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Soil microbial community diversity and distribution characteristics under three vegetation types in the Qilian Mountains, China

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Abstract: Qilian Mountains in Northwest China is a significant ecological security barrier due to its distinctive geographic setting, which has significant biological resource and gene pool. In order to assess the soil quality and ecosystem health in this area, we identified the structural characteristics and functional groups of soil microbial communities. This study focused on Amidongsuo, a typical watershed of the Qilian Mountains, and researched the vertical distribution and dominant populations of soil microorganisms in different habitats, and the relationship between soil microorganisms and environmental factors. Soil microorganisms from three grassland plots, five shrubland plots, and five forest plots in Amidongsuo were studied using high-throughput sequencing. The Venn diagram showed that the types of bacteria were fewer than those of fungi in Amidongsuo. Soil bacteria Acidobacteriota, Proteobacteria, and Methylomirabilota as well as fungi Basidiomycota, Ascomycota, and Mortierellomycota played dominant roles in Amidongsuo, according to the LEfSe (linear discriminant analysis (LDA) effect size) and community structure analyses. According to the ANOSIM (analysis of similarities) result, for both bacteria and fungi, *R* values of grassland and shrubland were small (R^2=0.045 and R^2=0.256, respectively), indicating little difference between these two ecosystems. RDA (redundancy analysis) showed a closer relationship between soil nutrients and fungi, and a gradually decreasing correlation between soil nutrients and microorganisms with increasing soil depth. Bacteria were mainly affected by pH, nitrogen (N), and potassium (K), while fungi were mainly affected by K. Overall, fungi had more effect on soil quality than bacteria. Therefore, adjustment of soil K content might improve the soil environment of Amidongsuo in the Qilian Mountains.

Keywords: fungi; bacteria; diversity; soil nutrient; Qilian Mountains

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1 Introduction

Soils harbor an enormous diversity of microorganisms, including bacteria, archaea, and fungi, all of which play critical roles in ecosystem function, such as regulation of organic matter decomposition and soil carbon dynamics, and mediation of nutrient cycling (Nannipieri et al.,

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2017). Soil quality is significantly related to the microbial community structure and functional diversity (Kan et al., 2022), both of which are critical to the ecological environment's sustainability (Wang et al., 2014; Wan et al., 2015). Fungi and bacteria can effectively degrade complex components and litter in the soil (Gu et al., 2022), and promote the conversion and absorption of soil nutrients, decomposition of organic matter, and humus formation (Wang et al., 2020). Interactions between fungal and bacterial activities can significantly affect soil microbial ecosystem functions.

As an important ecological barrier in western China, the Qilian Mountains have important ecological functions, such as water conservation, forest protection, climate regulation, biodiversity, and ecosystem balance (Li et al., 2018). There have been many ecological restoration projects in this area, such as the Natural Forest Protection project, Grain for Green project, and the efforts to restore water, forest, farmland, lake, and grassland. However, there has been insufficient characterization of this ecosystem, making it difficult to determine the best strategies to protect this area. Determination of the distribution of soil microorganisms in this area can facilitate the ecological restoration and protection of the area.

Recent investigations of soil microorganisms in the Qilian Mountains characterized specific ecosystems such as alpine grassland (Ma, 2009; Kang, 2013; Li et al., 2018), meadow (Kang et al., 2020; Ma et al., 2020), and shrubland (Wang, 2006). Additionally, most studies focused only on bacterial communities with little attention to fungal or archaeal communities and their effects on various soil processes. Few studies have compared soil microbial communities in different ecosystems and characterized the coupling relationships between soil microbial communities and their living environments. Comprehensive studies are needed to determine the differences in soil microbial communities in different ecosystems of alpine regions to better understand the circulation of matter and energy throughout the region and to protect and conserve the environment. In this study, a typical small watershed in the Qilian Mountains, Amidongsuo, was selected as the research area. High-throughput sequencing technology was employed to analyze the characteristics of soil bacterial and fungal communities in three ecosystems, forest, grassland, and shrubland, to address the following three questions: (1) what are the major bacterial and fungal phyla that affect the three ecosystems? (2) how do the structures of soil bacterial and fungal communities differ in the three ecosystems? and (3) which soil factor regulate the communities and diversity of soil bacteria and fungi in the three ecosystems? The answers to these questions can provide data support for habitat quality evaluation and the implementation of effective ecological engineering strategies in the study area.

2 Materials and methods

2.1 Study area

The Amidongsuo watershed, located in the Qilian Mountains in China, covers a total area of 120.6 km^2 (38°02′–38°06′N, 100°14′–100°22′E; Fig. 1). The average annual temperature is 1°C, and the annual precipitation is about 420 mm. The watershed has an east-west valley with a total length of 14 km, an altitude of 2800–4667 m a.s.l. The main peak of the Tuolai Mountain is dominated by acidic igneous rock, conglomerate, and sandstone. The vegetation in this area includes forest (*Picea crassifolia* Kom. and *Sabina chinensis* (L.) Ant.), shrub (*Dasiphora fruticosa* (L.) Rydb.), and grass (*Achnatherum splendens* (Trin.) Nevski).

2.2 Sample collection

The study area is dominated by forest (LD), grassland (CD), and shrubland (GC). According to their actual distribution, area distribution ratio, and altitude (100 m intervals), a total of 13 plots were selected, including three grassland plots (CD1, CD2, and CD3), five shrubland plots (GC1, GC2, GC3, GC4, and GC5), and five forest plots (LD1, LD2, LD3, LD4, and LD5) (Table 1). A 20 m×20 m square was set up for each type of sample plot, and three 1 m×1 m square samples were randomly placed in each sample plot. We carried out the sampling according to the national

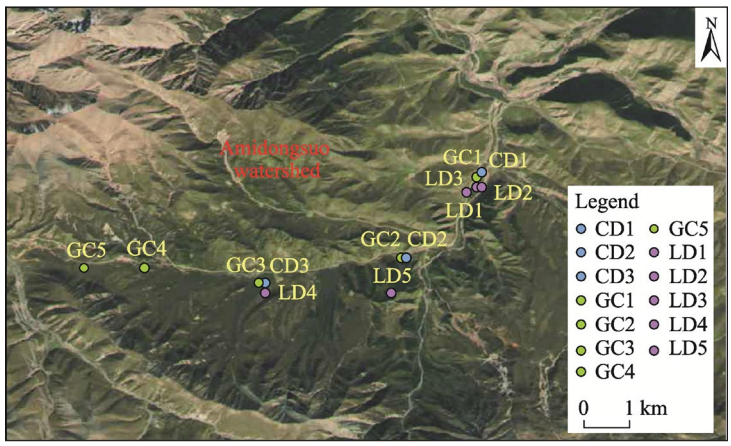

Fig. 1 Sample points in the Amidongsuo watershed, Qilian Mountains, China. Grassland plots: CD1, CD2, and CD3; shrubland plots: GC1, GC2, GC3, GC4, and GC5; forest plots: LD1, LD2, LD3, LD4, and LD5.

standards (technical specification for soil environmental monitoring, HJ/T 166-2004; guidelines for the design of soil sampling procedures for soil quality, GB/T 36199-2018). The sampling time, location, vegetation coverage, and orientation were recorded. In each sample plot, holes with diameter of 5 cm were drilled, and soil samples from 0–10, 10–20, and 20–30 cm soil layers were collected. After the removal of gravel and grass roots, samples were mixed and air dried before nutrient testing.

For microbial sampling, samples were taken from soils at 0–10, 10–20, and 20–30 cm layers. The fresh soil samples were immediately sieved to separate the soil and roots using a 2-mm sieve, which was disinfected with 75% alcohol. After sieving, soil samples were then loaded into sterilized tubes, labeled, and stored in liquid nitrogen tanks for subsequent analysis.

