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Effects of Simulated Nitrogen Deposition on Soil Microbial Community Structure in Temperate Forest Based on PLFA Method

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Authors' contributions

All authors made significant contributions to this study. All authors contributed to the article and approved the submitted version.

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ABSTRACT

Soil microorganisms play a crucial role in the biogeochemical cycling of terrestrial ecosystems. However, previous studies on the effects of nitrogen deposition on microorganisms have primarily focused on nitrogen-sensitive tropical forest ecosystems. This study focused on soil in a temperate Korean pine plantation and conducted a field simulated nitrogen deposition experiment. The effects of different nitrogen application rates on the microbial community structure were analyzed using the

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phospholipid fatty acid (PLFA) method. The experiment included four nitrogen application rates: control (CK: 0 kg N ha^{-1} yr^{-1}), low nitrogen treatment (LN: 20 kg N ha^{-1} yr^{-1}), medium nitrogen treatment (MN: 40 kg N ha^{-1} yr^{-1}), and high nitrogen treatment (HN: 80 kg N ha^{-1} yr^{-1}). After seven years of continuous application of ammonium nitrate solution, the soil microbial community structure was determined using the PLFA method. The results showed that all nitrogen application rates significantly reduced the PLFA concentration of fungi and AM fungi (P < 0.05), while bacterial biomass significantly decreased in the high nitrogen treatment group. The biomass of Gram-positive bacteria, Gram-negative bacteria (G^-), and actinomycetes also significantly decreased in the HN treatment group. Furthermore, long-term application of high nitrogen concentration (80 kg N ha^{-1} yr^{-1}) significantly reduced soil microbial biomass and changed the fungal to bacterial ratio, thus affecting the soil microbial community structure. Redundancy analysis (RDA) of soil microbial and soil chemical properties found that the long-term simulated nitrogen deposition experiment affected the soil microbial community structure by changing the content of soil N, P elements, and soil pH values. In summary, long-term simulated N deposition can negatively affect soil microbial biomass and its community structure, and the main reason for this analysis is related to long-term N application leading to soil acidification and changes in the conversion of soil N and P elements.

Keywords: Nitrogen addition; Korean pine plantation; phospholipid fatty acid; microbial biomass.

1. INTRODUCTION

Since the Industrial Revolution, human activities such as agricultural and industrial practices, as well as the burning of fossil fuels, have led to a significant increase in the release of reactive nitrogen into the atmosphere, which has resulted in a rapid rise in nitrogen deposition in both terrestrial and aquatic ecosystems [1-5]. Nitrogen deposition provides a new source of nutrients for plants, but excessive nitrogen input can also affect biogeochemical cycling and alter ecosystem structure and function. The continued increase in nitrogen deposition has far-reaching impacts on terrestrial ecosystems, including soil acidification, loss of biodiversity, and degradation of forest function [6-8]. The effects of nitrogen deposition on grassland ecosystems have long been a hot topic in the field of ecology, while research on forest ecosystems in northern temperate regions is relatively scarce. Forest ecosystems have important ecological functions, such as climate regulation and protection of biodiversity [9,10], and the stability and function of these ecosystems depend on the contribution of soil microorganisms. Soil microorganisms are a key component of soil ecosystems and are involved in important ecological processes such as organic matter decomposition, nutrient transformation, and plant growth [11]. Changes in soil microbial community structure and biomass can directly affect the health and productivity of forest ecosystems [12]. However, excessive nitrogen deposition may have a negative impact on soil microbial biomass and community structure, thus affecting soil ecological function. Therefore, studying the impact of nitrogen deposition on the soil microbial community structure in Korean pine plantation in the northern temperate zone is of great significance for us to deepen our understanding of the stability and sustainable development of forest ecosystems. This article aims to explore the effects of nitrogen deposition on the soil microbial community structure in Korean pine plantation in the northern temperate zone through simulated experiments, in order to provide scientific basis for the protection and management of forest ecosystems.

