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Dynamic effects of soil mineral ion-microorganism interactions on phosphorus-sulfur cycling revealed by spatio-temporal data mining techniques

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ABSTRACT

Soil microorganisms are the main drivers in maintaining soil health. This paper focuses on the process of soil mineral ions and microorganisms involved in regulating the phosphorus-sulfur cycle, and systematically investigates the repair and improvement mechanism of soil microorganisms. Relying on an experimental area of a typical grassland in Inner Mongolia, we set up experiments with different nitrogen addition treatments, and combined with one-way analysis of variance (ANOVA) to investigate the distribution of soil phosphorus and sulfur fractions under various scenarios. Then, structural equation modeling was applied to explore the dynamic role between microbial action and phosphorus-sulfur cycle under N addition. Under different nitrogen addition scenarios, Ca10-P accounted for the largest proportion of inorganic phosphorus fractions, which were all greater than 40%. The percentage of inorganic sulfur in the soil was relatively small, less than 3% of total sulfur, and the response of inorganic and total sulfur to the gradient of nitrogen addition, nitrogen frequency, and different grassland management practices was not obvious. Fungal communities were important drivers of changes in functional genes for interleaf phosphorus and sulfur cycling at different N application levels, i.e., N fertilizer application altered the interleaf fungal communities by affecting soil physicochemical properties, which significantly regulated the interleaf bacterial communities, phosphorus and sulfur cycling functional gene abundance, and pathogenic fungal abundance.

Keywords: structural equation modeling, one-way ANOVA, soil microorganisms, phosphorus-sulfur cycle

1. Introduction

As an important component of terrestrial ecosystems, soil microorganisms play an important role in

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regulating soil ecological functions and driving biogeochemical cycles [1-2]. At the same time, soil microorganisms are extremely sensitive to external environmental changes and soil perturbations, and they will influence important ecological processes related to soil biogeochemical cycles by changing their own community composition, structure and function [3-6]. Most of the soil minerals with diverse content and species and particle size composition not only affect soil water transport, but also profoundly influence soil microbial community composition and population changes, which have far-reaching effects on soil physicochemical properties and soil evolution [7-9].

In particular, it is rich in mineral elements such as phosphorus and sulfur, which have important potential in enhancing soil fertility and improving soil water retention capacity [10]. The process of soil mineral ion-microbe interaction occurs widely in nature, which not only plays an important role in the material cycle between soil nutrients and plant growth, but also soil microorganisms, as a sensitive indicator of environmental change, are of great indicative significance in soil ecological monitoring and evaluation [11-12]. In addition, the study of soil mineral ions and microbial interactions can help to grasp the changing law of microbial activity and metabolic activity in soil farming environment, and provide important theoretical guidance and technical support for the sustainable development of agricultural soils [13-14].

In this paper, the mechanism of microorganisms in soil improvement and restoration was synthesized from the direct and indirect effects of microorganisms on soil. An experiment was conducted in a test area of a typical grassland in Inner Mongolia, where soil phosphorus and sulfur fraction content data and microbial-related data were collected using soil physical and chemical analyses, high-throughput sequencing, and high-throughput quantitative PCR under different nitrogen application gradients, two nitrogen addition frequencies, and two management modes (fencing and sealing or mowing). Statistical methods and one-way analysis were used to analyze soil phosphorus fractions and soil sulfur fractions under multiple modes to investigate the effects of N addition on soil phosphorus and sulfur fractions. Structural equation modeling was then used to analyze the driving mechanism of the interleaf microbial community and the functional genes of the phosphorus-sulfur cycle under nitrogen addition, and to clarify the dynamic roles of nitrogen addition and microbial action on the phosphorus-sulfur cycle.

2. Remediation and amelioration mechanisms of soil microorganisms

Soil is rich in microbial resources with a wide variety of metabolic functions, which are both drivers of soil nutrient cycling and major contributors to the degradation, metabolism and transformation of various environmental pollutants. Within the soil profile, microorganisms can produce and consume atmospheric trace gases, influence soil acidity, regulate soil carbon dynamics and regulate nutrient cycles such as iron (Fe), sulfur (S), phosphorus (P) and nitrogen (N). This in turn indirectly promotes plant growth through nutrient cycling and structural changes, and the growth of the plant root system feeds back the soil nutrients as well as improves the microbial community and composition, thus achieving the purpose of restoration and improvement.

