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# Responses of Bacterial Communities in Soils under Winter Wheat to Nightly Warming and Nitrogen Addition

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**Abstract:** Understanding soil bacterial diversity under global warming is necessary because of its crucial role in soil nitrogen cycling. However, the interaction effect of warmer temperatures and nitrogen application on bacterial communities in the soils of winter wheat fields is unclear. In this study, the air temperature was increased with infrared heating, and this heating treatment was combined with nitrogen fertilizer application. The two-year continuous temperature increase significantly decreased the soil's pH and nitrate nitrogen content, but significantly increased the content of soil available nutrients. Warming changed the community structure of the soil bacteria, and significantly increased the bacterial richness and diversity by 17.77% and 3.52%, respectively. The changes in the physical and chemical properties of the soil caused by the increased nighttime temperature decreased the percentage abundance of *Pseudomonadota*, which is the largest bacterial phylum, and plays an important role in the global carbon, sulfur, and nitrogen cycles. The structural equation model demonstrated that the influence of soil temperature on bacterial diversity was mediated through soil moisture. Nitrogen application rate directly affected soil bacterial diversity and was the most significant parameter influencing bacterial diversity.

**Keywords:** global warming; nitrogen; soil bacteria; soil physicochemical properties; community diversity



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## 1. Introduction

Global climate warming has taken place; the impact of this increase in atmospheric temperature has been widely considered by researchers around the world [1,2]. Climate warming may affect soil function, soil moisture and heat, nutrients, and plant physiology and growth, as well as microbial species, abundance, and activity [3–5]. It is estimated that the global average surface temperature will increase significantly by 1.0–3.9 °C by the end of the 21st century, and will rise by 0.6–7.8 °C by the end of the 23rd century [6]. Asymmetric diurnal warming is one of the main features of global warming. In the past 35 years, the trend of daytime and nighttime warming in Jiangsu province is obvious, and the rate of daytime and nighttime warming is asymmetric [7]. The rate of increase in global warming in the daytime was lower than that at night, this asymmetric warming is likely to intensify in the coming decades, ultimately leading to a drop in the day and night temperature range [8–10]. At the same time, agricultural production still relies heavily on chemical fertilizers [11]. The excessive application of chemical fertilizers, especially nitrogen fertilizers, is a serious and widespread issue that has caused problems such as poor quality of cultivated land, and low fertilizer utilization and crop yield reduction [12], and it has severely disrupted the ecological balance of the soil biological system, leading to a decline in the diversity of soil biological communities [13].

Soil bacteria play a very important role in terrestrial ecosystems, and they are the main participants in the formation, transformation, and turnover of soil organic matter [14]. Many important ecosystem functions, including soil carbon and nitrogen cycles, are realized by soil bacteria [15]. The change of soil bacterial community structure caused by climate

warming will further affect the decomposition of organic matter, soil carbon cycle and biodiversity, and then it will affect the stability of the ecosystem. In recent years, there have been many reports on the effects of warming and nitrogen fertilization on soil bacteria. However, due to the complexity of the soil bacteria, the differences in warming time and amplitude, and the diversity of research methods, the research results are inconsistent. Zi et al. [16] demonstrated that short-term warming did not obviously impact upon the composition, structure, and diversity of the soil bacterial community, which is consistent with the research results of Hayden et al. [17]. Li et al. [18] found that bacterial alpha diversity significantly increased in an alpine meadow after 3 years of infrared heating. Studies have also shown that warming significantly reduces the richness and diversity of bacterial communities and alters bacterial community composition [19]. Zhou et al. [20] concluded that warming does not always lead to a loss of microbial diversity. Many findings have suggested that nitrogen addition reduces soil bacterial biomass and diversity [21–24]. Dai et al. [25] reported that long-term nitrogen fertilization increases soil available nitrogen content but decreases soil microbial biomass carbon and bacterial diversity. Pan et al. [26] reported that both inorganic and organic fertilization treatments significantly increased the sizes of bacterial and fungal populations compared to controls with no fertilizer treatment. Short-term climate warming and nitrogen application had no significant effect on the soil bacterial community, but the interaction between warming and nitrogen application had a profound effect on bacterial beta diversity [27]. These different results indicate that the response of the soil bacterial community to warming and nitrogen application is complex.

