nitrogen

MDPI

Review

Effect of Different Synthetic Nitrogen Forms and Levels on Nitrification and Denitrification Key Genes Abundances: Implications for Oligotrophic Forest Soil Management

Muhammad Jamal Ameer, Yushan Liu, Xiaoting Zhao, Siyu Yan and Tongbao Qu *

College of Forestry and Grassland, Jilin Agricultural University, Changchun 130118, China; jameer6524@gmail.com (M.J.A.); 18943478100@163.com (Y.L.); 13072255117@163.com (X.Z.); 16643179269@163.com (S.Y.)
* Correspondence: qvtb@jlau.edu.cn

Abstract: Climate change and anthropogenic nitrogen addition alter the soil physicochemical properties and microbial activity in oligotrophic forest soil. Unbalanced and non-selective nitrogen fertilizer application is lost as gas emissions (N_2O, NO) and also contributed to eutrophication through NO_3^- leachate. Similarly, NO_3^- infiltrates and contaminated drinking water sources lead to human thyroid dysfunction. In order to protect depleting timber growth due to nitrogen deficiency and increasing ecological concerns from nitrogen misapplication, we reviewed the effects of different synthetic nitrogen forms and levels on the biogeochemical process. In this review, we focused on the most recent findings from research articles, review articles, and meta-analyses on forest soil and also followed the complementary insights from agricultural soil so that we may be able to highlight how these observations contribute to the understanding of the forest soil nitrogen cycle. Firstly, we elaborated the role of nitrification and denitrification in the nitrogen transformation process. Secondly, we discussed the effect of different nitrogen forms and levels on nitrification and denitrification functional gene abundances. Thirdly, we analyzed the possible effect of gene abundances on the nitrogen conversion process. Finally, we revealed that different forms and levels of synthetic nitrogen not only alter the nitrogen conversion pathways by increasing the gene abundances through substrate availability but also shift the gene dominance, thereby modifying soil physicochemical properties, such as pH. This collectively changes the conditions, which are critical for gene expression potential involved in the nitrogen conversion process. These findings may create a direction for sustainable and eco-friendly fertilizer application in nitrogen-deficient soil.

Keywords: synthetic nitrogen; gene abundances; ecological niche; nitrogen transformation; forest soil

Academic Editor: Concepción Avila

Received: 9 December 2024
Revised: 7 January 2025
Accepted: 8 January 2025
Published: 10 January 2025

Citation: Ameer, M.J.; Liu, Y.; Zhao, X.; Yan, S.; Qu, T. Effect of Different Synthetic Nitrogen Forms and Levels on Nitrification and Denitrification Key Genes Abundances: Implications for Oligotrophic Forest Soil Management. *Nitrogen* **2025**, *6*, 4. https://doi.org/10.3390/nitrogen6010004

1. Introduction

Nitrogen is an essential nutrient that is abundant as N_2 in the atmosphere [1]. Despite the high content of nitrogen in the atmosphere, it is limited in an available form [2]. The human input of synthetic nitrogen has increased over the past decades to enhance the yield of crops and timber production; however, this increase has been miscalculated and caused unintended consequences and contributed to climate change as air pollution through the emission of N_2O gas [3].

Globally, N_2O emissions due to N addition are 2.2–3.7 TgNyear-1 from forest soil [4,5]. Forest trees are commercial timber trees that need nitrogen for vegetative growth [6],

but balanced nitrogen utilization for forest trees has become a challenge due to climate change [7]. Nitrification and denitrification are the main processes that perform nitrogen transformation in the soil, and for the nitrogen transformation pathway, different functional genes are the responsible drivers of the nitrification and denitrification processes [8,9]. The nitrification process comprises two parts: the first part is ammonia oxidation driven by the AOA (amoA) and AOB (amoA) genes [10]; and the second part is nitrite oxidation, which is led by NOB as nitrobacter or nitrospira [11]. In acidic soil, nitrospira outcompetes nitrobacter and is the most affected by the changing soil pH [12].

Previously, different studies have focused on the inorganic nitrogen addition effect on nitrification functional gene abundances but at a small level [13,14]. For instance, Carey et al. (2016) demonstrated AOA (amoA) abundance increases due to a low level of NH_4^+ nitrogen application [15]. Similarly, Dong et al. described that low NH_4^+ increases AOA (amoA) abundances [16]. Xu et al. (2022) described that $CO(NH_2)_2$ at a high level of application increases AOB (amoA) abundances and decreases AOA (amoA) abundances [17]. AOA and AOB (amoA) separately have a limited capacity to reflect the impact of each form and level of nitrogen addition on ammonia oxidation due to ecological niche differentiation [15].

For example, a study by Rutting et al. (2021) described that a low to high level of NH_4^+ addition shifted the ammonia oxidation dominance from AOA (amoA) to AOB (amoA) [10], and in the case of an extreme condition, AOA (amoA) outcompeted AOB (amoA) in abundance [18]. Similarly, the denitrification functional genes, mainly nirS and nirK, are functionally the same but ecologically belong to different niches [19] that are responsible for the rate-limiting step [20] of denitrification [21,22]. Although the study described that organic nitrogen increases the denitrification functional gene abundances due to the carbon content, which may be utilized by the denitrifier as a substrate [23]. For instance, few studies have elaborated that only organic nitrogen or a mix with inorganic nitrogen increase the nirS, nirK, and nosZ abundances [14]. Similarly, You et al. (2022) described in a meta-analysis that nitrogen addition only increased the AOA and AOB (amoA) abundances in forest soil and the denitrification gene abundances were unaffected [13,24]. Some studies also described that the inorganic nitrogen NO_3^- form increases the abundances of the nirS and nirK genes [25], but it is also under debate whether NO_3^- effect is a substrate for the denitrifier or the denitrification process because the role of NO_3^- is yet unexplored. For instance, a study by Saleh-Lakha et al. (2009) described that as the NO_3^- concentration increases, the nirS and nosZ abundances increase [26], but Xu et al. (2021) described that the denitrification rate increases as the NO_3^- level increases and the denitrifier abundances are not related to the denitrification rate [25]. Under the contradictions of the genes' abundances and their activity rates, this indicates a possible role of nitrogen fertilizer as an abiotic factor besides the substrate. Therefore, it is necessary to further explore the inorganic nitrogen effect on the nitrification and denitrification gene abundances on a transcriptional basis and their reflecting effect on the nitrogen conversion process in forest soil.

We aim to study the following unsolved questions in the coming sections of this review: 1, whether nitrogen addition changes the key gene abundances of nitrification and denitrification, thereby effecting the nitrogen conversion process; 2, whether different forms of nitrogen addition have different effects; and 3, whether the effect is related to the level of nitrogen added.

2. Nitrification

Nitrification is the primary step in the nitrogen cycle, which is the pathway of N availability and loss. The nitrification process is divided into two types: 1. autotrophic nitrification; and 2. heterotrophic nitrification, in which autotrophic nitrification is mainly

carried out by ammonium oxidizing bacteria (AOB), ammonium oxidizing archea (AOA), and nitrite oxidizing bacteria (NOB) [27].

Autotrophic nitrification contributed to 54.6 to 96.9% of the nitrification [28]; however, the combined contribution of autotrophic and heterotrophic nitrification in forest soil is yet unexplored.

The main ammonium transfer pathway is autotrophic nitrification in forest soil [29], rather than heterotrophic nitrification due to the fast process. But some studies have elaborated that AOB (amoA), which is a main player of autotrophic nitrification and has affinity for NH_4^+ as a substrate, is not activated in forest soil nitrification [30] and is suppressed in acidic conditions [31]. Meanwhile, it is described in other studies that synthetic N fertilization addition increased the abundances of AOB (amoA) in forest soil more efficiently as compared with AOA (amoA) [28]. The genes shift its pathway by changing the form and level of nitrogen addition according to its distinct ecological niche; therefore, the exact role of genes in autotrophic nitrification is crucial to understand the nitrifier pathway under synthetic nitrogen application [32].

