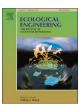
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Research Paper

The linkage between vegetation and soil nutrients and their variation under different grazing intensities in an alpine meadow on the eastern Qinghai-Tibetan Plateau



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ABSTRACT

The grassland degradation caused by overgrazing is the result of imbalance of energy flow and material cycle under grazing pressure in the ecosystem, and numerous ecological restoration and ecological engineering techniques widely used to restore some ecosystem. However, little research known focused on the relationship (energy flow and material cycle) between plant and soil under different grazing intensity, especially at the rhizosphere which is the most powerful area of plant and soil interaction. In this research we conducted a 5-year grazing experiment including 4 different grazing intensities (no grazing, UG; light grazing, LG; moderate grazing, MG: and heavy grazing, HG) in an alpine meadow on the northeastern margin of Oinghai-Tibetan Plateau (OTP). Plants and soil materials were sampled in July 2015, and to examine the nutrients concentration and ecological stoichiometric of vegetation and soil, the soil microbial biomass and activity, as well as their relationship with vegetation and soil characteristics. We found that grazing increased vegetation and soil nutrient concentrations and increased the ratio of microbial biomass carbon (MBC) to microbial biomass nitrogen (MBC), but the grazing intensity did not significantly influence the nutrients enrichment ratio of rhizosphere soil. There was significant relationship between total carbon (TC) concentration of vegetation and soil, and between vegetation total phosphorus (TP) and soil PO₄ 3--P concentration. These results suggested that vegetation and soil nutrient concentrations respond differently to the grazing intensity. Coupling relationship exists in specific nutrients of vegetation and soil and rhizosphere is a powerful tool to understanding the linkage between plant and soil.

1. Introduction

Livestock grazing is not only the main land use from, but also the human distrbution of grassland (Luo et al., 2009; Yang et al., 2016; Zhou et al., 2006) which influence the ecosystem structure and energy flow and nutrients (material) cycle (Gao et al., 2009; McNaughton et al., 1988). It is widely known that overgrazing contributes the grassland degradation (Concostrina-Zubiri et al., 2016; Dong et al., 2013; Listopad et al., 2017; Yang et al., 2016), and which is the result of imbalance of energy flow and material cycle in the grassland ecosystem (Wang et al., 2013) and performmance as the output of grass and animal products from the grassland ecosystem reduced the grassland

nutrients content and soil fertility, even affect the growth of grass (Wang et al., 2013). A micro-cell zone of soil adjacent to plant roots where the root activity significantly affects the soil biological properties and plays a significant role in plant health and soil fertility called rhizosphere (Chaudhary et al., 2015; Zhang et al., 2012), is the most powerful area of plant and soil interaction and affect the energy flow and material cycle of ecosystem (Zhang et al., 2004, 2012).

Rhizosphere is different from the bulk soil in physical, chemical and microbiological characteristics, including the soil pH, cation-exchange capacity, nutrient availability and content, microbial biomass, turnover, activity and community structure, and enzyme activity (Chaudhary et al., 2015). Functionally, rhizosphere cycles the

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nutriments between the plant and environment through the roots releasing carbon (C) into soil as root exudation, and interaction of plant and mycorrhizal fungi (Zhang et al., 2010). For example, plant roots release a part of photosynthate into soil in the form of root exudation, which is the important source of soil C (Chaudhary et al., 2015; Zhang et al., 2010; Zhang et al., 2012). Since the rhizosphere processes are a critical bottleneck controlling nutrient transformation and nutrient flow from soils to plants (Zhang et al., 2010), they are often used to understand the influence and feedback between the plant community and belowground soil (Bell et al., 2014; Zhang et al., 2012). Researchers also use ecological stoichiometric (element ratio of C, nitrogen (N) and phosphorous (P)) as a powerful tool for understanding the plant and soil interaction (Fan et al., 2015; Yang et al., 2015; Zechmeister-Boltenstern et al., 2015), especially that at the rhizosphere (Bell et al., 2014).

The Qinghai-Tibetan Plateau (QTP, average elevation 4000 m a.s.l.) is the largest plateau in the world but a relatively fragile system (Chen et al., 2014; Feng et al., 2010; Zhang et al., 2015). As its dominant vegetation type (more than 40%), alpine meadow provides fundamental sources of livelihood for local residents and ecosystem services to the human population living downstream (Zhang et al., 2016). Recently, this important ecosystem has degraded (25% of the total area of this region), resulting in serious social and ecological problems (Dong et al., 2013; Feng et al., 2010; Zhang et al., 2016). To explore the management practices for sustainable development of degraded alpine meadows, numerous difference appropriative ecological restoration and ecological engineering techniques widely used in this area according the actual situation of different alpine meadows (Benayas and Bullock, 2009; Jaunatre et al., 2014), such as fencing to exclude grazers (the retire livestock and restore grassland program) (Shi et al., 2010; Wu et al., 2010), re-vegetation and reestablishment (Feng et al., 2010; Wang et al., 2006), soil fertilization (Ma et al., 2014) and other methods. The previous studuies are mainly focused on the response of plant and soil to livestock grazing (Dong et al., 2013; Gao et al., 2007, 2009; Shi et al., 2010; Yang et al., 2016) or other animals (Zhang et al., 2016) and the effective measures to restore the degraded meadow (Feng et al., 2010; Ma et al., 2014; Wu et al., 2010) in the alpine meadow in the QTP. Howere, little in known about the linkage between plant and soil under different grazing intensity in the QTP region. In order to prevent and treat the degradation of the alpine meadows in the QTP, we need to recognize the effect of grazing on the interaction between plant and soil of this special ecosystem. In this study, we examined with 4 different grazing treatments the differences in vegetation and soil nutrients, soil elements ratios, and their relationships. We hypothesized a correlation between concentrations of C, N and P both in plant and soil, respectively. The objectives of this study were (i) to determine the variations of vegetation (shoots and roots) and soil (rhizosphere and bulk) nutrients; (ii) to examine the variations of soil (rhizosphere and bulk) microbial biomass and activity; (iii) to seek the relationship between vegetation and soil nutrients; and (iv) to recognize the role of rhizosphere in the nutrients cycle under different grazing intensity.

