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Abstract:

Background Grassland patchiness degradation poses an escalating threat to grassland ecosystem functions and biodiversity, particularly in alpine ecosystems. The patches of native poisonous plants are rapidly expanding on the Qinghai-Tibet Plateau, forming degraded grasslands dominated by poisonous plants, leading to the loss of grassland ecological functions. However, the impact of *Ligularia virgaurea* patches expansion on soil microbial communities and the key driving factors remain unclear. In response to this concern, we analyzed the soil physicochemical properties and soil microbial communities within *L. virgaurea* patches of different densities and explored the impacts of *L. virgaurea* invasion and patches expansion on soil nutrients, enzyme activities, and soil microbial communities.

Results The results of this study indicated that the expansion of *L. virgaurea* patches enriched more available resources within the patches. Specifically, the soil organic carbon (SOC) content increased by 4.3%, soil total nitrogen (TN) by 9.5%, and soil total phosphorus (TP) by 11.3% within the patches. *L. virgaurea* patches enhanced soil resource heterogeneity, indirectly affecting the abundance and diversity of soil microorganisms. The expansion of *L. virgaurea* patches promoted the diversity of soil microbial communities. Furthermore, we identified pH, NO_3^--N, and cellulase as common drivers of bacterial and fungal community changes.

Conclusion Overall, *L. virgaurea* patches enhance the soil resources heterogeneity, changing the composition of soil microbial communities within the patches and increasing soil microbial diversity by regulating the availability of soil resources. This study clarifies the changing characteristics and interrelationships of abiotic and biological soil properties during the expansion of native poisonous plants in alpine meadows, providing a theoretical foundation for the management of grasslands affected by poisonous plants.

Keywords: Grassland degradation, Plant invasion, *Ligularia virgaurea*, Poisonous plant patches, Soil microbial community

1. Introduction

Grassland degradation is a critical issue in discussions of global ecological security. Around 49% of the world's grasslands have undergone varying levels of degradation, largely due to climate change and overgrazing. [1, 2]. Grassland degradation diminishes their capacity to essential ecosystem services, and human livelihoods, posing a significant threat to global ecological stability and the sustainability of human development. [3]. Particularly in high-altitude regions such as the Qinghai Tibet Plateau (QTP), due to its high sensitivity to climate change, grassland degradation lead to loss of biodiversity, decreased grassland productivity, and soil function degradation, which severely threaten the livelihoods of approximately 5 million herders and farmers on the QTP [4-6]. Currently, grasslands are gradually developing fragmented vegetation and degraded patches driven by grazing [7], particularly forming grasslands dominated by poisonous plants [8], which exacerbate grassland degradation and severely impacts the sustainable use of grassland ecosystems. Therefore, understanding the expansion of poisonous plant patches and their effects on grassland ecosystems is of great importance for grassland management and reversing degradation.

Irrational grassland utilization methods may disrupt the stability of grassland communities and weaken the ecosystem's resistance, creating ecological niches for poorly palatable or poisonous plant species, thereby altering the composition of the grassland plant community [9, 10]. *Ligularia virgaurea* is a perennial herbaceous plant of the genus *Ligularia* in the Asteraceae family, widely distributed in the QTP and its peripheral regions in China. Commonly regarded as indicator species of alpine grassland degradation, and native invasive species due to their strong reproductive ability and extensive ecological adaptability [11, 12]. Recent reports show that the expansion of the *L. virgaurea* population is intensifying, and rapidly forming grasslands dominated by *L. virgaurea* [8, 13]. The formation of degraded patches and the invasion of poisonous species have largely weakened grassland productivity and sustainable utilization value [14, 15]. Therefore, understanding the impacts of poisonous plant patches expansion on grassland ecosystems and their ecological consequences is particularly important, as existing research has not fully addressed these complex ecological processes.

Ecological theory suggests that soil heterogeneity promotes species coexistence and diversity by providing unique plant ecological niches [16]. However, the impact of soil heterogeneity on species coexistence and diversity is not always positive. Studies indicate that although the fertile islands

created by Artemisia annua patches enhance soil resource heterogeneity, community richness within these patches is consistently reduced compared to surrounding areas [9]. Therefore, understanding the relationship between plant communities and soil resource heterogeneity is crucial for studying the expansion of toxic plant patches and their ecological effects. In interspecific competition, species with a competitive advantage are typically able to prioritize and acquire limited resources efficiently and sustainably [17, 18]. They also enrich effective nutrients around them through the mutual feedback relationship between vegetation and soil, thereby promoting soil resource heterogeneity [19]. For example, the net primary productivity (NPP) of invaded ecosystems increases [20], and the litter decomposition rate of invasive species is faster than that of native species [21], to some extent accelerating the ecosystem nutrient cycling rate. This suggests that plant invasions may create positive feedback for further invasions. And this positive feedback enhances both abiotic (water and nutrients) and biotic (microbial activity and litter decomposition) processes under vegetation, promoting the spatial heterogeneity of soil resources [19, 22]. However, the intensification of soil resource heterogeneity can affect both inter- and intraspecific competition within communities, and under the dual influence of allelopathy, this can lead to changes in plant community composition and a reduction in diversity [23, 24] , thereby amplifying the impact of soil resource heterogeneity on grassland ecosystems [25].

Soil microbes are crucial for nutrient cycling, soil multifunctionality and plant resource acquisition [26, 27]. There is a two-way feedback relationship between soil properties and the microbiome [28]. On the one hand, soil properties shape the composition and function of the microbiome; on the other hand, as key drivers of element cycling, microbial processes in turn affect soil properties by regulating nutrient availability [29]. The increase in resource availability (water and available nutrients) within plant patches can indirectly affect soil microbial communities. Sha *et al* [30] found that woody plant invasion, by changing the soil properties beneath the canopy, enhanced soil microbial community diversity and microbial activity. The decomposition of plant litter significantly increases the carbon and nitrogen content in the soil, creating ecological niches for the growth and reproduction of microbial communities [31], thereby driving changes in the composition of soil microbial communities [30]. At the same time, patches expansion can alter soil pH and moisture content, which in turn affects soil microbial communities [19, 32]. Additionally, allelopathy plays a crucial role in driving changes in soil microbial communities within plant patches, as it can

regulate microbial assembly and succession by altering plant community structure and the rhizosphere microenvironment [33, 34]. The formation and expansion of plant patches cause changes in soil microbial communities, accelerating nutrient accumulation and further promoting soil resource heterogeneity. However, some studies suggest that the expansion of toxic plants can reduce the number of microorganisms [8]. Therefore, the impact of plant patches expansion on soil microbial communities varies across different ecosystems. There is still insufficient understanding of the mechanisms behind the expansion of poisonous plant patches in grassland ecosystems and their impact on soil microbial communities.

Therefore, we contend that it is highly necessary to study the effects of *L. virgaurea* expansion on the composition and structural changes of soil microbial communities. This study selected three density gradients *L. virgaurea* patches (low-density patches, medium-density patches and high-density patches) and non-patch grassland to conduct vegetation composition investigation and soil sample collection, to simulate different stages of *L. virgaurea* invasion in grasslands, in order to study the impact of its expansion on alpine meadows. We hypothesized that 1) *L. virgaurea*, by utilizing its competitive advantage to create soil resource heterogeneity, optimizes its own living environment, thereby promoting population expansion; 2) The heterogeneity of soil resources affects the structure of soil microbial communities, leading to an increase in microbial activity and diversity.

2. Materials and methods

2.1 Site description

Soil samples were collected in August 2022 from a permanent grassland subjected to moderate grazing by yak in Maqin County, Qinghai Province, China (34°28′47.83″N, 100°12′4.99″E; 3758 m asl; Fig. 1a). The sampling site features flat terrain. The site is characterized by a typical plateau continental climate. The annual mean temperature is 0.8℃, with lowest monthly mean temperature being -26.9℃ in January and highest monthly mean temperature 23.3℃ in July. The annual mean precipitation is 541 mm (based on data from 2000 to 2022), 85% of which is concentrated within the plant growing season from May to September [35]. The soil is classified as alpine meadow soil. The soil of alpine meadow is clay loam of Mat-Cryic Cambisols and corresponds to Cryrendolls in the USDA soil taxonomy system [36]. The vegetation is typical alpine meadows, dominated by *Carex alatauensis*, *Carex capillifolia* and *Elymus nutans* as well as some species of Gentianaceae and Ranunculaceae.