3. Denitrification

Denitrification occurs in anaerobic conditions and uses NO_3^- nitrogen as a substrate source causing NO_3^- reduction as NO_2, NO, N_2O, and N_2 are driven by the functional genes narG/napA, nirK/nirS, norB, and nosZ, respectively [31]. Hallin et al. (2009) described that the denitrification functional gene abundances are not likely correlated with denitrification activity due to the inhibitory effect of low- or high-level nitrogen addition [33]. While in contrast to this, Ouyang et al. (2018) described that N addition increases the denitrification functional gene abundances and activity consequently [14]. Organic sources are very crucial to explain the denitrifying abundances and denitrification activity.

Cellulose and lignin are two abundant organic resources on earth that describe the decomposition status of carbon compounds [29]. Some studies have described that synthetic N addition decreased the litter decomposition rate in temperate forest by reducing the ligninolytic enzyme activity [29,30]. For instance, litter decomposition is slowed down by NH_4^+ addition, while $CO(NH_2)_2$ does not have an effect on litter decomposition. Similarly, conifer leaf litter is more affected by N addition than broad leaf litter, which shows that decomposition is mainly affected according to the type of organic litter and applied nitrogen form [31]. Previous studies have discussed that high NH_4^+ nitrogen availability will favor AOB (amoA) dominance, while high carbon availability will favor AOA (amoA) ammonia oxidizer dominance in ammonia oxidation [34]. In this context, besides the AOA and AOB change by altering C/N%, applied synthetic N also has an effect on the denitrification process in two ways, either through the available form of NO_3^- as a denitrification substrate or by carbon content availability (if C/N% is low), which is a denitrifier substrate [35,36].

4. Different Synthetic Nitrogen Forms and Levels Effect on Functional Gene Abundances

Different forms and levels of synthetic nitrogen NH_4^+, $CO(NH_2)_2$, and NO_3^- affect gene abundances differently due to the gene substrate affinity.

4.1. Low- and High-Level (NH_4^+) Nitrogen Effect on Functional Gene Abundances

Ammonia oxidation in forest has become complex due to ammonia oxidizers ecological niche differentiation [9]. Che et al. (2015) and Lin et al. (2021) observed that AOB (amoA) has increased abundances in acidic soil [37,38], while Gubry-Ranjin et al. (2011) and Zhang et al. (2012) elaborated that AOA (amoA) has increased abundances in acidic soil [39,40]. The NH_3^+ level has long been considered as a key factor in the shift of AOA (amoA)

and AOB (amoA) abundances [41,42]. Previous studies have discussed that a high NH_4^+ nitrogen addition will increase the AOB (amoA) abundances, while low NH_4^+ will influence the AOA (amoA) abundances [15,43]. Similarly, Sterngren et al. (2015) also concluded that AOA (amoA) is active in ammonia oxidation in poor NH_4^+ nitrogen concentration conditions and the input of high NH_4^+nitrogen favors AOB (amoA) abundances [34]. Not only does the level of nitrogen differentiate the AOA (amoA) and AOB (amoA) abundances but the NH_4^+ form of nitrogen also affects them differently. For instance, a trial was conducted to analyze the effect of the N fertilizer form. AOA and AOB (amoA) abundances were analyzed, which resulted in more abundances of AOA (amoA) over AOB (amoA) in unfertilized soil (CK) treatment but after NH_4^+ fertilizer application, AOA (amoA) abundances decreased by AOB (amoA), and upon amino acid addition, AOA (amoA) again dominated [34].

Similarly, a microcosm experiment was conducted that analyzed the low- and high-level NH_4^+ effect on ammonia oxidizer abundances and the respective nitrogen conversion process using the 15 N-Tracer model method for quantifying gene abundances by Rutting et al. (2021) [10].

According to Figure 1, low- and high-level NH_4^+ addition shifted the nitrogen transformation pathway. Figure 1 reflects the gene affinity to form and level correlated with the nitrogen conversion process. In contrast to the study [10] results, another study was conducted in which the genes AOA (amoA) were insensitive to the fertilization effect over ecological niche conditions. A recent field trial elaborated that despite high NH_4^+ application, AOA (amoA) dominated over AOB (amoA), which was an analysis using the MPN method [44] as compared with the 15N-Trace model method used in experiment [10].

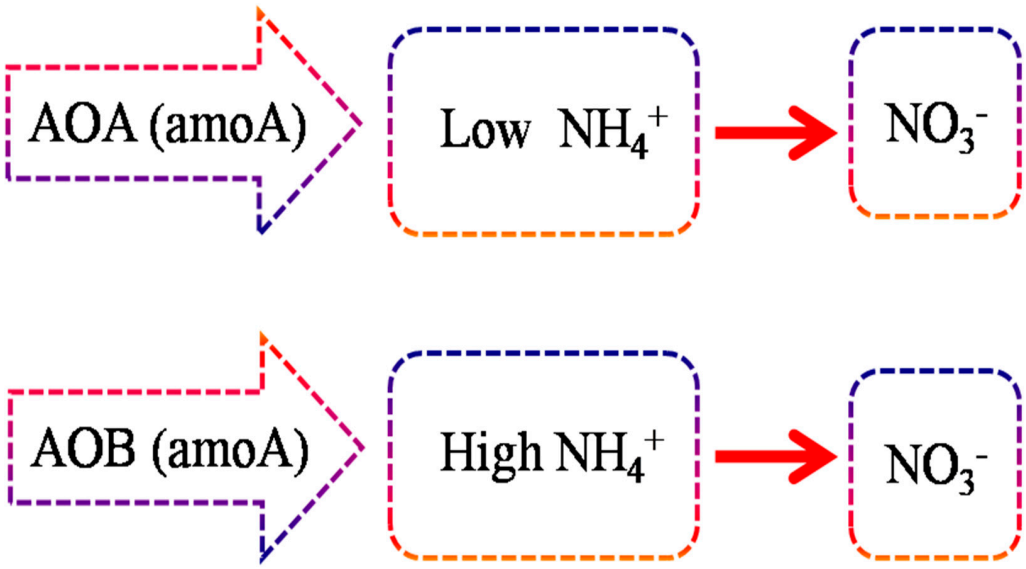

Figure 1. Above arrow indicates the contribution of ammonia oxidizers AOA (amoA) in gross nitrification with a low NH_4^+ level, while below arrow describes the AOB (amoA) dominated effect on gross nitrification at a high NH_4^+ level [10].

There may be two possibilities for AOA (amoA) dominance despite high NH_4^+ addition: one is a sample site containing organic cattle manure irrigated soil with wet conditions, which favors the AOA (amoA) niche [45], containing high NH_3^+ that is an AOA (amoA) substrate [42] over AOB (amoA) [46]; secondly, the gene abundances were measured using the MPN (Most Probable Number) method in this experiment, which is less efficient in identifying relative abundances [40,47]. Therefore, in experiment [44], it was indicated that AOA (amoA) abundances correlated with nitrification potential may be due to the overall

gene AOA (amoA) expression in this ecological niche and, in these conditions, high NH_4^+ was acting as a non-limiting factor.

For further elaboration, we analyzed the results of gene abundances using a standard analytical tool (qPCR) by the low and high NH_4^+ addition effect in Table 1.

Table 1. NH_4^+ fertilizer effect on ammonia oxidizers and denitrifier abundances.

Fertilizer Level	Functional Genes	Sources
Low NH_4^+	AOB ↑, AOA ↓	[37]
High NH_4^+	AOB ↑	[27]
Low NH_4^+	AOA ↑	[48,49]
High NH_4^+	nirS ↓, nirK ↓, nosZ ↓, AOB ↑	[50,51]
Low NH_4^+	AOA ↑	[10]
High NH_4^+	AOB ↑	[52]
Low NH_4^+	AOB ↑, AOA ↓	[43]
High NH_4^+	AOB ↑, AOA ↓	[53,54]
Low NH_4^+	AOB ↑	[55]
High NH_4^+	AOB ↑	[56,57]
Low NH_4^+	AOA ↑	[58,59]
High NH_4^+	AOB ↑, AOA ↓	[10,49]
Low NH_4^+	AOB ↑	[60]
High NH_4^+	AOA ↓, AOB ↑, nirS ↓, nirK ↓ and nosZ ↓	[54,61]
Low NH_4^+	AOA ↑	[62]
High NH_4^+	↓ nirS ↓ nirK ↓ nosZ	[33]
Low NH_4^+	AOA ↑	[49,63]
High NH_4^+	AOB ↑, AOA ↓	[64,65]

↑: Increase; ↓: Decrease; AOA: Ammonia oxidizing archea; AOB: Ammonia oxidizing bacteria.

