



Grazing-to-fencing conversion affects soil microbial composition, functional profiles by altering plant functional groups in a Tibetan alpine meadow

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ABSTRACT

Information on the effect of anthropogenic activities on soil microbial communities can improve our ability to formulate restoration strategies in the Tibetan Plateau, where meadow degradation has been an increasing concern. In this study, we evaluated plant functional groups, soil properties, microbial communities, and their functional groups in four types of meadows (grazed, fenced, fenced + reseeded, and intact) in the Tibetan Plateau. Based on amplicon sequencing data, both the bacterial and fungal composition, their potential functions and their possible drivers have been investigated. Fencing was the most effective approach to restore degraded alpine meadows, characterized by higher aboveground biomass, soil nutrient levels, and microbial diversity. The soil carbon, ammonium nitrogen, and the above- and below-ground biomass (mainly sedge and grass groups) were closely related to the changes in bacterial and fungal composition, while the diversity of sedge, grass, legumes, and forbs was largely associated with N cycling and fungal functional guilds. Grazing-to-fencing conversion also caused changes in the microbial community composition, specifically resulting in a shift from oligotrophs to copiotrophs, an increased abundance of diazotrophs and plant pathogens, and a decreased abundance of nitrifiers. Our results revealed the effects of grazing-to-fencing conversion on an alpine meadow and can provide significant guidance for the sustainable management of degraded grassland ecosystems.

1. Introduction

Alpine meadow, a major vegetation type that is widely distributed over the Middle Eastern Tibetan Plateau, spanning more than approximately 700,000 km², plays multitudinous roles in maintaining the ecosystem, including carbon sequestration, biogeochemical cycle stabilization, and food supply for livestock (Pepin et al., 2015; Yang et al., 2017). Despite its importance in the ecosystem, the alpine meadow has been facing serious degradation in the past two decades owing to overgrazing, which poses a serious threat to human existence and the ecological environment (Li et al., 2016; Ma et al., 2016). Grazing exclusion by fencing and reseeded has recently been applied to restore degraded meadow areas (Feng et al., 2010; Wu et al., 2009). These land-use changes have been reported to improve aboveground productivity, maintain plant diversity, and increase soil nutrient levels (Laliberte et al., 2010); however, the impact of grazing-to-fencing conversion on

the soil microbial community remains poorly understood. Microorganisms play essential roles in maintenance of the structure and function of ecosystems (Zhang et al., 2017; Liu et al., 2021). Therefore, addressing microbial response patterns during grazing-to-fencing conversion, including community composition, functional taxa, and their interactions with the plant community is necessary to understand the mechanisms of land-use effects on ecosystems and provide important information for research on the sustainable management of degraded grasslands.

Most microorganisms are heterotrophs and require carbon from organic substances as a source of energy, which comes mainly from plant residues, litters, and root exudates (Cline et al., 2018; Eldridge and Delgado-Baquerizo, 2018). As different plants have different physiological characteristics and chemical compositions, changes in plant biomass, diversity, or the composition of plant functional groups (PFGs) are likely to alter the quantity and quality of these organic substances,

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with consequences on changes in the quantity, composition, activity, and function of the soil microbial community (Cortois et al., 2016; Gomoryova et al., 2013). However, the mechanisms underlying soil microbial feedback in response to plant community changes are unclear. The effect of plant community on microbes in grassland soil has been reported to be positive (Francioli et al., 2020; Wang et al., 2020a) or neutral (Schops et al., 2018) and is expected to be driven by various forces. For example, Eisenhauer et al. (2010) reported that a plant richness reduction resulted in a significant decrease in the bacterial and fungal biomass, abundance, and functional diversity. In our previous study, based on pot experiments, we demonstrated that increased plant diversity could increase bacterial and fungal diversity of the grassland soil owing to the high diversity of root exudates, but it had no effect on microbial biomass (Zhang et al., 2019). Other studies also found no indispensable association between soil microbial communities and plant richness/PFG diversity because of the difficult incorporation of organic substances from plants into the soil (Habekost et al., 2008; Marshall et al., 2011). Thus, further research should be performed on the response of soil biota to changes in plant richness/PFGs.

Plants also influence the structure of soil microbes by altering the surrounding soil conditions (Lozano et al., 2014). For example, soil communities under conditions of high nutrients tend to be bacteria-dominated, whereas low-nutrient soils tend to support fungal-dominated communities (Marshall et al., 2011). Field observations indicated that reductions in plant biomass and PFG diversity can lead to a decrease in C and N allocation to soils and consequently weaken the growth and activity of bacterial communities (Bastida et al., 2017; Gebhardt et al., 2017). Plants also alter the microbial diversity and activities by changing the aboveground coverage, which decreases evaporation and helps to retain moisture; they also create varying microhabitats suitable for different microbes using their roots (Wang et al., 2020a). Collectively, plant traits such as coverage, diversity, roots, and PFG composition are likely to influence soil attributes, which could provide feedback to the soil microbial community structure and function.

Microorganisms play essential roles in biochemical processes, such as those associated with N cycling. Previous studies have demonstrated that the functional group called diazotrophs performs biological N-fixation, which reduces gaseous N to ammonium N available to plants (Levy-Booth et al., 2014). Nitrifying and denitrifying microbes are responsible for regulating N availability in the soil, reflecting the uptake of N by organisms and some N losses in the environment (Merloti et al., 2019; Srikanthasamy et al., 2018). Many known functions of fungi are also mediated by specific fungal guilds, such as saprotrophs (decomposers related to recalcitrant organic matter), symbionts (mycorrhizal fungi), or pathogens (population related to plant disease) (Eldridge and Delgado-Baquerizo, 2018). In addition, various species of fungi and bacteria live together to form a complex co-occurrence network (de Menezes et al., 2017). These inter-species networks play an important role in the maintenance of soil functions and the establishment of a plant community. However, microbial co-occurrence patterns are not well understood, especially in the alpine meadow ecosystem. Although the importance of microbes in ecosystem processes is recognized, only one study has attempted to investigate how grazing-to-fencing conversion can affect the soil microbial community in an alpine meadow and determine the factors affected by it (Yin et al., 2021). The complicated interactions among plants, soil, and microorganisms make it difficult to understand these microbial communities in detail.

In this study, we aimed to evaluate the effect of grazing-to-fencing conversion on the soil microbial community composition and functional profiles in a Tibetan alpine meadow. To better understand these effects, we sequenced bacterial 16S rRNA and fungal ITS coupled with the quantification of their abundance to assess soil communities in four types of alpine meadows of the Tibetan Plateau (grazed, fenced, fenced + reseeded, and intact). We hypothesized that grazing-to-fencing

conversion would affect the microbial communities, especially the functional profiles. We hypothesize that: (1) fencing is an effective approach to restore degraded alpine meadows by improving above-ground biomass, soil nutrient levels, and microbial diversity; (2) changes in the composition of soil microbial communities and their functional profiles are closely associated with nutrient availability and above- and below-ground biomasses. To test these hypotheses, the following questions were addressed: (1) the main changes in the structure and composition of bacterial and fungal communities caused by meadow land-use change; (2) how land-use change affects soil-specific microbial groups, such as oligotrophic and copiotrophic microbes, diazotrophs, nitrifiers, saprotrophs, and pathogens; (3) the main drivers of the changes in the microbial composition and functional profiles in these areas.

2. Materials and methods

2.1. Study area description

Our experiments were conducted in the northwest Qinghai-Tibetan Plateau region at the Bangjietang experimental site of the Alpine Meadow Ecosystem Research Station, Chinese Academy of Science at 32°21' N, 91°40' E, 4672 m a.s.l, in Tibet Province, PR China. The plateau has a continental climate and is characterized by a mean annual temperature of -3.0 °C, ranging from -15.0 °C in January to 7.9 °C in July. The mean annual precipitation is 409.0 mm, with peak levels observed from July to August. The major soil type is Mattic-Cryic Cambisol (Alpine meadow soil, Cambisols in FAO/UNESCO taxonomy). The vegetation is typical alpine meadow dominated by *Kobresia robusta*, *Kengyilia thoroldiana*, *Kobresia pygmaea*, *Stipa purpurea*, and *Elymus nutans*. Generally, plateau pika (*Ochotona curzoniae*) are the natural small mammals and widely distributed in these meadows.

