on the 8th day after N fertilizer application, and the content reached 55 mg kg<sup>-1</sup>. For the NH<sub>4</sub><sup>+</sup>-N in the soil, the NPI maintained minor fluctuations, implying that it can continuously and stably supply N fertilizer to crops and reduce the loss of nitrate (Figure 3b). After applying N fertilizer, the CI treatment reached its peak on the 4th day and then dropped rapidly. On the 12th day after N application, the NH<sub>4</sub><sup>+</sup>-N of the NPI improved by an average of 88% compared with CI (Figure 3b).



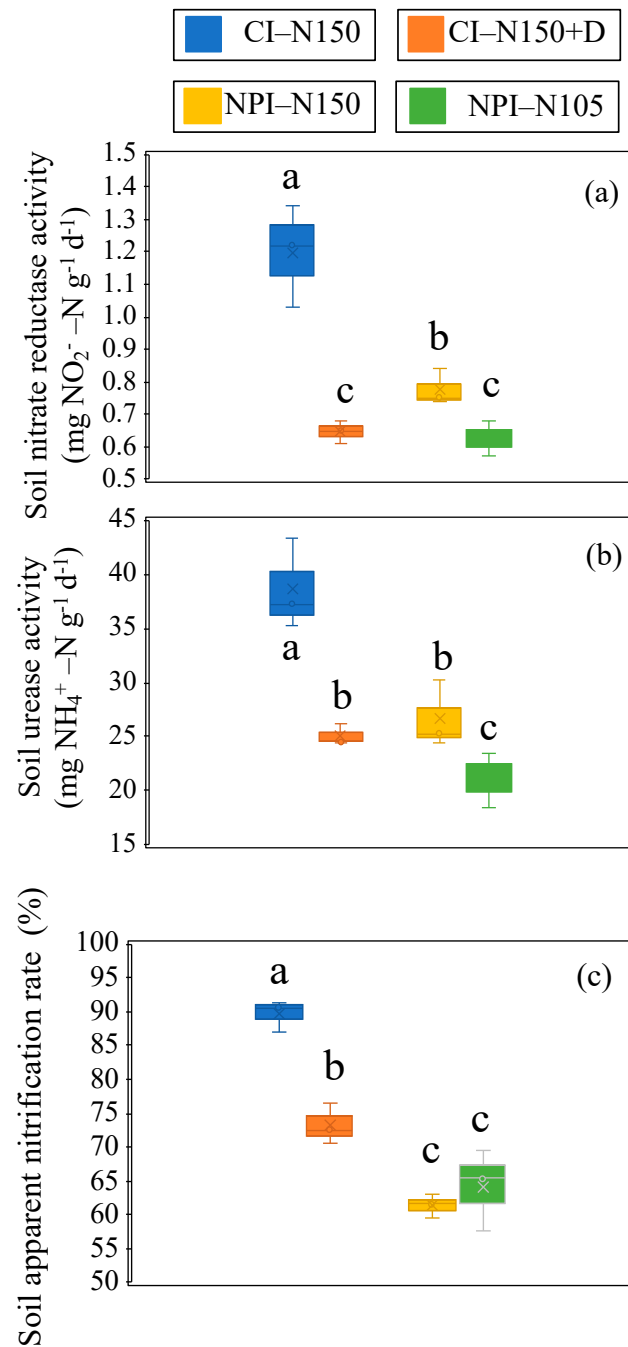
**Figure 2.** WUE and PFP under different irrigation and N treatments. (a) Water use efficiency (WUE); (b) Partial factor productivity (PFP) of the applied N. CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>; D is DCD addition. All of the data are the means of three replicates  $\pm$  SE, and different letters indicate significant differences between the treatments ( $p < 0.05$ ) by Duncan's tests.



**Figure 3.** Dynamic changes in soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N contents under different irrigation and N treatments. (a) Soil NO<sub>3</sub><sup>-</sup>-N; (b) Soil NH<sub>4</sub><sup>+</sup>-N. CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>; DCD is DCD addition. All of the data are the means of three replicates. DAF is the day after fertilization.