2.3 Determination of soil nutrients

Soil pH was measured by a pH meter at a ratio of 1:5 (soil weight: volume distilled water). The potassium dichromate hydration heating method was used to determine the soil organic matter (SOM) content. Total nitrogen (TN) was measured using an elemental analyzer (Vario EL III, Elementary Co. Ltd., Hanau, Germany). Total phosphorus (TP) was analyzed by the molybdenum-antimony anti-spectrophotometric method (Zhu et al., 2018). Total potassium (TK) was analyzed by the sodium hydroxide fusion-flame photometric method (Zhu et al., 2018).

Table 1 Sample information in this study

Ecological system	Code	Latitude	Longitude	Altitude (m)	Slope (°)	Aspect (°)	Vegetation coverage (%)
Grassland	CD1	38°05′08″N	100°19′08″E	2928	3.0	53	93
Grassland	CD2	38°04′04″N	100°18′14″E	3043	11.0	76	87
Grassland	CD3	38°03′46″N	100°16′33″E	3187	14.5	70	76
Forest	LD1	38°04′55″N	100°19′04″E	2980	30.5	328	72
Forest	LD2	38°04′55″N	100°19′08″E	3018	32.0	330	68
Forest	LD3	38°04′51″N	100°18′57″E	2958	35.5	345	57
Forest	LD4	38°03′39″N	100°16′33″E	3208	23.5	22	51
Forest	LD5	38°04′01″N	100°18′03″E	3078	18.5	29	56
Shrubland	GC1	38°05′02″N	100°19′04″E	2929	7.5	47	60
Shrubland	GC2	38°04′04″N	100°18′10″E	3041	2.0	46	58
Shrubland	GC3	38°03′46″N	100°16′33″E	3187	14.0	62	67
Shrubland	GC4	38°03′57″N	100°15′07″E	3064	17.5	67	46
Shrubland	GC5	38°03′57″N	100°14′24″E	3530	20.5	51	49

2.4 Microbial sequencing

DNA (deoxyribonucleic acid) concentration of samples was measured using the Qubit® dsDNA HS Assay Kit. Next-generation sequencing library construction and sequencing were completed by GENEWIZ, Inc. (South Plainfield, New Jersey, USA). The 16S rRNA gene fragment was amplified from samples by PCR (polymerase chain reaction) with primers CCTACGGRRBGCASCAGKVRVGAAT and GGACTACNVGGGTWTCTAATCC and the ITS rRNA gene fragment was PCR amplified with primers GTGAATCATCGATC and TCCTCCGCTTATTGAT. PE250/FE300 paired-end sequencing was performed by Illumina MiSeq/Novaseq (Illumina, San Diego, CA, USA) to obtain raw sequencing data.

2.5 Species annotation and diversity analysis

Sequence clustering was performed using VSEARCH v.1.9.6 (sequence similarity was set to 97%), the 16S rRNA reference database for alignment was Silva 138, and the ITS rRNA reference database for alignment was the UNITE ITS database (https://unite.ut.ee/). Then, the RDP (ribosomal database program) classifier Bayesian algorithm was used to perform species taxonomic analysis on the representative sequences of operational taxonomic units (OTUs), with determination of the community composition of each sample at different levels of species classification.

2.6 Data processing

SPSS v.22.0 software was used for statistical analysis of the data, and LEfSE (linear discriminant analysis (LDA) effect size) v.1.0 software was used to compare different species among multiple groups. One-way analysis of variance (ANOVA) was used to analyze the variance and test the significance of differences in soil nutrients. We used non-metric multidimensional scaling analysis (NMDS) based on Bray-Curtis distance to analyze the similarity and significance of the composition of community structures in different ecosystems. RDA (redundancy analysis) was used to explain the correlation between soil microbes and soil nutrients. QIIME (quantitative insights into microbial ecology) software was used for β-diversity analysis and dilution curve construction. Species abundance was expressed by ACE (abundance-based coverage estimator) and Chao1 indices, and the diversity index was expressed by Simpson and Shannon indices. ANOSIM (analysis of similarities) was used to examine whether the differences between groups were significantly greater than within-group differences, and to determine whether grouping was meaningful.

3 Results

3.1 Analysis of soil nutrient characteristics

Soil nutrient data were analyzed (Table 2) and the results showed that, compared with TN, TP and TK, the data of SOM were relatively discrete. On the one hand, it could be due to experimental error, but it could also be due to SOM accumulation in the humus layer of the three ecosystems. The coefficient of variation between TK and pH was below 10%, with variation between 10% and 100% for the other coefficients, indicating that the distribution change of TK was relatively stable. Soil nutrients were generally higher in grassland than in shrubland, and higher in shrubland than in forest. The soils in the study area are mainly neutral, weakly acidic, and weakly alkaline.

It can be seen from Figure 2 that soil pH and soil TK content increased slightly with the increase in soil depth, and soil TN, TP, and SOM decreased significantly with the increase in soil depth. There was no significant difference in soil pH or TP content across different ecosystems or different soil layers. There was a significant difference between forest and grassland in soil TK content in 0–10 cm soil layer. There was a significant difference in TN content in the forest and grassland between 20–30 and 0–10 cm soil layers, and a significant difference in TN content among grassland, forest, and shrubland. For SOM content, there was a significant difference

Table 2 Soil nutrient characteristics in the study area

Ecological systems	Soil nutrient	Min (g/kg)	Max (g/kg)	Mean (g/kg)	SD (g/kg)	Variance	Skewness	Kurtosis	CV (%)
Grassland	TN	4.65	9.69	6.75	2.02	4.09	0.46	–1.63	29.96
	TP	1.31	2.53	1.92	0.39	0.16	0.01	–0.83	20.54
	TK	20.45	24.37	22.13	1.17	1.38	0.74	0.50	5.31
	SOM	111.12	213.70	157.81	31.83	1013.04	0.24	–0.25	20.17
	pH	6.60	8.10	7.24	0.55	0.30	0.67	–1.34	7.56
Shrubland	TN	1.00	11.99	5.46	3.58	12.80	0.57	–1.10	65.48
	TP	1.16	2.38	1.61	0.37	0.13	0.74	–0.39	22.72
	TK	17.67	25.29	21.45	2.09	4.35	–0.25	–0.32	9.72
	SOM	24.90	205.87	118.54	61.49	3781.13	0.10	–1.44	51.87
	pH	6.54	8.26	7.33	0.57	0.33	0.40	–1.60	7.79
Forest	TN	1.08	7.28	3.44	1.39	1.93	1.30	3.86	40.37
	TP	1.17	1.91	1.49	0.23	0.05	0.52	–1.00	15.40
	TK	17.85	21.96	20.15	1.09	1.20	–0.44	0.09	5.43
	SOM	24.22	172.12	88.43	34.31	1177.42	0.53	1.87	38.80
	pH	6.39	7.96	7.15	0.48	0.23	0.01	–1.11	6.65

Note: TN, total nitrogen; TP, total phosphorus; TK, total potassium; SOM, soil organic matter; SD, standard deviation; CV, coefficient of variation.