2.2 Experimental design

To unravel the shifts in the soil microbiome during the succession of *Ligularia virgaurea* patches, soil samples were collected from three invasion density classes: low-density (LD, 20~30 ind/0.25m^2), medium-density (MD, 50~65 ind/0.25m^2), and high-density (HD, 85~100 ind/0.25m^2) *L. virgaurea* patches. All sampling patches were standardized to be 6-8 m^2 in size and more than 30 m between each patch to eliminate errors caused by patch size and distance between patches (Fig. 1b-e). In addition, to compare the effects of the formation of *L. virgaurea* patches on grassland, we collected soil samples from non-patch healthy grasslands located more than 30 m away from the *L. virgaurea* patches as control (CK) for this study. For each density treatment, we selected six independent patches as replicates of the experiment. As *L. virgaurea* patches underwent succession and expansion, they eventually developed into severely degraded forb grasslands dominated by this invasive species (Fig. 1f). Detailed information on density classes and vegetation characteristics of *L. virgaurea* was provided in the supplementary information (Table S1).

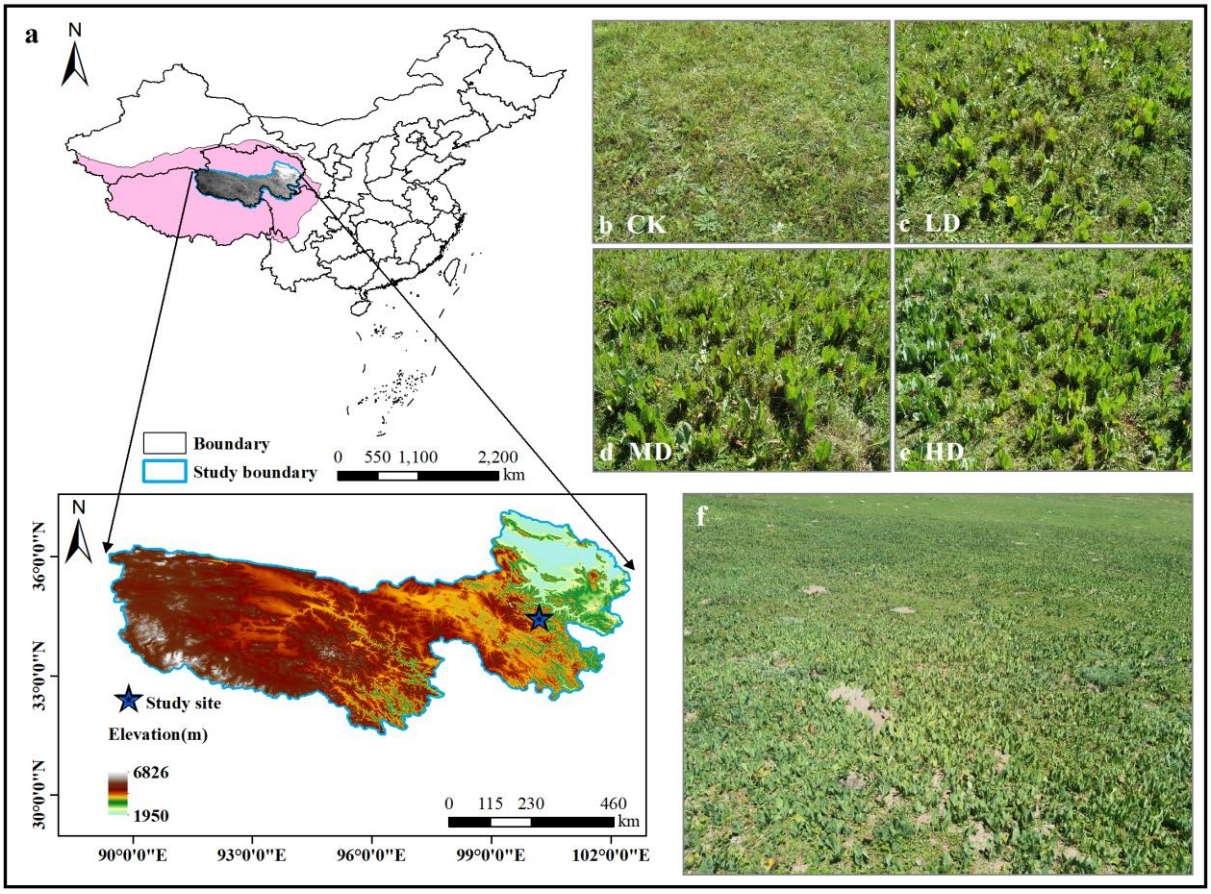

Fig. 1 Maps showing the location of the Sanjiangyuan region in China and distribution of sampling sites. (b) CK: non-patches; (c) LD, low-density patches; (d) MD, medium-density patches; (e) HD, high-density patches. (f) Terminal degradation landscape resulting from ecological succession of *L. virgaurea* patches.

2.3 Soil sampling and physico-chemical properties analysis

During peak plant growth in 2022 (August), we sampled soils and investigated vegetation characteristics in 24 patches (6-8 m^2) on the grassland. Homogenized surface soil samples (0-10 cm depth) were collected from each patch using a 3.5 cm diameter soil auger following a five-point sampling method. Once collected, the 5 subsamples from the patch were processed in the field to form a composite sample. All composite soil samples were sieved with a 2 mm sieve to remove any stones and roots, thoroughly mixed, and then divided each sample into three parts for analysis. The first portion was placed in an insulated box with dry ice (i.e. frozen CO_2) and transported to the laboratory for storage at – 80°C for DNA extraction. The second portion stored at – 20°C and analyzed for microbial biomass (MBC, MBN, and MBP, mg kg^{-1}), available inorganic N (NH_4^+-N and NO_3^--N, mg kg^{-1}). After air-drying, the third portion was analyzed for soil physicochemical properties and

soil pH. The density of *L. virgaurea* and vegetation species composition of patch were also measured within these sub-patches.

Soil water content (SWC, %) was determined by drying oven 10 g fresh soil to constant weight at 105℃ and calculating the weight loss. The pH was measured in a 1:5 soil–water ratio (w/v) suspension, using a E-201-C pH composite electrode (Leici, Shanghai, China). The soil organic carbon (SOC, g kg^{-1}) was determined using the $K_2Cr_2O_7$–H_2SO_4 oxidation method. Soil available inorganic N (NH_4^+-N and NO_3^--N, mg kg^{-1}) extracted from the soil by 2 M KCl (solid/liquid ratio of 1/5 (w/v)) were measured using automated discrete analyzer (Smartchem 450, AMS, Italy). Soil total N (TN, g kg^{-1}) was measured by the semi-micro Kjeldahl method with 98% H_2SO_4 digestion, catalyzed by Se, $CuSO_4$, and Na_2SO_4. Soil total phosphorus (TP, g kg^{-1}) was determined using the antimony-molybdenum blue colorimetric method after soils were digested. Available phosphorus (AP, mg kg^{-1}) was determined by the antimony-molybdenum blue colorimetric method after extraction with 0.5 M $NaHCO_3$. Soil microbial biomass C (MBC, mg kg^{-1}), microbial biomass N (MBN, mg kg^{-1}) and microbial biomass P (MBP, mg kg^{-1}) were determined using chloroform-fumigation extraction method. The soil physico-chemical properties were determined using standard laboratory methods [37].

2.4 Soil enzymatic activity

Soil enzyme activities associated with three nutrient acquisition pathways were measured, including one enzyme involved in C-acquisition: cellulase activity (C_enzy, mg $g^{-1}d^{-1}$) was determined using the 3,5-dinitrosalicylic acid colorimetric method; one enzyme involved in N-acquisition: urease activity (N_enzy, mg $g^{-1}d^{-1}$) was measured with the phenol-sodium hypochlorite colorimetric method; one enzyme involved in P-acquisition: phosphatase activity (P_enzy, mg $g^{-1}d^{-1}$) was assayed via the disodium phenyl phosphate colorimetric method.

2.5 DNA extraction, library construction and sequencing

DNA was extracted from soil samples using the cetyltrimethylammonium bromide (CTAB) method. DNA quality and quantity were assessed using Agilent 5400 (Agilent Technologies Co. Ltd., USA) to evaluate degree of degradation, potential contamination and DNA concentration.

