Response of arbuscular mycorrhizal fungal community in soil and roots to grazing differs in a wetland on the Qinghai-Tibet plateau

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ABSTRACT

Grazing as one of the most important disturbances affects the abundance, diversity and community composition of arbuscular mycorrhizal (AM) fungi in ecosystems, but the AM fungi in response to grazing in wetland ecosystems remain poorly documented. Here, we examined AM fungi in roots and soil in grazing and non-grazing plots in Zoige wetland on the Qinghai-Tibet plateau. Grazing significantly increased AM fungal spore density and glomalin-related soil proteins, but had no significant effect on the extra radical hyphal density of AM fungi. AM fungal richness was significantly lower in roots than in soil, but not significantly influenced by grazing. AM fungal community composition was significantly different between roots and soil, and was significantly influenced by grazing in soil but not in roots. This finding may enhance our understanding of the AM fungi in response to grazing in the wetland on the Qinghai-Tibet plateau.

Keywords: Arbuscular mycorrhizal fungal abundance, Community, Diversity, Grazing, Wetland

INTRODUCTION

Wetlands cover about 6% of the land surface on the earth and have high species diversity, including many endemic species (*Junk et al., 2013*). In China, wetlands account for 7% of the wetland on the world (*Junk et al., 2013*) and have about 225 families, 815 genera and 2,276 species of higher plants (*Yan & Zhang, 2005*). Wetlands provide important ecological functions in water resource conservation and quality purification, climate regulation, substance circulation and regional ecological balance maintenance (*Barbier, Acreman & Knowler, 1997; Li, 2001; Chmura et al., 2003; Green et al., 2017*). Moreover, as an important carbon (C) pool, wetlands can reduce the impact of increased greenhouse gases on global climate change (*Frolking et al., 2011*). However, wetland ecosystems have suffered severe degradations in recent decades due to global warming, intense resource exploitation, changes in hydrology and human disturbance (*Xiang et al., 2009; Junk et al., 2013; Meng et al., 2016*). For example, in human activities over-grazing has affected biodiversity, productivity, community stability and soil C cycling in wetlands (*Wang et al., 2012; Hoffmann et al., 2016; Zhou et al., 2017*).

Arbuscular mycorrhizal (AM) fungi, as one of the key components of soil microorganisms, form symbiotic associations with most terrestrial plant species (*Smith & Read, 2008*). In the AM associations, plants provide C source for the growth and function of fungi, thereby affecting the community of AM fungi (*Bonfante & Genre, 2010*). On the contrary, AM fungi can increase the nutrient and water absorption of host plants, then affecting plant community and productivity (*van der Heijden, Bardgett & van Straalen, 2008*). Furthermore, glomalin-related soil protein (GRSP) produced by AM fungi can stably exist in soil and play an important role in soil C pool (*Rillig, Field & Allen, 2001; Godbold et al., 2006*). In addition, AM fungi can improve plants to

tolerate grazing and other stresses from the environment (*Bennett & Bever, 2007; Sharifi, Ghorbanli & Ebrahimzadeh, 2007*). Thus, revealing the AM fungi in response to grazing is of great importance for understanding the diversity maintenance and community stability of plants in ecosystems.

Previous studies have demonstrated that the effect of grazing on AM fungi is depended on grazing intensity (*García & Mendoza, 2012; Shi et al., 2017; Kusakabe et al., 2018; Yang et al., 2019*). For instance, the light and moderate grazing intensity positively influenced AM fungal spore density in grasslands in Jilin province, China (*Ba et al., 2012*) and in British Columbia, Canada (*van der Heyde et al., 2017*). In contrast, over-grazing negatively affected AM fungal spore density in a semi-arid grassland in China (*Su & Guo, 2007*). Moderate grazing intensity did not influence AM fungal extra radical hyphal density in an alpine meadow in China (*Yang et al., 2013a*), but high grazing pressure negatively affected the extra radical hyphal density of AM fungi in a semi-arid grassland in China (*Ren et al., 2018*). Besides, moderate grazing had a neutral effect on AM fungal richness in a meadow in China (*Ba et al., 2012*). Moderate grazing significantly affected the community composition of AM fungi in soil and roots in grassland ecosystems (*Bai et al., 2013; Yang et al., 2013a; Kusakabe et al., 2018*). In contrast, others found that moderate grazing did not influence the community composition of AM fungi in roots in alpine meadow (*Jiang et al., 2018*) and in soil in mountain grassland (*van der Heyde et al., 2017*) ecosystems. However, previous studies have mainly focused on semi-arid, arid, alpine and mountain grassland ecosystems (*Epelde et al., 2017; Jiang et al., 2018; Kusakabe et al., 2018; Ren et al., 2018*). So far, we know little about how grazing affects AM fungi in wetland ecosystems.

Zoige wetland is a typical representative of the alpine wetland ecosystem on the Qinghai-

Tibet plateau in China, and has high plant species diversity and an important C sink function (*Guo et al., 2013*). However, Zoige wetland has suffered severe ecosystem degradations since the 1970s, due to global warming, low precipitation and human disturbance, such as ditching for grassland enlargement, peat exploitation and over-grazing (*Xiang et al., 2009; Guo et al., 2013; Wang et al., 2017*). Previous studies have mainly focused on plant diversity, microbial community (archaea group), and ecosystem conservation and restoration in the Zoige Wetland (*Wang, Bao & Yan, 2002; Zhang et al., 2008; Xiang et al., 2009*). However, the grazing effect on AM fungi has never been studied.

In order to reveal the AM fungi in response to grazing in wetland ecosystem, we established non-grazing (natural) and moderate grazing plots in Zoige wetland on the Qinghai-Tibet plateau. AM fungal spore density, extra radical hyphal density and GRSP content were examined in grazing and non-grazing plots. We examined the communities of AM fungi in roots and soil by Illumina MiSeq sequencing of 18S rDNA region. We hypothesize that: (H1) moderate grazing increases AM fungal spore density and GRSP content, but does not change the extra radical hyphal density of AM fungi, and (H2) moderate grazing changes the community composition of AM fungi but not richness in roots and soil in the Zoige wetland.

