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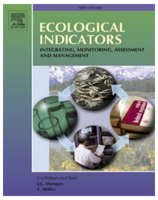

The dominance of *Ligularia* spp. related to significant changes in soil microenvironment

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ABSTRACT

Exploring how plants adapt to and change the surrounding environment has become essential to understanding their survival strategies and co-evolution mechanisms. *Ligularia virgaurea* and *Ligularia sagitta* are the two most common species in the alpine grazing ecosystems of the eastern Qinghai-Tibetan Plateau (QTP) and becoming increasingly dominant. Studies have suggested that overgrazing has allowed *Ligularia* to gain a competitive advantage by changing plant community structure, which is often closely related to the soil environment. However, we don't fully understand the soil environment changes during this process, and the underlying mechanisms have not been explored. Therefore, we investigated plant community characteristics, soil fertility and soil microbial diversity in the *L. virgaurea* and *L. sagitta* communities on the eastern QTP. *Ligularia* spp. significantly changed the plant community by reducing biomass, vegetation coverage, abundance, and biodiversity, and the effect of *L. sagitta* on the plant community was stronger than that of *L. virgaurea*. In the plant communities dominated by *L. virgaurea* and *L. sagitta*, soil nutrients and soil microbial communities changed significantly. Aggregated boosted trees analysis revealed that soil Mg levels had the greatest relative influence on the structure and diversity of the soil microbial community. Our study provides data and a theoretical basis for revealing the survival strategies of *L. sagitta* and *L. virgaurea* and, provides a basis for weed management in grazed ecosystems.

1. Introduction

In the process of natural selection or evolution, environmental factors can affect plant survival in various ways. Temperature changes can alter plant phenology, morphology and even gene frequency (Root et al., 2003), and even lead to plant extinction (Dullinger et al., 2012). Reich et al. (2018) argued that changes in soil moisture caused by climate change may ultimately affect plant photosynthesis. Dubois and Inzé (2020) proposed that osmotic stress could cause plant growth stagnation in a short period. Light can also regulate the activity of many enzymes and stomata in the process of carbon assimilation, which affects plant growth and metabolism, and the transportation of substances (Kami et al., 2010). A lack of flexibility to avoid extreme environments has caused plants to evolve a series of extraordinary environmental adaptations (Barfield and Norman, 1983), such as altering the life cycle, changing physiological morphology, etc., and these adaptations can also have an impact on the environment. As a novel adaptation to biological invasion, plants can also release allelochemicals into the surrounding environment, changing soil physical and chemical properties and nutrient status, and affecting plant growth (Oliva et al., 2002). Many plants can free root border cells from the root cap epidermis to gather around the root tips. These root border cells are similar to human neutrophils and can be used to construct a layer of extracellular traps (Carreras et al., 2019) to resist external stress and regulate the rhizosphere environment to protect roots (Hawes et al., 2000). Examples from paleoecology also demonstrate the transformation of the environment by plants. For example, the rapid expansion of carboniferous terrestrial plants led to a significant increase in atmospheric oxygen content (Berner, 2006), with a series of subsequent ecological consequences. During the long progress of evolution, plants not only need to overcome the stress caused by the surrounding environment, but also species competition. The mutual response between plant and environment widely exists (Wu et al., 2007), and the various plant abilities to adapt or change their environment may have enhanced their survival. Therefore,

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exploring how plants adapt and change the surrounding environment has become an essential way to understand their survival strategies and co-evolution mechanisms.

The Qinghai-Tibetan Plateau (QTP) supports the largest grassland in Eurasia with over 66% covered by alpine meadows (Qin et al., 2015). However, this ecosystem has been affected by climate change (Liu and Chen, 2000), wildlife activity (Du et al., 2004), and grazing activity (Zhang et al., 2005) since the 1970s, and has suffered serious degradation (Wang et al., 2006). Over this time, the original dominant species of Cyperaceae and Gramineae have been replaced through interspecific competition by other plants such as *Ligularia* (Wu et al., 2013). In a large-scale field investigation, we found that *L. virgaurea* and *L. sagitta* are common in the grasslands of the eastern QTP. They have spread along the Yellow River–Rierlang Mountain area; *Ligularia sagitta* mainly in the north and *L. virgaurea* in the south; and both species have become dominant across their distributional range. Ecological studies on *Ligularia* in the area have focused mainly on their morphological characteristics (Lei et al., 2018), reproductive strategy (Wu et al., 2010), and allelopathic potential (Wu et al., 2011). Shi et al. (2018) conducted a limited study on the environmental impact of *L. virgaurea*, and concluded that soil nitrogen limitation was one of the dynamic factors controlling its expansion. Unfortunately, other factors, apart from total, and available soil nitrogen, were not measured. So, while these two species may play important roles in local grazing ecosystems, the underlying relationship between them and environmental factors is unclear.

Some studies have suggested that overgrazing allows some species to gain a competitive advantage by changing plant community structure (Nunes da Cunha and Junk, 2004; Báez et al., 2008), which is often closely related to the soil environment (Peng et al., 2020). However, presently we don't fully understand the soil environment changes during this process, and the underlying mechanisms have not been explored. Therefore, we propose two hypotheses: (1) *Ligularia sagitta* and *L. virgaurea* dominance indicates significant soil microenvironment changes, but these changes differ because of different species habitats, and (2) While these two species likely attain dominance due to their toxicity to herbivores, they are associated with different soil chemical and microbial communities that may contribute to their subsequent community persistence. To test these hypotheses, we investigated plant communities containing *L. virgaurea* and *L. sagitta* located in Xiahe County in Gansu Province (north) to Hongyuan County, in Sichuan Province (south) in the eastern QTP. Our results will provide data and a theoretical basis for revealing the survival strategies of two *Ligularia* species and other plants, and a scientific framework for similar studies. This study also provides a reference for weed management in grazing ecosystems on the QTP.

2. Materials and the methods

2.1. Research area and sampling strategy

The research site is located on the southeast edge of the QTP, between Xiahe County, Gansu Province (102°31′6.26″ E, 35°11′56.37″ N) and Hongyuan County, Sichuan Province (102°21′59.97″ E, 32°26′32.85″ N) at altitudes between 3,100 and 3,400 m (Fig. 1). The climate is a typical continental plateau climate, with an average annual temperature of 1.1–2.6 °C, annual rainfall of 512–729 mm, and annual potential evapotranspiration of 1200–1304 mm. In the peak plant growth period, the average vegetation coverage is over 80%, with a maximum height of 45–60 cm. Major plant species include Cyperaceaeous plants (*Kobresia setchwanensis*, *Kobresia pygmaea*), grasses (*Elymus nutans*, *Poa annua*), legumes (*Oxytropis kansuensis*, *Gueldenstaedtia diversifolia*), and forbs (*Ligularia*, *Saussurea,* and *Potentilla*) (Li et al., 2011). The soils have been classified as Mat Cry-gelic Cambisols, using the Chinese soil classification (Gao et al., 2007).

