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Interactions of soil bacteria and fungi with plants during long-term grazing exclusion in semiarid grasslands

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ABSTRACT

Microbial succession has been extensively investigated during the restoration of degraded environments, but the interactions of microbes with plants and soils have not been well documented. We examined changes in the plant communities, soil variables, and microbial communities of grasslands after different periods of grazing exclusion (0, 10, 25, and 35 y) on the Loess Plateau in China. The microbial communities were characterized based on their biomass, enzymatic activities, quantity of functional microbes, and composition using high-throughput sequencing. Grazing exclusion increased the plant diversity, above- and belowground biomass, organic carbon content, total nitrogen content, microbial biomass, enzymatic activities, abundance of ammonia-oxidizing microbes, and diversities of the bacterial and fungal communities; however, the highest values of these variables occurred at the 25-y exclusion site and subsequently declined, indicating that long-term exclusion could have a negative effect on this grassland. Decreases in the abundances of *Alphaproteobacteria* and *Leotiomycetes* and increases in *Acidobacteria* and *Sordariomycetes* along the chronosequence indicated different successional patterns in the microbial communities. The patterns of change in the composition and diversity of the plant, bacterial, and fungal communities suggest that plant and bacterial succession occurred in parallel and proceeded faster than fungal succession. Indicators of the bacterial and fungal communities, including their biomass, enzymatic activities, and community composition and diversity, were affected by the plant diversity and organic carbon, total nitrogen, and nitrate nitrogen contents. Fungal succession was also susceptible to changes in the soil moisture content. These results suggest that plant diversity plays an important role in shaping the microbial communities, likely by altering the levels of soil nutrients and moisture.

1. Introduction

An estimated 30% of the terrestrial area around the world is arid or semiarid, and these regions offer many ecosystem services (Zhan et al., 2007). Many grasslands in these regions have been degraded and are gradually disappearing due to anthropogenic interference, especially overgrazing (Slimani et al., 2010). Nearly 4 million km^2 of grassland in China covers > 40% of the land area (Ren et al., 2008), and approximately 90% of the grasslands have been degraded by long-term livestock overgrazing and overexploitation, which has become a serious environmental problem for the Chinese government (Cheng et al., 2016). Restoring degraded grasslands has been a serious concern in recent decades (Bai et al., 2004; Simmons et al., 2007; Jing et al., 2014). Microorganisms are important contributors to the structure and function of ecosystems, so addressing microbial successional patterns and their interactions with plants and soils is essential for increasing our understanding of the mechanisms of restoration, improving our capacity to predict the responses of ecosystems to human disturbance, and optimizing the design of large-scale restoration projects (Kardol et al., 2013).

The exclusion of grazing is an efficient approach for restoring degraded semiarid grasslands in China (Cheng et al., 2016). Grassland restoration is a long-term and complex process (Millard and Singh, 2010; Zeng et al., 2017a; Zhang et al., 2016) and mainly focuses on three essential elements: vegetation structure, soil variables, and microbial communities (Liu et al., 2018; Fry et al., 2016). Plants affect microbial communities during restoration and drive changes in soil physicochemical properties through the decomposition of litter, the turnover of roots, and root exudation (Haichar et al., 2008). Soil microorganisms in turn can either positively or negatively affect plant growth through the maintenance and transformation of soil nutrients, which further influence the plant-community composition (Philippot et al., 2013, van der Putten et al., 2013). Interactions between plants and soil microbes may therefore have important consequences for the

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Table 1
Geographical characteristic of study sites. The soil of all the sites is a montane gray-cinnamon soil.

Sites	Latitude (N)	Longitude (E)	Altitude (m)	Slope gradient(∘)	Slope aspect	Dominant species
GE0	36°17′06″	106°23′28″	2017	18	E26°N	*Potentilla bifurca* Linn., *Stipa przewalskyi* Roshev., *Carex tangiana* Ohwi.
GE10	36°16′57″	106°23′28″	2034	20	E38°N	*Leymus secalinus* Tzvel., *Carex tangiana* Ohwi, *Stipa grandis* P. Smirn.
GE25	36°16′31″	106°23′27″	2070	18	W21°N	*Stipa grandis* P. Smirn. *Artemisia sacrorum* Ledeb, *Oxytropis bicolor.*
GE35	36°15′05″	106°23′10″	2071	21	E29°N	*Stipa przewalskyi* Roshev., *Carex tangiana* Ohwi.

dynamics of plant communities, becoming determining factors of community assemblages and ecosystem functioning (Kardol et al., 2013). Previous studies of the effect of grazing exclusion on degraded grassland, however, have almost exclusively focused on the dynamics of plant communities (coverage, biomass, and diversity) (Jing et al., 2013) and soil variables (nutrient levels, aggregates, and enzymes) (Cheng et al., 2016), and the few studies of soil microorganisms have focused only on microbial activity and community composition (Zeng et al., 2017a; Hu et al., 2017). The relationships between aboveground plant communities and belowground microbial communities are thus poorly understood. Experimental evidence of predictable patterns in microbial community structure during the natural restoration of grassland where grazing has been excluded is lacking, particularly compared to the amount of evidence for plant community patterns.

