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Nitrogen Addition Effects on Wetland Soils Depend on Environmental Factors and Nitrogen Addition Methods: A Meta-Analysis

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Abstract: Identifying the effects of nitrogen (N) addition under key environmental factors and N addition methods can aid in understanding the paradigm of N addition in wetland ecosystems. In this study, we conducted a meta-analysis of 30 field studies of wetland ecosystems and selected 14 indicators. We found that the changes in soil TN and SOC contributed significantly to the changes in microbial community structure under N additions. The environmental factors and N addition methods altered the direction or size of N addition effects on wetland soil properties, microbial diversity and key C and N cycling genes. N-limited conditions and climate conditions determined the N addition effect direction on SOC, and saline-alkali conditions determined the N addition effect direction on microbial diversity and AOB abundance. Environmental heterogeneity and N addition methods determine the response of wetland soil to nitrogen application. Therefore, it is crucial to study the effects of environmental factors and N addition methods on the N deposition of wetland soils.

Keywords: nitrogen addition; meta-analysis; wetlands; soil microbial diversity; functional genes

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1. Introduction

Atmospheric nitrogen deposition under the interference of human activities has become an important part of the global N biogeochemical cycle and an important driving factor of global change. Predictable increase of N deposition is an inevitable development trend [1,2]. At the same time, the trend of N deposition cannot be ignored since it can affect soil ecosystems significantly in temporal and spatial scales [3,4]. N deposition promotes plant growth, leads to soil acidification [5,6], changes in microbial community structure [7,8], and enzyme activity [9,10]. So, it is necessary to study the effects of N deposition on soils.

N addition can affect soil biochemical properties, such as soil pH, TN content, microbial diversity [11], and the abundance of microbial functional genes [12], etc. It could reduce microbial biomass and influence microbial community structure, which can form a more active copiotrophic microbial community. Moreover, N addition could result in soil acidification and decrease the decomposition of SOC. It is generally believed that N addition decreases forest and grassland soil pH [13], which is caused by H^+ input, NH_4^+ nitrification, NO_3^- leaching loss and plant absorption [14]. In most terrestrial ecosystems, N addition reduced microbial biomass and microbial diversity [11]. The decrease of soil

microbial diversity caused by the competition of other restricted resources between plants and microorganisms and soil acidification [14]. Soil salinization will affect soil properties and reduce soil TN [14]. N addition can increase N content and soil microbial biomass in saline soil. Most ecosystems are affected by N limitation, and N addition can greatly alleviate the N limitation, increase soil nutrients, promote plant growth, and increase microbial diversity [11]. Studies on the effects of N addition on key functional genes of soil C and N cycling are not systematic at present, mainly focusing on CH_4 and N_2O generation [15]. Carey [15] reported the impact of N fertilization on the abundance of ammonia oxidizing archaea (AOA) and bacteria (AOB), and found that AOB are more responsive than AOA to N fertilization.

A wetland is an ecosystem with many unique functions. Wetlands play an important role in maintaining ecological balance and biodiversity species resources, reducing pollution and regulating climate. However, previous studies mainly focus on the forests [16], grassland [17] and farmland ecosystem. The study of N addition affect wetland ecosystem is relative lack. In addition, wetland soils have more spatial and temporal heterogeneities of N-limited conditions [18], water level, and salinity [19]. Thus, the way in which environmental factors and N addition methods affected wetland ecosystems and the degree of this effect needs to be clarified [20]. We need to determine and quantify these key environmental factors and N addition methods as well as the N addition effect.

Based on this concept, we integrated 54 N addition treatments from 30 articles to study the effects of N addition on microbial diversity and key functional genes of C and N cycling [21] in wetland ecosystems. We aimed to address the following questions: (1) What are the relationships among soil chemical properties, microbial diversity, and abundance of key functional genes of C and N cycling in wetlands under N addition conditions? (2) Do the effects of N addition on soil chemical properties, microbial diversity and abundance of key functional genes of C and N cycling depend on the environmental factors and N addition methods? Which factors affect wetlands soil?