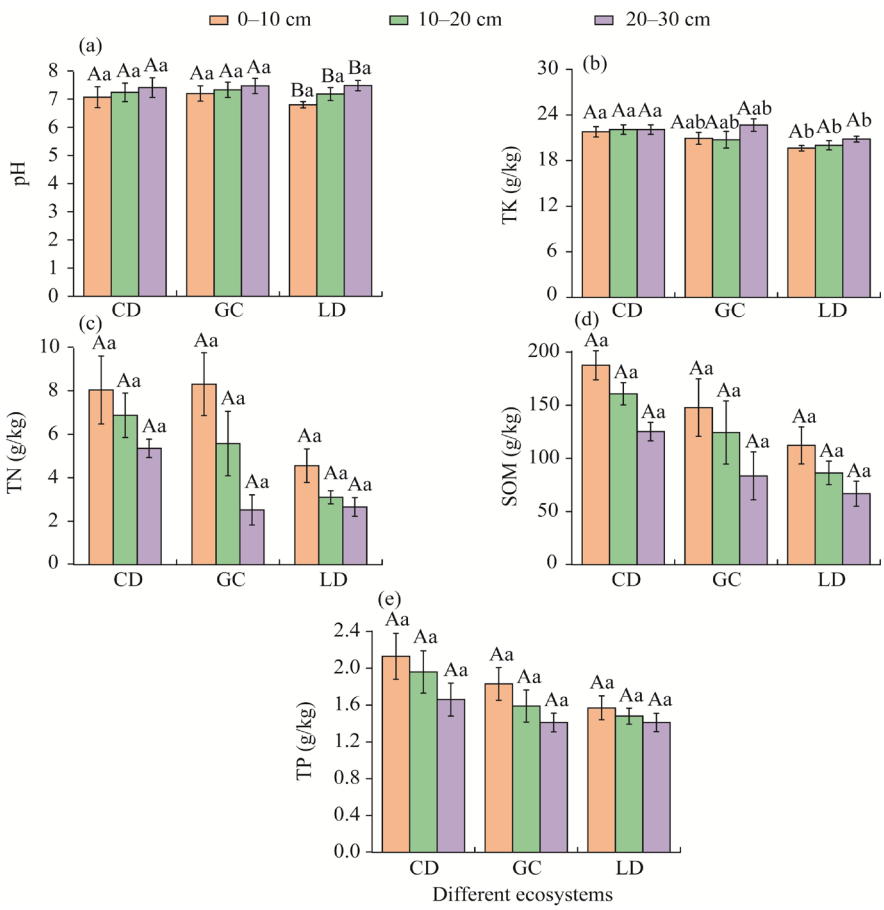

Fig. 2 Physical-chemical characteristics of soil in different ecosystems. Different uppercase letters within the same ecosystem indicate significant differences among different soil layers at $P<0.05$ level; different lowercase letters within the same soil layer indicate significant differences among different ecosystems at $P<0.05$ level. CD, grassland; GC, shrubland; LD, forest; TK, total potassium; TN, total nitrogen; SOM, soil organic matter; TP, total phosphorus. The abbreviations are the same in the following figures. (a), pH; (b), TK; (c), TN; (d), SOM; (e), TP. Bars are standard errors.

between 20–30 and 0–10 cm soil layers in grassland, and no significant difference was found across different ecosystems. These results suggested that in the study area, soil TP content and pH value were less affected by changes in ecosystem type and depth, SOM was primarily affected by soil depth, soil TK content was affected by ecosystem type, and soil TN content was affected by both soil depth and ecosystem type.

3.2 Dilution curve analysis

Quality control and filtering were performed on the sequences to obtain a total of 1012 bacterial OTUs and 1811 fungi OTUs at a level of 97% sequence similarity. The number of bacteria was less than that of fungi. The bacterial dilution results showed that the number of OTUs increased sharply and then increased more gradually with the increase in sample size. The dilution curves of LD1-3, LD2-1, and LD2-2 were significantly lower than those corresponding to the rest of the samples, indicating fewer species in these three samples (Fig. 3). In contrast, the curves for fungi exhibited a more uniform distribution than the curves for bacteria, with a consistent variation. The volume of sequencing data should be sufficient to characterize microbial changes in the study area.

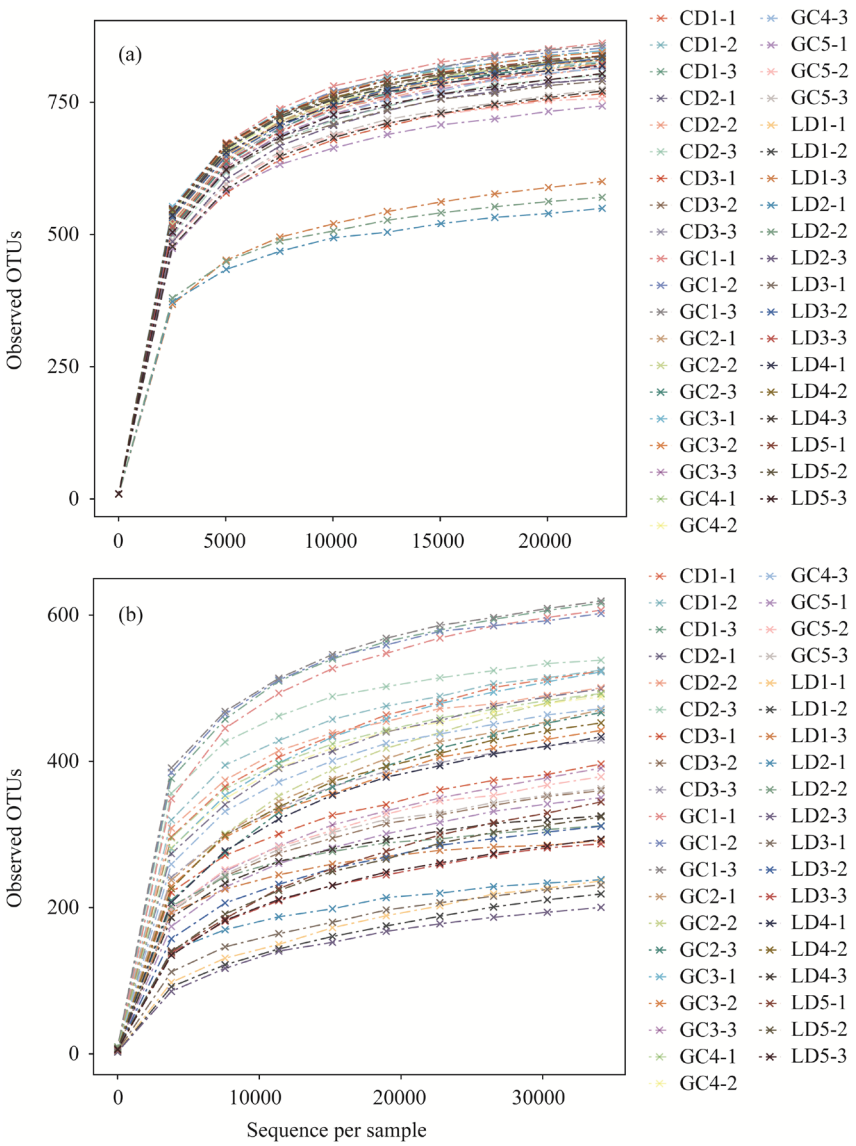

Fig. 3 Dilution curves of soil microbial samples. (a), bacteria; (b), fungi. OTUs, operational taxonomic units

3.3 Venn diagrams of different ecosystems

As shown in Figure 4, for bacteria, analysis of grassland samples revealed 981 OTUs, shrubland samples contained 995 OTUs, forest samples contained 978 OTUs, grassland and forest shared 951 OTUs, grassland and shrubland shared 979 OTUs, and shrubland and forest shared 961 OTUs. For fungi, grassland contained 1305 OTUs, shrub land contained 1457 OTUs, forests contained 1024 OTUs, grassland and shrubland shared 1145 OTUs, shrubland and forest shared 749 OTUs, and grassland and forest shared 705 OTUs. Among the three ecosystems, most of the soil bacterial OTUs were shared, with few unique OTUs. Compared with bacteria, fungi have more ecosystem-specific OTUs.

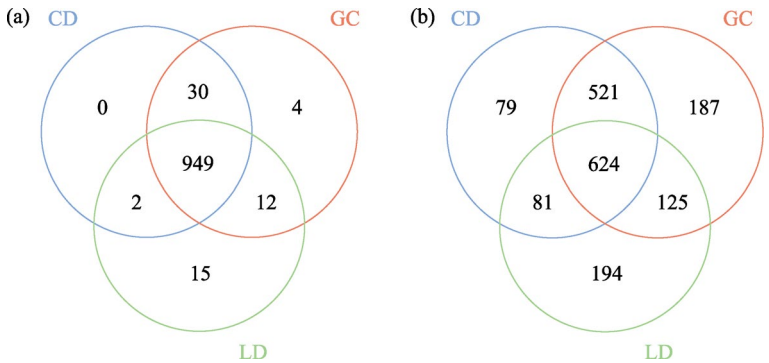

Fig. 4 Venn diagram of OTUs (operational taxonomic units). (a), bacteria; (b), fungi.

