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主编点评文章

Molecular ecological network analyses revealing the effects of livestock grazing on soil microbial community in the Tibetan grassland

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Abstract: [Objective] To unveil effects of livestock grazing in Tibet on microbial interaction at the functional gene level. **[Methods]** We applied a recently developed network inference tool (Random Matrix Theory-based molecular ecological network) on GeoChip data related to carbon (C) and nitrogen (N) cycling with or without livestock grazing. **[Results]** C and N cycling gene networks in both control and grazing conditions had topological features of scale-free, small-world, modularity and hierarchy. Key genes in the grazing networks (hubs and connectors) differed substantially from those in the control. The grazing effects on soil microbial interactions were revealed by smaller, denser networks in the grazing samples, suggestive of environmental stress. In support of close linkages between aboveground plants and microbial community at this site, aboveground plant biomass was significantly (P=0.001) linked to grazing network topology. **[Conclusion]** Livestock grazing significantly altered microbial interaction at the functional gene level.

Keywords: Molecular ecological network, Soil microbial interactions, Functional genes, Grazing in Tibet, C and N cycling, GeoChip

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基于分子生态学网络探究西藏草地放牧对土壤微生物群落的影响

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摘 要:【目的】揭示西藏地区放牧对在功能基因层面上的微生物相互作用的影响。【方法】利用最近发明的网络工具(基于随机矩阵理论的分子生态学网络)分别在对照和放牧条件下分析基因芯片中碳循环和氮循环基因。【结果】碳和氮循环基因网络在对照和放牧条件下都具有无标度、小世界、模块性和层次性的拓扑学特征。放牧条件下的网络关键基因(模块枢纽和连通者)与对照显著不同。放牧导致网络变得小而紧密,暗示环境压力的存在。地上植物生物量与微生物基因网络显著相关(P=0.001),证实了研究样地地上植物与地下微生物紧密相连。【结论】放牧显著改变了西藏草地在功能基因层面上的微生物相互作用关系。

关键词: 分子生态学网络,土壤微生物相互作用,功能基因,西藏放牧,碳氮循环,基因芯片

1 Introduction

Livestock grazing is a world-wide anthropogenic activity with significant impacts on economy and environment, such as plant diversity and species composition[1-2], spatial and temporal dynamics of soil nutrient availability[3], and ecosystem functions[4-5]. It is the dominant land use activity in the Tibetan grassland, which has a very fragile ecosystem that was difficult to restore when damaged[6-10]. With the increase of residence and domestic livestock in the Tibetan plateau, the grazing pressure on grasslands has increased accordingly[11], necessitating the assessment for its environmental impacts.

A number of studies have been conducted to analyze effects of grazing on aboveground vegetation and soil nutrition cycling in the Tibetan grassland. Grazing was shown to increase average soil temperatures[12], decrease vegetation canopy height[12-13], decrease grassland yield and litter[13-14], change vegetation composition by decreasing palatable grass species[13], increase standing death quality[12] and soil dissolved organic C in different depths[12,15], and modify C and N cycling by increasing soil inorganic N and decreasing soil organic N. By contrast, depsite the central role of microbial communities in driving biogeochemical cycling[16], the effects of grazing on soil microbial communities were poorly documented, which was partially due to their high complexity as well as technical barriers in analyzing microbes[17-19]. To tackle it, we have recently used a high-throughput metagenomics tool named GeoChip to examine the response of microbial functional composition to grazing in the Tibetan grassland[20]. We found that grazing altered microbial community composition, increased microbial functional potentials in N mineralization and nitrification but decreased those in denitrification. We also found that the presence of livestock increased soil pathogen and virulence in the grassland.

Understanding interactions among different microbes and their responses to environmental perturbation is a central goal in microbial ecology. However, it is challenging because of lack of theoretical framework and experimental data. Recently, several studies investigated microbial interactions by the Bayesian approach[21], the effective metabolic

overlap algorithm based approach[22] and co-occurrence networks[23-24]. In addition, we have developed a Random Matrix Theory-based algorithm to construct microbial association networks named molecular ecological networks[25-27]. Statistical analyses showed that the approach was reliable, sensitive and robust in withstanding noise inherent in high-throughput data set[27].

Here we report a network analysis of existing GeoChip data to compare microbial interactions at the functional gene level between samples collected from grazing sites and their controls. Since the whole dataset of GeoChip goes beyond the computational capacity of network reconstruction, we focus on important subsets of GeoChip, namely, functional genes involved in C and N cycling. Accordingly, we constructed four molecular ecological networks to analyze the overall topological characteristics and important network nodes. To our knowledge, this is the first network study to reveal the grazing effects on microbial interactions at the functional gene level in Tibetan grasslands.

2 Materials and Methods

2.1 Experimental sites and GeoChip data collection

Experimental sites were located at the Haibei Alpine Meadow Ecosystem Research Station in a Tibetan grassland (37.37°N, 101.12°E). We were granted permission to conduct the study at the station by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. Historically, alpine grasslands at the elevation of 3 600 m and 3 800 m above the sea level have been exposed to free livestock grazing during the growth season (from April to September) on the yearly basis. To create controls for the grazing study, six sites (Three sites at 3 600 m and three at 3 800 m, respectively) of 1 m×1 m size have been fenced since May 2006 to prevent grasslands from grazing. Samples were collected in August 2009. For control sites, five soil cores with 1.5 cm diameter at the depth of 0−20 cm were collected at each fenced site and mixed into one composite sample. For the treatment of livestock grazing, samples were collected in the unfenced area of 1 m×1 m size with the same procedure. Soil sample transport, storage, DNA extraction, purification, GeoChip hybridization and scanning were described in our recent publications[20,28-29].

2.2 GeoChip data preprocessing

Spots with the ratio of signal to noise less than 2.0 were discarded. The signal intensity of detected gene was divided by the total signal intensity of each sample, followed by natural logarithm transformation. Only genes detected in all biological replicates were retained for network construction.

