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Effects of Water Supply Mode on Nitrogen Transformation and Ammonia Oxidation Microorganisms in a Tea Garden

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Abstract: Drought limits tea yield and can also negatively impact its quality. In this study, constant humidity and dry–wet alternating modes were compared to determine their impacts on soil nitrogen transformation and ammonia-oxidizing microorganisms. Drought was found to reduce the soil NH_4^+-N concentration under the constant humidity mode, and the NO_3^--N concentration was highest in 60% water-holding capacity (WHC) soil. Soil NO_3^--N content increased rapidly after rewatering, and increasing the frequency of dry–wet watering resulted in a higher accumulation of NO_3^--N. In the constant humidity mode, drought reduced the abundance of ammonia-oxidizing archaea (AOA), whereas that of ammonite-oxidizing bacteria (AOB) increased. Increases in drought duration and the frequency of dry–wet watering inhibited the activity of AOA under the dry–wet alternating mode, whereas the relative activity of AOB increased after rehydration. The water supply mode did not change the community structure of AOA or AOB at the genus level but affected their relative abundance. In the constant humidity mode, the contribution rate of AOA to nitrification potential (PNR) was 42.75–49.72%, whereas that of AOB was 50.28–57.25%. In the dry–wet alternating mode, the contribution rate of AOA to PNR increased, and the contribution rate of AOB decreased. Taken together, these findings indicate that ammonia oxidation might be primarily driven by AOA and AOB in weakly acidic and neutral soil. This study reveals the effects of different water supply modes on soil nitrogen transformation and ammonia-oxidizing micro-organisms and provides a scientific basis for improving nitrogen use efficiency.

Keywords: moisture supply; nitrogen transformation; ammonia-oxidizing microorganisms

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Citation: Wang, H.; Hou, J.; Zhou, B.; Han, X. Effects of Water Supply Mode on Nitrogen Transformation and Ammonia Oxidation Microorganisms in a Tea Garden. *Agronomy* **2023**, *13*, 1279. https://doi.org/10.3390/agronomy13051279

Academic Editor: Wenxu Dong

Received: 26 March 2023
Revised: 23 April 2023
Accepted: 27 April 2023
Published: 29 April 2023

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1. Introduction

Tea plants (*Camellia sinensis* (L.) O. Kuntze) are grown for their leaves and require sufficient nitrogen to achieve high yields. Higher levels of soil nitrogen increase the photosynthetic rate, thereby improving both yield and quality [1]. The annual average precipitation in the Shandong tea growing area is less than 1000 mm, and rainfall often occurs intermittently. The dry–wet alternations presented by these pulses of rainfall could affect the soil nitrogen cycle and impact plant growth [2].

Soil moisture is one of the important factors affecting soil nitrogen mineralization rate. There was an approximately linear relationship between soil water content and nitrogen mineralization. With the increase in water content, soil nitrogen mineralization increased [3]. However, past a certain point, increasing soil moisture could result in a rapid decrease in nitrogen mineralization [4]. The soil nitrogen transformation varied greatly under different water supply modes. The humification processes of soil organic matter were also known to be affected in well-ventilated dry–wet alternating modes, resulting in differences in soil organic matter quality, and promoting the transformation of non-acidolysis nitrogen in complex soil structures to relatively simple acidolysis ammonia nitrogen and amino sugar nitrogen [1].

Microorganisms were the main drivers behind the biogeochemical cycle of soil elements. Soil microorganisms played a vital role in agricultural ecosystems, as they were involved in several soil functions and ecological services [5,6]. Soil microorganisms were known to be affected by changes in soil moisture, which could lead to alterations in nitrogen transformation. Additionally, drought had been shown to reduce the activity of soil microorganisms in tea gardens, thereby inhibiting the utilization of soil inorganic nutrients by microorganisms [7,8], and further affecting the release of nitrogen in soil. Although soil mineralization still occurs during drought, these conditions reduced the abundance of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) and slowed the transformation of ammonium nitrogen to nitrate nitrogen [9]. Unlike bacteria and fungi, archaea were known to have higher diversity in relatively arid regions. This phenomenon might be due to the unique niches created by more extreme environments, which archaea were able to fill. Additionally, Pett-Ridge et al. [10] found that their higher affinity for oxygen made ammonia-oxidizing archaea more competitive than ammonia-oxidizing bacteria in low-oxygen environments. Furthermore, ecological studies have shown that ammonia-oxidizing archaea were more adaptable to hypoxia than ammonia-oxidizing bacteria. In the soil ecosystem, the number of ammonia-oxidizing archaea was inversely proportional to the oxygen concentration in the soil, whereas the number of ammonia-oxidizing bacteria was positively correlated with oxygen concentration.

Tea garden soil is typically acidic, aluminum-rich, and high in polyphenols, forming a very unique chemical environment. The soil nitrogen transformation process and the microbial population driving nitrogen transformation were significantly different from other soil ecosystems. Although a large number of studies have been conducted on soil nitrogen transformation in tea gardens [1,3], the effects of water supply mode on soil nitrogen transformation and microbial communities in tea gardens had not been reported. In this study, the response of soil nitrogen transformation to different water supply modes was studied to better understand the mechanism of soil nitrogen transformation in Shandong tea gardens. We employed the ammoxidation inhibitors 1-octyne and acetylene, in addition to a quantitative real-time polymerase chain reaction (PCR) and high-throughput sequencing technology, to study the quantitative and structural characteristics of ammonia-oxidizing bacterial communities under different watering modes. We sought to understand the microbial driving mechanism of nitrogen transformation in tea garden soil under different water supply modes to reveal the interaction mechanism between soil nitrogen and microorganisms under different water supply modes. Our results provide new insights into the nitrogen transformation of tea garden soil, provide a scientific basis for improving nitrogen availability, and provide a scientific explanation of soil molecular ecology for the application of tea garden management.