3.4 Composition of biomes in different ecosystems

As shown in Figure 5, the composition and distribution of soil bacterial communities in different ecosystems were regionally different, but there was no significant difference in bacterial dominant groups. At the phylum level, a total of 31 bacterial species and unidentified taxa were detected. Acidobacteria, Proteobacteria, Gemmatimonadetes, and Methylomirabilota were present at high relative abundances of 31.89%–37.31%, 26.36%–36.06%, 5.11%–7.10%, and 2.38%–5.67%, respectively, and these four groups accounted for 76.53%, 75.42% and 77.43% of the relative abundances of bacteria in grassland, shrubland and forest, respectively. At the phylum level, 13 fungal species were detected. Basidiomycota, Ascomycota, k__Fungi_Unclassified, and Mortierellomycota were present at higher relative abundances of 34.64%–78.49%, 16.50%–36.31%, 2.9%–20.53%, and 1.53%–3.14%, respectively, and these four groups accounted for 94.62%, 97.45% and 99.92% of the relative abundances of fungi in grassland, shrubland and forest, respectively.

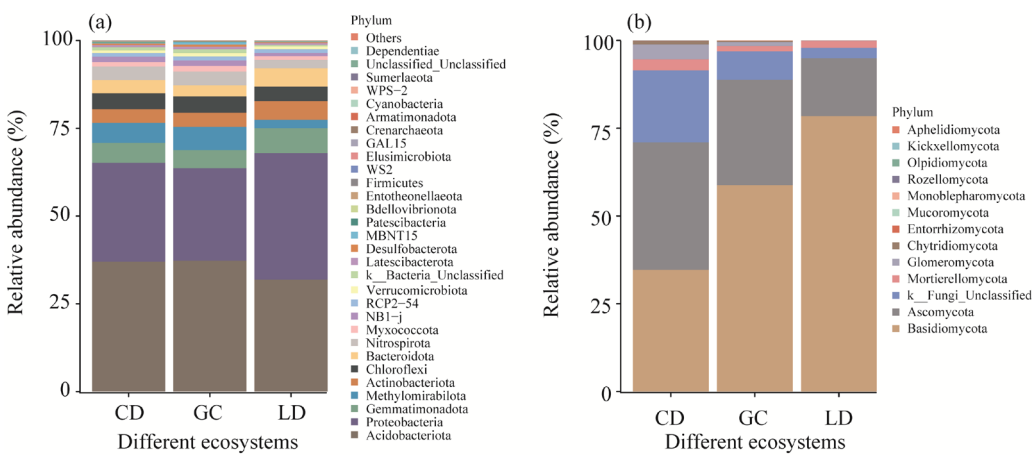

Fig. 5 Community characteristics of different ecosystems at the phylum level. (a), bacteria; (b), fungi.

3.5 Examination of microbial differences in different ecosystems

LEfSe difference analyses were performed for different ecosystems, and species with LDA values greater than 3.0 are shown in Figures 6 and 7. Forest, shrubland, and grassland had 26, 13, and 9 abundant bacterial clades, respectively. At the phylum level, forest had the most different taxa, and showed enrichment of Protobacteria (from class to genus), Bacteroidota (from class to order), and Acidobacteriota (from class to genus). Grassland had the lowest variation, with enrichment of Acidobacteriota (from class to genus) and Nitrospiota (from class to genus). Shrubland was enriched for Acidobateriota (from class to genus) and Methylomirabilota (from class to genus).

Forest, shrubland and grassland had 17, 13 and 39 rich fungal clades, respectively. At the phylum level, grassland had the most differential taxa, with enrichment of Ascomycota (from class to genus), Basidiomycota (from family to genus), Mortierellomycota (from class to genus), and Chytridiomycota (from class to family). Shrubland had the fewest differential fungi, with enrichment of Basidiomycota (from order to genus), Mortierellomycota (order), and Ascomycota (from class to genus). The forest samples showed enrichment of Basidiomycota (from order to genus) and Ascomycota (from order to genus).

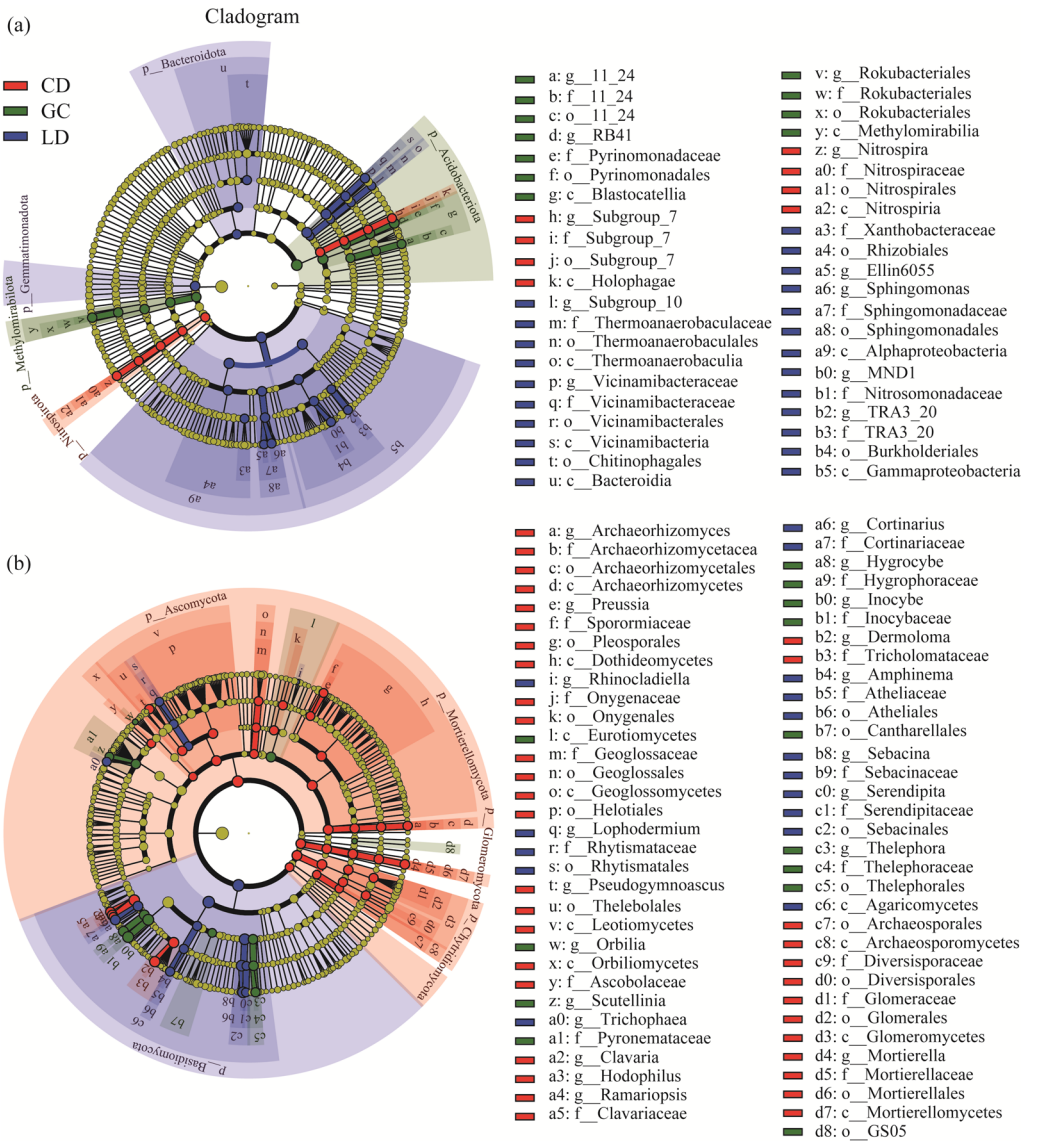

Fig. 6 LEfSe (linear discriminant analysis (LDA) effect size) diagram. (a), bacteria; (b), fungi.