MATERIALS AND METHODS

Study site and sampling

The study was carried out in the centre of Zoige Swamp in the Zoige National Nature Reserve on the Qinghai-Tibet plateau (33°25'-34°80'N, 102°29'-102°59'E, 16,671 ha, 3365 m above sea level). The site has a plateau cold temperate humid monsoon climate, with a mean

annual temperature (MAT) of 1.1 °C, and a mean annual precipitation (MAP) of 660 mm. The site begins to freeze in late September and is completely thawed in mid-May (*Wang, Bao & Yan, 2002*). The abundant plant species are *Blysmus sinocompressus*, *Potentilla anserina*, *Carex enervis*, *Caltha scaposa*, *Elymus nutans* and *Leontopodium wilsonii* in the site (*Wang, Bao & Yan, 2002*).

We established 20 plots (each 1 m × 1 m), > 20 m away from each other, in non-grazing (natural grass) and grazing area, respectively (Supplementary Fig. S1). The average species number and height of vegetation were 7.8 ± 0.495 (mean ± SE) and *ca.* 31 cm in the non-grazing plots and 5.4 ± 0.255 and *ca.* 7 cm in the grazing plots. This site was mainly grazed by yak. The grazing intensity in this study site was described as moderate (*He et al., 2000*). In July 2018, we randomly collected five soil cores (3 cm in diameter; 15 cm in depth; *ca*. 300 g) and mixed into one composite sample from each plot. A total of 40 samples were obtained, packed in an ice box and transported to our laboratory. Soil samples were sieved (1-mm sieve) to remove debris and roots. Subsoil samples were kept at −80 °C until the extraction of fungal hyphae and DNA, and the remaining subsoil samples were air dried and kept at 10 °C until the analysis of AM fungal spore density, GRSP content and soil properties. We manually collected the mixed roots (< 2 mm in diameter) from each sample, washed with sterilized deionized water and kept at −80 °C until DNA extraction.

Soil property analysis

Soil moisture was measured using oven-drying at 105 °C for 24 h. Soil pH was measured at a ratio of 1:2.5 (w/v, soil: water) with a glass electrode (Thermo Orion T20, Columbia, USA). Soil total nitrogen (N) and C were determined by CHNOS Elemental Analyser (Vario EL Ⅲ

Elementar Analysensysteme GmbH, Germany). Soil total phosphorus (P) was extracted using the $HClO_4$-H_2SO_4 digestion method and determined with a spectrophotometer (UV-2550, Shimadzu, Japan). The soil properties analyzed are shown in Supplementary Table S1.

EE-GRSP and T-GRSP

Total GRSP (T-GRSP) and easily extracted GRSP (EE-GRSP) were measured according to *Wright & Upadhyaya (1998)* and the modification method of *Janos et al. (2008)*. We extracted EE-GRSP from 0.1 g air dried soil using sodium citrate buffer (8 mL, 0.02 M, pH 7.0) at 121 °C for 90 min in an autoclave (Yamato SQ810C, China). We repeatedly extracted T-GRSP from 0.1 g air dried soil using sodium citrate buffer (8 mL, 0.05 M, pH 8.0) at 121 °C for 90 min until no obvious color in the supernatant was observed. Supernatants were separated by centrifugation at 6000 g for 15 min to remove the soil particles and saved in a plastic tube (4 °C). Then 0.5 mL of supernatant of EE-GRSP and T-GRSP was stained with 5 mL of Coomassie Brilliant Blue G-250 and was read in a micro-plate reader (Biotek Synergy H4, Winooski, VT, USA) at 595 nm. The bovine serum albumin was used as a standard solution with Coomassie Brilliant Blue method and a standard curve was drawn to determine the content of EE-GRSP and T-GRSP.

AM fungal extra radical hyphal density and spore density

We extracted fungal hyphae from soil according to the membrane filter method (*Rillig, Field & Allen, 1999*). In total, 4.0 g of frozen soil from each sample was mixed with 12 mL sodium hexametaphosphate (35 g L^{-1}) and 100 mL distilled deionized water in a flask, and then blended for 30 s, settled for 30 min and sieved (38-μm sieve). The fungal hyphae on the sieve were washed into a flask with 200 mL distilled water, and then 2 mL aliquot was filtered through

a 25-μm Millipore filter. The fungal hyphae on the filter were stained with 1% acid fuchsine and distinguished into AM and non-AM fungi on the basis of morphological characteristics and staining color (*Miller, Jastrow & Reinhardt, 1995*). We measured the hyphal length of AM fungi according to the grid-line intersect method (*Tennant, 1975*). We extracted AM fungal spores from 20 g air dried soil from each sample according to the wet-sieving and decanting method (*Daniels & Skipper, 1982*) and counted the spore numbers under 40 × magnification (Nikon 80i, Japan).

DNA extraction, PCR and Illumina Miseq sequencing

We extracted DNA from 0.2 g frozen roots and soil using the PowerSoil® DNA isolation kit (MOBIO Laboratories, Inc., Carlsbad, USA) in accordance with the manufacturer's instructions, and measured the DNA concentration using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, USA). We amplified the fungal 18S rDNA region using a two-step PCR procedure. The first PCR using primers AML2 (*Lee, Lee & Young, 2008*) and GeoA2 (*Schwarzott & Schüßler, 2001*) was conducted in a final 25 μL reaction mixture, including *ca*. 10 ng of template DNA, 0.75 μM of each primer, 250 μM of each dNTP, 0.5 U KOD-plus-Neo polymerase (Toyobo, Tokyo, Japan), 1.5 mM $MgSO_4$, and 2.5 μL 10 × buffer. The thermal cycling conditions were performed as follows: an initial denaturation at 95 °C for 5 min, 30 cycles for denaturation at 94 °C for 1 min, annealing at 58 °C for 50 s and extension at 68 °C for 1 min, and a final extension at 68 °C for 10 min. The products of the first amplification were diluted 100 times, and 1 μL of the diluted DNA template was used for the second amplification. The thermal cycling conditions for the second amplification were the same as first amplification, except that the primers NS31 (*Simon, Lalonde & Bruns, 1992*) and AMDGR (*Sato et al., 2005*)