2. Materials and Methods

2.1. Sample Collection

Soil samples were taken from a tea planting location at Shandong Agricultural University (N-36°28′, E-116°83′), which contained sandy loam soil. The surface vegetation of sampling points was removed, and the top 0–20 cm of soil was sampled with a soil drill at multiple random locations. Fresh soil samples were then passed through a 2-mm sieve. The soil water content was maintained at 20% of the maximum water holding capacity (WHC) by natural drying prior to bottling the samples.

2.2. Experimental Design

A total of 100 g dry weight of soil was placed into a culture bottle. Acetylene (Ace, 0.1% v/v) and 1-octyne (1-Oct, 5 μM aqueous solution) [11] were used as selective nitrification inhibitors for soil microcosm culturing. The culture temperature was maintained at 25 °C, and the relative humidity was maintained at 40%. The samples were placed in incubators set at 25 °C, with 40% relative humidity, in the dark. A summary of the experimental conditions is shown in Table 1. Samples were taken after 7 d, 14 d, and 21 d of culturing

and utilized for nitrogen content measurement. After 21 d of incubation, soil DNA was extracted from six treatments of 20% WHC and 60% WHC in constant humidity mode and nine treatments in dry–wet alternating mode. The samples were then used to determine the abundance and diversity of soil ammonia-oxidizing microorganisms. Destructive sampling was used in all experiments, and each bottle of soil was used as a biological replicate, with three replicates per condition. During the incubation period, sterile deionized water was used to adjust the soil water content every 1–2 d.

Table 1. Experimental design.

	Water Supply Mode	No-Inhibitor Culture	Acetylene Culture	1-Octyne Culture
Constant humidity mode	soil water content was set to 20% WHC	T_{20}	T_{20}-Ace	T_{20}-Oct
	soil water content was set to 40% WHC	T_{40}	T_{40}-Ace	T_{40}-Oct
	soil water content was set to 60% WHC	T_{60}	T_{60}-Ace	T_{60}-Oct
	soil water content was set to 80% WHC	T_{80}	T_{80}-Ace	T_{80}-Oct
Dry–wet alternation mode	20% WHC for 1–7 d, 60% WHC for 8–21 d	D7W14	D7W14-Ace	D7W14-Oct
	20% WHC for 1–14 d, 60% WHC for 15–21 d	D14W7	D14W7-Ace	D14W7-Oct
	20% WHC for 1–7 d, 60% WHC for 8–14 d, 20% WHC for 15–21 d	D7W7D7	D7W7D7-Ace	D7W7D7-Oct

2.3. Determination of Ammonium Nitrogen and Nitrate Nitrogen in Soil

In brief, 10 g of cultivated soil sample was added to 50 mL of 2 mol/L KCl solution, followed by 1 h of shaking and then filtration. The contents of ammonium nitrogen and nitrate nitrogen were determined by a continuous flow analyzer (AA3, SEAL Analytic, Norderstedt, Germany), and the soil nitrification potential (PNR) was calculated.

2.4. Extraction of Total DNA, Real-Time Fluorescent Quantitative PCR

Soil total DNA was extracted with an "Omega Soil DNA Kit" (Omega Bio-tek, Norcross, Georgia, USA). The concentrations and purity of DNA were determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Extracted DNA was stored at −80 °C prior to the determination of the abundance and diversity of soil ammonia-oxidizing microorganisms.

The copy numbers of AOA-*amoA* and AOB-*amoA* genes were determined by quantitative real-time PCR. The primer sequences of target genes are shown in Table 2. The reaction was performed with an ABI7500 quantitative real-time PCR instrument (Thermo Fisher Scientific, Waltham, MA, USA). The 20-μL reaction system contained 2 μL DNA template, 1 μL each forward and reverse primers, 6 μL double-distilled H_2O, and 10 μL 2× SYBR® Green qPCR Master Mix (Applied Biosystems, Foster City, CA, USA). Each reaction was repeated three times, and the results were expressed as the copy number of genes per gram of dry soil weight.

Table 2. The primer sequence of the target gene.

Target Gene	Primer Name	Sequence(5′-3′)	Length of Amplicon (bp)
AOA	amoA F	5′-STAATGGTCTGGCTTAGACG-3′	635
	amoA R	5′-GCGGCCATCCATCTGTATGT-3′	
AOB	amoA1F	5′-GGGGTTTCTACTGGTGGT-3′	491
	amoA2R	5′-CCCCTCKGSAAAGCCTTCTTC-3′	

2.5. High-Throughput Sequencing of Soil Ammonia-Oxidizing Microorganisms

An ABI GeneAmp® 9700 PCR instrument (Thermo Scientific, USA) was used to amplify *amoA* genes of AOA and AOB. The primers were the same as those used for quantitative real-time PCR. The PCR products were detected by 2% agarose gel electrophoresis

and recovered using an AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The PCR products were quantitatively detected by a QuantiFluor™-ST (Promega, Madison, WI, USA) blue fluorescence quantitative system, followed by the construction of a Miseq sequencing library. Sequencing was performed on the Illumina Novaseq 6000 sequencing platform at Biomarker Technologies Co., Ltd. (Beijing, China).