According to Table 1, gene abundances/copy numbers increase correlating with the form and level of NH_4^+ application as a substrate for AOA and AOB (amoA) and an abiotic agent for nirS, nirK, and nosZ, changing the soil pH.

4.2. Low- and High-Level $CO(NH_2)_2$ Nitrogen Effect on Functional Gene Abundances

Many past studies have concerned the role of $CO(NH_2)_2$ application in N_2O emission. $CO(NH_2)_2$ hydrolysis is a set stage in nitrifier denitrification [66]. Some studies have described that $CO(NH_2)_2$ causes more N_2O emission as compared with NH_4^+ [67,68] and it is also described that N_2O emission from AOA (amoA) is less than AOB (amoA)-derived ammonia oxidation because AOA (amoA) lacks homologous NO reductase [69]. A study was conducted in 2022, in which AOB (amoA) abundances increased due to CO $(NH_2)_2$ addition in acidic soil [52] and AOA (amoA) abundances increased in both acidic and alkaline soil [70]. Abdo et al. (2022) described that $CO(NH_2)_2$ addition increased AOA (amoA) and AOB (amoA) abundances by low and high levels, respectively [71]. Similarly, Tan et al. (2013) described that high $CO(NH_2)_2$ increases the AOB (amoA) abundances, while low $CO(NH_2)_2$ increased the AOA (amoA) abundances [72]. The effect of low and high levels of $CO(NH_2)_2$ addition on gene abundances (quantified using qPCR) from previous studies is discussed in Table 2.

Table 2. $CO(NH_2)_2$ fertilizer effect on ammonia oxidizers and denitrifier abundance.

Low $CO(NH_2)_2$	**AOB ↑, AOA ↓**	[67,73]
Moderate $CO(NH_2)_2$	↑ AOB, ↑ nirS, ↑ nirK	[74]
High $CO(NH_2)_2$	AOB ↑, AOA ↓	[73,75]
Low $CO(NH_2)_2$	AOB ↑,AOA ↓	[76]
High $CO(NH_2)_2$	AOB ↑, nirS ↑, nirK ↑	[74]
Low $CO(NH_2)_2$	AOA (amoA) ↓, narG ↓, nosZ ↓	[77]
High $CO(NH_2)_2$	AOA (amoA) ↓, narG ↓, nosZ ↓, AOB (amoA) ↑	[77,78]
Low $CO(NH_2)_2$	AOA (amoA) ↑, AOB (amoA) ↓	[79]
High $CO(NH_2)_2$	nirS ↓, nirK ↑, nosZ ↑, AOB ↑	[80]
Low $CO(NH_2)_2$	AOA (amoA) ↑, AOB ↑ (amoA)	[81]
High $CO(NH_2)_2$	nirK ↑	[82]

Abbreviations and symbols as Table 1.

$CO(NH_2)_2$ low and high levels impacted and shifted the ammonia oxidizers pathway as a substrate factor. Although a previous meta-analysis study stated that only nitrogen application affects ammonia oxidizer abundances and left the denitrifier unaffected [13], but it may be a reflection of the nitrogen form effect only as a substrate factor for the denitrifier. Rather than as a denitrifier substrate, low and high $CO(NH_2)_2$ has a significant distinct effect on the nirK and nirS pathways by changing the soil condition and affinity of the NO_3^- substrate [25].

A study was conducted in Hebei province, China in 2017. This study indicated that a high level of $CO(NH_2)_2$ fertilizer application increases the nirK gene abundances while the nirS gene decreases [80], and the nirK gene is also correlated with the denitrification rate (Figure 2).

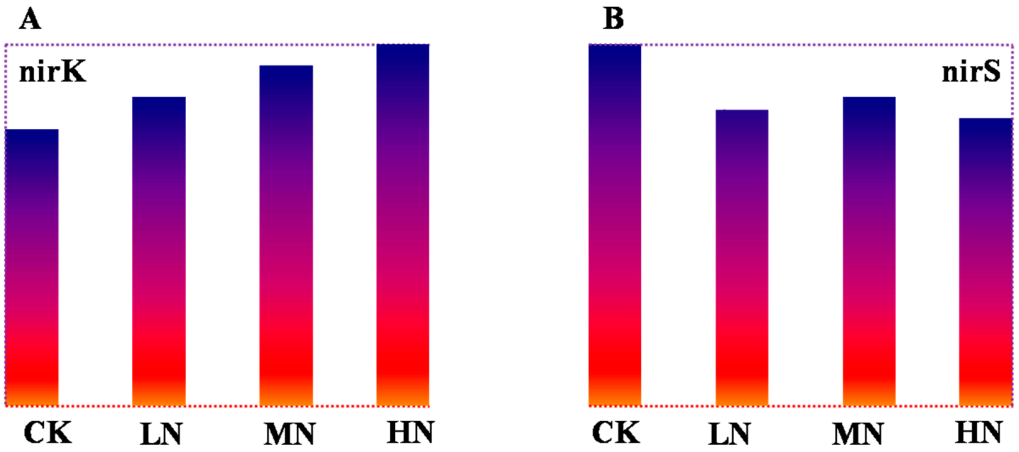

Figure 2. Abundance of (**A**) cu-type nitrite reductase gene and (**B**) cd1-type nitrite reductase gene represented in letters under different levels of $CO(NH_2)_2$ addition. CK (control), LN (low N level), MN (medium N level), and HN (high N level) reflect the N levels of 0, 75, 150, and 225 N ha^{-1} yr^{-1}, respectively [80].

Do fertilizer-induced changes in soil physicochemical properties reflect denitrification process alteration thereby affecting gene activity? According to this experiment, nirS and nirK expression changes due to low and high levels of $CO(NH_2)_2$ addition. We may observe that in all the treatments, the nirS gene abundances/copy number increase in alkaline soil (8.5 pH) but the $CO(NH_2)_2$ form and levels shift the soil composition

in favor of nirK gene expression. The results of this experiment indicated that despite the gene abundances, changes in the fertilizer application levels may shift the nitrogen conversion potential by altering the soil physicochemical properties and gene substrate level. Therefore, the gradient factor in nirS and nirK changes collectively increases the total NO_3^- concentration [83,84], which is directly related to the denitrifier abundances and the denitrification and soil pH is not limiting factor due to denitrification potential relevance with nirK activity [85]. It indicated that gene activity and substrate concentration may more important factor for denitrification and nirS and nirK distinction than soil pH.

Following this study result, we compared the distinction of nirS and nirK in transcribed abundances for further elaboration.

A long-term experiment conducted in 2022 in north-east China [77] described the low and high $CO(NH_2)_2$ fertilizer effect on the genes' transcribed abundances (Table 3), this experiment was 29 years as compared with a previous short-term experiment [80].

Table 3. $CO(NH_2)_2$ fertilizer effect on denitrifier abundances and soil physicochemical properties [77].

Treatments	Nitrite Reductase (Genes)	Soil pH	SOC (g kg^{-1})	C/N	NH_4^+ (mg kg^{-1})	NO_3^- (mg kg^{-1})	TN (g kg^{-1})
CK	nirS ↑, nirK ↓	5.97	7.39	9.82	0.64	6.19	0.75
LCF	nirK ↑, nirS ↓	5.31	7.25	9.39	1.23	21.29	0.77
HCF	nirK ↓, nirS ↓	5.16	7.04	9.11	1.65	22.12	0.77
CMF	nirK ↓, nirS ↓	5.86	8.40	9.28	1.30	15.71	0.89

CK: control; LCF: low N chemical fertilizer; HCF: high N chemical fertilizer; CMF: chemical+ manure fertilizer.