Six sampling sites were established, including three *L. sagitta* sampling areas: SCK/LS-1 (102°27′27.78″ E, 35°3′51.86″ N), SCK/LS-2 (102°26′52.89″ E, 34°43′33.31″ N) and SCK/LS-3 (102°30′3.11″ E, 34°10′51.67″ N), mainly in the northern Rierlang Mountains, and three *L. virgaurea* sampling sites: VCK/LV-1 (102°55′48.11″ E, 33°37′46.72″ N), VCK/LV-2 (102°41′12.85″ E, 102°21′11.03″ N) and VCK/LV-3 (33°8′39.73″ E, 32°26′24.50″ N), mainly in the southern Rierlang Mountains. All sampling areas were located on the alpine grassland, with flat terrain and a uniform vegetation community structure.

2.2. Community survey and soil sample collection

Six 1 m × 1 m quadrats were selected in each sampling area, three containing one of the *Ligularia* species, and three where *Ligularia* species were absent (controls). Consequently, there were four treatments: LS

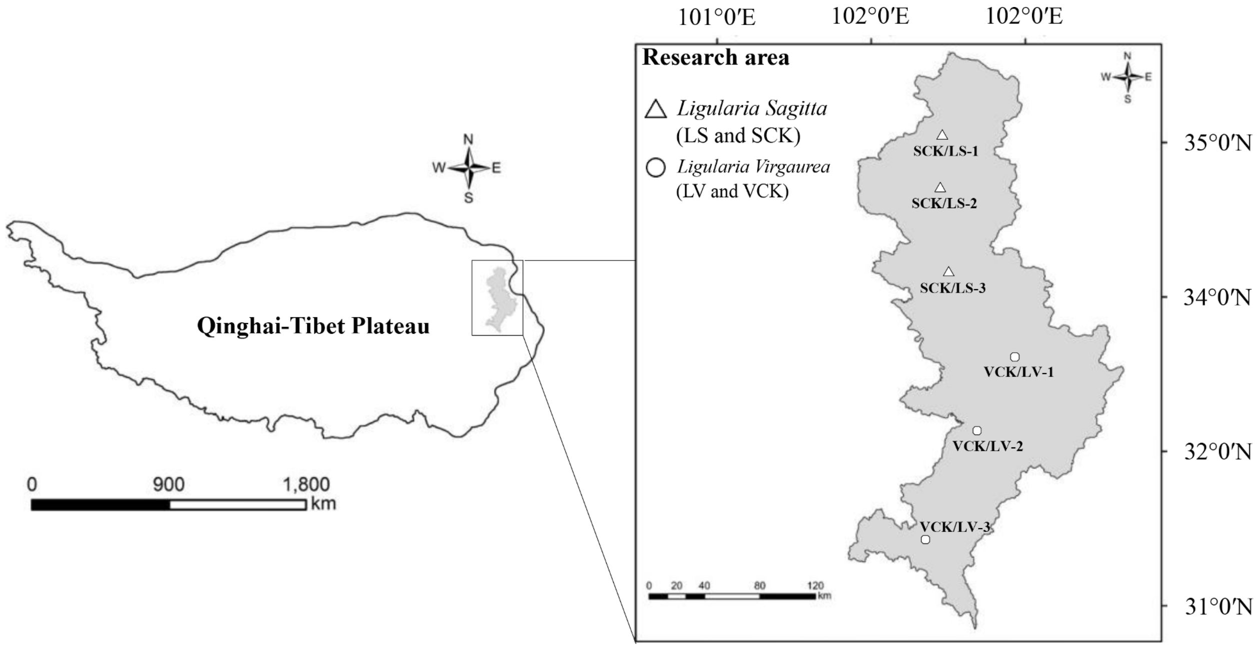

Fig. 1. Geographical location of the research area.

(*L. sagitta*), SCK (non-*L. sagitta* control), LV (*L. virgaurea*), VCK (non-*L. virgaurea* control), and each treatment contained three sub-treatments (for example, LV consists of LV-1, LV-2, and LV-3). To investigate plant community traits such as coverage and height, aboveground vegetation in the quadrats was cut and stored at low temperature before drying to a constant weight (105 °C for 30 min, 80 °C for at least 24 h), to measure biomass. To quantify species richness and diversity, the Simpson index, Shannon-Wiener index, and Pielou-J index were calculated for each quadrat (Hu et al., 2019). Soil cores (5 cm diameter; 20 cm depth) were taken along both diagonals in each quadrat; a total of 36 soil samples were collected. Soil samples were immediately cooled (-20 °C) and transported to a laboratory for analysis. Each sample was divided into two sub-samples: the first was stored at −80 °C and used for genomic DNA extraction, and the second was stored at 4 °C for the determination of physical and chemical properties. To minimize the influence of phenology and meteorological conditions, the vegetation survey and sample collection were completed over a relatively short period (August 10–12, 2019) when weather in the area was stable (clear air, average daily temperature of 15–16 °C).

2.3. Determination of soil chemical properties

Soil pH was measured using a pH meter (Fe20-Five Easy pH, Mettler Toledo, Germany) at a soil-to-distilled water ratio of 1:5 (weight/volume). Total carbon (C) and total nitrogen (N) contents were determined using an element analyzer (Vario MACRO Cube, Elementar, Germany). Soil organic matter (SOM) was determined using the potassium bichromate titrimetric method. Available nitrogen (AN) was determined using an automatic Kjeldahl apparatus (Kieltec$^{\text{TM}}$ 2300, Foss, Denmark) and total phosphorus (P) and available phosphorus (AP) were determined using the molybdenum–antimony anti-spectrophotometric method. Total potassium (K) and available potassium (AK) were determined using sodium hydroxide-flame photometry and ammonium acetate-flame photometry and an atomic absorption spectrophotometer (Z-2000, Hitachi, Japan), respectively. Soil Ca, Na, Mg, S, Fe, Cu, Zn, B, and Mn contents were determined using an inductively coupled plasma emission spectrometer (P-9000, Analytik Jena, Germany). Mercury (Hg) content was also determined using a cold vapor atomic fluorescence spectrophotometer (AFS-230E, Haiguang Instrument, China).