2. Materials and Methods

2.1. Data Collection

Peer-reviewed articles reporting the effects of N addition on soil microbes and functional genes in wetland ecosystems were collected globally by searching the Web of Science (http://apps.webofknowledge.com, accessed on 27 February 2021), Scopus (https://www.scopus.com, accessed on 27 February 2021), Wiley (https://onlinelibrary.wiley.com, accessed on 27 February 2021) and China National Knowledge Infrastructure (CNKI) databases before January 2021. The keywords and terms used for the literature online-searching were (N addition OR N application OR N enrichment OR N fertilizer OR N amendment OR N elevated) AND (microbial biomass OR microbial communities OR functional genes) AND (wetland OR marsh OR swamp OR everglade OR moist soil OR quagmire OR humidly). Articles satisfying the following criteria were included in this meta-analysis: (1) N was directly added to the wetland ecosystem, and at least one of the considered indicators was measured. (2) If the experiment included treatments other than N addition, only control and N treatment data were selected. (3) The amount and duration of N addition were recorded. (4) The mean value and sample size of the selected indicators are available or can be calculated from relevant publications. There are 89 articles corresponding to our subject were obtained. All raw data were extracted from the body of the publication, tables, charts, and appendices. When the data were presented graphically, GetData Graph Digitizer 2.24 was used to retrieve the digital data. We aimed to collect all available functional genes, but only *nifH*, archaeal *amoA*, bacterial *amoA*, *nirK*, *nirS*, *nosZ*, *mcrA* and *pmoA* had sufficient data for this meta-analysis. There are only 30 articles could finally obtain effective data successfully. So, we selected the 30 papers as our meta-analysis objects in the study.

A total of 54 N addition treatments from 30 articles were collected in this study (Tables S1–S3; Text S1) [22–51], and a total of 103 data points were identified, including

18 Shannon index observations and 13 Chao1 index observations (Table S1). Greater than half of the data points were released in the past five years (2017–2021). Soil organic carbon (SOC) (2.31–139.25), total nitrogen (TN) (0.37–65.01) and pH (4.46–8.2) also showed wide variation ranges. Urea and ammonium nitrate (NH_4NO_3) are the most commonly used N fertilizers. N addition type of included in our database were N (66.3%), NP (12.6%), NK (2%), and NPK (19.1%). Nitrogen addition methods include nitrogen addition rate and time. According to the amount of N addition, the following groups were established: low N addition rate (0–50 kg N ha^{-1} $year^{-1}$), medium N addition rate (50–200 kg N ha^{-1} $year^{-1}$) and high N addition rate (>200 kg N ha^{-1} $year^{-1}$). Among them, 50% of the experiments applied N at a rate of less than 50 kg N ha^{-1} $year^{-1}$, 31.4% applied between 50 and 200 kg N ha^{-1} $year^{-1}$, and 16.6% applied >200 kg N ha^{-1} $year^{-1}$. According to the length of N addition time, the following groups were established: short-term N addition (0–10 years), medium N addition (10–20 years) and long-term N addition (>20 years). The time of N addition was also short-term (0–10 years) and long-term (>20 years) N addition was noted in 55.5% and 22.2% of studies, respectively. The hydrological condition is a large important characteristic of wetland ecosystems. Generally, wetlands can be classified into flooded wetlands and non-flooded according to hydrological conditions. Moreover, based on climate zone, N-limited conditions, and saline conditions, we divided the wetlands into temperate wetlands and subtropical wetlands, N-limited wetlands and N-unlimited wetlands, and saline wetlands and freshwater wetlands. We used a meta-analysis [52] to determine the effects of N addition on soil properties (pH, soil organic C (SOC) and soil total N (TN)), soil microbial diversity (Shannon, Simpson, Chao1, ACE) [53], key soil C and N cycling microbial functional genes (*nifH*, *nirK*, *nirS*, *nosZ*, AOA, AOB, *mcrA*, *pmoA*) [54] and soil greenhouse gas (CH_4, CO_2) [55] emissions.

2.2. Statistical Analyses

Microbial diversity (Shannon index, H; Simpson index, D) and richness (Chao1 index) [53] were calculated using the following equations:

$$\text{Shannon index(H)} = -\sum_{i=1}^{s} p_i \ln p_i, \quad (1)$$

$$\text{Simpson}^{'}\text{s diversity index(D)} = 1 - \sum_{i=1}^{s} {p_i}^2, \quad (2)$$

where p_i is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), and s is the number of species.

$$\text{Chao1} = S_{obs} + \frac{F_1^2}{2F_2}, \quad (3)$$

where S_{obs} is the total number of species observed in a sample; F_1 is the number of singleton species; and F_2 is the number of doubleton species. Chao1 represents microbial richness, whereas the Shannon index considers both richness and the relative abundance of different groups. Therefore, Chao1 is more sensitive to rare species in the community. It could be possible that the Shannon index increases while Chao1 decreases under the same treatment, which generally would suggest the potential loss of rare species.