2. RESEARCH PLOTS AND RESEARCH METHODS

2.1 Overview of the Research Plot

The experimental site is located in the Korean pine plantation within Liangshui National Nature Reserve, Heilongjiang Province (47°10′50″N, 128°53′20″E), with a natural nitrogen deposition of 12.93 kg N ha^{-1} yr^{-1} [13]. The region exhibits a distinct temperate continental monsoon climate, with an annual average temperature of -0.3℃, a daily average maximum temperature of 7.5℃, a daily average minimum temperature of -6.6℃, an annual average precipitation of 676 mm, an annual average evapotranspiration of 805 mm, a frost-free period of 100-120 days, and a snow season of 130-150 days. The Korean pine plantation at the experimental site was established in 1954 after the mixed broad-leaved Korean pine forest was cut down. The soil at the site is classified as dark brown forest soil according to the Chinese soil classification system. The dominant vegetation in the area is

Pinus koraiensis, with accompanying herbaceous plants such as *Oxalis corniculata* and *Mitella nuda* growing under the forest canopy.

2.2 Experimental Design

The nitrogen addition experiment started in 2014, with nitrogen treatments applied to the study site from mid-June to mid-September. The NH_4NO_3 required for each treatment was dissolved in 20 L of water and evenly sprayed onto the ground using a backpack sprayer. A completely randomized design was used, with each plot measuring 5 m x 30 m and separated by a 10 m wide buffer zone. There were four gradients, including a control (CK: 0 kg N ha^{-1} yr^{-1}), low nitrogen (LN: 20 kg N ha^{-1} yr^{-1}), medium nitrogen (MN: 40 kg N ha^{-1} yr^{-1}), and high nitrogen (HN: 80 kg N ha^{-1} yr^{-1}) [14], with three replicates per treatment.

2.3 Experimental Methods

2.3.1 Soil sampling and preparation

Soil samples were collected in mid-July 2020 using a soil sampler (5 cm inner diameter, 0-15 cm depth) from five random locations within each plot. The collected soil samples were mixed to form one composite sample per plot. The composite samples were divided into two portions: one portion was stored at −80°C for soil microbial analysis and the other portion was air-dried for soil chemical analysis.

2.3.2 Soil chemical analysis

Air-dried soil samples were used to measure soil pH by mixing with a 2.5:1 ratio of deionized water using a pH meter (FiveEasy FE20). The samples were ground with a ball mill and sieved through a 200-mesh sieve. Total carbon (TC) and total nitrogen (TN) were measured using a multi N/C 2100 TOC/TN analyzer (Analytikjena, Germany). Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were measured using the magnesium oxide diesel alloy distillation method, and total phosphorus (TP) and available phosphorus (AP) were measured using the molybdenum antimony colorimetric method.

2.3.3 Measurement of soil microbial community structure

The soil microbial community was analyzed using the modified phospholipid fatty acid (PLFA) method described by Koranda [15]. Frozen soil samples were extracted with a mixture of methanol, chloroform, and citrate buffer in a ratio of 2:1:0.8 (v/v/v), and the phospholipids were separated using a silica gel column (in the methanol fraction). The extract was then dried under a stream of N_2 and mildly alkaline methanolysis was performed. The resulting fatty acid methyl esters (FAMEs) were re-dissolved in hexane and analyzed using a gas chromatograph (Agilent 7890B). Different PLFAs were classified into different microbial groups (Table 1) [16,17] and expressed as concentrations (nmol g^{-1} soil). The concentration of the target PLFA was calculated using the following formula:

$$\text{Target PLFA concentration} = \frac{\text{PLFA peak area}}{\text{internal standard peak area}} \times \frac{\text{internal standard concentration}}{\text{PLFA molar mass}} \times \frac{\text{volume of extracted sample}}{\text{sample mass}}$$