2.1 Direct effects on soil

2.1.1. Promoting nutrient cycling. Soil microorganisms participate in the cycling of elements such as carbon, nitrogen, phosphorus, iron and sulfur in the soil through their own metabolism, which is of great significance to the material and energy cycle of the ecosystem. Soil microorganisms are involved in the decomposition of organic matter, such as plant residual roots and leaves and organic fertilizers applied to the soil, which decay and decompose through the action of soil microorganisms, releasing nutrient elements for plants to utilize and forming humus to improve the soil structure. In addition, soil microorganisms are the engine of the biogeochemical cycle of soil elements, such as the nitrogen cycle, as one of the core of the elemental cycle of the soil ecosystem, whose four main

processes, i.e., biological nitrogen fixation, ammonification, nitrification, denitrification, are all driven by microorganisms.

2.1.2. Improvement of soil structure and texture. Soil microorganisms play a key role in promoting aggregation of soil particles and maintaining the stability of aggregates. Macromolecules formed by microbial decomposition of organic matter such as humic acids, cellulose, lignin, and polysaccharide carboxylic acids are adsorbed onto soil colloids and contribute to the formation of large soil aggregates. In addition, root hairs and fungal hyphae, especially those produced by tufted mycorrhizal fungi, are particularly important for the formation of large aggregates. Some metabolites of soil microorganisms may act as colloids to contribute to the formation of soil agglomerate structures, or soil microagglomerates formed through extracellular polymers that attach cells to the surface of minerals and promote the aggregation of mineral particles. The formation of these aggregates also contributes to the metabolic activity of soil microorganisms, which further promotes nutrient cycling in the soil.

2.1.3. Mitigating soil contamination hazards. Microorganisms can also degrade the residual organic pesticides, urban dirt and factory wastes in the soil, decomposing these into low-harm or even harmless substances, reducing the residual toxicity hazard, and a large number of soil microorganisms can be used for the remediation of contaminated soil.

2.2 Indirect effects on soil

Soil microorganisms alter the microenvironment around the soil root zone and promote plant growth, thereby enhancing the microbial role in soil repair and improvement. Some microorganisms directly increase the ability of plants to obtain nutrients from the soil; for example, nitrogen is a core nutrient needed by plants, and nitrogen-fixing bacteria in the soil provide plants with an available source of organic and inorganic nitrogen. Microorganisms in the soil can secrete hormones such as gibberellin, cytokinin and indoleacetic acid that are conducive to promoting plant growth during growth and reproduction, and these exogenously secreted growth hormones can also regulate plant growth and promote plant growth and development, as well as increase the uptake of nitrogen, phosphorus, and potassium nutrients by the plant root system. Microorganisms can also secrete organic acids, coccidiomycin and other substances to change the inter-root microenvironment, which in turn reduces the uptake of heavy metals by plants. The growth of the root system results in more organic matter in the soil, and the roots release a variety of compounds that serve as substrates for microbial growth and metabolism.

3. Materials and methods

Nitrogen fertilizer application is an important agronomic measure in crop production and has a profound impact on the associated microbial communities. In this paper, we used nitrogen in soil mineral ions as an example through experimental design to explore the effects of different nitrogen elements on phosphorus and sulfur compositions in soil, as well as the dynamic effects of nitrogen-microbe interactions on phosphorus and sulfur cycling.