To facilitate the comparison of N addition effects among different studies, the responses of these indicators involved in soil properties, soil microbial diversity and soil C and N cycling genes to N addition were standardized. Meta-analysis methods are as follows: For each study, the response ratio (lnR), which was defined as the "effect size", was thus used to estimate the responses of soil microbial diversity and soil C and N cycling genes to N addition effects. The lnR was calculated as follows:

$$\text{lnR} = \ln(X_t/X_c) = \ln(X_t) - \ln(X_c), \quad (4)$$

Where X_t and X_c are the mean values for the N addition treatment and control, respectively. The variance of effect size was calculated using the following equation:

$$v = \frac{{s_t}^2}{n_t {X_t}^2} + \frac{{s_c}^2}{n_c {X_c}^2}, \quad (5)$$

where s_t and s_c represent the standard deviation of the treatment and control groups, respectively; n_t and n_c are the sample sizes for the treatment and control groups, respectively. For each study, we calculated the weighting factor (w) with the following formula:

$$w_{ij} = \frac{1}{v}, \quad (6)$$

The weighted mean response ratio (lnR_{++}) was calculated from the RR of individual pairwise comparisons between the treatment and control:

$$w_{ij} = \frac{1}{v}, \quad (7)$$

$$lnR_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij} lnR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}, \quad (8)$$

where m is the number of groups (e.g., N addition rates), and k is the number of comparisons in the ith group. The SE of the lnR_{ij} (s (lnR_{++})) was calculated as follows:

$$s(lnR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}}, \quad (9)$$

If the number of data points used for assessing lnR_{++} of a concerned variable was greater than 20, the 95% CI was calculated as follows:

$$95\%\ CI = lnR_{++} \pm 1.96 s(lnR_{++}) \quad (10)$$

If the number of data points was less than 20, the bootstrapping method was used to obtain the lowest and highest 2.5% values as the bootstrap confidence based on 5000 iterations. If the 95% CI overlapped with zero, then it was considered an insignificant N-induced response. The percentage changes in the variables induced by N addition were measured as follows:

$$\text{Effect size}(\%) = \left(\exp(lnR_{++}) - 1\right) \times 100\% \quad (11)$$

2.3. Structural Equation Modelling

We constructed a structural equation model (SEM) to determine the relationship among wetland soil properties, soil microbial diversity and microbial function. We compared the covariance matrix of implicit variance and observed variance. The maximum likelihood estimation method is used to fit the data into the model. Given that some of the variables introduced are not normally distributed, the probability that the path coefficient is different from zero is tested using a bootstrap method. To simplify the model, we deleted the unimportant path with low path coefficient. Then, the model was recalculated. Chi-square (χ^2) was used to test the overall goodness of fit of structural equation models. When the χ^2/DF model fitting index was between 0.00 and 3.00 and the p value was greater than 0.50, the structural equation model was considered acceptable.

3. Results

3.1. The Effects of N Addition on Wetland Soil Properties Subsection

Firstly, across all of the studies, we found that N addition promoted soil acidification, decreased pH by 28% (95% CI: −0.758, 0.088), increased SOC by 34% (95% CI: −0.1, 0.688) and increased TN by 32% (95% CI: −0.283, 0.844) (Figure 1).