### 3.5. Soil Apparent Nitrification Rate, Nitrate Reductase and Urease Activities

As shown in Figure 4, the NPI system and DCD addition prominently reduced the activity of soil nitrate reductase (SNR) and urease (SUA). Soil SNR, compared with CI-N150 and NPI-N150 with DCD, decreased by 35% and 46%, respectively. In addition, by reducing the N application under NPI-N105, the soil SNR was also reduced by 19% compared with that under NPI-N150 (Figure 4a). There was a similar trend for SUA; CI-N150 reached the highest activity, while NPI-N105 had the lowest activity. NPI significantly reduced the soil apparent nitrification rate (ANR), but there was no difference between the two N treatments of NPI (Figure 4c). For example, compared with CI-N150 treatment, the NPI-N150 conspicuously decreased by 32%, and the addition of DCD reduced it by 18% for CI.

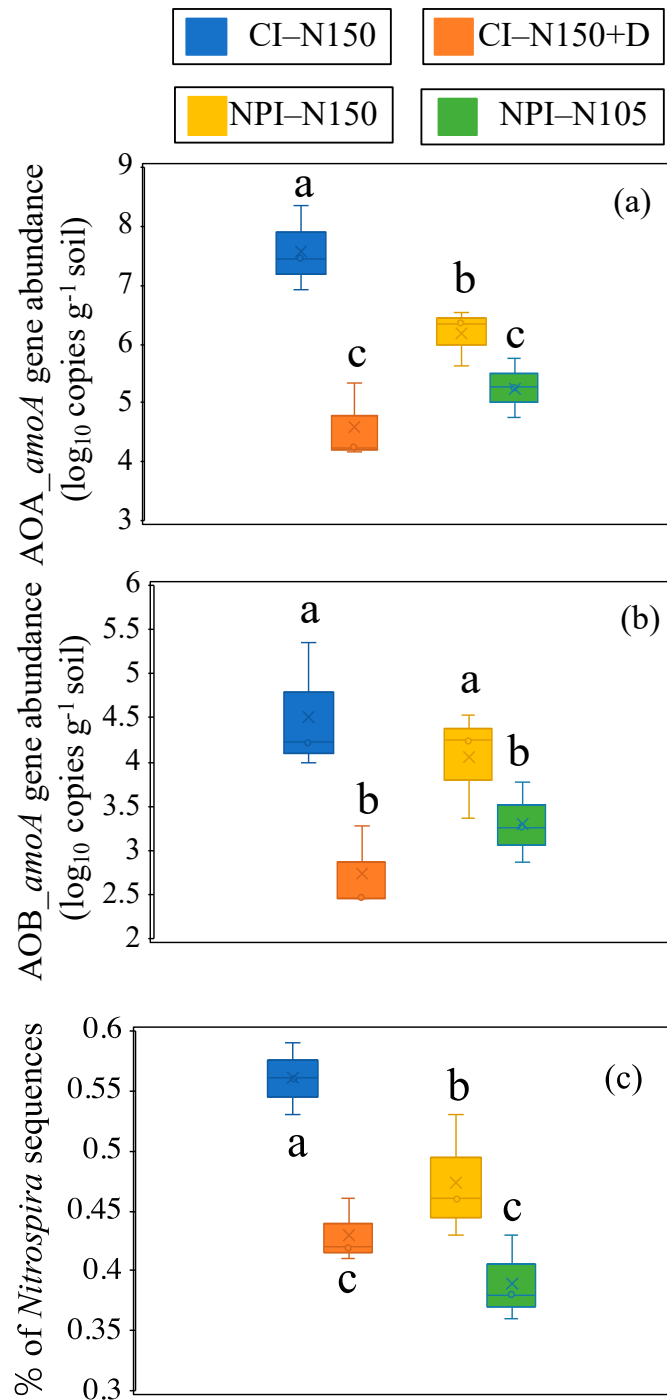


**Figure 4.** Soil nitrate reductase and urease activities, soil apparent nitrification rate under different irrigation and N treatments. (a) Soil nitrate reductase activity; (b) Soil urease activity; (c) Soil apparent nitrification rate. CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>; D is DCD addition. All of the data are the means of three replicates  $\pm$  SE, and different letters indicate significant differences between the treatments ( $p < 0.05$ ) by Duncan's tests.