2.3 Network construction and visualization

Construction of molecular ecological networks was carried out in an open-access pipeline (http://ieg2.ou.edu/mena/) based on a Random Matrix Theory-based algorithm as previously described[25,27]. Thresholds in the network construction were automatically chosen, and module separation was based on the fast greedy modularity optimization[27,30].

2.4 Network analyses

In order to test the general network characteristics, random networks with the same number of nodes and links were constructed to compare with the original network, which was described previously[27]. Key genes, including module hubs and connectors, were identified by values of connectivity within module (Z_i) and connectivity among modules (P_i). Module hubs with Z_i values larger than 2.5 and P_i values smaller than 0.62 were more connected with nodes inside the module than nodes in other modules. Connectors with P_i values larger than 0.62 and Z_i values smaller than 2.5 served as linkers among different modules. Peripherals had Z_i values smaller than 2.5 and P_i values smaller than 0.62[25]. Cytoscape 3.1.1 was used to visualize networks.

Linkages between network topology and environmental variables were examined. Gene significance (GS) was defined as the square of Pearson correlation coefficient between relative abundance of genes in networks and environmental variables. Then Mantel tests were used to measure the correlation between GS and connectivity[27].

3 Results

3.1 General network characteristics

The network of a complex system usually possesses four topological characteristics of scale-free, small-world, modularity and hierarchy[31-32]. Scale-free means that the majority of nodes in a

network have limited connections with other nodes while a small number of nodes have many connections, which can be measured by how the connectivity of the network fits with a power law curve[25]. The correlation r^2 values of power-law for all of the four networks in our study ranged from 0.838 to 0.904 (Table 1), demonstrating that they were scale-free. Small-world means the majority of nodes can be connected by a small number of links[33-34], which is characterized by high clustering compared with random networks, and the logarithmic increase of the average shortest path length with the number of nodes[35]. Our results showed that clustering coefficient values of all four networks were clearly larger than those of random networks (Table 1), and average path distance of each network was positively correlated with the logarithmic values of the number of nodes (n=4, r=0.927, P=0.025), suggesting that all four networks were small-world. Modularity means that a module is comprised of nodes more closely connected with other nodes inside the module than connected with nodes outside the module. Our results revealed significantly higher modularity than random networks (Table 1), demonstrating that the networks were modular. Hierarchy means that networks form tree structures, which could be examined by whether the logarithmic values of clustering coefficients linearly correlate with the logarithmic values of connectivity[32]. The correlation values (r= from −0.466 to −0.263) for all four networks, albeit significant (P<0.001), showed poor linear regression visualization (Figure 1), suggesting they were weakly hierarchical.

3.2 Distinct characteristics of C cycling gene networks between grazing and control conditions

The same threshold (0.980) was applied for C cycling gene networks generated from control and grazing samples (Table 1), resulting in a substantially smaller C cycling gene network from grazing samples (1 455 nodes) than that from control samples (2 830 nodes). However, the C cycling gene network in the grazing condition was denser than the network in the control condition, as demonstrated by the lower average path distance (4.761 vs. 8.440) and the higher connectivity (5.511 vs. 5.133).

Table 1 Overall C and N cycling gene network topological characteristics in control and grazing samples
表 1 对照和放牧样品的整体碳氮循环基因网络的拓扑学特性

Topological characteristics 拓扑学特性	Control C network 对照的碳循环网络	Grazing C network 放牧的碳循环网络	Control N network 对照的氮循环网络	Grazing N network 放牧的氮循环网络
Threshold	0.980	0.980	0.980	0.980
Total nodes	2 830	1 455	1 976	983
Total links	7 263	4 009	3 623	2 205
Positive link percentage (%)	57.2	58.3	63.4	61.0
r^2 of power-law	0.864	0.904	0.838	0.887
Connectivity	5.133	5.511	3.667	4.486
Clustering coefficient	0.336	0.316	0.305	0.316
Random clustering coefficient	0.005±0.001	0.015±0.002	0.003±0.001	0.009±0.002
Average path distance	8.440	4.761	5.846	4.410
Random average path distance	4.455±0.035	3.785±0.055	5.100±0.061	4.034±0.084
Modularity	0.769	0.710	0.840	0.730
Random modularity	0.432±0.002	0.401±0.003	0.560±0.003	0.471±0.004