2.4. Determination of soil microbes

A MoBio PowerSoil DNA kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) was used to extract the total DNA from 0.25 g of soil. The concentration and purity of the extracted DNA were detected using an ultra-microspectrophotometer (NanoDrop 2000C, Thermo Scientific, USA), then the DNA sample was diluted to 10 ng/L and stored at −20 °C for subsequent analysis. The V3–V4 region of the 16S rRNA was amplified using primers 338F-(5′-ACTCCTACGGGAGGCAGCA-3′) and 909R (5′-CCCCGYCAATTCMTTTRAGT-3′) with a 12 nt long barcode (Tamaki et al., 2001) to evaluate soil microbial community. The polymerase chain reactions and other experimental procedures are described in detail by Ade et al. (2018). All of the qualified samples (concentration and purity) were mixed at the same molar ratio for MiSeq PE250 sequencing and sequenced using MiSeq Reagent Kit V3 (600 cycles) and an Illumina MiSeq sequencing platform (MiSeq 2500, Illumina, USA) from Shanghai Personal Biotechnology Co., Ltd. The sequencing library used was from Illumina's TruSeq Nano DNA LT Library Prep Kit. The VCK only had 8 replicates due to the failure of one sample.

The processing of the NovaSeq sequencing data was mainly performed using QIIME 1.8.0 software (Caporaso et al., 2010a). The original sequences were sorted based on their unique sample barcodes and trimmed for sequence quality using the QIIME pipeline (length > 300 bp, average base quality score > 30). Chimera sequences were removed with the UCHIME algorithm (Edgar et al., 2011). Sequences were clustered using the complete-linkage clustering method incorporated in the QIIME pipeline and clustered into operational taxonomic units (OTUs) based on a sequence similarity threshold of 97% (Wang et al., 2007), and a typical sequence was compared with the local reference database (Greengenes core set). Sequence alignment was performed using the Python Nearest Alignment Space Termination (PyNAST) algorithm (Caporaso et al., 2010b). Prior to building the phylogenetic trees, the quality of the sequence alignment was checked, and then phylogenetic trees were reconstructed using general time-reversible (GTR) models, the maximum likelihood (ML) method, and the FastTree 2.1.1 software (Price et al., 2010). QIIME was used to build the OTU table, followed by α and β diversity (NMDS, Weighted UniFrac) analyses. The α diversity analysis included the Shannon-Wiener Index, the Faith Phylogenetic-Diversity (Faith-PD), the Observed-Species, and the Chao 1 index.

The original sequence data used in this study were deposited in the National Center for Biotechnology Information (NCBI), Accession No. PRJNA639691 (https://www.ncbi.nlm.nih.gov/sra/PRJNA639691).

2.5. Statistical analysis

One-way analysis of variance was performed to test for differences in plant community characteristics (vegetation coverage, biomass, abundance, Simpson index, Shannon-Wiener index, and Pielou-J index), soil physicochemical properties (SOM, C, N, AN, P, AP, K, AK, Ca, Na, Mg, S, Fe, Cu, Zn, B, Mn, Hg, and pH), diversity indexes (Shannon-Wiener index, Faith-PD, Observed-Species, and Chao 1 index), and the relative abundance of soil bacteria (Phyla) using SPSS software (SPSS statistics 22.0, IBM, USA). When significant differences between the treatments ($P < 0.05$) were observed, the least significant difference (Tukey) test was applied. The Circos species relation diagram was used to assess differences in the relative abundances of soil bacteria (www.omicshare.com/tools). Pearson correlation analyses were performed using SPSS. The aggregated boosted tree analysis (ABT) was conducted using R.2.9.1 (Gemplus package) to quantify the relative influences of soil physicochemical factors on soil bacterial community structure and diversity (Kuang et al., 2013). Redundancy analysis (RDA) in CANOCO (Windows 4.5) was used to determine correlations between soil physicochemical properties and dominant bacteria phyla.

3. Results

3.1. Plant community characteristics

Ligularia virgaurea and *L. sagitta* had similar effects on neighboring plant communities (Fig. 2). Both reduced plant coverage, biomass, abundance, Shannon-Werner index, and Pielou-J index. The LS plant community coverage and the Simpson index were significantly lower than those of SCK ($P < 0.05$). Furthermore, biomass, abundance, Simpson index, and Shannon-Werner index of quadrats with *L. virgaurea* (LV and VCK) were significantly higher than in *L. sagitta* (LS and SCK) quadrats, although the Pielou-J index in *L. virgaurea* quadrats was slightly lower. The change of each sampling area was similar to the above results (Supplementary Table 1), but some differences should be noted, for example, LS-1 plant community biomass was higher than in SCK-1.

3.2. Soil environment

Ligularia virgaurea and *L. sagitta* effects on soil properties were similar (Fig. 3). Both species reduced soil C, SOM, N, AN, AP, AK, Ca, Na, S, Hg, and pH, but increased P, Mg, Fe, Cu, B, and Mn ($P < 0.05$). However, *L. virgaurea* reduced soil Zn content ($P < 0.05$), while *L. sagitta* did not affect it. Differences between the control and LV treatments for AP, Mg, Fe, and Mn were −57.02%, 28.36%, 74.66%, and 86.51%, respectively, while differences between the control and LS treatments were −25.92%, 7.84%, 28.95%, and 16.70%, respectively. Differences in sampling area did not affect the overall results of the analysis, and changes in K were