2.2. Experimental design, sampling, and soil property analysis

Four types of managed meadows, including continuous grazed meadow (GM), fenced meadow (FM), fenced + reseeded meadow (FRM), and intact meadow (IM), were established in the experimental site with an area of 200 hm² (Wang et al., 2020b). Five replicated plots covering approximately 1 hm² for each plot were randomly set up in each meadow treatment, and a total of 20 plots was established. These meadows had the same soil types (Mattic-Cryic Cambisol) and similar altitudes (ranging from 4550 m to 4600 m). The detailed information about these meadows is as follows: (1) GM was grazed by four yaks (*Bos grunniens*) per hectare since 1988, it has been subjected to grazing for 30 years and the grazing intensity was calculated according to the annual biomass of the experimental meadow, feed intake of yaks (*Bos grunniens*), pasture area, and grazing time. (2) FM was established in 2009, and the meadow was fenced using iron to prevent herbivore access into sites. Before fencing, the site had the same grazing management as that of the GM treatment. (3) FRM had the same management as the FM and had additional seeding of native plant species. Reseeding was conducted in June 2009, and seeding holes were 3 cm deep and spaced 20 cm apart. Three native species, *Elymus nutans* Griseb, *Poa cymophila* Keng, and *K. thoroldiana* (Oliver) J. L. were seeded at seeding rates of 3, 5, and 8 g•m⁻², respectively. (4) The IM was an original and intact alpine meadow without any human interference or grazing.

In October 2018, nine subplots (10 × 10 m²) were set up along a diagonal line in each plot, and each subplot was 10–20 m apart. In each subplot, three quadrats (1 × 1 m²) were randomly established. Plant investigation and soil sampling were performed in each quadrat. After removing litter, stone, and debris, 0–15 cm soil samples were collected along an S-shaped pattern (5 soil cores) and then mixed to form one composite sample. Finally, soil samples from nine subplots were mixed to form one sample as a replicate for each treatment. This sampling approach eliminated the biases and errors caused by soil heterogeneity

(Lozano et al., 2014; Yang et al., 2017). Each soil sample was divided into two parts. One was stored at -80°C for molecular analysis, whereas the other was air-dried for physicochemical measurements. Soil organic carbon (OC), pH, total nitrogen (TN), $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, total phosphorus, available phosphorus (AP), and soil water content were determined by the methods described in our previous study (Wang et al., 2020a, b, see details in Appendix S1).

Plant coverage, above-ground/below-ground biomass, and species number were determined in each quadrant. Aboveground parts of each species were collected and dried at 70°C for 48 h to measure above-ground biomass; the roots were washed using tap water and dried at 70°C for 48 h to measure below-ground biomass. The Shannon–Wiener index of a plant community was determined by Zhang et al. (2016).

2.3. Microbial DNA extraction and quantitative PCR analyses

DNA was extracted from 0.25 g of homogenized soil subsamples using the FastDNA® SPIN Kit for Soil (MP Biomedicals, Cleveland, USA). The concentrations of DNA extracted from both sample replicates were independently measured using a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The copy numbers of 16S rDNA and ITS were examined using 7500 Real-Time PCR System (Applied Biosystems, ABI, USA). The universal primer set used for the amplification of 16S rDNA was 515F (5'-GTGCCAGMCCCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') (Yusoff et al., 2013), and that for ITS DNA was ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATGCATGC-3') (Adams et al., 2013). The reaction mixture contained 10 μL of ChamQ SYBR Color qPCR Master Mix (2 \times , Vazyme Biotech Co., Ltd. Nanjing, China), 1 μL of template DNA, 7.4 μL of double distilled water, and 0.8 μL each of forward and reverse primers (5 μM). The PCR cycling conditions were as follows: denaturation (95°C for 5 min), amplification and quantitation (95°C for 5 s, 55°C for 30 s, and 72°C for 40 s) repeated 40 times. Specific PCR products were examined by a melting curve analysis. Standard curves, which were characterized by $R^2 = 0.99$ and an amplification efficiency of 99.83–105.55%, were constructed using plasmids containing the 16S rDNA and ITS fragments for bacteria and fungi, respectively.

2.4. MiSeq sequencing and bioinformatics analysis

For taxonomical profiling of bacteria and fungi, the universal primer set 515F-907R and ITS1-ITS2 was used to amplify the V4-V5 hyper-variable regions of the bacterial 16S rDNA gene and ITS 1 region of the fungal genes, respectively. The PCR mixtures contained 4 μL of 5 \times TransStart FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL TransStart FastPfu DNA Polymerase, 10 ng template DNA, and finally ddH₂O up to 20 μL . The PCR cycling conditions were as follows: 98°C for 60 s; 30 cycles at 98°C for 10 s, 50°C for 30 s, and 72°C for 30 s; 72°C for 5 min; hold at 4°C . Successful PCR amplification was verified by 2% agarose gel electrophoresis. The PCR products were mixed in equidense ratios and purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). Sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). The library quality was assessed using the Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system, according to the manufacturer's protocol. Finally, the library was sequenced on the Illumina MiSeq PE300 platform, and 300 bp paired-end reads were generated. The raw reads were deposited into the NCBI Sequence Read Archive database (accession number: SRP198617 and SRP292450).

Raw sequence data were de-multiplexed, quality-filtered, and chimera-checked using the QIIME (v1.7.0) workflow and quality control process (Caporaso et al., 2010). The bacterial and fungal tags were compared with reference databases using the UCHIME algorithm to detect chimeric sequences (Edgar, 2013). Chimera sequences were then

removed. The remaining sequences were clustered with UPARSE software (v7.0.1001) and assigned to operational taxonomic units (OTUs) at similarities of 97%. The bacterial taxonomic identity was confirmed using the Silva reference database (v132) via the RDP classifier (Release 11.1 <http://rdp.cme.msu.edu/>), and fungi were identified in the Unite database (v8.2) with BLAST using QIIME (v1.7.0). The community diversity indicators, including observed OTUs, the Shannon–Wiener index, and the Chao1 estimator, were calculated using 20,566 and 38,696 reads per sample for bacteria and fungi, respectively. LefSe analysis indicated significant differences in sequencing depth among sample groups, characterized by LDA scores of 3.69 and 2.38 for bacteria and fungi, respectively. The proportions of the copiotrophic and oligotrophic prokaryotic lineages were calculated based on the study by Zhou et al. (2017) and Che et al. (2019). Finally, given the relatively limited information available from 16S rRNA or ITS analyses, further analysis is generally difficult to conduct; to better understand the potential functional contributions of the observed microbes in these meadows, FAPROTAX database 1.1 (Louca et al., 2016) and FUNGuild database 1.1 (Nguyen et al., 2016) were used for the functional profiles of bacteria and fungi, respectively; 47.78% (range from 37.24% to 58.92%) and 80.71% (range from 64.45% to 98.54%) of sequences from bacteria and fungi were aligned to different functional groups, respectively.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) followed by a post Duncan multiple comparison were used to evaluate the differences in soil properties and vegetation characteristics among the four treatments; LefSe analysis was performed as a linear discriminant analysis of samples from different grouping conditions according to their taxonomic composition, as well as potential functional groups, to determine the communities that exhibit significant differences in four treatments. $P < 0.05$ was considered statistically significant. Principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity and permutational multivariate ANOVA (PERMANOVA) based on OTUs were performed to examine the overall differences in structures of microbial communities. Variance partitioning analysis (VPA), structural equation modeling (SEM), and aggregated boosted tree (ABT) analyses were conducted to reveal the driver of the microbial community composition and potential function (PCA1 axis, Fig. S1a–b). First, VPA was used to verify the contribution of plant community and soil properties to the changes in the microbial community. Second, based on the results of VPA that confirmed a greater effect of plant community, fitted SEM was established to illustrate how the plant community affects the properties of bacterial and fungal communities (direct or indirect). The first step in SEM requires establishing a prior model based on the known effects and hypothetical relationships between the drivers of microbial properties. In this study, we assumed that the plant community affects the microbial community directly by altering the plant biomass, diversity, and species richness and indirectly by changing the soil nutrient contents. Finally, based on the results of SEM, ABT analysis was employed to clarify the plant groups and properties of these groups that determine the bacterial and fungal communities. In addition, owing to the strong collinearity among environmental factors, before performing VPA and SEM, we performed variable clustering to assess the redundancy of environmental variables with the VARCLUS procedure. The variables with higher correlations (Spearman's $\rho^2 > 0.7$) were removed from the VPA and SEM analyses. Finally, AP, $\text{NH}_4^+\text{-N}$, and OC were used for the next analysis (Fig. S2). All analyses were performed using “*agricolae*” (de Mendiburu, 2020), “*vegan*” (Oksanen et al., 2017), “*Hmisc*” (Harrell, 2013), “*gbmpls*” (De'ath, 2007), and “*lavaan*” (Rosseeel, 2012) in the R software system (version 3.5.3, <https://www.r-project.org/>).