3.1 Experimental design

The study site was an experimental area in the Inner Mongolia grassland, covering an area of 4 hm^2 in a completely randomized block design with 10 main zones. There were 9 nitrogen addition gradients with nitrogen addition rates of 0, 1, 2, 3, 5, 10, 15, 20 and 50gN m-2a-1, respectively, and 4 treatments: NTF (encapsulation twice a year nitrogen application), NMF (enclosure once a month nitrogen application), NTM (mowing twice a year nitrogen application) and NMM (mowing once a month nitrogen application). Biannual nitrogen additions were added semi-annually, monthly

nitrogen applications were made at the beginning of each month, and mowing treatments were made once a year at the end of August with a stubble height of 10 cm. no fertilizer was applied prior to the experimental setup.

The nitrogen used to simulate nitrogen deposition was 99.5% NH4NO3, and the simulated nitrogen deposition was carried out in the form of simulated wet deposition (spraying) and simulated dry deposition (dry spreading). To simulate wet deposition during the growing season (May~October), ammonium nitrate was proportionally dissolved in distilled water, and then evenly sprayed in the sample plots with a sprayer. In the non-growing season (November~April), ammonium nitrate was mixed with 500g of roasted fine sand, and then spread evenly in the experimental plots. The fine sand used was sieved through 1mm, soaked in dilute hydrochloric acid for 2d, washed with water, placed in an oven at 105℃, and then dried for 48 hours before being taken out for spare.

After 6 years of simulated nitrogen deposition test (nitrogen addition), soil samples were collected in 380 experimental plots in the first half of August 2014 by random sampling method, 5 sample points were collected in each plot, and then mixed to make 1 sample, the sampling depth was 0-10cm, and a soil auger with an inner diameter of 5cm was used to take the samples, and a total of 200g of soil was collected.Soil samples were placed in a sealed bag to dry naturally after collection, and after drying, the soil was removed. After air-drying, the root system and withered material were removed from the soil, part of which was sieved through a 2mm sieve, and the other part was ground with a ball mill and prepared for use.

3.2 Measurement methods

3.2.1. Determination of physical and chemical properties of soil. The air-dried soil samples were sieved through 2 mm sieve and deionized water were mixed with soil-water ratio of 2:5 (w/v) for 30 min, and then left to stand. The total nitrogen and total sulfur of the soil were determined by an elemental analyzer, and the effective phosphorus of the soil was determined by molybdenum antimony antimony colorimetric assay after leaching with 0.5 mol/L sodium bicarbonate solution.

3.2.2. Determination of leaf nutrient content. A portion of the collected rice leaves was first killed at 105°C for 30 min and then dried at 75°C until constant weight. The leaf samples were ground into powder using a high-speed pulverizer for leaf nutrient content determination. Leaf total nitrogen and total sulfur were determined by elemental analyzer, and total phosphorus was determined by concentrated sulfuric acid-hydrogen peroxide ablation method and molybdenum antimony antimony colorimetric method.

3.2.3. Soil and Interleaf Microbial DNA Extraction. Rice leaf samples were collected at different levels of nitrogen application to elute the interleaf microorganisms from the rice leaf surface. The oscillated phosphate buffer was then filtered using a vacuum filtration device, and the interleaf microorganisms were collected onto a 0.22 μm filter membrane, which was sheared with sterile scissors, and the interleaf microbiome DNA was extracted using the MP FastDNA® Spin Kit.0.5000 g of a fresh non-root soil sample was weighed, and the same kit was used to extract the non-root soil microbial DNA, and all of the DNA samples were stored at -20°C.

3.2.4. Microbial 16S rRNA and ITS high-throughput sequencing. The V5-V7 variable regions of bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR), and the fungal ITS1 region was amplified and sequenced in high throughput on the Illumina platform.

3.2.5. Determination of functional genes. High-throughput fluorescence quantitative PCR (HT-qPCR) method was used to detect the functional genes of microbial nitrogen, phosphorus and

sulfur cycling in combination with SmartChip.The amplification process of HT-qPCR was as follows: pre-denaturation at 95°C for 10 min, followed by denaturation at 95°C for 30 s, annealing at 58°C for 30 s for a total of 40 amplifications, and finally, extension at 72°C for 30 s, and the extension in the 72°C stage was kept for 5min.

