 doi:10.20944/preprints202109.0128.v1

Title: Soil Microbial Differences under Unpalatable *Stellera Chamaejasme* and Neighboring Palatable *Elymus Nutans* in Alpine Meadows

Names of authors: Jianguo Ma*

Affiliation of authors: *College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China*

Address information: *State Key Laboratory of Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China*

***Correspondence**

Jianguo Ma, *College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China.*

Email: majg18@lzu.edu.cn

 doi:10.20944/preprints202109.0128.v1

Highlights

- Distinct bacteria and fungi were in *Stellera* and *Elymus* soils.
- Soil properties under *Stellera* and *Elymus* were different
- Significantly greater Actinobacteria and Verrucomicrobia bacteria were in *Stellera* soils
- Significantly greater Basidiomycota and Glomeromycota fungi were in *Stellera* soils

ABSTRACT

Stellera chamaejasme L. is a fast-spreading unpalatable poisonous plant that grows in the alpine grasslands of the Qinghai-Tibetan Plateau (QTP). The impacts of unpalatable plant species spread on animal health and plant community have been well studied, but studies into their effects on belowground organisms and processes are rare. We carried out a soil metabarcoding study using Illumina MiSeq sequencing to investigate whether the soil bacteria and fungi communities of *Stellera* are different to the soil microbiome of neighboring palatable grass *Elymus* nutans Griseb. Total carbon and nitrogen, the ratio of carbon to nitrogen, ammonium nitrogen, and microbial biomass carbon were all significantly greater in *Stellera* soil compared to *Elymus* soil, while no significant differences were observed for gravimetric soil moisture, pH or nitrate nitrogen. There were no significant differences in bacterial and fungal abundance between *Stellera* and *Elymus* soil. The bacterial species richness was significantly lower

 doi:10.20944/preprints202109.0128.v1

in *Stellera* soil but no significant difference was observed for fungal species richness. The beta diversity and community composition of bacteria and fungi were markedly different between soils. The presence of bacterial phyla Actinobacteria and Verrucomicrobia, and fungal phyla, Basidiomycota and Glomeromycota, were significantly greater under *Stellera* soil. This study demonstrated that the spread of undesirable unpalatable plants can potentially disrupt existing plant-soil-microbe associations with potential consequences for grassland soil biodiversity and ecosystem functioning.

Key words**:** Poisonous plants; soil microbial communities; *Stellera chamaejasme*; *Elymus nutans*

INTRODUCTION

A greater proportion of unpalatable poisonous plants in the vegetation can have a profound impact on both livestock health and grassland productivity (Zhang *et al*., 2017). The impacts of unpalatable plant spread on animal health (Holechek, 2002), plant community characteristics and grassland degradation have been well studied and defined (Gao *et al*., 2019; Wu *et al*., 2015), but studies on their effects on belowground organisms and processes are rare and, hence, poorly understood (Sanon *et al*., 2009). Such studies are important because plant species can have a significant influence on below ground processes such as soil carbon sequestration (Lange *et al*., 2015), soil nitrogen transformations and soil physical and chemical properties (Groenigen *et al*.,

 doi:10.20944/preprints202109.0128.v1

2015; Schlatter *et al*., 2015; Gould *et al*., 2016).

In recent years, the rapid spread of unpalatable plants into alpine grasslands in the Qinghai-Tibetan Plateau (QTP) in China has resulted in a serious threat to plant community diversity and productivity (Shi, 1997; Wang *et al*., 2015) The spread of unpalatable plants in the alpine grasslands of the QTP is thought to be due to ongoing grassland degradation triggered by climate change (Li *et al*., 2014; Ganjurjav *et al*., 2016), frequent freeze and thaw cycles and human disturbances such as overgrazing by domestic animals (Shang and Long, 2007; Valkó *et al*., 2016). In fact, some reports suggest about 90% of grassland on the QTP has been degraded, and 35% has been seriously degraded into a so-called "black soil beach" covered only by unpalatable poisonous plants (Dong *et al*., 2012). Although they are associated with the degradation of grasslands, it should be noted that the ability of most unpalatable poisonous plants to endure adverse growth environments is considered beneficial for restoration of degraded grassland because it can prevent desertification and complete collapse of the grassland ecological environment (Callaway *et al*., 2005).

Stellera chamaejasme (henceforward *Stellera*), known as Duanchangcao in Chinese, is one of the common unpalatable poisonous plants that is wide spreading in alpine grasslands in the QTP (Zhao *et al*., 2010). It is a perennial herbaceous plant belonging to the Thymelaeaceae family that produces toxins in both above ground herbage and roots (Shi, 1997). The plant releases an odor that deters livestock, resulting in the rapid replacement of palatable species (Liu *et al*., 2004). It is now recognized that *Stellera* spread into the natural grasslands of the QTP is a serious threat to grassland

 doi:10.20944/preprints202109.0128.v1

productivity, on the other hand, it's reported can provide benefits to the environment by *Stellera* expansion as well as, such as the ability on reducing N_2O emissions potentially (Ma *et al*., 2020) and improving soil nutrient levels (Sun *et al*., 2009). Hence, it was recognized widely as a major challenge for conservation and ecological sustainability (Liu *et al*., 2004; Xing *et al*., 2002).

Sun *et al*. (2009) examined changes in soil nutrients, carbon and nitrogen pool sizes and turnover rates caused by the spread of *Stellera* under two topographic habitats in an alpine meadow ecosystem on the Tibetan Plateau of China. Their study revealed that *Stellera* patches create islands of fertility (i.e. greater soil nutrient availability), which promotes its establishment and spread within the alpine meadows. In addition to higher total nutrient pools, soil microbial biomass, the cycling of carbon and nitrogen, and the availability of nitrogen were all higher within *Stellera* patches. As the composition, diversity and function of soil microbial communities play a significant role in soil biogeochemical processes (Miransari, 2013), we hypothesize that the root zone of *Stellera* plant patches may be colonized by a different microbial community to that of neighboring palatable plant species. Therefore, we carried out a soil metabarcoding study using Illumina MiSeq sequencing analysis to investigate the soil bacteria and fungi in the root zone soils of *Stellera*. To establish differences between *Stellaria* and palatable species we compared the microbiome of *Stellera* soil to that of the palatable grass *Elymus nutans* (henceforward *Elymus*), a dominant perennial forage grass that grows naturally in the alpine meadow grasslands of the QTP.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

MATERIALS AND METHODS

Study area

Soil sampling of *Stellera* and *Elymus* patches was carried out in an alpine meadow grassland at the Azi Research station in Maqu county (33° 59' N, 102°00' E, 3500 m a.s.l.) on the eastern QTP. Mean annual precipitation at the site is 620 mm and mean annual temperature is 1.2 ℃. The grassland is generally grazed year-round by yak. The soil type of the grassland is Mattic Cryic Cambisols (alpine meadow soil, Cambisols in FAO/UNESCO taxonomy). The dominant palatable plants in the vegetation are *Elymus*, *Kobresia graminifolia* C. B. Clarke, *Poa annua* L., *Koeleria macrantha* Schultes and *Poa poophagorum* Bor. The dominant unpalatable poisonous plants at the site include *Stellera*, *Ligularia virgaurea* (Maxim.) and *Thermopsis lanceolate* R. Br. (Xie *et al*., 2014; Sun, 2015).

