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Long-term grazing exclusion effects on vegetation characteristics, soil properties and bacterial communities in the semi-arid grasslands of China

Jimin Cheng[a,1], Guanghua Jing[b,1], Lin Wei[b], Zhaobin Jing[c,*]

[a] Institute of Soil and Water Conservation, Northwest A&F University, Yangling, Shaanxi Province, China
[b] Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resource, Yangling, Shaanxi Province, China
[c] College of Horticulture, Northwest A&F University, Yangling, Shaanxi Province, China

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ABSTRACT

Grazing exclusion is regarded as an effective way to restore degraded grasslands. However, it remains unclear if grazing exclusion could improve soil bacterial communities and how the soil bacteria affect soil organic carbon (SOC) in semi-arid grasslands over 33 years of continuous grazing exclusion. We studied the effects of 33 years of grazing exclusion on vegetation characteristics, soil properties, and the soil bacterial communities in the semi-arid grasslands. Our results showed that grazing exclusion significantly increased species diversity and richness, coverage, above- and belowground biomass and litter biomass. Total nitrogen (TN), soil organic carbon (SOC), total phosphorus (TP), soil available potassium (AK), and soil available phosphorus (AP) significantly increased. Grazing exclusion also improved the diversity and abundance of soil bacteria, which had a significant positive correlation with SOC. The dominant taxonomic groups of soil bacteria in grazed and grazing exclusion grasslands included *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Planctomycetes*, *Chloroflexi*, *Gemmatimonadetes* and *Bacteroidetes*. There was an interaction between SOC, TN, AK, AP and the relative abundances of some dominant groups. Long-term grazing exclusion had a negative effect on diversity and the abundance of soil bacteria. Our results may provide new insights for grassland management in the semi-arid regions.

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1. Introduction

Grasslands cover 20% of the terrestrial surface and play an important role in preventing soil erosion and supporting animal husbandry in semi-arid regions (Jing et al., 2013, 2014). Owing to human disturbance (especially over-grazing) and climate change, grasslands are severely deteriorating, which further accelerates soil nutrient degradation (Jiao et al., 2011), ecological function loss and social-economic damage (Jing et al., 2014). China has ~400 million hectares of various grasslands that account for approximately 41.7% of the country's land area (Ren et al., 2008). Recently, grassland degradation has become a serious problem in China (Ren et al., 2008; Jing et al., 2013, 2014). Therefore, restoration of degraded grassland ecosystems has become a central scientific issue in ecological engineering (Bai et al., 2004; Jing et al., 2013).

Grazing exclusion is regarded as the most effective method for restoring the degraded semi-arid grasslands of China (Jing et al., 2013). Many recent studies have addressed the influence of grazing exclusion on vegetation (Jing et al., 2013, 2014), soil properties (Jiao et al., 2011; Shi et al., 2013; Jing et al., 2014) and soil microbes (Jiang et al., 2009). However, these studies were based on short-term methods. Soil microbial communities play an important role in most soil nutrient transformations and influence plant diversity and productivity (Will et al., 2010). Furthermore, the variability of soil bacterial communities has been correlated with different soil properties (Lauber et al., 2008). For example, soil pH was found to determine microbial diversity and composition (Zhalnina et al., 2013), and bacterial diversity was higher in neutral soils and lower in acidic soils (Fierer and Jackson, 2006). While several studies have shown that soil bacterial structure and diversity are influenced by management practices (such as tillage, fertilization, and fire) (Mbuthia et al., 2015; Mikita-Barbato et al., 2015), there are minimal long-term effects of continuous grazing exclusion on the soil bacterial composition and activity. Therefore, understanding

* Corresponding author.
E-mail address: 353813638@qq.com (Z. Jing).
[1] Jimin Cheng and Guanghua Jing contributed equally to this study.

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the changes in the soil bacterial community is crucial for soil and vegetation restoration.

This study aimed to analyze the effects of 33 years of continuous grazing exclusion on plant diversity, productivity, soil properties and soil bacteria in the semi-arid grasslands of China. We proposed the following hypotheses: (1) long-term grazing exclusion significantly increases plant diversity, productivity, and soil nutrients and further improves soil bacterial diversity and abundance; (2) the changes in the dominant phylum of the soil bacterial community may support or limit some soil properties after 33 years of continuous grazing exclusion. To test our hypotheses, we selected the largest semi-arid grassland regions of the Loess Plateau to investigate the characteristics of vegetation and soil. This study contributes to our understanding of restoration of vegetation and soil nutrients, especially soil bacteria, and provides new insights for grassland management in semi-arid regions.