linked with 12-base barcode sequences were used. The size of amplified fragment was about 300 base pairs (bp). We purified the PCR products using a PCR Product Gel Purification Kit (Omega Bio-Tek, USA), and pooled the purified PCR products with the same amount (100 ng) from each sample and adjusted the concentration to 10 ng μL^{-1}. We constructed a sequencing library by addition of an Illumina sequencing adaptor (5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTC TGCTTG-3') to the products using the Illumina TruSeq DNA PCR-Free LT Library Prep Kit (Illumina, CA, USA) according to the manufacturer's instructions. We sequenced the library by an Illumina MiSeq PE 250 platform using the paired-end (2 × 250 bp) option in the Chengdu Institute of Biology, Chinese Academy of Sciences, China.

Bioinformatics analysis

We filtered the raw sequences using Quantitative Insights into Microbial Ecology (QIIME) v.1.7.0 (*Caporaso et al., 2010*) to eliminate low-quality sequences, such as read length < 200 bp, no valid primer sequence or barcode sequence, containing ambiguous bases, or an average quality score < 20. We checked and deleted the potential chimeras against the Maarj*AM* database (*Öpik et al., 2010*) using the 'chimera.uchime' command in Mothur version 1.31.2 (*Schloss et al., 2009*). High quality sequences were subjected to de-replication and de-singleton, and then clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level using the cluster_otus command in USEARCH v8.0 (*Edgar, 2013*). Using a basic local alignment search tool (BLAST) (*Altschul et al., 1990*), we selected the most abundant sequence of each OTU and searched against the Maarj*AM* database and National Center for Biotechnology Information (NCBI) nt database. We identified OTUs as the AM fungi based on the closest BLAST hit

annotated as 'Glomeromycotina' and *E* values < e^{-50}. Furthermore, we normalized the sequence number of each sample to the smallest sample size using the 'sub.sample' command in Mothur. We have submitted the representative sequence of each AM fungal OTU to the European Molecular Biology Laboratory (EMBL) database (accession no. LR736402-LR736557). The identified AM fungi are shown in Supplementary Table S2.

Statistical analysis

We conducted all statistical analyses in R version 3.3.2 (*R Development Core Team, 2017*). Tukey's honestly significant difference (HSD) test or Conover's test was used to examine the significant difference of soil moisture, pH, total N, total C and total P in the grazing and non-grazing plots at $P < 0.05$. One-way analysis of variance (ANOVA) was conducted to evaluate the effect of grazing on AM fungal spore density, extra radical hyphal density, T-GRSP and EE-GRSP, and then Tukey's HSD test was used to examine the significant difference between treatments at $P < 0.05$. Two-way ANOVA was conducted to evaluate the effect of grazing, sample type (soil and root) and their interaction on the OTU richness and the relative abundance of abundant OTUs (relative abundance > 1%) and orders of AM fungi, and then Tukey's HSD test was used to examine the significant difference between treatments at $P < 0.05$. As these data (except for the relative abundance of OTU141) did not satisfy homogeneity of variance after log and square root transformation, nonparametric Kruskal–Wallis test was carried out, and then Conover's test was conducted for comparisons between grazing and non-grazing treatments in soil and roots using the post-hoc.kruskal.conover.test function in the PMCMR package (*Pohlert, 2014*).

The distance matrices of AM fungal community composition (Hellinger-transformed OTU

read data) in roots and soil were established by the Bray-Curtis method (*Clarke, Somerfield & Chapman, 2006*). Permutational multivariate analysis of variance (PerMANOVA) was conducted to examine the effect of sample type, grazing and their interaction on AM fungal community composition, using the 'adonis' function in the vegan with 999 permutations (*Oksanen et al., 2013*). Redundancy analysis (RDA) was conducted to reveal the significant correlation of AM fungal community composition and soil variables using the Monte Carlo permutation test with 999 permutations.

RESULTS

EE-GRSP and T-GRSP contents

The EE-GRSP content was 28.96 ± 3.73 μg g^{-1} (mean ± SE) and 25.71 ± 2.26 μg g^{-1} in grazing and non-grazing treatments, respectively. The T-GRSP content was 96.7 ± 18.82 μg g^{-1} and 71.87 ± 12.87 μg g^{-1} in grazing and non-grazing treatments, respectively. One-way ANOVA showed that grazing significantly influenced EE-GRSP ($F_{1,38}$ = 9.907, P = 0.003) and T-GRSP ($F_{1,38}$ = 23.57, P < 0.001). For example, EE-GRSP and T-GRSP contents were significantly lower in non-grazing than in grazing treatments (Fig. 1a and b).

AM fungal spore density and extra radical hyphal density

The spore density of AM fungi was 25.89 ± 12.17 g^{-1} (mean ± SE) and 15.03 ± 5.88 g^{-1} in grazing and non-grazing treatments, respectively. The extra radical hyphal density of AM fungi was 4.00 ± 2.51 m g^{-1} and 3.10 ± 1.56 m g^{-1} in grazing and non-grazing treatments, respectively. One-way ANOVA revealed that grazing significantly affected AM fungal spore density ($F_{1,38}$ =

10.71, $P = 0.002$) but not extra radical hyphal density ($F_{1,38} = 2.000$, $P = 0.165$). For example, the spore density of AM fungi was significantly lower in non-grazing than in grazing treatments (Fig. 1c). No significant difference of the extra radical hyphal density of AM fungi in non-grazing and grazing treatments was observed (Fig. 1d).