Soil sampling

During the peak plant growth period in August 2018, *Stellera* and *Elymus* patches (0.5 m x 0.5 m) were identified in 6 areas (each area approximately 50 m x 50 m) of the grassland. The selected areas were approximately 20-50 m apart, and the coverage of target plant was over 95% on each patch of targeted plant. Soil cores were collected from randomly identified *Stellera* and *Elymus* patches in each area. In each patch, after removing above ground herbage, a root zone soil sample was taken from the middle of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

the patch using a 10 cm diameter soil corer to a depth of 10 cm. In total there were 12 soil samples (2 plant species x 6 replicates). After removing roots, litter, stones and other debris, the fresh soil sample was passed through a 2 mm sieve. The sample was then divided into three sub samples and transfer to lab within 24 h for analysis, for soil moisture determination, for soil chemical analysis (stored at 4 ℃) and for the soil microbial analysis (stored at -20 ℃). The soil microbial analysis was carried out using only 3 replicate samples due to minimize sequencing costs.

Soil physiochemical analysis

Gravimetric soil moisture content was determined by drying to a constant weight at 105 ℃ in an electrothermal blowing oven (DHG - 9240 - a, Shanghai Jing Macro, Shanghai, China). Soil pH was determined using a slurry of soil-to-water ratio 1:5 with a pH meter (PE-10, Sartorious, Germany). Total soil carbon (TC) and total soil nitrogen (TN) was determined using an elemental analyzer (Elementar Vario EL/micro cube, Hanau, Germany). Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were measured using the soil extracts collected by mixing 5 g soil with 50 mL 2 M KCl and shaking for 60 minutes. The extracts were filtered with 0.45 μm filter and the NH_4^+-N and NO_3^--N of the extracts were analyzed colorimetrically as described by He *et al*. (2017) using an ultraviolet spectrophotometer (Cary 6 UV-Vis, Agilent Technologies, USA). Soil microbial biomass carbon (MBC) was determined according to the procedure of Vance *et al*. (1987).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Soil microbial community analysis

Soil DNA extraction

Total soil genome DNA from three replicate fresh soil samples was extracted from 0.5 g soil using a FastDNA ® spin kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. Cell lysis was performed by vigorous shaking in a Bioprep-24 Homogenizer (MP Biomedicals, Ohio, USA) at an intensity of 6 m $\cdot s^{-1}$ for 45s. The extracted DNA was dissolved in 100 µL of the DNA elution solution. DNA quantity and purity were determined using Nanodrop® ND-1000 UV-visible spectrophotometer (Nanodrop technologies, Delaware, USA) and stored in a -20°C freezer until required for further analysis.

Microbial community abundance analysis

The absolute abundance of the microbial community was measured by quantitative real-time polymerase chain reaction (qPCR) using a CFX96TM Thermal Cycler (Bio-Rad laboratories, Hercules, USA) targeting the 16S rDNA gene for bacteria and the 18S rDNA gene for fungi. Quantification was performed in triplicate using a 20 µL reaction mixture with TB Green premix Ex Taq (TaKaRa Biotech, Dalian, China), Forward and Reverse primers were 515F and 907R targeted 16S, FF390 and FR1 targeted 18S respectively, and sterile ultra-pure water. The details of primers and thermal cycle condition are described in Table 1. The standards were generated by 10-fold dilutions of plasmids containing targeted gene fragments.

 doi:10.20944/preprints202109.0128.v1

(Table 1)

Microbial composition analysis

The Illumina MiSeq sequencing libraries for bacteria and fungi were prepared by PCR (Table 1). The PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN, USA) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step paired-end 2x300 bp sequencing was performed using the Illumina MiSeq platform with MiSeq Reagent Kit v3 at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was employed to process the sequencing data (Caporaso *et al*., 2009). The sequencing processing was as described in Kang et al. (2021). High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity using UCLUST software (Edgar, 2010). OTUs containing less than 0.001% of total sequences across all samples were discarded.

The rarefied OTUs matrix at a consistent sequencing depth was used to identify composition and diversity. The relative abundance distribution of Phylum, Class, Order, Family and Genus in each sample was used to describe microbial community composition. Alpha diversity (diversity within each sample) of soil bacterial and fungal communities was tested using the Chao 1 and ACE (Richness) and Shannon and Simpson diversity indices (Evenness) using QIIME software (Kuczynski *et al*., 2012). Beta diversity (dissimilarity of community structure between samples) of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

communities and their relationship to soil properties were assessed by principal component analysis using a weighted UniFrac distance matrix in R software (R Core Team, 2016).

Statistical analysis

Mann-Whitney U test was used to compare the relative abundance of bacterial and fungal phyla, genera and the soil parameters in *Stellera* and *Elymus* soils, $P \leqslant 0.05$ was recognized sstatistically significant. The difference between microbial communities between *Stellera* and *Elymus* soils was statistically tested by ANOSIM (analysis of similarities) at OUT level with 999 permutations using QIIME software.

Nucleotide sequence accession numbers

The nucleotide sequences from this study have been deposited in the NCBI GenBank under the SRA accession PRJNA589249.

RESULTS

Soil physicochemical properties

There were no significant differences in gravimetric soil moisture, pH and NO_3^--

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

N, between *Stellera* and *Elymus* soils. The TC, TN, C/N, NH_4^+-N and MBC were significantly higher ($P < 0.05$) in *Stellera* soil compared to *Elymus* soil (Table 2).

Soil microbial community abundance

There were no significant differences in bacterial and fungal abundance between *Stellera* and *Elymus* soil (Table 2)

(Table 2)

Soil microbial community diversity and composition

Soil bacterial community diversity and composition of *Stellera* and *Elymus* soils were assessed by a total of 125, 517 high quality sequencing reads with an average read of 20920 per replicate sample. A total of 9694 bacterial operational taxonomic units (OTUs) were detected in this study. Of these, 3584 were shared by *Stellera* and *Elymus* soils; 3288 were unique to *Elymus* soil and 2822 were unique to *Stellera* soil.