2.6. Data Processing

Acetylene was used to completely inhibit the ammonia oxidation of autotrophic nitrification, and the PNR of AOA + AOB was calculated by subtracting the PNR in the control from the PNR after acetylene inhibition. Additionally, 1-octyne was used to specifically inhibit AOB activity without affecting AOA activity. The PNR of AOA was determined by subtracting the PNR after 1-octyne inhibition from the PNR after acetylene inhibition. The PNR of AOB was determined by subtracting the PNR of AOA + AOB from the PNR of AOA.

Statistical analysis was conducted using SPSS v16.0 for Windows. One-way analysis of variance (ANOVA) and least significant difference (LSD) tests were used to compare the averages between treatments. The significance cutoff was set at $p = 0.05$. Origin 8.0 software was used to create graphs.

3. Results

3.1. Effects of Water Supply Model on Soil Mineralization in Tea Garden

Changes in the relative amounts of soil NH_4^+-N and NO_3^--N during cultivation reflect nitrogen transformation. During the constant humidity mode, the NH_4^+-N concentration first increased and then decreased, whereas the NH_4^+-N content in T_{20} decreased. After 21 d of incubation, the NH_4^+-N level was highest in T_{40}, followed by T_{80}, $T_{60,}$ and then T_{20} ($p < 0.05$) (Figure 1a). Compared with the no-inhibitor culture, the relative levels of NH_4^+-N in samples treated with 1-octyne were T_{80}-Oct > T_{60}-Oc > T_{40}-Oct > T_{20}-Oct, with increases of 42.71%, 57.70%, 9.85%, and 5.11% respectively at the end of the culture period. However, T_{20}-Ace, T_{40}-Ace, T_{60}-Ace and T_{80}-Ace increased by 17.45%, 61.12%, 184.54% and 152.10%, respectively. At the end of the culture period, the relative amounts of NO_3^--N in the no-inhibitor culture and inhibitor-treated culture were 60% WHC > 80% WHC > 40% WHC > 20% WHC, (Figure 1c), with the two inhibitors lower than that of the no-inhibitor culture. Compared with 1-octyne, T_{20}-Ace, T_{40}-Ace, T_{60}-Ace and T_{80}-Ace decreased by 5.22%, 14.61%, 9.38% and 7.59% respectively.

In the dry–wet alternation mode, the content of NH_4^+-N and NO_3^--N increased after rewatering and decreased after drought (Figure 1b,d). After 21 d of incubation, the NH_4^+-N content in D14W7 was the highest, followed by D7W14 and then D7W7D7, whereas the NO_3^--N content was D14W7 > D7W14 > D7W7D7. The NH_4^+-N content in D7W14-Oct, D14W7-Oct, and D7W7D7-Oct increased by 12.14%, 10.68%, and 57.03%, whereas NH_4^+-N content in D7W14-Ace, D14W7-Ace, and D7W7D7-Ace increased by 59.64%, 23.55%, and 88.58% compared to the no-inhibitor culture, respectively. At the end of culture, the NO_3^--N content in D7W14-Oct, D14W7-Oct, and D7W7D7-Oct decreased to 26.14, 27.86, and 18.03 mg/kg respectively, and those of D7W14-Ace, D14W7-Ace, and D7W7D7-Ace decreased to 21.24, 20.99 and 16.17 mg/kg, respectively.

3.2. Relative Contribution of Ammonia-Oxidizing Microorganisms to Soil Nitrate Nitrogen

In the constant humidity mode, the contribution rate of AOA to PNR was less than 50%, with the largest proportion detected in T_{80}. The contribution rate of AOB was higher than that of AOA, which remained between 50.28% and 57.25%, whereas the contribution rate of AOB was the highest in T_{60} (Table 3). Compared with the constant humidity mode, the contribution rate of AOA to PNR increased under the dry–wet alternation mode, especially D7W14 (54.34%) and D14W7 (51.63%). The contribution rate of AOB decreased, with the highest proportion detected in D7W7D7 (53.8%).