Wheat is one of the most important food crops in the world. Research on the effects of temperature or fertilization on wheat generally focus on crop growth and yield, and there are very few studies on soil nutrient and microbial properties [28], which would have deep effects on microbial activity, community composition, and ecosystem function. Moreover, most studies on the impacts of climate change on soil microbes have examined single-factor manipulations [17]; the interactive effects of warming and nitrogen application have rarely been reported. Due to the shortage of studies, the combined effects of warming and nitrogen application on soil nutrients and their relation to microbial properties remain uncertain.

In this study, we investigated the response of the bacterial community structure to warming and nitrogen application treatments. Three specific questions are addressed: (i) how do nighttime warming and different nitrogen application rates affect soil physical and chemical properties; (ii) how do nighttime warming and different nitrogen application rates affect soil bacterial communities; and (iii) what are the main drivers affecting soil bacterial communities?

## 2. Materials and Methods

### *2.1. Site Description*

The study site was conducted at the Jiangpu Farm Agricultural Experiment Station of Nanjing Agricultural University in Nanjing, Jiangsu Province (32°01′–32°03′ N, 118°36′–118°38′ E), from October 2014 to June 2016. The climate of the test station is controlled by subtropical monsoons with abundant sunshine and rainwater. The annual average temperature is 15.3 °C, and the highest and lowest temperatures are 39.2 °C and −9 °C, respectively. The average annual precipitation is 1063.7 mm. The 0–20 cm soil layer was identified as a Typic Paleudult. The soil organic matter (SOM), pH, total nitrogen (TN), available potassium (AK), and available phosphorus (AP) at a soil depth of 0–20 cm were 18.89 g $kg^{-1}$, 6.86, 2.56 g $kg^{-1}$, 102.73 mg $kg^{-1}$, and 28.08 mg $kg^{-1}$, respectively.

### *2.2. Experimental Design*

The Yangmai 13 wheat cultivar was used as the test material. We set up two temperature treatments, a nighttime warming (NW) treatment using a continuous active warming device with a warming time from 18:00 to 06:00, and an ambient (AMB) treatment, and repeated each treatment three times. The heating source was an electric tube that released

infrared radiation, and the height of the warming device was continuously adjusted to always maintain a distance of 1.5 m between the crop canopy and the device before the wheat flowering period. An empty base without a heating tube was set up to offset the possible shading effect in the ambient treatment (Figure 1). The soil temperature increased by approximately 1 °C. The test layout is shown in Figure 2. We set up treatments with three different nitrogen levels using a randomized block method. The amounts of pure nitrogen applied were 150 kg $ha^{-1}$ (N1 treatment), 225 kg $ha^{-1}$ (N2 treatment), and 375 kg $ha^{-1}$ (N3 treatment). The nitrogen fertilizer was urea, which was applied twice at a 1:1 base and topdressing ratio. The topdressing was applied before the wheat jointing stage. Phosphorus and potassium fertilizers were applied as base fertilizers in the form of $P_2O_5$: 170 kg $ha^{-1}$ (14% superphosphate) and $K_2O$: 170 kg $ha^{-1}$ (60% potassium chloride), respectively. The plot area was 2 m × 2.4 m, the spacing of the wheat rows was 20 cm, and the management of the wheat complied with local conventional high-yield cultivation management practices.