Soil fungal community diversity and composition of *Stellera* and *Elymus* soils were assessed by a total of 234596 high quality sequencing reads with an average read of 39099 per replicate sample. A total of 2609 fungal OTUs were detected in this study. Of these, 625 were shared by *Stellera* and *Elymus* soils; 1122 were unique to *Elymus* soil and 862 were unique to *Stellera* soil.

The rarefaction analysis of richness index; Chao1 (Fig. 1), showed a saturation of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

the rarefaction curves for bacteria and fungi indicating the sequencing depth in our study was sufficient to capture the characteristics of the soil microbial communities. Both rarefaction curves, for the bacteria and fungi, tended to be consistently lower for *Stellera* soil indicating a lower diversity compared to *Elymus* soil. Differences in alpha diversity indices - species richness (Chao1 and ACE) and evenness (Simpson and Shannon-wiener) - are shown in Table 3. Bacteria richness were significantly lower ($P<0.05$) in *Stellera* compared to *Elymus* soils but no significant difference was observed for fungal species richness. There were no significant differences between the two soils for Simpson and Shannon diversity indices for bacteria or fungi.

(Fig. 1)

(Table 3)

Beta diversity, explored using PCA analysis, clearly indicated dissimilar bacterial and fungal communities for *Stellera* and *Elymus* soils (Fig. 2). The first and second axis of PCA ordination plots explained the variation of 76.9 and 13.9 % for bacteria (Fig. 2a) and 96.92 and 1.27 % for fungi (Fig. 2b) respectively. The PCA analysis also showed that apart from NO_3^--N, all other measured soil properties were positively related to the *Stellera* bacterial and fungal communities.

(Fig. 2)

The top 10 dominant bacterial and fungal phyla found in *Stellera* and *Elymus* soils are shown in Table 4 and 5. The prominent bacterial and fungal phyla in both *Stellera* and *Elymus* soils were similar (Table 4, 5). The most abundant bacterial phyla in *Stellera* soil was Actinobacteria (46%) while both Actinobacteria (29%) and

 doi:10.20944/preprints202109.0128.v1

Proteobacteria (29%) were dominant in *Elymus* soil. The relative abundance of bacterial phyla Actinobacteria and Verrucomicrobia were significantly higher in *Stellera* soil while Proteobacteria, Bacteroidetes, and Firmicutes were significantly lower in *Stellera* soil (Table 4). The relative abundance of bacterial phyla Acidobacteria, Chloroflexi, Planctomycetes, Gemmatimonadetes and Nitrospirae were not significantly different between the two soils (Table 4).

(Table 4)

Ascomycota was the dominant fungal phylum in both soils. The relative abundance of the fungal phyla Basidiomycota and Glomeromycota were significantly higher in *Stellera* soil while the relative abundance of the fungal phyla Mortierellomycota, Rozellomycota, Chytridiomycota, Olpidiomycota and Blastocladiomycota and GS19 were significantly lower in *Stellera* soil. The relative abundance of Ascomycota and Mucoromycota were not significantly different between the two soils (Table 5).

(Table 5)

At lower taxonomic levels, we examined differences among the most abundant 20 bacterial and fungal genera (Fig. 3) identified in *Stellera* and *Elymus* soils. In Fig. 3, the genera were ordered by their abundance in *Stellera* soil. The bacterial genera *Solirubrobacter*, *RB41* and *Peseudonocardia* were the most abundant in *Stellera* soil and their abundance was significantly greater than in *Elymus* soil. The least abundant bacterial genera (among the top 20 genera) in *Stellera* soil (*Brevundimonas*, *Chryseobacterium* and *Flavobacterium*) were more abundant in *Elymus* soil (Fig. 3a).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Among the top 20 fungal genera, the abundance of 15 genera was significantly different between the two soils, 8 genera had significantly higher and 7 significantly lower abundance in *Stellera* soil compared to *Elymus* soil (Fig. 3b). The fungal genera *Archaeorhizomyces*, *Melanospora* and *Didymella* were the most abundant in *Stellera* soil and their abundance was significantly higher than in *Elymus* soil. The least abundant bacterial genera (among the top 20 genera) in *Stellera* soil (*Schizothecium*, *Plectosphaerella* and *Wardomyces*) were significantly more abundant in *Elymus* soil (Fig. 3b). The fungal genus *Microglossum* was only found in *Stellera* soil.

(Fig. 3)

DISCUSSION

Our study contributes evidence towards demonstrating that soil microbial community composition is closely associated with the identity of associated plant species (Zheng *et al*., 2016; DeSouza *et al*., 2017; Haruna *et al*., 2018). More importantly, our study demonstrated the soil bacterial and fungal community associated with *Stellera* and *Elymus* were different. The differences in soil properties (Table 2 and Fig. 2), litter decomposition (Hobbie, 2015), together with different quantity and quality of root exudations (Sun *et al*., 2009; Guo *et al*., 2019; Brunel *et al*., 2019) are likely the reasons for the different soil microbial community found in *Stellera* patches. The alpha diversity indices and the rarefaction analysis (Fig. 1) indicated microbial species richness was lower under *Stellera* patches, perhaps due to the biological inhibition of

 doi:10.20944/preprints202109.0128.v1

certain microbial groups by the toxic carbon compounds released by *Stellera* root exudates (Guo *et al*., 2015). In our study only 37% and 24% of bacterial and fungal OTUs respectively, were shared between *Stellera* and *Elymus* soils, while this value may rise slightly as the sample size increases. The dissimilarity of the soil microbial community composition was further highlighted by the PCA analysis (Fig. 2).

Our results are consistent with Sun *et al*., (2009) who found significantly different soil properties under *Stellera* patches compared to cooccurring species in an alpine grassland in the eastern Tibetan plateau. As regards our hypotheses, we found total soil carbon, total soil nitrogen, the ratio of carbon to nitrogen, soil microbial biomass and the diversity and identity of the soil microbial community were significantly different underneath *Stellera* patches compared to adjacent *Elymus* patches. As pointed out by Sun *et al*. (2009), the soil carbon, nitrogen and microbial biomass differences may have been driven by the higher above ground litter production and litter quality of *Stellera*. Other studies have also shown that high aboveground litter production can contribute to high nitrogen and carbon, and higher turnover rate of soil nutrients (Berendse *et al*., 1987; Koukoura *et al*., 2003). *Stellera* can transfer soil nutrients to deeper soil layers through its long and widely distributed root system resulting in a "fertility island" effect; this results in improved soil nutrient availability and gives *Stellera* a competitive advantage over neighboring plant species leading to expansion of *Stellera* in the grassland community (Sun *et al*., 2009).