Table 1. Main PLFA for characterizing microbes

Microbiome	PLFA-labeled
Bacterial	15:0, 17:0, i13:0, i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, 12:0 OH, 14:1ω5c, 15:1ω6c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω7c
Gram-positive bacteria	i13:0, i14:0, i15:0, a15:0, i16:0, i17:0, a17:0
Gram-positive bacteria	12:0 OH, 14:1ω5c, 15:1ω6c, 16:1ω7c, 16:1ω9c, 17:1ω8c, 18:1ω7c
Fungi	16:1 w5c, 18:1ω9c, 18:2ω6c, 20:1ω9c
——AM fungi	16:1 w5c
Actinomycetes	16:0 10Me, 17:0 10Me, 18:0 10Me
Anaerobe	15:0 DMA, 16:2 DMA, 17:0 DMA, 16:1 ω7c DMA, 16:1 ω9c DMA, 18:1 ω9c DMA, 18:1 ω7c DMA

2.4 Data Processing

The experimental data was analyzed using IBM SPSS Statistics 26 software to perform one-way ANOVA and Duncan's multiple range test (P <0.05). The correlation between soil environmental factors was calculated using Pearson's test (two-tailed) at two significant levels (P <0.05 and P <0.01). The impact of soil properties on soil microbial communities was revealed using RDA analysis. Graphpad software was used for data visualization.

3. RESULTS

3.1 Effect of Simulated Nitrogen Deposition on Basic Physical and Chemical Properties of Soil

As the nitrogen addition concentration increased, the pH of the soil in the artificial Korean pine plantation gradually decreased, with the pH of the HN group significantly reduced to 5.18 (P <0.05) (Table 2). The total phosphorus (TP) content in the soil significantly increased by 14.5% in the MN group, while there was no significant difference in other treatment groups (P >0.05). The available phosphorus (AP) content significantly decreased after nitrogen addition. The content of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) in the soil showed significant changes, with NH_4^+-N content increasing with increasing nitrogen addition, and significant increases observed in the MN and HN groups. The nitrate nitrogen content was significantly increased in the LN and MN groups, while the HN group showed a significant decrease in nitrate nitrogen content, but the overall fluctuation did not exceed 0.68 mg kg^{-1}.

3.2 Correlation Analysis of Soil Physical and Chemical Factors under Nitrogen Addition

As shown in Table 3, soil NH_4^+-N content was significantly negatively correlated with soil NO_3^--N content (P <0.05), and was also significantly negatively correlated with soil pH value. Soil NO_3^--N content was significantly positively correlated with soil TP content. Soil total carbon (TC) and soil organic carbon (SOC) content were significantly positively correlated (P <0.01).

3.3 Effects of Simulated Nitrogen Deposition on Different Types of Soil Microorganisms

The simulated nitrogen deposition had a significant effect on the soil microorganisms of the Korean pine plantation (P <0.05). The soil fungi, bacteria, actinomycetes and total PLFA were the highest in the CK group, and the HN group was the lowest, but the responses of various microorganisms to nitrogen addition were different.

With the increase of nitrogen application rate, fungi and bacteria showed a trend of decreasing first, then slightly increasing and then decreasing. Compared with the control group, the concentration of fungal PLFA in the soil of LN group, MN and HN groups decreased significantly (P <0.05), decreased by 33.6%, 27.5% and 46.0% respectively (Fig. 1-A), bacteria decreased by 14.2%, 10.2% and 25.2% respectively (Fig. 1-B), by It can be seen that nitrogen addition will reduce the biomass of soil fungi and bacteria. The ratio of fungi to bacteria also had a similar trend. Compared with the CK group, the fungi/bacteria in the LN, MN, and HN groups were significantly reduced in varying degrees (P <0.05).

Both Gram-positive bacteria(G^+) and Gram-negative bacteria(G^-) showed a trend of decreasing first, then slightly increasing and then decreasing. Compared with the control group, the G^+ concentration of PLFA decreased significantly (P <0.05), the LN, MN, and HN groups decreased by 22.2%, 21.1% and 31.38% respectively (Fig. 2-A), and the G^- decreased by 17.1%, 11.1% and 17.7% (Fig. 2-B), which shows that nitrogen addition will reduce the biomass of soil G^+ and G^-. The ratio of G^+ to G^- decreased in different degrees with the decrease of nitrogen application rate, but there was no significant difference among the groups (P >0.05).