3.3 Data analysis

Differences in soil phosphorus and sulphur among treatments were compared using one-way ANOVA, and the significance of differences among different nitrogen addition gradients was compared using Duncan's method of multiple comparisons, with a significance level of $P<0.05$. Structural equation modeling (SEM) was constructed using SPSS AMOS 24 to investigate direct and indirect influences on the interleaf microbial community, phosphorus and sulphur cycling functional genes, and pathogenic bacteria.

3.3.1. Structural equation modeling. Structural equation modeling (SEM) is a statistical method for analyzing the relationships between variables based on their covariance matrices and is an important tool for multivariate data analysis. It is used to analyze the interrelationships among variables and combines traditional path analysis and factor analysis.

Structural equation modeling consists of a structural model and a measurement model. The interrelationships between latent variables are reflected by the structural model with the following expression:

$$\eta = B\eta + \Gamma\xi + \zeta \tag{1}$$

Where, η is the endogenous latent variable, ξ is the exogenous latent variable, B is the coefficient array of the endogenous latent variable, Γ is the coefficient array of the exogenous latent variable, and ζ is the random perturbation term.

The relationship between the latent and observed variables is expressed by the measurement model with the following expression:

$$X = \Lambda x\xi + \delta \tag{2}$$

$$Y = \Lambda v\eta + \varepsilon \tag{3}$$

where X is the observed variable for ξ, Y is the observed variable for η, Λx and Λv are factor loading matrices, δ is the error term for X, and ε is the error term for Y.

3.3.2. Model analysis process:

1) Modeling. Structural equation modeling is based on structural equation modeling, based on different specialties and research purposes, the relationship between each variable is analyzed and tested with actual data.

2) Model fitting. The fitting of structural equation modeling is to try to approximate the covariance matrix to the sample covariance matrix. In the model,there are many parameter estimation problems,and each of them has its own advantages and uses. Based on this, a new parameter estimation algorithms such as generalized least squares and great likelihood methods are proposed. The most common method is parameter estimation.

3) Model Evaluation. Model evaluation refers to the comprehensive evaluation of the collected information and the preliminary constructed model according to various fitness indexes, in order to ensure that the constructed model conforms to both the reality and the theoretical requirements, and has a strong practical value. After the fitness test of the model, the various parameters obtained are evaluated to determine whether the fitness of the model is qualified. When evaluating the model fitness, GFI, AGFI, RMSEA, CMIN/DF, etc. are used as evaluation indexes for evaluation.

4) Model Correction. After parameter estimation of the model, if the theoretical model and the

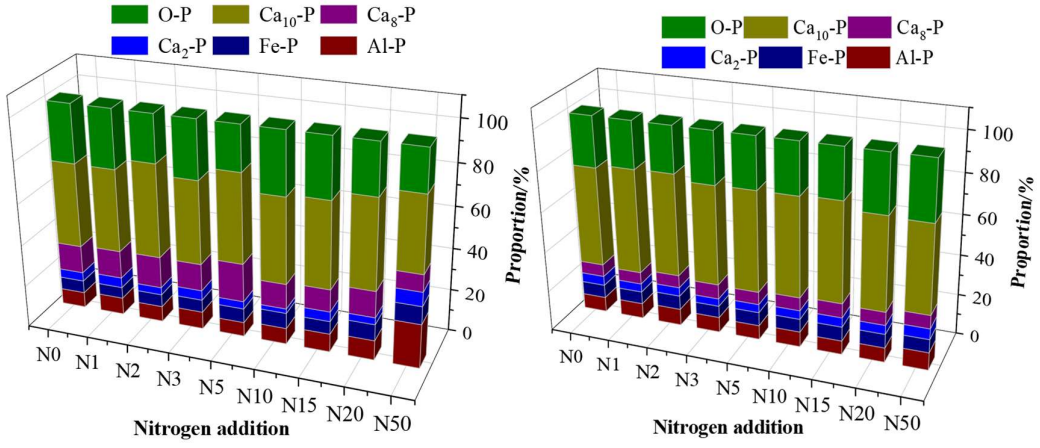
measured data are inconsistent, and the overall fitting index of the model cannot meet the requirements, then it needs to be adjusted accordingly to achieve a better matching effect. In order for the correction to be reasonably interpreted, there must be sufficient theoretical basis. When the model correction is carried out, the specific calculation results of the structural equation model can be referred to to modify the path relationship, increase the path relationship, improve the fitting degree of the model, and ensure that the final model is the best.