**Figure 1.** System structure of Free Air Temperature Increased (FATI) in winter wheat field.

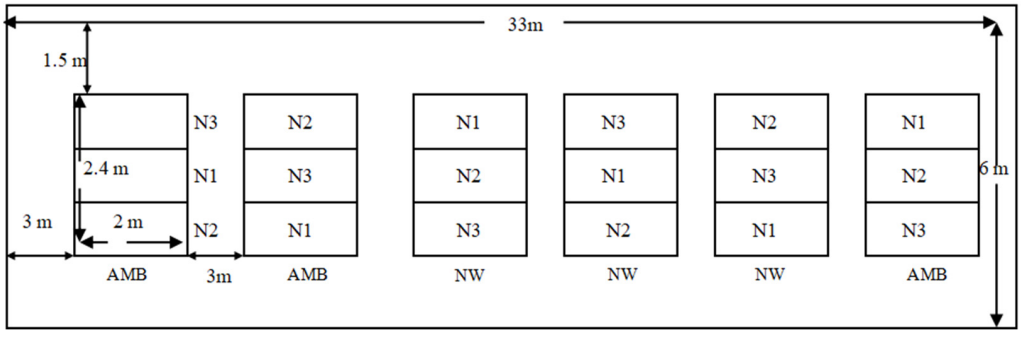


**Figure 2.** Experimental plot layout.

### *2.3. Soil Sampling and Physicochemical Analysis*

Soil samples were collected from the surface layer (0–20 cm) using a five-point sampling method at the anthesis stages. After weeds and other litter were removed, one portion of the soil was passed through a 2 mm mesh sieve and stored in a 4 °C refrigerator for soil physicochemical property analysis, and the other soil portion was stored at −70 °C for DNA extraction and subsequent analysis.

The test plots were equipped with temperature recorders (ZDR-41, Hangzhou Zeda Instrument Co., Ltd., Hangzhou, China). Each recorder had four temperature sensor

probes, which were placed in the upper canopy, middle canopy, ground surface, and at a soil depth of 5 cm, to record the temperature. The temperature was automatically recorded every 20 min. The pH was measured using a pH meter, ammonium nitrogen was determined using the indophenol blue colorimetric method, nitrate nitrogen was analyzed via ultraviolet spectrophotometry, total nitrogen was determined via the Kjeldahl method, soil organic matter was determined using the potassium dichromate method, available phosphorus was measured via sodium bicarbonate–ultraviolet spectrophotometry, available potassium was determined using a flame photometer, and soil microbial biomass carbon and nitrogen were tested via the chloroform fumigation-extraction method [29].

### *2.4. DNA Extraction and Analysis of Sequencing Data*

DNA was extracted from 0.5 g of soil using a fast DNA SPIN kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions, and the quality of the extracted DNA was checked via 1% agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the 338F primer (5′-ACTCCTACGGGAGGCAGCAG-3′) and the 806R primer (5′-GGACTACHVGGGTWTCTAAT-3′) [30]. The PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 33 cycles for 30 s at 95 °C, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Agarose gel electrophoresis (2% agarose) was used to extract the PCR products, an AxyPrepDACBA Gel Extraction Kit (AXYGEACB, San Francisco, CA, USA) was used for purification, and the products were quantified using QuaACBtiFluor™-ST (Promega, Madison, WI, USA) according to the manufacturer's protocol.

The original sequencing sequence was quality controlled and assembled using Trimmomatic and FLASH software, with the following criteria. First, reads with an average quality score <20 were truncated over a 50 bp sliding window, and reads shorter than 50 bp were discarded. Then, primers were exactly matched, allowing 2 mismatches, and reads containing ambiguous bases were removed. Third, sequences with an overlap longer than 10 bp were merged according to their overlap sequence. Sequences with $\geq$ 97% similarity were clustered into the same OTU using Usearch (Version 7.1, Tiburon, CA, USA), and chimeric sequences were removed with UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed using an RDP Classifier against the Silva 16S rRNA database, with a 70% confidence threshold. Mothur software (Version 1.30.1, Ann arbor, MI, USA) was used to analyze the diversity.

### *2.5. Statistical Analysis*

Rarefaction analysis based on Mothur was conducted to reveal the bacterial alpha diversity [31]. The bacterial alpha diversity was expressed using the Ace index, the Chao index, the Shannon index, and the Simpson index. By comparing the Silva database (SSU123) (setting the comparison threshold to 70%), the species classification of each sequence was annotated using the RDP classification classifier (http://rdp.cme.msu.edu/ (accessed on 13 November 2021)), and the community composition of each sample was counted at each classification level. Redundancy analysis (Canoco 5.0 software, New York, NY, USA, http://www.microcomputerpower.com/ (accessed on 25 November 2021)) was applied to evaluate the influence of edaphic factors on bacterial community diversity. Structural equation modeling (SEM) was used to gain a mechanistic understanding of how soil properties mediate alterations in soil bacterial diversity under warming and nitrogen application conditions [32]. Two-way ANOVA was used to examine the effects of warming, nitrogen application, and their interaction on the diversity of soil bacteria. Multiple comparisons of the measured indicators were performed using the least squares difference (LSD) method. The data presented in the text are the average of three repetitions. All data were statistically analyzed using SPSS 16.0.