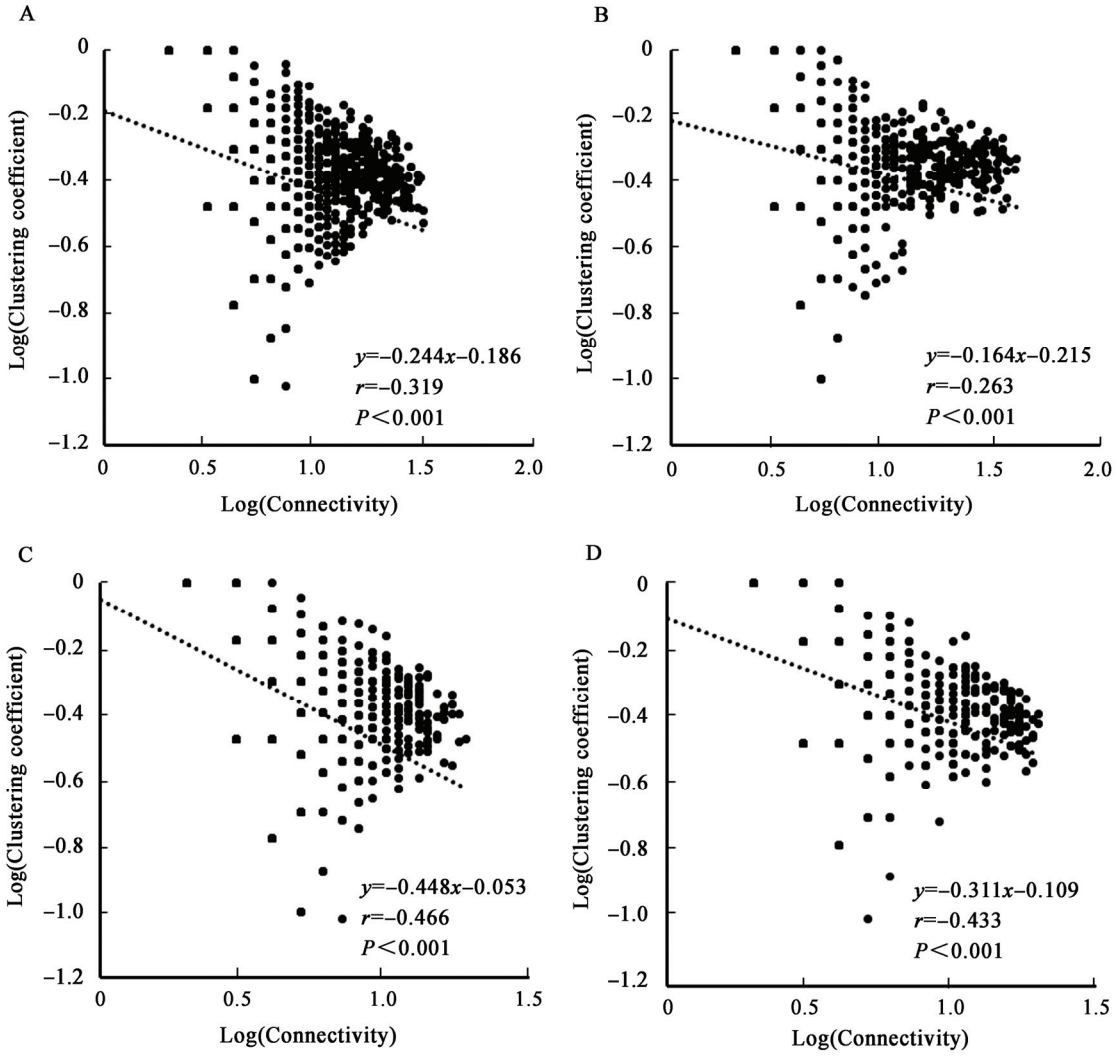

Figure 1 Linear regression of Log(Connectivity) and Log(Clustering coefficient) of nodes

图 1 连通度对数和聚类系数对数的线性回归

Note: A: The C cycling gene network in control samples; B: The C cycling gene network in grazing samples; C: The N cycling gene network in control samples; D: The N cycling gene network in grazing samples.

注：A：对照样地的碳循环网络；B：放牧样地的碳循环网络；C：对照样地的氮循环网络；D：放牧样地的氮循环网络.

There were more positive links than negative links in C cycling gene networks (Table 1). Grazing slightly increased the positive link percentage in C cycling gene networks from 57.2% to 58.3%.

3.3 Modules and key genes of C cycling gene networks

In the grazing samples, there was a group of 19 modules with sizes ranging from 177 to 3 nodes (Figure 2). By contrast, there was a group of 35 modules with sizes ranging from 375 to 3 nodes in the control samples (Figure 3).

As described in the Materials and Methods section, module hubs and connectors were identified from C cycling gene networks (Table 2 and 3). A total of 20 and 28 module hubs were present in the grazing and control samples, respectively. Among them, only one chitin degradation gene of *endochitinase* with sequence similar to *Vibrio* sp. RC586 ortholog was shared as a module hub by both networks, suggesting that module hubs substantially differed between grazing and control networks. Only one C fixation gene (*CODH*) was identified as a module hub in the grazing samples, while four C fixation genes (two *CODH* genes and two *pcc* genes) were module hubs in

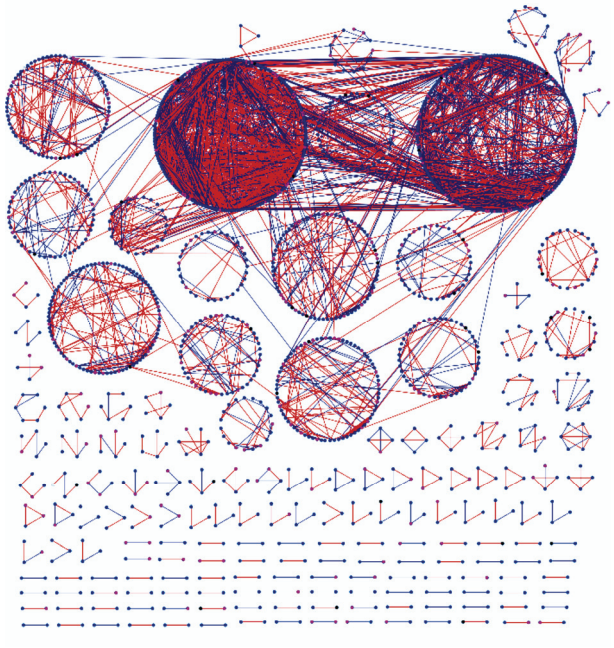

Figure 2 Overall distribution of nodes in C cycling gene network modules of grazing samples
图 2 放牧的碳循环基因网络模块的分布
Note: Red lines indicate positive correlations, and blue lines indicate negative correlations.
注：红线代表正相关，蓝线代表负相关.