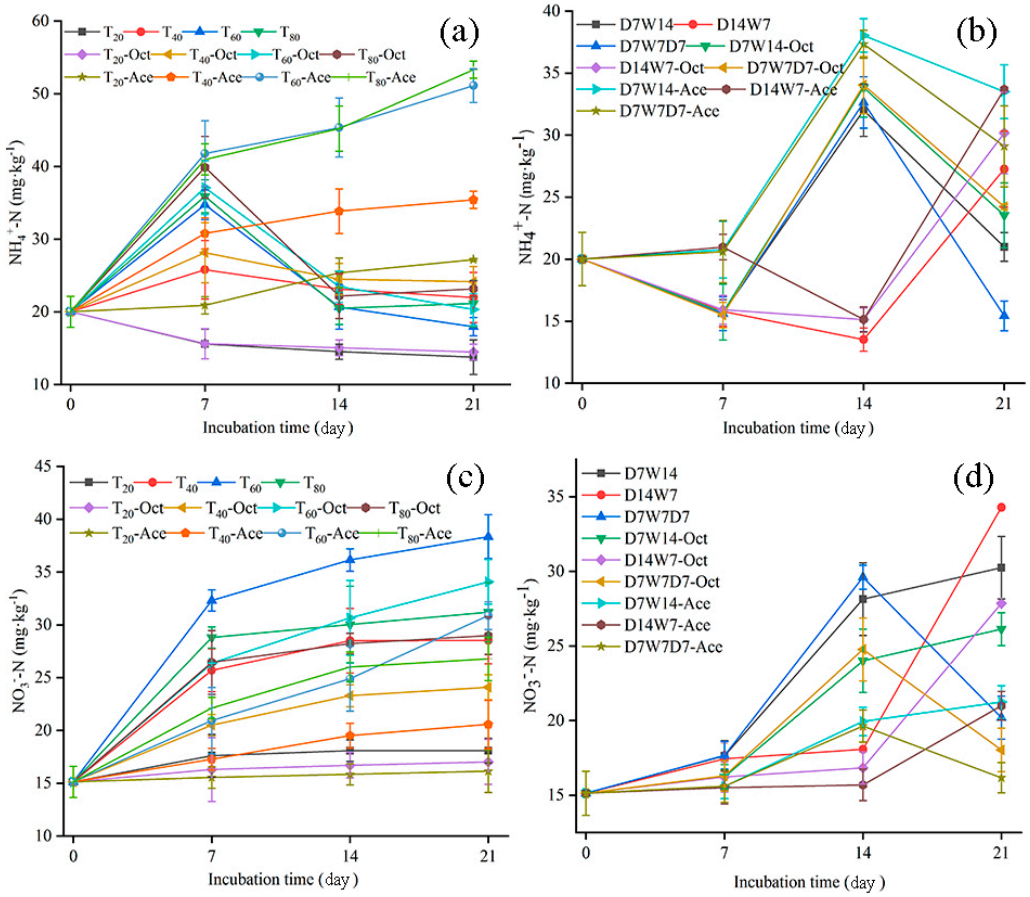

Figure 1. The content of soil NH_4^+–N, NO_3^-–N under different water supply modes. (**a**,**c**) were constant humidity mode; (**b**,**d**) were dry–wet alternation mode. Values were the mean of three replicates ± S.D. T_{20}: 20% WHC; T_{40}: 40% WHC; T_{60}: 60% WHC; T_{80}: 80% WHC; D7W14: 20% WHC for 1–7 d, 60% WHC for 15–21 d; D14W7: 20% WHC for 1–14 d, 60% WHC for 15–21 d; D7W7D7: 20% WHC for 1–7 d, 60% WHC for 8–14 d, 20% WHC for 15–21 d. Treatments with added acetylene and 1-octane were labeled as Oct and Ace respectively.

Table 3. The relative contribution rate of ammonia-oxidizing microorganisms to PNR. T_{20}: 20% WHC; T_{40}: 40% WHC; T_{60}: 60% WHC; T_{80}: 80% WHC; D7W14: 20% WHC for 1–7 d, 60% WHC for 15–21 d; D14W7: 20% WHC for 1–14 d, 60% WHC for 15–21 d; D7W7D7: 20% WHC for 1–7 d, 60% WHC for 8–14 d, 20% WHC for 15–21 d.

Water Supply Mode		Relative Contribution Rate(%)	
		AOA	AOB
Constant humidity mode	T_{20}	45.62	54.38
	T_{40}	44.22	55.78
	T_{60}	42.75	57.25
	T_{80}	49.72	50.28
Dry–wet alternation mode	D7W14	54.34	45.66
	D14W7	51.63	48.37
	D7W7D7	46.17	53.83

3.3. Effects of Different Water Supply Modes on Ammonia Oxidizing Microorganisms

3.3.1. Response of Gene Abundance of Ammonia Oxidizing Microorganisms to Different Water Supply Modes

The abundance of AOA-*amoA* steadily decreased in response to the constant humidity mode, whereas the level of AOB-*amoA* increased. The abundance of AOA-*amoA* and AOB-

amoA in T_{60} (1.85×10^7–2.85×10^7 copies/g; 6.04×10^6–7.89×10^6 copies/g) were significantly higher than those in T_{20} (1.06×10^7–1.95×10^7 copies/g; 4.40×10^6–5.86×10^6 copies/g) (Figure 2a,c). And the addition of 1-octyne did not impact the abundance of AOA-*amoA*. At the end of the culture period, the AOB-*amoA* abundance in T_{20}-Oct and T_{60}-Oct decreased by 43.10% and 30.48%, whereas that in T_{20}-Ace and T_{60}-Ace decreased by 44.47% and 36.55%, respectively. In the dry–wet alternating mode, the abundance of AOA-*amoA* in D7W14 increased by 12.99%, whereas that in D14W7 and D7W7D7 decreased by 13.08% and 10.52% (Figure 2b); the abundance of AOB-*amoA* increased, and D14W7 increased the most (63.44%) (Figure 2d) at the end of culture, compared to after 7 d of culture.