Sequencing libraries were prepared with the NEBNext Ultra DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to each sample. Briefly, the genomic DNA was sheared by sonication to a size of 350 bp,

then DNA fragments were end-polished, A-tailed, and ligated with the full-length adaptor for Illumina sequencing with further PCR amplification. Finally, PCR products were purified (AMPure XP system) and libraries were analyzed for size distribution by Agilent 2100 Bioanalyzer (Agilent Technologies Co. Ltd., USA) and quantified using real-time PCR.

The clustering of the index-coded samples was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA) and paired-end reads were generated. The Raw Data of bacteria and fungi in soil samples were obtained by metagenomic sequencing using Illumina Novaseq high-throughput sequencing platform. The clean sequences of all samples were annotated and categorized, and Bracken was used to predict the actual relative abundance of species. Sequencing service was provided by Wekemo Tech Group Co., Ltd. Shenzhen China.

2.6 Statistical analysis

The significance of soil physiochemical properties, enzymatic activity and microbial community diversity were conducted using IBM SPSS Statistics 26 with Tukey's post-hoc test at a 0.05 significance level. Prior to ANOVA, normality was assessed for each group using Shapiro-Wilk tests, and homogeneity of variance was evaluated using Levene's test. The graphics were performed using Origin 2021 software (OriginLab Corporation, Northampton, MA, USA) for soil physicochemical properties, enzyme activity, and microbial community composition. The relationship between patches density and soil properties were analyzed using liner and quadratic regression models. Beta diversity was evaluated via principal coordinate analysis (PCoA) based on Bray-Curtis distances, implemented in the “vegan” R package. Bacteria and fungi at all phylum levels with relative abundances exceeding 0.1% were selected for network analysis. Firstly, process the raw OTU data with “reshape2” R package to compute network metrics, including the number of nodes and edges. Then, export the processed data from “reshape2” R package and visualize the network using Gephi 0.10.0. Redundancy analysis (RDA) was used to analyze the relationship between soil microbial community composition and environmental factors. To further explore the data, hierarchical partitioning analysis with the “rdacca.hp” R package was employed to determine the key environmental factors influencing changes in microbial community. We employed the “piecewiseSEM” package to construct a structural equation model (SEM) for assessing the effects of *L. virgaurea* patches, environmental factors,

240 microbial activity, and soil nutrients on soil bacterial and fungal diversity.

241 **3. Results**

242 3.1 Soil environmental factors and enzyme activities

243 Regarding the physico-chemical properties of the soil, compared with the control, soil water
244 content (SWC), soil organic carbon (SOC), total nitrogen (TN), NH_4^+-N, and total phosphorus (TP)
245 initially increased and then decreased with increasing density of *L. virgaurea* patches (Fig. S1). SWC,
246 SOC, TN, NH_4^+-N, and TP exhibited quadratic relationships in response to increasing density of *L.*
247 *virgaurea* (Fig. 2). In particular, the formation of *L. virgaurea* patches significantly reduced soil pH
248 and NO_3^--N content by 0.9%~1.6% and 10.8~28.7%, respectively, but had no effect on soil available
249 phosphorus content (Fig. S1). Soil pH and NO_3^--N declined linearly with increasing density of *L.*
250 *virgaurea* (Fig. 2). In addition, *L. virgaurea* patches also led to 28.5%~39.9% reduction in soil
251 microbial biomass nitrogen (MBN). The contents of soil microbial biomass carbon (MBC) and
252 microbial biomass phosphorus (MBP) exhibited an initial increase followed by a decrease during the
253 succession of *L. virgaurea* patch communities (Fig. S2). The soil nutrient content in low-density and
254 medium-density *L. virgaurea* patches was significantly higher than that in CK and HD treatments,
255 indicating that the invasion of low-density and medium-density *L. virgaurea* populations promoted
256 nutrient enrichment within the patches and caused heterogeneity in soil resources inside and around
257 the *L. virgaurea* patches.

258 In low-density (LD) *L. virgaurea* patches, the soil nutrient-acquiring enzymes activities
259 (cellulase, C_enzy; urease, N_enzy; phosphatase, P_enzy) were significantly higher than those in the
260 medium-density (MD), high-density (HD), and control (CK) treatments (Fig. S2). Compared with
261 control, low-density treatment significantly decreased cellulase, urease and phosphatase activities by
262 31.2%, 72.7% and 36.7%, respectively.

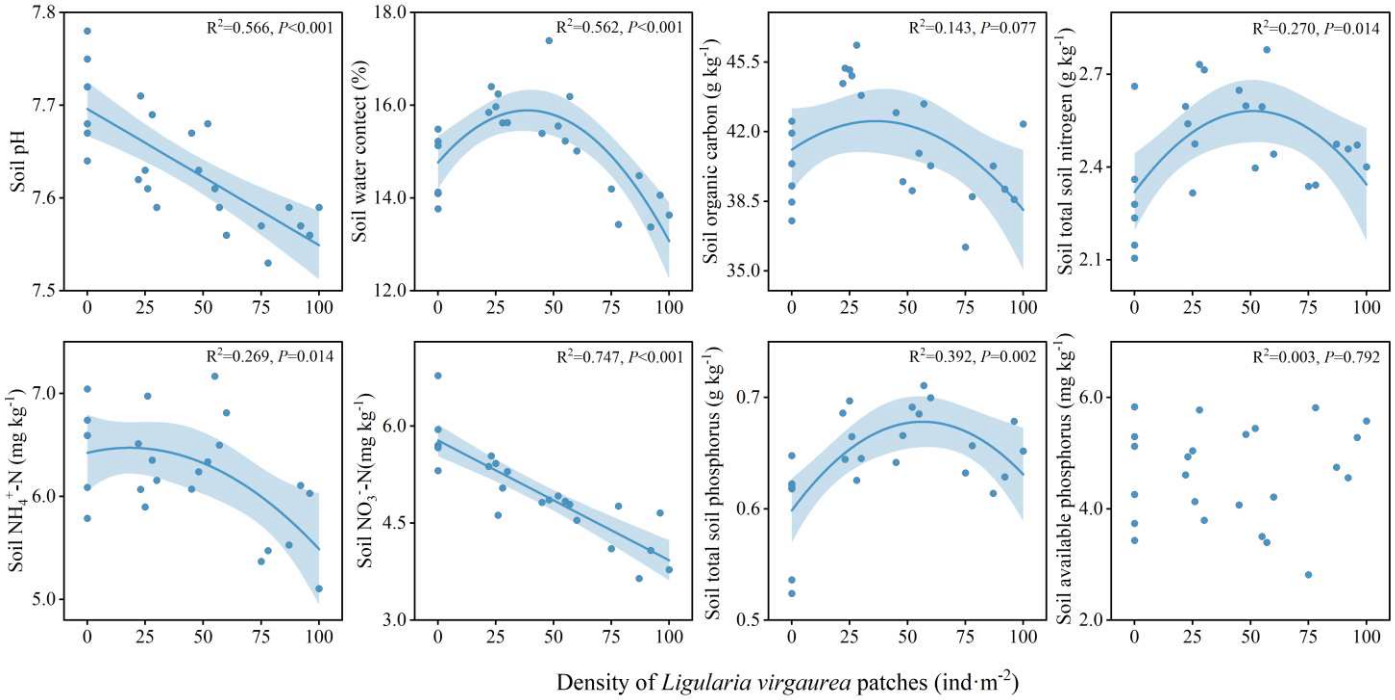

Fig. 2 Relationship between soil characteristics and patches density of *L. virgaurea*

3.2 Compositions of soil bacterial and fungal communities

During the formation of *L. virgaurea* patches, higher common species in soil bacteria (1447) than soil fungi (91) (Fig. S3a, c), and significant differences in dominant species among different density patches (Fig. S3b, d). Proteobacteria (61.33%–64.21%), Actinobacteria (32.50%–35.41%), Acidobacteria (1.17%–1.63%) were the three dominant bacterial phyla across all treatments (Fig. 3a), the dominant fungal phyla were Ascomycota (47.59%–63.60%), Mucoromycota (27.87%–38.39%) and Basidiomycota (6.68%–12.85%) (Fig. 3c). *L. virgaurea* patches affected the abundance of dominant bacterial and fungal phyla; compared with the control, the invasion of *L. virgaurea* resulted in a significant increase in the abundance of Proteobacteria, Verrucomicrobia, and Bacteroidetes by 2.99%, 58.92%, and 84.78% respectively, and an average decrease in the abundance of Actinobacteria, Acidobacteria, and Firmicutes by 4.84%, 13.53%, and 7.74%, respectively. At the genus level of the bacterial, the abundance of *Bradyrhizobium*, *Microvirga*, *Variovorax*, *Streptomyces*, *Mesorhizobium* were higher, and have a significant difference between patches of different densities (Fig. 3b). The relative abundance of *Variovorax*, *Streptomyces*, *Mesorhizobium* exhibited decreasing trend with increasing density of patches. The invasion of *L. virgaurea* resulted in a significant decrease in the abundance of Ascomycota by 13.58%, conversely, the abundance of Mucoromycota increased by 20.05%. At the genus level of the fungal, the abundance of *Rhizophagus*, *Pseudogymnoascus*,

Beauveria, *Fusarium*, *Ilyonectria* were higher (Fig. 3d). The relative abundance of *Rhizophagus* exhibited increasing trend with increasing density of patches.