4. Experimental results and analysis

4.1 Effect of nitrogen addition on soil phosphorus and sulfur fractions

The aim of this section is to investigate the effects of different N application gradients, different N application frequencies and different grassland management practices on soil phosphorus fractions.

4.1.1. Effects on soil phosphorus fractions. The effects of nitrogen addition on the proportion of soil inorganic phosphorus fractions are shown in Fig. 1, (a) to (d) the results of NTF (sealing biannual nitrogen application), NTM (mowing biannual nitrogen application), NMF (sealing once a month nitrogen application) and NMM (mowing once a month nitrogen application). Ca10-P accounted for the largest proportion of inorganic phosphorus in each of the N addition treatments, followed by O-P. Al-P, Fe-P, Ca2-P, Ca8-P, Ca10-P, and O-P accounted for 9.42, 6.70, 4.21, 12.87, 41.01, and 25.79% of the total inorganic phosphorus in the soil of sealed biannual nitrogen application, respectively. The percentage of various inorganic phosphorus in mowed biannual nitrogen application, sealed monthly nitrogen application and mowed monthly nitrogen application soils were all not much different from that of sealed biannual nitrogen application. The more active and soluble inorganic phosphorus fractions in the soil accounted for a smaller proportion of the inorganic phosphorus, and the insoluble inorganic phosphorus fractions in the soil accounted for a larger proportion of the inorganic phosphorus, and the sealing treatment significantly increased the content of inorganic phosphorus fractions in the soil relative to the mowing treatment.



(a)NTF (b)NTM

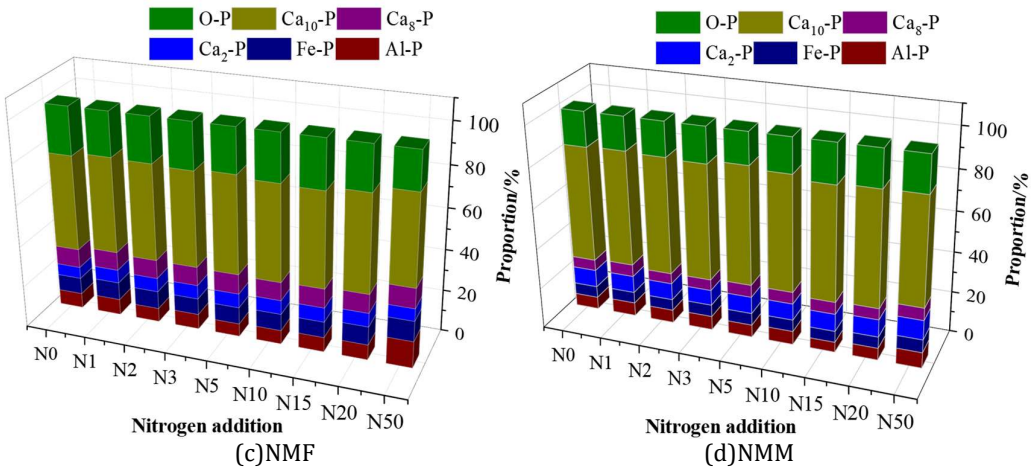


Fig. 1. Effects of nitrogen addition on proportion of inorganic P fractions

4.1.2. Effects on soil sulphur fractions. The sum of three forms of inorganic sulfur, water-soluble sulfur, adsorbed sulfur and insoluble sulfur is the total inorganic sulfur content in the soil solution, and the effect of nitrogen addition on total inorganic sulfur in the soil is shown in Fig. 2, with (a) and (b) indicating the results of the two cases of sealing and mowing, where different letters indicate significant differences between treatments ($P<0.05$), and F2 denotes biannual nitrogen application, and F12 denotes 12 times of nitrogen application in a year. Total inorganic sulphur was significantly higher in the N20 and N50 treatments with biannual N application in sealing and monthly N application in mowing, 6.31 and 5.85 mg/kg above the mean in sealing, and 3.99 and 4.37 mg/kg above the mean in mowing. Inorganic sulfur was not significantly different across the N addition gradient between monthly N application in closed and biannual N application in mowing, while no significant difference was observed in other N application gradients compared to control.