2. Materials and methods

2.1. Study site and experimental design

We conducted the study in an alpine meadow located in the Mamo pasture of Amu Township (Science and Technology Park of Sichuan Academy of Grassland Science) in Hongyuan County, in the northwest of Sichuan Province and on the northeastern margin of QTP, China (Latitude $34^\circ54'$ N , Longitude $102^\circ06'$ E and 3480 m a.s.l.; Fig. 1). In the period of 1980-2010, the mean annual temperature in this area was $1.7\,^\circ\text{C}$; the annual precipitation was $746\,\text{mm}$ (data from the Chinese National Meteorological Information Center, http://www.nmic.gov.cn/ . Precipitation data included monthly accumulation of rain and snowfall). According to the Chinese soil taxonomic classification, the typical

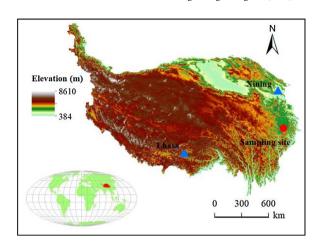


Fig. 1. Location of study area in Qinghai-Tibetan Plateau.

soil type is alpine meadow soil.

We designed 4 different grazing intensity treatments (no grazing, UG, with forage utilization rate 0%; light grazing, LG, forage utilization rate of 30%; moderate grazing, MG, forage utilization rate of 50% and heavy grazing, HG, forage utilization rate of 70%), based on factors including acreage, aboveground biomass, theoretical intakes of yak and grazing time. The UG area was $1.6~\rm hm^2$, LG $4.2~\rm hm^2$, MG $2.5~\rm hm^2$, and HG $1.9~\rm hm^2$; the grazing intensity was 0, 0.7, 1.2 and 1.6 yaks hm $^{-2}$, respectively (Table S1); 3 replicates experiment areas for each of LG, MG and HG treatments and 1 experiment areas for UG were randomly designed. The female yaks (body weight about 200 kg) were left to graze in the corresponding experiment plots from May to October 2010–2015 (153 days every year, 24 h a day).

2.2. Sampling and measurement

In July 2015, when the aboveground biomass peaked, we used random sampling method to ensure representative sampling from every treatment (Chaudhary et al., 2015). We randomly chose 3 plots from each of LG, MG and HG treatments, each plot $(1 \text{ m} \times 1 \text{ m})$ at least 20 m away from each other. For UG, 9 plots $(1 \text{ m} \times 1 \text{ m})$ at least 10 m from each other were randomly selected, every 3 plots being regarded as a replicate. Therefore all together 36 plots of $1 \text{ m} \times 1 \text{ m}$ were selected for sampling. Plants in each sampling plot were dug up at the depth of 15 cm and transported to the laboratory to collect the bulk and rhizosphere soil by shaking the plants. The soil not tightly adhering to the roots was defined as the bulk soil (BS) and that attached to the roots as rhizosphere soil (RS) (Chaudhary et al., 2015). The soil samples were stored at -20 °C before further analyses. Then the plants were cut at root collar to separate the aboveground parts (shoots) and belowground parts (roots).

Fresh soil samples from each plot were sieved through a 2-mm mesh. Then one part was immediately analyzed for $\mathrm{NH_4}^+$ -N (ammonium nitrogen), $\mathrm{NO_3}^-$ -N (nitrate nitrogen), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and fluorescein diacetate (FDA) concentration; the other part was air-dried and ground, with part of it passing through a 0.25-mm mesh for $\mathrm{PO_4}^{3-}$ -P (available phosphorus) concentration determination, and the other part through a 0.15-mm mesh for total carbon (TC), nitrogen (TN) and phosphorus (TP) determination. The above- and belowground parts of the plants were dried to constant weight at 60 °C after belowground parts were washed, and ground to fine powder by a miller (d < 0.15 mm) for TC, TN and TP determination.

The concentration of soil $\mathrm{NH_4}^+$ -N and $\mathrm{NO_3}^-$ -N was measured with continuous flow analytical system (Auto Analyzer Bran + Luebbe, Germany) after extracting soil with 2 M KCL (m:v = 1: 10) on a shaker under ambient temperature for l h (200 r min $^{-1}$) (Ma et al., 2015). The

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