### 3.6. AOA and AOB Gene, *Nitrospira* Abundance

The results show that the NPI and DCD addition treatments noticeably decreased the AOA and AOB gene copies as well as abundance of *Nitrospira* at genus level in the rhizosphere soil of lettuce (Figure 5). For example, for AOA gene abundance, N150 showed the highest copy number in the CI treatment, followed by NPI-N150, while the values were the lowest with DCD addition. The DCD treatment was significantly reduced by 23%

compared with CI under the same N150 condition (Figure 5a). The abundance of AOB had a similar trend; the highest abundance was in the CI-N150 treatment, and the lowest was in the DCD treatment (Figure 5b). The abundance of *Nitrospira* was 16% lower under NPI-N150 than CI-N150.

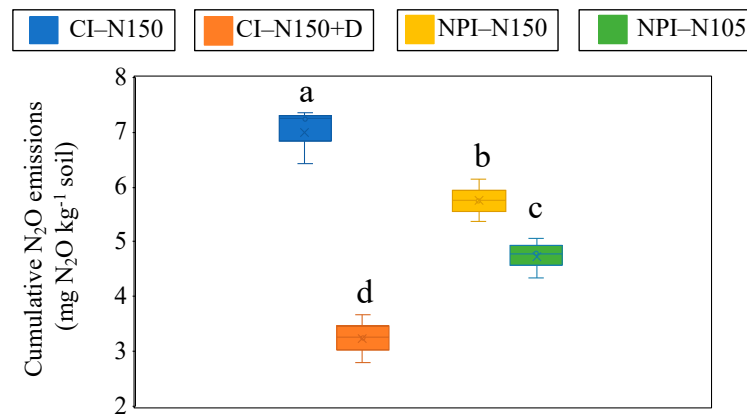


**Figure 5.** Abundance of AOA and AOB *amoA* genes, *Nitrospira* under different irrigation and N treatments. (a) AOA\_amoA gene abundance; (b) AOB\_amoA gene abundance; (c) % of *Nitrospira* sequences. CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>; D is DCD addition. All of the data are the mean of three replicates  $\pm$  SE, and different letters indicate significant differences between the treatments ( $p < 0.05$ ) by Duncan's tests.



### 3.7. Cumulative N<sub>2</sub>O Emissions

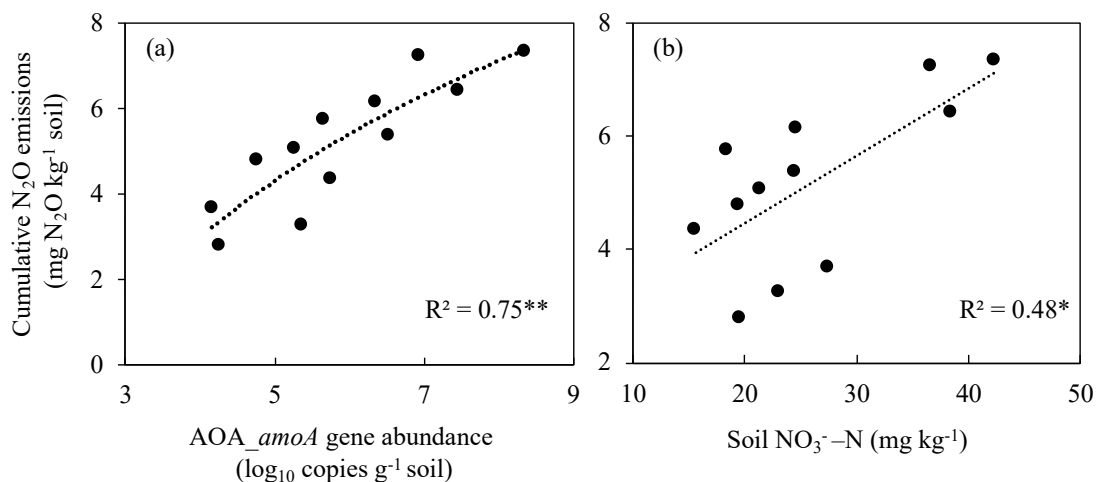
The soil incubation experiment showed that the DCD addition had the most prominent inhibitory effect on N<sub>2</sub>O emissions (Figure 6). Under the CI-150 treatment, the addition of DCD reduced the emissions by 54%, and NPI-N150 notably decreased the emissions by 18%. Moreover, by reducing the amount of N applied in the NPI system, the emission of N<sub>2</sub>O was also reduced by 32% compared with CI-N150.