PLFA of actinomycetes, AM fungi and anaerobic bacteria in soil to varying degrees (Fig. 3). Compared with the blank control group, the LN, MN, and HN groups all significantly reduced the AM fungal biomass (P <0.05), which decreased by 38.9%, 35.8%, and 55.9%, respectively (Fig. 3-A). For actinomycetes in the soil, only the high nitrogen treatment pair had a significant reduction (P <0.05), which decreased by 35.8% (Fig. 3-B). Nitrogen addition had no significant effect on anaerobic bacteria (P >0.05) (Fig. 3-C).

The total soil PLFA concentration was shown in Fig. 4. With the increase of nitrogen concentration, the total soil microbial biomass showed a downward trend, and the total biomass of H N treatment decreased significantly (P <0.05), which decreased by 27.9%.

Table 2. Effects of nitrogen addition on soil chemical properties of Korean pine plantation

	CK	LN	MN	HN
pH	5.77±0.10 a	5.51±0.07 b	5.40±0.03 b	5.18±0.11 c
TP (g kg^{-1})	0.55 ±0.03 b	0.59 ±0.04 ab	0.63 ±0.02 a	0.56±0.03 b
AP（mg kg^{-1}）	1.25±0.17 a	0.79±0.12 b	0.81±0.05 b	0.95±0.03 b
TN（g kg^{-1}）	6.67±0.09 a	6.58±0.10 a	6.80±0.14 a	6.77±0.07 a
NH_4^+-N（mg kg^{-1}）	5.38±0.64 c	5.83±0.14 c	8.44±0.45 b	9.44±0.49 a
NO_3^--N（mg kg^{-1}）	3.69±0.09 b	4.08±0.13 a	3.99±0.07 a	3.40±0.17 c
TC（g kg^{-1}）	72.10±8.94 a	66.87±6.60 a	73.27±2.18 a	71.47±11.50 a
SOC（g kg^{-1}）	64.07±6.77 a	61.00±6.70 a	64.23±2.47 a	62.47±5.33 a

Table 3. Correlation analysis of soil physical and chemical factors in Korean pine plantations under nitrogen addition

	pH	AP	NH_4^+-N	TP	TN	NO_3^--N	TC	SOC
pH	1							
AP	.540	1						
NH_4^+-N	-.870**	.290	1					
TP	-.154	.550	.100	1				
TN	-.380	.240	.510	.147	1			
NO_3^--N	.361	.410	-.51*	.585*	-.378	1		
TC	-.106	.010	.150	.255	.396	-.126	1	
SOC	-.009	.120	.080	.223	.130	.053	.915**	1

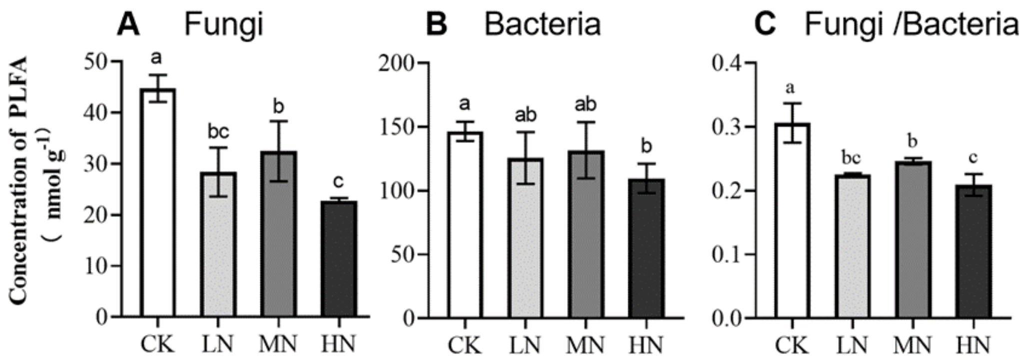

Fig. 1. Effects of Nitrogen Addition on Fungal, Bacterial and Fungal/Bacterial PLFA Concentrations in Soil of Korean Pine Plantation