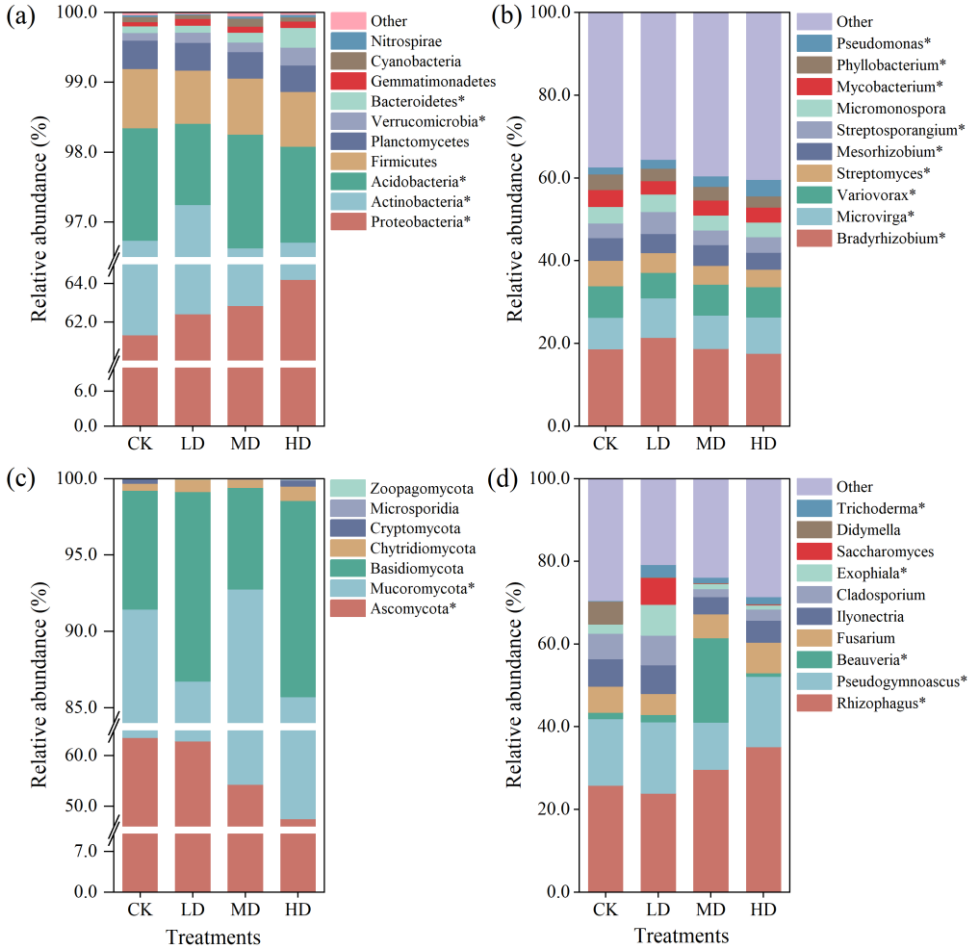

Fig. 3 The relative abundance of the dominant bacterial (a, b) and fungal (c, d) communities at the phylum (top 10) and genus (top10) level in grassland patches with different density levels of *L. virgaurea*. The asterisk (*) indicates the significant differences ($P < 0.05$) among the four treatments. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

LEfSe analysis indicated that significant differences in bacterial and fungal communities across the treatments of different density patches (Fig. 4). In HD, Gammaproteobacteria (e.g., *Pseudomonas*) was enriched substantially, while the LD treatments showed a higher abundance of Actinobacteria (e.g., *Streptomyces*). CK-specific taxa included Solirubrobacterales, whereas MD was characterized by *Bradyrhizobium*. In fungal communities, CK was enriched in Ascomycota lineages (e.g., Hyaloscyphaceae), whereas HD showed dominance of Mucoromycota taxa (*Rhizophagus*). LD and MD exhibited intermediate signatures, including *Apiotrichum* and *Beauveria*, respectively.

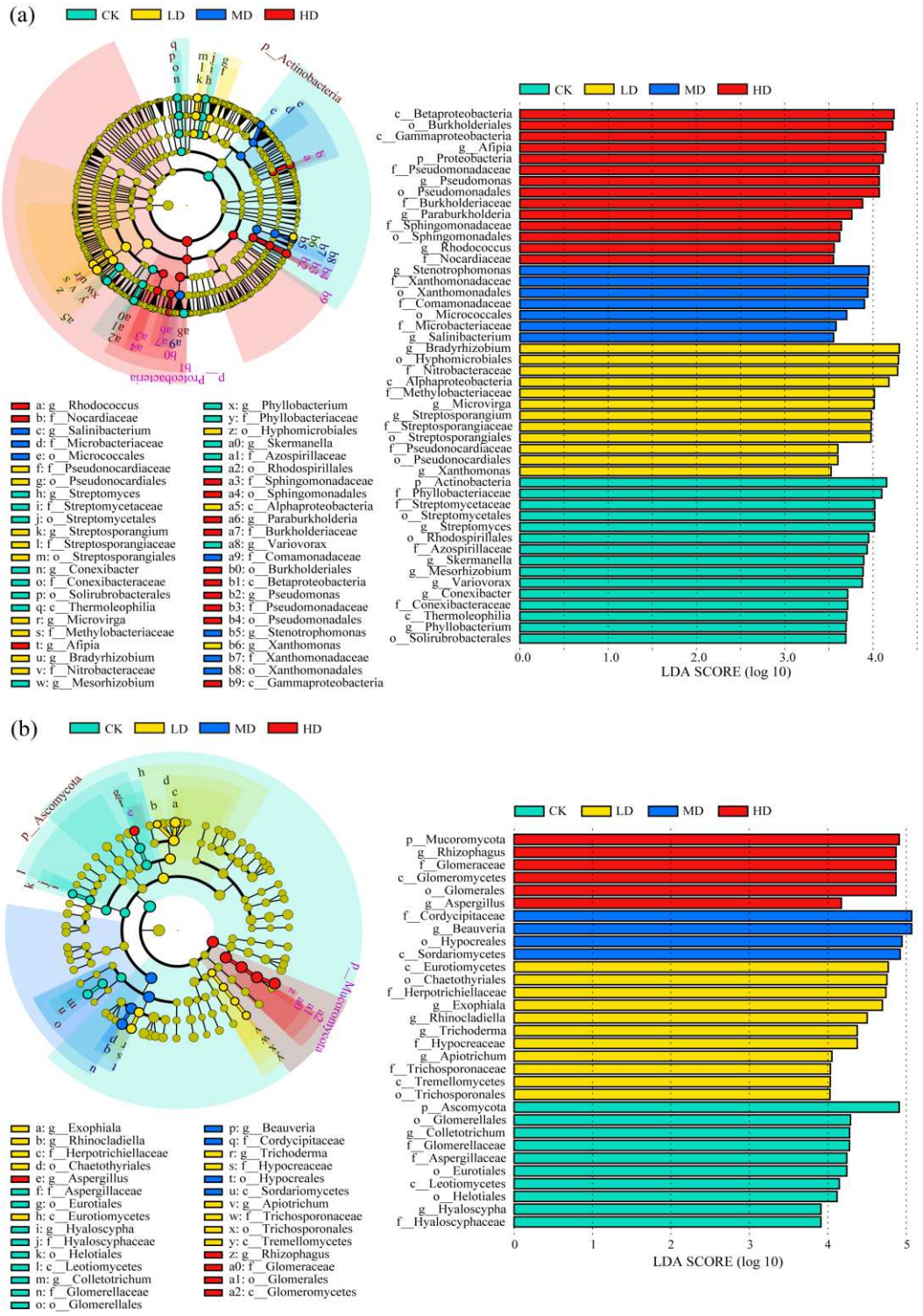

Fig. 4 Cladograms illustrated the phylogenetic distribution of the bacteria (a) and fungi (b) lineages in grassland patches with different density levels of *L. virgaurea*. Histograms of LDA scores (threshold > 3.5) were evaluated for differently abundant species in the bacterial (a) and fungal (b) communities in patches grassland with different density levels of *L. virgaurea*. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