3. Results

3.1. Plant and soil properties

Grazing-to-fencing conversion caused significant changes in the plant community composition (Fig. S3; Table 1). In general, FRM and FM had higher plant diversity, coverage, and above- and below-ground biomass than GM. Except for GM, in which no Leguminosae species were found, the other three treatments comprised four plant functional groups, including sedge, grass, legumes, and forbs. The plant community of IM was dominated by sedge groups (relative abundance, 83.20%), whereas grass dominated the GM, FM, and FRM plant communities, accounting for 36.93%, 50.77%, and 77.09%, respectively. FM presented the highest diversity, coverage, and above- and below-ground biomass of legumes and grass, followed by FRM, GM, and IM. IM had the highest coverage and above- and below-ground biomass of sedge and

Table 1

Effect of grazing-to-fencing conversion on plant community. SEs are shown in parentheses ($n = 5$). Values within the same row not followed by the same letter differ significantly (Duncan's test, $P < 0.05$). H is plan Shannon diversity. AGB and BGB are above- and belowground biomass, respectively. PFGs: Plant functional groups; GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow. “-” indicates the absence of plant species.

	PFGs	GM	FM	FRM	IM	
H	Total	1.27 ± 0.12 b	1.70 ± 0.09 a	1.65 ± 0.07 ab	0.77 ± 0.12 c	
	Sedge	0.31 ± 0.02 ab	0.38 ± 0.02 a	0.42 ± 0.06 a	0.21 ± 0.05 b	
	Grass	0.62 ± 0.09 b	0.72 ± 0.06 a	0.64 ± 0.11 b	0.10 ± 0.03 c	
	Legume	-	0.11 ± 0.03 a	0.04 ± 0.01 b	0.06 ± 0.01 b	
	Forbs	0.67 ± 0.08 a	0.49 ± 0.05 ab	0.28 ± 0.01 b	0.41 ± 0.04 b	
	Total	27.80 ± 1.77 c	85.40 ± 3.33 a	58.80 ± 3.43 b	92.80 ± 2.11 a	
	Sedge	7.90 ± 0.57 d	16.70 ± 1.07 b	12.68 ± 1.37 c	76.60 ± 3.01 a	
	Grass	14.12 ± 1.80 c	60.50 ± 4.32 a	49.70 ± 3.67 b	4.20 ± 1.24 d	
	Legume	-	9.40 ± 0.58 b	2.70 ± 0.92 b	3.91 ± 0.85 a	
	Forbs	9.80 ± 1.89 b	11.12 ± 0.89 b	10.04 ± 1.69 b	17.98 ± 1.79 a	
Coverage (%)	Total	4.33 ± 0.67 b	16.31 ± 1.69 a	15.29 ± 1.99 a	16.58 ± 0.84 a	
	Sedge	1.15 ± 0.39 c	3.86 ± 0.36 b	2.88 ± 0.72 b	7.61 ± 1.64 a	
	Grass	1.15 ± 0.34 b	10.25 ± 0.87 a	8.33 ± 0.85 a	0.40 ± 0.03 b	
	Legume	-	1.91 ± 0.09 a	0.44 ± 0.02 b	1.54 ± 0.07 ab	
	Forbs	3.00 ± 0.71 b	3.20 ± 0.30 b	3.60 ± 0.14 b	5.40 ± 0.88 a	
	Total	24.50 ± 4.39 c	129.18 ± 10.95 b	72.36 ± 12.35 d	250.01 ± 21.73 a	
	Sedge	12.79 ± 4.44 d	50.46 ± 7.57 b	25.74 ± 2.80 c	214.81 ± 23.73 a	
	Grass	9.65 ± 2.85 c	70.11 ± 5.73 a	38.58 ± 5.32 b	5.72 ± 1.78 c	
	Legume	-	4.17 ± 0.03 a	2.44 ± 0.04 b	2.6 ± 0.03 b	
	Forbs	2.06 ± 0.90 d	6.39 ± 0.36 b	5.44 ± 0.01 c	25.31 ± 3.54 a	
BGB (g/m ²)	Sedge	33.13 ± 1.85 b	26.86 ± 3.25 c	13.90 ± 1.83 d	83.20 ± 2.62 a	
	Grass	36.93 ± 6.41 c	50.77 ± 3.06 b	77.09 ± 3.04 a	3.17 ± 1.13 d	
	Legume	-	3.77 ± 0.38 a	0.85 ± 0.02 ab	1.71 ± 0.35 ab	
	Forbs	29.94 ± 1.76 a	18.61 ± 2.74 b	8.16 ± 1.97 d	11.92 ± 3.25 c	
	Relative abundance (%)	Total	12.79 ± 4.44 d	50.46 ± 7.57 b	25.74 ± 2.80 c	214.81 ± 23.73 a
		Sedge	9.65 ± 2.85 c	70.11 ± 5.73 a	38.58 ± 5.32 b	5.72 ± 1.78 c
		Grass	36.93 ± 6.41 c	50.77 ± 3.06 b	77.09 ± 3.04 a	3.17 ± 1.13 d
		Legume	-	3.77 ± 0.38 a	0.85 ± 0.02 ab	1.71 ± 0.35 ab
		Forbs	29.94 ± 1.76 a	18.61 ± 2.74 b	8.16 ± 1.97 d	11.92 ± 3.25 c

forbs. Grazing-to-fencing conversion also caused changes in soil properties (Table 2). The OC, TN, NO₃⁻-N, and moisture content of FM soil were higher than those of GM but lower than those of IM. Soil pH values ranged from 8.45 to 8.82, and FRM showed the highest pH value. FM soil also had the highest content of NH₄⁺-N and lowest C/N among the four meadows.

3.2. Microbial community diversity and composition

Grazing-to-fencing conversion changed the abundance of bacteria (Fig. 1). The bacterial abundance in FM and FRM was higher than that in GM but lower than that in the IM site. Fungal quantity did not differ significantly among the four meadows. Grazing-to-fencing conversion led to an increase in taxonomic diversity of bacteria and fungi (Table 3). Specifically, FM and FRM had the highest observed OTUs, diversity (Shannon and phylogenetic), and Chao's estimator of bacterial and fungal communities. No significant difference in these indices were observed between FM and FRM for both bacterial and fungal communities.