The effect of different N application frequencies on soil inorganic sulfur was significant in the closed treatment, and the two N application frequencies in the mowing treatment differed significantly at N1, N3, and N50. The rest of the treatments had no significant changes on inorganic sulfur changes, indicating that single application of nitrogen fertilizer, with no significant changes in inorganic sulfur at medium and high additions, could maintain the cycling and dynamic equilibrium of inorganic sulfur in the soil solution.

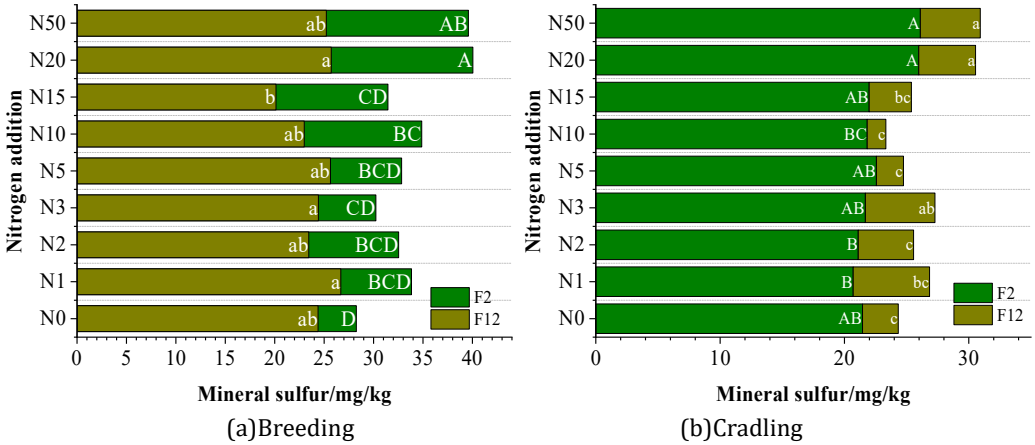


Fig. 2. Effects of nitrogen addition on soil total inorganic S

The effects of nitrogen addition on soil total sulfur are shown in Fig. 3, with (a) and (b) indicating the results under both sealing and mowing conditions. Soil total sulfur content did not respond significantly to the three influencing factors of simulated nitrogen deposition gradient, nitrogen

frequency, and mowing, and the simulated nitrogen deposition for 6 years had no significant effect on the total sulfur in the soil, indicating that a series of migratory transformation processes of sulfur existed in the soil solution, and that organic sulfur would be transformed to inorganic sulfur when there was a shortage of inorganic sulfur. Most of the sulfur in soil exists in organic form, and organic sulfur accounts for more than 97% of the total sulfur, while inorganic sulfur accounts for less than 3% of the total sulfur.