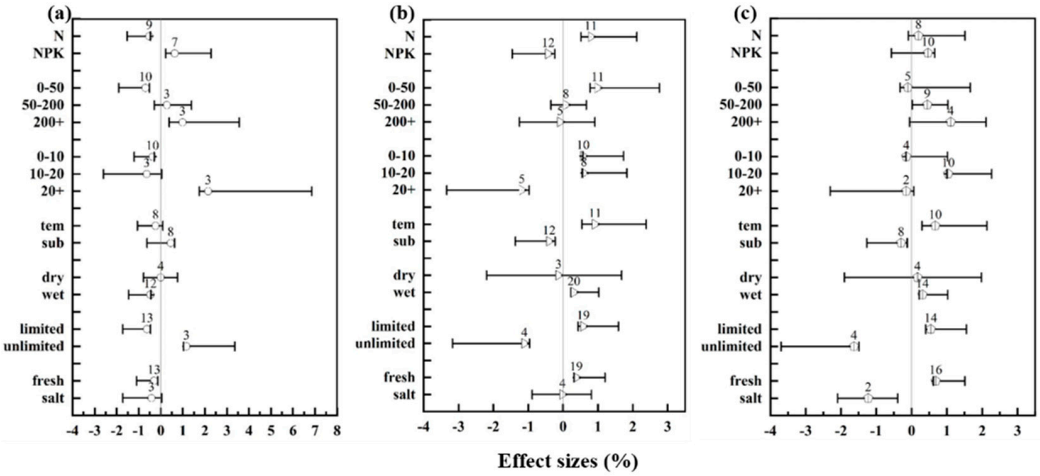

Figure 1. Effect of N addition on soil properties (**a**) pH; (**b**) SOC; (**c**) TN and 95% confidence intervals (CIs). Vertical lines are drawn when the effect size is zero. Special symbols represent the effect sizes. The sample size is noted above each value. N, N fertilizer only; NPK, N P K fertilizer; 0–50, N addition rate is 0–50 kg N ha^{-1} $year^{-1}$; 50–200, N addition rate is 50–200 kg N ha^{-1} $year^{-1}$; 200+, N addition rate is >200 kg N ha^{-1} $year^{-1}$; 0–10, N addition time is 0–10 years; 10–20, N addition time is 10–20 years; 20+, N addition time is >20 years; tem, temperate wetlands; sub, subtropical wetland; dry, non-flooded wetland; wet, flooded wetland; limited, N-limited wetlands; unlimited, N-unlimited wetlands; fresh, freshwater wetlands; salt, saline wetlands.

Secondly, we analyzed the effects of N addition on soil properties under different environmental conditions and different N addition methods. The specific results are as follows. SOC and TN increased by 151% and 96% in temperate wetlands but reduced by 33% and 26% in subtropical wetlands. SOC and TN increased by 78% and 72% in N-limited wetland but decreased by 67% and 80% in N-unlimited wetland (Figure 1).

Under low N addition rate, soil pH and TN decreased by 50% and 10%, and SOC increased by 170%; under medium N addition rate, soil pH, SOC and TN increased by 32%, 8% and 56%; under high N addition rate, SOC decreased by 8%, and soil pH and TN increased by 168% and 203%. Under short-term N addition, soil pH and TN decreased by 31% and 14%, whereas SOC increased by 78%; under medium-term N addition, soil pH decreased by 47%, and SOC and TN increased by 82% and 182%; under long-term N addition, soil TN and SOC decreased by 69% and 14%, and soil pH increased by 758% (Figure 1).

3.2. The Effects of N Addition on Wetland Soil Microbial Diversity

Firstly, across all of the studies, N addition reduced the soil microbial diversity: the Shannon index decreased by 8% (95% CI: −0.475–0.303), the Simpson index decreased by 49% (95% CI: −1.144–−0.2), the Chao1 index decreased by 53% (95% CI: −1.19–−0.2), and the ACE index decreased by 24% (95% CI: −1.89–1.33) (Figure 2).