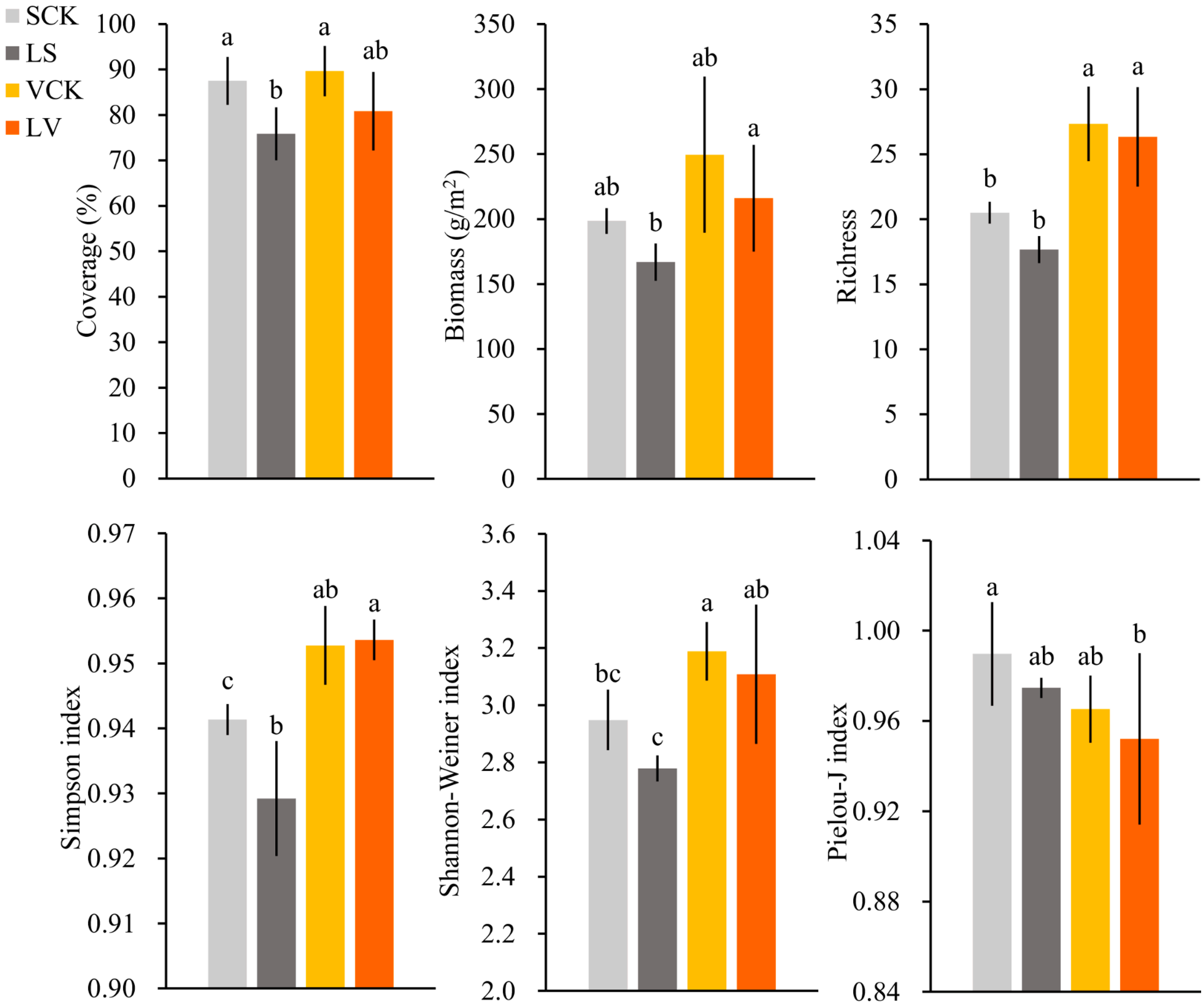

Fig. 2. Effects of different treatments on alpine meadow plant community on Qinghai-Tibet plateau (mean ± SE, n = 6). SCK is control treatment near the sampling points of *Ligularia sagitta*, LS is the treatment of *Ligularia sagitta*, VCK is control treatment near the sampling points of *Ligularia virgaurea*, LV is the treatment of *Ligularia virgaurea*. Different letters on the pillar indicate significant differences between treatments at 0.05 level, the same below.

related to latitude. (Supplementary Table 2).

3.3. Soil bacterial community

The soil bacterial community was assessed using y16S rRNA sequencing which revealed a total of 29 phyla; the 14 most abundant phyla are shown in Fig. 4a (Supplementary Table 3). Overall, both *L. virgaurea* and *L. sagitta* reduced the relative abundances of Proteobacteria, Bacteroidetes, Chloroflexi, Rokubacteria, and Planctomycetes in the soil, while they increased the relative abundances of Actinobacteria, Verrucomicrobia, Gemmatimonadetes, and Firmicutes. However, *L. virgaurea* increased the relative abundance of Acidobacteria, while *L. sagitta* decreased Acidobacteria and *L. virgaurea* significantly affected Toxobacteria, Verrucomicrobia, Bacteroidetes, and Firmicutes ($P < 0.05$), while *L. sagitta* only affected Firmicutes ($P < 0.05$). Overall, *L. virgaurea* had a greater impact on the soil bacterial community than *L. sagitta*. Moreover, differences in sampling area did not significantly affect soil microbial community structure (Fig. 4b, Supplementary Table 4).

Ligularia sagitta did not influence the α-diversity of the soil bacterial community, whereas *L. virgaurea* significantly ($P < 0.05$) increased the Chao1, observed-species, Shannon-Weiner index, and Faith-PD (Table 1, Supplementary Table 5).

The β-diversity of the soil microbes was analyzed using the Weighted UniFrac NMDS (Fig. 5, Stress = 0.0457). LS and SCK bacterial community structures were similar and were significantly different from LV and VCK quadrats (LV and VCK quadrats also differed significantly).

3.4. Correlation between relative biomass of L. virgaurea or L. sagitta, coverage and various other factors

There were more significant ($P < 0.05$) or extremely significant ($P < 0.01$) negative correlations between *L. sagitta* and plant community characteristics, while only relative biomass was significantly negatively correlated with *L. virgaurea* plant community coverage ($P < 0.01$; Fig. 6). There were moderate ($P < 0.05$) or strong ($P < 0.01$) positive correlations between *L. sagitta* and Actinobacteria and Firmicutes, while there were moderate ($P < 0.05$) or strong ($P < 0.01$) negative correlations between *L. sagitta* and Acidobacteria and Bacteroidetes. *Ligularia virgaurea* was correlated ($P < 0.05$) or strongly correlated ($P < 0.01$) with Firmicutes, Actinobacteria, Verrucomicrobia, Acidobacteria, and Gemmatimonadetes, but negatively correlated with Bacteroidetes ($P < 0.05$) and Chloroflexi ($P < 0.01$). There was a moderate ($P < 0.05$) or strong ($P < 0.01$) positive correlation between *L. virgaurea* and α-diversity of the soil microbial community but there was no correlation with *L. sagitta*. Both *L. virgaurea* and *L. sagitta* were moderately ($P < 0.05$) or strongly (P