Despite the high carbon content and soil pH, nirK and nirS are insensitive to expression as compared with CK but the denitrification potential continues to increase and reached highest at (CMF) treatment which directed to unidentify genes pathways may be contributing to denitrification besides nirS and nirK. Low and high NO_3^- remained constant where nirS gene expressed under low and nirK in high NO_3^- level. Previously, different studies reflected (nirS) affinity to lower NO_3^- level and complete anaerobic condition as compared to nirK [86,87]. NH_4^+-based $CO(NH_2)_2$ fertilizer performs as a substrate of ammonia oxidation and impacts the nirS and nirK abundances by increasing the NO_3^- concentration. But it is necessary to further explore the nitrite reducers' gene expression behavior under the effect of non-organic synthetic nitrogen fertilizer application to know whether low and high NO_3^- differently affect the nirS and nirK abundances and by doing so, whether this shifts the nitrite reduction pathway [88].

4.3. Low- and High-Level (NO_3^-) Nitrogen Effect on Functional Gene Abundances

In previous studies, it has been described that the denitrification functional genes are affected by NO_3^- application, in which the nirK gene increases in both fluctuating aerobic and anaerobic conditions, while nirS is increased only under anaerobic conditions [89,90]. nirS and nirK denitrification functional genes belong to different ecological niches [91]; therefore, the NO_3^- effect on the nirS and nirK gene abundances depends on the source of nitrogen addition and the content of carbon available [92]. While in some studies, it is elaborated that water-filled pour spaces (WFPS) and soil organic carbon are the main reason for denitrifying gene abundances, rather than only inorganic NO_3^- addition [35]. A study described that the nirS gene may be active in anaerobic conditions without NO_3^- for a short time [26], as some studies have also described that there is not necessarily a correlation between the denitrification functional gene abundances and inorganic NO_3^- addition [93]. But in the denitrification process, NO_3^- is a required entity for the denitrifier to complete denitrification as a better substitute of atmospheric O_2 [94,95]. NO_3^- is highly

mobile and reactive, which causes concerning effects; therefore, for its application, in order to avoid nitrogen cycle waste, we need a balanced approach [96].

To understand the low and high NO_3^- effect on nirS and nirK is crucial because the nirK gene is less prevalent in organisms that can completely reduce nitrite to dinitrogen [97,98] as compared with nirS [89]. Barta et al. (2010) experimented that NO_3^- increased the nirK gene abundances as compared with nirS [99]. A in situ experiment conducted in terrestrial forest soil (2017) explained that high NO_3^- decreases the nirS gene abundances [100]. Another study was also consistent regarding the nirK increase and nirS decrease upon the application of KNO_3 [101]. Similarly, in drought conditions, NO_3^- application will affect the denitrifier, which decreases the nirS abundances and leaves nirK and nosZ unaffected [35]. A study conducted with low and high levels of NO_3^- addition and NH_4^+ addition to analyze the effect on nirS and nirK abundances and the denitrification process is discussed in Table 4 [25].

Table 4. nirS and nirK abundance responses to NH_4^+ and NO_3^- fertilizer.

Nitrate (Low)	10–30 kg N $ha^{-1}yr^{-1}$	**nirS ↑, nirK ↓**
Nitrate (High)	≥30 kg N $ha^{-1}yr^{-1}$	**nirK ↑**
Ammonium (Low)	≤30 kg N $ha^{-1}yr^{-1}$	**nirS ↑, nirK ↓**
Ammonium (High)	≥30–50 kg N $ha^{-1}yr^{-11}$	**nirS ↑, nirK ↓**

(≥): high or equal; (≤): low or equal.

In this experiment, NO_3^- nitrogen affected the gene abundances by increasing at a specific rate until the threshold level (30 kg N $ha^{-1}yr^{-1}$) and above this level of concentration (>30 kg N $ha^{-1}yr^{-1}$), it decreases the gene abundances but denitrification continues to increases. According to this experiment, the NO_3^- level is more linked to denitrifier abundances than the denitrification process. Similarly, a study was conducted to find the low- and high-level effect of NO_3^- on the denitrifying gene abundances and denitrification activity in culture media. In this experiment, the nirS, norB, and nosZ abundances were investigated upon the application of different NO_3^-concentrations (0, 10, 100, and 1000 mg of KNO_3/L) for a short-term analysis with a duration of 0, 2, 4, 6, 8, and 24 h. Genes quantified using qRT-PCR in this experiment were compared with previous studies [25] and Table 5, which used qPCR for the gene quantification (DNA copies).

Table 5. NO_3^- fertilizer effect on (NIR) gene abundances.

Low Level NO_3^-	**nirS ↑**	**[36]**
High Level NO_3^-	nirK ↑	**[99]**
Low Level NO_3^-	nirS ↑	**[102,103]**
High Level NO_3^-	nirK ↑, nirS ↓	**[101]**
Low Level NO_3^-	nirS ↓	**[35]**
High Level NO_3^-	nirK ↑, nirS ↓	**[80,104]**

This experiment concluded that the denitrifier gene abundances and denitrification activity are not equally correlated because denitrification continues to occur at a high level of NO_3^-addition (1000 mg of KNO_3/L) [26] indicated the consistency with study [25] and at 2 h with 0 mg of KNO_3 /liter, nirS and norB will remain expressed under complete anaerobic conditions, which shows NO_3^- level insensitivity toward nirS nitrite reductase gene abundances. For further elaboration, we compared the denitrifier gene abundances using a qPCR analysis.

A study by Wittorf et al. (2018) elaborated that the main distinct factor between nirS and nirK is O_2 availability, as in oxic conditions nirK was correlated to NO_3^- high level while in anoxic condition nirS gene was expressed regardless of NO_3^- level [89].

Consistently, another study by Sarrenheimo et al. (2015) described that in high NO_3^- levels, nirK abundances increases, while at a low level, nirS dominated in complete anaerobic condition in the lake [105]. Xie et al. (2014) stated that the nirK gene correlated with NO_3^- concentration and nirS deviated [84]. A study by Cantarel et al. (2021) disclosed that low and high NO_3^- addition increases nirK gene abundances as compared with nirS [106]. According to Pold et el. (2024), nirS-dominated pathways lead to complete denitrification [107], while nirK gene pathways related to incomplete denitrification [97]. Although, by interpreting different studies regarding the effect of different forms and levels on nirS and nirK abundances, we cannot neglect the influence of each factor but we can understand the niche of the genes toward the level of NO_3^- application and the correlated effect on the denitrification process.

5. Conclusions and Future Directions

In this review, we studied the different synthetic nitrogen forms and levels impacted as substrate factor, thereby affecting the nitrogen transformation pathway and similarly as abiotic factors according to applied level. As discussed, many studies focused on the N addition effect on gene abundances (population size) of nitrification and denitrification, while the genes' ecological response and niches study remained unexplored. We found that short-term experiment results are quite opposite to long-term experiments, which indicates significant climatic effects on the nitrogen conversion process. This is why gene niches are more important to define gene activity in dynamic soil rather than the size of the population, which indeed may contribute to the pre-expression potential reference point in cropping soil or stable soil. Therefore, it is an urgent call that, in future, the focus should shift from the broader N effect to a microbial gene substrate level, which may offer better insight on gene nitrogen transformation pathways. Additionally, it is necessary to consider long-term in situ trials and microbial activities should be examined through a holistic approach, i.e., a metagenomic analysis in order to understand the unidentified pathways of nitrogen transformation in oligotrophic forest soil.

Author Contributions: Conceptualization, M.J.A., T.Q. and Y.L.; validation, M.J.A. and T.Q.; formal analysis, T.Q.; data curation, M.J.A. and S.Y.; writing—review and editing, M.J.A. and X.Z.; supervision, T.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Jilin Provincial Science and Technology Department project (20220101180JC).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data availability statement: This review does not include any new data. All data discussed are cited.

Conflicts of Interest: Not applicable.