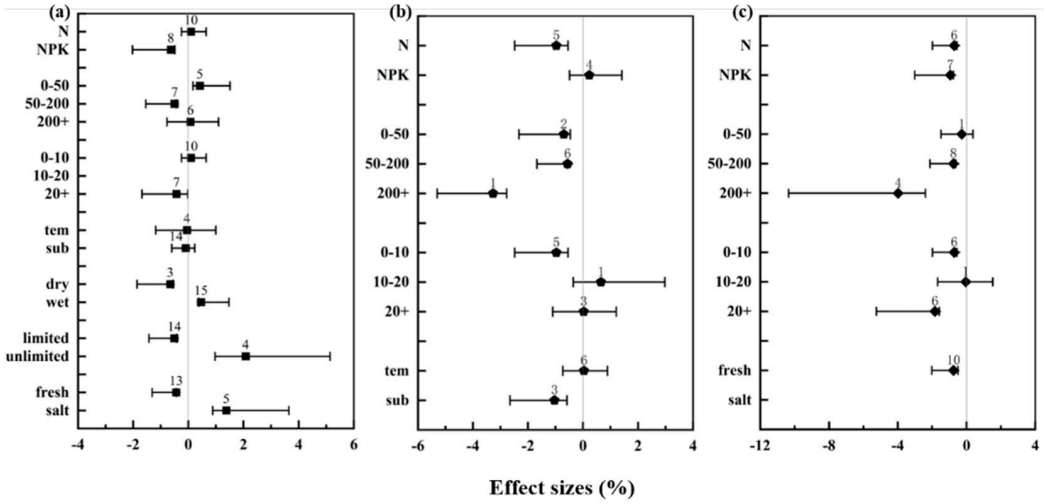

Figure 2. Effect of N addition on microbial diversity (**a**) Shannon index; (**b**) Simpson index; (**c**) Chao1 index and 95% confidence intervals (CIs). Vertical lines are drawn when the effect size is zero. Special symbols represent the effect sizes. The sample size is noted above each value. N, N fertilizer only; NPK, N P K fertilizer; 0–50, N addition rate is 0–50 kg N ha^{-1} $year^{-1}$; 50–200, N addition rate is 50–200 kg N ha^{-1} $year^{-1}$; 200+, N addition rate is >200 kg N ha^{-1} $year^{-1}$; 0–10, N addition time is 0–10 years; 10–20, N addition time is 10–20 years; 20+, N addition time is >20 years; tem, temperate wetlands; sub, subtropical wetland; dry, non-flooded wetland; wet, flooded wetland; limited, N-limited wetlands; unlimited, N-unlimited wetlands; fresh, freshwater wetlands; salt, saline wetlands.

Secondly, we analyzed the effects of N addition on soil microbial diversity under different environmental conditions and different N addition methods. The specific results are as follows. The Simpson index increased by 4% and Chao1 index decreased by 4% in temperate wetlands; the Shannon index, Simpson index, and Chao1 index decreased by 9%, 64%, and 57% in subtropical wetlands. The Shannon index of wetland soil decreased by 39% in N-limited wetland but increased by 703% in N-unlimited wetland (Figure 2).

Under low N addition rate, the Simpson index and Chao1 index decreased by 50% and 24%, whereas the Shannon index increased by 52%; under medium N addition rate, the Shannon index, Simpson index and Chao1 index decreased by 39%, 43% and 53%; under high N addition rate, the Simpson index and Chao1 index decreased by 96% and 98%, and the Shannon index increased by 9%. Under short-term N addition, the Simpson index and Chao1 index decreased by 62% and 51%, and the Shannon index increased by 11%; under medium-term N addition, the Shannon index and Chao1 index decreased 85% and 4%, whereas the Simpson index increased 93%; under long-term N addition, the Shannon index and Chao1 index decreased by 35% and 84%, whereas the Simpson index increased by 3% (Figure 2).

3.3. The Effects of N Addition on Key Wetland Soil C and N Cycling Genes

Firstly, across all of the studies, N addition increased the abundance of most of the key microbial functional genes in C and N cycling in wetlands. The abundance of *nifH*, AOA, AOB, *nirK*, *nirS*, *nosZ* and *mcrA* functional genes increased by 394% (95% CI: 0.725–2.481), 1080% (95% CI: 0.893–4.043), 21% (95% CI: −0.750–1.135), 4% (95% CI: −0.811–0.898), 69% (95% CI: −0.367–1.414) and 60% (95% CI: −0.43–1.37). Only the gene abundance of *pmoA* decreased by 92% (95% CI: −4.094–−1.067) (Figure 3).