The bacterial community of *Stellera* was dominated by members of the Actinobacteria (47%) phylum (Table 4). Actinobacteria was also dominant in *Elymus*

 doi:10.20944/preprints202109.0128.v1

but its relative abundance was 18% lower compared to *Stellera* soil. A study using 28 soils from a broad range of ecosystems in North America by Ramirez *et al*. (2012) found a positive relationship between Actinobacteria and nitrogen amendment, suggesting this was a major reason for the greater abundance of Actinobacteria. The ability to produce antibacterial substances by Actinobacteria may have contributed to the lower microbial diversity in *Stellera* soil (Miao and Davis, 2010). Jin *et al*. (2018) found Actinobacteria and Proteobacteria were dominant in rhizosphere and root communities in *Stellera* plants collected along an altitude gradient of 3664 m to 4741 m in the Tibetan Plateau. However, we found both Proteobacteria and Bacteroidetes were significantly lower in *Stellera* soil. This finding was surprising as both these phyla are widely considered to have a copiotroph life strategy, preferentially consuming labile soil organic carbon and having high nutritional requirements and growth rates (Fierer *et al*., 2007). Given the high nutrient availability, organic carbon and nitrogen levels and greater net mineralization in *Stellera* soil (Sun *et al*., 2009), we would expect these two phyla to be more abundant in *Stellera* soil compared to *Elymus* soils.

It is well recognized that the abundance of the Phylum Acidobacteria is pH dependent (Sait *et al*., 2006), and in our study, soil pH was not significantly different between the two soils and the relative abundance of Acidobacteria between the two soils was also not significantly different (Table 2, 5). Acidobacteria are ubiquitous in many environments suggesting they have a wide tolerance for different conditions (Eichorst *et al*., 2018).

Soil fungi contribute to multiple biological functions including carbon and

 doi:10.20944/preprints202109.0128.v1

nitrogen cycles and soil health (Frąc *et al*., 2018). Plant community diversity in grasslands can have significant influence on the soil fungal community (Chen *et al*., 2017; Li *et al*., 2018). In our study we found overall fungal community composition was different between *Stellera* and *Elymus* soils (Fig. 3b). The fungal phylum Ascomycota was the most dominant (average 78%) in both soils. This is consistent with the study of Chen *et al*. (2017) who found Ascomycota dominant in 312 soil samples collected from 52 sites of meadow steppe, typical steppe, desert steppe and deserts of Northern China. At the phylum level, the relative abundance of Ascomycota was not significantly different between two soils but at the lower taxonomic level a number of Ascomycota genera had significantly different abundance between the soils as did a number of less dominant fungal phyla (Table 5). Basidiomycota, which are generally considered to be important decomposers (Šťursová *et al*., 2012), were more abundant in *Stellera* soil which is consistent with the greater input of litter in *Stellera* patches. The phylum Glomeromycota, of which most members are arbuscular mycorrhizal fungi (AMF), was more abundant in *Stellera* soil. While both *Elymus* and *Stellera* plants form AMF associations (Wang *et al*., 2019), greater resource availability might have resulted in increased AMF associations in *Stellera* soil. In our study Archaeorhizomyces was the most abundant fungal genera in both soils with significantly greater numbers in *Stellera* soil. The plant growth promoting capability of Archaeorhizomyces may play a role in the competitive success of *Stellera* in this grassland community (Zhang *et al*., 2018).

IMPLICATIONS

 doi:10.20944/preprints202109.0128.v1

Our results demonstrate that changes in the botanical composition of natural grassland can potentially change existing plant-soil-microbe associations with potential consequences for ecosystem functioning (Ma *et al*., 2020). This study also provides important information on controls on soil biodiversity in natural grasslands. Wolf and Klironomos (2005) argued that land managers should consider incorporating information on below ground traits such as soil biota in the restoration process following plant invasions, and suggest monitoring the structure and function of the soil microbial community in relation to a benchmark community, such as the community that existed before exotic plant invasion, or nearby communities where invasion has not occurred. Here we provide the information necessary to develop such an approach for one of the fastly spreading undesirable plants in alpine grasslands in the QTP that threatens grazing land's productivity. In this study we focused only on the soil microbial impact during the peak growth period of plants, but it is important to explore temporal responses in future studies.

CONCLUSIONS

Characterization of the soil microbes of *Stellera* and *Elymus* root zone soils in the QTP revealed distinct differences in the composition and diversity of bacteria and fungi. These differences in microbial communities likely arise from both the direct and indirect effects of the two plant species. This study provides direct evidence of the

 doi:10.20944/preprints202109.0128.v1

consequences of undesirable plant spread in grasslands on soil biodiversity.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

This study was supported by the Project of the Second Tibetan Plateau Scientific Expedition (2019QZKK0302), the National Natural Science Foundation of China (31672472), and the program for Changjiang Scholars and Innovative Research Team in University (IRT_17R50).

DATA AVAILABILITY STATEMENT

If more details are needed on the data, please contact the first author to obtain at majg18@lzu.edu.cn.

REFERENCES

AanderudZ T, Saurey S, Ball B A, Wall D H, Barrett J E, Muscarella M E, Griffin N A, Virginia R A, Barberán A, Adams B J. 2018. Stoichiometric shifts in soil C: N: P promote bacterial taxa dominance, maintain biodiversity, and deconstruct

community assemblages. *Front. Microbiol*. **9**: 1401. doi: 10.3389/fmicb.2018.01401

Altschul S F, Madden T L, Schäffer A A, Zhang J, Zhang Z, Miller W, Lipman D J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic. Acids. Res*. **25**, 3389-3402. doi: 10.1093/nar/25.17.3389

Berendse F, Berg B, Bosatta E. 1987. The effect of lignin and nitrogen on the decomposition of litter in nutrient-poor ecosystems: a theoretical approach. *Can. J. Bot*. **65**: 1116-1120. doi: 10.1139/b88-063

Brunel C, Beifen Y, Pouteau R, Li J, Kleunen M V. 2019. Responses of rhizospheric microbial communities of native and alien plant species to *cuscuta parasitism*. *Microb. Ecol*. **5**: doi: 10.1007/s00248-019-01438-z

Callaway R M, Kikodze D, Chiboshvili M, Khetsuriani L. 2005. Unpalatable plants protect neighbors from grazing and increase plant community diversity. *Ecology*. **86**: 1856-1862. doi: 10.1890/04-0784

Caporaso J G, Bittinger K, Bushman F D, DeSantis T Z, Andersen G L, Knight R. 2009. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*. **26**: 266-267. doi: 10.1093/bioinformatics/btp636

Chen Y L, Xu T L, Veresoglou S D, Hu H W, Hao Z P, Hu, Y J, Liu L, Deng Y, Riling M C, Chen B D. 2017. Plant diversity represents the prevalent determinant of soil fungal community structure across temperate grasslands in northern China. *Soil Biol. Biochem*. **110**: 12-21. doi: 10.1016/j.soilbio.2017.02.015