Amplicon sequencing generated 1,766,205 and 2,528,106 high-quality sequences for bacteria and fungi from 20 soil samples, respectively. The ordination of PCoA showed distinct fungal and bacterial community compositions in the different meadows, characterized by a separate cluster for each treatment (Fig. 2a-b). These differences in microbial composition were further demonstrated by PERMANOVA analyses (bacteria and fungi; $R^2 = 0.61$, $P = 0.001$ and $R^2 = 0.34$, $P = 0.008$, respectively). Overall, the bacterial community comprised 27 phyla based on the SILVA database (Fig. 3a). Proteobacteria was the dominant phylum, accounting for 29.40% of sequences on average, followed by Actinobacteria (23.80%), Acidobacteria (19.87%), Chloroflexi (8.45%), and Planctomycetes (6.32%). The abundance of Proteobacteria was higher in FM and FRM than in GM but lower than that in IM. An opposite trend was observed for the abundance of Actinobacteria and Acidobacteria. All identified fungal OTUs were assigned to 14 phyla based on the Unite database (Fig. 3b). The relative abundance of Ascomycota was higher in GM than in FM and FRM but lower than that in IM. Basidiomycota relative abundance was the highest in FM, followed by FRM, GM, and IM. IM had the highest abundance Mortierellomycota, followed by FRM, GM and FM.

LefSe analysis was performed to identify microbes specifically enriched at the family level in the four meadows, and 33 bacterial families and 12 fungal families presented statistically significant differences with an LDA score > 2.0, among treatments (Fig. 4a-b); these

Table 2

Effect of grazing-to-fencing conversion on the characteristics of soil physico-chemical properties (Wang et al., 2020b). OC: organic carbon; TN: total nitrogen; TP: Total phosphorus; SM: soil moisture. The SEs are shown in parentheses ($n = 5$). Values within the same row not followed by the same letter differ significantly (Duncan's test, $P < 0.05$). GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow.

Properties	GM	FM	FRM	IM
OC (g kg ⁻¹)	4.04 ± 0.15 c	5.58 ± 0.12 b	4.25 ± 0.06 c	12.91 ± 0.61 a
TN (g kg ⁻¹)	0.48 ± 0.02 c	0.82 ± 0.02 b	0.44 ± 0.00 c	1.25 ± 0.05 a
TP (g kg ⁻¹)	0.20 ± 0.00 b	0.21 ± 0.00 b	0.20 ± 0.01 b	0.29 ± 0.01 a
NO ₃ ⁻ -N (mg kg ⁻¹)	2.06 ± 0.02 bc	3.28 ± 0.03 b	1.35 ± 0.08 c	7.86 ± 0.94 a
NH ₄ ⁺ -N (mg kg ⁻¹)	2.68 ± 0.10 b	4.33 ± 0.28 a	2.07 ± 0.01 c	2.48 ± 0.08 bc
C/N	8.41 ± 0.25 c	6.83 ± 0.22 d	9.66 ± 0.10 b	10.36 ± 0.12 a
SM (%)	4.07 ± 0.64 c	8.20 ± 0.93 b	5.80 ± 0.45 c	14.88 ± 2.83 a
pH	8.70 ± 0.02 b	8.69 ± 0.04 b	8.82 ± 0.04 a	8.45 ± 0.04 c

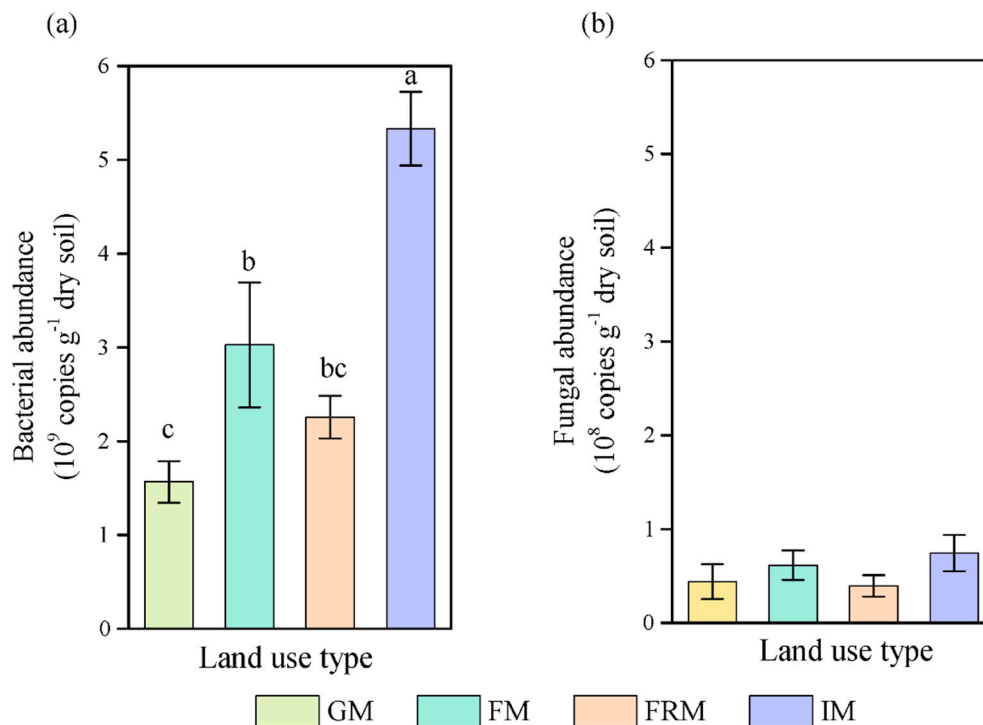


Fig. 1. Soil microbial abundance in different treatments. Data was log transformed to improve normality before variance analysis. Lowercase letter above the bar plots indicated the significance difference established by the Duncan's test at the 0.05 significance level. GM: grazed meadow, FM: fenced meadow, FRM: fenced + reseeded meadow, IM: intact meadow.

Table 3

Soil microbial diversity in different treatments. All the data are present as mean \pm SE ($n = 5$). The lowercase letter above the bar plots indicated the significance difference established by the Duncan's test at the 0.05 significance level. GM: grazed meadow, FM: fenced meadow, FRM: fenced + reseeded meadow, IM: intact meadow.

Parameters		GM	FM	FRM	IM
Observed OTU	Bacteria	2219.2 \pm 42.08 b	2378.4 \pm 24.94 a	2343.8 \pm 22.08 a	2026 \pm 59.64 b
	Fungi	351 \pm 44.6 b	443.2 \pm 20.6 a	437.6 \pm 14.37 a	461.2 \pm 17.5 a
Shannon index	Bacteria	6.48 \pm 0.04 a	6.57 \pm 0.03 a	6.56 \pm 0.03 a	6.28 \pm 0.04 b
	Fungi	2.22 \pm 0.62 b	3.21 \pm 0.34 b	3.14 \pm 0.32 b	3.84 \pm 0.07 a
Chao1 estimator	Bacteria	2883.04 \pm 45.4 b	3102.23 \pm 38.57 a	2971.96 \pm 14.18 ab	2730.37 \pm 78.18 c
	Fungi	406.91 \pm 50.45 b	474.39 \pm 22.58 a	483.71 \pm 14.16 a	497.24 \pm 12.69 a
Phylogenetic index	Bacteria	132.76 \pm 2.39 b	142.01 \pm 2.12 a	140.72 \pm 1.74 a	121.99 \pm 2.98 c
	Fungi	91.92 \pm 9.58 b	120.19 \pm 6.86 ab	111.23 \pm 3.69 ab	129.34 \pm 4.51 a

taxa accounted for 22.96% and 9.00% of sequences of bacteria and fungi, respectively. For bacteria, the genomic features in GM identified the families Vicinamibacteraceae, Anaerolineaceae, Nitrosococcaceae, Roseiflexaceae, and Entothionellaceae as important taxonomic contributors; Flavobacteriaceae was the most differential taxon in FM. Micrococcaceae, Chitinophagaceae, and Phormidiaceae were the most differential taxa in FRM, while Gaiellaceae, Solirubrobacteraceae, and Pirellulaceae were the most differential taxa in IM. For fungi, Sarcoscyphaceae and Niessliaceae were the most differential taxon in GM, Symponenturiaceae and Lycoperdaceae were the most differential taxa in FRM, and Coniochaetaceae and Pseudeurotiaceae were the most

differential taxa in IM.

