Correlation Model Analysis of Nitrogen Addition and Tan Sheep Grazing Effects on Soil Bacterial Community in the Loess Plateau , China

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***Abstract*—In recent years, the rapid development of high-throughput sequencing technologies and bioinformatics methods has greatly facilitated the monitoring of soil microbial diversity. Monitoring microbial diversity in soil could lead to a number of applications for example a better understanding of the characteristics of spatial and temporal variation of microbial community in soil and the identification of the driving mechanisms of soil microbial composition, diversity, and functional genes in different ecosystems, such as forests, grasslands, wetlands, and farmland. However，there are still no universally adaptable models to explain the relationships between microbial diversity change and ecosystem functions. This study is based on the special geographical environment of the Loess Plateau. Control experiments for grazing and nitrogen addition were set up and , through to analysis soil bacterial community was analyzed through high-throughput sequencing technology. A joint model of soil bacterial community diversity, soil physical and chemical properties, and human disturbance such as grazing has been established. The results show that grazing and nitrogen addition have different effects on soil bacterial community diversity. Within a certain range, nitrogen addition can balance the effects of grazing.**

Keywords—Loess Plateau; grazing; nitrogen addition; high-throughput sequencing; soil bacterial community diversity; structural equation model

I. INTRODUCTION

Soil microbes are one of the most diverse and species-rich biotas on Earth and a key driver of biogeochemical processes [1], [2]. Soil microbial diversity including species diversity and genetic diversity, and the formation of them is influenced by community succession evolution and geographic distribution factors [3], [4], and threatened by natural and human activities [5]. Revealing the mechanisms and spatial-temporal distribution patterns of the diversity within and between species plays an important role in understanding of the structures and functions of underground ecosystems and protecting4 microbial diversity resources.

Compared with plant and animal research, recent years have seen a growing interest in investigating microbial diversity in soil. The rapid development of high-throughput sequencing technology and bioinformatics methods has greatly promoted the depth and breadth of soil microbial diversity monitoring research. A number of research projects in soil biology have been carried out in various countries around the world, and many new research methods have been developed to give people a deeper understanding of the composition and energy diversity of soil microbes and their temporal and spatial distribution [6]. The Global Soil Biodiversity Initiative (www.globalsoilbiodiversity.org), which began in 2011, aims to promote understanding of soil biodiversity and soil ecosystem services, and provide a scientific basis for developing environmental policies. Studies of soil bacterial diversity in North and South America have shown that soil pH is the most important environmental factor affecting bacterial diversity and abundance on a large scale [7]. At different geographic scales, the key drivers of soil microbial diversity change will be different, showing regional dependence. As in the arid and semi-arid grassland soils of western China, the soil bacterial diversity and composition are mainly affected by the degree of drought. Meanwhile, the climate and geographical distance also affect the biogeographical distribution pattern of bacterial communities [8]. Soil microbial diversity monitoring was carried out in the Qinghai-Tibet Plateau and the Gongga Mountain. The study revealed the response patterns of different bacterial groups to nitrogen deposition [9] and warming [10]. It is very attractive to carry out microbial diversity monitoring on the Loess Plateau where soil erosion is severe.

Main landform structure of the Loess Plateau is composed of large thickness and wide area loess that is an aeolian sediment formed by the accumulation of wind-blown silt. As the soil is loose and the vertical joints are developed, it is easy to seep. Because the loess has weak anti-erosion ability, coupled with unreasonable human use, vegetation degradation and soil erosion in this area are increasingly serious [11], and the Loess Plateau has become one of the regions with the lowest nitrogen storage in China [12]. Studies on soil erosion in the Loess Plateau have shown that excessive and unreasonable human activities are the main factors that aggravate loss of soil and nutrient and promote grassland primary productivity decline [13]. Long-term overgrazing leads to a reduction of grassland vegetation coverage and species diversity. Moreover, overgrazing will reduce the primary productivity of the ecosystem and destroy the maintenance of soil nutrients. In recent years, due to the serious degradation of grassland, exploring the mechanism of grassland degradation and ecological restoration has become a research hotspot of grassland ecology. Soil microbes regarded as sensitive indicators for evaluating ecosystem functions and ecological health [14], can highlight the changes in the process, function, and environment of the grassland ecological ecosystems timely. So research on the effects and mechanisms of grazing on soil microbial community

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composition has important theoretical and practical implications for grassland ecosystem protection, restoration, and reconstruction.