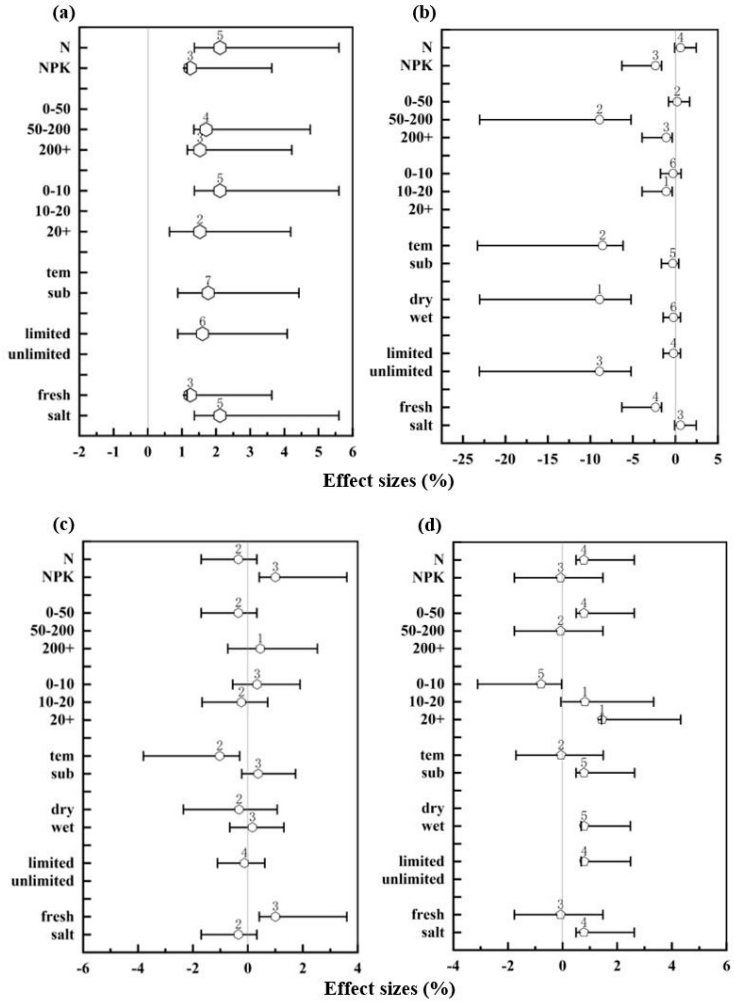

Figure 3. Effect of N addition on functional genes (**a**) *nifH*; (**b**) AOB; (**c**) *nirS*; (**d**) *nosZ* in microbial C and N cycles and 95% confidence intervals (CIs). Vertical lines are drawn when the effect size is zero. Special symbols represent the effect sizes. The sample size is noted above each value. N, N fertilizer only; NPK, N P K fertilizer; 0–50, N addition rate is 0–50 kg N ha^{-1} $year^{-1}$; 50–200, N addition rate is 50–200 kg N ha^{-1} $year^{-1}$; 200+, N addition rate is >200 kg N ha^{-1} $year^{-1}$; 0–10, N addition time is 0–10 years; 10–20, N addition time is 10–20 years; 20+, N addition time is >20 years; tem, temperate wetlands; sub, subtropical wetland; dry, non-flooded wetland; wet, flooded wetland; limited, N-limited wetlands; unlimited, N-unlimited wetlands; fresh, freshwater wetlands; salt, saline wetlands.

Secondly, we analyzed the effects of N addition on key wetland soil C and N cycling genes under different environmental conditions and different N addition methods. The specific results are as follows. AOB gene abundance increased by 100% in temperate wetlands, and *nifH* increased by 489% in subtropical wetlands. In addition, *nifH* gene abundance increased by 397% in N-limited wetlands, and *pmoA* decreased by 100% in N-unlimited wetlands. Moreover, *nifH* and AOB increased by 729% and 81%, in saline wetlands (Figure 3).

Under low N addition rate, the abundance of *nirS* and *pmoA* genes decreased by 29% and 87%, whereas the abundance of *nosZ* AOB and *mcrA* genes increased by 118%, 24% and 110%; under the medium N addition rate, the abundance of *nosZ* and *mcrA* genes decreased by 7% and 17%, whereas the abundance of *nifH* and AOB genes increased by 450% and 100%; under high N addition rate, the abundance of *nifH*, *nirS* and AOB genes increased by 360%, 57% and 66%. Under short-term N addition, the abundance of *nosZ* and AOB genes decreased by 54% and 24%, whereas the abundance of *nifH* and *nirS* genes increased by

729% and 40%; under medium-term N addition, the abundance of *nirS* and AOB decreased by 21% and 66%, whereas the abundance of *nosZ* increased by 126%; under long-term N addition, the abundance of *nifH* and *nosZ* genes increased by 360% and 325% (Figure 3).