**Figure 6.** Cumulative N<sub>2</sub>O emissions under different irrigation and N treatments. CI is conventional irrigation, and NPI is negative pressure irrigation. N150: 150 kg N ha<sup>-1</sup>, N105: 105 kg N ha<sup>-1</sup>, D is DCD addition. All of the data are the means of three replicates ± SE, and different letters indicate significant differences between the treatments ( $p < 0.05$ ) by Duncan's tests.

### 3.8. Relationship between N<sub>2</sub>O Emissions and AOA and Soil NO<sub>3</sub><sup>-</sup>-N

Figure 7 shows that the cumulative N<sub>2</sub>O emissions were directly positively correlated with AOA abundance ( $R^2 = 0.75$ ,  $p < 0.01$ ) and soil NO<sub>3</sub><sup>-</sup>-N in the soil ( $R^2 = 0.48$ ,  $p < 0.05$ ). This implies that the most direct way to mitigate the emission of N<sub>2</sub>O is to reduce the content of nitrate in the soil, and this suggests that the use of NPI can limit the content of AOA and nitrate in soil. Moreover, the main contribution of NPI has been supplying nitrogen fertilizer from subsurface irrigation, and the root system can directly uptake N, reduce the nitrate formation and the copies of nitrifying bacteria, and ultimately alleviate N<sub>2</sub>O emissions.



**Figure 7.** Correlation analysis of cumulative N<sub>2</sub>O emissions with AOA and soil NO<sub>3</sub><sup>-</sup>-N: (a) between cumulative N<sub>2</sub>O emissions and AOA,  $n = 12$ ; (b) between cumulative N<sub>2</sub>O emissions and soil NO<sub>3</sub><sup>-</sup>-N,  $n = 12$ . For correlation analysis,  $R^2$  values with asterisks \* and \*\* denote  $r^2 < 0.05$  and  $r^2 < 0.01$ , respectively.

## 4. Discussion

### 4.1. NPI Improved the Performance of Lettuce

NPI has the ability to improve water and nutrient efficiency and has been used in greenhouse and field trials [1–6,36]. This study certified that the yield and quality of lettuce can be significantly advanced (Tables 1 and 2), as well as WUE and PFP, under NPI treatments (Figure 2). The NPI-N105 treatment also reduced the N fertilizer dosage by 30%, which could achieve enhanced results compared with CI-N150 (Table 2). This is primarily because the NPI is able to transport water and nutrients directly to the rhizosphere to accurately and constantly supply water according to the needs of crops [1–6], thereby reducing deep seepage and the evaporation of soil surface water [6,30,37]. Moreover, appropriate amounts of water and nutrients are beneficial to increase stomatal conductance and the chlorophyll content, reduce osmoregulation in the pericarp, promote VC content, and increase the concentration of sugar entering the phloem [38–40], thus promoting photosynthesis and dry matter accumulation in plants [2,39,40]. This is reflected by the fact that NPI increases the dry matter accumulation, soluble sugar, and VC content of crops (Table 2). In addition, relying on the NPI system and reducing N application could reduce the nitrate content in shoots; simultaneously, by increasing the uptake of potassium with N, the NPI system promoted the metabolism of nitrate and resulted in increased contents of soluble sugar and VC in plants [2,40]. Although DCD addition was able to effectively increase the yield of lettuce under CI, its application still caused potential pollution to the environment and vegetables [13,18]. Taken together, these results further confirm that the NPI system not only promoted water and nutrient use efficiency but also reduced the potential pollution of the environment by fertilizers.