**Different lowercase letters indicate significant differences among different nitrogen addition treatments (P <0.05)*

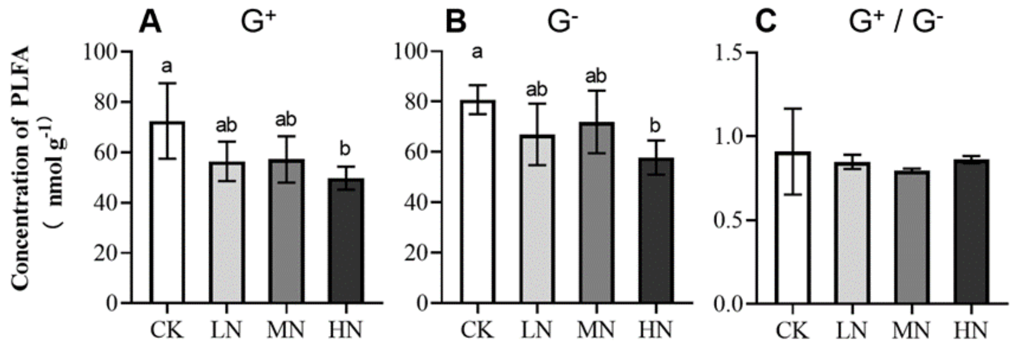

Fig. 2. Effects of nitrogen addition on Gram-positive bacteria(G^+), Gram-negative bacteria(G^-) and G^+/G^- of PLFA concentration in Korean pine plantation soil

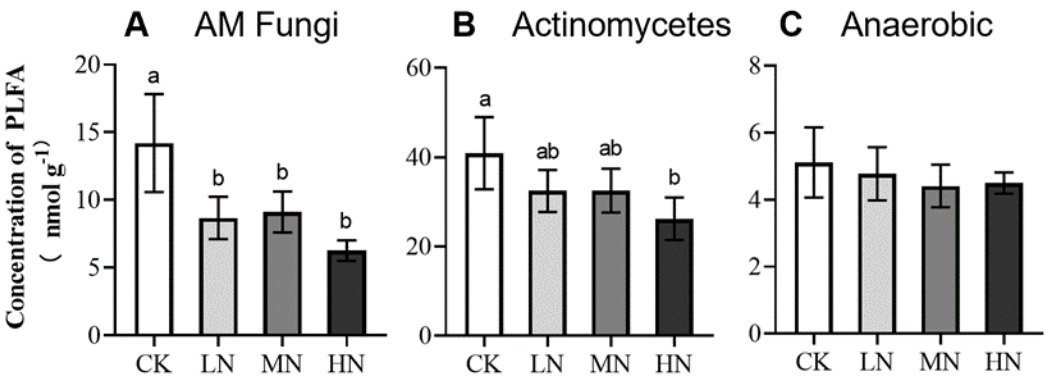

Fig. 3. Effects of nitrogen addition on the concentration of A M fungi, actinomycetes and anaerobic bacteria P LFA in the soil of Korean pine plantation