4. Discussion

4.1. Relationships among Different Indicators under the Effects of N Addition

To reveal the relationships among indicators under N addition, a SEM was constructed (Figure 4). We found that the changes in soil TN and SOC could explain the changes in soil microbial community structure better than pH under N addition. The change in SOC explained the change in microbial diversity [54] and the change in abundance of key microbial functional genes (except denitrifying functional genes) in the C and N cycle. The change in soil pH only explained the change in C sequestration gene abundance. These results indicated a sensitive feedback relationship between SOC and TN and soil C and N cycling after N addition. Compared with pH, the changes in nutrients and C sources were important factors determining the changes in microbial community structure under N addition. This finding also explains why the SOC and microbial diversity we found in 4.2 have the same response pattern to wetland environmental factors (Figures 1 and 2; Table 1). The change in *mrcA* gene abundance could not explain the change in SOC, and the change in SOC under N addition might be mainly determined by plant biomass accumulation. Besides TN and SOC, the changes in microbial diversity also resulted from the changes in the abundance of nitrification [55] and denitrification functional genes. Changes in *nifH* abundance did not contribute to changes in microbial diversity but contributed to methane emission to a certain extent since methanogenic archaea were the main hosts and expression groups of *nifH* in swamps [56]. N fixation is closely related to methane production.

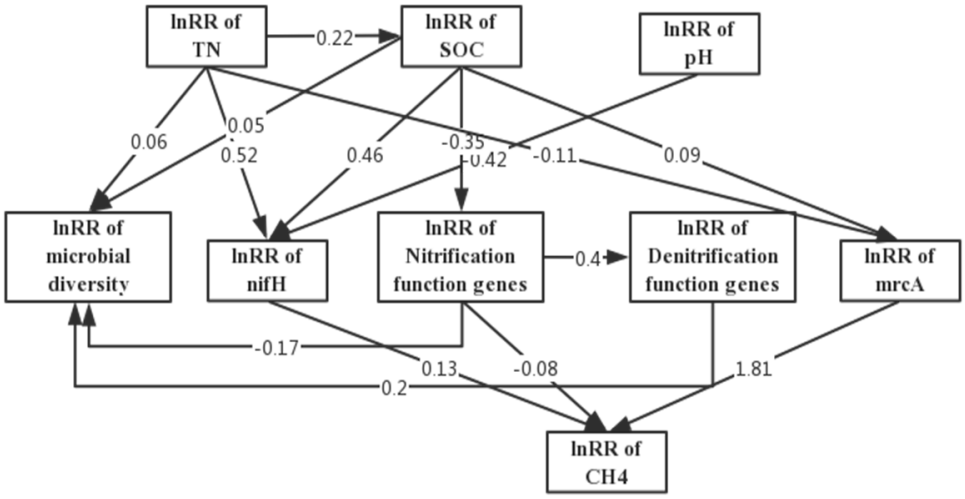

Figure 4. Structural equation model (SEM) depicting the effect of multiple drivers on the response ratio of soil microbial diversity and key functional genes. SOC, TN, and pH are reported as the response ratios of SOC, TN, and pH under N addition. R^2 represents the ratio of microbial diversity to the response ratio of C/N function explained by these drivers. The number next to the arrow is the normalized path coefficient, which is similar to the relative regression weight and indicates the extent of the influence of the relationship. $\chi 2$, chi-square DF; DF, degree of freedom; *p*, probability level; nonsignificant $\chi 2$ test ($p > 0.05$) and CFI values greater than 0.90 are considered acceptable.

Table 1. Effect of N addition on wetland microbial community structure under wetland environmental factors and N addition methods.

	Wetland Environmental Factors			N Addition Methods	
	Climate Conditions	**Saline-Alkali Conditions**	**NLC [1]**	**NAT [2]**	**NAR [3]**
Different effect directions	SOC, Simpson	AOB, Shannon	SOC, Shannon	Simpson	pH
Significant different effect size	*nifH*, AOB, Chao1	*nifH*	*pmoA*	*nifH*	*nifH*, Chao1
No Significant effect	pH, TN, Shannon, *nirS*, *nosZ*, *mcrA*	pH, SOC, TN, *nirS*, *nosZ*, *mcrA*	pH, TN, *nirK*	pH, SOC, TN, Shannon, Chao1, *nirK*, *nirS*, *nosZ*	SOC, TN, Shannon, Simpson, *nirK*, *nirS*, *nosZ*, AOB, *mcrA*

[1] N limited condition. [2] N addition time. [3] N addition rate.