Chu H Y, Neufeld J D, Walker V K, Grogan P. 2011. The influence of vegetation type on the dominant soil bacteria, archaea, and fungi in a low Arctic tundra landscape. *Soil Sci. Soc. Am. J*. **75**: 1756-1765. doi: 10.2136/sssaj2011.0057

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., and Andersen, G.L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol*. **72**, 5069-5072. doi: 10.1128/AEM.03006-05

DeSouza, T.A.F., DeAndrade, L.A., Freitas, H., and Aline, D.S.S. (2017). Biological invasion influences the outcome of plant-soil feedback in the invasive plant species from the brazilian semi-arid. *Microb. Ecol*. **76**, 102-112. doi: 10.1007/s00248-017-0999-6

Dong, S.K., Wen, L., Li, Y.Y., Wang, X.X., Zhu, L., and Li, X.Y. (2012). Soil-quality effects of grassland degradation and restoration on the Qinghai-Tibetan Plateau. *Soil Sci. Soc. Am. J*. **76**, 2256-2264. doi: 10.2136/sssaj2012.0092

Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*. **26**, 2460-2461. doi: 10.1093/bioinformatics/btq461

Eichorst, S.A., Trojan, D., Roux, S., Herbold, C., Rattei, T., and Woebken, D. (2018). Genomic insights into the *Acidobacteria* reveal strategies for their success in terrestrial environments. *Environ. Microbiol*. **20**, 1041-1063. doi: 10.1111/1462-2920.14043

Fierer, N., Bradford, M.A., and Jackson, R.B. (2007). Toward an ecological classification of soil bacteria. Ecology. **88**, 1354-1364. doi: 10.1890/05-1839

Frąc, M., Hannula, S.E., Bełka, M., and Jędryczka, M., 2018. Fungal biodiversity and their role in soil health. *Front. Microbiol*. **9**, 707. doi: 10.3389/fmicb.2018.00707

Ganjurjav, H., Gao, Q., Gornish, E.S., Schwartz, M.W., Liang, Y., Cao, X.J., Zhang, Y., Li, W.H., Wan, Y.F., Li, Y., Danjiu, L.B., Guo, H.B., and Lin, E. (2016). Differential response of alpine steppe and alpine meadow to climate warming in the central Qinghai–Tibetan Plateau. *Agric. For. Meteorol*. **223**, 233-240. doi: 10.1016/j.agrformet.2016.03.017

Gao, F.Y., Li, K., Zhao, C.Z., Ren, H., Nie, X.Y., Jia, D.Y., Li, L.F., and Li, Q.F. (2019). The expansion process of a *Stellera chamaejasme* population in a degraded alpine meadow of Northwest China. Environ. *Sci. Pollut. Res*. **26**, 20469-20474. doi: 10.1007/s11356-019-05421-6

Gould, I.J., Quinton, J.N., Weigelt, A., DeDeyn, G.B., and Bardgett, R.D. (2016). Plant diversity and root traits benefit physical properties key to soil function in grasslands. *Ecology letters*. **19**, 1140-1149. doi: 10.1111/ele.12652

Groenigen, J.W., Huygens, D., Boeckx, P., Kuyper, T.W., Lubbers, I.M., Rütting, T., and Groffman, P.M. (2015). The soil N cycle: new insights and key challenges. *Soil*. **1**, 235-256. doi: 10.5194/soil-1-235-2015

Großkopf, R., Janssen, P.H., and Liesack, W. (1998). Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Appl. Environ. Microbiol*. **64**, 960-969. doi: 10.1128/AEM.64.3.960-969.1998

Guo, H.R., Cui, H.Y., Jin, H., Yan, Z.Q., Ding, L., and Qin, B. (2015). Potential allelochemicals in root zone soils of *Stellera chamaejasme* L. and variations at different geographical growing sites. *J. Plant Growth Regul*. **77**, 335-342. doi: 10.1007/s10725-015-0068-4

Guo, L.Z., Li, J.H., He, W., Huang, D., and Wang, K. (2019). High nutrient uptake efficiency and high water use efficiency facilitate the spread of *Stellera chamaejasme* L. in degraded grasslands. *BMC Ecology*. **19**, 50. doi: 10.1186/s12898-019-0267-3

Haruna, E., Zin, N.M., Kerfahi, D., and Adams, J.M. (2018). Extensive Overlap of Tropical Rainforest Bacterial Endophytes between Soil, Plant Parts, and Plant Species. *Microb. Ecol*. **75**, 88-103. doi: 10.1007/s00248-017-1002-2

He, P.P., He, C.S., Liu, Q., and Mu, Y. (2017). Dehalogenation of diatrizoate using nanoscale zero-valent iron: impacts of various parameters and assessment of aerobic biological post-treatment. *RSC Adv*. **7**, 27214-27223. doi: 10.1039/C7RA03750C

Hobbie, S.E. (2015). Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends Ecol. Evol. **30**, 357-363. doi: 10.1016/j.tree.2015.03.015

Holechek, J.L. (2002). Do Most Livestock Losses to Poisonous Plants Result from "Poor" Range Management? *J. Range Manage*. **55**, 270-276. doi: 10.2307/4003134

Jin, H., Yang, X.Y., Liu, R.T., Yan, Z.Q., Li, X.D., Li, X.D., Li, X.Z., Su, A.X., Zhao, Y.H., and Qin, B. (2018). Bacterial community structure associated with the

rhizosphere soils and roots of *Stellera chamaejasme* L. along a Tibetan elevation gradient. *Ann. Microbiol*. **68**, 273-286. doi: 10.1007/s13213-018-1336-0

Kang, B.T., Bowatte, S., and Hou, F.J. (2021). Soil microbial communities and their relationships to soil properties at different depths in an alpine meadow and desert grassland in the Qilian mountain range of China. *J. Arid Environ*. **184**, doi: 10.1016/j.jaridenv.2020.104316

Koukoura, Z., Mamolos, A.P., and Kalburtji, K.L. (2003). Decomposition of dominant plant species litter in a semi-arid grassland. *Appl. Soil Ecol*. **23**, 13-23. doi: 10.1016/S0929-1393(03)00006-4

Kuczynski, J., Stombaugh, J., Walters, W.A., González, A., Caporaso, J.G., and Knight, R. (2012). Using QIIME to analyze 16S rRNA gene sequences from Microbial communities. *Curr. Protoc*. **27**. doi: 10.1002/0471250953.bi1007s36

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Perla, G., Vázquez, M., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.T., and Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nat. Commun*. **6**, 6707. doi: 10.1038/ncomms7707