References

1. Rennenberg, H.; Dannenmann, M. Nitrogen Nutrition of Trees in Temperate Forests—The Significance of Nitrogen Availability in the Pedosphere and Atmosphere. *Forests* **2015**, *6*, 2820–2835. [CrossRef]
2. Aczel, M.R. What is the nitrogen cycle and why is it key to life? *Front. Young Minds* **2019**, *7*, 41. [CrossRef]
3. Geisseler, D.; Scow, K.M. Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biol. Biochem.* **2014**, *75*, 54–63. [CrossRef]
4. Tian, H.; Xu, R.; Canadell, J.G.; Thompson, R.L.; Winiwarter, W.; Suntharalingam, P.; Davidson, E.A.; Ciais, P.; Jackson, R.B.; Janssens-Maenhout, G. A comprehensive quantification of global nitrous oxide sources and sinks. *Nature* **2020**, *586*, 248–256. [CrossRef] [PubMed]
5. Melillo, J.; Steudler, P.; Feigl, B.; Neill, C.; Garcia, D.; Piccolo, M.; Cerri, C.; Tian, H. Nitrous oxide emissions from forests and pastures of various ages in the Brazilian Amazon. *J. Geophys. Res. Atmos.* **2001**, *106*, 34179–34188. [CrossRef]

6. Qu, L.; Wang, Y.; Masyagina, O.; Kitaoka, S.; Fujita, S.; Kita, K.; Prokushkin, A.; Koike, T. *Larch: A Promising Deciduous Conifer as an Eco-Environmental Resource*; Conifers—Recent advances; Intech Open: London, UK, 2022; pp. 1–37.
7. McLellan, E.L.; Cassman, K.G.; Eagle, A.J.; Woodbury, P.B.; Sela, S.; Tonitto, C.; Marjerison, R.D.; Van Es, H.M. The nitrogen balancing act: Tracking the environmental performance of food production. *Bioscience* **2018**, *68*, 194–203. [CrossRef]
8. Zhang, Y.; Cheng, X.; van Groenigen, K.J.; García-Palacios, P.; Cao, J.; Zheng, X.; Luo, Y.; Hungate, B.A.; Terrer, C.; Butterbach-Bahl, K. Shifts in soil ammonia-oxidizing community maintain the nitrogen stimulation of nitrification across climatic conditions. *Glob. Change Biol.* **2024**, *30*, e16989. [CrossRef] [PubMed]
9. Canfield, D.E.; Glazer, A.N.; Falkowski, P.G. The evolution and future of Earth's nitrogen cycle. *Science* **2010**, *330*, 192–196. [CrossRef] [PubMed]
10. Rütting, T.; Schleusner, P.; Hink, L.; Prosser, J.I. The contribution of ammonia-oxidizing archaea and bacteria to gross nitrification under different substrate availability. *Soil Biol. Biochem.* **2021**, *160*, 108353. [CrossRef]
11. Ramanathan, B.; Boddicker, A.M.; Roane, T.M.; Mosier, A.C. Nitrifier gene abundance and diversity in sediments impacted by acid mine drainage. *Front. Microbiol.* **2017**, *8*, 2136. [CrossRef] [PubMed]
12. Han, S.; Luo, X.; Liao, H.; Nie, H.; Chen, W.; Huang, Q. Nitrospira are more sensitive than Nitrobacter to land management in acid, fertilized soils of a rapeseed-rice rotation field trial. *Sci. Total Environ.* **2017**, *599*, 135–144. [CrossRef] [PubMed]
13. You, L.; Ros, G.H.; Chen, Y.; Yang, X.; Cui, Z.; Liu, X.; Jiang, R.; Zhang, F.; de Vries, W. Global meta-analysis of terrestrial nitrous oxide emissions and associated functional genes under nitrogen addition. *Soil Biol. Biochem.* **2022**, *165*, 108523. [CrossRef]
14. Ouyang, Y.; Evans, S.E.; Friesen, M.L.; Tiemann, L.K. Effect of nitrogen fertilization on the abundance of nitrogen cycling genes in agricultural soils: A meta-analysis of field studies. *Soil Biol. Biochem.* **2018**, *127*, 71–78. [CrossRef]
15. Carey, C.J.; Dove, N.C.; Beman, J.M.; Hart, S.C.; Aronson, E.L. Meta-analysis reveals ammonia-oxidizing bacteria respond more strongly to nitrogen addition than ammonia-oxidizing archaea. *Soil Biol. Biochem.* **2016**, *99*, 158–166. [CrossRef]
16. Dong, L.; Berg, B.; Gu, W.; Wang, Z.; Sun, T. Effects of different forms of nitrogen addition on microbial extracellular enzyme activity in temperate grassland soil. *Ecol. Process.* **2022**, *11*, 36. [CrossRef]
17. Xu, A.; Li, L.; Xie, J.; Gopalakrishnan, S.; Zhang, R.; Luo, Z.; Cai, L.; Liu, C.; Wang, L.; Anwar, S. Changes in ammonia-oxidizing archaea and bacterial communities and soil nitrogen dynamics in response to long-term nitrogen fertilization. *Int. J. Environ. Res. Public Health* **2022**, *19*, 2732. [CrossRef] [PubMed]
18. Zhou, L.; Wang, S.; Zou, Y.; Xia, C.; Zhu, G. Species, abundance and function of ammonia-oxidizing archaea in inland waters across China. *Sci. Rep.* **2015**, *5*, 15969. [CrossRef] [PubMed]
19. Chen, Z.; Ma, J.; Liu, Y.; Zhao, J.; Ma, J.; Yu, Q.; Zou, P.; Lin, H.; Wang, Q. Differential responses of soil nirS-and nirK-type denitrifying microbial communities to long-term application of biogas slurry in a paddy soil. *Appl. Soil Ecol.* **2023**, *182*, 104711. [CrossRef]
20. Zhao, G.; He, H.; Yue, M.; Wang, H.; Shao, H.; Wang, M. Differential responding patterns of the nirK-type and nirS-type denitrifying bacterial communities to an Ulva prolifera green tide in coastal Qingdao areas. *Front. Mar. Sci.* **2022**, *9*, 1063585. [CrossRef]
21. Wang, X.; Li, Y.; Ciampitti, I.A.; He, P.; Xu, X.; Qiu, S.; Zhao, S. Response of soil denitrification potential and community composition of denitrifying bacterial to different rates of straw return in north-central China. *Appl. Soil Ecol.* **2022**, *170*, 104312. [CrossRef]
22. Wei, W.; Isobe, K.; Nishizawa, T.; Zhu, L.; Shiratori, Y.; Ohte, N.; Koba, K.; Otsuka, S.; Senoo, K. Higher diversity and abundance of denitrifying microorganisms in environments than considered previously. *ISME J.* **2015**, *9*, 1954–1965. [CrossRef] [PubMed]
23. Wan, Z.; Wang, L.; Huang, G.; Rasul, F.; Awan, M.I.; Cui, H.; Liu, K.; Yu, X.; Tang, H.; Wang, S.; et al. nirS and nosZII bacterial denitrifiers as well as fungal denitrifiers are coupled with N_2O emissions in long-term fertilized soils. *Sci. Total Environ.* **2023**, *897*, 165426. [CrossRef] [PubMed]
24. Karimi, T.; Stöckle, C.O.; Higgins, S.S.; Nelson, R.L. Impact of climate change on greenhouse gas emissions and water balance in a dryland-cropping region with variable precipitation. *J. Environ. Manag.* **2021**, *287*, 112301. [CrossRef]
25. Xu, M.; Li, T.; Liu, W.; Ding, J.; Gao, L.; Han, X.; Zhang, X. Sensitivity of soil nitrifying and denitrifying microorganisms to nitrogen deposition on the Qinghai–Tibetan plateau. *Ann. Microbiol.* **2021**, *71*, 6. [CrossRef]
26. Saleh-Lakha, S.; Shannon, K.E.; Henderson, S.L.; Zebarth, B.J.; Burton, D.L.; Goyer, C.; Trevors, J.T. Effect of nitrate and acetylene on nirS, cnorB, and nosZ expression and denitrification activity in Pseudomonas mandelii. *Appl. Environ. Microbiol.* **2009**, *75*, 5082–5087. [CrossRef] [PubMed]
27. De Boer, W.; Kowalchuk, G.A. Nitrification in acid soils: Micro-organisms and mechanisms. *Soil Biol. Biochem.* **2001**, *33*, 853–866. [CrossRef]
28. Wertz, S.; Leigh, A.K.; Grayston, S.J. Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiol. Ecol.* **2012**, *79*, 142–154. [CrossRef]
29. Carreiro, M.; Sinsabaugh, R.; Repert, D.; Parkhurst, D. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* **2000**, *81*, 2359–2365. [CrossRef]