Overgrazing could cause the degradation of natural grassland resources and reduces the biodiversity of ecosystems while inhibiting the circulation of nitrogen, such as the gradual reduction of species with high nitrogen contents. The current research shows that the reasonable addition of nitrogen and moisture can greatly improve the physical and chemical properties of the soil，leading to the restoration of degraded grassland. In this study, we conducted a controlled nitrogen addition-grazing experiment to study changes in the response of soil bacterial community structure characteristics to grazing and nitrogen addition. Soil samples were collected across a period of times and 16s high-throughput sequencing technology was used to analyze the abundance and diversity of soil bacterial communities, and a structural equation model was established to explain the relationships between grazing， nitrogen addition and soil bacterial communities.

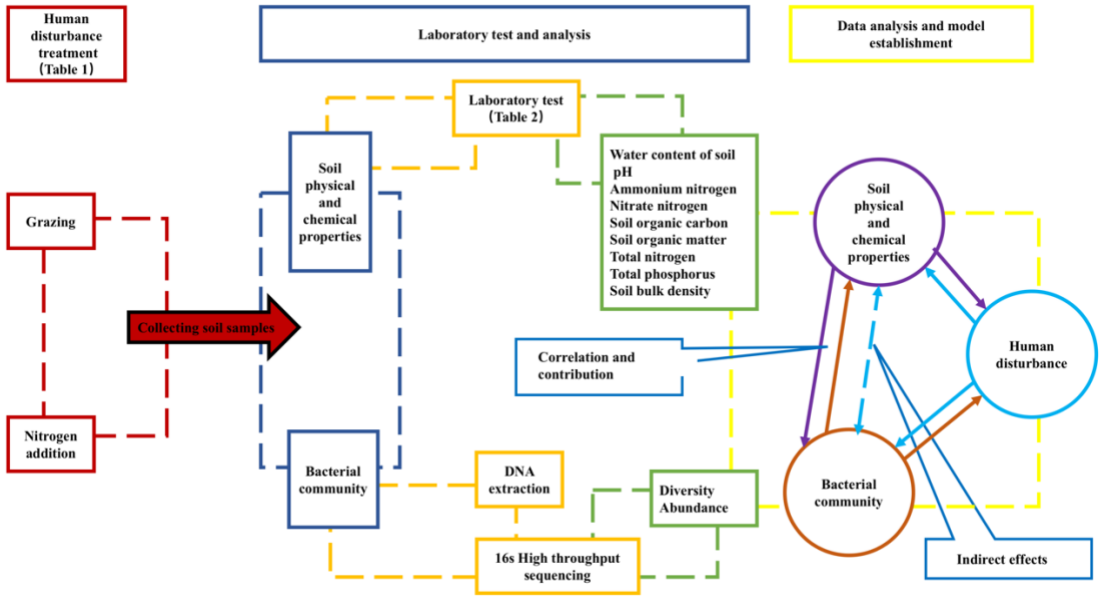

Fig. 1. An illustration of the framework used in this study

II. Materials and Methods

The overall framework used in this study is illustrated in Fig. 1.

A. Study Site and Sampling

The study site is located in Huan County, Gansu province, LongDong on the Loess Plateau of China (37.12°N, 106.82°E). Since 2001, the summer pastures have been grazing from June to September each year, with a rotational grazing cycle of 30 days and a grazing interval of 20 days. The rotational grazing cycle repeated three times each year. In 2012-2017, four 2m × 2m test plots were randomly placed in each of the three grazing rates (2.7 sheep/ha, 5.3 sheep/ha and 8.7 sheep/ha). Each plot is divided into 2 split zones, one of which is enclosed (five years of enclosure), and the other (12 years of grazing) is still grazing by the Tan-sheep（Table 1）. The sheep used for the grazing all have similar body condition and age. The NH_4NO_3 was used as a nitrogen supplement which was dissolved in water and sprayed evenly on the plot. The amount of water is equivalent to 1*mm* of rainfall, and the nitrogen addition levels are 0 (water only), 50, 100, 200kg/ha; In 2012-2017, nitrogen was added twice a year before grazing and after grazing （May and July）respectively.