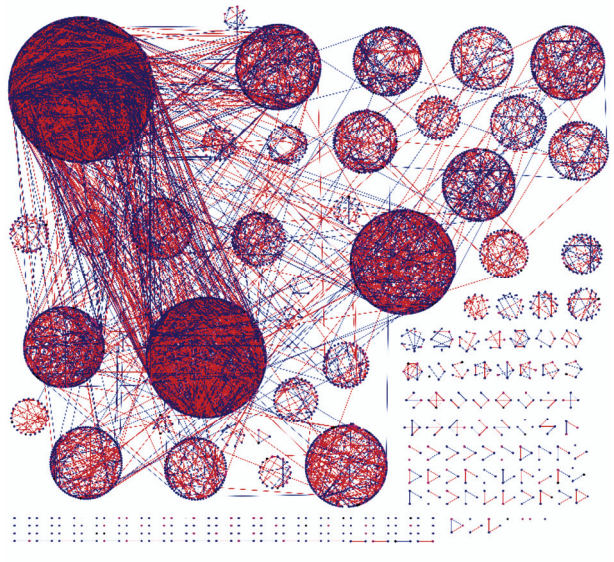

Figure 3 Overall distribution of nodes in C cycling gene network modules of control samples
图 3 对照的碳循环基因网络模块的分布
Note: Red lines indicate positive correlations, and blue lines indicate negative correlations.
注：红线代表正相关，蓝线代表负相关.

the control samples. As for C degradation genes, only three chitin degradation genes were identified as module hubs in the grazing samples, but seven chitin degradation genes constituting the largest category of module hubs in the control samples.

No connectors were shared between two C cycling gene networks. There were only 5 connectors in the grazing samples, including an acetogenesis gene (*FTHFS*), one C fixation gene (*pcc*), two chitin degradation genes (one *acetylglucosaminidase* and one *exochitinase*) and one *aceA* encoding isocitratelyase. By contrast, a total of 27 connectors were identified in the control samples. The majority were 22 C degradation genes. The rest were four C fixation genes (three *pcc* genes and one *aclB* gene), and a methane production gene (*mcrA*).

3.4 Distinct characteristics of N cycling gene networks between grazing and control conditions

Although the same threshold (0.980) was applied for N cycling gene networks generated from control samples and grazing samples (Table 1), the number of nodes in the grazing network (983 nodes) was substantially fewer than that in the control network (1 976 nodes). Similar to the findings in C cycling gene networks, the N cycling gene network from grazing samples was also denser, with lower average path distance (4.410 vs. 5.846) and higher connectivity (4.486 vs. 3.667).

There were more positive links than negative links in N cycling gene networks (Table 1). However, in contrast to the result in C cycling gene networks, grazing slightly decreased the positive link percentage in N cycling gene networks from 63.4% to 61.0%.

3.5 Modules and key genes of N cycling gene networks

There was a group of 29 modules with sizes ranging from 161 to 3 nodes in the grazing samples (Figure 4A). As for control, there was a group of 27 modules with sizes ranging from 235 to 3 nodes (Figure 5A). Notably, *hzo* genes related to anaerobic ammonium oxidation (anammox) were also detected in N cycling gene networks (Figure 4B and 5B).

A total of 2 and 13 module hubs were present in the grazing and control networks, respectively (Table 4). No hubs were shared between two networks, which might be attributed to the robustness of correlation

Table 2 Module hubs of C cycling gene networks
表 2 碳基因网络的模块枢纽

ID	Gene name	Sub-category	ID	Gene name	Sub-category
In control			21219500	*aceB*	Others
84494737	*amyA*	Starch	62424587	*aceB*	Others
213691058	*amyA*	Starch	163757532	*aceB*	Others
222447724	*amyA*	Starch	194540305	*aceB*	Others
225185314	*amyX*	Starch	In grazing		
39625	*cda*	Starch	72121507	*vanA*	Others
118021972	*glucoamylase*	Starch	254389458	*aceB*	Others
3560021	*exoglucanase*	Cellulose	iegcoxL63	*CODH*	Carbon fixation
117164748	*acetylglucosaminidase*	Chitin	218240655	*ara*	Hemicellulose
161785006	*acetylglucosaminidase*	Chitin	184154878	*xylA*	Hemicellulose
31414751	*endochitinase*	Chitin	144753	*endoglucanase*	Cellulose
92908605	*endochitinase*	Chitin	148501120	*vanA*	Others
115375557	*endochitinase*	Chitin	53759164	*aceA*	Others
133778514	*endochitinase*	Chitin	151360705	*aceB*	Others
262402570	*endochitinase*	Chitin	221156336	*aceB*	Others
77687829	*CODH*	Carbon fixation	116742440	*cda*	Starch
218750970	*CODH*	Carbon fixation	127512815	*cda*	Starch
170781052	*pcc*	Carbon fixation	227873316	*nplT*	Starch
227549966	*pcc*	Carbon fixation	228557049	*nplT*	Starch
147735311	*ara*	Hemicellulose	5453414	*pectinase*	Pectin
251799172	*ara*	Hemicellulose	256361016	*acetylglucosaminidase*	Chitin
257479579	*ara*	Hemicellulose	92908605	*endochitinase*	Chitin
113934988	*xylanase*	Hemicellulose	262404309	*endochitinase*	Chitin
197123496	*aceA*	Others	505629	*lip*	Lignin
227400024	*aceA*	Others	150034875	*mnp*	Lignin