Bacterial and fungal communities are important functional groups in soils that play different roles in regulating ecosystem function and soil biogeochemistry, and these communities are differentially affected by environmental factors (Sun et al., 2017; Geisseler and Scow, 2014; Rinnan and Bååth, 2009). Bacteria are regarded as important mediators of the rapid pathways of carbon cycling in soil, and their growth tends to be approximately 10-fold greater than that of fungi (Rousk and Bååth, 2007). Fungi generally have more symbiotic relationships with plants, and their dispersal may be more limited because of their larger size (Schmidt et al., 2014). Given the differences in phenotype, phylogeny, and life history between bacteria and fungi, a comparison of their patterns of succession and their responses to plants and soils should enhance our understanding of the responses of various components of soil microbial communities to ecosystem restoration and help our assessment of the environmental impacts of land-use changes.

In the present study, four grasslands that had experienced different periods of grazing exclusion (0, 10, 25, and 35 years) in a typical semiarid area, the Chinese Loess Plateau, were selected to investigate the effects of long-term exclusion on the above- and belowground communities. We hypothesized that (i) grazing exclusion is beneficial for restoring degraded grassland, including the aboveground productivity (biomass), soil nutrient level, and microbial activity and diversity; (ii) there are different succession patterns between the plant and microbial communities, and bacterial succession likely proceeds more rapidly than fungal succession; and (iii) plant diversity plays a significant role in structuring the bacterial and fungal communities. To test these hypotheses, we evaluated the changes in plant communities, soil variables, and microbial communities (bacteria and fungi) and determined their relationships along a 35-year grazing-exclusion chronosequence. The microbial community compositions were analyzed by sequencing the bacterial 16S ribosomal RNA (rRNA) and a fungal internal transcribed spacer (ITS) gene. The functional microbes involved in nitrification, denitrification, and N_2 fixation were determined using real-time quantitative PCR.

2. Materials and methods

2.1. Study sites

Our experiment was conducted in the largest grassland on the Loess Plateau, which occurs in the Yunwushan National Natural Grassland Protection Zone (106°21′–106°27′E, 36°10′–36°17′N) in the Ningxia Autonomous Region of China. This region has been protected since 1982 to monitor the long-term restoration of degraded grassland. The climate in this area is semiarid, with a mean annual precipitation of 425 mm, > 60% of which falls from July to September. The mean annual temperature is 7 °C, with an average minimum of −8.2 °C in January and an average maximum of 25.2 °C in August. The soil is a montane gray-cinnamon soil.

2.2. Experimental design and sampling

We studied four grassland sites along a chronosequence of grazing exclusion in August 2017, when the aboveground biomass was the highest. Three of these sites have not been grazed by livestock, allowing natural restoration, since 1982, 1992, and 2007, corresponding to grazing exclusion for 35 year (GE35), 25 y (GE25), and 10 y (GE10), respectively. Meanwhile, the remaining site has been continuously grazed (4 sheep/ha) throughout the year and served as a reference (GE0). Before grazing exclusion, all the investigated sites had been intensively grazed (> 60 sheep/ha) (Jing et al., 2014). The sites have similar soil types, altitudes, slope gradients, slope aspects, and previous management practices (Table 1). Three 50 m × 100 m plots were established in each site and were separated by 80–100 m. Soil samples were collected from the top 20 cm of the soil profile using an auger (5 cm in diameter and 20 cm long) after the litter layer was removed. Ten soil cores were collected from each plot along a sigmoidal transect and then combined to make one sample. Roots, stones, litter, and debris were removed, and each bulked sample was divided into three subsamples. One subsample was immediately stored at −80 °C for DNA extraction, another was stored at 4 °C for the determination of microbial biomass and enzymatic activities, and the third was air dried for physicochemical analysis. Five 1 × 1 m subplots were randomly established within each plot for the measurement of vegetation coverage, above- and belowground biomass, maximum/mean height, and number of species. The Shannon-Wiener index ($H = -\Sigma P_i \ln P_i$) was used to estimate the diversity of the plant communities, where P_i is the ratio of the number of each species to the total number of all species. The aboveground biomass was determined by drying the aboveground tissues, including the shoots, leaves, and litter, at 60 °C for 36 h. The belowground biomass was measured by washing the roots in distilled water and then drying them at 60 °C for 36 h.

2.3. Analysis of soil physicochemical properties

The amount of soil organic carbon (OC) in the samples was determined using dichromate oxidation. The total nitrogen (TN) content was measured using an automatic Kjeldahl instrument (Kjeltec 8400, FOSS Corporation, Denmark). The available phosphorus (AP) content was determined using the Olsen method. The NH_4^+-N and NO_3^--N contents were determined using a continuous-flow auto-analyzer (Alpkem, OI Analytical, USA) after sample extraction with 2 M KCl at a soil: KCl ratio of 1:5. The soil pH was measured using a 1:2.5 soil: water mixture. The soil moisture was measured using the oven-drying method.