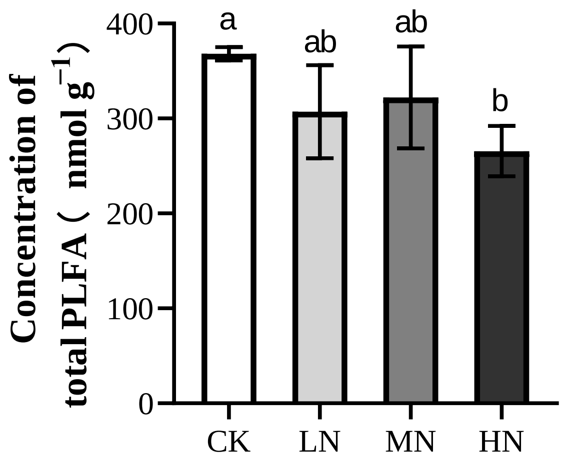

Fig. 4. Effect of nitrogen addition on the concentration of total PLFA

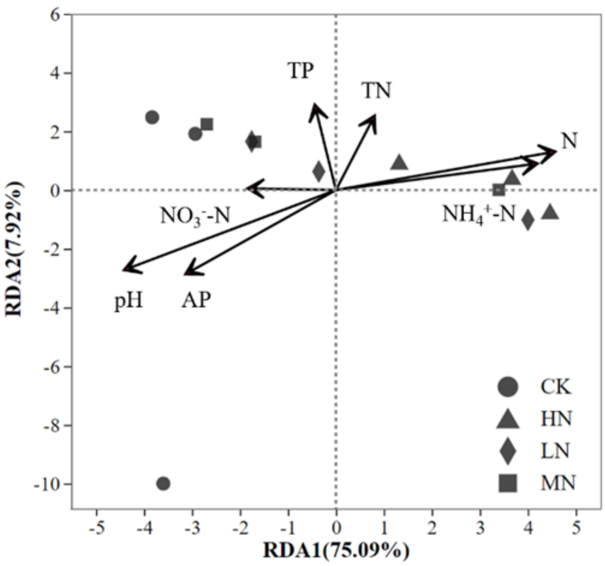

Fig. 5. Redundancy Analysis (RDA) of Soil Microbial Community and Environmental Characteristics

3.4 Influencing Factors of Soil Microbial Community Structure under Nitrogen Addition

The RDA analysis is shown in Fig. 5. The length of the arrow can represent the degree of influence of environmental factors on the structure of the microbial community. The six types of soil chemical factors can explain 83.01% of the changes in the soil microbial community. Soil AP and NH_4^+-N content significantly affected soil microbial community composition, and soil pH value and nitrogen addition (N) concentration significantly affected soil microbial community composition at *P* <0.01 level. In summary, the dynamic changes of nitrogen and phosphorus in soil and soil pH are the main environmental factors affecting microbial communities.

4. DISCUSSION

4.1 Effects of Nitrogen Addition on Soil Physicochemical Properties

The results of this experiment showed that with the increase of nitrogen addition concentration, the soil pH value gradually decreased, indicating that nitrogen addition may lead to soil acidification. Many existing research results show that nitrogen addition can lead to soil acidification [18-22]. In addition, experiments by Hong et al. revealed the response of soil acidification to different forms of nitrogen deposition in plantations of different tree species. Nitrate nitrogen directly reduces soil pH, while ammonium nitrogen affects soil pH indirectly mainly by affecting soil inorganic carbon [23]. In terms of the distribution of soil phosphorus elements, the total phosphorus content in this experiment fluctuated, and the available phosphorus content decreased after nitrogen application. Zhang et al.'s research demonstrated that nitrogen deposition affects the dynamic transformation of soil phosphorus, which can be explained by soil pH and soil microbial characteristics [24]. Nitrogen addition did not significantly change the total nitrogen content of the soil, but compared with the control group, it increased the content of ammonium nitrogen and nitrate nitrogen in the soil, which is consistent with Li et al.'s research results in temperate forest soils. In addition, Preeti et al.'s research on the effects of nitrogen deposition on soil nitrogen transformation showed that nitrogen deposition causes soil acidification, leading to soil aluminum ion toxicity, which in turn affects soil nitrogen transformation [25].

4.2 Effects of Nitrogen Addition on Soil Microbial Community Structure

The impact of nitrogen deposition on soil microorganisms has been studied, and a meta-analysis by Zhang et al. summarized the negative effects of nitrogen deposition on soil microorganisms. The total microbial biomass, bacterial biomass, and fungal biomass decreased by 13.2%, 16.6%, and 19.2%, respectively [26]. In this study, the PLFA method was used to measure soil microbial biomass, and compared with the control group, nitrogen addition significantly reduced the soil bacterial, fungal, and total PLFA amounts. Chen et al. found that nitrogen deposition can reduce soil microbial biomass, especially in acidic soil [27]. For AM fungi in the soil, we found that nitrogen addition significantly reduced their biomass. The reason for this may be that nitrogen deposition causes soil acidification or alters potassium cycling, which in turn reduces plant diversity and abundance [28,29], leading to a decrease in AM fungi biomass that mutually benefit with plants. The PLFA concentration of soil actinomycetes was significantly reduced in the high-nitrogen treatment group, possibly due to the significant decrease in pH in the high-nitrogen treatment group, which is not conducive to the survival of actinomycetes.