Table 3 Connectors of C cycling gene networks
表 3 碳基因网络的模块连通者

ID	Gene name	Sub-category	ID	Gene name	Sub-category
In control			219852885	*mcrA*	Methane production
154351876	*aclB*	Carbon fixation	21106322	*aceA*	Others
72117264	*pcc*	Carbon fixation	83645136	*aceA*	Others
83364899	*pcc*	Carbon fixation	221632522	*aceA*	Others
119448637	*pcc*	Carbon fixation	71736436	*aceB*	Others
118468324	*CDH*	Cellulose	108804884	*aceB*	Others
151360998	*cellobiase*	Cellulose	114570174	*aceB*	Others
210630336	*cellobiase*	Cellulose	255106588	*aceB*	Others
145304813	*exoglucanase*	Cellulose	54026163	*limEH*	Others
90419809	*xylA*	Hemicellulose	11066196	*acetylglucosaminidase*	Chitin
254788460	*xylA*	Hemicellulose	115260360	*acetylglucosaminidase*	Chitin
258524352	*amyA*	Starch	In grazing		
224950322	*cda*	Starch	71156382	*FTHFS*	Acetogenesis
218458458	*pulA*	Starch	84498092	*pcc*	Carbon fixation
145603717	*glx*	Lignin	221721956	*acetylglucosaminidase*	Chitin
169846094	*phenol_oxidase*	Lignin	227273381	*exochitinase*	Chitin
169848689	*phenol_oxidase*	Lignin	242804264	*aceA*	Others

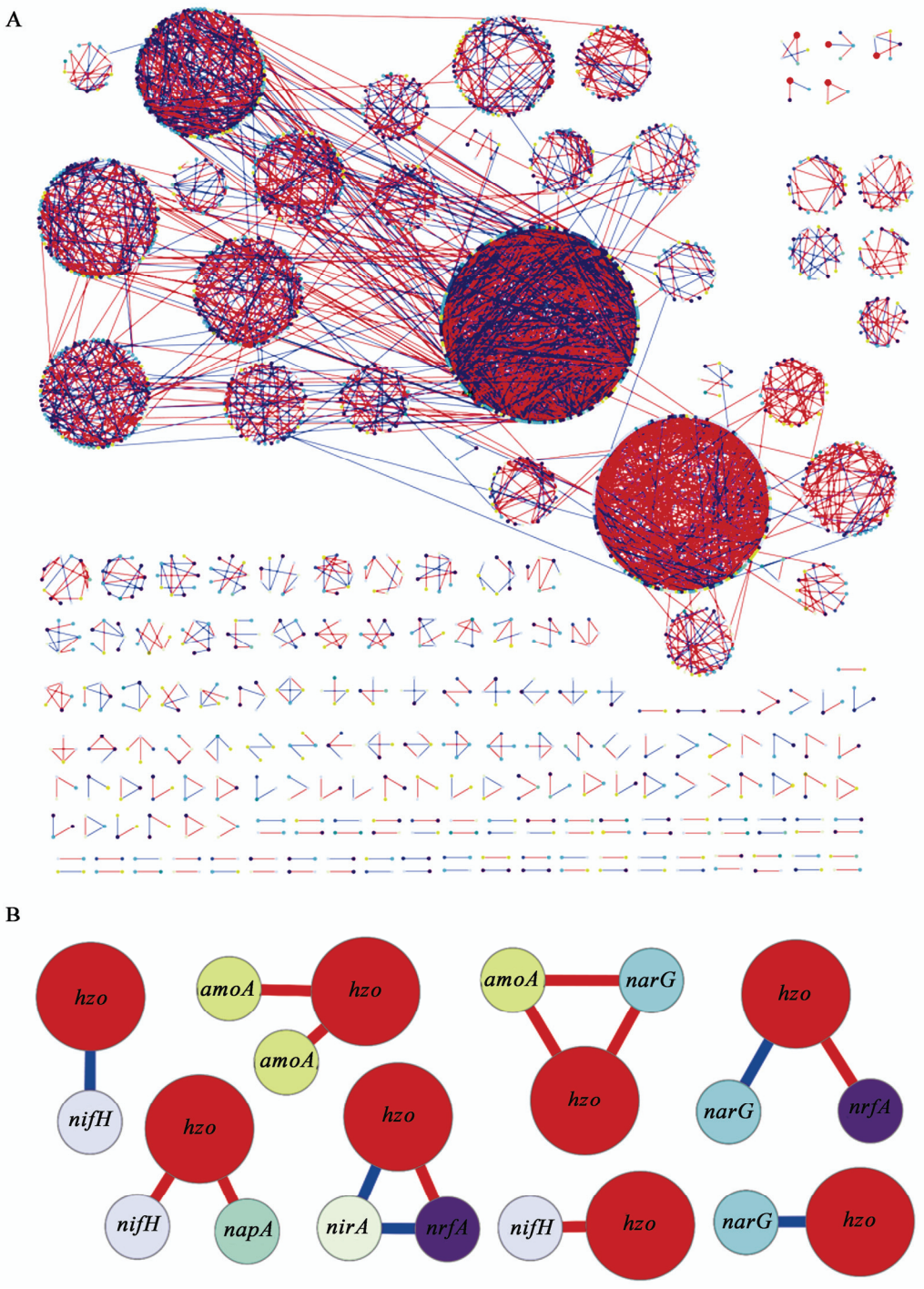

Figure 4 The N cycling gene network modules of grazing samples

图 4 放牧的氮循环基因网络模块的分布

Note: A: All nodes; B: *hzo* genes and nodes directly connecting with them. Red lines indicate positive correlations, and blue lines indicate negative correlations.

注：A：所有节点；B：*hzo* 基因以及与其直接相连的节点. 红线代表正相关，蓝线代表负相关.