3.3. Microbial predictive functional profiles

The functional prediction based on FAPROTAX databases suggested that grazing-to-fencing conversion increased the relative abundance of nitrogen-fixers and aerobic ammonia-oxidizing microbes but decreased the relative abundance of nitrifiers (Table 4). FM soil had the highest abundance of diazotrophs, whereas GM had the highest abundance of nitrifiers and aerobic ammonia-oxidizing microbes. The abundance of denitrifiers and nitrate reducers did not differ across the four meadows. In general, grazing-to-fencing conversion significantly increased the proportion of copiotrophs and decreased the proportion of oligotrophs, and their rate of change ranged from 27.65% to 48.86% and 11.59% to 26.96%, respectively (Table 4). Moreover, grazing-to-fencing conversion significantly increased the relative abundance of pathogenic fungi, especially plant pathogens, as shown by the FUNGuild-based predictions (Table 4). Specifically, FM presented a significantly higher abundance of plant pathogens than IM, FRM, and GM sites. GM presented the highest abundance of dung saprotrophs across the four meadows.

3.4. Possible drivers of microbial community composition and functional profiles

The results of VPA indicated that plant and soil properties were the significant factors affecting bacterial and fungal community composition, and they together accounted for 83.36% and 75.66% of variation in bacterial and fungal composition, respectively (Fig. 5a, b). Plants showed a greater contribution to the changes in bacterial (45.39% explanation) and fungal community composition (42.93%) than soil properties (16.97% and 10.22%). The results of SEM further revealed the direct and indirect effects of plants and soils on the microbial community compositions (Fig. 5c, d); 85% and 79% of variance in bacterial and fungal composition, respectively, was observed. Clearly, plants had a direct effect on the bacterial and fungal composition by altering the

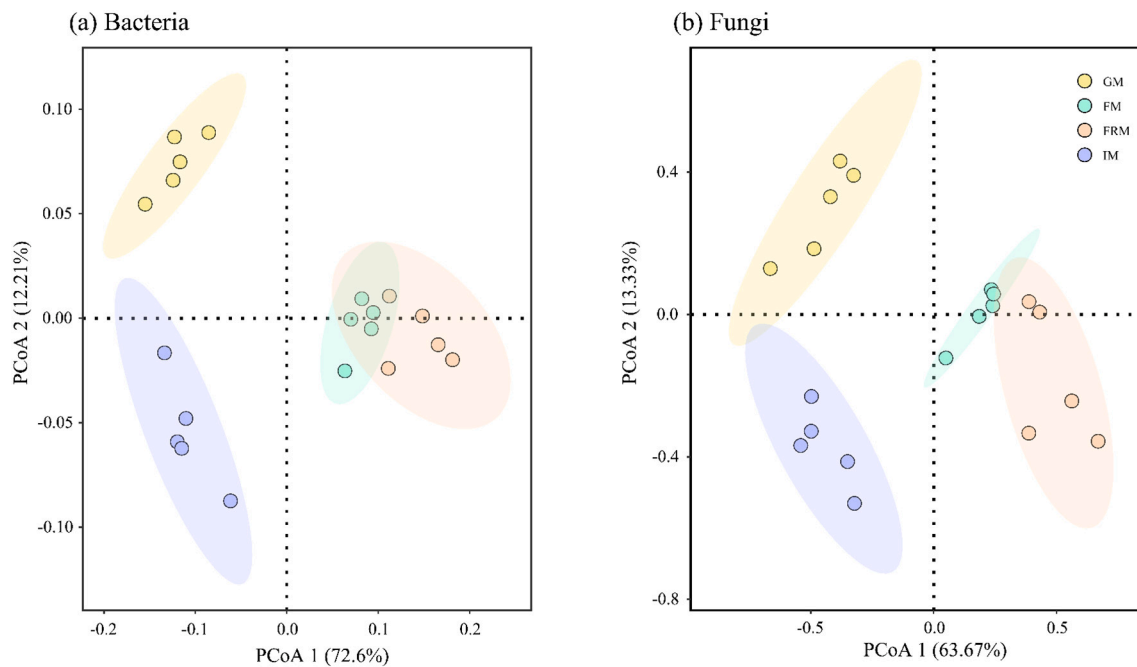


Fig. 2. Principal coordinates analysis (PCoA) based on Bray-Curtis dissimilarity at OTU level illustrated changes of the soil bacterial (a) and fungal (b) community composition in Tibetan alpine meadow. Ellipses were represented 95% confidence interval. GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow.

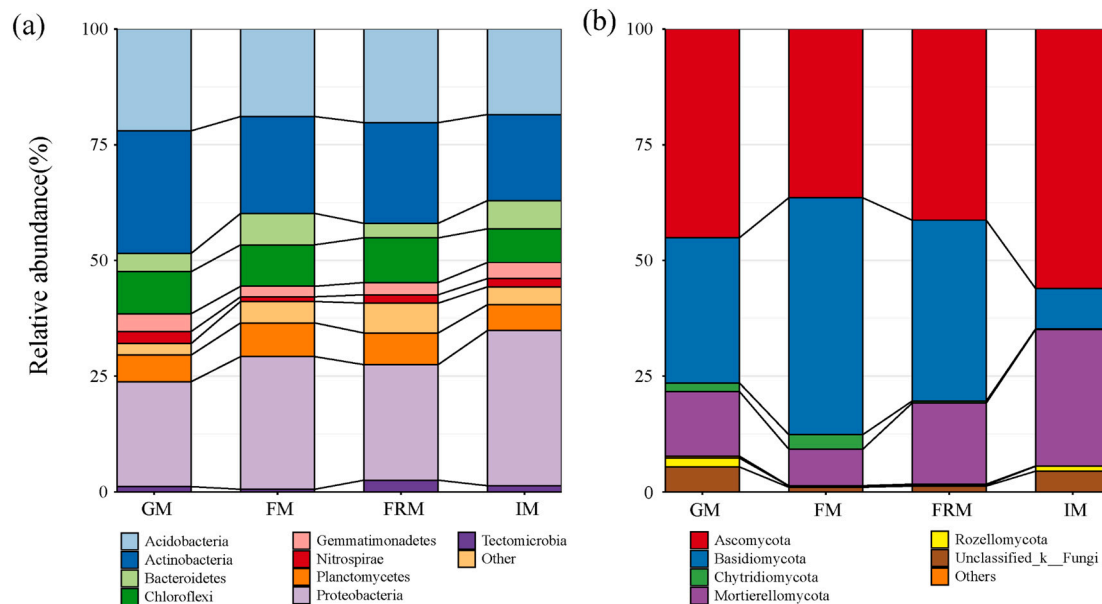


Fig. 3. The effect of grazing-to-fencing conversion on the relative abundance of dominant bacterial (A) and fungal taxa (B) in Tibetan alpine meadow. GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow.

community diversity and species richness. In addition, plants affected the input of OC and NH₄-N into soil and further led to changes in bacterial and fungal composition. Given the predominant regulation of the microbial community by plants, the effects of specific PFGs on microbial community composition and functional profiles were further evaluated using ABT analysis (Fig. 6a-d). Obviously, changes in grass and sedge were associated with the shift in the composition of bacterial and fungal communities; the biomass of these two groups together accounted for more than 60% of the variation, and their biomass had the greatest impact (Fig. 6a, b). Legume and forb diversity had a major effect on bacterial potential function related to N cycling, whereas the diversity of

grass and sedge had a greater effect on fungal functional guilds (Fig. 6c, d).