3.3 α-diversity and β-diversity of soil bacterial and fungal communities

The patch formation of *L. virgaurea* significantly affected (excluding the Shannon, Chao1, and Ace indices of soil bacteria) soil microbial alpha diversity (Table 1). The diversity indices (Shannon-Wiener and Simpson) of soil bacteria increased with higher densities of *L. virgaurea* in the patches, and the Simpson index was significantly higher in LD treatment than in CK ($P < 0.05$); the bacterial richness indices (Chao1 and Ace) exhibited a unimodal pattern, initially increasing then decreasing with escalating *L. virgaurea* densities in the patches. For the fungal communities, the invasion of *L. virgaurea* increased the alpha diversity indices of soil fungal communities, and the alpha diversity indexes under MD treatment were significantly higher than that in CK ($P < 0.05$). The alpha diversity indexes of soil fungal community were highest in MD and lowest in CK. The diversity indices and richness indices of soil fungal community exhibited an initial increase followed by a decrease with the increase of the density of *L. virgaurea* in the patches. The soil microbial alpha diversity exhibited a unimodal pattern, indicating that the low-density invasion by *L. virgaurea* increased the diversity of soil microbial communities, but higher invasion intensities significantly reduced the diversity of soil microbial communities, consequently altering the structure of soil microbial communities. Principal coordinate analysis (PCoA) based on the Bray-Curtis distances revealed that the community distribution of soil bacterial and fungal differed significantly among different density patches (Fig. 5).

Table 1 The characteristics of soil bacterial and fungal diversity and richness indices in grassland patches with different density levels of *L. virgaurea*

	Treatments	Richness	Shannon	Simpson	Chao1	Ace
Bacteria	CK	1220.33±22.14	5.75±0.03	0.89±0.00b	1278.28±19.60	1262.91±18.24
	LD	1211.17±19.41	5.82±0.03	0.90±0.00a	1264.43±20.92	1253.31±20.49
	MD	1276.50±27.53	5.87±0.04	0.90±0.00ab	1322.79±28.64	1314.57±26.78
	HD	1264.67±23.24	5.87±0.03	0.90±0.00ab	1311.05±29.52	1302.43±26.40
	ANOVA					
	F	1.921	3.055	5.714	1.189	1.636
	P	0.159	0.052	0.005	0.339	0.213
Fungi	CK	65.17±2.04	2.81±0.09b	0.68±0.02b	83.69±6.74b	87.20±6.30b
	LD	71.83±1.38	3.16±0.06ab	0.75±0.01a	94.76±3.86ab	96.59±3.20ab
	MD	73.33±2.88	3.24±0.05a	0.77±0.01a	114.40±8.75a	105.92±4.67a
	HD	69.00±2.22	3.19±0.08ab	0.75±0.01a	89.29±4.92ab	88.41±3.97ab
	ANOVA					
	F	2.675	7.725	7.781	4.429	3.434
	P	0.75	0.001	0.001	0.015	0.037

Different lowercase letters indicate significant differences between treatments at $P < 0.05$. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

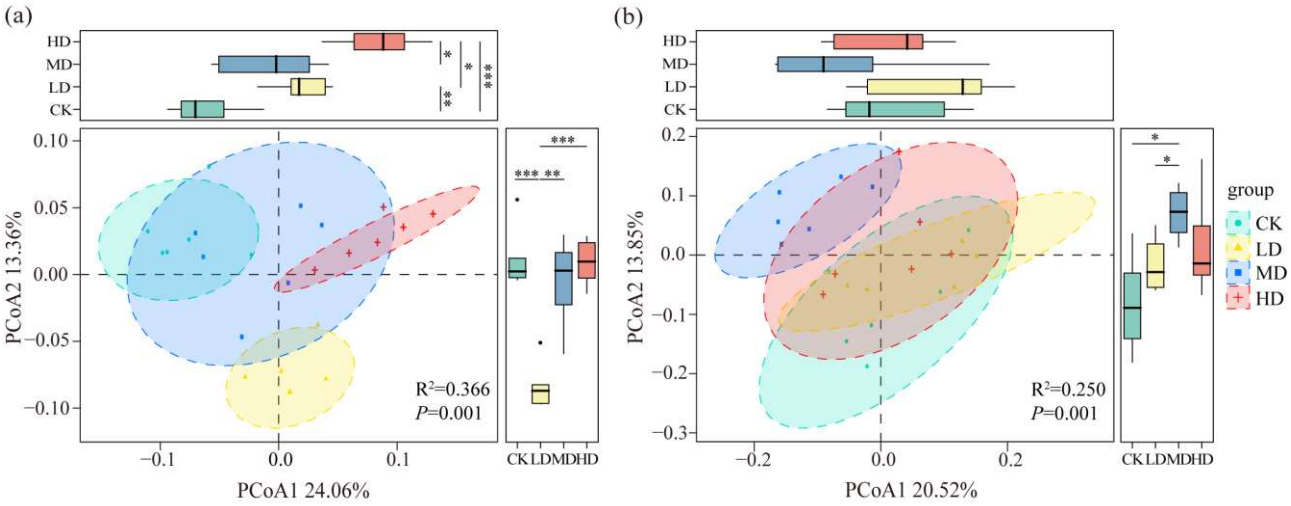

Fig. 5 Principal coordinate analysis (PCoA) of bacterial (a) and fungal (b) communities in grassland patches with different density levels of *L. virgaurea* based on the Bray-Curtis distance. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

3.4 Co-occurrence network of soil bacterial and fungal

Correlation networks were constructed to reveal the co-occurrence patterns of soil bacteria and fungi in *L. virgaurea* patches with different density levels. The overall topological properties indicated

the bacterial and fungal network indices of *L. virgaurea* patches with different density levels were different (Fig. 6 and Table S2). Analysis of the co-occurrence networks based on the phylum level soil microorganisms revealed that the proportion of negative correlations, modularity, average clustering coefficient and average path length were higher in the bacterial network under LD patch than those under non-patch. Subsequently, the topological parameters of bacterial network exhibited a decreasing trend with increasing densities of *L. virgaurea* patches. The number of edges and nodes in the bacterial network were highest in MD. For soil fungi, the proportion of negative correlations were higher in the fungal network under LD patch than those under non-patch. The number of edges and nodes in the fungal network were highest in HD. The modularity and average clustering coefficient were higher in the fungal network under MD patch than those under non-patch, and the lowest average path length was observed under LD treatment. The networks result clearly showed that compared to non-patch, soil microorganisms in low-density patches have shifted from cooperation to competition, and microbial members exhibited higher network connectivity and structural complexity.

Fig. 6 Co-occurrence network of soil bacterial (a) and fungal (b) in grassland patches with different density levels of *L. virgaurea*. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

3.5 The relationship between soil microbial community structure and soil environmental factors

Redundancy analysis revealed that environmental variables in the primary and second RDA dimensions explained 51.74% (RDA1: 33.03 %, RDA2: 18.71 %) and 57.65% (RDA1: 32.87 %, RDA2: 24.78 %) of the total variance in the soil bacterial and fungal communities, respectively (Fig. 7). CK and LD had a positive impact on the activity of Actinobacteria and Planctomycetes, respectively. MD promoted the activity of Acidobacteria and Nitrospirae, while Verrucomicrobia and Proteobacteria exhibited higher activity under HD treatment. In contrast to bacteria, fungal communities had a weaker response to different density of *L. virgaurea* patches. The key determinants influencing the soil bacterial and fungal community structures exhibited significant differences

among *L. virgaurea* patches of varying densities. The factors affecting bacterial community structure in CK treatment were C_enzy, MBC, MBP and SOC. In LD treatment, pH, SWC, NH_4^+-N, NO_3^--N and MBN were the main factors affecting bacterial community structure. Soil nutrients and environmental factors drove changes in microbial (bacterial and fungal) community composition in non-patch and low-density *L. virgaurea* patch. The hierarchical partitioning analysis further showed that NO_3^--N, pH, and C_enzy jointly drove changes in bacterial and fungal community structure.