Li, X.Y., Wang J., Zhang S.P., Wang H.H., Li X., Li X., and Zhang H.W. (2018). Distribution of fungal endophytes in roots of *Stipa krylovii* across six vegetation types in grassland of northern China. *Fungal Ecol*. **31**, 47-53. doi: 10.1016/j.funeco.2017.11.001

Li, Y.Y., Dong, S.K., Liu, S.L., Wang, X.X., Wen, L., and Wu, Y. (2014). The interaction between poisonous plants and soil quality in response to grassland degradation in the alpine region of the Qinghai-Tibetan Plateau. *Plant ecol*. **215**, 809-819. doi: 10.1007/s11258-014-0333-z

Liu, Y., Long, R.J., and Yao, T. (2004). Research progress on *Stellera chamajasme* L. in grassland. *Pratacult. Sci*. **6**, 55-61. (in Chinese with English abstract). doi: 10.3969/j.issn.1001-0629.2004.06.017

Ma, J.G., Bowatte, S., Wang, Y.F., Newton, P.C.D., and Hou, F.J. (2020). Differences in

soil ammonia oxidizing bacterial communities under unpalatable (*Stellera chamaejasme* L.) and palatable (*Elymus nutans* Griseb.) plants growing on the Qinghai Tibetan Plateau. *Soil Biol. Biochem*. **144**. doi: 10.1016/j.soilbio.2020.107779

Miao, V., and Davies, J.L. (2010). Actinobacteria: the good, the bad, and the ugly. *Antonie Van Leeuwenhoek*. **98**, 143-150. doi: 10.1007/s10482-010-9440-6

Miransari, M. (2013). Soil microbes and the availability of soil nutrients. *Acta physiol. Plant*. **35**, 3075-3084. doi: 10.1007/s11738-013-1338-2

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www. R-project.org/

Ramirez, K.S., Craine, J.M., and Fierer, N. (2012). Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biol*. **18**, 1918-1927. doi: 10.1111/j.1365-2486.2012.02639.x

Sait, M., Davis, K.E., and Janssen, P.H. (2006). Effect of pH on isolation and distribution of members of subdivision 1 of the phylum *Acidobacteria* occurring in soil. *Appl. Environ. Microbiol*. **72**, 1852-1857. doi: 10.1128/AEM.72.3.1852–1857.2006

Sanon, A., Andrianjaka, Z.N., Prin, Y., Bally, R., Thioulouse, J., Comte, G., and Duponnois, R. (2009). Rhizosphere microbiota interfers with plant-plant interactions. *Plant Soi*l **321**, 259-278. doi: 10.1007/s11104-009-0100-4

Schlatter, D.C., Bakker, M.G., Bradeen, J.M., and Kinkel, L.L. (2015). Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*. **96**, 134-142. doi: 10.1890/13-1648.1

Shang, Z.H., and Long, R.J. (2007). Formation causes and recovery of the “Black Soil Type” degraded alpine grassland in Qinghai-Tibetan Plateau. *Frontiers of Agriculture in China*. **1**, 197-202. doi: 10.1007/s11703-007-0034-7

Shi, Z.C. (1997). Important Toxic Plants of China Grassland. China Agriculture Ture Press, Beijing.

Štursová, M., Žifčáková, L., Leigh, M.B., Burgess, R., and Baldrian, P. (2012). Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiol. Ecol*. **80**, 735-746. doi: 10.1111/j.1574-6941.2012.01343.x

Sun, G., Luo, P., Wu, N., Qiu, P.F., Gao, Y.H., Chen, H., and Shi, F.S. (2009). *Stellera chamaejasme* L. increases soil N availability, turnover rates and microbial biomass in an alpine meadow ecosystem on the eastern Tibetan Plateau of China. *Soil Biol. Biochem*. **41**, 86-91. doi: 10.1016/j.soilbio.2008.09.022

Sun, Y. (2015). Interaction of soil-herbage-livestock in alpine meadow-tibetan sheep grazing system, Qinghai-Tibetan plateau. Thesis (Ph.D.) Lanzhou University, College of Pastoral Agriculture Science and Technology. 14 pp

Valkó, O., Zmihorski, M., Biurrun, I., Loos, J., Labadessa, R., and Venn, S. (2016). Ecology and conservation of steppes and semi-natural grasslands. *Hacquetia*. **15**, 5-14. doi: 10.1515/hacq-2016-0021

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem*. **19**, 703-707. doi: 10.1016/0038-0717(87)90052-6

Wang, P., Lassoie, J.P., Morreale, S.J., and Dong, S.K. (2015). A critical review of socioeconomic and natural factors in ecological degradation on the Qinghai-Tibetan Plateau, China. *The Rangeland Journal*. **37**, 1-9. doi: 10.1071/RJ14094

Wang, X.J., Wang, Q., Jin, L., Sun, L., Wang, Q., Zhang, L., and Chen, Y.L. (2019). Arbuscular mycorrhizal fungi in the rhizosphere soil of poisonous plants depressed the growth of pasture grasses in the Tibetan Plateau Alpine meadow. *Ecosyst. Health Sust*. doi: 10.1080/20964129.2019.1673215

Wolfe, B.E., and Klironomos, J.N. (2005). Breaking new ground: soil communities and exotic plant invasion. *Bioscience*. **55**, 477-487. doi: 10.1641/0006-3568(2005)055[0477:BNGSCA]2.0.CO;2

Wu, J.S., Yang, P.W., Zhang, X.Z., Shen, Z.X., and Yu, C.Q. (2015). Spatial and climatic patterns of the relative abundance of poisonous vs. non-poisonous plants

across the Northern Tibetan Plateau. *Environ. Monit. Assess*. **187**, 491. doi: 10.1007/s10661-015-4707-z

Xie, Z., Le, R.X., Wang, C.P., Gu, Z.K., An, M., Nan, H.Y., Chen, B.Z., Li, F., Liu, Y.J., Du, G.Z., Feng, H.Y., and Ma. X.J. (2014). Identifying response groups of soil nitrifiers and denitrifiers to grazing and associated soil environmental drivers in Tibetan alpine meadows. *Soil Biol. Biochem*. **77**, 89-99. doi: 10.1016/j.soilbio.2014.06.024

Xing, F., Song, R., Qi, B.L., and Guo, J.X. (2002). Analysis to the interspecific association of *Stellera chamaejasme* population and other main plant species in *Cleistogenes squarrosa* steppe. *Acta Prataculturae Sinica*. **11**, 46–51 (in Chinese with English abstract). doi: 10.3321/j.issn:1004-5759.2002.04.007

Zhang, F.G., Huo, Y.Q., Sun, Y., Xiao, Y., Yang, G., and Zhang, Y. (2018). Effect of consecutive biofertilizer application on aboveground biomass and management of soil microflora in grassland. *J. Nanjing Agric. Univ*. **41**, 382-388. doi: 10.7685/jnau.201708004