TABLE I. Sample Composition Collected on The Loess Plateau of China

		Grazing					
		2.7 sheep/ha		*5.3 sheep/ha*		*8.7 sheep/ha*	
		grazing	*5 years enclosure*	*grazing*	*5 years enclosure*	*grazing*	*5 years enclosure*
Nitrogen addition	***0 g/m²***	3	3	3	3	3	3
	5 g/m²	3	3	3	3	3	3
	10 g/m²	3	3	3	3	3	3
	20 g/m²	3	3	3	3	3	3

[a.] Numbers in the table indicate the number of samples collected under different treatments.

TABLE II. SOIL PHYSICAL AND CHEMICAL PROPERTIES DETERMINATION METHOD

Soil physical and chemical indicators	Method
The Water content of soil (WC)	Aluminum box method [15]
Soil bulk density(SBD)	Cutting ring method [16]
pH	pH meter （soil: water =1:5）[17]
Ammonium nitrogen(NH_4^+)	Spectrophotometer colorimetry [18]
Nitrate nitrogen (NO_3^-),	UV spectrophotometry [19]
Total nitrogen (TN)	Kjeldahl determination [20]
Total phosphorus(TP)	Molybdenum antimony colorimetric method [21]
Soil organic carbon (SOC)	Potassium dichromate-sulfuric acid external heating method [22]

B. Soil Physical and Chemical Properties Analysis

0-10cm soil samples were collected in each experimental plot to determine the physical and chemical properties of the soil which were selected basic earth element of soil microbial living environment[23]. They include Water content of soil (WC), pH, Ammonium nitrogen (NH_4^+), Nitrate nitrogen (NO_3^-), Soil organic carbon (SOC), Soil organic matter (SOM), Total nitrogen (TN), Total phosphorus (TP), and Soil bulk density (SBD) as listed in Table 2.

C. DNA Extraction and Sequencing Analysis.

Soil samples were stored in a refrigerator at -40℃ for 16s high-throughput sequencing. DNA was extracted from 0.5 g of soil using a Fast DNA spin kit for soil (MP Biomedical, Carlsbad, CA, USA) following the manufacturer's instructions. DNA quality assessment and quantification were conducted using a Nano-Drop ND-1000 Spectrophotometer (Nano Drop Technologies Inc., Wilmington, DE, USA). Then, the DNA extractions were diluted to 10 ng/*u*L and stored at –80◦ C. The extracted DNA was amplified with bacterial specific forward 515F and reverse 806R. The PCR product was detected by electrophoresis using a 2% agarose gel. The samples were mixed according to the concentration of the PCR product. After thorough mixing, the PCR product was purified by electrophoresis using a 1×TAE concentration of 2% agarose gel band. The product purification kit uses the Thermo Scientific GeneJET Glue Recovery Kit to recover the product. The library was constructed using Thermofisher's Ion Plus Fragment Library Kit 48 rxns library. The constructed library was subjected to Qubit quantification and library testing and then sequenced using Thermoscher's Ion S5TMXL.

D. OTU clustering and species annotation

Cutadapt [26] was used to cut the low-quality parts of the reads, and then separate the sample data from the obtained reads according to the barcode. Then the raw reads were obtained by cutting off the barcode and the primer sequence. The processing of the reads sequence is performed by comparing the chimeric sequences with the species annotation database [27]. The final clean reads were derived by removing the chimeric sequences therein [28].

The Uparse software clusters all Clean Reads for all samples were clustered by using Uparse software [29]. The sequences were then clustered into OTUs (Operational Taxonomic Units) with 97% identity. At the same time, according to the principle of the algorithm, the sequence with the highest frequency of occurrence in the OTUs is screened as a representative. Species annotation of OTUs representative sequences based on the Mothur method and SILVA's SSUrRNA database[30](set threshold of 0.8~1).

E. Community analysis and model building

The diversity of a community was estimated using Shannon and Simpson indices. The Chao1 and abundance-based coverage estimators (ACE) were used to describe community openness. The mathematical representation of these indices were given below. The calculation of these indices was done using the Qiime software (Version 1.9.1)[24][25]. Alpha diversity index analysis was performed using R software on both parametric and nonparametric tests.

Community diversity:

Shannon-Wiener diversity index is defined as:

$$H = -\sum_{i=1}^{S}(p_i \log_2 p_i) \quad (1)$$

Simpson's index is defined as 1 - dominance:

$$1 - \sum p_i^2 \quad (2)$$

Community richness:

Chao1 richness estimator. The bias-corrected version is defined as:

$$chao1 = S_{obs} + \frac{F_1(F_1-1)}{2(F_2+1)} \quad (3)$$

where F_1 and F_2 are the count of singletons and doubletons, respectively.