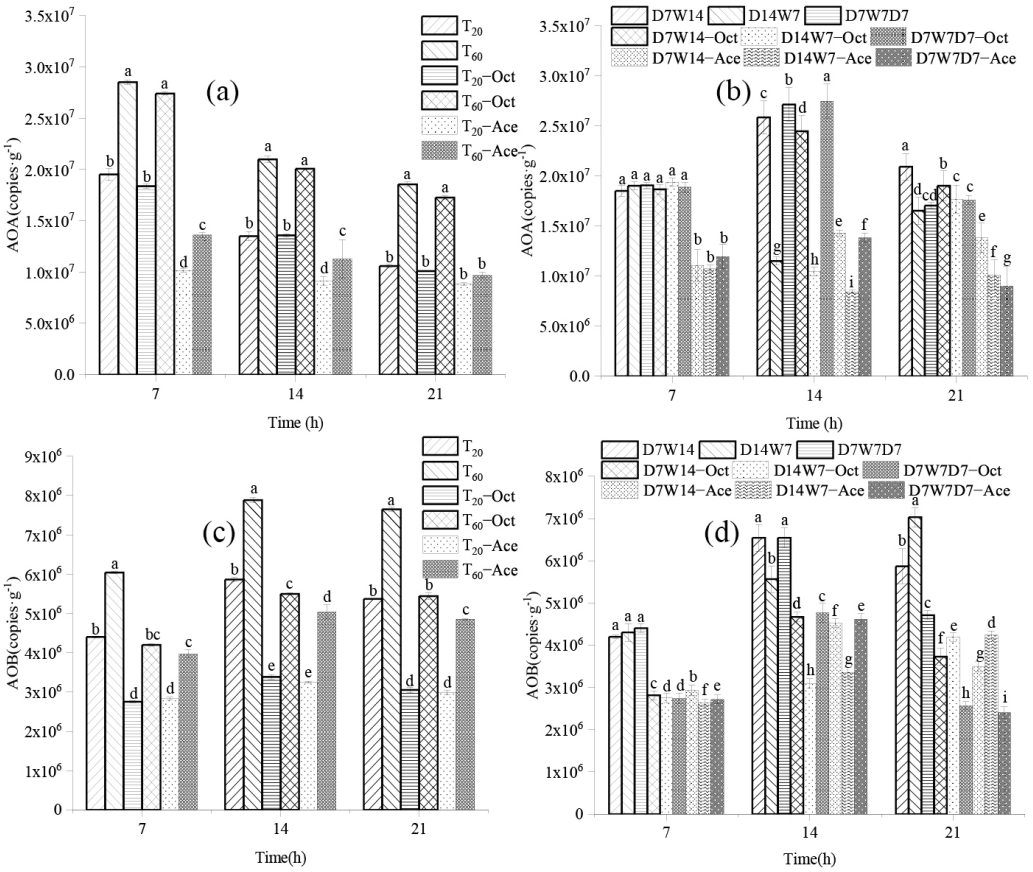

Figure 2. The abundance of ammonia-oxidizing microorganisms under different water supply modes. (**a**,**c**) were constant humidity mode; (**b**,**d**) were dry–wet alternation mode. Data represent mean ± SE (n = 3) and statistically differences ($p < 0.05$), indicated by letters. T_{20}: 20% WHC; T_{60}: 60% WHC; D7W14: 20% WHC for 1–7 d, 60% WHC for 15–21 d; D14W7: 20% WHC for 1–14 d, 60% WHC for 15–21 d; D7W7D7: 20% WHC for 1–7 d, 60% WHC for 8–14 d, 20% WHC for 15–21 d. Treatments with added acetylene and 1-octane were labeled as Oct and Ace respectively.

3.3.2. The Community Composition of Ammonia Oxidizing Microorganisms

The AOA community was mainly composed of Candidatus Nitrosocaldus, Candidatus Nitrosocosmicus, Nitrososphaera, Nitrosoarchaeum, Nitrosopumilus, Candidatus Nitrosotalea, Candidatus Nitrosotenuis. Candidatus Nitrosocaldus had the highest abundance (constant humidity mode, 73–82%; dry–wet alternation mode, 75–80%) (Figure 3), but its abundance was unaffected by water content in the constant humidity mode. The relative abundance of Nitrososphaera, Nitrosoarchaeum, Nitrosopumilus, Candidatus Nitrosotalea, and Candidatus Nitrosotenuis in T_{60} was significantly higher than those in T_{20} (Table S1). At the genus level, the AOB community was mainly composed of Nitrosospira and Nitrosovibrio, which belong to β-Proteobacteria and γ-Proteobacteria, respectively.

In the constant humidity mode, changes in water content had no significant effect on the relative abundance of AOB (Table S3).