Zhang, H.X., Zhang, M.L., and Wang, Y. (2017). Distribution pattern of poisonous plant species in arid grasslands: a case from Xinjiang, Northwestern China. *The Rangeland Journal*. **39**, 279-287. doi: 10.1071/rj16018

Zhao, B.Y., Liu, Z.Y., Hao, L., Wang, Z.X., Sun, L.S., Wan, X.P., Guo, X., Zhao, Y.T., Wang, J.J., and Shi, Z.C. (2010). Damage and control of poisonous weeds in western grassland of China. *Agric. Sci. China*. **9**, 1512-1521. doi: 10.1016/S1671-2927(09)60242-X

Zheng, Y., Chen, L., Luo, C.Y., Zhang, Z.H., Wang, S.P., and Guo, L.D. (2016). Plant identity exerts stronger effect than fertilization on soil arbuscular mycorrhizal fungi in a sown pasture. *Microb. Ecol*. **72**, 647-658. doi: 10.1007/s00248-016-0817-6

 doi:10.20944/preprints202109.0128.v1

List of figure captions

Fig. 1. Rarefaction analysis of richness index; Chao1 in soils originating under *Stellera* or *Elymus*

Fig. 2. Principal component analysis (PCA) of soil microbial communities and soil environment factors

Fig. 3. Relative abundance of soil microbial genera in soils originating under *Stellera* or *Elymus*. *indicates statistical significance at $P<0.05$

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Table 1 Quantitative PCR thermal cycle conditions and primer details

Gene	Primer	Sequence (5′–3′)	Thermal cycles profile	References
16S rDNA	515F	GTGCCAGCMGCCGCGG	94°C,2min;20cycles(94°C,45s; 55°C,45s,72,1min);	(Großkopf et al.
	907R	CCGTCAATTCMTTTRAGTTT	72°C,6min	1998)
18S rDNA	FF390	CGATAACGAACGAGACCT	95 °C,8min; 30cycles(95 °C,30s; 50°C,45s; 72 °C,2min);	(Chu et al. 2011)
	FR1	AICCATTCAATCGGTAIT	72 °C,10min	

 doi:10.20944/preprints202109.0128.v1

Table 2 Differences of **s**oil physicochemical properties and soil microbial community abundance in soils originating under *Stellera* or *Elymus*. Different lowercase letters indicate statistical significance at $P<0.05$

Plant	Moisture (%)	pH	TN ($g \cdot kg^{-1}$)	TC ($g \cdot kg^{-1}$)	C/N	MBC ($mg \cdot kg^{-1}$)	NH_4^+-N ($mg \cdot kg^{-1}$)	NO_3^--N ($mg \cdot kg^{-1}$)	Bacteria ($lg \cdot copies \cdot g^{-1}$)	Fungi ($lg \cdot copies \cdot g^{-1}$)
Elymus	51.83±1.13a	5.73±0.14a	5.3±0.27b	60.02±2.27b	11.38±0.36b	479±64b	22.82±3.51b	20.92±2.35a	8.66±0.08a	8.01±0.04a
Stellera	53.80±2.97a	5.97±0.08a	7.32±0.33a	105.65±11.82a	14.4±1.41a	1177±276a	43.21±4.13a	16.47±1.7a	8.18±0.19a	7.85±0.13a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Table 3 Differences of soil microbial alpha diversity in soils originating under *Stellera* or *Elymus.* Different lowercase letters indicate statistical significance at $P<0.05$ within bacteria and fungi.

Index	Bacteria		Fungi	
	Elymus	*Stellera*	*Elymus*	*Stellera*
Chao1	4157±92a	3422±71b	784.33±84.94a	726±66a
ACE	4379±90a	3449±72b	784.33±84.94a	729±68a
Simpson	0.9976±0.0006a	0.9977±0.0001a	0.9714±0.0072a	0.9531±0.0125a
Shannon-wiener	10.50±0.19a	10.49±0.07a	6.85±0.29a	6.26±0.39a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Table 4 Differences of relative abundance of soil bacterial phyla in soils originating under *Stellera* or *Elymus*

Phylum	*Elymus* (%)	*Stellera* (%)	*P* value
Actinobacteria	28.95±3.71	46.93±3.61	0.026
Proteobacteria	29.57±2.55	19.8±0.89	0.022
Acidobacteria	15.52±1.39	13.73±1.45	0.423
Chloroflexi	5.65±0.4	5.75±0.42	0.869
Bacteroidetes	6.88±0.73	2.25±0.42	0.005
Planctomycetes	3.42±0.43	3.45±0.12	0.951
Gemmatimonadetes	3.38±0.62	2.37±0.39	0.242
Nitrospirae	2.18±0.07	1.72±0.21	0.103
Firmicutes	1.63±0.07	1.11±0.04	0.002
Verrucomicrobia	0.85±0.09	1.24±0.07	0.028

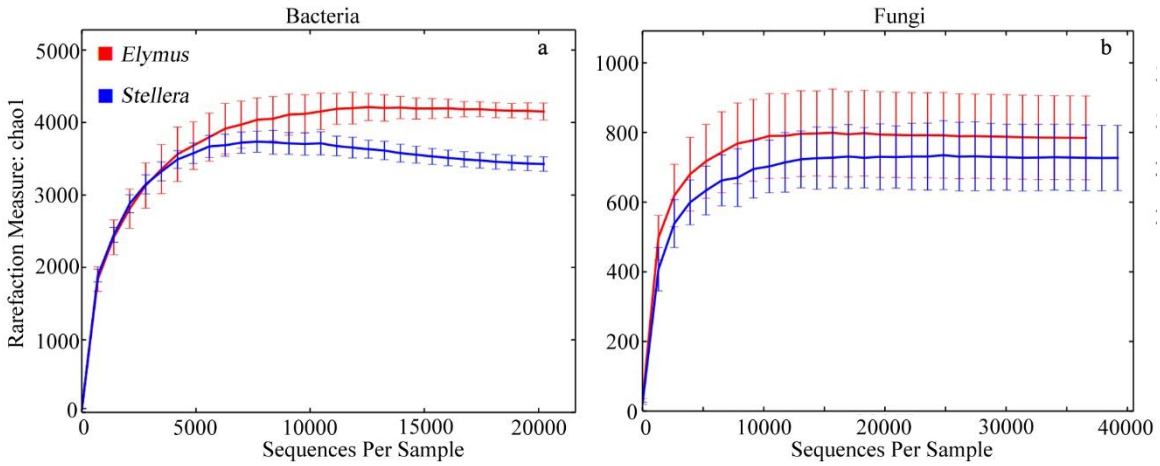
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Table 5 Differences of relative abundance of soil fungal phyla in soils originating under *Stellera* or *Elymus*