## 3. Results

### *3.1. Soil Physicochemical Properties and Microbial Biomass*

The effects of nighttime warming and nitrogen application on soil physicochemical properties are shown in Table 1. The results showed that increasing the temperature significantly reduced soil pH under different nitrogen levels, indicating that warming led to soil acidification, while nitrogen application counteracted the acidification effect to a certain extent. Nighttime warming significantly increased the contents of ammonium nitrogen and total nitrogen under different nitrogen levels, but significantly decreased the content of nitrate nitrogen. The nitrate nitrogen content in the soil increased with increasing nitrogen application in the order of N3 > N2 > N1. With the increase in the nitrogen application rate, the content of ammonium nitrogen in the soil first increased, and then decreased; that is, the content of ammonium nitrogen was the highest at the N2 level. The contents of available potassium and available phosphorus in the soil significantly increased with increasing temperature. With the increase in the nitrogen application rate, available potassium and available phosphorus first decreased and then increased, but this change did not reach a significant level. Soil organic matter did not have any significant change.

**Table 1.** Effects of nighttime warming and nitrogen application on soil physical and chemical properties.

| Treatment | | pH | Total N (g kg$^{-1}$) | AK (mg g$^{-1}$) | AP (mg kg$^{-1}$) | SOM (g kg$^{-1}$) | $NH_4^+$-N (mg kg$^{-1}$) | $NO_3^-$-N (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| AMB | N1 | 6.74 ± 0.06 a | 2.41 ± 0.15 b | 0.13 ± 0.02 b | 15.95 ± 1.14 ab | 18.85 ± 0.04 a | 2.23 ± 0.18 c | 18.59 ± 2.56 b |
| | N2 | 6.75 ± 0.01 a | 2.79 ± 0.07 a | 0.13 ± 0.02 b | 13.43 ± 3.27 b | 20.46 ± 0.54 a | 2.97 ± 0.24 abc | 19.35 ± 3.36 b |
| | N3 | 6.70 ± 0.03 a | 2.96 ± 0.40 a | 0.13 ± 0.02 b | 16.85 ± 2.42 ab | 20.39 ± 0.76 a | 2.50 ± 0.95 bc | 27.95 ± 0.76 a |
| NW | N1 | 6.56 ± 0.02 b | 2.99 ± 0.22 a | 0.22 ± 0.02 a | 20.30 ± 2.83 a | 20.38 ± 2.25 a | 3.55 ± 0.34 abc | 12.37 ± 0.53 c |
| | N2 | 6.56 ± 0.02 b | 2.84 ± 0.34 a | 0.19 ± 0.01 a | 18.43 ± 3.27 a | 18.68 ± 2.80 a | 3.75 ± 0.88 a | 16.00 ± 2.19 b |
| | N3 | 6.59 ± 0.06 b | 3.03 ± 0.11 a | 0.23 ± 0.03 a | 18.87 ± 4.27 b | 21.77 ± 1.92 a | 3.52 ± 0.51 ab | 16.19 ± 1.83 b |
| F value | W | 113.391 ** | 8.585 * | 66.446 ** | 7.743 * | 0.178 | 13.23 ** | 59.238 ** |
| | N | 0.135 | 6.063 * | 1.397 | 0.763 | 2.178 | 1.261 | 17.871 ** |
| | W×N | 4.237 * | 3.769 | 0.948 | 2.014 | 1.345 | 0.109 | 8.481 ** |

Different lowercase letters in the above columns indicate significant differences among the treatments at the 0.05 level. The last three lines indicate the significance of the influence of the two factors and their interactions; W: warming effect; N: nitrogen application effect; W × N: interaction effect between the temperature and nitrogen application; * $p < 0.05$; ** $p < 0.01$. AMB: ambient; NW: nighttime warming; N1: 150 kg ha$^{-1}$ nitrogen application; N2: 225 kg ha$^{-1}$ nitrogen application; N3: 375 kg ha$^{-1}$ nitrogen application; Total N: total nitrogen; AP: available phosphorus; AK: available potassium; SOM: soil organic matter; $NH_4^+$-N: ammonium nitrogen; $NO_3^-$-N: nitrate nitrogen.

The analysis of variance showed that the warming had significant effects on all environmental factors except organic matter in the soil, and had extremely significant effects on soil pH and available potassium, ammonium nitrogen, and nitrate nitrogen contents. Nitrogen application had a significant effect on total nitrogen. There was an interaction effect between increasing temperature and nitrogen application on soil pH and nitrate nitrogen.