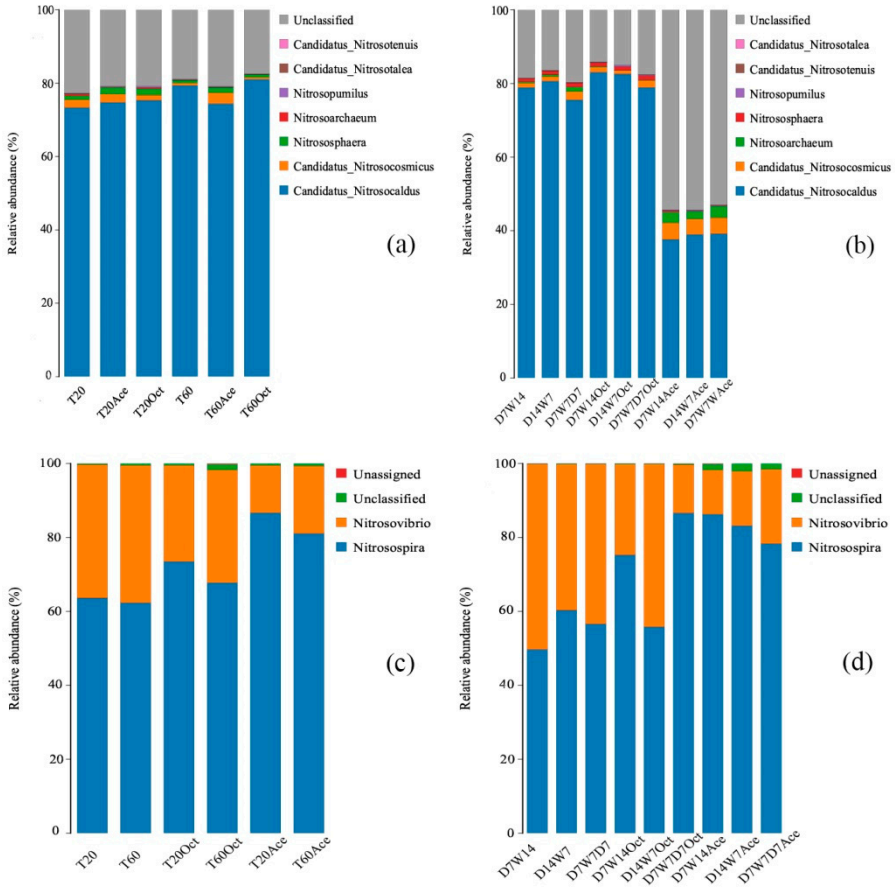

Figure 3. Structure composition of ammonia-oxidizing microorganisms with different water supply modes at the genus level. (**a**,**c**) were the structure of AOA, AOB in constant humidity mode; (**b**,**d**) was the structure composition of AOA, AOB in dry–wet alternate mode. T_{20}: 20% WHC; T_{60}: 60% WHC; D7W14: 20% WHC for 1–7 d, 60% WHC for 15–21 d; D14W7: 20% WHC for 1–14 d, 60% WHC for 15–21 d; D7W7D7: 20% WHC for 1–7 d, 60% WHC for 8–14 d, 20% WHC for 15–21 d. Treatments with added acetylene and 1-octane were labeled as Oct and Ace respectively.

In the dry–wet alternative mode, the relative abundance of *Candidatus Nitrosocaldus* was significantly lower in D7W7D7 than that in D7W14 and D14W7 after incubation for 21 days, whereas the relative abundance of *Candidatus Nitrosocosmicus*, *Nitrososphaera*, *Nitrosorchaeum*, *Nitrosopululus*, *Candidatus Nitrosotalea* was significantly higher than those in D7W14 and D14W7 ($p < 0.05$). Except for *Candidatus Nitrosocosmicus* and *Nitrosopumilus*, the relative abundance of other genera in D14W7 was significantly higher than those in D7W14 ($p < 0.05$) (Table S2). Analysis of the AOB community structure indicated that there were significant differences between different drought-rewatering treatments ($p < 0.05$). The relative abundance of *Nitrosospira* was highest in D14W7, followed by D7W7D7 and then D7W14, whereas the relative abundance of *Nitrosovibrio* was highest in D14W7, followed by D7W7D7 and then D7W14 (Table S4).

3.4. The Correlation Analysis

3.4.1. Spearman Correlation Analysis

There was a significant positive correlation between PNR and NH_4^+, NO_3^- in both the constant humidity mode and dry–wet alternation mode ($p < 0.01$) (Table 4). In the constant humidity mode, the AOA abundance was positively correlated with NH_4^+ ($p < 0.01$), PNR ($p < 0.05$), and NO_3^- ($p < 0.05$). The AOB abundance was positively correlated with NH_4^+

($p < 0.05$), PNR ($p < 0.01$), and NO_3^- ($p < 0.01$). In the dry–wet alternation mode, AOA and AOB were significantly positively correlated with NH_4^+, NO_3^-, and PNR ($p < 0.01$) (Table 4). To sum up, AOA and AOB might jointly lead the ammonia oxidation.

Table 4. Correlation analysis between ammonia-oxidizing microbial abundance and nitrogen under constant humidity mode and dry–wet alternation mode. *, $p < 0.05$; ** $p < 0.01$.