30. Zhong, Z.; Makeschin, F. Differences of soil microbial biomass and nitrogen transformation under two forest types in central Germany. *Plant Soil* **2006**, *283*, 287–297. [CrossRef]
31. Wu, P.-P.; Zhang, Z.; Li, R.; Ji, J.-H.; Mao, R. Impact of nitrogen addition on single and mixed tree leaf litter decomposition depends on N forms in subtropical China. *Appl. Soil Ecol.* **2023**, *190*, 104970. [CrossRef]
32. Zhang, X.; Liu, W.; Schloter, M.; Zhang, G.; Chen, Q.; Huang, J.; Li, L.; Elser, J.J.; Han, X. Response of the abundance of key soil microbial nitrogen-cycling genes to multi-factorial global changes. *PLoS ONE* **2013**, *8*, e76500. [CrossRef]
33. Hallin, S.; Jones, C.M.; Schloter, M.; Philippot, L. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *ISME J.* **2009**, *3*, 597–605. [CrossRef]
34. Sterngren, A.E.; Hallin, S.; Bengtson, P. Archaeal ammonia oxidizers dominate in numbers, but bacteria drive gross nitrification in N-amended grassland soil. *Front. Microbiol.* **2015**, *6*, 168618. [CrossRef] [PubMed]
35. Hammerl, V.; Kastl, E.-M.; Schloter, M.; Kublik, S.; Schmidt, H.; Welzl, G.; Jentsch, A.; Beierkuhnlein, C.; Gschwendtner, S. Influence of rewetting on microbial communities involved in nitrification and denitrification in a grassland soil after a prolonged drought period. *Sci. Rep.* **2019**, *9*, 2280. [CrossRef]
36. Cai, M.; Hong, Y.; Wu, J.; Moore, S.S.; Vamerali, T.; Ye, F.; Wang, Y. Nitrate Addition Increases the Activity of Microbial Nitrogen Removal in Freshwater Sediment. *Microorganisms* **2022**, *10*, 1429. [CrossRef] [PubMed]
37. Che, J.; Zhao, X.Q.; Zhou, X.; Jia, Z.J.; Shen, R.F. High pH-enhanced soil nitrification was associated with ammonia-oxidizing bacteria rather than archaea in acidic soils. *Appl. Soil Ecol.* **2015**, *85*, 21–29. [CrossRef]
38. Lin, Y.; Hu, H.-W.; Ye, G.; Fan, J.; Ding, W.; He, Z.-Y.; Zheng, Y.; He, J.-Z. Ammonia-oxidizing bacteria play an important role in nitrification of acidic soils: A meta-analysis. *Geoderma* **2021**, *404*, 115395. [CrossRef]
39. Zhang, L.-M.; Hu, H.-W.; Shen, J.-P.; He, J.-Z. Ammonia-oxidizing archaea have more important role than ammonia-oxidizing bacteria in ammonia oxidation of strongly acidic soils. *ISME J.* **2012**, *6*, 1032–1045. [CrossRef]
40. Gubry-Rangin, C.; Hai, B.; Quince, C.; Engel, M.; Thomson, B.C.; James, P.; Schloter, M.; Griffiths, R.I.; Prosser, J.I.; Nicol, G.W. Niche specialization of terrestrial archaeal ammonia oxidizers. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 21206–21211. [CrossRef]
41. Prosser, J.I.; Nicol, G.W. Archaeal and bacterial ammonia-oxidisers in soil: The quest for niche specialisation and differentiation. *Trends Microbiol.* **2012**, *20*, 523–531. [CrossRef]
42. Qin, W.; Wei, S.P.; Zheng, Y.; Choi, E.; Li, X.; Johnston, J.; Wan, X.; Abrahamson, B.; Flinkstrom, Z.; Wang, B. Ammonia-oxidizing bacteria and archaea exhibit differential nitrogen source preferences. *Nat. Microbiol.* **2024**, *9*, 524–536. [CrossRef] [PubMed]
43. Ouyang, Y.; Norton, J.M.; Stark, J.M.; Reeve, J.R.; Habteselassie, M.Y. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. *Soil Biol. Biochem.* **2016**, *96*, 4–15. [CrossRef]
44. Saukoly, S.A.; Meitiniarti, V.I.; Nugroho, R.A.; Krave, A.S. Ammonium enrichment reduces the diversity and changes the composition of ammonia-oxidizing microbial communities in agricultural soil media. *Biodiversitas J. Biol. Divers.* **2024**, *25*, 3. [CrossRef]
45. Le Châtelier, H.L. Chemical equilibrium. *Ann. Mines* **1888**, *13*, 157.
46. Fudjoe, S.K.; Li, L.; Jiang, Y.; Karikari, B.; Xie, J.; Wang, L.; Anwar, S.; Wang, J. Soil amendments alter ammonia-oxidizing archaea and bacteria communities in rain-fed maize field in semi-arid Loess Plateau. *Land* **2021**, *10*, 1039. [CrossRef]
47. Cochran, W.G. Estimation of bacterial densities by means of the "most probable number". *Biometrics* **1950**, *6*, 105–116. [CrossRef] [PubMed]
48. Assémien, F.L.; Pommier, T.; Gonnety, J.T.; Gervaix, J.; Le Roux, X. Adaptation of soil nitrifiers to very low nitrogen level jeopardizes the efficiency of chemical fertilization in west african moist savannas. *Sci. Rep.* **2017**, *7*, 10275. [CrossRef] [PubMed]
49. Zhaoming, C.; Qiang, W.; Yanli, L.; Jinping, Z.; Jiang, F.; Tao, L.; Qiaogang, Y.; Junwei, M. Effects of nitrogen levels on ammonia oxidizers and nitrification in fluvo-aquic soil. *Acta Agric. Zhejiangensis* **2022**, *34*, 2004.
50. Shang, S.; Song, M.; Wang, C.; Dou, X.; Wang, J.; Liu, F.; Zhu, C.; Wang, S. Decrease of nitrogen cycle gene abundance and promotion of soil microbial-N saturation restrain increases in N_2O emissions in a temperate forest with long-term nitrogen addition. *Chemosphere* **2023**, *338*, 139378. [CrossRef] [PubMed]
51. Chen, D.; Li, Y.; Wang, C.; Liu, X.; Wang, Y.; Shen, J.; Qin, J.; Wu, J. Dynamics and underlying mechanisms of N_2O and NO emissions in response to a transient land-use conversion of Masson pine forest to tea field. *Sci. Total Environ.* **2019**, *693*, 133549. [CrossRef]
52. Hu, L.; Dong, Z.; Wang, Z.; Xiao, L.; Zhu, B. The contributions of ammonia oxidizing bacteria and archaea to nitrification-dependent N_2O emission in alkaline and neutral purple soils. *Sci. Rep.* **2022**, *12*, 19928. [CrossRef] [PubMed]
53. Di, H.J.; Cameron, K.C.; Shen, J.-P.; Winefield, C.S.; O'Callaghan, M.; Bowatte, S.; He, J.-Z. Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nat. Geosci.* **2009**, *2*, 621–624. [CrossRef]
54. Xingchen, D.; Zhang, J.; Huizhen, Q.; Zhang, H.; Chaoyue, L.; Delei, D.; Qirong, S.; Zhongjun, J. Chronic nitrogen fertilization modulates competitive interactions among microbial ammonia oxidizers in a loess soil. *Pedosphere* **2019**, *29*, 24–33.