### *3.2. Analysis of High-Quality Sequences*

The diversity index results of the bacterial community (Table 2) showed that nighttime warming significantly increased the soil bacterial community richness index, with the Ace (3101.09) and Chao (3111.83) indices increasing by 17.77% and 16.44% on average, respectively. The increase in nitrogen fertilizer application significantly reduced the soil richness index and reached a significant level. The richness index was highest at N1 under the warming. The effects of nighttime warming and nitrogen application on the soil diversity index were similar to those of the soil richness index. Warming increased the Shannon index (6.8462) by 3.52% on average, and increased the Simpson index (0.9976) by 0.10% on average. With the increase in the nitrogen application rate, the soil bacterial community diversity decreased but did not reach a significant level.

**Table 2.** Effects of nighttime warming and nitrogen application on the diversity index of the soil bacterial community.

| Treatment | | Ace | Chao | Shannon | Simpson |
|---|---|---|---|---|---|
| AMB | N1 | 2718.60 ± 129.11 cd | 2760.33 ± 133.39 b | 6.6902 ± 0.06 ab | 0.9971 ± 0.0001 a |
| | N2 | 2673.54 ± 194.97 d | 2720.99 ± 167.53 bc | 6.6354 ± 0.16 b | 0.9969 ± 0.0004 a |
| | N3 | 2506.34 ± 156.91 d | 2533.91 ± 162.84 c | 6.5163 ± 0.04 b | 0.9960 ± 0.0007 b |
| NW | N1 | 3225.19 ± 137.46 a | 3257.53 ± 145.09 a | 6.8582 ± 0.11 a | 0.9977 ± 0.0003 a |
| | N2 | 3143.45 ± 112.06 ab | 3148.86 ± 110.16 a | 6.8512 ± 0.06 a | 0.9976 ± 0.0002 a |
| | N3 | 2934.62 ± 239.12 bc | 2929.09 ± 228.17 b | 6.8293 ± 0.17 a | 0.9976 ± 0.0006 a |
| F value | W | 55.361 ** | 61.567 ** | 24.743 ** | 23.204 ** |
| | N | 5.755 * | 8.747 ** | 1.65 | 2.808 |
| | W×N | 0.129 | 0.288 | 0.835 | 2.244 |

Different lowercase letters in the above columns indicate significant differences among the treatments at the 0.05 level. The last three lines indicate the significance of the influence of the two factors and their interactions; W: warming effect; N: nitrogen application effect; W × N: interaction effect between the temperature and nitrogen application; * $p < 0.05$; ** $p < 0.01$. AMB: ambient; NW: nighttime warming; N1: 150 kg ha$^{-1}$ nitrogen application; N2: 225 kg ha$^{-1}$ nitrogen application; N3: 375 kg ha$^{-1}$ nitrogen application.

The analysis of variance showed that the warming had a significant effect on the species richness and diversity, and nitrogen application had a significant effect on the richness of the soil bacterial community, indicating that the soil bacterial community was more complex and had more species under the warming and nitrogen application treatments, while the bacterial community in the control remained relatively simple.

### *3.3. Soil Bacterial Community Composition*

As shown in Figure 3, the dominant bacterial phyla in the soil were *Pseudomonadota*, *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, and *Bacteroidetes*. *Pseudomonadota* was the most predominant phylum and constituted 30.42%, 37.12%, and 30.44% of the bacteria in the three AMB samples, and 28.43%, 33.22%, and 27.36% in the three NW samples. At the N2 level, the relative abundance of *Pseudomonadota* was the highest, while that of *Acidobacteria* was the lowest (14.55% under AMB treatment and 16.37% under NW treatment). Nighttime warming decreased the relative abundance of *Pseudomonadota* but increased that of *Acidobacteria* and *Chloroflexi* under the same nitrogen application level. With the increase in the nitrogen application rate, the relative abundance of *Pseudomonadota* first increased and then decreased, which was opposite to the change trend of *Acidobacteria* and *Chloroflexi*. Meanwhile, the relative abundance of *Actinobacteria* increased under AMB treatment but decreased under NW treatment with the increase in nitrogen application. Warming and nitrogen application had little impact on the relative abundance of *Bacteroidetes*.