4. Discussion

4.1. Effect of grazing-to-fencing conversion on plant community and soil properties

The prohibition of grazing by fencing in the Tibetan alpine region is considered a major anthropogenic activity that facilitates restoration and has an impact on the plant community. In our study, higher plant

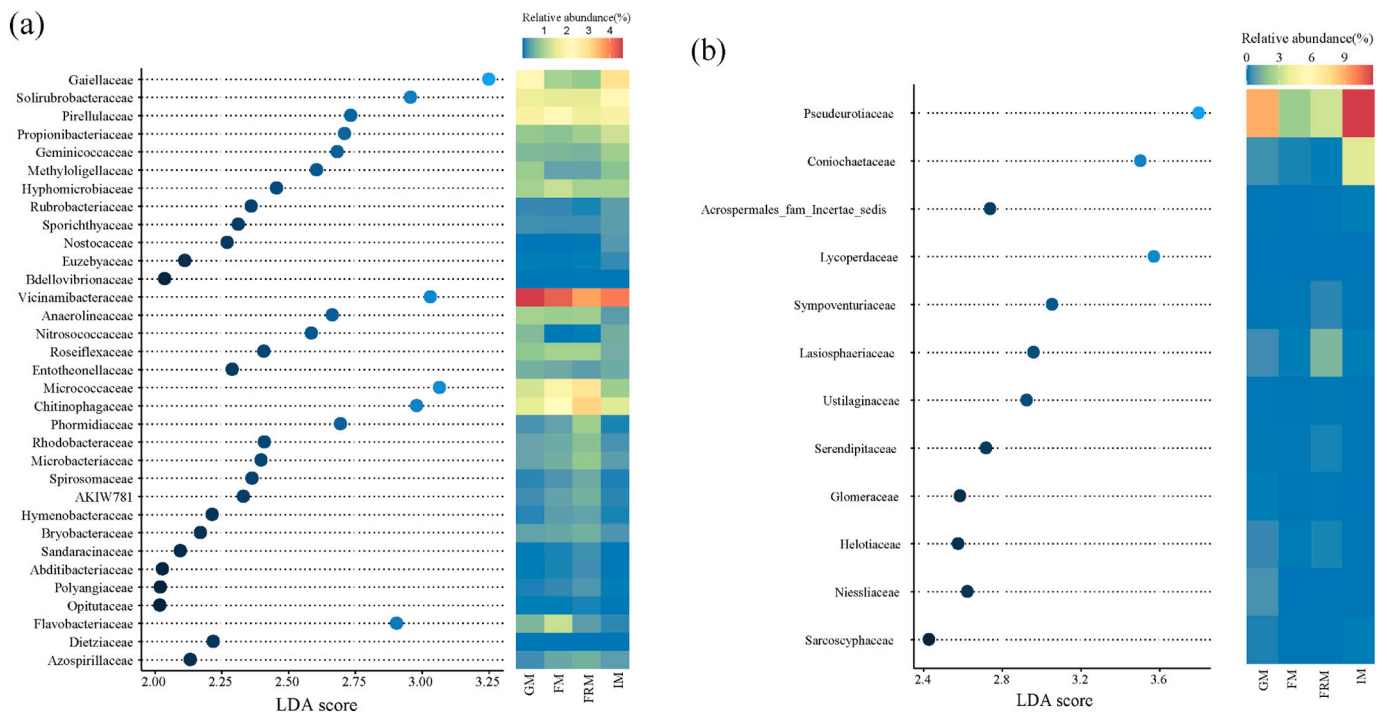


Fig. 4. Linear discriminate analysis (LDA) of effect size (LEfSe) to identify differential taxa at the family level in each alpine meadow. GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow.

Table 4

Putative functional groups of soil microbes enriched among microbiomes in different land use type. * *p*-value lower than 0.05 means that putative functional groups are significantly different. Change in relative abundance of copiotrophic and oligotrophic in four alpine meadows. The copiotrophic lineages included Proteobacteria, Bacteroidetes, and Firmicutes. Actinobacteria, Acidobacteria, Armatimonadetes, Chloroflexi, Fibrobacteres, Gemmatimonadetes, Nitrospirae, Planctomycetes, Rokubacteria, Verrucomicrobia, FBP were classified as oligotrophic categories (Zhou et al., 2017; Che et al., 2019). GM: grazed meadow; FM: fenced meadow; FRM: fenced + reseeded meadow; IM: intact meadow.

Functional groups	Effect size (LDA score)	<i>P</i> -value	Relative abundance (%)			
			GM	FM	FRM	UM
Copiotrophic	3.86	0.003296	29.34	38.00	37.78	43.20
Oligotrophic	3.82	0.003296	67.09	59.43	58.79	53.26
Nitrifiers			9.43	7.23	17.17	5.68
Diazotrophs			1.44	1.76	1.37	1.58
Denitrifiers			0.30	0.37	0.26	0.23
Aerobic ammonia oxidizing microbes			1.82	2.30	3.50	1.74
Nitrate reducers			2.11	1.86	1.76	1.73
Animal pathogen	3.72	0.042575	3.33	1.62	4.26	6.20
Endophyte			15.51	8.35	17.81	29.99
Dung saprotroph	4.08	0.047023	17.54	2.27	2.69	3.13
Plant pathogen	3.82	0.015745	5.82	7.93	6.32	3.59

coverage, biomass (above- and below-ground), and diversity in fenced and fenced-reseeded sites, compared with those in grazed sites, suggested that grazing-to-fencing conversion improved the aboveground productivity and community diversity of the alpine meadow (Table 1). The enhanced diversity in fenced sites could be explained by the “intermediate disturbance assumption,” which states that a moderate disturbance could provide more habitats for species and cause an increase in community diversity (Wu et al., 2009). Consistent with previous studies (Feng et al., 2010; Mou et al., 2018), our study showed that the Tibetan alpine meadow mainly consists of sedge, grass, legume, and forbs groups, and these communities varied differently after land-use

changes (Table 1). Grazing-to-fencing conversion increased the diversity and biomass of grass and leguminous species, which could be associated with selective ingestion by yaks (Bi et al., 2018). Yaks in the alpine meadows exhibit a strong preference for grass and leguminous species, especially grass species such as *Poa annua*, *Stipa purpurea*, and *K. thoroldiana* (Diaz et al., 2007). Previous studies have shown that species decreased their aboveground productivity or even disappeared from a community due to their lower competitive abilities for resources, such as water, nutrients, and light, than other species (Bi et al., 2018; Grime, 1998). In the present study, reseeded increased the relative abundance of grass species but did not increase their diversity and biomass (Table 1). A possible reason could be that the original native plants possessed a higher competitive ability for resources (e.g., canopy and nutrients) than the seeded species (Feng et al., 2010; Wu et al., 2009). Grazing-to-fencing conversion also improved the soil nutrient levels, such as OC, TN, NO₃⁻-N, and NH₄⁺-N. Compared to that in the grazed sites where the leaves and shoots were ingested by yaks, the higher above- and below-ground biomass in fenced sites could increase the nutrient input into the soil through litter decomposition and root exudate release. However, these nutrient contents (except for NH₄⁺-N) in fencing sites were lower than those in the IM sites. This was because undegraded meadows have existed for hundreds of years and therefore have abundant C and N contents owing to the long-term organic matter accumulation and mineralization supply (Yang et al., 2017). The high soil moisture in fenced sites could be ascribed to the high aboveground coverage that reduces evaporation. Notably, we found that fenced-reseeded sites did not improve the soil nutrients (e.g., OC, NH₄⁺-N) compared to those in the grazed sites, but they had a higher above- and below-ground biomass. A possible reason is that reseeded species decreased the ability of microbes to decompose organic matter (e.g., cellulose, hemicellulose, and lignin); however, this inference should be further investigated.