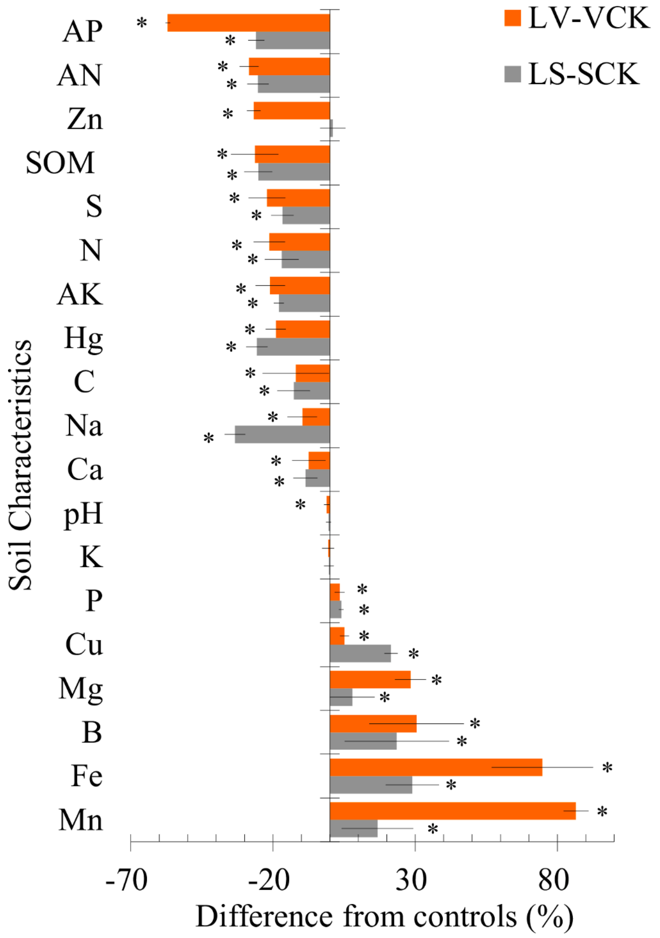

Fig. 3. Effects of different treatments on soil environment of alpine meadow on Qinghai-Tibet plateau (mean ± SE, n = 9). LV-VCK is the change from VCK to LV, and LS-SCK is the change from SCK to LS. C: Soil total Carbon; SOM: Soil organic matter; N: Soil total Nitrogen; AN: Soil available Nitrogen; P: Soil total phosphorus; AP: Soil available Phosphorus; K: Soil total Potassium; AK: Soil available Potassium; Ca: Soil Calcium; Na: Soil Sodium; Mg: Soil Magnesium; S: Soil Sulfur; Fe: Soil Iron; Cu: Soil Copper; Zn: Soil Zinc; B: Soil Boron; Mn: Soil Manganese; Hg: Soil Mercury; pH: Soil pH, the same below. * on the pillar indicate significant differences between LS and SCK or LV and VCK at 0.05 level.

< 0.01) positively correlated with Mg, Fe, Cu, B, Mn, and P and were moderately positively ($P < 0.05$) or strongly ($P < 0.01$) negatively correlated with SOM, N, AN, AP, AK, Ca, Na, S, Zn, and Hg. Total carbon was positively correlated ($P < 0.05$) with *L. sagitta*, while soil Zn and pH were moderately ($P < 0.05$) or strongly ($P < 0.01$) positively correlated with *L. virgaurea*.

3.5. *Soil microenvironment effects on the soil bacterial community*

The aggregated boosted tree analysis revealed that soil Mg content (49.9%) had the greatest influence on soil bacteria community structure (Fig. 7a), followed by P (11.9%), AP (7.3%), C (5.8%), and zinc (5.1%) contents. The soil Mg content (53.18%) had the greatest influence on soil bacterial diversity (Fig. 7b), followed by SOM (9.1%), AN (9.1%), B (4.8%), and K (3.8%).

Redundancy analysis was also conducted on the relative abundance of soil bacteria and environmental factors (Fig. 7c). Axis 1 and Axis 2 accounted for 43.2% and 11.7% of the variation, respectively. Gemmatimonadetes, Actinobacteria, and Acidobacteria were positively correlated with Mg, Zn, pH, Ca, Cu, Ap, and B. Chloroflexi, Proteobacteria, Planctomycetes, Patescibacteria, Firmicutes, and Bacteroidetes were positively correlated with S, N, and K. Planctomycete abundance was positively correlated with Fe, and Verrucomicrobia were positively correlated with Na.

4. Discussion

4.1. *Plant level competition*

Both *L. virgaurea* and *L. sagitta* have a negative influence on grassland plant communities (Wang et al., 2018) and their presence indicates alpine meadow degradation (Shi et al., 2018). Similarly, in our study *L. virgaurea* and *L. sagitta* negatively influenced plant coverage, biomass, abundance, and local plant community diversity, demonstrating an ecologically competitive advantage.

Ecological theory suggests that some plants gain an advantage over others by: (1) better utilizing ecological opportunities caused by changes in the external environment (niche opportunism), or (2) having a “competitive advantage’’ (Sol et al., 2012). From a niche opportunism perspective, *L. virgaurea* and *L. sagitta* were able to gain a competitive advantage due to the sharp increase in the number of livestock in the QTP area through increased pastoral farming in recent years (Zhang et al., 2005). *Ligularia virgaurea* and *L. sagitta* emit strong, pungent odors and are poisonous to livestock, consequently, they are avoided. Therefore, previously dominant species in alpine meadows including grasses and sedges have gradually lost their advantage due to excessive livestock grazing. However, from a competitive advantage perspective, *L. virgaurea* and *L. sagitta* also have significant advantages over other species. Wu et al. (2011) and Ma et al. (2007) showed that *L. virgaurea* and *L. sagitta* can affect the growth of other plants by releasing an allelopathic substance into the surrounding environment. Moreover, Reich et al. (2014) proposed that leaf traits are closely related to a plant’s ecological processes, and the relatively large leaf areas of *L. virgaurea* and *L. sagitta* in the alpine meadow plant community were conducive to high light interception, reducing photosynthesis in species of lower stature.

4.2. *Changes in the soil environment*

The dominance of *L. virgaurea* and *L. sagitta* also indicates significant changes in the soil microenvironment (Fig. 2, Supplementary Table 1). Plants need to obtain almost all of their essential elements from the soil, except for C, H, and O. Plants may experience growth retardation or reproductive abnormalities in the absence of essential elements, and plant community structure is also greatly affected by changes in soil nutrient composition (Peng et al., 2020). On the other hand, the changes *L. virgaurea* and *L. sagitta* caused in the environment would eventually, directly or indirectly, affect the growth of other plants in the community, changing the ecology of the soil/plant system.

The soil carbon sink is an important node in the carbon cycle and has a large influence on the soil microbial community. We found that a decline in soil carbon reserves was accompanied by the presence of *L. virgaurea* and *L. sagitta*, similar to the effects of *Spartina alterniflora* reported by Zeng et al. (2011). This may be due to lower carbon input caused by biomass decline.