The ACE metric is defined as:

$$S_{ace} = S_{abund} + \frac{S_{rare}}{C_{ace}} + \frac{F_1}{C_{ace}}\gamma_{ace}^2 \quad (4)$$

where S_{abund} is the number of abundant OTUs (with more than rare threshold individuals) when all samples are pooled, S_{rare} is the number of rare OTUs (with less than or equal to rare_threshold individuals) when all samples are pooled, C_{ace} is the sample abundance coverage estimator, F_1 is the frequency of singletons, and γ_{ace}^2 is the estimated coefficient of variation for rare OTUs. The estimated

coefficient of variation is defined as (assuming rare threshold is 10, the default):

$$\gamma_{ace}^2 = max\left[\frac{S_{rare}}{C_{ace}}\frac{\sum_{i=1}^{10} i(i-1)F_i}{(N_{rare})(N_{rare}-1)} - 1,0\right] \quad (5)$$

Interaction effect analysis method: General Regression Models (GLM) was used to explore the interaction effect between factors of Two-factor interaction test. For the dependent variable *Y*, there are 2 independent variables X_1 and X_2. In order to analyze the interaction of the two independent variables, a regression model with an interaction term (X_1X_2) can be constructed as depicted in equation (6).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \varepsilon \quad (6)$$

Y is the dependent variable; X_1 and X_2 are the main effect terms; X_1X_2 is the interaction term; β_1, β_2 and β_3 are variable coefficients; ε is a random error. The null hypothesis is H_0 : $\beta_3 = 0$, a t-test is performed on the null hypothesis to judge whether the interaction effect of X_1 and X_2 is significant ($\beta_3 \neq 0$, the interaction effect is significant). To establish a statistic for the H_0 test, the sum of the squared deviations is decomposed into the sum of the squares of the regression and the sum of the squares of the residuals.

$$S_T = \sum_{i=1}^{n}(y_i - \overline{y})^2 = \sum_{i=1}^{n}(\acute{y}_i - \overline{y})^2 + \sum_{i=1}^{n}(y_i - \acute{y}_i)^2 \quad (7)$$

Corresponding to the dependent variable *Y*, y_i is the real number of the dependent variable *Y* at the *i* level; $\bar{y}$ is the mean of the population; $\acute{y}_i$ is the estimated value at the *i* level. Build function $F = \frac{\sum_{i=1}^{n}(\acute{y}_i - \overline{y})^2/P}{\sum_{i=1}^{n}(y_i - \acute{y}_i)^2/P} \sim F(p, n-p-1)$, for a given significance level α, when the calculated *F* value satisfies $F > F_\alpha(p, n-p-1)$, H_0 is negative, and it is considered that there is significant linearity between the dependent variable and the independent variable at the significance level α.

Structural Equation Modeling, SEM: The measurement equation is used to describe the relationship between the indicator and the latent variable, and is represented by the following model:

$$\begin{cases} X_m = A_x\xi + \delta \\ Y_n = A_\gamma\eta + \varepsilon \end{cases} \quad (8)$$

$X = (x_1, x_2, x_3, \ldots\ldots, x_m)^T$ is a column vector composed of *m* exogenous indicators; $\xi = (\xi_1, \xi_2, \xi_3, \ldots\ldots, \xi_u)^T$ is a column vector composed of *u* exogenous latent variables; A_x is a matrix of $m \times u$ dimensions, as the factor load matrix of *X* on ξ, describe the relationship between exogenous indicators and exogenous latent variables; $\delta = (\delta_1, \delta_2, \delta_3, \ldots\ldots, \delta_m)^T$ is the *m*-dimensional error term column vector, $Y = (y_1, y_2, y_3, \ldots\ldots, y_m)^T$ is a column vector composed of *n* endogenous indicators; $\eta = (\eta_1, \eta_2, \eta_3, \ldots\ldots, \eta_v)^T$ is a column vector consisting of v endogenous variables; A_γ is a matrix of $n \times v$ dimensions, as the factor load matrix of *Y* on η, describe the relationship between endogenous indicators and endogenous latent variables; $\varepsilon = (\varepsilon_1, \varepsilon_2, \varepsilon_3, \ldots\ldots, \varepsilon_m)^T$ is the error term column vector of the dimension

The structural equation（Fig.2）used to describe the relationship between exogenous latent variables and endogenous latent variables was expressed by the following model: $\eta = \beta_{vu}\eta + \kappa_{vu}\xi + \gamma.\beta$ is $v \times u$ dimensional matrix describing the relationship between endogenous latent variables; κ is $v \times u$ dimensional matrix describing exogenous latent variables; $\gamma = (\gamma_1, \gamma_2, \gamma_3, \ldots\ldots, \gamma_m)^T$ is a *v* dimensional structural model residual term column vector, explain the part of the model that is not explained by η.