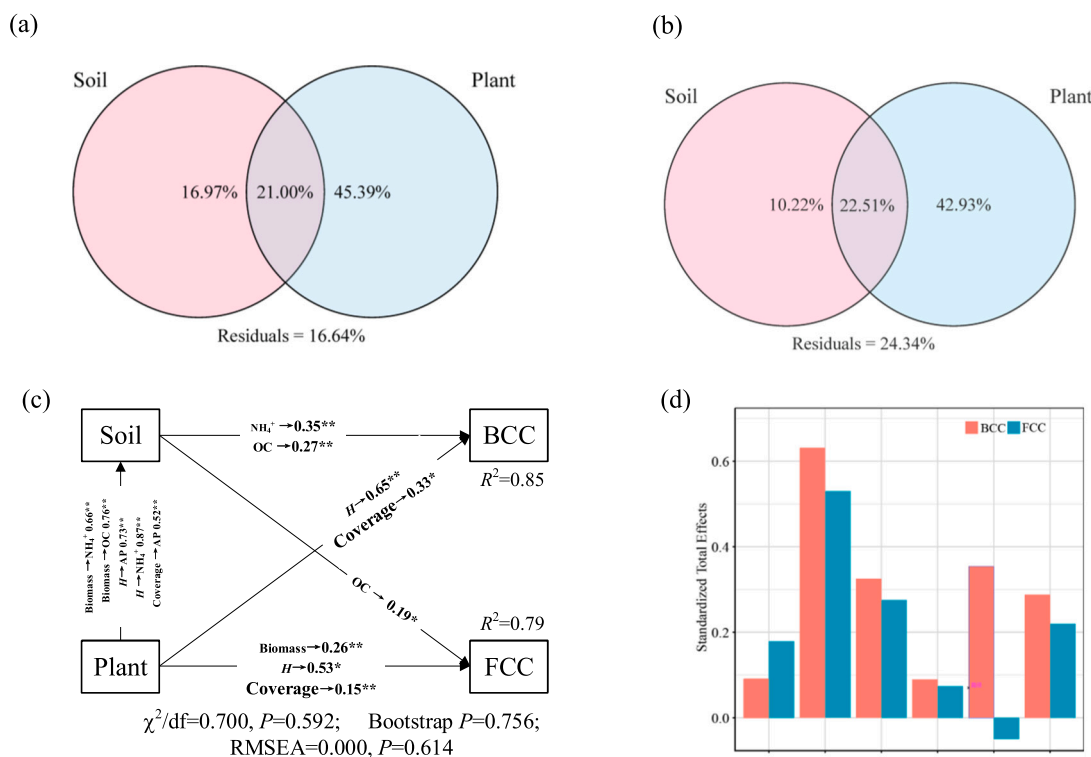


Fig. 5. Variance partitioning analysis (VPA) and structural equation modeling (SEM) revealed the effect of soil variables (NH_4^+ , OC, AP) and plant characteristics (biomass, Shannon diversity and species richness) on the bacterial (a, c) and fungal (b, d) community compositions in Tibetan alpine meadow. BCC or FCC is bacterial or fungal community composition and was represented by the first component from the PCoA. *H* is plan Shannon diversity, coverage is plant coverage, AP is soil available phosphorus and OC is soil organic carbon. Biomass is the sum of aboveground biomass and belowground biomass. The components within plant and soil physicochemical properties are included in the model as independent observed variables, but in this figure are grouped for simplicity. Numbers within the arrows show standardized path coefficients and indicate the effect size of the relationship among variables. Only significant relationships are shown (* $p < 0.05$; ** $p < 0.01$). The goodness-of-fit of the model is shown in the bottom the figure. Abbreviation: RMSEA = root mean square error of approximation.

4.2. Effect of grazing-to-fencing conversion on microbial diversity, community composition, and putative functional profiles

Consistent with previous studies (Cheng et al., 2016; Yin et al., 2021), our study showed that grazing-to-fencing conversion increased soil bacterial and fungal diversity, and this could be due to the increased plant diversity in the fenced sites. Plant diversity has been reported to determine the quality and quantity of substrates (e.g. C, N) that are released into the soil (Eldridge and Delgado-Baquerizo, 2018). A more diverse plant community is thus usually accompanied by a more diverse organic substrate and higher microbial diversity (Wang et al., 2020a). The bacterial and fungal community compositions were also strongly affected by grazing-to-fencing conversion, showing a community conversion from an oligotrophic-dominated community to a copiotrophic-dominated community (Table 4). Grazing-to-fencing conversion significantly altered fungal community composition but had no significant effect on fungal abundance (Figs. 1b and 2b). Fungal community composition is closely associated with microbe-mediated ecological functions, such as C mineralization and P solubilization (Boer et al., 2005). Therefore, grazing-to-conversion probably adjusts the microbe-mediated ecological processes by altering fungal community compositions rather than changing their abundance.

In our study, fenced soils had a higher abundance of diazotrophs than grazed soil, suggesting an improved biological N-fixation potential caused by grazing-to-fencing conversion. This could be attributed to the appearance of leguminous species in the fenced sites, such as *Astragalus strictus* Grah. Ex Bend and *Astragalus arnoldii* Hemsl. These species are capable of converting gaseous N to ammonium N that can be directly utilized by plants. Consistent with previous study findings (Ding et al., 2015; Song et al., 2019), our study showed that the relative abundance

of nitrifiers was decreased by grazing-to-fencing conversion. This could be explained by several factors. First, as a chemoautotrophic population, most nitrifiers are oligotrophic and usually more competitive in poor nutrient environments (Che et al., 2019; Verhamme et al., 2011). Compared with those in grazed sites, where the aboveground vegetation was removed by yak ingestion, fencing greatly promoted plant growth and increased the C and N input into soil through litter decomposition. This nutrient-rich environment is not conducive to nitrifiers. Second, nitrifiers usually prefer aerobic environments (Levy-Booth et al., 2014; Merloti et al., 2019), which is provided in the degraded meadow by the low vegetation coverage. Poor aeration conditions by grazing-to-fencing conversion could weaken nitrification (Sahrawat, 2008). This speculation was supported by the changes in aerobic ammonia oxidation that occurred consistently with nitrification as the pioneer step of nitrification (Table 4). Third, some nitrifiers are sensitive to chemicals secreted by plant roots that inhibit the activities of nitrifiers (Srikanthasamy et al., 2018). Decreased nitrification potential by conversion can impede the transformation of ammonium N to nitrate N, consequently decreasing inorganic N loss by leaching. N has been reported to be a limiting factor for the alpine meadow on the Tibetan Plateau (Feng et al., 2010). The potentially reduced N leaching induced by the microbial community after grazing-to-fencing conversion would promote the restoration of degraded meadows.