Both *L. virgaurea* and *L. sagitta* reduced most key macronutrient concentrations except Mg and P, albeit marginally. Macronutrients are crucial for many essential plant functions, particularly N (Ogawa et al., 2016) and S (Ravilious and Jez, 2012) which are essential for organic compound synthesis. Similarly, P is important for plant respiration and energy metabolism. Sardans et al. (2017) showed that phosphorus content is usually high in the leaves of rapidly expanding plants, which indicates that they are obtaining more phosphorus from the soil, thus reducing soil P availability for the growth of other plants. Calcium is a key regulator of plant growth and development (Hepler, 2005), and soil calcium limitation may lead to the death of other young meristem regions of the plants. Mg is an important nutrient for photosynthesis (Chen

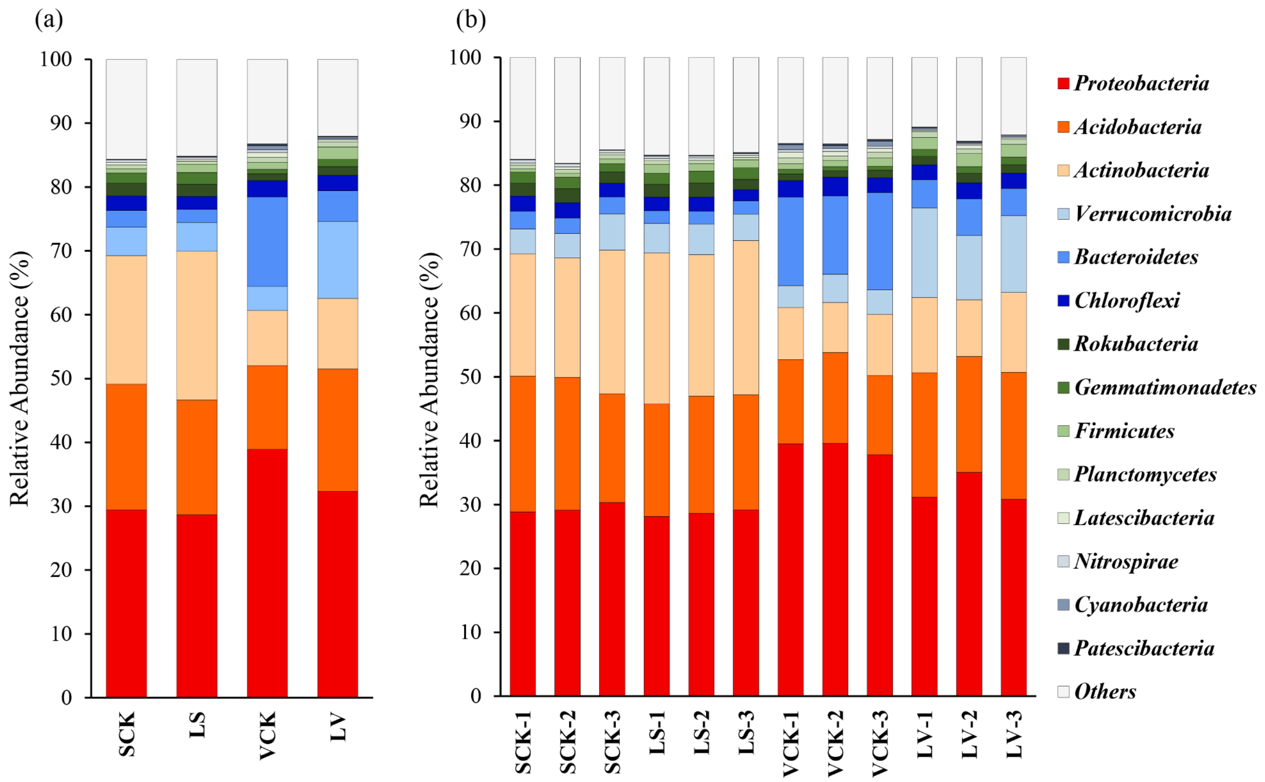

Fig. 4. Effects of different treatments on soil microbial community structure of alpine meadow on Qinghai-Tibet plateau (mean ± SE, n = 8 ~ 9). At the phylum level, Fig. 4 (a) intuitively shows the differences between different treatments at the phylum level, and Fig. 4 (b) shows the differences between different treatments and different sampling sites in more detail.

Table 1
Effects of different treatments on α-diversity of the soil bacterial community (Mean ± SE, n = 8 ~ 9).

	Chao 1	Shannon-Weiner	Observed-species	Faith-PD
SCK	2424.38 ± 124.51a	10.13 ± 0.07a	2194.84 ± 90.87a	116.24 ± 4.99bc
LS	2349.46 ± 193.88a	10.08 ± 0.13a	2134.22 ± 159.47a	112.92 ± 5.2c
VCK	1489.66 ± 128.75b	8.46 ± 0.15c	1453.86 ± 95.93b	122.83 ± 3.7b
LV	2252.42 ± 204.19a	9.82 ± 0.22b	2161.06 ± 170.95a	143.14 ± 11.21a

* indicates significant differences between LS and SCK or LV and VCK ($P = 0.05$).

et al., 2017), but soil Mg levels were not influenced by *L. virgaurea* and *L. sagitta*, although soil Mg was associated with decreased total community biomass (Fig. 6).

Soil micronutrients were also affected by *L. virgaurea* and *L. sagitta*. Potential implications include restricted carbon sequestration due to lower Na availability in C4 plants (Subbarao et al., 2003) and reduced zinc effects on chlorophyll synthesis (Broadley et al., 2007). Mélières et al. (2003) suggested that an appropriate mercury concentration is beneficial to plant growth, consequently, the reduction in soil mercury content we observed could potentially reduce plant growth. Conversely, *L. virgaurea* and *L. sagitta* increased soil Fe, B, Mn, and Cu contents, and these nutrients can be toxic to plants above critical concentrations (Antonovics et al., 1971). Soil pH was reduced by *L. virgaurea* and *L. sagitta* (Shi et al., 2018) and may result from changes in the levels of soil cations such as Ca and the allelochemicals released by *L. virgaurea* and *L. sagitta*.