### *3.4. Correlation Analysis between Soil Bacterial Communities and Environmental Factors*

The redundancy analysis (RDA) (Figure 4) showed that there was a certain correlation between the environmental factors and the bacterial communities. The first two axes of the RDA accounted for 76.75% of the total variance in the bacterial community composition, and the corresponding interpretation of the first axis accounted for 76.69%. There was a positive correlation among soil temperature, pH, and bacterial community diversity. There was a negative correlation among soil moisture, nitrate nitrogen, soil organic matter, ammonium nitrogen, and bacterial community diversity. Soil pH and soil temperature had the greatest impact on the diversity of the bacterial communities.

### *3.5. Structural Equation Model of Soil Bacterial Community*

Structural equation modeling (SEM) was established to further explore the direct and indirect effects of nighttime warming, different nitrogen application rates, and soil properties (soil moisture, pH, and soil organic matter) on soil bacterial diversity (Figure 5). The SEM demonstrated that the influence of soil temperature on bacterial diversity was mediated through soil moisture. Nitrogen application rate directly (β = 0.646, standardized

coefficient) affected soil bacterial diversity, and was the most significant parameter influencing bacterial diversity. The strongest relationship observed in the SEM analysis was between soil temperature and soil pH (β = −0.824, standardized coefficient). There was also a weaker and negative relationship between soil pH (β = −0.434, standardized coefficient), soil organic matter (β = −0.233, standardized coefficient), and soil bacterial diversity.