Identification of AM fungi

In total, 3,205,557 high-quality sequences were filtered from 3,335,816 raw sequences and clustered into 882 OTUs at a 97% sequence similarity level. Among 882 OTUs, 156 (2,919,706 sequences) belonged to AM fungi. As the sequence number of AM fungi varied from 20,408 to 48,572 in the 80 samples, the number of sequence was normalized to 20,408. The normalized dataset contained 156 AM fungal OTUs (1,632,640 sequences). Of the 156 AM fungal OTUs obtained, 154 were from soil, 152 from roots, and 150 shared both soil and roots. Among 156 AM fungal OTUs, 153 were detected from more than three samples (frequency ≥ 3.75%) (Supplementary Fig. S2a). Furthermore, the 21 abundant AM fungal OTUs (relative abundance > 1%) occupied 83.85% of the total sequences (Supplementary Fig. S2b). Among 156 AM fungal OTUs, 109 were identified to Glomerales (79.52% of sequences), 22 to Diversisporales (10.84%), 21 to Archaeosporales (8.75%), and 4 to Paraglomerales (0.89%). In addition, the rarefaction curves indicated that the sample numbers were sufficient to detect the most AM fungi in this study (Supplementary Fig. S3).

AM fungal OTU richness

AM fungal OTU richness in grazing and non-grazing treatments was 123.70 ± 2.96 (mean ± SE) and 122.85 ± 3.01 in soil, and 117.55 ± 2.26 and 118.00 ± 4.38 in roots, respectively.

Kruskal–Wallis test revealed that AM fungal OTU richness was influenced by sample type (root and soil; $\chi^2 = 35.01$, $P < 0.001$), but not by grazing ($\chi^2 = 0.045$, $P = 0.832$). For example, AM fungal OTU richness was significantly lower in roots than in soil in both grazing and non-grazing treatments (Fig. 2). However, no significant difference of AM fungal OTU richness between grazing and non-grazing treatments in roots and soil was observed (Fig. 2).

AM fungal community

Two-way ANOVA and Kruskal–Wallis tests revealed that grazing had significant effect on the relative abundance of abundant AM fungal OTU13 and OTU141 (Glomerales), and sample type had significant effect on the relative abundance of abundant AM fungal OTU4, OTU5, OTU7, OTU12, OTU14, OTU15, OTU18, OTU25 and OTU141 (Glomerales), OTU8 and OTU17 (Diversisporales) and OTU23 (Archaeosporales) (Fig. 3). For example, the relative abundance of OTU5, OTU8, OTU15, OTU17, OTU23, OTU25 and OTU141 was significantly lower in roots than in soil, and that of OTU141 was significantly lower in non-grazing treatment than in grazing treatment (Fig. 3; Supplementary Table S3). By contrast, the relative abundance of OTU4, OTU7, OTU12, OTU14 and OTU18 was significantly lower in soil than in roots (Fig. 3; Supplementary Table S3). In the grazing treatment, the relative abundance of OTU5, OTU8, OTU15, OTU17, OTU23 and OTU141 was significantly higher in soil than in roots, while that of OTU4, OTU7, OTU14 and OTU18 was significantly lower in soil than in roots (Fig. 3; Supplementary Table S3). In the non-grazing treatment, the relative abundance of OTU5, OTU8, OTU17, OTU23 and OTU141 was significantly higher in soil than in roots, while that of OTU4, OTU7, OTU12, OTU14 and OTU18 was significantly lower in soil than in roots (Fig. 3; Supplementary Table S3). Besides, the relative abundance of soil OTU13 and OTU141 was

significantly lower in non-grazing treatment than in grazing treatment (Fig. 3; Supplementary Table S3).

Kruskal–Wallis test revealed that sample type but not grazing significantly influenced the relative abundance of Glomerales, Diversisporales and Archaeosporales, but not on Paraglomerales (Fig. 4). The relative abundance of Glomerales was significantly lower in soil than in roots; by contrast, the relative abundance of Diversisporales and Archaeosporales was significantly lower in roots than in soil, regardless of non-grazing and grazing treatments (Fig. 4).

The PerMANOVA demonstrated that the community composition of AM fungi was significantly influenced by sample type (soil and root; $F = 15.49$, $R^2 = 0.149$, $P = 0.001$) and grazing ($F = 2.617$, $R^2 = 0.025$, $P = 0.008$). Furthermore, the community composition of AM fungi was significantly influenced by grazing in soil ($F = 2.639$, $R^2 = 0.055$, $P = 0.001$), but not in roots ($F = 0.998$, $R^2 = 0.025$, $P = 0.419$). Furthermore, RDA showed that the community composition of AM fungi in soil and roots was significantly correlated with soil pH, moisture, total C, total N and total P (Fig. 5).

DISCUSSION

We found that grazing had positive effect on AM fungal spore density, EE-GRSP and T-GRSP, in consistent with some previous studies (*Hammer & Rillig, 2011; Yang et al., 2013a; van der Heyde et al., 2017*). Previous findings suggest that moderate removal of aboveground biomass may increase the allocation of C to the roots and exudation from roots to soil (*Eom, Wilson & Hartnett, 2001; Hamilton et al., 2008; Soka & Ritchie, 2018*), which could be

beneficial for the sporulation of AM fungi (*Ba et al., 2012; van der Heyde et al., 2017*). Furthermore, since about 80% of GRSP is produced by the AM fungi, grazing increased AM fungal spore density, resulting in increasing GRSP content in soil (*Driver, Holben & Rillig, 2005*). However, grazing did not significantly influence AM fungal extra radical hyphal density, as reported in a previous study (*García & Mendoza, 2012*). Although moderate grazing may increase C allocation to the roots, this increase may be ephemeral (*van der Heyde et al., 2019*) and not be sufficient to promote the growth of AM fungal hyphae.