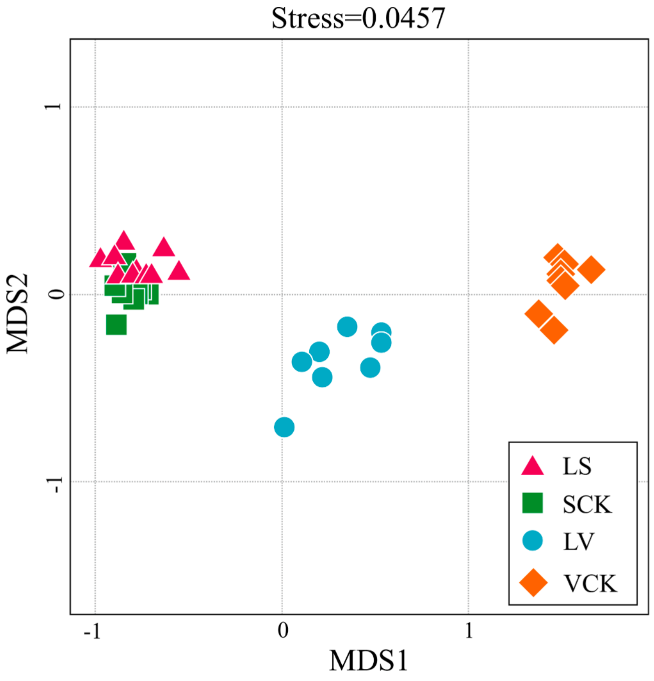

Fig. 5. Samples plotted in the plane of NMDS1 and NMDS2 from a principal coordinate analysis of the bacterial in the different treatment soil samples based on Weighted UniFrac for all samples.

4.3. Soil bacterial community

Soil microbes are an important ecosystem component (Zhong et al., 2010) and an important link between vegetation and soil interactions

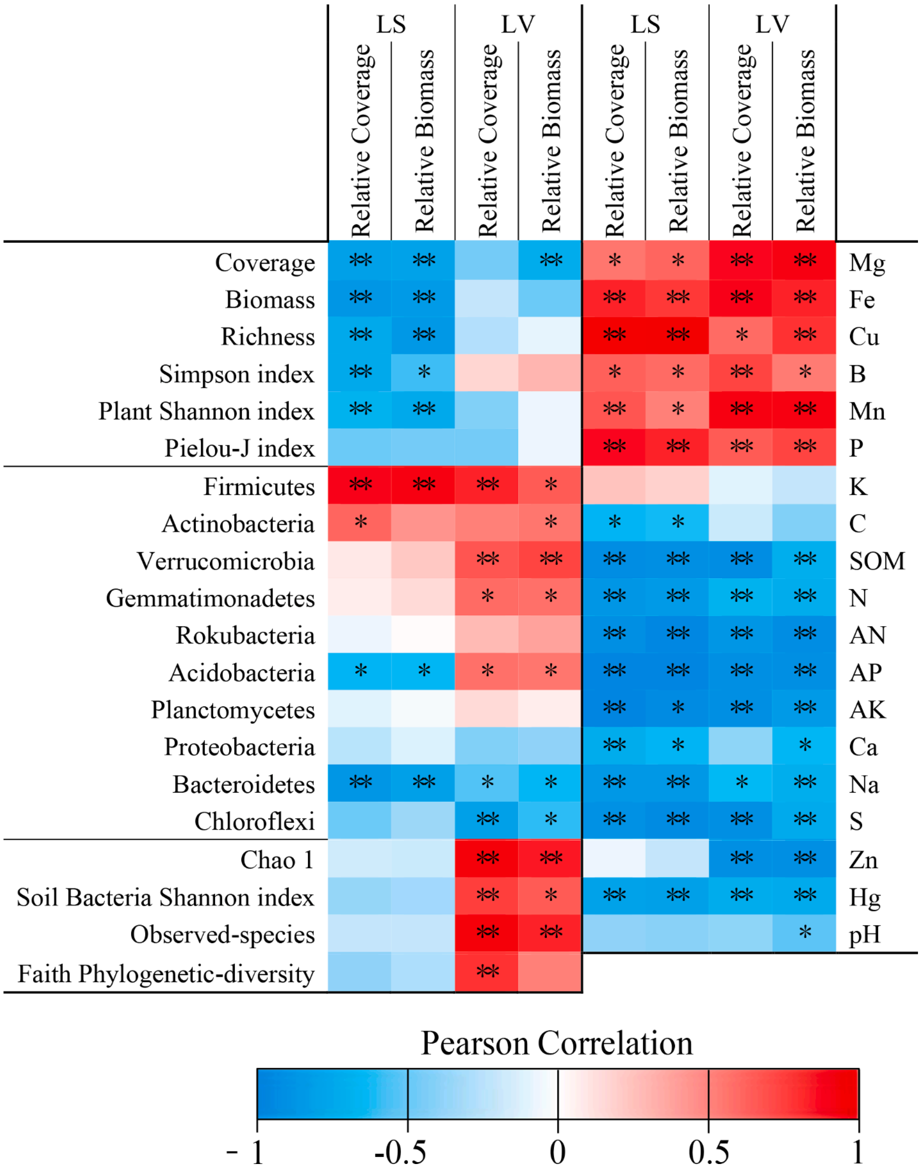

Fig. 6. Pearson correlation matrix between the relative coverage and relative biomass of vegetation in different quadrants, and plant community traits, abundance of different soil bacterial and soil nutrient levels. $^{**}P < 0.01$, $^{*}P < 0.05$.

(Valencia et al., 2018). Plants change soil bacterial communities by releasing energy and nutrients (Paterson et al., 2007), through litter decomposition (Koller et al., 2013), for example and bacterial communities can promote plant growth in various ways (Eisenhauer et al., 2012). This interaction has resulted in the co-evolution of plants and the soil microbial community (Eisenhauer et al., 2012; Lambers et al., 2009). Previous studies have confirmed that *L. virgaurea* (Wu et al., 2011) and *L. sagitta* (Ma et al., 2007) produce allelochemicals, especially in their root exudates. These allelochemicals may influence the characteristics of associated soil microbial communities, favoring those bacteria that are advantageous to *L. virgaurea* and *L. sagitta*.

Soil physical and chemical properties are also important factors affecting soil microbial community characteristics, but most studies have focused on the effects of macronutrients (Heuck et al., 2015). For example, Shi et al. (2018) suggested that *L. virgaurea* affected the soil microbial community by regulating soil C and N. In contrast, our knowledge of the effects of plants on soil micronutrients is relatively weak. Magnesium plays an important role as an essential component of chlorophyll present in some bacteria (Tamiaki et al., 2011), and is important for stabilizing and regulating bacterial ribosomes (Lee et al., 2019), membrane structure (Thomas and Rice, 2014), and reproduction in soil microbes. Our results showed that soil Mg levels had the greatest relative influence on soil microbial community structure and diversity, reaching 49.88% and 53.17%, respectively (Fig. 7a–b).