55. Okano, Y.; Hristova, K.R.; Leutenegger, C.M.; Jackson, L.E.; Denison, R.F.; Gebreyesus, B.; Lebauer, D.; Scow, K.M. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Appl. Environ. Microbiol.* **2004**, *70*, 1008–1016. [CrossRef] [PubMed]
56. Tian, X.-F.; Hu, H.-W.; Ding, Q.; Song, M.-H.; Xu, X.-L.; Zheng, Y.; Guo, L.-D. Influence of nitrogen fertilization on soil ammonia oxidizer and denitrifier abundance, microbial biomass, and enzyme activities in an alpine meadow. *Biol. Fertil. Soils* **2014**, *50*, 703–713. [CrossRef]
57. Luchibia, A.O.; Lam, S.K.; Suter, H.; Chen, Q.; O'Mara, B.; He, J.-Z. Effects of repeated applications of urea with DMPP on ammonia oxidizers, denitrifiers, and non-targeted microbial communities of an agricultural soil in Queensland, Australia. *Appl. Soil Ecol.* **2020**, *147*, 103392. [CrossRef]
58. Sauder, L.A.; Peterse, F.; Schouten, S.; Neufeld, J.D. Low-ammonia niche of ammonia-oxidizing archaea in rotating biological contactors of a municipal wastewater treatment plant. *Environ. Microbiol.* **2012**, *14*, 2589–2600. [CrossRef]
59. Zheng, L.; Zhao, X.; Zhu, G.; Yang, W.; Xia, C.; Xu, T. Occurrence and abundance of ammonia-oxidizing archaea and bacteria from the surface to below the water table, in deep soil, and their contributions to nitrification. *MicrobiologyOpen* **2017**, *6*, e00488. [CrossRef] [PubMed]
60. Giguere, A.T.; Taylor, A.E.; Myrold, D.D.; Bottomley, P.J. Nitrification responses of soil ammonia-oxidizing archaea and bacteria to ammonium concentrations. *Soil Sci. Soc. Am. J.* **2015**, *79*, 1366–1374. [CrossRef]
61. Hu, Y.; Jiang, H.; Chen, Y.; Wang, Z.; Yan, Y.; Sun, P.; Lu, X. Nitrogen addition altered the microbial functional potentials of carbon and nitrogen transformation in alpine steppe soils on the Tibetan Plateau. *Glob. Ecol. Conserv.* **2021**, *32*, e01937. [CrossRef]
62. Verhamme, D.T.; Prosser, J.I.; Nicol, G.W. Ammonia concentration determines differential growth of ammonia-oxidising archaea and bacteria in soil microcosms. *ISME J.* **2011**, *5*, 1067–1071. [CrossRef]
63. Guo, Y.; Geng, J.; Cheng, S.; Fang, H.; Li, X.; Yang, Y.; Li, Y.; Zhou, Y. Soil acidification and ammonia-oxidizing archaeal abundance dominate the contrasting responses of soil N_2O emissions to NH_4^+ and NO_3^- enrichment in a subtropical plantation. *Eur. J. Soil Biol.* **2023**, *116*, 103491. [CrossRef]
64. Hink, L.; Gubry-Rangin, C.; Nicol, G.W.; Prosser, J.I. The consequences of niche and physiological differentiation of archaeal and bacterial ammonia oxidisers for nitrous oxide emissions. *ISME J.* **2018**, *12*, 1084–1093. [CrossRef]
65. Hink, L.; Nicol, G.W.; Prosser, J.I. Archaea produce lower yields of N_2O than bacteria during aerobic ammonia oxidation in soil. *Environ. Microbiol.* **2017**, *19*, 4829–4837. [CrossRef] [PubMed]
66. Oliveira, B.G.; Lourenço, K.S.; Carvalho, J.L.N.; Gonzaga, L.C.; Teixeira, M.C.; Tamara, A.F.; Cantarella, H. Soil pH does not interfere with nitrification inhibitor efficiency for reducing N_2O emissions from soils treated with concentrated vinasse and urea. *Geoderma* **2022**, *426*, 116087. [CrossRef]
67. Fu, Q.; Xi, R.; Zhu, J.; Hu, H.; Xing, Z.; Zuo, J. The relative contribution of ammonia oxidizing bacteria and archaea to N_2O emission from two paddy soils with different fertilizer N sources: A microcosm study. *Geoderma* **2020**, *375*, 114486. [CrossRef]
68. van der Weerden, T.J.; Luo, J.; Di, H.J.; Podolyan, A.; Phillips, R.L.; Saggar, S.; de Klein, C.A.M.; Cox, N.; Ettema, P.; Rys, G. Nitrous oxide emissions from urea fertiliser and effluent with and without inhibitors applied to pasture. *Agric. Ecosyst. Environ.* **2016**, *219*, 58–70. [CrossRef]
69. Prosser, J.I.; Hink, L.; Gubry-Rangin, C.; Nicol, G.W. Nitrous oxide production by ammonia oxidizers: Physiological diversity, niche differentiation and potential mitigation strategies. *Glob. Change Biol.* **2020**, *26*, 103–118. [CrossRef] [PubMed]
70. Wang, Q.; Zhao, Z.; Yuan, M.; Zhang, Z.; Chen, S.; Ruan, Y.; Huang, Q. Impacts of urea and 3, 4-dimethylpyrazole phosphate on nitrification, targeted ammonia oxidizers, non-targeted nitrite oxidizers, and bacteria in two contrasting soils. *Front. Microbiol.* **2022**, *13*, 952967. [CrossRef]
71. Abdo, A.I.; Xu, Y.; Shi, D.; Li, J.; Li, H.; El-Sappah, A.H.; Elrys, A.S.; Alharbi, S.A.; Zhou, C.; Wang, L. Nitrogen transformation genes and ammonia emission from soil under biochar and urease inhibitor application. *Soil Tillage Res.* **2022**, *223*, 105491. [CrossRef]
72. Tan, H.; Xu, M.; Li, X.; Zhang, H.; Zhang, C. Effects of chlorimuron-ethyl application with or without urea fertilization on soil ammonia-oxidizing bacteria and archaea. *J. Hazard. Mater.* **2013**, *260*, 368–374. [CrossRef] [PubMed]
73. Xu, A.; Li, L.; Xie, J.; Zhang, R.; Luo, Z.; Cai, L.; Liu, C.; Wang, L.; Anwar, S.; Jiang, Y. Bacterial Diversity and Potential Functions in Response to Long-Term Nitrogen Fertilizer on the Semiarid Loess Plateau. *Microorganisms* **2022**, *10*, 1579. [CrossRef]
74. Hu, J.; Richwine, J.D.; Keyser, P.D.; Yao, F.; Jagadamma, S.; DeBruyn, J.M. Urea fertilization and grass species alter microbial nitrogen cycling capacity and activity in a C4 native grassland. *PeerJ* **2022**, *10*, e13874. [CrossRef] [PubMed]
75. Dai, Y.; Di, H.J.; Cameron, K.C.; He, J.-Z. Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N_2O emissions in a grazed pasture soil. *Sci. Total Environ.* **2013**, *465*, 125–135. [CrossRef]
76. Fan, F.; Yang, Q.; Li, Z.; Wei, D.; Cui, X.a.; Liang, Y. Impacts of organic and inorganic fertilizers on nitrification in a cold climate soil are linked to the bacterial ammonia oxidizer community. *Microb. Ecol.* **2011**, *62*, 982–990. [CrossRef] [PubMed]