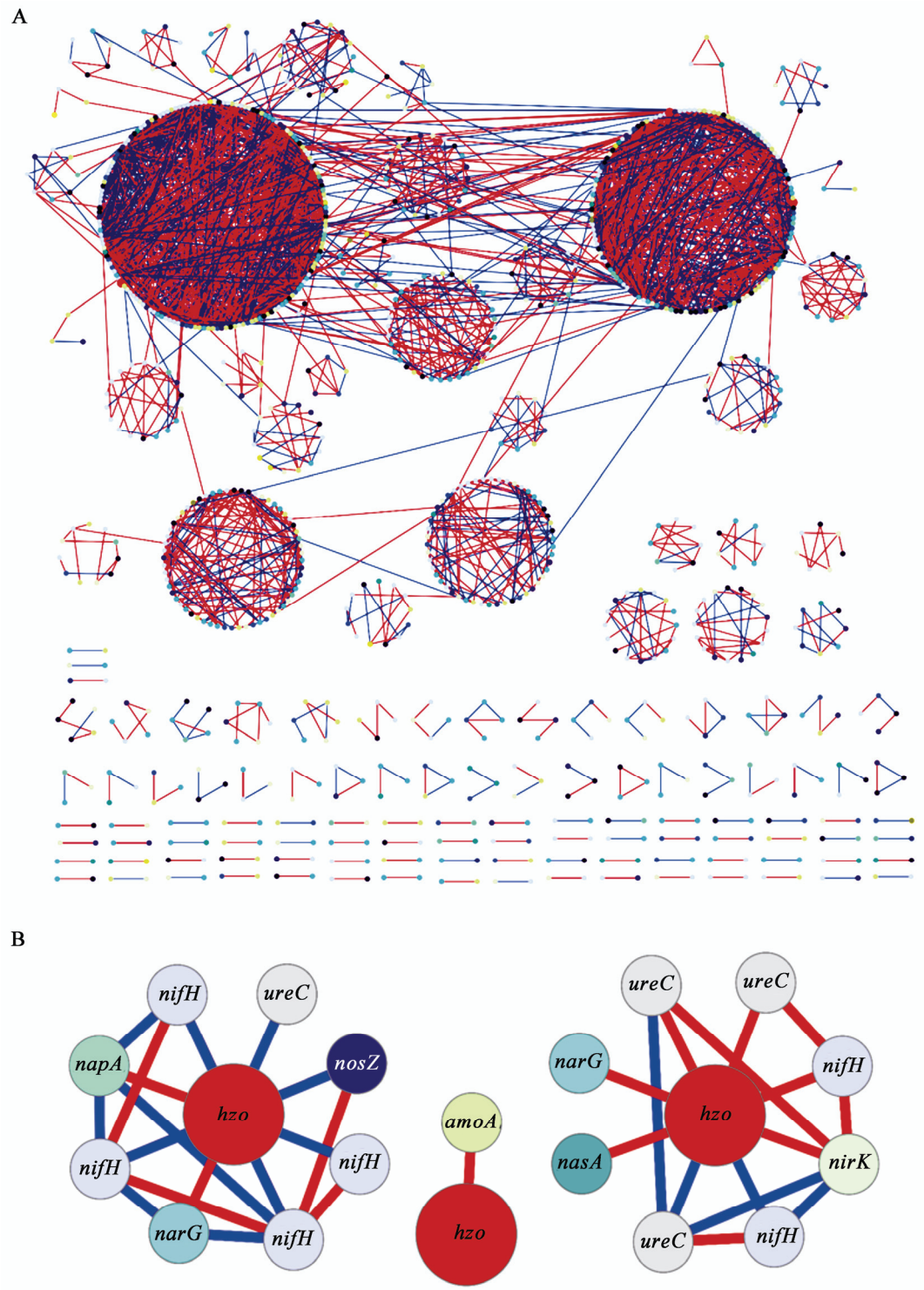

Figure 5 The N cycling gene network modules of control samples

图 5 对照的氮循环基因网络模块的分布

Note: A: All nodes; B: *hzo* genes and nodes directly connecting with them. Red lines indicate positive correlations, and blue lines indicate negative correlations.

注：A：所有节点；B：*hzo* 基因以及与其直接相连的节点. 红线代表正相关，蓝线代表负相关.

networks in removing inherent noise of high-throughput data set[36] and consequently manifesting the differences between grazing and control samples. Only two *narG* genes were detected to be hubs in the grazing network. By contrast, there were seven *nifH* genes related to fixing N_2 into ammonium, two *nrfA* genes, two *narG* genes, one *norB* gene and one *nasA* gene detected as hubs in the control network. No connectors were shared between the networks (Table 5). There were only 4 genes identified as connectors in the grazing network, while there were 11 genes identified as connectors in the control network.

3.6 The relationship between network topology and environmental variables

The importance of environmental variables on network topology was examined as described previously[27]. First, gene significance (GS) was defined as the square of Pearson correlation coefficients between relative abundance of genes in a network and environmental variables. Then, Mantel tests were performed to examine the linkages between gene connectivity and GS, which identified environmental variables significantly correlated with network connectivity.

In the C cycling gene network from the grazing samples, node connectivity was significantly (P=0.001) correlated with the GS of aboveground plant biomass and diversity. Consistently, node connectivity in N cycling gene network from the grazing samples was also significantly correlated with the GS of aboveground plant biomass (P=0.001), and marginally significantly correlated with aboveground plant diversity (P=0.089). In the C cycling gene network from the control samples, node connectivity was significantly correlated with GS of soil temperature

Table 4 Module hubs of N cycling gene networks
表 4 氮基因网络的模块枢纽

ID	Gene name	Sub-category	ID	Gene name	Sub-category
In control			78093546	*narG*	Denitrification
56809467	*nifH*	Nitrogen fixation	160917390	*narG*	Denitrification
76781065	*nifH*	Nitrogen fixation	260598199	*nasA*	Assimilatory N reduction
84711534	*nifH*	Nitrogen fixation	196173236	*nrfA*	Dissimilatory N reduction
89512536	*nifH*	Nitrogen fixation	196226334	*nrfA*	Dissimilatory N reduction
89512984	*nifH*	Nitrogen fixation	In grazing		
114537112	*nifH*	Nitrogen fixation	78093522	*narG*	Denitrification
116812191	*nifH*	Nitrogen fixation	169793492	*narG*	Denitrification
4454060	*norB*	Denitrification			