AM fungal richness was significantly lower in roots than in soil, as previous studies reported in alpine and meadow ecosystems (*Hempel, Renker & Buscot, 2007; Liu et al., 2012; Yang et al., 2013a*). This may be due to the seasonal nature of AM fungal communities (*Clark, Rillig & Nowak, 2009; Liu et al., 2009; Martínez-García et al., 2011*). Furthermore, the currently and formerly active propagules of AM fungi could remain in soil, but only currently active AM fungi could occur in the roots (*Liu et al., 2009; Martínez-García et al., 2011*). However, we found that grazing did not significantly influence AM fungal richness in roots and soil. Similarly, a previous study showed that moderate grazing could maintain the AM fungal diversity (*Dudinszky et al., 2019*). In general, AM fungi have low specificity (*Smith & Read, 2008*), thus AM fungal richness may not be influenced by the low plant species diversity caused by moderate grazing, as some studies found that AM fungal richness was not related to plant species diversity (*Wolf et al., 2003; Xiang et al., 2014*).

The community composition of AM fungi significantly differed between roots and soil in this study, as previous studies reported in grassland (*Yang et al., 2013a*), farmland (*Liu et al., 2016*) and temperate (*Saks et al., 2014*) and subtropical forest (*Maitra et al., 2019*) ecosystems. This may be explained by the difference in AM fungal abundance in roots and soil (*Hempel,*

Renker & Buscot, 2007; Varela-Cervero et al., 2015; Maitra et al., 2019). Indeed, our result found that some AM fungi were abundant in roots and soil, respectively. In addition, AM fungal phenology may produce different communities in soil and roots (*Pringle & Bever, 2002; Liu et al., 2012*).

Grazing significantly affected the AM fungal community composition in soil, in consistent with some previous studies reported in desert steppe and grassland ecosystems (*Murray, Frank & Gehring, 2010; Bai et al., 2013*). Grazing may influence the AM fungal community composition by changing soil properties through animal trampling and fecal deposition (*McNaughton, Banyikwa & McNaughton, 1997; Yang et al., 2013b; Liu et al., 2015; Yang et al., 2019*). For example, animal trampling may make the soil tight and alter soil bulk density (*Kobayashi, Hori & Nomoto, 1997; Kauffman, Thorpe & Brookshire, 2004; Byrnes et al., 2018*), thereby influencing AM fungal community (*Yang et al., 2018*). Moreover, dung and urine produced by animals, as soil fertilization, may decrease soil pH and increase soil nutrients as shown in this and previous studies (*McNaughton, Banyikwa & McNaughton, 1997; Kohler et al., 2005*), thus altering AM fungal community composition. Indeed, our result showed that the community composition of AM fungi was significantly related to soil pH, moisture, total C, total N and total P, as previous studies reported in semi-arid, alpine and temperate grassland and subtropical forest ecosystems (*Zheng et al.,2014; Gao et al., 2016; Zhang et al., 2016; Goldmann et al., 2019; Maitra et al., 2019*). However, the AM fungal community composition in roots was not significantly influenced by grazing, as previous studies reported in semi-arid and alpine grassland ecosystems (*González et al., 2018; Jiang et al., 2018*). It is possible that moderate grazing does not much change the allocation of carbohydrates to roots, thereby without altering AM fungal community. Furthermore, although grazing may alter the AM fungal function,

it does not necessarily alter the community in roots (*González et al., 2018*).

CONCLUSIONS

In conclusion, we examined the AM fungi in response to grazing in the Zoige wetland on the Qinghai-Tibet plateau for the first time. AM fungal spore density and GRSP content positively responded to grazing. The extra radical hyphal density and OTU richness of AM fungi had neutral response to grazing. The community composition of AM fungi significantly differed between roots and soil, and was significantly influenced by grazing in soil but not in roots. This finding may enhance our understanding of the AM fungi in response to grazing in the wetland ecosystem on the Qinghai-Tibet Plateau.

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Figure legend

Fig. 1. Easily extracted glomalin-related soil protein (EE-GRSP, a), total extracted GRSP (T-GRSP, b), spore density (c) and extra radical hyphal (ERH) density (d) of arbuscular mycorrhizal (AM) fungi in grazing and non-grazing treatments. One-way ANOVA showed the effect of grazing on AM fungal variables. Data are means ± SE (n = 20). Bars with different letters denote significant difference in grazing and non-grazing treatments according to Tukey's HSD test at $P < 0.05$.

Fig. 2. The operational taxonomic unit (OTU) richness of arbuscular mycorrhizal (AM) fungi in soil and roots in grazing and non-grazing treatments. Kruskal-Wallis test showed the effect of grazing and sample type (soil and root) on the OTU richness. Data are means ± SE (n = 20). Bars with different letters denote significant difference in grazing and non-grazing treatments according to Conover's test at $P < 0.05$.

Fig. 3. Relative abundance of arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) in soil and roots in grazing and non-grazing treatments. Two-way ANOVA and Kruskal-Wallis tests showed the effect of grazing and sample type (soil and root) on the relative abundance of AM fungal OTUs (ns; $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The rare AM fungal OTUs (< 1% of total AM fungal reads) and abundant AM fungal OTUs (> 1% of total AM fungal reads) that was not significantly affected by grazing and sample type were all assigned to "Others". SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG, root grazing.

670

671 **Fig. 4.** Relative abundance of arbuscular mycorrhizal (AM) fungi at the order level in soil and
672 roots in grazing and non-grazing treatments. Kruskal-Wallis test showed the effect of grazing
673 and sample type (soil and root) on the relative abundance of AM fungal orders (ns; $P \geq 0.05$, ***
674 $P < 0.001$). Different letters are significantly different at $P < 0.05$, as indicated by Conover's test.
675 SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG, root grazing.

676

677 **Fig. 5.** Redundancy analysis (RDA) biplots showing arbuscular mycorrhizal (AM) fungal
678 community composition in soil and roots (a), soil (b) and roots (c). Significant soil variables
679 were presented as vectors on the RDA biplot graphs using the 'envfit' (based on 999
680 permutations) at $P < 0.05$. SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG,
681 root grazing; N, soil total nitrogen; C, soil total carbon; P, soil total phosphorus.