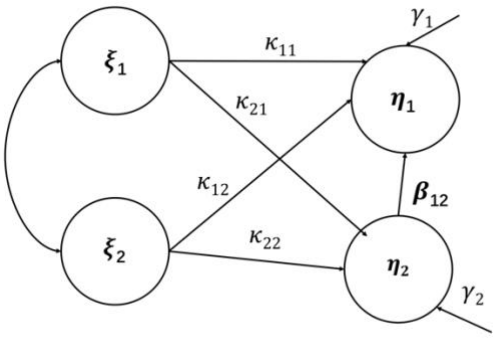

Fig. 2. Structural model

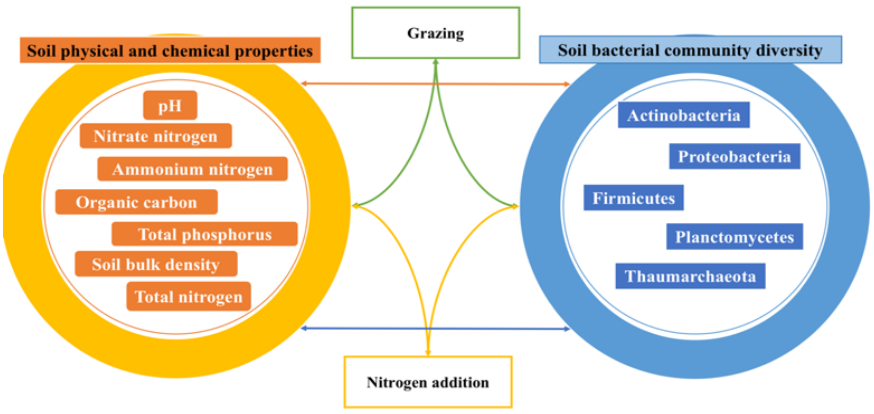

Fig. 3. Structural equation model framework

III. RESULTS

A. *Effects of nitrogen addition and grazing on soil physicochemical properties*

Grazing or nitrogen addition often affects soil bacterial community indirectly by affecting soil physical and chemical properties. This study analyzed soil physicochemical properties (Table III). Grazing significantly (p<0.05) affected soil water content(WC) and Soil total phosphorus(TP). Nitrogen addition significantly (p<0.05) affected soil nitrate nitrogen content(NO_3^-). The interaction between grazing and nitrogen addition significantly (p<0.05) affected soil nitrate nitrogen content(NO_3^-). Grazing, nitrogen addition, and the interaction between them have no significant (p>0.05) effects on soil total nitrogen(TN), soil organic carbon(SOC) and ammonium nitrogen（NH_4^+）.

TABLE III. ANALYSIS OF MAIN EFFECTS AFFECTING SOIL PHYSICAL AND CHEMICAL PROPERTIES

	Soil Physicochemical Properties	Contribution rate	df	F	Sig.
Grazing	WC	44.46%	3	15.498	0.000***
	pH	2.63%	3	0.605	0.614
	NH_4^+	1.31%	3	0.305	0.822
	NO_3^-	0.98%	3	1.216	0.313
	SOC	1.51%	3	0.325	0.807
	SOM	1.51%	3	0.326	0.807
	TN	8.69%	3	2.111	0.109
	TP	21.43%	3	6.202	0.001***
	SBD	6.99%	3	1.8	0.158
Nitrogen addition	WC	0.53%	3	0.198	0.897
	pH	4.99%	3	1.154	0.336
	NH_4^+	9.35%	3	2.18	0.101
	NO_3^-	62.40%	3	77.811	0.000***
	SOC	0.03%	3	0.007	0.999
	SOM	0.03%	3	0.007	0.999
	TN	6.42%	3	1.555	0.211
	TP	0.71%	3	0.391	0.76
	SBD	2.74%	3	0.679	0.569
Grazing*Nitrogen addition	WC	2.06%	9	0.243	0.986
	pH	9.81%	9	0.756	0.656
	NH_4^+	11.22%	9	0.873	0.555
	NO_3^-	5.42%	9	2.252	0.032***
	SOC	12.95%	9	0.93	0.507
	SOM	12.96%	9	0.93	0.507
	TN	11.66%	9	0.943	0.469
	TP	14.29%	9	1.439	0.194
	SBD	18.69%	9	1.586	0.143

a. "*" indicates significant p value <0.05，"**" indicates significant p value <0.01，"***" indicates significant p value <0.001. "df" is the degree of freedom of the F test, "F" is the F statistic of the F test, "Sig." is the p-value of F test. "Contribution rate" is calculated by covariance analysis.