77. Wang, F.; Liang, X.; Ding, F.; Ren, L.; Liang, M.; An, T.; Li, S.; Wang, J.; Liu, L. The active functional microbes contribute differently to soil nitrification and denitrification potential under long-term fertilizer regimes in North-East China. *Front. Microbiol.* **2022**, *13*, 1021080. [CrossRef] [PubMed]
78. Yang, X.; Ni, K.; Shi, Y.; Yi, X.; Ji, L.; Ma, L.; Ruan, J. Heavy nitrogen application increases soil nitrification through ammonia-oxidizing bacteria rather than archaea in acidic tea (*Camellia sinensis* L.) plantation soil. *Sci. Total Environ.* **2020**, *717*, 137248. [CrossRef]
79. Shen, X.-Y.; Zhang, L.-M.; Shen, J.-P.; Li, L.-H.; Yuan, C.-L.; He, J.-Z. Nitrogen loading levels affect abundance and composition of soil ammonia oxidizing prokaryotes in semiarid temperate grassland. *J. Soils Sediments* **2011**, *11*, 1243–1252. [CrossRef]
80. Yang, Y.; Zhao, J.; Jiang, Y.; Hu, Y.; Zhang, M.; Zeng, Z. Response of bacteria harboring nirS and nirK genes to different N fertilization rates in an alkaline northern Chinese soil. *Eur. J. Soil Biol.* **2017**, *82*, 1–9. [CrossRef]
81. Guo, D.; Bayu, B.; Pan, K.; Shen, S.; Zhang, J.; Jiang, X.; Yu, Z.; Li, J.; Luo, H. Response of nitrification and nitrifying microorganisms to different nitrogen sources in the acid Ultisols of Jinyun Mountain. *Soil Sci. Plant Nutr.* **2021**, *67*, 576–584. [CrossRef]
82. Sun, R.; Wang, F.; Hu, C.; Liu, B. Metagenomics reveals taxon-specific responses of the nitrogen-cycling microbial community to long-term nitrogen fertilization. *Soil Biol. Biochem.* **2021**, *156*, 108214. [CrossRef]
83. Niu, Y.; Hu, W.; Zhou, T.; He, B.; Chen, X.; Li, Y. Diversity of nirS and nirK denitrifying bacteria in rhizosphere and non-rhizosphere soils of halophytes in Ebinur Lake Wetland. *Biotechnol. Biotechnol. Equip.* **2022**, *36*, 209–219. [CrossRef]
84. Xie, Z.; Le Roux, X.; Wang, C.; Gu, Z.; An, M.; Nan, H.; Chen, B.; Li, F.; Liu, Y.; Du, G.; et al. Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows. *Soil Biol. Biochem.* **2014**, *77*, 89–99. [CrossRef]
85. Sun, H.; Jiang, S. A review on nirS-type and nirK-type denitrifiers via a scientometric approach coupled with case studies. *Environ. Sci. Process. Impacts* **2022**, *24*, 221–232. [CrossRef]
86. Yoshida, M.; Ishii, S.; Otsuka, S.; Senoo, K. Temporal shifts in diversity and quantity of nirS and nirK in a rice paddy field soil. *Soil Biol. Biochem.* **2009**, *41*, 2044–2051. [CrossRef]
87. Avrahami, S.; Conrad, R.; Braker, G. Effect of Soil Ammonium Concentration on N_2O Release and on the Community Structure of Ammonia Oxidizers and Denitrifiers. *Appl. Environ. Microbiol.* **2002**, *68*, 5685–5692. [CrossRef]
88. Aslam, M.; Huffaker, R.C. Role of nitrate and nitrite in the induction of nitrite reductase in leaves of barley seedlings. *Plant Physiol.* **1989**, *91*, 1152–1156. [CrossRef] [PubMed]
89. Wittorf, L.; Jones, C.M.; Bonilla-Rosso, G.; Hallin, S. Expression of nirK and nirS genes in two strains of *Pseudomonas stutzeri* harbouring both types of NO-forming nitrite reductases. *Res. Microbiol.* **2018**, *169*, 343–347. [CrossRef] [PubMed]
90. Deng, M.; Dai, Z.; Senbati, Y.; Li, L.; Song, K.; He, X. Aerobic denitrification microbial community and function in zero-discharge recirculating aquaculture system using a single biofloc-based suspended growth reactor: Influence of the carbon-to-nitrogen ratio. *Front. Microbiol.* **2020**, *11*, 1760. [CrossRef]
91. Jones, C.M.; Hallin, S. Ecological and evolutionary factors underlying global and local assembly of denitrifier communities. *ISME J.* **2010**, *4*, 633–641. [CrossRef] [PubMed]
92. Lu, M.; Cheng, S.; Fang, H.; Yang, Y.; Guo, Y.; Li, Y.; Zhou, Y. Contrasting response of soil N_2O release to ammonium, nitrate, and urea addition rates is determined by substrate availability and microbial community abundance and composition. *Eur. J. Soil Biol.* **2022**, *109*, 103393. [CrossRef]
93. Mergel, A.; Schmitz, O.; Mallmann, T.; Bothe, H. Relative abundance of denitrifying and dinitrogen-fixing bacteria in layers of a forest soil. *FEMS Microbiol. Ecol.* **2001**, *36*, 33–42. [CrossRef]
94. Levy-Booth, D.J.; Prescott, C.E.; Grayston, S.J. Microbial functional genes involved in nitrogen fixation, nitrification and denitrification in forest ecosystems. *Soil Biol. Biochem.* **2014**, *75*, 11–25. [CrossRef]
95. Xu, M.; Zhang, Q.; Xia, C.; Zhong, Y.; Sun, G.; Guo, J.; Yuan, T.; Zhou, J.; He, Z. Elevated nitrate enriches microbial functional genes for potential bioremediation of complexly contaminated sediments. *ISME J.* **2014**, *8*, 1932–1944. [CrossRef] [PubMed]
96. Zweig, D. The Role of Nitrate in Controlling Denitrification in Denitrification Bed Substrates. Ph.D. Thesis, University of Waikato Hamilton, Hamilton, New Zealand, 2013.
97. Graf, D.R.; Jones, C.M.; Hallin, S. Intergenomic comparisons highlight modularity of the denitrification pathway and underpin the importance of community structure for N_2O emissions. *PLoS ONE* **2014**, *9*, e114118. [CrossRef] [PubMed]
98. Nadeau, S.A.; Roco, C.A.; Debenport, S.J.; Anderson, T.R.; Hofmeister, K.L.; Walter, M.T.; Shapleigh, J.P. Metagenomic analysis reveals distinct patterns of denitrification gene abundance across soil moisture, nitrate gradients. *Environ. Microbiol.* **2019**, *21*, 1255–1266. [CrossRef]
99. Bárta, J.; Tahovská, K.; Kaňa, J. The effect of nitrate addition on abundance of nirK, nirS and gln genes in acidified Norway spruce forest soil. In Proceedings of the EGU General Assembly Conference Abstracts, Vienna, Austria, 1–6 May 2010; p. 10333.
100. Lennon, E.F.; Houlton, B.Z. Coupled molecular and isotopic evidence for denitrifier controls over terrestrial nitrogen availability. *ISME J.* **2017**, *11*, 727–740. [CrossRef]

101. Castellano-Hinojosa, A.; González-López, J.; Bedmar, E.J. Distinct effect of nitrogen fertilisation and soil depth on nitrous oxide emissions and nitrifiers and denitrifiers abundance. *Biol. Fertil. Soils* **2018**, *54*, 829–840. [CrossRef]
102. Castellano-Hinojosa, A.; Correa-Galeote, D.; González-López, J.; Bedmar, E.J. Effect of nitrogen fertilisers on nitrous oxide emission, nitrifier and denitrifier abundance and bacterial diversity in closed ecological systems. *Appl. Soil Ecol.* **2020**, *145*, 103380. [CrossRef]
103. Etchebehere, C.; Tiedje, J. Presence of Two Different Active *nirS* Nitrite Reductase Genes in a Denitrifying *Thauera* sp. from a High-Nitrate-Removal-Rate Reactor. *Appl. Environ. Microbiol.* **2005**, *71*, 5642–5645. [CrossRef]
104. Veraart, A.J.; Dimitrov, M.R.; Schrier-Uijl, A.P.; Smidt, H.; de Klein, J.J. Abundance, activity and community structure of denitrifiers in drainage ditches in relation to sediment characteristics, vegetation and land-use. *Ecosystems* **2017**, *20*, 928–943. [CrossRef]
105. Saarenheimo, J.; Rissanen, A.J.; Arvola, L.; Nykänen, H.; Lehmann, M.F.; Tiirola, M. Genetic and environmental controls on nitrous oxide accumulation in lakes. *PLoS ONE* **2015**, *10*, e0121201. [CrossRef]
106. Cantarel, A.A.; Rouifed, S.; Simon, L.; Bourg, J.; Gervaix, J.; Blazère, L.; Poussineau, S.; Creuzé des Châtelliers, C.; Piola, F. In nitrate-rich soil, fallopia x bohemica modifies functioning of N cycle compared to native monocultures. *Diversity* **2020**, *12*, 156. [CrossRef]
107. Pold, G.; Bonilla-Rosso, G.; Saghaï, A.; Strous, M.; Jones, C.M.; Hallin, S. Phylogenetics and environmental distribution of nitric oxide-forming nitrite reductases reveal their distinct functional and ecological roles. *ISME Commun.* **2024**, *4*, 1. [CrossRef] [PubMed]