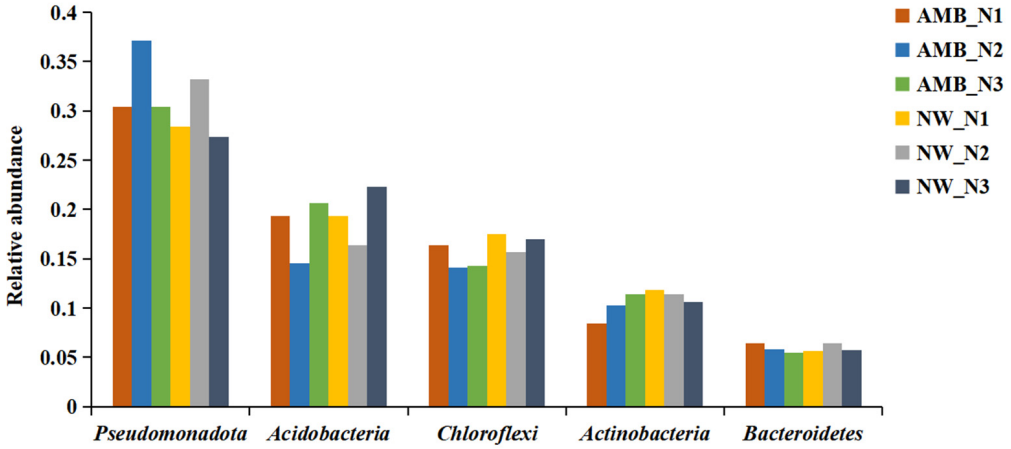


**Figure 3.** Relative abundance of the dominant soil bacterial phyla under different warming and nitrogen treatments.

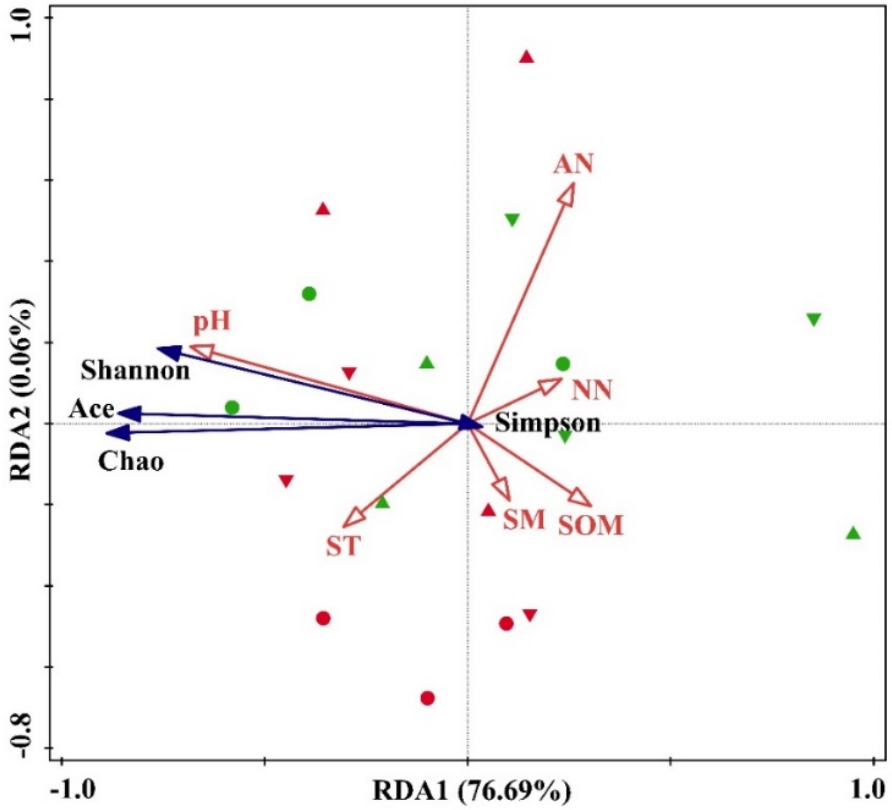


**Figure 4.** Redundancy analysis (RDA) was used to investigate the relationships between the diversity of bacterial communities (expressed as blue arrows) and soil environmental factors (expressed as red arrows). ST: soil temperature; SM: soil moisture; pH: soil acidity; SOM: soil organic matter; AN: ammonium nitrogen; NN: nitrate nitrogen.

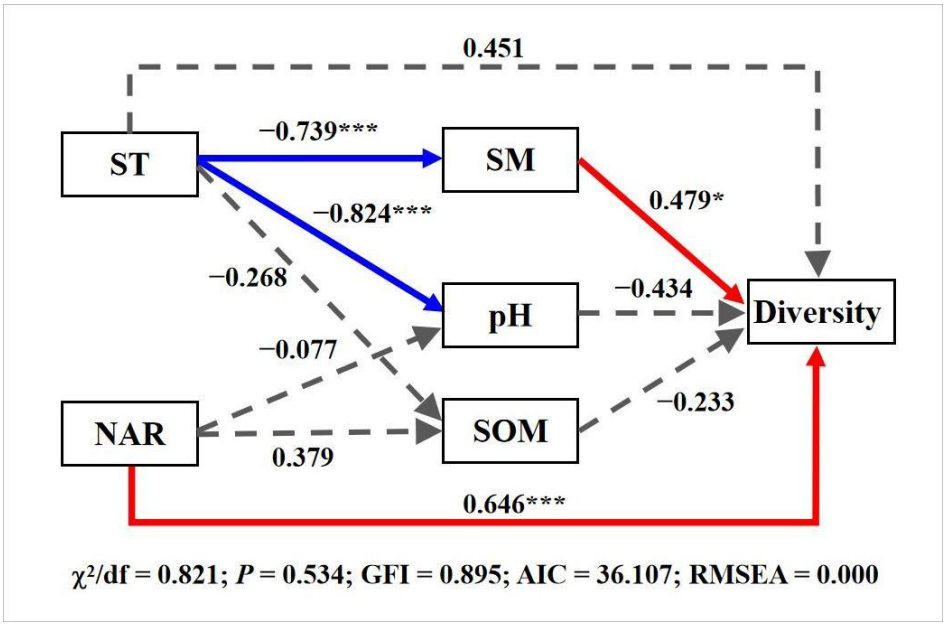


**Figure 5.** Structural equation models of the soil temperature, nitrogen application rate, and soil properties as predictors of soil bacterial diversity. Solid red arrows represent positive paths ($p < 0.05$), solid blue arrows represent negative paths ($p < 0.05$), and dotted grey arrows represent nonsignificant paths ($p > 0.05$). The estimated value of the path coefficient represents the size of the impact scale. * $p < 0.05$; *** $p < 0.001$; ST: soil temperature; NAR: nitrogen application rate; SM: soil moisture; pH: soil acidity; SOM: soil organic matter.