Figure 1

Grazing significantly affected AM fungal biomass.

Fig. 1. Easily extracted glomalin-related soil protein (EE-GRSP, a), total extracted GRSP (T-GRSP, b), spore density (c) and extra radical hyphal (ERH) density (d) of arbuscular mycorrhizal (AM) fungi in grazing and non-grazing treatments. One-way ANOVA showed the effect of grazing on AM fungal variables. Data are means ± SE (n = 20). Bars with different letters denote significant difference in grazing and non-grazing treatments according to Tukey's HSD test at $P < 0.05$.

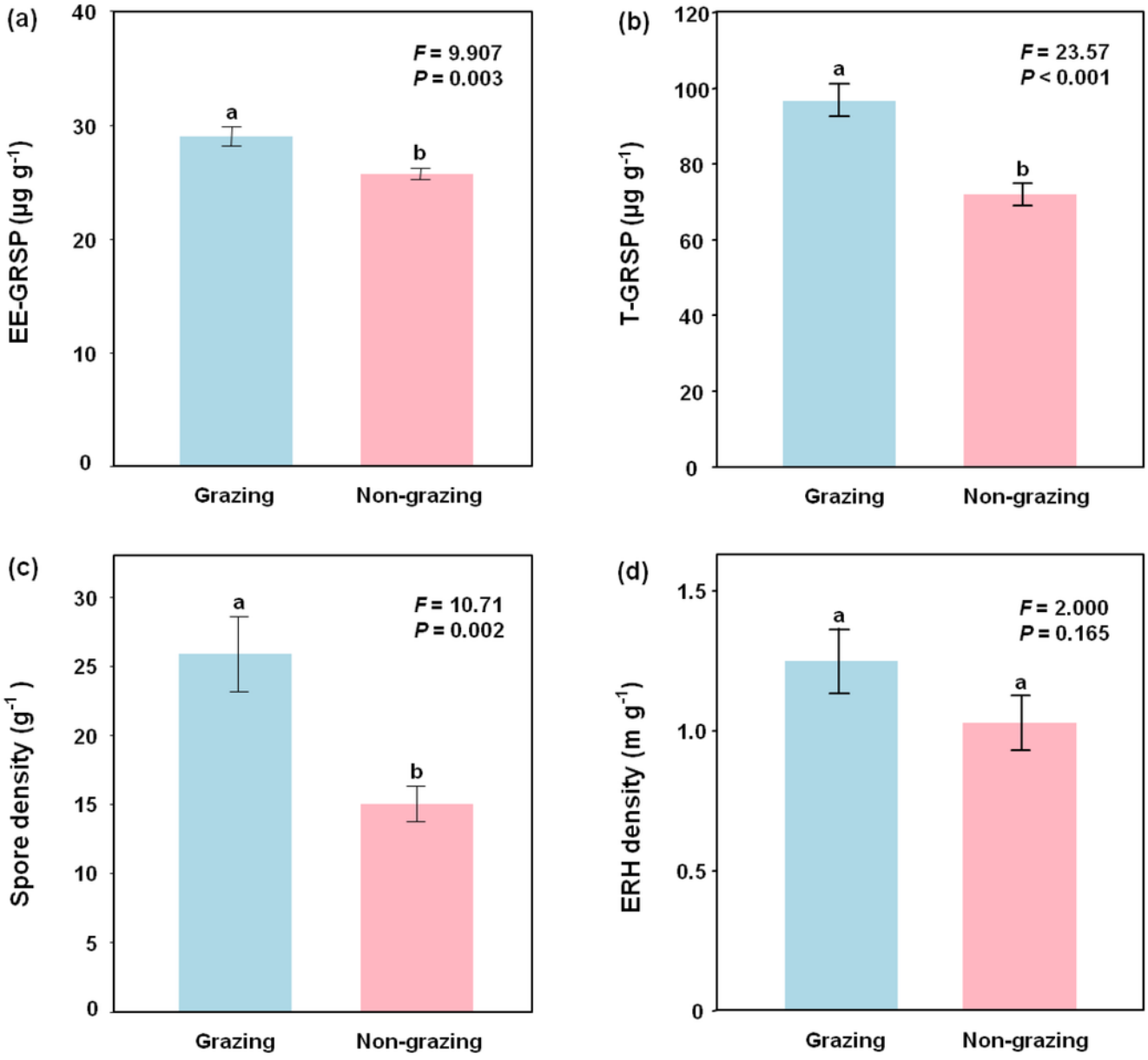
(a)
EE-GRSP (μg g-1)
F = 9.907
P = 0.003
a
b
Grazing
Non-grazing
(b)
T-GRSP (μg g-1)
F = 23.57
P < 0.001
a
b
Grazing
Non-grazing
(c)
Spore density (g-1)
F = 10.71
P = 0.002
a
b
Grazing
Non-grazing
(d)
ERH density (m g-1)
F = 2.000
P = 0.165
a
a
Grazing
Non-grazing

Figure 2

Grazing did not significantly affected AM fungal richness.

Fig. 2. The operational taxonomic unit (OTU) richness of arbuscular mycorrhizal (AM) fungi in soil and roots in grazing and non-grazing treatments. Kruskal-Wallis test showed the effect of grazing and sample type (soil and root) on the OTU richness. Data are means ± SE (n = 20). Bars with different letters denote significant difference in grazing and non-grazing treatments according to Conover's test at $P < 0.05$.