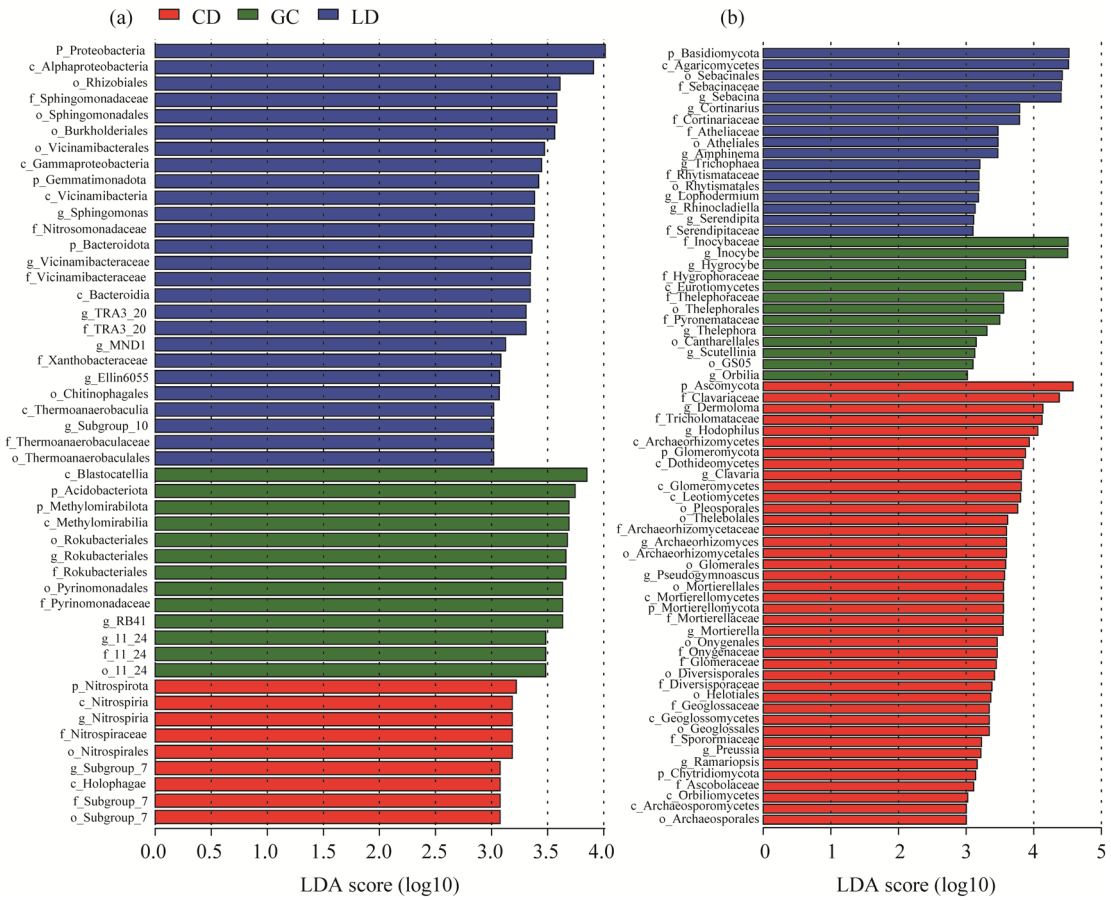

Fig. 7 LDA (linear discriminant analysis) discriminant results. (a), bacteria; (b), fungi.

3.6 Alpha diversity index

Diversity was further investigated by the indices of the ACE and Chao1. As shown in Tables 3 and 4, the ACE and Chao1 indices of bacteria in different ecosystems had little difference in grassland and shrubland, but was different from that in forest ecosystem. The ACE and Chao1 indices of at 0–10 cm soil layer in forest and grassland were different from those of 10–30 cm layer, and those of 10–20 cm layer in shrubland were different from those of other two soil layers. The Shannon and Simpson indices of bacteria were generally similar. For fungi, the four indices were similar for shrubland, but was different for forest. Different ecosystems showed different changing trends in different soil layers.

Table 3 Alpha diversity index of bacteria in different ecosystems

Ecological systems	Soil layer (m)	ACE	Chao1	Shannon	Simpson
Grassland	0–10	848.87±16.42^{a}	860.46±19.15^{a}	8.26±0.03^{a}	0.99±0.00^{a}
	10–20	878.71±8.89^{a}	896.87±9.72^{a}	8.24±0.09^{a}	0.99±0.00^{a}
	20–30	871.36±13.03^{a}	891.86±14.21^{a}	8.21±0.07^{a}	0.99±0.00^{a}
Shrubland	0–10	865.72±16.99^{a}	874.30±16.69^{a}	8.32±0.03^{a}	0.99±0.00^{a}
	10–20	862.82±17.68^{a}	868.80±19.62^{a}	8.24±0.06^{ab}	0.99±0.00^{a}
	20–30	865.69±12.19^{a}	877.63±9.56^{a}	8.11±0.03^{b}	0.99±0.00^{a}
Forest	0–10	806.85±51.89^{a}	818.34±53.11^{a}	8.13±0.11^{a}	0.99±0.00^{a}
	10–20	827.86±50.60^{a}	851.80±47.64^{a}	8.17±0.09^{a}	0.99±0.00^{a}
	20–30	814.32±36.01^{a}	832.69±37.94^{a}	7.90±0.08^{a}	0.99±0.00^{a}

Note: Different lowercase letters within the same column indicate significant differences among different ecological systems and soil layers at $P<0.05$ level. Mean±SE. ACE, abundance-based coverage estimator.

Table 4 Alpha diversity index of fungi in different ecosystems

Ecological systems	Soil layer (cm)	ACE	Chao1	Shannon	Simpson
Grassland	0–10	563.03±35.14[a]	569.07±32.27[a]	5.60±0.56[a]	0.94±0.03[a]
	10–20	532.40±47.18[a]	542.06±55.10[a]	5.43±1.04[a]	0.85±0.11[a]
	20–30	576.54±52.63[a]	582.42±52.11[a]	6.22±0.76[a]	0.94±0.04[a]
Shrubland	0–10	566.65±39.65[a]	570.91±38.30[a]	5.49±0.44[a]	0.92±0.03[a]
	10–20	554.05±28.73[a]	559.62±27.26[a]	5.33±0.40[a]	0.92±0.02[a]
	20–30	541.54±43.03[a]	548.26±45.57[a]	4.96±0.48[a]	0.86±0.03[a]
Forest	0–10	395.9±52.24[a]	390.96±49.98[a]	3.73±0.55[a]	0.77±0.09[a]
	10–20	404.26±38.15[a]	403.06±37.07[a]	3.98±0.67[a]	0.78±0.11[a]
	20–30	327.51±18.77[a]	327.25±20.63[a]	3.96±0.57[a]	0.81±0.07[a]

Note: Different lowercase letters within the same column indicate significant differences among different ecological systems and soil layers at $P<0.05$ level. Mean±SE. ACE, abundance-based coverage estimator.

3.7 ANOSIM of the soil microbial community

ANOSIM is a nonparametric test, usually represented by a box-plot. The *P*-value indicates the confidence of the analysis, and $P<0.05$ indicates statistical significance. As can be seen from Figure 8, there was no significant difference in bacteria between grassland and shrubland, but there were significant differences between grassland and forest, shrubland and forest ($P<0.01$). There were significant differences ($P<0.05$) of fungi between grassland and shrubland, shrubland and forest, and forest and grassland, and extremely significant differences ($P<0.01$) between shrubland and forests and between forest and grassland. The *R* value can be obtained by analyzing the distance matrix between samples, with *R* value close to 0 indicating no significant difference between or within groups, and *R* value close to 1 indicating the difference between groups is greater than the difference within groups. For both bacteria and fungi, *R* values of grassland and shrubland were small ($R^2=0.045$ and $R^2=0.256$, respectively), indicating that the two ecosystems had little difference.