### 4.2. Reduction of the Formation of $\text{NO}_3^-$ -N with NPI

Nitrogen is one of the macrolelements required for plant growth and development, but rapid nitrification after N is applied to the soil has a strong negative effect on agricultural ecosystems [20,41–43]. For example, the high-mobility form of  $\text{NO}_3^-$ -N is prone to leaching and runoff pollution, resulting in severe N inefficiency and environmental toxicity [41,44–46]. Hence, suppression of the formation of highly motile  $\text{NO}_3^-$ -N is the key to reducing N pollution. The N fertilizer is provided by NPI directly to the rhizosphere zone, which explains its inhibition of  $\text{NO}_3^-$ -N formation manifested by the relatively low content of  $\text{NO}_3^-$ -N, while the highest content was observed under CI (Figure 3a). This suggests NPI reduces the probability of N conversion to nitrate, as well as reducing leaching and GHG. Moreover, it is capable of retaining a high and stable  $\text{NH}_4^+$ -N content for a long period (Figure 3b) and is associated with lower levels of nitrifying populations (Figure 5), which means that NPI can provide more available N sources for the beneficiary crop and prevent N from coming into contact with nitrifying bacteria. This view is supported by Subbarao and Searchinger [41], who claimed that more ammonium in solution would mitigate N pollution and boost the crop yield. This result is consistent with previous experiments, which found that a continuous and stable water supply can ensure a stable distribution uniformity of soil N when a subsurface irrigation system is employed [2,3]. Consequently, the nitrate reductase activity and soil urease activity were reduced (Figure 4). This result means there was a reduction in the formation of nitrate in the rhizosphere zone under NPI conditions, as well as reduced activity of soil-nitrifying bacteria and enzymes, thus increasing the utilization efficiency of N.

### 4.3. Mitigation of Nitrifying Activity and $\text{N}_2\text{O}$ Emissions

Nitrogen fertilizers prominently contribute to the emission of the greenhouse gas  $\text{N}_2\text{O}$  from agricultural soils, which is catalyzed by ammonia oxidants in oxygenated soils [47–49], and the main source of  $\text{N}_2\text{O}$  is nitrate in soil [47,50]. NPI produced low levels of nitrate (Figure 3a), which had the potential to reduce  $\text{N}_2\text{O}$  emissions and showed prominently positive correlations (Figure 7b). The current study also found that N application led to higher AOA and AOB abundances in the soil, resulting in  $\text{N}_2\text{O}$  emissions (Figure 6),

especially for CI-N150 (which had the highest levels of emissions), but the NPI system could alleviate N<sub>2</sub>O emissions. This result is supported by high N application being an important driving force for nitrification [20,25], which increases the abundance of AOA; simultaneously, lower NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations are favored by AOA over AOB [23,35,51]. Moreover, AOA plays a dominant role in regulating the fate of N, and in many habitats it exceeds AOB, particularly in acidic soils [23,25,47]. This validates our finding that a higher abundance of AOA than AOB may reflect that AOA mainly mediates ammonia oxidation and that NPI significantly inhibits both AOA and AOB (Figure 5). Similarly, the greater soil NO<sub>3</sub><sup>-</sup> content under CI reflects higher copy numbers of AOA and AOB and promotes N<sub>2</sub>O fluxes. Therefore, the irrigation situation was the main variable that mediated most of the N transformation in the soil, affecting the abundance of nitrifying bacteria and the production of N<sub>2</sub>O [27,52]. Although DCD addition can effectively inhibit the copies of nitrifying bacteria and has the lowest N<sub>2</sub>O emissions (Figure 6), the measurement results prove that the NPI system has this function without any food or environmental safety issues [13,18]. Therefore, NPI performs well at altering the nitrifying activity in the rhizosphere through its irrigation function; when applied with N fertilizer, the nitrification and emission of N<sub>2</sub>O will be reduced.

## 5. Conclusions

Overall, the NPI system significantly increased the yield and quality of lettuce, improved the WUE and PFP, and reduced the formation of nitrate over different sampling days compared with CI. Furthermore, the ANR, SNR, and SUA in the rhizosphere soil decreased, and the abundance of AOA, AOB, and *Nitrospira* at genus level was reduced. Additionally, NPI mitigated N<sub>2</sub>O emissions. Therefore, NPI is a feasible strategy to promote lettuce performance and mitigate N<sub>2</sub>O emissions. Our study provides a scientific theoretical basis for supporting the sustainable development of agriculture.

## 6. Patents

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

**Author Contributions:** X.G., S.Z. and H.L. designed the experiments. X.G. and Y.S. conducted the experiments. X.G. and S.Z. wrote the manuscript. All authors have contributed to the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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