Grazing-to-fencing conversion also reduced the relative abundance of dung saprotrophic fungi. Grazed meadow soil can favor dung saprotrophs, because livestock manure is a rich habitat that offers rich resources to these microbes. Another interesting finding is the increased abundance of plant pathogenic fungi, which could be attributed to two reasons. First, diverse plants provide varying habitats and a diverse range of resources for different pathogens (Eldridge and Delgado-

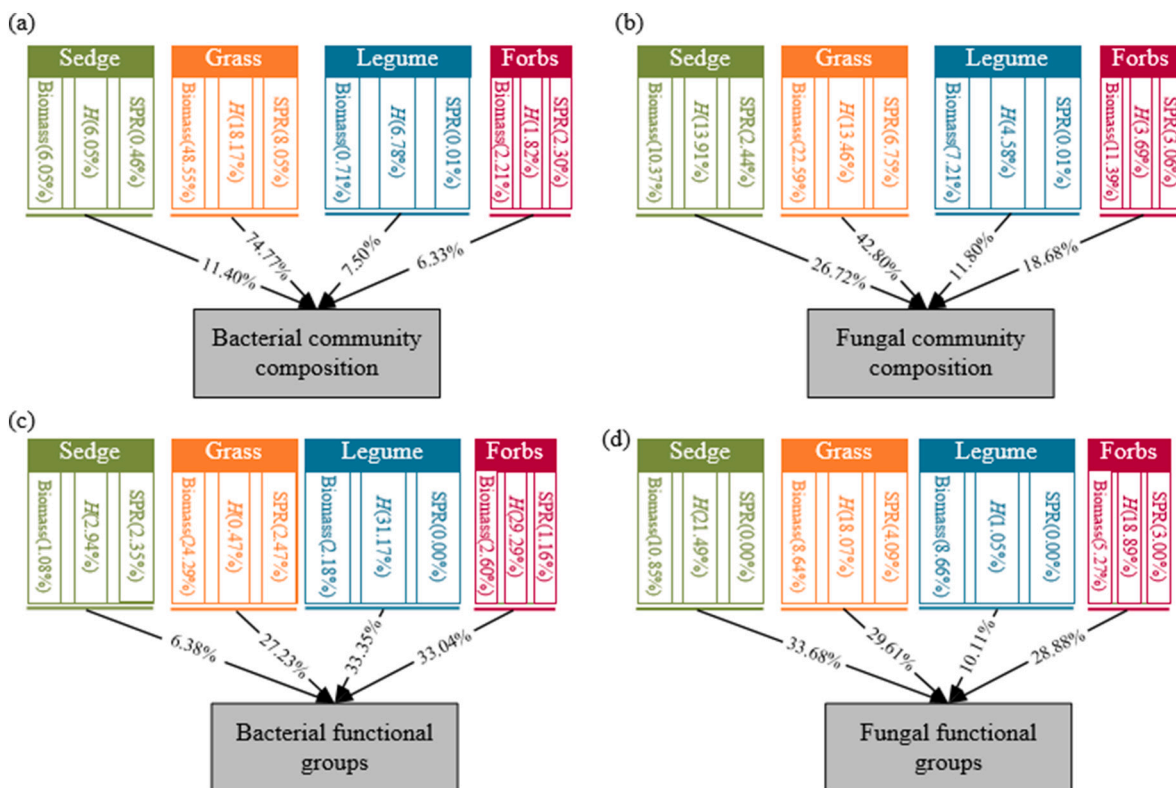


Fig. 6. Results from aggregated boosted tree (ABT) analysis revealed the effect of plant functional groups on bacterial and fungal community (composition and function). *H* is plan Shannon diversity and coverage is plant coverage. Bacterial or fungal community (composition and functional groups) was represented by the first component from the PCA. Biomass is the sum of aboveground biomass and belowground biomass.

Baquerizo, 2018). The increase in plant diversity due to conversion provided the favorable substrates for these pathogens and consequently caused an increase in their abundance (Tables 1 and 4). Additionally, pika activity contributed to the enhanced relative abundance of plant pathogens in Tibetan alpine meadows. Indeed, we found many pika holes in the fenced and fenced-reseeded sites (Che et al., 2019). Therefore, pika activity might be another reason for the increased plant pathogens. As beneficial fungi commonly found in plants, endophytic fungi have become a potential source of resistance to pathogens (Zanne et al., 2020). However, the abundance of endophytic fungi was lower in the fenced site than in grazed sites, suggesting a negative effect of conversion on this functional microbe. Together with the increased abundance of plant pathogenic fungi, our findings indicated that grazing-to-fencing conversion was likely to increase the risk of plant diseases. Fungal abundance was unaffected by the land-use changes. The increased and decreased proportions of plant pathogens and endophytes implied an increase and decrease in the number of plant pathogenic and endophytic fungi by grazing-to-fencing conversion, respectively, which could hinder the restoration of degraded meadows and should be taken into account.

4.3. Possible drivers of soil microbial community

In line with our hypothesis, grazing-to-fencing conversion drives the changes in microbial community composition and functional profiles by altering PFGs (Figs. 5 and 6). Our results showed that there are two pathways for this effect; one is the indirect effect of soil nutrient content on microbes induced by changes in the plant community. Specifically, changes in soil OC and $\text{NH}_4^+\text{-N}$ contents were the largest contributors to this effect, and they mainly affected the bacterial and fungal community composition. These results are consistent with those of previous studies documenting the importance of organic matter for the formation of microbial community structures (Francioli et al., 2020; Wang et al.,

2020a). Because most heterotrophic bacteria and saprotrophic fungi depend on C and N substrates as living resources, changes in these nutrient components could lead to a shift in bacterial and fungal community composition (Boer et al., 2005). Fungal composition was not sensitive to the changes in $\text{NH}_4^+\text{-N}$ content owing to their wide adaption to N availability. In contrast to our results, (Marshall et al., 2011) showed the resistance of soil microbial community composition to the changes in PFG composition. This discrepancy might be because of the fact that their study was based on a coarse measure of phospholipid fatty acid data, which provide a coarse level of taxonomic analysis of the community, and only examined the most dominant community members. Because grass and sedge had the largest above- and below-ground biomass, they contributed the most to the changes in bacterial and fungal community composition. Our results showed that the plant community also had a direct effect on the microbial community by mediating community diversity and richness and microbial functional profiles (Figs. 5 and 6). This result was inconsistent with the study by Zhang et al. (2017) who observed a weak effect of PFG composition on microbial functional diversity based on 5-year PFG removal field experiments. One important aspect that could explain the different results is the retardation effect of plants on the microbial community. Plant diversity is expected to drive microbial diversity by providing more diverse types of carbon resources (Cline et al., 2018); however, this did not occur immediately. For example, in a 6-year Jena Experiment, Eisenhauer et al. (2010) found that there might be as long as a 4-year lag for the effect of plant species on soil microbial communities after the manipulation of plant species richness and PFG richness. Our previous study also found a delayed influence of plants on bacterial and fungal communities during secondary succession (Zhang et al., 2018). In other words, microbial taxonomic diversity might not have had sufficient time to respond to changes in plant diversity. Thus, the short-term experiment (only a 5-year treatment) by Zhang et al. (2017) resulted in weak effects of PFGs on microbial functional diversity. Notably, we found that

bacterial function related to N cycling and fungal function guilds responded differently to PFG identity (Fig. 6c-d). Because of the important roles of legume species in soil N availability (e.g., biological N fixation), the diversity of legume groups exerted a predominant influence on the potential function related to N cycling (Merloti et al., 2019). It is not well understood why the diversity of sedge, grass, and forbs had a major effect on fungal functional guilds. The possible mechanism might be related to differences in components (lignin, cellulase, and hemicellulose) and nutrient stoichiometry in litter composition or the specific chemicals in root exudates processed by these species that are favorable for pathogens and saprotrophs. Nevertheless, this speculation needs to be further confirmed. It is worth mentioning that the results obtained in our study were based on samples pooling from a wide area. Although this approach has been used to investigate soil microbe in natural ecosystem (Bastida et al., 2017; Yang et al., 2017; Cui et al., 2021), it is not perfect as it is difficult to avoid the risk of microbial heterogeneity and cause compositions that might not be represented in natural ecosystem. Thus, investigation based on more soil sampling should be further conducted.

5. Conclusions

Grazing-to-fencing conversion could increase the aboveground biomass, soil nutrient level, and microbial diversity. Changes in the biomass of sedge and grass were the major contributors to the variation in bacterial and fungal composition, whereas the diversity of sedge, grass, legumes, and forbs largely explained the variation of potential functions related to N cycling and fungal guilds. Grazing-to-fencing conversion resulted in an increase in copiotrophs, diazotrophs, and plant pathogens and a decrease in nitrifiers and dung saprotrophs. Taken together, our results revealed that fencing is an effective approach to restore the degraded alpine meadows and highlighted the importance of PFGs in shaping the soil microbial community.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2021.104008>.

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