Water Supply Mode		NH_4^+	NO_3^-	PNR	AOA	AOB
Constant humidity mode	NH_4^+	1	0.571 *	0. 643 **	0.938 **	0.631 *
	NO_3^-		1	0.955 **	0.640 *	0.849 **
	PNR			1	0.615 *	0.724 **
	AOA				1	0.406
	AOB					1
Dry–wet alternation mode	NH_4^+	1	0.572 **	0.645 **	0.689 **	0.626 **
	NO_3^-		1	0.915 **	0.786 **	0.748 **
	PNR			1	0.658 **	0.649 **
	AOA				1	0.185
	AOB					1

3.4.2. Redundancy Analysis of Ammonia Oxidizing Microorganisms

Redundancy analysis (RDA) showed that NO_3^- and NH_4^+ had significant effects on soil microorganisms ($p < 0.05$), the contribution rate of NO_3^- and NH_4^+ was higher, reaching 44.90% and 43% respectively. It means that NO_3^- and NH_4^+ play a leading role in affecting soil microorganisms. Spearman correlation analysis found that NO_3^- was significantly positively correlated with *Candidatus Nitrosocaldus* and *Nitrosovibrio* ($p < 0.01$), and significantly negatively correlated with *Candidatus Nitrosocosmicus*, *Nitrosopumilus*, *Candidatus Nitrosotalea* and *Nitrosospira*. NH_4^+ was positively correlated with *Nitrosospira* ($p < 0.05$), and negatively correlated with *Nitrososphaera*, *Candidatus Nitrosotalea*, *Nitrosovibrio* (Figure 4).

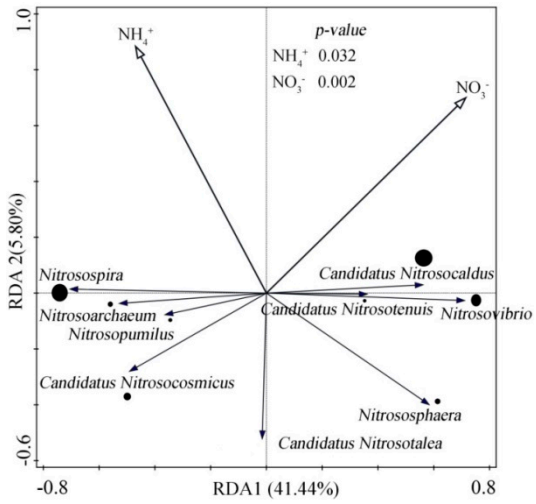

Figure 4. Redundancy analysis (RDA) of soil bacteria (phylum lever) with soil environmental factors, *viz*. NH_4^+ and NO_3^-.

4. Discussion

4.1. Effects of Different Moisture Modes on Nitrogen Conversion

The inorganic nitrogen absorbed by tea plants was mainly NH_4^+ and NO_3^-. Changes in water content could have an impact on nitrogen conversion in soil. In this paper, it was found that the NH_4^+ content of soil increased with the increase of water content in the early stage of constant humidity culture, but the NH_4^+ content of all treated soils showed a decreasing trend in the later stage of culture (Figure 1a). The soil NO_3^- content maintained

a trend of 60% WHC > 80% WHC > 40% WHC > 20% WHC throughout the culture period (Figure 1c). This might be because suitable water conditions were conducive to the release of soil nutrients due to the increased reproduction of microorganisms involved in mineralization and nitrification [12,13]. However, with the increase of soil water content, oxygen content, and soil permeability were reduced, resulting in the solidification of NH_4^+ stronger than nitrification [14], and the denitrification effect was enhanced, which in turn weakens the nitrogen mineralization amount and nitrogen mineralization rate [14]. This led to a gradual decrease in NH_4^+ content and an increase in ammonia volatilization [15,16].

Nitrification increased with higher soil moisture, increasing the conversion of NH_4^+ to NO_3^- [17,18], but very high moisture levels had the opposite effect [19]. Studies had shown that there was a significant positive correlation between soil moisture and soil nitrogen mineralization, with an optimum range of moisture [20]. When the soil moisture content was lower than field water holding capacity, the soil primary nitrogen mineralization rate and primary nitrogen fixation rate were mainly affected by substrate transport [21], and the diffusion of substrates to microorganisms was limited in low-water-content soils [22]. When the soil moisture content was in the range of 40–100% WHC, the effective carbon content of the soil increased, providing more energy and substrate for microorganisms [23,24]. Increases in the water level result in higher abundance and activity of microorganisms, up to a certain point, thereby increasing the primary nitrogen mineralization rate of soil [25]. In our experiments, 60% WHC was found to be optimal for Shandong tea plantation soil. Additionally, inhibition of nitrification by 1–octane and acetylene, the increase rate of NH_4^+ in 60% WHC soil was the largest. Changes in soil NO_3^- concentration were the result of the combined effects of nitrification, leaching loss, and denitrification [26,27]. These three factors affected the balance between NO_3^- production and consumption after 7 d of incubation, indicating that the NO_3^- content remained relatively stable across treatments (Figure 1c).

In the wet-dry alternating mode, the wet-dry conversion process destroyed soil aggregates, exposed more soil organic matter, provided a large number of nutrients for microorganisms [28], and increased the nitrogen conversion rate after soil rewatering [29]. Dijkstra et al. found that the total ammoniation rate and total nitrification rate increased rapidly 1–3 d after water input in dry grassland, and microbial activity was quickly activated after soil rewatering [30]. In this experiment, soil NH_4^+ and NO_3^- also showed "pulse" changes, whereas overall NH_4^+ decreased over time (Figure 1b). The alternation of dry and wet conditions also drove the conversion of NH_4^+ to NO_3^- in the soil, and the soil NO_3^- content increased rapidly after drought and rehydration (Figure 1d).