Table 5 Connectors of N cycling gene networks
表 5 氮基因网络的模块连通者

ID	Gene name	Sub-category	ID	Gene name	Sub-category
In control			209957260	*napA*	Dissimilatory N reduction
56692284	*nifH*	Nitrogen fixation	253700771	*nrfA*	Dissimilatory N reduction
56809495	*nifH*	Nitrogen fixation	7595786	*amoA*	Nitrification
99083367	*nifH*	Nitrogen fixation	In grazing		
56962489	*narG*	Denitrification	56809407	*nifH*	Nitrogen fixation
192764344	*narG*	Denitrification	209401648	*narG*	Denitrification
22252768	*nirK*	Denitrification	126709873	*amoA*	Nitrification
77378479	*nirS*	Denitrification	42782712	*ureC*	Ammonification
84503105	*ureC*	Ammonification			

(*P*=0.039), soil moisture (*P*=0.045), soil NH_4^+ (*P*=0.001), soil total organic carbon (*P*=0.008), and aboveground plant biomass (*P*=0.001) (Table 6). In the N cycling gene network from the control samples, node connectivity was significantly (*P*=0.008) correlated with the GS of soil NH_4^+.

4 Discussion

Here we use a correlation-based association network approach to infer microbial interaction at the functional gene level in response to livestock grazing. Unlike networks whose structures are already known, such as food webs characterized by relationships among tropical levels[37-38], microbial networks are more complex and difficult for interpretation. To date, microbial interaction remains largely elusive[27]. While mathematical relationships are certainly no direct proof of interactions, they imply either direct or indirect connections with ecological implication[39]. Networks in our study are based on the Random Matrix Theory, which has several notable advantages. Thresholds are automatically chosen, which avoids ambiguity in the network reconstruction. Since thresholds are higher than networks generated by other approaches (all four thresholds were 0.980 in our study), rendering network robust to noise[25-26].

All four networks were scale-free, small-world and modular, which were typical topology characteristics in networks of complex systems[25,27,40-41]. The hierarchical characteristic of networks in our study was significant but weak. A previous work has reported different hierarchical characteristics among different habitats, with strongly hierarchical networks from lake sediment samples and weakly hierarchical networks from grassland soil samples[27]. Consistently, our study was conducted with grassland soil samples.

Positive and negative links among nodes are important characteristics, but difficult to interpret. Positive links could be attributed to niche overlap and cross-feeding, while negative relationship could be attributed to competition and amensalism[42]. All four networks in our study had more positive links than negative ones, which implied more cooperation than competition. Alternatively, it might be attributed to the mutualism among genes in long-term co-evolution processes[43]. It was interesting to note that grazing slightly increased the percentage of positive links in the C cycling gene network, but slightly decreased that in the N cycling gene network. As the removal of aboveground plant biomass decreased the relative abundance of C cycling genes[20], it may force microbes to cooperate more in order to cope with less C input to soil. By contrast, manure deposition at the

Table 6 Mantel tests on connectivity and GS of environmental variables
表 6 连通度和环境因子 GS 之间的 Mantel 检验

Environmental variables	Control C network		Grazing C network		Control N network		Grazing N network	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
T	0.012	0.039	−0.006	0.799	0.003	0.340	−0.029	1.000
NO_3^-	−0.040	1.000	−0.036	1.000	−0.011	0.936	−0.019	0.982
NH_4^+	0.048	0.001	−0.039	1.000	0.025	0.008	−0.006	0.791
TOC	0.018	0.008	−0.044	1.000	0.004	0.263	−0.026	1.000
TN	0.008	0.120	−0.039	1.000	0.000	0.464	−0.021	1.000
Moisture	0.012	0.045	−0.057	1.000	0.003	0.376	−0.020	0.992
Biomass	0.040	0.001	0.095	0.001	0.007	0.235	0.156	0.001
Diversity	−0.008	0.919	0.069	0.001	−0.015	0.994	0.011	0.089

Note: T: Soil temperature; TOC: Soil total organic C; TN: Soil total N; Biomass: Aboveground plant biomass; Diversity: Aboveground plant diversity. Degree of freedom: Control C network (2 828), grazing C network (1 453), control N network (1 974), grazing N network (981).
注：T：土壤温度；TOC：土壤总有机碳；TN：土壤总氮；Biomass：地上植物生物量；Diversity：地上植物多样性. 自由度：对照样品的碳网络(2 828)，放牧样品的碳网络(1 453)，对照样品的氮网络(1 974)，放牧样品的氮网络(981).

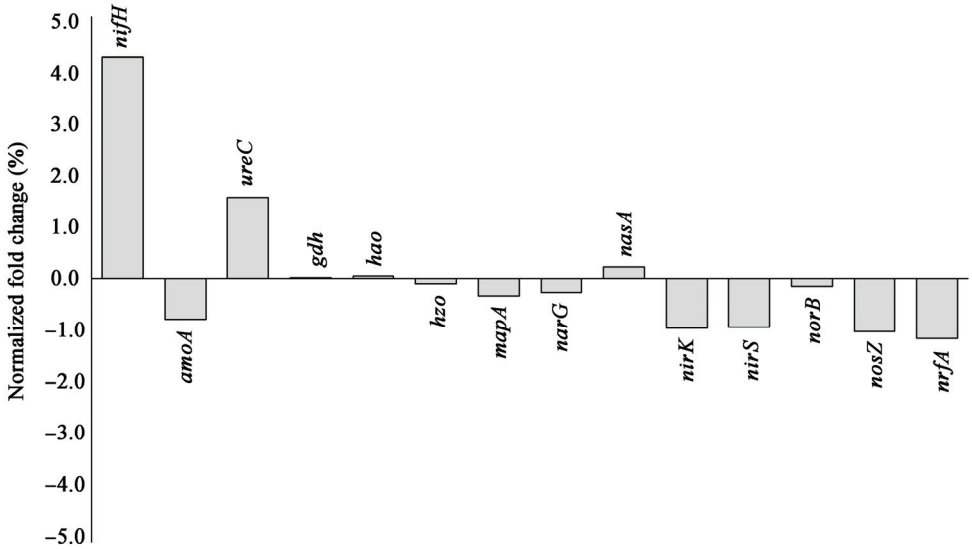

Figure 6 Changes of node number in N cycling gene networks in grazing samples

图 6 放牧的氮基因网络节点数的变化

Note: The *Y* axis is the normalized fold change which results from the calculation of node number in each gene family multiplied by the ratio of node number in this gene family to total node number of N cycling gene networks.