3.8 Edaphic factors affecting soil microbial community composition

In this study, RDA was used to explore the coupling relationship between soil nutrients and soil microorganisms. The top 17 bacteria species and the top 13 fungi species and soil nutrients were selected for RDA, and a two-dimensional ranking map was constructed (Fig. 9). The correlations between bacterial communities and environmental factors are shown in Figure 9a, c, and e. With increasing soil depth, the ratio of the eigenvalues of the first two axes to the eigenvalues of the total sorting axis decreased from 65.36% to 41.47%. TP, SOM, and TN in 0–10 cm soil layer were mainly affected by Patescib, Chloroft, Myxococc ($P=0.360$, $P=0.074$, and $P=0.130$, receptively), TK was affected by Entorrhz, Kickxell, and Ascomyct ($P=0.060$), TK was mainly affected by Aphelidi and Chytridi ($P=0.012$), and TN, SOM, and TP were mainly and positively affected by Olpidiom, Rozellom, K_Fungi, Mortierl, and Monobiep ($P=0.150$, $P=0.110$, and $P=0.410$, respectively). TN, SOM, and TP in the 10–20 cm soil layer were mainly affected by Entorrhz, Rozellom, Olpidiom, Aphelidi, and Monoblep ($P=0.840$, $P=0.210$, and $P=0.640$, respectively), and TK and pH were mainly affected by Kickxell, Ascomyct, and Mortierl ($P=0.008$ and $P=0.120$, respectively), all with positive correlation. TN, SOM and TP in the 20–30 cm soil layer were mainly affected by Mucoromc, Rozellom, Monoblep, Olpidiom, Chytridi, and K_Fungi ($P=0.204$, $P=0.910$, and $P=0.710$, respectively), TK was mainly affected by Mortierl and Glomerom ($P=0.046$), and pH was mainly affected by Ascomyct and Kickxell ($P=0.710$), all with positive correlations.

The correlations between fungal communities and environmental factors are shown in Figure 9b, d, and f. The ratio of the eigenvalues of the first two axes to the eigenvalues of the total sorting axis decreased from 71.12% to 52.01% (Fig. 9b, d, and f). The pH in the 0–10 cm soil layer was mainly and positively affected by MBNT15 and Acidobac ($P=0.600$), and pH was mainly affected by RCP2_54 and NB1-j ($P=0.026$). TP, SOM, and TN in the 10–20 cm soil layer were mainly affected by Nitrospr, Desulfob, Myxococc, RCP-54, K_Bacte, and MBnt15 ($P=0.130$,

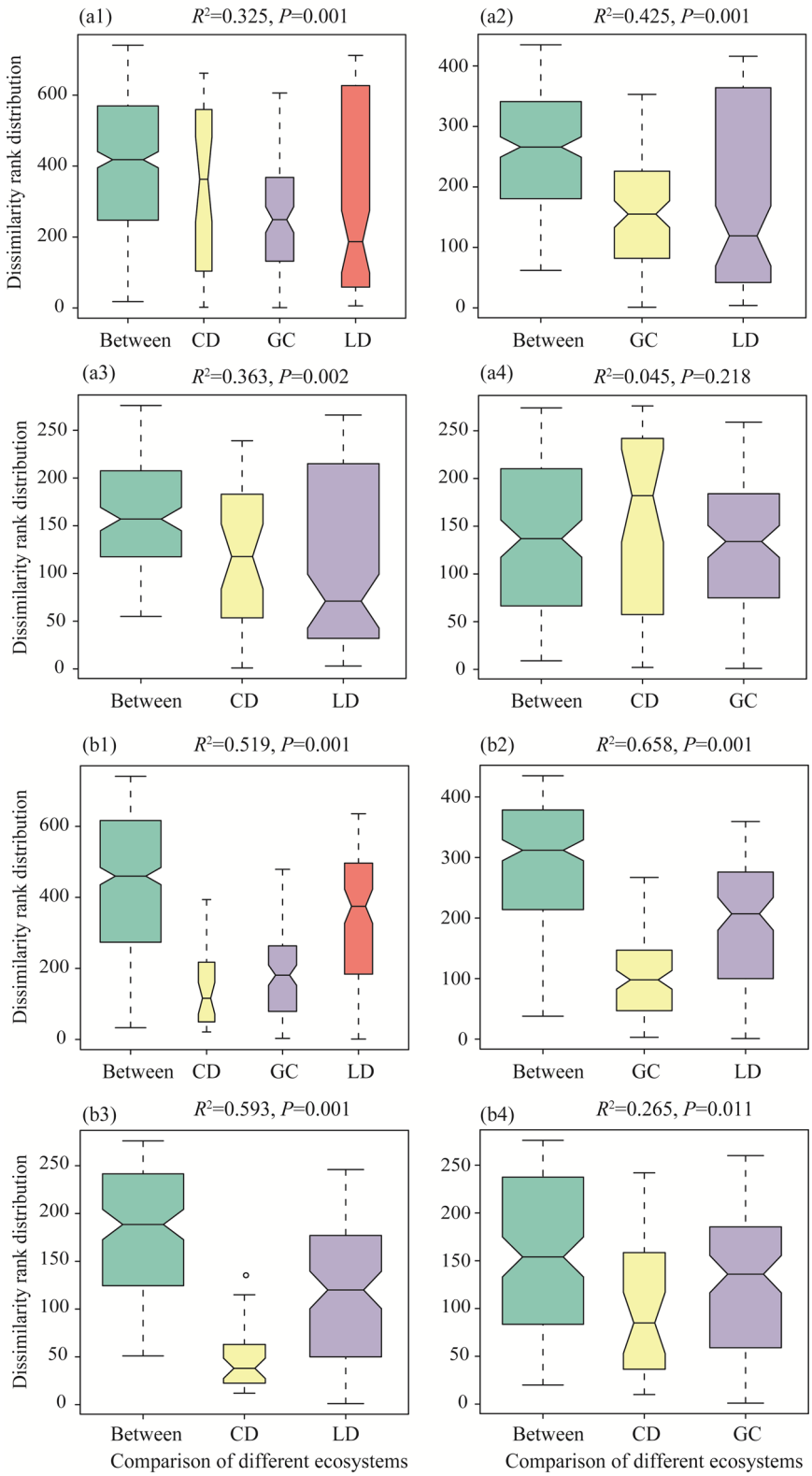

Fig. 8 ANOSIM (analysis of similarities) of the soil microbial community. (a1–a4), bacteria; (b1–b4), fungi. In Figure 8, boxes indicate the IQR (interquartile range, 75th to 25th of the data). The median value is shown as a line within the box. Whiskers extend to the most extreme value within 1.5×IQR. Outlier is shown as circle.