Through SEM analysis of environmental variables and microbial diversity (Fig. 8), it was revealed how environmental variables shape soil microbial diversity through direct and indirect pathways during the succession of *L. virgaurea* patches. It was found that *L. virgaurea* invasion impacted soil microbial diversity through regulating environmental factors and soil nutrients (Fig. 7a and c). The density of *L. virgaurea* patches exhibited a positive effect on environmental factors and soil nutrients. The invasion of *L. virgaurea* primarily affected soil bacterial diversity through direct influences environmental factors and soil nutrients. Fungal diversity was mainly influenced by environmental factors. pH and SWC had a significant effect on soil bacterial and fungal diversity, while TP and N_enzy also exerted an impact on fungal diversity (Fig. 8b and d). Specifically, environment, microbial activity and soil nutrients explained 17%, 22% and 45% of the total variance in the soil bacterial diversity, and 20%, 36% and 26% of the total variance in the soil fungal diversity, respectively.

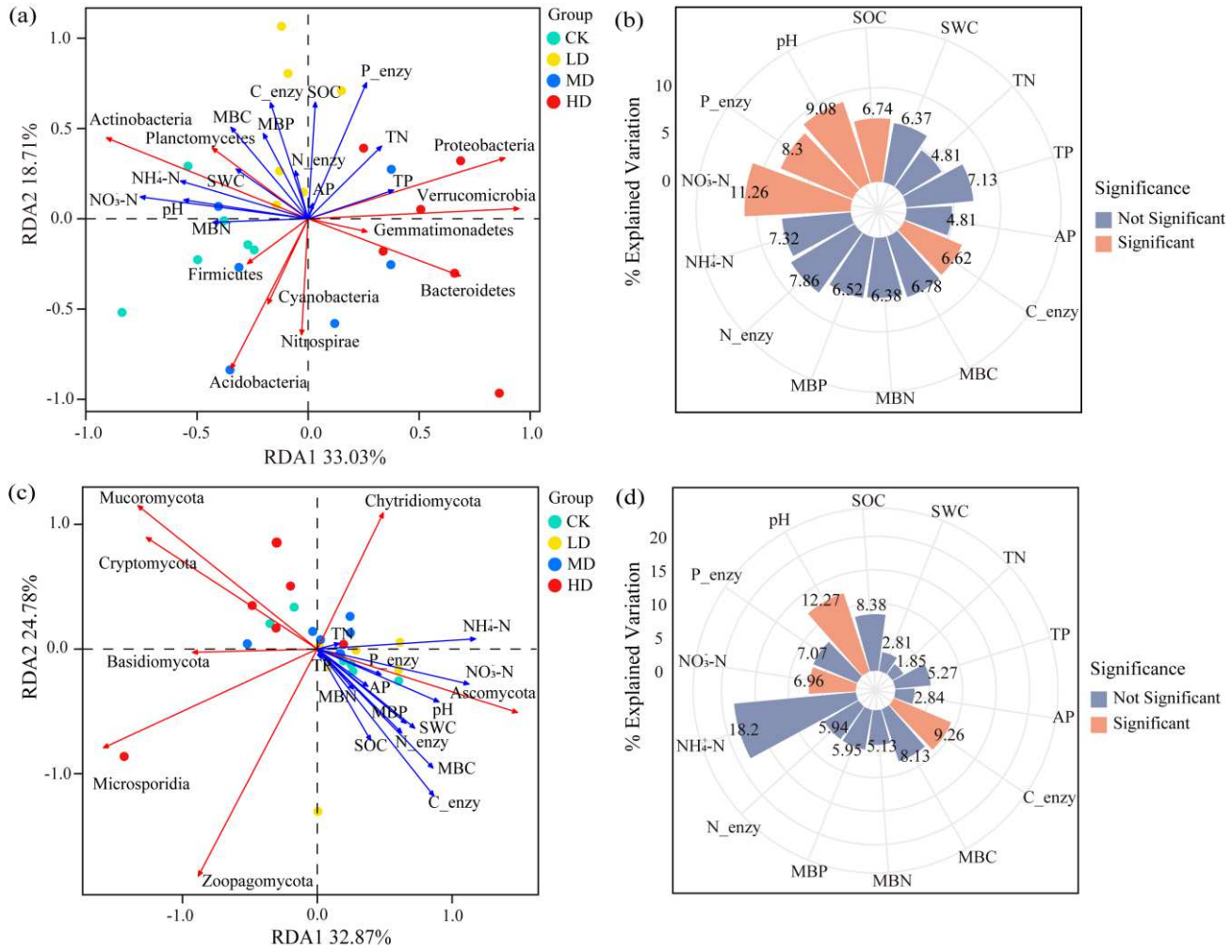

Fig. 7 Redundancy analysis (RDA) based on the correlations between soil bacteria (a) and fungi (c) at the phylum level and environmental factors under different density levels of *L*. virgaurea patches, and the explanatory power of individual factors on the total variation in bacterial (b) and fungal (d) community structures. Red arrows represent species, blue solid lines represent soil environmental factors. CK, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L*. *virgaurea* patches in grassland, respectively.

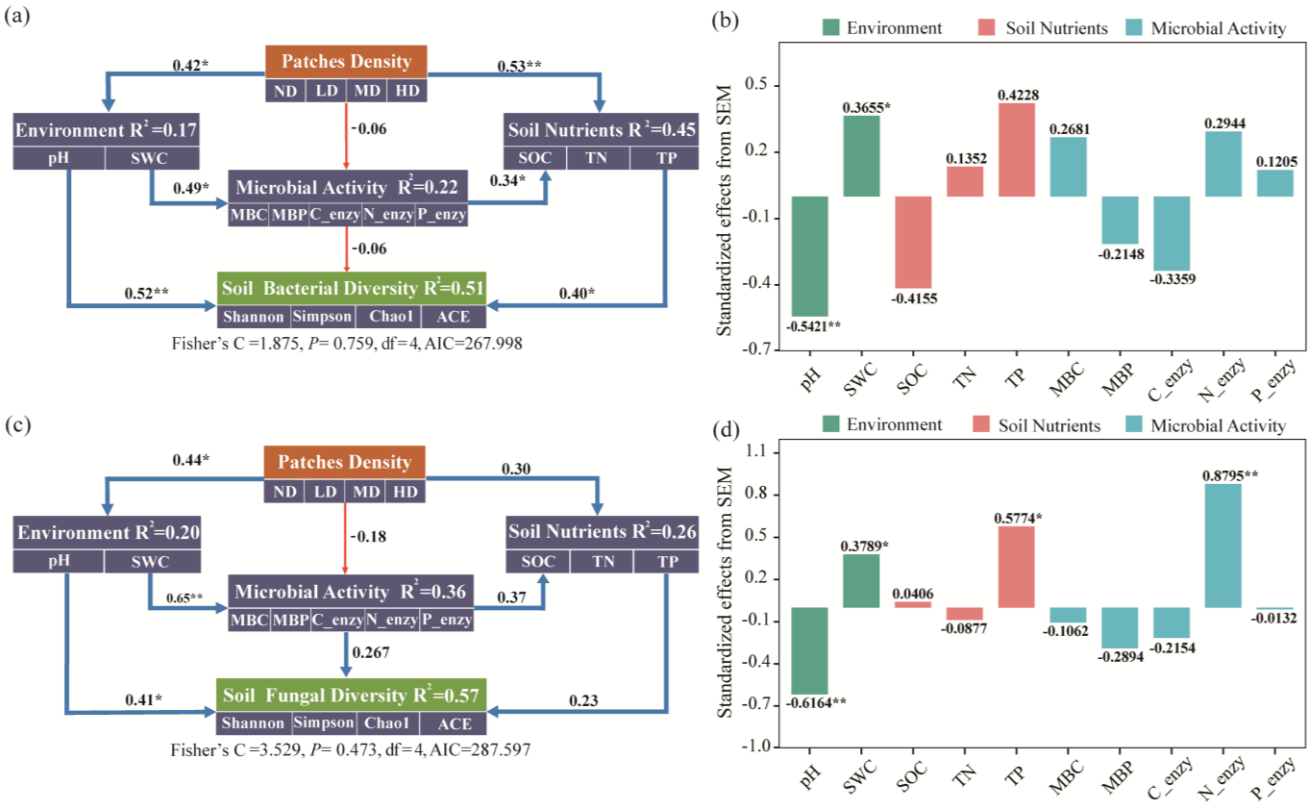

Fig. 8 Structural equation model (SEM) and the standardized effect of each indicator visualized the relationship between *L. virgaurea* patches, environmental factors, microbial activity and soil nutrients and soil bacterial diversity (a and b) and fungal diversity (c and d). The blue and red solid arrows indicate directly positive and negative effects, respectively. The arrow thickness represents the strength of the relationship. The numbers adjacent to the solid arrows represent standardized path coefficients. ** and * indicate significant differences at the $P < 0.01$ and $P < 0.05$ levels, respectively. ND, LD, MD and HD represent non-patch, low-density, medium-density and high-density *L. virgaurea* patches in grassland, respectively.