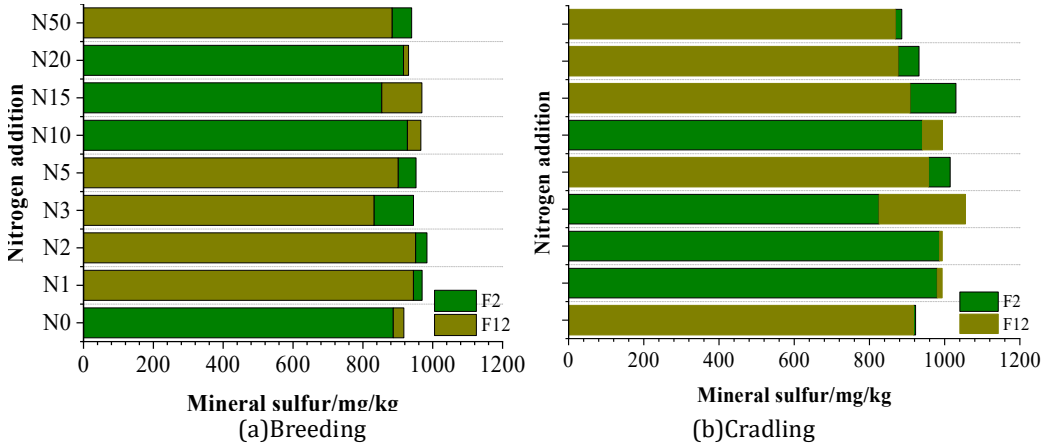


Fig. 3. Effects of nitrogen addition on soil total S

4.2 Dynamic impacts on the phosphorus-sulfur cycle

In order to further investigate the driving mechanisms of the interleaf microbial community and functional genes of nitrogen, phosphorus, and sulfur cycling, this study investigated the relationships between the interleaf microbial community and functional genes of the interleaf microbes and the soil physicochemical properties and microbial community under nitrogen fertilizer application using structural equation modeling (SEM). The results of the structural equation modeling of nitrogen addition and microbial action on phosphorus-sulfur cycling are shown in Table 1, with *, **, and *** denoting significance at the $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively. Overall, the model explained 57.7% of the changes in interleaf carbon, nitrogen, phosphorus, and sulfur functional genes under nitrogen fertilizer application.The SEM results indicated that nitrogen fertilizer application could indirectly affect the interleaf phosphorus and sulfur cycling functional genes by altering soil properties. In addition, the interleaf phosphorus-sulfur cycle functional genes were significantly and positively correlated with the interleaf diversity (Estimate=0.768, P=0.007), in which nitrogen fertilizer application could indirectly affect the phosphorus-sulfur cycle functional genes by influencing soil physicochemical properties and interleaf fungal diversity. Meanwhile, under N fertilizer application, the structure of the interleaf bacterial community was significantly affected by the structure of the interleaf fungal community (Estimate=0.443, P=0.031), which indirectly affected the interleaf phosphorus and sulfur cycle functional genes positively.

Table 1. The model results of P and S cycle by nitrogen and microorganism

Path	Estimate	R2	P
Nitrogen addition-->Interleaf bacterial diversity	0.116	0.185	0.128
Nitrogen addition--> Interleaf fungi diversity	-0.348	0.254	0.201
Nitrogen addition--> Soil bacterial diversity	-0.053	0.005	0.134
Nitrogen addition--> Soil fungi diversity	-0.328	0.124	0.217
Nitrogen addition--> Soil chemical properties	-0.476	0.235	0.024*
Soil bacterial diversity--> Interleaf bacterial diversity	-0.055	0.162	0.334
Soil bacterial diversity--> The community structure of the interleaved bacteria	0.255	0.116	0.205
Soil fungi diversity--> Interleaf fungi diversity	-0.104	0.254	0.347
Soil fungi diversity--> The community structure of the interleaved fungi	0.101	0.557	0.251
Interleaf bacterial diversity--> The community structure of the interleaved bacteria	-0.543	0.222	0.033*
Interleaf fungi diversity--> The community structure of the interleaved fungi	0.591	0.237	0.009**
Interleaf bacterial diversity--> P and S function gene	0.418	0.244	0.334
Interleaf fungi diversity--> P and S function gene	0.768	0.271	0.007**
Soil chemical properties--> Interleaf bacterial diversity	0.467	0.114	0.241
Soil chemical properties--> Interleaf fungi diversity	-0.529	0.105	0.015*
Soil chemical properties--> The community structure of the interleaved bacteria	0.308	0.122	0.313
Soil chemical properties--> The community structure of the interleaved fungi	-0.248	0.161	0.469
Soil chemical properties--> P and S function gene	-0.561	0.577	0.017*
The community structure of the interleaved bacteria--> P and S function gene	0.546	0.118	0.025*
The community structure of the interleaved fungi --> P and S function gene	-0.905	0.321	***
The community structure of the interleaved fungi --> The community structure of the interleaved bacteria	0.443	0.174	0.031*