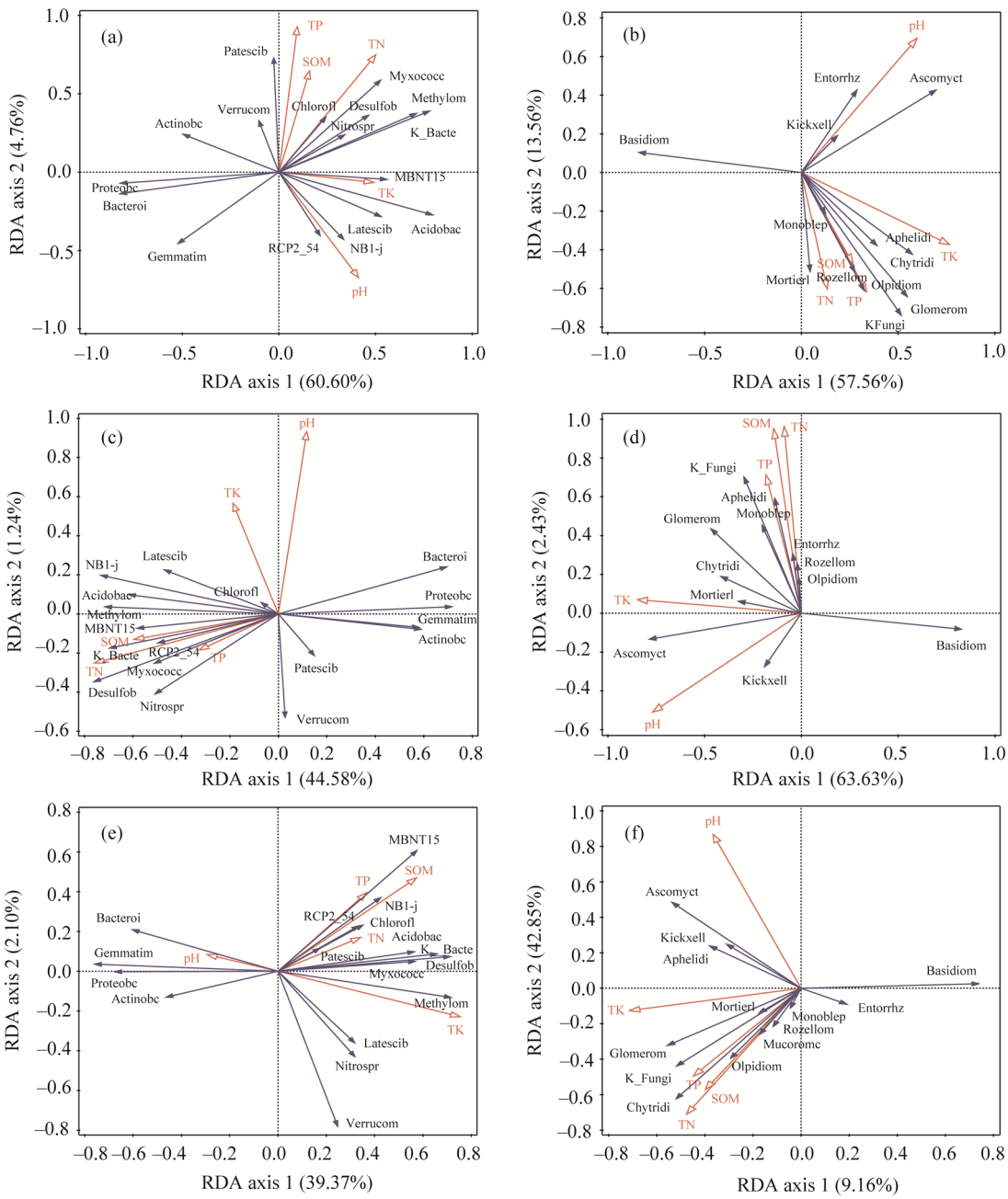

Fig. 9 RDA (redundancy analysis) of soil nutrients and microorganisms at the phylum level. (a), (c), and (e) are the RDA of bacteria and soil nutrients; (b), (d), and (f) are the RDA of fungi and soil nutrients; (a) and (b), 0–10 cm soil layer; (c) and (d), 10–20 cm soil layer; (e) and (f) , 20–30 cm soil layer.

P=0.710, and *P*=0.034, respectively), and TK and pH were significantly affected by Chlorofl, Latescib, and Bacteroi (*P*=0.980 and *P*=0.430, respectively). TP, SOM, and TN in the 20–30 cm soil layer were mainly affected by RCP2-54, MBNT15, NB1-j, Chlorofl, and Patescib (*P*=0.750. *P*=0.270, and *P*=0.510, respectively), TK was mainly affected by Methylom and Latescib (*P*=0.050), and pH was mainly affected by Bacteroi and Gemmatim (*P*=0.750).

The analysis showed that soil nutrients are more closely related to fungi. The influence of soil nutrients on microbial communities gradually decreased with the increase in soil layers. Soil nutrients in different soil layers were differently affected by soil microbial species. TN, SOM, and

TP were affected by similar soil microorganisms, and TK and pH were affected by similar microorganisms. For bacteria, pH was the primary factor affecting soil microbial community in e 0–10 cm soil layer, with an explanation rate of 30.1% (P<0.05), TN was the primary factor affecting soil microbial community in 10–20 cm soil layer, with an explanation rate of 25.9% (P<0.05), and TK was the primary factor in 20–30 cm soil layer, with an explanation rate of 22.6% (P<0.05). For fungi, K was the primary factor affecting the soil microbial community in all soil layers, with explanation rates in the range of 22.6%–45.7% (P<0.05).

4 Discussion

4.1 Effects of different ecosystems on microbial community structure and diversity

Variation in vegetation is an important environmental factor that causes microbial differences (Wu and Ai, 2008; Chu et al., 2016; Zhu et al., 2017; Zhao et al., 2021). Changes in the aboveground vegetation, including changes in the number, composition, and decomposition rate of fallen leaves and fine roots affects the structure of soil microbial communities (Yang et al., 2015; Li et al., 2016; Liu et al., 2016; Kim et al., 2018). The trends of soil TN, TP, SOM, TK, pH, and microbial diversity in this three ecosystems were in the order of grassland>shrubland>forests, indicating nutritional differences of soil microenvironment. The lowest microbial abundances were found in forest, with only a minor difference between grassland and shrubland. Due to the differences in soil environments of different ecosystems and their different effects on the relative abundances of soil microbial communities (Tables 5 and 6), there were differences in the bacterial diversity and fungal richness of grassland, shrubland, and forest (Tables 5 and 6; Fig. 10). This difference may be due to the important role of plant root exudates in the distribution of soil microorganisms (Nayyar et al., 2009; Zhao et al., 2013). Plant composition may alter the range of litter inputs and root exudates (Reynolds et al., 2003; Wardle et al., 2004), and plant-microbe interactions (Martinez-Garcia et al., 2015). With low vegetation coverage (Table 1) in the forest ecosystems, there is less photosynthetic area and nutrients stored in the root system. In addition, the litter decreases and the root systems are relatively sparse, resulting in reduced nutrients for the soil and reduced enzyme activities of soil microorganisms (Sheng et al., 2009). In the grassland, vegetation is dense, there is low evaporation of soil water, and abundant litter, biomass, and plant roots. Therefore, soil nutrient content is high, and microbial diversity and richness are high.

Table 5 Correlation between bacterial diversity and soil nutrients

Ecological systems	Bacteria	ACE	Chao1	Shannon	Simpson
Grassland	TN	–0.73*	–0.73*	0.34	0.72*
	TP	–0.80*	–0.79*	0.11	0.46
	TK	–0.38	–0.31	0.09	0.45
	SOM	–0.37	–0.41	0.26	0.48
	pH	0.82**	0.77*	–0.10	–0.53
Shrubland	TN	0.01	–0.04	0.31	0.26
	TP	–0.05	–0.11	0.11	0.20
	TK	0.46	0.46	0.08	–0.08
	SOM	0.01	–0.07	0.12	0.11
	pH	0.53*	0.52*	0.24	0.03
Forests	TN	–0.08	–0.14	0.22	0.47
	TP	–0.75**	–0.76**	–0.54*	–0.06
	TK	0.10	0.15	–0.19	–0.24
	SOM	–0.25	–0.28	0.12	0.50
	pH	–0.10	–0.04	–0.39	–0.44

Note: **, P<0.01 level; *, P<0.05 level; ACE, abundance-based coverage estimator.

Table 6 Correlation between fungal diversity and soil nutrients

Ecological systems	Fungi	ACE	Chao1	Shannon	Simpson
Grassland	TN	–0.60	–0.56	–0.66	–0.42
	TP	–0.47	–0.44	–0.41	–0.20
	TK	–0.45	–0.41	–0.34	–0.12
	SOM	–0.48	–0.48	–0.56	–0.36
	pH	0.73*	0.70*	0.60	0.38
Shrubland	TN	0.04	0.02	–0.29	–0.25
	TP	–0.04	–0.04	–0.34	–0.41
	TK	0.22	0.23	0.34	0.11
	SOM	0.04	0.04	–0.40	–0.42
	pH	0.46	0.44	0.72**	0.62*
Forest	TN	–0.05	–0.05	0.09	0.11
	TP	–0.00	–0.02	0.02	0.06
	TK	–0.18	–0.17	–0.29	–0.35
	SOM	–0.23	–0.24	0.02	0.05
	pH	0.09	0.12	0.05	–0.15

Note: **, $P<0.01$ level; *, $P<0.05$ level; ACE, abundance-based coverage estimator.