The addition of nitrogen has been found to significantly decrease the fungal to bacterial ratio in soil microbial structure. Tian et al. found that nitrogen deposition significantly decreased the fungal to bacterial ratio in tropical forest soils, while the effect on temperate forests was not significant [30]. The difference in results may be due to differences in experimental conditions, such as nitrogen application rate and duration. We predict that long-term and high levels of nitrogen deposition will exacerbate soil acidification and decrease the fungal to bacterial ratio. Short-term nitrogen addition reduces the biomass of G^+ and G^- in soil, consistent with the findings of Hong et al. [31], and significantly decreases the G+/G- ratio, altering soil bacterial community structure. According to RDA analysis (Fig. 5), the main factors affecting soil microbial community structure are changes in soil N and P elements and soil pH caused by nitrogen application.

5. CONCLUSION

Long-term nitrogen addition led to a significant decrease in the biomass of soil fungi, AM fungi and bacteria. Gram-positive bacteria, Gram-negative bacteria and actinomycetes were significantly reduced in the high nitrogen treatment. In addition, simulated nitrogen deposition significantly reduced the total soil microbial biomass and altered the fungal-to-bacterial ratio, thereby affecting the soil microbial community structure. Through RDA analysis, it was found that the long-term simulated nitrogen deposition test affected the soil microbial community structure by changing the transformation of soil N and P elements and reducing soil pH value. In summary, long-term simulated nitrogen deposition will have a negative impact on soil microorganisms, and the main reason is related to soil acidification and changes in the transformation of soil N and P elements.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ackerman D, Millet DB, Chen X. Global estimates of inorganic nitrogen deposition across four decades. Glob. Biogeochem. Cycle. 2019;33(1):100-107.
2. Yu G, Jia Y, He N, Zhu J, Chen Z, Wang Q, et al. Stabilization of atmospheric nitrogen deposition in China over the past decade. Nat. Geosci. 2019;12(6):424-429.
3. Liu L, Xu W, Wen Z, Liu P, Xu H, Liu S, et al. Modeling global oceanic nitrogen deposition from food systems and its mitigation potential by reducing overuse of fertilizers. Proc. Natl. Acad. Sci. U. S. A. 2023;120(17):e2073508176.
4. Zhu H, Chen Y, Zhao Y, Zhang L, Zhang X, Zheng B, et al. The response of nitrogen deposition in china to recent and future changes in anthropogenic emissions. J. Geophys. Res.-Atmos. 2022; 127(e2022JD03743723).
5. Altieri KE, Fawcett SE, Hastings MG. Reactive nitrogen cycling in the atmosphere and ocean. Annu. Rev. Earth Planet. Sci. 2021;49:523-550.
6. Gao Y, Jia Y, Yu G, He N, Zhang L, Zhu B, et al. Anthropogenic reactive nitrogen deposition and associated nutrient limitation effect on gross primary productivity in inland water of China. J. Clean. Prod. 2019;208:530-540.
7. Hong S, Gan P, Chen A. Environmental controls on soil pH in planted forest and its response to nitrogen deposition. Environ. Res. Lett. 2019;172:159-165.
8. Du E, Xia N, Guo Y, Tian Y, Li B, Liu X, et al. Ecological effects of nitrogen deposition on urban forests: an overview. Front. Agric. Sci. Eng. 2022;9(3):445-456.
9. Qiao X, Hautier Y, Geng Y, Wang S, Wang J, Zhang N, et al. Biodiversity contributes to stabilizing ecosystem productivity across spatial scales as much as environmental heterogeneity in a large temperate forest region. For. Ecol. Manage. 2023; 529:120695.
10. Deng O, Li Y, Li R, Yang G. Estimation of forest ecosystem climate regulation service based on actual evapotranspiration of New Urban Areas in Guanshanhu District, Guiyang, Guizhou Province, China. Sustainability. 2022;14(16):10022.
11. Coban O, De Deyn GB, van der Ploeg M. Soil microbiota as game-changers in restoration of degraded lands. Science. 2022;375(6584):e725.
12. Zhou Z, Wang C, Luo Y. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. Nat. Commun. 2020;11(1).
13. Song L, Tian P, Zhang J, Jin G. Characteristics of nitrogen deposition in heilongjiang liangshui national nature reserve. Huanjing kexue. 2018;39(10), 4490-4496.
14. Song L, Tian P, Zhang J, Jin G. Effects of three years of simulated nitrogen deposition on soil nitrogen dynamics and greenhouse gas emissions in a Korean pine plantation of northeast China. Sci. Total Environ. 2017;609:1303-1311.
15. Koranda M, Schnecker J, Kaiser C, Fuchslueger L, Richter A. Microbial processes and community composition in