注：纵轴代表变化百分数的标准化，它等于每个基因的节点数乘以此基因数与整氮基因节点数的比例.

grazed sites accelerates N cycling by efficiently recycling through the animal excreta pathway, which provides additional available N to microbes that may stimulates competition among microbial members.

Disturbance on a smaller, denser network will have larger influence on the overall network equilibrium[44]. Grazing resulted in smaller, denser gene networks, thus the networks were more vulnerable to disturbance. In addition, grazing increased the numbers of *nifH* and *ureC* genes in the N cycling gene network (Figure 6). Consistently, their relative abundances were increased by grazing[20]. *hzo* genes, whose enzymatic product plays an essential role in anammox, were also detected in N cycling gene networks. Since recent studies have shown that it is a common process in wet environments[45-51], it is likely that anammox is active in Tibetan alpine grasslands, which are cold and wet environments in general.

Grazing removed aboveground plants, which was closely linked to the underground microbial community structure[20]. Consistently, network connectivity was significantly correlated with aboveground plant variables in the grazing networks, suggesting that genes with higher connectivity were closely linked to aboveground plant variables. Since disturbance on genes with higher connectivity has larger influence on the overall network equilibrium[44], it is likely that the grass removal by grazing imposed strong influence on microbial community interactions.

5 Conclusions

C and N cycling gene networks in this Tibetan grassland were characterized as scale-free, small-world, modular and hierarchical. Livestock grazing resulted in smaller, denser gene networks, reflecting higher vulnerability to the grazing disturbance. Consistently, grazing substantially changed hubs and connectors in the networks. Grazing also increased numbers of *nifH* and *ureC* genes in the N cycling gene network, suggesting that ammonification potential was increased. The grass removal by grazing appeared to have a significant impact on microbial community interactions, since aboveground plants were significantly linked to network connectivity under the grazing condition. This study unveiled grazing effects on microbial interactions at the functional gene level in the Tibetan grassland by network reconstruction, which was an importance step towards an integrated understanding of grazing effects on Tibetan ecosystems.

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科技信息摘录

海底 2 400 m 下采集到微生物

如果你想要找到一些奇怪的尚未被发现的生物体，海底 2 000 m 以下的沉积层应该是一个不错的选择。那里的热量和压力都非常大，而食物则供不应求。研究人员如今在这一深度获得了首批微生物样本，然而它们却出乎意料的普通。这些细胞与生活在陆地上的一个不太苛刻的栖息地——森林中的土壤的微生物非常类似。

从炙热的温泉到南极冰下的湖泊，微生物往往能够在一些非常艰难的环境中生存。然而在海底多深的地方依然能够有微生物生存却一直没有答案。研究人员曾经从海底下方 1 900 多米深处采集过细胞，同时它们也曾在海底 4 000 m 深处探测到生命存在的化学迹象。

为了寻找其他被深埋的微生物，一个国际研究团队在远离日本东北海岸的沉积层中向下钻探了 2 400 多米。这项研究的联合作者、德国不来梅大学生物地球化学家 Kai-Uwe Hinrichs 介绍说，大约 2 300 万年前，这片海域曾是包含湿地与泻湖的沿海环境，有点类似于今日美国佛罗里达州的一部分。然而随着陆地位置的改变，该地区逐渐下沉并最终被沉积层覆盖。如今，这个深层含有丰富的煤炭资源。

研究人员采取了多个防范措施以免有其他微生物污染他们的样本。在钻探船上，他们用一部 X 射线 CT 扫描仪仔细检查了沉积岩心，并挑选了最坚实的部分。研究人员随后对岩心的中间部分进行了分析。

在实验室中，科学家对岩心中的微生物以及可能污染样本的微生物进行了基因测序。随后他们又利用一种统计方法纠正了当闯入者存在时的测量值。

研究人员日前在《科学》杂志上报告说，他们的分析表明，少量的微生物来自于最深的沉积层——距离海底 2 466 m。1 cm^3 的深海沉积层中大约含有 10 到 1 万个微生物细胞。Hinrichs 说，相比之下，你家后院相同大小的泥土中大约有几十亿个微生物。他说，在这样的深度，"生命依然存在，但只是很少的生命"。

当研究人员在 40 ℃ 培育这些微生物并将其投入少量煤尘中后，他们检测到代谢活动的迹象，这意味着来自如此深度的微生物以煤炭为食并且会释放甲烷。

这项研究的共同作者、日本横须贺海洋地球科学与技术机构地球微生物学家 Fumio Inagaki 认为，这项研究表明"微生物能够在距离海底 2.5 km 的地方存活"。

地球上最重要的过程之一便是碳循环，即碳原子穿梭于生物与非生物的环境之间。而人类通过燃烧化石燃料、向大气中释放大量二氧化碳而扭曲了碳循环。Inagaki 表示，微生物以煤炭为食并释放甲烷表明它们同时扮演了"碳循环中的一个重要生态角色"。

为了搞清生活在沉积岩心中的微生物到底是什么，科学家将它们的基因序列与生活在其他环境中的微生物的基因序列进行了比较。

研究表明，远离海底的微生物组并不同于那些生活在浅层的微生物。但令研究人员感到吃惊的是，深海微生物更类似于那些生活在森林土壤中的微生物。

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