2. Materials and methods

2.1. Study area

The study area is located on the largest typical grasslands of the Loess Plateau at Yunwu Mountain (106°21′–106°27′E, 36°10′–36°17′N) in the Ningxia Hui Autonomous Region of China (Fig. 1). Yunwu Mountain has been protected as a long-term monitoring site since 1982. The temperatures range from 22 to 25 °C in July, and the mean annual temperature is 7.01 °C. The mean annual rainfall is 425.42 mm, with approximately 60%–75% of the annual rainfall falling between July and September. The cumulative temperature (≥0 °C) is 2847 °C–3592 °C, and the annual daylight hours range from 2300 to 2500. Annual evaporation ranges from 1017 to 1739 mm and the frost-free season averages 137 days (from 1981 to 2015). The vegetation types mainly include *Stipa grandis*, *S. przewalskyi*, *S. bungeana*, *Artemisia sacrorum*, and *Thymus mongolicus*. The vegetation consists of 297 plant species. Gentianaceae, *Stipa* and *Potentilla* are important plant components, and the main dominant species include *S. bungeana*, *S. grandis*, *T. mongolicus*, *A. sacrorum*, *Potentilla acaulis*, *and Androsace erecta*. The soil is a montane grey-cinnamon soil, Calci-Orthic Aridisol, according to the Chinese taxonomic system, which is equivalent to a Haplic Calcisol in the FAO/UNESCO system. The area is located at an elevation of 1800–2100 m and has a total area of 6660 ha. The grassland protection areas include core conservation areas (1000 ha), buffer conservation areas (1300 ha) and experimental areas (4360 ha). Starting in 1982, different grazing exclusion treatments were established at different times in the grassland protection areas, and no agricultural activities (such as fertilization or crop cultivation) have been carried out in these grazing exclusion regions since 1982. The climate of the study area is semi-arid within the middle temperate zone (Jing et al., 2013, 2014).

2.2. Study design and sample collection

2.2.1. Experimental design

This experiment includes two parts: grazing exclusion grasslands and grazing grasslands. An *S. bungeana* grassland, *T. mongolicus* grassland, and *A. sacrorum* grassland were established for grazing exclusion in core conservation areas in 1982. Before grazing exclusion, these grasslands were grazed by sheep, and the stocking rates were at a heavy density (>50 sheep/ha); since 1982, these grasslands have been excluded from livestock grazing. Three grazing grasslands were selected as controls in the experimental area, and these grazing grasslands have a medium density of sheep during the whole year (8 sheep/ha), and the dominant species is *S. bungeana* perennial bunchgrass.

2.2.2. Vegetation sampling and analysis

The vegetation investigations were conducted in grazing exclusion areas and grazing grasslands when aboveground biomass reached a peak value in 2015. Using the line transect method, three equal-sized replicated blocks (50 m × 10 m) were established in each grassland. Then, nine 1 m × 1 m quadrats were established with three replicates in three blocks, and a total of twenty-seven quadrats were established for grazing exclusion and grazing grasslands. The distance between quadrats was at least 15 m. In each quadrat, plant species identifications were completed in situ, and plant species numbers, plant coverage, plant height and plant biomass were measured separately for each species. The aboveground biomass was clipped and measured by drying at 80 °C for 48 h to a constant weight for each species, and the sum of all species of aboveground biomass was used to represent the whole community. According to Bai et al. (2004) methods, we classified the species into the following five plant functional groups: perennial rhizome grass (PR), perennial bunchgrasses (PB), perennial forbs (PF), shrubs and semi-shrubs (SS), and annual and biennials (AB). And Jing et al. (2013) methods were used to classify the species into four functional groups: Poaceae, Fabaceae, Asteraceae and weeds.

In this study, the richness index (R), Shannon-Wiener diversity index (H) and Evenness index (E) were calculated for grazing exclusion and grazing grasslands according to the methods of Jing et al. (2013, 2014).

The Richness Index (R) was $R = S$;

The Shannon-Wiener diversity index (H) was calculated as follows:

$$H = -\sum_{i-1}^{s} P_i \ \ln P_i$$

The Evenness index(E) was calculated as : $E = \dfrac{H}{\ln S}$

with S representing the total species number of the community, and Pi representing the relative importance value of species i.

2.3. Soil sampling and analysis

2.3.1. Soil sampling

After vegetation sampling, the topsoil litter was removed before soil sampling. The soil samples were taken at five points; that is, four corners along the diagonal and the center of the quadrat by bucket auger at three different depths: 0–20, 20–40 and 40–60 cm. Fifteen soil samples of three quadrats were mixed as a soil sample of block, and three soil samples were collected for each grassland. A total of nine soil samples per depth were collected for grazing exclusion and grazing grasslands. All samples were sieved quickly on-site through 1.4 mm mesh. The samples were divided into two parts: 20 g was transported on dry ice to the laboratory and then stored at −80 °C for DNA extraction, and the remaining parts were air-dried and used to analyze the physical and chemical properties.

2.3.2. Physical and chemical properties analysis

From each sampling quadrat, a section (1 m × 0.8 m × 1.2 m) was dug to measure the soil bulk density (BD), and the soil cores were dried at 105 °C for 24 h and then weighed for calculating BD. All of the soil was air-dried and passed through a 1.4 mm sieve for soil nutrients analysis. The soil pH was measured using a 1:5 soil/water suspension. The soil organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), and available potassium (AK) were analyzed according to the methods of Jing et al. (2014).

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