4.2. The Driving Effect of Ammonia-Oxidizing Microorganisms on Ammonia Oxidation under Different Moisture Modes

Ammonia-oxidizing microorganisms drove the process of soil ammonia oxidation, and both AOA and AOB were involved in this process. A previous work showed that autotrophic ammonia oxidation could be completely inhibited by the addition of low concentrations of acetylene [31]. In this study, the nitrification rate was significantly reduced after adding acetylene to moist soil, indicating that autotrophic ammonia oxidation was the dominant ammonia oxidation process under this condition. The soil PNR was higher after inhibition of AOA than after inhibition of AOB (Figure 2), and the contribution rate of AOB to PNR was higher than that of AOA (Table 3), indicating that AOB plays a key role in nitrification of weakly acidic and neutral soil in constant humidity mode.

The contribution of AOA and AOB to ammonia oxidation was affected by the external environment, with pH playing a major role. In our assays, the soil pH was weakly acidic to neutral (approximately 6.8), and AOA was more likely to survive in an acidic environment, making the abundance higher than AOB (Figure 2), which was similar to the results of most acidic soils [32,33]. As the neutral soil environment inhibits the ammonia oxidation of AOA [34,35], the contribution rate of AOA to PNR was slightly lower than that of AOB. Song et al. also found that ammonia-oxidizing bacteria were the primary drivers of soil

nitrification in neutral and slightly acidic soils (pH 6.33), with nitrification contribution rates of 59.44% and 61.99%, respectively [36]. Our data showed that AOA and AOB were significantly positively correlated with NO_3^-, but the significance of AOB was higher than that of AOA in the constant humidity mode. However, the correlation between AOB and NO_3^- was not much different from that of AOA in the dry–wet alternation mode (Table 4). Although the abundance of AOA and AOB changed before and after incubation, the level of AOB was more highly correlated with the nitrification rate.

In addition, the soil nitrogen content affected the growth of ammonia-oxidizing microorganisms. Redundancy analysis showed that NO_3^- and NH_4^+ had significant effects on soil ammonia-oxidizing microorganisms ($p < 0.05$), with contribution rates of 44.90% and 43%, respectively (Figure 3), indicating that NO_3^- and NH_4^+ were major factors impacting soil microorganisms. In ammonia-oxidizing microorganisms, AOA tend to thrive in low nitrogen environments [32,37], whereas AOB prefer higher nitrogen settings [38,39]. In the constant humidity mode, the soil nitrogen content increased over the course of the culture, leading to the formation of the high nitrogen conditions favored by AOB. In the dry–wet alternating environment, soil osmotic potential increased during the drought period. These changes caused microorganisms to adapt to increasing osmotic potential by accumulating or producing osmotic substances [40], which leads to the fixation of a large amount of N [41]. After rewatering, the soil osmotic potential suddenly decreased, and microorganisms released the accumulated osmotic substances to avoid rupture. Recovery from low activity or dormancy resulted in enhanced nitrogen mineralization and nitrification [42,43], meaning that drought-rewatering cycles cause high $NH4^+$ concentrations in soil. This adversely affected AOA, but had no significant effect on AOB abundance [44]. We also found that frequent dry–wet alternations caused AOB to contribute more to ammonia oxidation than AOA (Table 3). Overall, the relative contribution rate of AOA (constant humidity mode, 42.75–49.72%; dry–wet alternation mode, 46.17–54.34%) and AOB (constant humidity mode, 50.28–57.25%; dry–wet alternation mode, 45.66–53.83%) were not much different (Table 3), implying that ammonia oxidation may be dominated by AOA and AOB in weak acid and neutral brown soil.

5. Conclusions

Drought was found to reduce the soil NH_4^+-N concentration under the constant humidity mode, and the NO_3^--N concentration was highest in 60% WHC soil. The increasing the frequency of dry–wet watering resulted in a higher accumulation of NO_3^--N. The influence of dry–wet alternation mode on soil nitrogen transformation was greater than that of constant humidity mode. In the constant humidity mode, drought reduced the abundance of AOA, whereas that of AOB increased. Increases in drought duration and the frequency of dry–wet watering inhibited the activity of AOA under the dry–wet alternating mode, whereas the relative activity of AOB increased after rehydration. The water supply mode did not change the community structure of AOA or AOB at the genus level but affected their relative abundance. In weakly acidic and neutral soils, ammonia oxidation might be mainly driven by AOA and AOB. This study reveals the effects of different water supply modes on soil nitrogen transformation and ammonia-oxidizing micro-organisms and provides a scientific basis for improving nitrogen use efficiency. This study reveals the effects of different water supply modes on soil nitrogen transformation and ammonia-oxidizing micro-organisms and provides a scientific basis for improving nitrogen use efficiency.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy13051279/s1, Table S1: Relative abundance of ammonia-oxidizing archaea at the genus level in constant humidity mode; Table S2: Relative abundance of ammonia-oxidizing archaea at the genus level in dry–wet alternate mode; Table S3: Relative abundance of ammonia-oxidizing bacteria at the genus level in constant humidity mode; Table S4: Relative abundance of ammonia-oxidizing bacteria at the genus level in constant humidity mode.

Author Contributions: Conceptualization, X.H.; methodology, H.W.; formal analysis, J.H. and B.Z.; investigation, H.W. and J.H.; writing—original draft preparation, H.W.; writing—review and editing, X.H.; project administration, X.H.; funding acquisition, X.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Natural Science Foundation Committee of Shandong Province (the Natural Science Foundation of Shandong Province, Grant number: ZR2019BC062).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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