4.2. Wetland Environmental Factors and N Addition Methods Affect the Direction and Extent of the N Addition Effects

N addition effects derived from all of the studies could not represent the effects under different environmental factors and N addition methods. Wetland environmental factors and N addition methods determine the direction of N addition effects on some indicators of soil properties, microbial diversity and the abundance of key microbial functional genes in C and N cycling (Figures 1–3; Table 1). N-limited conditions and climate conditions determined the N addition effect direction on SOC (Table 1). The wetland C sequestration function mainly depends on the trade-off between plant C input and SOC decomposition [57–61]. In general, N addition could both increase plant biomass and SOC decomposition. The changes in SOC induced by N addition showed that N addition had an asymmetric effect on plant biomass accumulation and SOC decomposition. Our results demonstrated that with the increase of N addition rate, the plant C input and SOC decomposition also changed, SOC content decreased. Plant biomass had greater response to N addition than SOC decomposition under N-limited conditions, which had a smaller response under N-unlimited conditions. Saline-alkali conditions determined the N addition effect direction on AOB abundance (Table 1). As ammonia-oxidizing bacteria (AOB) grow in neutral environments, soil acidification after N addition could inhibit the growth of AOB [62]. Saline-alkali wetlands can neutralize soil acidification caused by N addition and alleviate the adverse effects of N addition on soil microorganisms [63]. Opposite effects of N addition on SOC and the microbial diversity index were significant in temperate and subtropical wetlands. Therefore, to clarify the evolution of the wetland C sequestration function under the background of global change, it is necessary to reveal the interaction between N deposition, N-limited conditions and climate conditions, instead of studying N deposition and the ecological effect as a single factor. In the aspect of N addition methods, soil acidification just occurred under medium and high N addition rate (Figure 1). The pH did not significantly decrease under low N addition rate which might be due to the large uptake of inorganic N by plant roots and microorganisms and the buffering properties of the soil itself.

Besides the above, environmental factors and N addition methods did not change the direction of the effect but significantly changed the size of the effect on some indicators (Table 1). As the N addition rate increased the decrease in the Chao1 index increased by 74%. The Chao1 index is indicative of the richness of microorganisms, especially for rare species [64–66]. This finding indicates that the increase in the N addition rate is more detrimental to rare soil microorganism species [66,67].

Considering all of the environmental factors and N addition methods, N addition increased the soil *nifH* abundance. The *nifH* is a functional gene encoding nitrogenase reductase during N fixation [68–71]. It is generally believed that biological N fixation is a high energy consumption process [72–74], and the biological N fixation process will be

reduced when N is sufficient in terrestrial ecosystems [75–77]. However, methanogens are the main N-fixers in wetlands. The methane generation process of the C cycle is directly related to the N fixation process [78–80]. In addition, plant root exudates, sugars, and litters promote nonsymbiotic N fixation in wetlands [80,81]. N addition can increase the N fixation process by increasing the available C in wetlands.

5. Conclusions

The changes in soil TN and SOC contributed significantly to the changes in microbial community structure under N additions. Environmental factors and N addition altered the direction or size of N enrichment roles, soil physical and chemical properties, microbial diversity, and key C and N cycling genes in wetlands. The N-limited conditions and climate conditions determined the effect of N addition on SOC content. The saline-alkali conditions determined the effect of N addition on soil microbial diversity and AOB abundance. This study clarified the type of wetlands by environmental factors. This study enriched the cognition of effects of N addition on wetland soils under different wetland classification. It is of great significance to guide wetland protection and restoration under the background of global change. Due to the fact that N and OC play important roles in wetland soils under N addition, it is strongly recommended that future studies focus on the dynamics of N species and SOC.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w14111748/s1. Table S1: Effects of nitrogen (N) addition on soil pH, SOC, TN, Table S2: Effects of nitrogen (N) addition on microbial Shannon index, microbial Chao 1 index, microbial Simpson index, microbial ACE index, Table S3: Effects of nitrogen (N) addition on the abundance of nitrogen cycling and carbon cycling genes, Table S4: Effects of nitrogen (N) addition on greenhouse gas emissions. (GHG), Text S1: A list of 30 primary studies from which the data were extracted for this meta-analysis, refs [25–51] are part of Text S1.

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