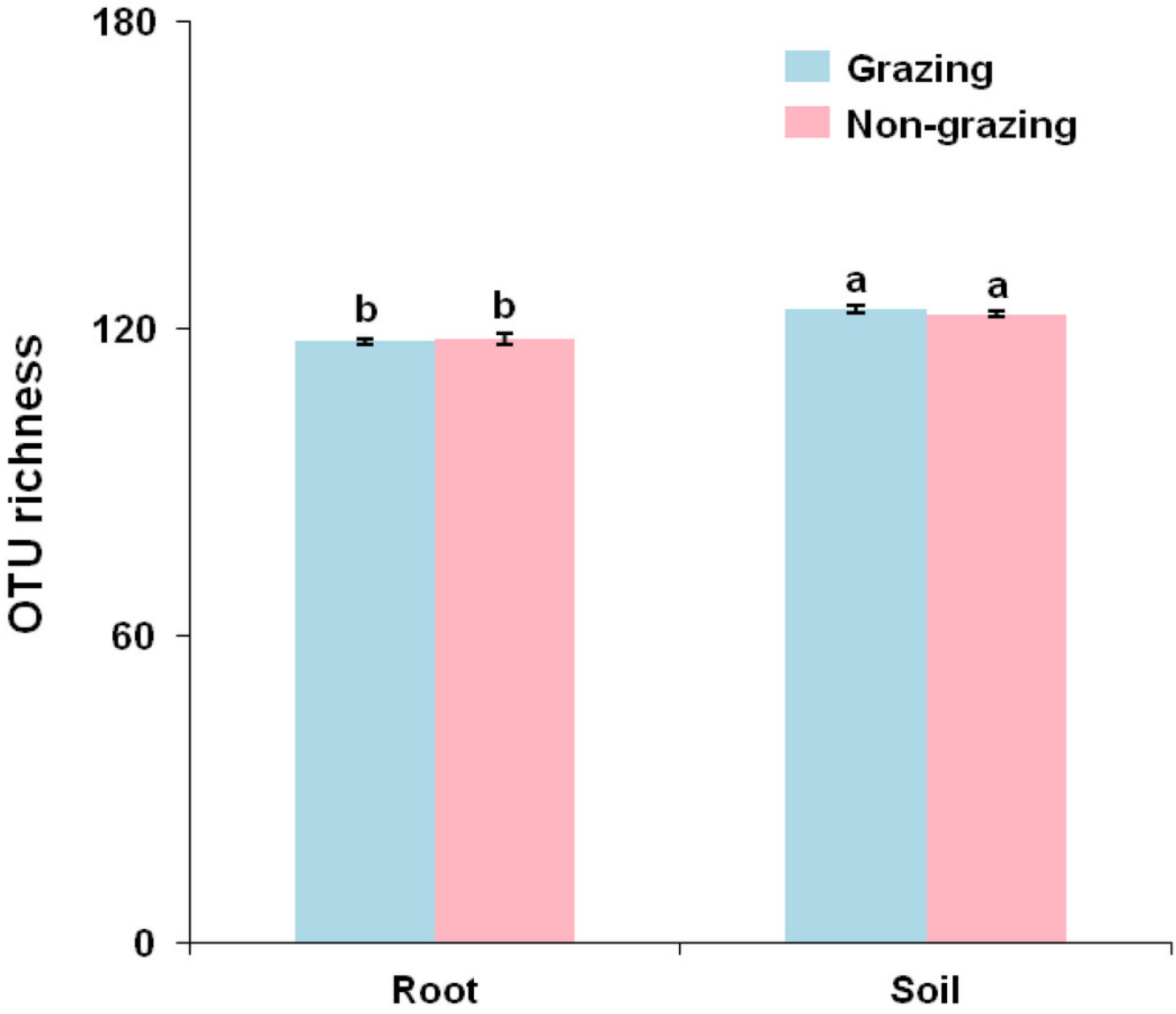
180
120
60
0
OTU richness
Grazing
Non-grazing
b
b
a
a
Root
Soil

Figure 3

Relative abundance of arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) in soil and roots in grazing and non-grazing treatments.

Fig. 3. Relative abundance of arbuscular mycorrhizal (AM) fungal operational taxonomic units (OTUs) in soil and roots in grazing and non-grazing treatments. Two-way ANOVA and Kruskal-Wallis tests showed the effect of grazing and sample type (soil and root) on the relative abundance of AM fungal OTUs (ns; $P \geq 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). The rare AM fungal OTUs (< 1% of total AM fungal reads) and abundant AM fungal OTUs (> 1% of total AM fungal reads) that was not significantly affected by grazing and sample type were all assigned to “Others”. SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG, root grazing.