Ligularia virgaurea significantly changed the relative abundance of Toxobacteria, Verrucomicrobia, Bacteroidetes, and Firmicutes (Fig. 4), but Acidobacteria are the dominant bacteria in QTP alpine meadow soils (Ade et al., 2018). Madigan et al. (2008) found that Acidobacteria were affected by pH, SOM, and other factors, consistent with our results (Fig. 7c). Verrucomicrobia was ubiquitous in the soil and its relative abundance was increased significantly by *L. virgaurea*, which may be due to its lower nutrient requirements (Bergmann et al., 2011). Environmental Bacteroidetes are considered to be specialized in the degradation of complex organic matter, especially polysaccharides (Wolińska et al., 2017), and so the decline in SOM that we observed may explain their decrease in relative abundance. Similarly, *L. sagitta* also significantly increased the relative abundance of Firmicutes in the soil, which may be due to their greater competitiveness in a stressful environment (Filippidou et al., 2016).

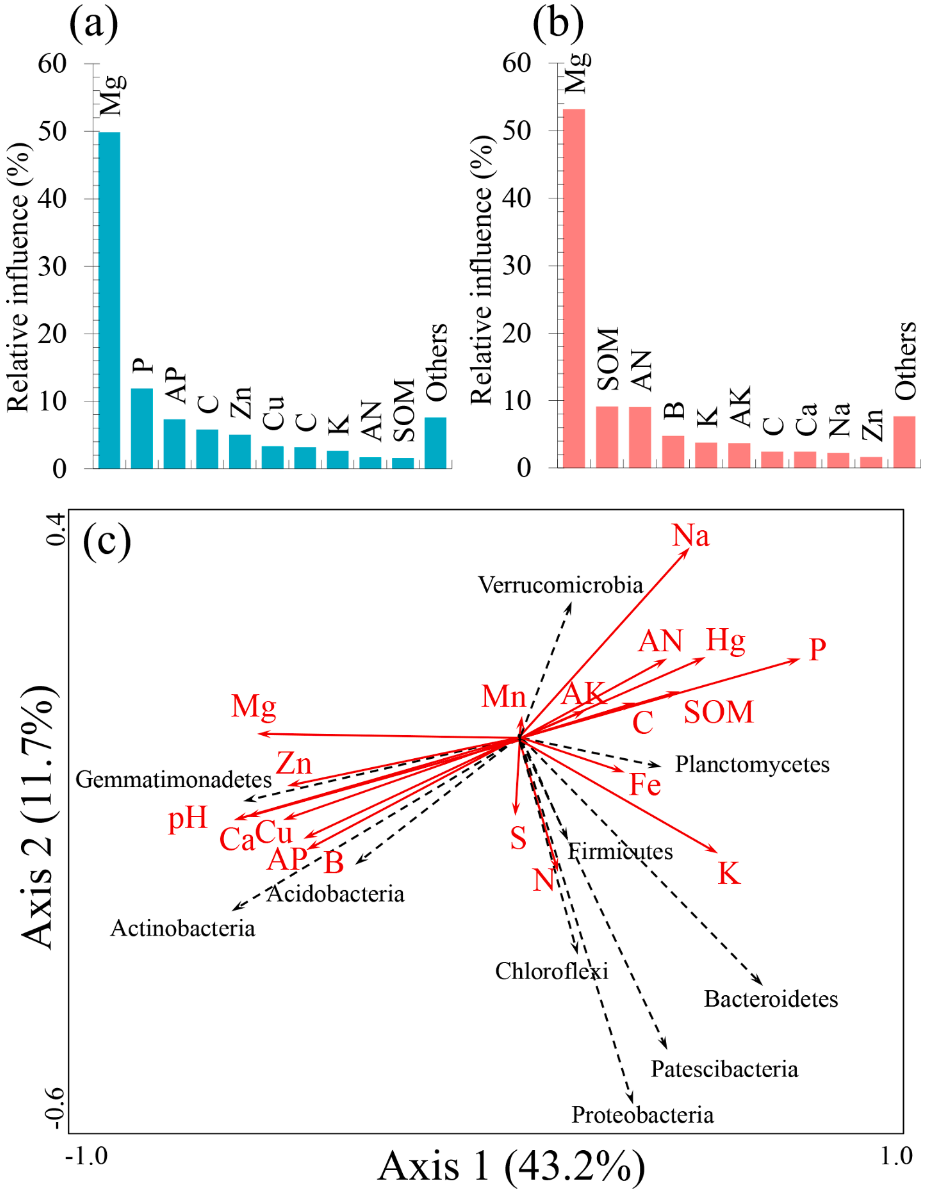

Fig. 7. Relative influence (%) for the soil environmental factors to soil bacterial composition (a) or Soil bacterial diversity (b), and redundancy analysis (RDA) for soil physicochemical properties and 311 soil bacterial phylum (c).

4.4. Ecological effects

The significant increase in *L. virgaurea* and *L. sagitta* biomass and cover indicated that they are highly competitive species in QTP alpine meadows. Their increase in dominance may be due to their toxicity to livestock during overgrazing (Zhang et al., 2020), and strong environmental adaptability (Colautti and Barrett, 2013). At the same time, *L. virgaurea* and *L. sagitta* release allelopathic substances (Wu et al., 2011), inducing changes in the plant and soil microbial communities and influencing soil nutrient levels, creating a positive ecological feedback loop. The way in which *L. virgaurea* and *L. sagitta* regulate allelochemical effects on soil microorganisms is still unknown but will be the focus of future research.

Finally, our results revealed that almost all soil indicators measured in this study, were significantly affected. This could mean that the impact of grassland degradation is so pervasive that it may be almost irreversible using existing technology.

5. Conclusions

Ligularia virgaurea and *L. sagitta* significantly changed QTP alpine plant communities by reducing biomass, vegetation coverage, abundance, and biodiversity, and the effect of *L. sagitta* on the plant community was stronger than that of *L. virgaurea*. *L. virgaurea* and *L. sagitta* dominance indicated significant changes in soil nutrients, lowering soil C, SOM, N, AN, AP, AK, Ca, Na, S, Hg, and pH but increasing P, Mg, Fe, Cu, B, and Mn. The soil microbial communities were also affected by the presence of both species; the relative abundance of Proteobacteria, Bacteroidetes, Chloroflexi, Rokubacteria, and Planctomycetes in the soil was reduced whereas the relative abundance of Actinobacteria, Verrucomicrobia, Gemmatimonadetes, and Firmicutes was increased. The dominance of *L. virgaurea* indicates significant increase in α-diversity of the soil bacterial community. Aggregated boosted trees analysis revealed that soil Mg levels had the greatest relative influence on the structure and diversity of the soil microbial community.

CRediT authorship contribution statement

Luji Ade: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft. **James P. Millner:** Writing – review & editing. **Fujiang Hou:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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.ecolind.2021.108183.

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