4. Discussion

Although many studies have reported the mechanisms of plant invasion and expansion, as well as how soil microbial drive plant invasion and patch expansion [17, 30, 38], it remains unclear about the expansion mechanism of *L. virgaurea* patches and whether soil microbial communities respond dynamically to invasion and expansion over time. Our results indicate that at low and medium population densities, *L. virgaurea* successfully establishes due to its biological traits (including strong competitiveness, broad adaptability, reproductive capacity, and allelopathic effects), thereby shaping the spatial heterogeneity of soil resources (such as nutrients, water, and organic matter) through its

presence and activities. This self-generated heterogeneous environment, while suppressing the recovery and growth of native plants, simultaneously creates more favorable soil conditions for the further expansion of *L. virgaurea*, thus forming a self-reinforcing positive feedback loop that continues to intensify its invasion process into grasslands. Meanwhile, the succession of *L. virgaurea* patches significantly influenced the composition of the soil microbial community. Specifically, the succession of low- to medium-density *L. virgaurea* patches enhanced soil microbial diversity.

4.1 Expansion of *Ligularia virgaurea* patches enhance soil resource heterogeneity in alpine meadows

The patchy degradation of grassland vegetation has become one of the primary characteristics of alpine meadow degradation on the Qinghai-Tibet Plateau [39]. However, the feedback between plant patches and soil resources promotes biological and non-biological processes under the patches, and the changes in these processes support the "fertility island effect" of poisonous plants, thereby promoting spatial heterogeneity of soil resources [19, 40]. Our results showed that *L. virgaurea* patches lead to significant changes in soil physicochemical characteristics, which is consistent with previous studies [9, 30]. In low- and medium-density *L. virgaurea* patches accumulated higher concentrations of nutrient resources (SWC, SOC, NH_4^+-N, TN, and TP). *L. virgaurea* gained an advantage in community competition from initial invasion (low density) to establishment (medium density), primarily manifested in its superior ability to intercept light and capture resources, which was stronger than other members in the community. According to recent reports, the differences in resource strategies between poisonous and non-poisonous plants suggest distinct patterns of resource allocation [8]. Because of this difference, the ability of poisonous plants to occupy and utilize resources was enhanced, which in turn increases the stress on non-poisonous plants by depriving them of growth space and increasing allelopathic secretion [41]. Therefore, *L. virgaurea* may enhance its advantage in resource acquisition by altering its resource allocation strategy, thereby further exacerbating the heterogeneity of soil resources (such as water and nutrients) within patches. During the expansion of poisonous plants, the shift in resource allocation strategy might have been a key mechanism driving the heterogeneity of patch soil resources [8].

Most studies also considered that the increases in soil nutrient availability and soil organic carbon(SOC) were attributable to the significant increase in aboveground biomass and litter input resulting from plant invasion [42, 43]. As demonstrated by Ehrenfeld [44], litter from native invasive plants decomposes faster than that of grass due to its higher quality, which increases the supply of

nutrient. Previous studies have reported that as the coverage of *L. virgaurea* increases in patches, functional traits such as plant height and above-ground biomass continue to increase [8, 45]. This indicates that *L. virgaurea* facilitates the return and enrichment of soil nutrients within patches through rapid decomposition of litter, further promoting soil resource heterogeneity. Soil extracellular enzymes are crucial for organic matter decomposition and nutrient release [46]. The results of this study indicated that *L. virgaurea* patches significantly affected the activity of soil urease, cellulase, and phosphatase. At low to medium densities, soil enzyme activity within *L. virgaurea* patches was higher than in non-patches grassland, indicating that the invasion and patches formation of *L. virgaurea* promote soil extracellular enzyme activity. Emerging evidence has revealed that invasive plants can reshape belowground nutrient cycling by modulating extracellular enzyme activities [47, 48]. Therefore, native plant invasion drives nutrient cycling in grassland ecosystems [43]. It suggests that the invasion and expansion of *L. virgaurea* may accelerate nutrient cycling and soil resources heterogeneity by promoting soil extracellular enzyme activity and decomposition of litter, which further supports its rapid establishment and expansion.

However, as the density of *L. virgaurea* increased, the soil nutrient content within the patches decreased, but the difference compared to non-patches grassland was not significant. This may be because the expansion of the *L. virgaurea* population accelerates the absorption of resources such as water and nutrients, leading to a reduction in available resources within patches. We suggest that if the density of *L. virgaurea* continues to increase, the uptake of available resources will keep rising, which could result in soil nutrient limitation. Recent report indicated that *L. virgaurea* enhanced plant diversity and functional diversity in degraded ecosystems at low to medium densities, but suppresses diversity at high densities [45]. Therefore, the reduction in available soil resources at high densities may be associated with decreased plant diversity and functional diversity in the ecosystem. This is because plant diversity and functional diversity within ecosystems are both closely linked to soil multifunctionality and nutrient cycling [49, 50]. Meanwhile, we observed that *L. virgaurea* patches significantly reduced soil pH, which is consistent with previous studies [45]. The decrease in soil pH may indirectly affect soil nutrient cycling by influencing soil biological processes such as soil enzyme and microbial activity [51]. The high-density expansion of *L. virgaurea* may limit species coexistence, reduce plant diversity, and gradually lead to the formation of a degraded grassland dominated solely by *L. virgaurea*.

During the succession of *L. virgaurea* patches, soil nutrient resources exhibit a "unimodal" response pattern. The shift in soil resource accumulation from promotion to inhibition under patch density regulation accelerates the expansion of the *L. virgaurea* population. The strong competitiveness of *L. virgaurea* is undoubtedly one of the main reasons for its population expansion. In low- to medium-density patches, by increasing soil resource heterogeneity, *L. virgaurea* consolidates its self-generated competitiveness and creates a favorable environment for its growth. These results support our first hypothesis: *L. virgaurea*, by utilizing its competitive advantage to create soil resource heterogeneity, optimizes its own living environment, thereby promoting population expansion.

4.2 *Ligularia virgaurea* patches alter soil microbial communities

Our results showed that the invasion and expansion of *L. virgaurea* significantly altered the composition and structure of soil microbial communities. The expansion of *L. virgaurea* significantly increased the relative abundance of Proteobacteria, Mucoromycota and Basidiomycota, while the relative abundance of Actinobacteria and Ascomycota significantly decreased. Notably, the phylum Glomeromycetes and genus *Rhizophagus* were significantly enriched in the high-density patch, suggesting a strong promotion of arbuscular mycorrhizal fungi(AMF) under high-density patch. Previous studies have reported that the abundance and diversity of soil bacterial communities in the center of plant fertile islands were higher than those at the edges and bare land [52]. The diversity and abundance of soil bacteria under the canopy of woody plants were higher than those in open areas between canopies [53]. The invasion of woody plants significantly enhances multiple microbial characteristics in grassland soil [30]. This evidence suggests that changes in available resources within patches alter the structure of soil microbial communities, which is consistent with our research findings. During the succession of *L. virgaurea* patches, the ecological strategies of the soil microbial community shifted from oligotrophic to copiotrophic, and high concentration of available resources favored the enrichment of copiotrophic microorganisms such as Proteobacteria [54]. For instance, microbial groups involved in carbon and nitrogen cycling, genera in maintaining plant growth such as *Microvirga*, *Bradyrhizobium* and *Rhizophagus* [55, 56], and carbon- decomposing microbes like *Pseudomonas* [57] increase in abundance within the patches. Meanwhile, network analysis showed that as the density of *L. virgaurea* patches increased, the number of edges and nodes between bacterial and fungal communities first increased and then decreased, and soil microorganisms in low-density

patches shifted from cooperation to competition. Microbial members exhibit higher network connectivity and structural complexity. The formation of complex networks typically requires a nutrient-rich environment [58], indicating that the soil resource heterogeneity caused by the expansion of *L. virgaurea* patches shapes the network structure complexity of soil microbial communities.