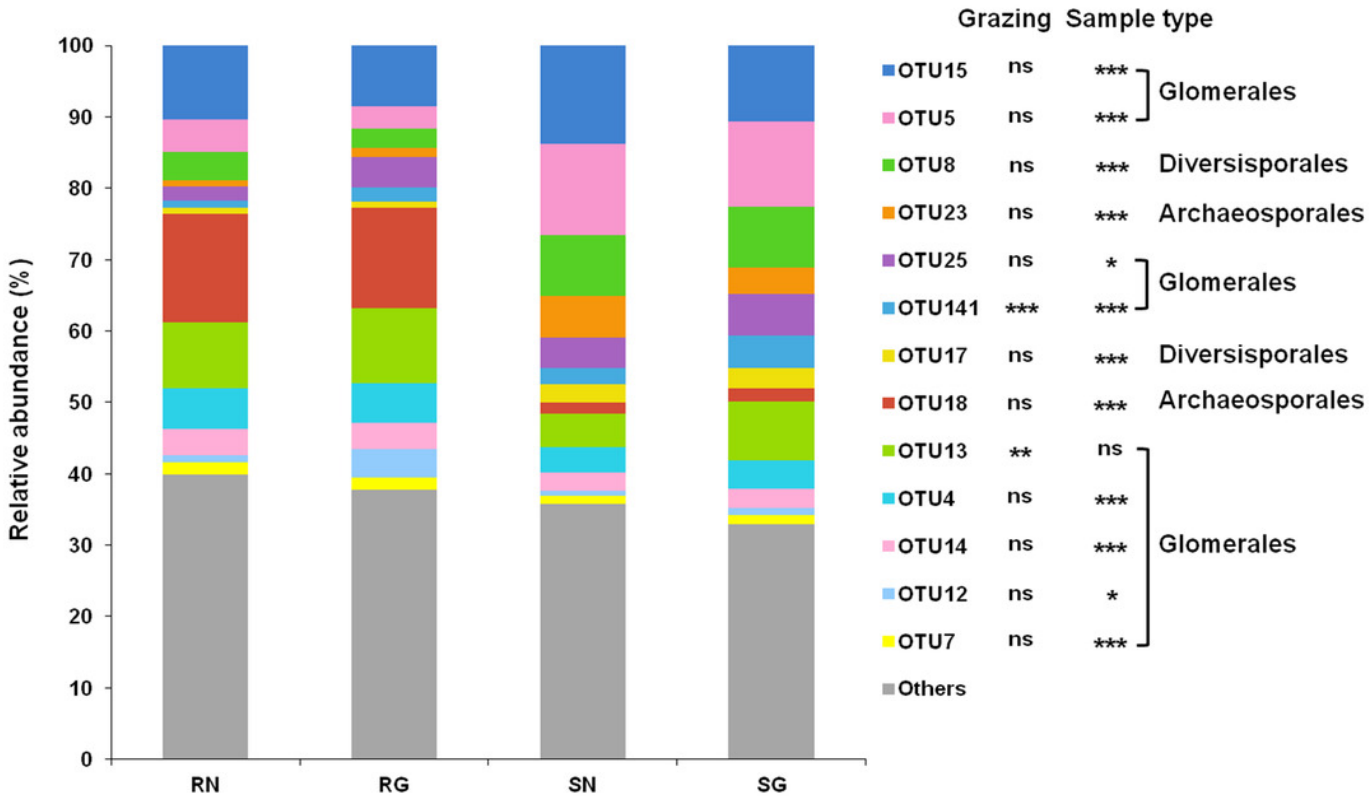

Figure 4

Relative abundance of arbuscular mycorrhizal (AM) fungi at the order level in soil and roots in grazing and non-grazing treatments.

Fig. 4. Relative abundance of arbuscular mycorrhizal (AM) fungi at the order level in soil and roots in grazing and non-grazing treatments. Kruskal-Wallis test showed the effect of grazing and sample type (soil and root) on the relative abundance of AM fungal orders (ns; $P \geq 0.05$, *** $P < 0.001$). Different letters are significantly different at $P < 0.05$, as indicated by Conover's test. SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG, root grazing.

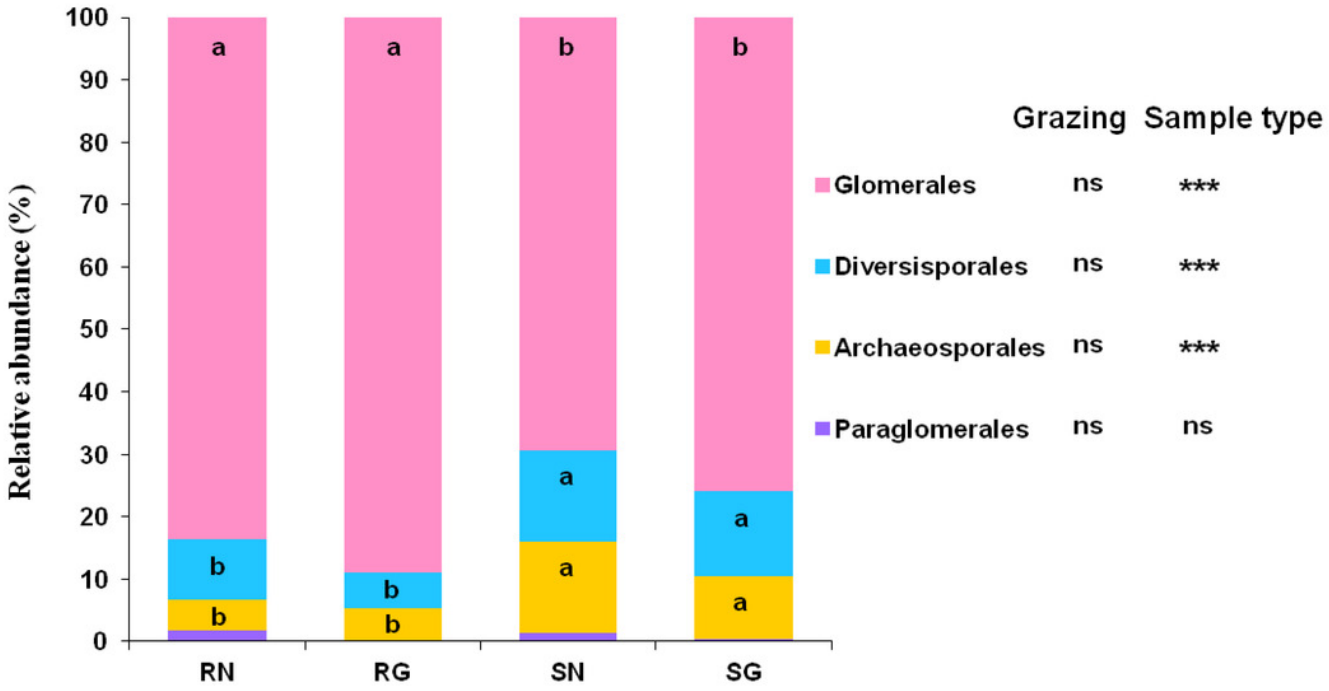

Figure 5

Redundancy analysis (RDA) biplots showing arbuscular mycorrhizal (AM) fungal community composition.

Fig. 5. Redundancy analysis (RDA) biplots showing arbuscular mycorrhizal (AM) fungal community composition in soil and roots (a), soil (b) and roots (c). Significant soil variables were presented as vectors on the RDA biplot graphs using the 'envfit' (based on 999 permutations) at $P < 0.05$. SN, soil non-grazing; SG, soil grazing; RN, root non-grazing; RG, root grazing; N, soil total nitrogen; C, soil total carbon; P, soil total phosphorus.