In addition, another SEM was constructed in this study to investigate the relationship between interleaf potential plant pathogens and soil physicochemical properties and interleaf microbial communities under N fertilizer application. The results of the structural equation modeling are shown in Table 2. Overall, the model explained 31.5% of the variation in interleaf pathogens under nitrogen fertilizer application. This SEM result suggests that nitrogen fertilizer application can significantly affect the abundance of potential plant pathogens in the interleaf by altering the soil physicochemical properties and thus positively or negatively affecting the diversity of interleaf fungi and the structure of the interleaf fungal community.

Table 2. Model results of the structural equation

Path	Estimate	R2	P
Nitrogen addition-->Interleaf bacterial diversity	-0.081	0.017	0.182
Nitrogen addition--> Interleaf fungi diversity	-0.342	0.253	0.163
Nitrogen addition--> Soil chemical properties	-0.473	0.217	0.011*
Interleaf bacterial diversity--> The community structure of the interleaved bacteria	-0.814	0.803	0.027*
Interleaf fungi diversity--> The community structure of the interleaved fungi	-0.655	0.644	0.014*
Soil chemical properties--> Interleaf bacterial diversity	0.054	0.177	0.478
Soil chemical properties--> Interleaf fungi diversity	-0.542	0.081	0.578
Soil chemical properties--> The community structure of the interleaved bacteria	-0.022	0.091	0.224
Soil chemical properties--> The community structure of the interleaved fungi	0.287	0.182	0.043*
Soil fungi diversity-->Pathogens	0.351	0.315	0.208
The community structure of the interleaved bacteria--> Pathogens	-0.349	0.175	0.453
The community structure of the interleaved fungi --> Pathogens	-0.636	0.132	0.032*
The community structure of the interleaved fungi --> The community structure of the interleaved bacteria	-0.317	0.125	0.411

5. Conclusion

Soil microorganisms are the core and key to maintain soil health. This project analyzes the repair and improvement mechanism of soil microorganisms. Through experimental tests, the effect of nitrogen addition on phosphorus and sulfur components in soil is studied. And the structural equation modeling was applied to explore the dynamic effects of the interaction between nitrogen and microorganisms in soil on the phosphorus-sulfur cycle. The results showed that the proportion of each component in the inorganic phosphorus system was different: Ca10-P>O-P>Ca8-P>Al-P>Fe-P>Ca2-P, and Ca10-P accounted for a larger proportion of the inorganic phosphorus components, all of which were above 40%. The inorganic phosphorus components can be transformed into each other to maintain the dynamic balance of inorganic phosphorus components in the soil solution. Soil inorganic sulfur in the sealing treatment responded significantly to different N application frequencies, soil total inorganic sulfur and total sulfur were not significantly affected by N addition gradient, N frequency and different grassland management practices, within a certain period of time, the total total amount of total sulfur and inorganic sulfur would not change significantly within a short period of time, and the soil inorganic sulfur content was small, less than 3% of total sulfur. The structural equation model, with an overall explanatory rate of 57.7%, found that changes in soil physicochemical properties induced by nitrogen fertilizer application further affected phosphorus and sulfur cycling functional genes by altering plant nutritional status, which is closely linked to interleaf microorganisms. Interleaf fungal communities were significantly correlated with functional genes at different levels of N application, and the diversity and structural composition of interleaf fungal communities and their regulatory effects on bacterial communities indirectly affected interleaf phosphorus-sulfur cycle functional genes. Overall, changes in functional genes of the interleaf phosphorus-sulfur cycle can be driven by the combined effects of N addition and microbial communities such as interleaf bacteria and fungi.

Making full use of microorganisms to optimize the internal function of soil is important to maintain the sustainable development of soil ecosystem. In the future, multifunctional research on soil mineral ions and microbial actions should be strengthened to achieve precise regulation of ecological functions, pollution remediation functions, and immune functions of the soil microbiome to enhance soil health.

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