B. *Units Effects of nitrogen addition and grazing on bacterial community diversity*

Under the different grazing intensities, nitrogen addition has a significant (p<0.05) effect on the number of OTU sequences and α-diversity of the bacterial community, contributing about 20% (Table IV)；the grazing intensity at each nitrogen addition level has no significant (p>0.05) effect on the number of OTU sequences and α-diversity of bacterial communities. As shown in Table IV, the contribution derived from the interaction between grazing and nitrogen addition is greater than the grazing, but neither affects the α-diversity of bacterial community structure significantly.

TABLE IV. ANALYSIS OF MAIN EFFECTS AFFECTING SOIL BACTERIAL COMMUNITY DIVERSITY

	Index	Contribution rate	df	F	Sig.
Grazing	OTU	2.29%	3	0.711	0.549
	shannon	2.28%	3	0.716	0.547
	simpson	3.33%	3	0.667	0.576
	chao1	2.78%	3	0.75	0.527
	ACE	2.62%	3	0.696	0.558
Nitrogen addition	OTU	26.20%	3	8.124	0.000***
	shannon	25.38%	3	7.968	0.000***
	simpson	13.33%	3	3.657	0.017**
	chao1	18.15%	3	4.898	0.004**
	ACE	15.77%	3	4.192	0.010**
Grazing*Nitrogen addition	OTU	11.29%	9	1.167	0.334
	shannon	12.88%	9	1.348	0.234
	simpson	10.00%	9	0.834	0.588
	chao1	9.89%	9	0.889	0.541
	ACE	11.13%	9	1.012	0.442

a. "*" indicates significant p value <0.05，"**" indicates significant p value <0.01，"***" indicates significant p value <0.001. "df" is the degree of freedom of the F test, "F" is the F statistic of the F test, "Sig." is the p-value of F test. "Contribution rate" is calculated by covariance analysis.

C. *The relationship between the shift in bacterial community composition and environmental variables*

Spearman rank correlation was used to study the relationship between environmental factors and microbial species richness (α-diversity) as shown Fig.4(A). There was a significant (p<0.05) negative correlation between nitrate nitrogen (NO_3^-) and OTU sequence number, and soil organic carbon (SOC) significantly (p<0.05) affected sample sequence coverage. VPA(variance partial analysis) indicated that nitrogen addition explained 2.5% of community changes; unexplained proportion reached 77.73% Fig.4(B). At the Phylum level shown in Fig.4(C), it can be seen that nitrate nitrogen has significant (p<0.05) correlation with Proteobacteria, Firmicutes, Planctomycetes, and Thaumarchaeota. Ammonium nitrogen(NH_4^+) has significant (p<0.05) positive or negative ?correlation with the abundance of Deinococcus-Thermus. Total nitrogen(TN) has significant (p<0.05) correlation with abundances such as Proteobacteria and Thermomicrobia, and total phosphorus(TP) has significant (p<0.05) correlation with abundances of Proteobacteria.