## 4. Discussion

### *4.1. Effects of Nighttime Warming and Nitrogen Application on Soil Physicochemical Properties*

Our studies found that nighttime warming significantly reduced the soil pH, leading to acidification of the soil, which is consistent with the results of Chen et al. [33]. Bryla et al. [34] showed that nitrogen application reduced soil pH. Our study showed that soil nitrate nitrogen contents increased with increasing nitrogen application, but the soil ammonium nitrogen content first increased and then decreased. Warming visibly increased the soil ammonium nitrogen content, which might be because the increase in temperature increased the soil mineralization and nutrient conversion rate [35,36], but with extended warming times, the rate of this mineralization shows a downwards trend [37]. Studies have shown that long-term warming accelerates the decomposition of soil organic carbon, leading to a decrease in the organic matter content of the soil [38]. However, we did not find such a result in our experiments, mainly due to the warming time and intensity [39]. Liu et al. [28] showed that the interaction between warming and nitrogen application played a significant role in the changes in the soil nitrate nitrogen content, which was consistent with the results of this study.

### *4.2. Effects of Nighttime Warming and Nitrogen Application on the Soil Bacterial Community*

Warming and nitrogen application can indirectly or directly affect the kinds, numbers, and functions of soil bacteria. McCaig et al. [40] found that fertilization reduced the diversity of soil bacteria. Upchurch et al. [41] believed that the microbial diversity of farmland ecosystems that use fertilizers for a long time will increase to a certain extent. Song et al.'s [42] research showed that 6 years of warming increased the bacterial abundance in soils from 0 to 15 cm. Studies have also shown that soil bacterial diversity and community composition do not change significantly in the short term [27]. The inconsistency of these results is mainly due to the differences in these warming studies [43]. The results of this study showed that the species richness and diversity of the bacterial community were sig-

nificantly affected by the warming treatment, and that warming increased the soil bacterial community richness and diversity indices, while with the increase in nitrogen application, the richness and diversity of the soil bacterial community had a downwards trend.

Bacteria are the most abundant soil microorganisms [44]. This study found that *Pseudomonadota*, *Chloroflexi*, *Actinobacteria*, and *Acidobacteria* were the dominant bacterial phyla in the soil, indicating that these bacterial phyla have strong vitality, which is basically consistent with other research [45–49]. *Pseudomonadota* is the largest bacterial phylum, and it has diverse metabolic pathways and plays an important role in the global carbon, sulfur, and nitrogen cycles [50]. This study showed that nighttime warming decreased the percentage abundance of *Pseudomonadota* under the N3 treatment, and decreased that of *Acidobacteria* under the N2 treatment, but significantly increased the relative abundance of *Acidobacteria* under the N3 treatment. With increasing temperature, the percentage abundance of *Chloroflexi* under different nitrogen conditions also increased.

### *4.3. Response of Soil Microorganisms to Nighttime Warming and Different Nitrogen Application Rates*

Warming and nitrogen application can change the soil's physicochemical properties, and thus affect the growth of soil microorganisms, which in turn affects the functions of soil ecosystems [51–53]. Bacteria are the most abundant microorganisms in the soil, and many changes in soil physical and chemical properties such as soil pH, soil moisture, and soil salt content affect the composition of the soil bacterial community [54–56]. In our research, pH and soil temperature had the greatest impacts on bacterial communities. Previous results on the effects of soil physical and chemical properties on soil microorganisms were not consistent. It is generally believed that soil pH will significantly affect the composition of soil microbial communities [57]. Rousk et al. [58] conducted a long-term liming experiment (pH 4.0–8.3), and the results showed that both the relative abundance and the diversity of soil bacteria were positively correlated with the soil pH. Similar to this result, our study indicated that bacterial community diversity was significantly and positively correlated to the soil pH. Climate change affects the life activities of soil microbia by changing the soil microclimate [59]. Based on the structural equation model, our study found that soil temperature indirectly affected soil bacterial diversity by changing soil moisture. Nitrogen application rate had an obvious effect on bacterial diversity, and was the most significant parameter influencing bacterial diversity.

## 5. Conclusions

We demonstrated that two consecutive years of nighttime warming and nitrogen application had different effects on the diversity of the soil bacteria and its associated communities in winter wheat. Warming increased the richness and diversity of soil bacteria, and the soil pH and temperature were the major drivers of the bacterial community diversity. The SEM demonstrated that the influence of soil temperature on bacterial diversity was mediated through soil moisture. However, nighttime warming and nitrogen application reduced pH, which led to a decrease in bacterial diversity, and the effect of warming on soil acidification was more pronounced.

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