However, some studies have also suggested that the spread of poisonous plants can alter the soil environment, reducing the abundance of soil microbial communities and nutrient uptake [33]. Ma *et al* [8] reported that during the formation of grasslands dominated solely by *L. virgaurea*, the number of gene and primary phyla's relative abundance of microbial communities showed a declining trend. Such reductions often help to maintain soil functions and accumulate nutrients by reducing competition for resources, increasing microbial residues, and altering the resource strategies of microbial communities [8, 59]. A meta-analysis summarized that the living roots of invasive plants have a negative effect on the biomass/abundance of soil biota and suggested that the allelopathic substances in the root tissues and exudates of invasive plants may inhibit native plant and microbial activity [33, 60]. This evidence contrasts sharply with the findings of our research, where the succession of *L. virgaurea* patches enhanced microbial activity (soil enzyme activity and microbial biomass). The results supported our second hypothesis well. The main reasons for this discrepancy are likely related to the duration and density of *L. virgaurea* invasion, as well as the allelochemicals in its plant tissues and root exudates. Because at a certain density, allelochemicals as carbon substrates in soil can attract specific microbial groups, thereby increasing the abundance of microorganisms involved in carbon and nitrogen metabolism [61, 62]. Soil nutrients play a critical role in microbial community responses to plant invasion and plant patches succession [9, 30]. Specifically, pH, SOC, and NO_3^--N drove the changes in bacterial communities, while pH and NO_3^--N drove the changes in fungal communities. RDA analysis revealed that under low-density patches, the increase in NH_4^+-N and NO_3^--N reflected an increase in Actinobacteria activity, whereas under high-density patches, the increase in TP and TN reflected an increase in Proteobacteria activity. Under low-density patches, the increase in soil nutrients reflected an increase in Ascomycota activity.

Regarding microbial diversity, the succession of *L. virgaurea* patches altered the diversity of bacterial and fungal communities. Specifically, the Simpson index of bacteria communities significantly increased , while the Shannon, Simpson, Chao1, and Ace indices of fungal communities

were all significantly elevated, which was consistent with previous studies [9, 63]. Furthermore, with increasing *L. virgaurea* patches density, the α-diversity indices of both bacterial and fungal communities demonstrated an initial increase followed by a decrease. The increase in microbial diversity is primarily driven by higher resource availability, and resource effectiveness expands the breadth of microbial ecological niche in the habitats by reducing interspecific competition. The reduction of microbial populations' dependence on limited resources enables more groups to coexist, promoting an increase in microbial diversity, which aligns with the theoretical framework of 'ecological release' or 'competitive release' effects. Therefore, the invasion and patch succession of *L. virgaurea* have increased the supply of available resources within patches, creating ecological niches for more microbial groups, which has led to an increase in bacterial and fungal diversity. In addition, the β-diversity of bacterial communities in low- and high-density *L. virgaurea* patches, and that of fungal communities in medium-density patches, were distinct from those in uninvaded grassland, which suggested that *L. virgaurea* patches caused heterogeneity of soil microorganisms. This may be related to soil resources heterogeneity, and the nonuniform succession in soil microorganisms could enhance microbial diversity [9, 64].

4.3 Soil resources heterogeneity accelerates the expansion of *Ligularia virgaurea* patches

The formation and succession of the *L. virgaurea* patches have enriched available soil resources and increased soil resource heterogeneity. In low-to-medium density patches, soil nutrients, SWC, and bacterial and fungal diversity have increased compared to non-patch grasslands. Through self-reinforcing mechanisms, the patches absorb more resources to support their establishment and expansion. Meanwhile, the increase in soil nutrient supply has altered the plant communities' traits and litter quality. Changes in plant communities and litter quality within patches will enhance soil resource heterogeneity, indirectly affecting microbial communities by influencing soil nutrients [33]. Overall, our results indicate that during the formation and succession of *L. virgaurea* patches, their strong competitiveness allowed them to accumulate substantial nutrient resources within patches, increasing soil resource heterogeneity and thereby stimulating microbial composition, diversity, biomass, activity, and enzyme activities. Soil resource heterogeneity indirectly influenced microbial diversity and reshaped belowground processes in grasslands. These changes imply an acceleration in nutrient cycling and an increase in soil nutrient accumulation, further strengthening the encroachment ability of *L. virgaurea*. Therefore, the abiotic and biotic heterogeneity of soil induced by *L. virgaurea*

may facilitate patch expansion.

4.4 Limitations and implications

Although we have elucidated the expansion of *L. virgaurea* patches alters the distribution pattern of soil resources, and investigated the effects of *L. virgaurea* patches on the composition and diversity of soil microbial communities, several limitations should be considered. We believe that the *L. virgaurea* patches are enriched with more available nutrient resources, leading to a differential distribution of soil resources. The subsequent abiotic and biotic changes driven by this heterogeneity may further accelerate the expansion of the patches. This conclusion is based on analyses of soil physicochemical properties and soil microbial communities, yet it does not account for the responses of plant community characteristics to the formation and succession of *L. virgaurea* patches. Additionally, this study did not conduct large-scale and multi-regional sample collection, which did not fully reflect the heterogeneity patterns under different habitats or geographical conditions. Future systematic sampling across larger spatial scales, combined with long-term fixed-site monitoring, will help to more comprehensively validate the ecological mechanism of *L. virgaurea* driving soil resources heterogeneity and promoting patches expansion.

Under certain conditions, maintaining an appropriate density of *L. virgaurea* may have a positive impact on biodiversity and contribute to the restoration of degraded ecosystems [45]. Our research results also validate this viewpoint, indicating that *L. virgaurea* is not merely a competitive weed but occupies a certain ecological niche within the community. It can provide microhabitats or resource complementarity for other species, thereby enhancing the diversity and ecological function of plant communities. However, when utilizing *L. virgaurea* populations to improve ecosystem diversity and function, particular consideration should be given to grassland types and grazing pressure. Overgrazing will reshape the structure of plant communities, and by avoiding grazing, *L. virgaurea* enhances its competitiveness, which may accelerate population expansion. Therefore, in future ecological restoration and grassland management, the population dynamics of *L. virgaurea* should be scientifically evaluated and reasonably guided to promote its positive ecological functions while avoiding potential negative impacts on the ecosystem.

5. Conclusion

Our study revealed the response and key driving factors of soil physicochemical properties and microbial communities in alpine meadows to the invasion and patches expansion of *L. virgaurea*. At

low to moderate density conditions, the invasion and patches expansion of *L. virgaurea* led to the enrichment of soil available nutrients and enhanced soil resource heterogeneity. The density of *L. virgaurea* patches drove changes in soil resource heterogeneity, which indirectly affected soil microbial abundance and diversity. To some extent, the invasion of *L. virgaurea* promoted the diversity of soil microbial communities. The heterogeneity of soil nutrient resources and soil microorganisms resulting from *L. virgaurea* patches may accelerate their population expansion. Furthermore, we identified pH, NO_3^--N, SOC, phosphatase, and cellulase as drivers of bacterial community changes, while fungal community changes were mainly influenced by pH, NO_3^--N, and cellulase. This study demonstrates the characteristics of changes in soil resources and soil microbial communities during the expansion of *L. virgaurea* patches and interrelationships. These results contribute to our understanding of the impacts of poisonous plant patches expansion on grassland ecosystems in the alpine meadows of the Qinghai-Tibet Plateau and provide theoretical support for grassland management in alpine meadow ecosystems.

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Author Contributions

JCS and DDL: Writing – original draft, Visualization, Methodology, Investigation. XJY and YST: Writing – review & editing, Resources, Methodology. AY, HY and XJZ: Visualization, Investigation. All authors reviewed the manuscript.

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Data availability

The raw sequencing data generated in this study have been deposited in the NCBI SRA under the accession number PRJNA1402964. The data is publicly accessible at: https://www.ncbi.nlm.nih.gov/sra/PRJNA1402964. All data sheets and images to process data are available upon request to the corresponding author.

Declarations

Ethics approval and consent to participate

The methods involved in this study were carried out in compliance with local and national

regulations. No materials from animals or humans were used in this research.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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