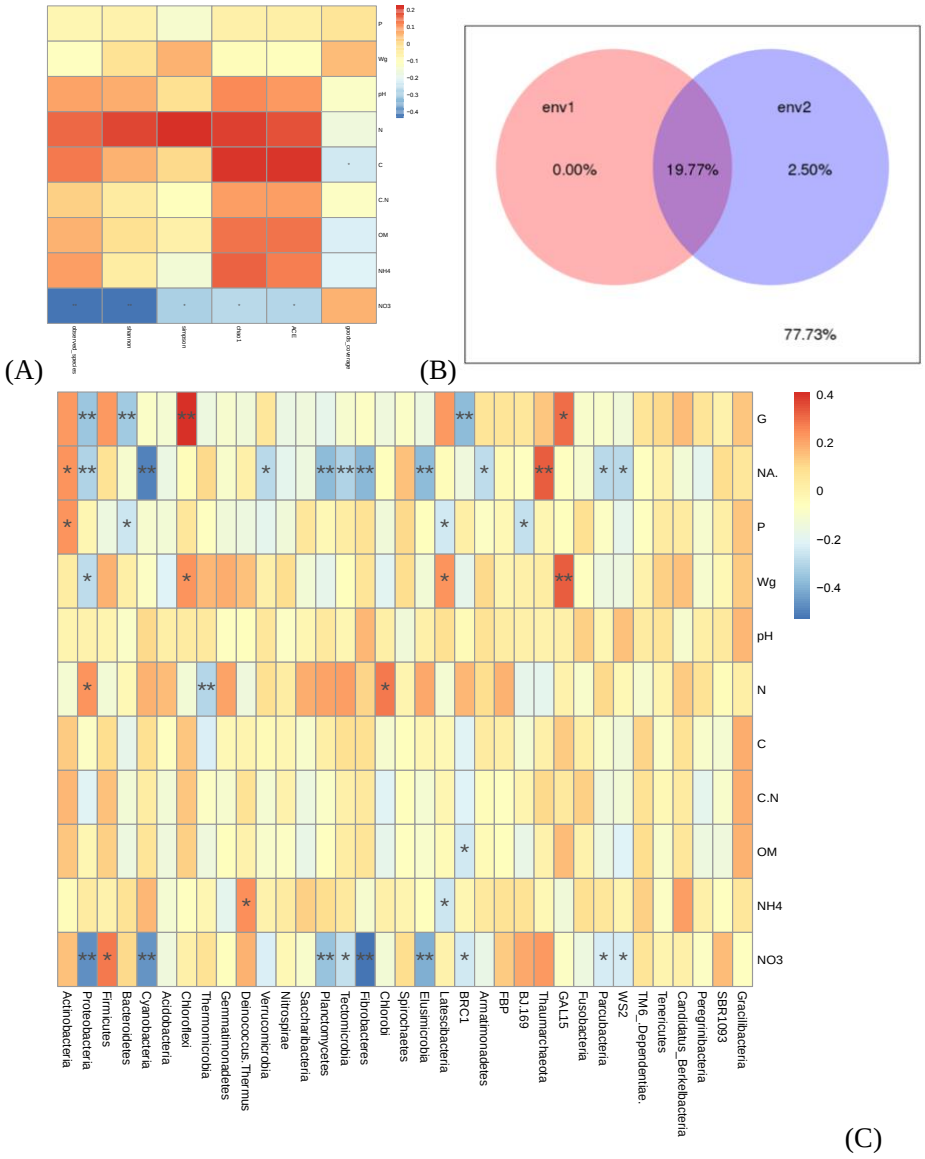

Fig. 4. (A)Environmental factor and alpha diversity related heatmap（*The longitudinal direction is the environmental factor information, the horizontal direction is the OTU sequence number and alpha diversity index, and the intermediate heat map corresponds to the Spearman correlation coefficient r, which is between -1,1, r<0 is a negative correlation, r>0 is a positive correlation, the"*"indicates significant p value <0.05，"**"indicates significant p value <0.01， (P=Total phosphorus,Wg=Water content,N=Total nitrogen,C=Carbon content,C.N= Carbon and nitrogen ratio,OM= Organic matter, NH_4^+= Ammonium nitrogen, NO_3^-= Nitrate nitrogen)*）(B)VPA analysis of bacterial communities by grazing and nitrogen addition (*env1=Grazing，env2=Nitrogen addition*) (C)Spearman correlation of environmental factors and phylum level species abundance heatmap（*The longitudinal direction is the environmental factor information, the horizontal direction is the species information, and the intermediate heat map corresponds to the Spearman correlation coefficient r, which is between -1,1, r<0 is a negative correlation, r>0 is a positive correlation, and the "*" indicates significant p value <0.05 ， "**" indicates significant p value <0.01，(G=Grazing，NA.= Nitrogen addition，P=Total phosphorus,Wg=Water content,N=Total nitrogen,C=Carbon content,C.N= Carbon and nitrogen ratio,OM= Organic matter, NH_4^+= Ammonium nitrogen, NO_3^-= Nitrate nitrogen)*）

D. *Structural equation model of the bacterial community by grazing and nitrogen addition*

This study established a structural equation model (R^2=0.21, Chi-square=35.78) to examine the indirect effects of grazing and nitrogen addition on soil bacterial community in terms of how soil physical and chemical properties were changed as illustrated in Fig.5. Grazing affects microbial diversity by affecting pH, nitrate nitrogen, ammonium nitrogen, organic carbon, total phosphorus, and soil bulk density, the significant indirect effect value is -0.048 (p<0.05). Nitrogen addition affected the microbial diversity through pH, nitrate nitrogen, organic carbon, and total phosphorus, the significant indirect value was 0.186 (p<0.05).