Phylum	*Elymus* (%)	*Stellera* (%)	*P* value
Ascomycota	79.16±1.63	77.09±6.43	0.771
Basidiomycota	4.89±0.54	11.3±0.55	0.001
Mortierellomycota	2.23±0.07	1.14±0.15	0.003
Rozellomycota	1.49±0.08	0.74±0.13	0.007
Chytridiomycota	0.72±0.11	0.19±0.01	0.043
Olpidiomycota	0.45±0	0.06±0.01	0.000
Mucoromycota	0.26±0.03	0.2±0.03	0.277
Blastocladiomycota	0.33±0.06	0±0	0.005
Glomeromycota	0.08±0.01	0.16±0.01	0.010
GS19	0.23±0.02	0±0	0.001

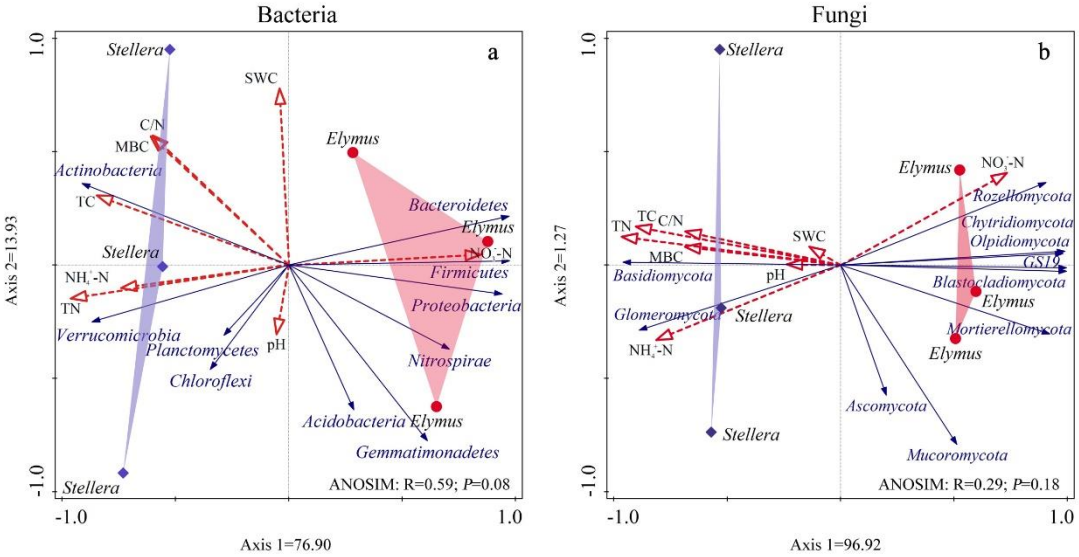
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Fig. 1.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

Fig. 2.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 7 September 2021 doi:10.20944/preprints202109.0128.v1

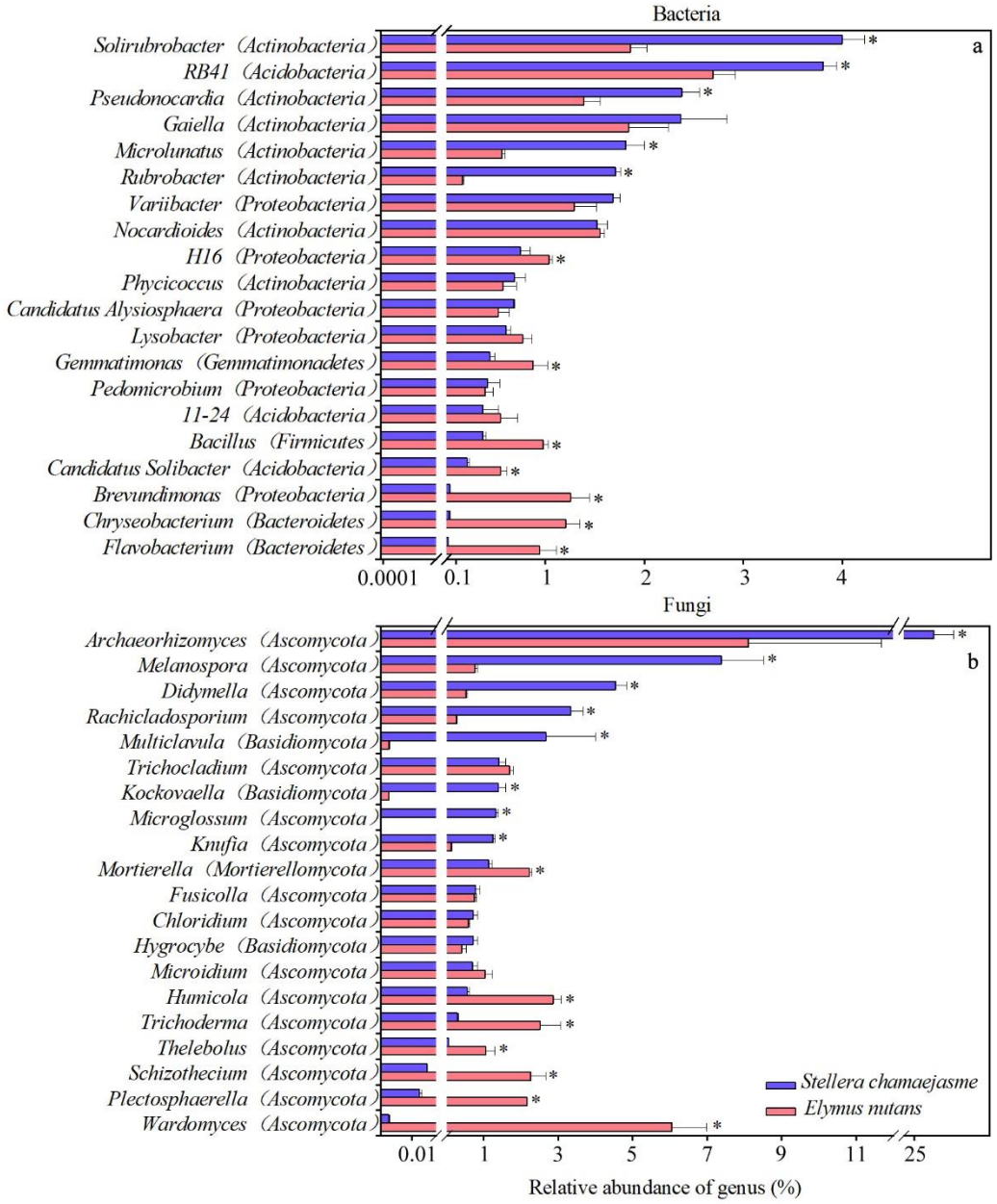


Fig. 3.