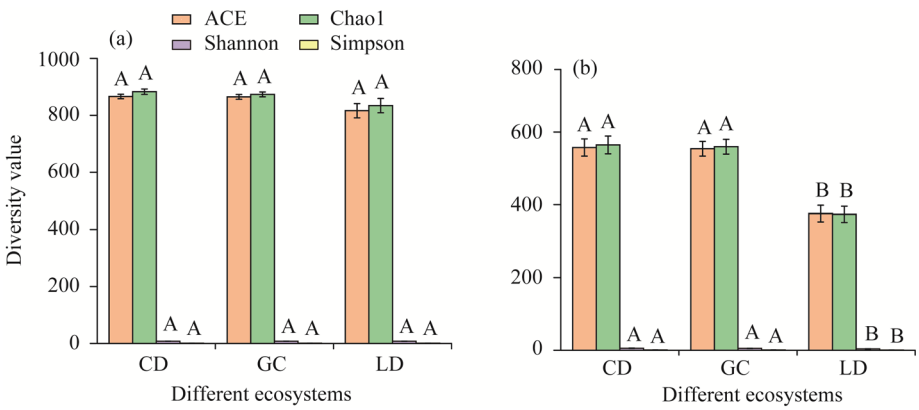

Fig. 10 Differences in diversity value across different ecosystems. (a), bacteria; (b), fungi. Different uppercase letters within the same diversity index indicate significant differences among different ecosystems at $P<0.05$ level. ACE, abundance-based coverage estimator.

In this study, three bacterial phyla, Acidobacteriota, Proteobacteria and Gemmatimonadetes were identified as the dominant soil bacteria in forest, shrubland, and grassland. This result is consistent with the findings of studies of the Qinghai-Tibet Plateau (Guan et al., 2013; Yuan et al., 2014; Chu et al., 2016). Proteobacteria prefer a nutrient-rich environment, and a carbon-rich environment can stimulate their rapid growth, contributing to the accumulation of soil nutrients and thus promoting the growth of eutrophic bacteria. Acidobacteria is a poor nutrient group (Morris et al., 2002; Sul et al., 2013) that can grow in an environment containing refractory carbon and degrade polymers of plant residues. Gemmatimonadetes are highly drought-resistant, and can oxidize organic and inorganic compounds. Ascomycota, Basidiomycota, and Mortierellomycota were identified as the dominant soil fungi, consistent with the results of most studies on the Tibetan Plateau (Guan et al., 2013; Yang et al., 2014; Zhang et al., 2016). Ascomycota and Basidiomycota play important roles in decomposing plant and straw residue (Yelle et al., 2008; Wu et al., 2018). Mortierellomycota can decompose organic matter, promote the absorption of mineral elements by plant roots, and secrete antibiotics that inhibit pathogenic bacteria (Gao, 2006; Liu and Li, 2007). As a result, these microorganisms can not only promote plant and animal decomposition and organic matter accumulation under drought conditions, but

also resist virus invasion, which is an important condition for ensuring the stability of the soil ecosystem.

4.2 Effects of different ecosystems on microbial community structures

The correlations of alpha diversity index and soil nutrients were determined (Tables 7 and 8). The correlations between ACE and Chao1 indices and soil nutrients were not significant, indicating that soil TN, TP, SOM, TK, pH, and other nutrients have little effect on soil bacterial richness. This is consistent with the findings of similar bacterial OTUs in different ecosystems, with only a few unique OTUs. There is a significant positive correlation between soil TN and Shannon index, with a correlation coefficient of 0.37. Soil TN, TP, and SOM are significantly and positively correlated with Simpson index, with the largest coefficient of correlation (0.52) between TN and Simpson index. Soil nutrients have a significant impact on bacterial diversity, particularly soil TN. The autotrophic microbial communities in the soil of the Qinghai-Tibet Plateau are dominated by nitrogen-fixing bacteria, primarily cyanobacteria and proteobacteria (Liu et al., 2016; Che et al., 2018). Characterization of the effects of environmental factors in different soil layers on microbial communities showed that pH and TK had a significant impact on microorganisms, a result that is consistent with previous studies of the Qilian Mountains conducted by Zhu (2017) and Zhang et al. (2014). This study area is primarily affected by denitrifying bacterial communities, and the abundance and activity of denitrifying bacteria in soil can be significantly affected by soil pH (Guo, 2013). Sang et al. (2020) found that TK input can significantly increase the abundance of Proteobacteria and Acidobacteria in soil. For fungi, our results showed that the ACE and Chao1 indices are significantly affected by TN, TK, and SOM, and changes in these three nutrients are substantially different for different ecosystems, thus explaining the unique OTUs in different ecosystems. In general, the stress of soil nutrients is greater on fungi than on bacteria. This may be because fungi are significantly affected by altitude and vegetation type (Liu et al., 2008; Pan et al., 2009).

Table 7 Correlation between soil nutrients and fungal alpha diversity index

Soil nutrient	ACE	Chao1	Shannon	Simpson
TN	0.33*	0.33*	0.11	0.13
TP	0.12	0.14	0.18	0.18
TK	0.34*	0.36*	0.36*	0.20
SOM	0.35*	0.35*	0.15	0.15
pH	0.20	0.21	0.42**	0.21

Note: **, $P<0.01$ level; *, $P<0.05$ level; ACE, abundance-based coverage estimator.

Table 8 Correlation between soil nutrients and bacterial alpha diversity index

Soil nutrient	ACE	Chao1	Shannon	Simpson
TN	0.10	0.06	0.37*	0.52**
TP	–0.21	–0.23	0.04	0.36*
TK	0.27	0.28	0.17	0.23
SOM	0.05	0.01	0.28	0.47**
pH	0.17	0.19	–0.05	–0.14

Note: **, $P<0.01$ level; *, $P<0.05$ level; ACE, abundance-based coverage estimator.

Soil nutrients shape the composition and distribution of microbial communities (Fierer, 2017), by affecting soil hydrothermal conditions and nutrient content (Ren et al., 2016; Yang et al., 2017; Qiu et al., 2019; Cai et al., 2022). The effects of environmental factors on microbial communities decreased with increased soil depth (Fig. 9). On the one hand, the surface root system is well developed, with root exudates and dense litter (Smith and Paul, 1990); on the other hand, the external environment affects the hydrothermal conditions of the soil surface, and increased soil water content can increase the activity of soil microorganisms (Xiang et al., 2008). Water content

in the Qilian Mountains decreases with the increase of soil depth (Yuan et al., 2019). Overall, the results show that the diversity of soil microbial community distribution in different soil layers is mainly due to coupling effects among different ecosystems, soil nutrients, and soil microorganisms (Figs. 6 and 9).

5 Conclusions

Analysis of the soil microbial communities in the study area revealed relatively high relative abundances of Acidobacteriota, Proteobacteria and Gemmatimonadetes, accounting for more than 75% of soil bacteria. Basidiomycota, Ascomycota, and Mortierellomycota have relatively high abundances, accounting for more than 94% of soil fungi. Diversity and richness of soil fungi and bacteria in different ecosystems show different distribution trends. According to the ANOSIM results, there was no significant difference in microbial community characteristics between grassland and shrubland (R=0.045, P>0.05), but there were significant or extremely significant differences between grassland and forest, and between shrubland and forest. RDA showed that the correlation between soil nutrients and microorganisms gradually decreased with the increase in soil depth, with a higher effect of soil TK content on soil microorganisms than those of other soil nutrients. Therefore, when improving soil environment, strategies to increase the abundance of microorganisms should improve the potassium content to promote energy flow in the ecosystem and improve soil fertility.

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