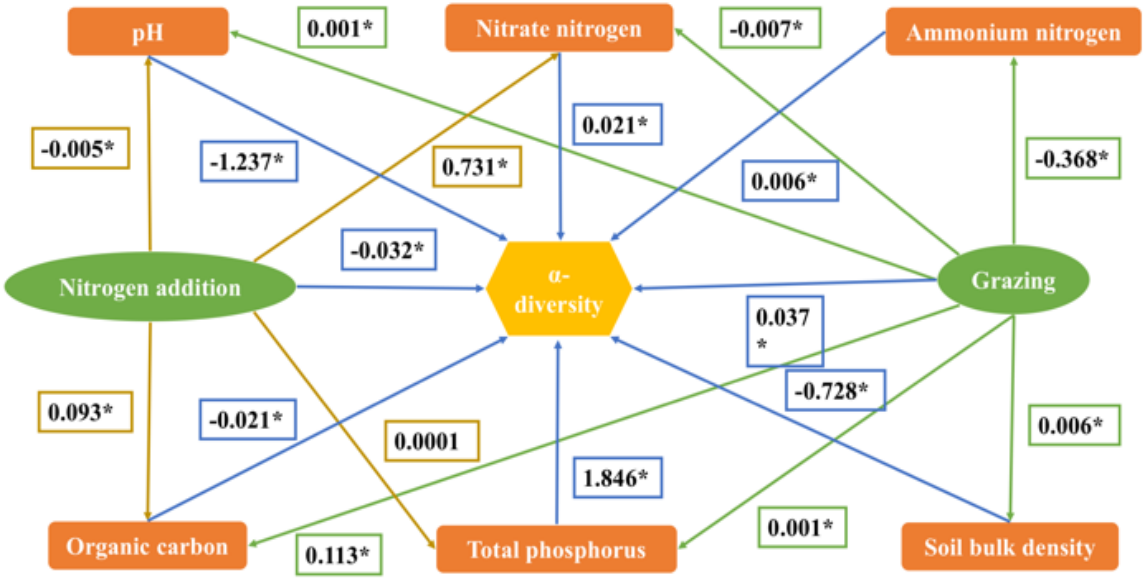

Fig. 5. Structural equation model for the response of soil bacterial community diversity*(the number in the box indicates corresponding path coefficient，"*" indicates significant p value <0.05)*

IV. Conclusion

The response of bacterial diversity to ecosystem changes has not been systematically addressed with multitudinous research subjects[31][32], although many studies have demonstrated that species diversity is closely related to ecosystem functions and biogeochemical processes [33]. It has been suggested that the relationship between disturbance, biodiversity, and ecosystem function are complicated, and a universally applicable model remains elusive [34]. In order to establish monitoring and management of soil microbial diversity, this study aims to construct a correlation model between human disturbance and bacterial community diversity, such as grazing and nitrogen addition, by using a structural equation model. The following conclusions have been obtained. Nitrogen addition significantly and positively (p<0.05) changes soil bacterial community diversity through changes in soil physicochemical properties. Grazing has a negative and significant (p<0.05) indirect effect on microbes; and the effect of nitrogen addition is greater than grazing. Finally, it has been shown that nitrogen addition significantly (p<0.05) changes soil bacterial community diversity by affecting the nitrate nitrogen content, which is closely related to the abundance of many species. This effect is weakened to a certain extent by grazing, but the grazing behavior itself does not bring about significant changes. The mechanism of microbial function operation behind these phenomena deserves further investigation and will be a very meaningful research direction in the future.

In this study, structural equation model was used to analyze the response of bacterial community in soil. However, the structural equation model is a kind of confirmatory analysis in which hypothetical variables are derived from normal distribution, rather than exploratory analysis. Thus the analysis limited by how the researcher organize the model structure and the sample size. Establishing a universally adaptive model to study the relationship between soil microbial diversity and ecosystem functions for predicting the evolution of soil microbes under changing global environmental conditions would be another direction for our future work.

V. Acknowledgment

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