the rhizosphere of European beech - The influence of plant C exudates. Soil Biology and Biochemistry. 2011;43(3):551-558.
16. Frostegrd, Tunlid A, Bth E. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods. 1991;14(3):151-163.
17. Bach, Elizabeth M, Williams, Ryan J, Hargreaves, Sarah K, et al. Greatest soil microbial diversity found in micro-habitats. Soil Biol. Biochem. 2018.
18. Zhang Q, Zhu J, Wang Q, Xu L, Li M, Dai G, et al. Soil acidification in China's forests due to atmospheric acid deposition from 1980 to 2050. Sci. Bull. 2022;67(9):914-917.
19. Tian Q, Yang L, Ma P, Zhou H, Liu N, Bai W, et al. Below-ground-mediated and phase-dependent processes drive nitrogen-evoked community changes in grasslands. J. Ecol. 2020;108(5):1874-1887.
20. Yang Z. The shadow price of nitrogen: a dynamic analysis of nitrogen-induced soil acidification in China. China Agric. Econ. Rev. 2019;11(3):489-506.
21. Yang Z. The shadow price of nitrogen: a dynamic analysis of nitrogen-induced soil acidification in China. China Agric. Econ. Rev. 2019;11(3):489-506.
22. Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, et al. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 2010;3(5):315-322.
23. Hong S, Gan P, Chen A. Environmental controls on soil pH in planted forest and its response to nitrogen deposition. Environ. Res. 2019;172:159-165.
24. Zhang L. Effects of nitrogen deposition and increased precipitation on soil phosphorus dynamics in a temperate forest. Geoderma: An International Journal of Soil Science. 2020;380(1).
25. Verma P, Sagar R. Effect of nitrogen (N) deposition on soil-N processes: a holistic approach. Sci Rep. 2020;10(1).
26. Zhang, Tian'An, Chen, Han Y, H, et al. Global negative effects of nitrogen deposition on soil microbes. The ISME Journal Emultidisciplinary Journal of Microbial Ecology. 2018.
27. Chen C, Chen X, Chen HYH. Mapping N deposition impacts on soil microbial biomass across global terrestrial ecosystems. Geoderma. 2023;433: 116429.
28. Han Y, Feng J, Han M, Zhu B. Responses of arbuscular mycorrhizal fungi to nitrogen addition: A meta-analysis. Glob. Change Biol. 2020;26(12):7229-7241.
29. Qin S, Yang G, Zhang Y, Song M, Sun L, Cui Y, et al. Mowing did not alleviate the negative effect of nitrogen addition on the arbuscular mycorrhizal fungal community. Front. Plant Sci. 2022:1986.
30. Tian D, Jiang L, Ma S, Fang W, Schmid B, Xu L, et al. Effects of nitrogen deposition on soil microbial communities in temperate and subtropical forests in China. Sci. Total Environ. 2017;607-608:1367-1375.
31. Hong P, Liu S, Yu H, Hao J. Effects of simulated nitrogen deposition on soil microbial biomass and microbial community structure of red vertebrium plantation young forest. Journal of Shandong University. 2016;51(5): 18-28.

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