Effects of long-term fencing on soil microbial community structure and function in the desert steppe, China Postprint

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Abstract

One of the goals of grazing management in the desert steppe is to improve its ecosystem. However, relatively little is known about soil microbe communities in the desert steppe ecosystem under grazing management. In this study, we investigated the diversity and aboveground biomass of Caragana korshinskii Kom. shrub communities in long-term fencing and grazing areas, combined with an analysis of soil physical-chemical properties and genomics, with the aim of understanding how fence management affects plant-soil-microbial inter-relationships in the desert steppe, China. The results showed that fence management (exclosure) increased plant diversity and aboveground biomass in C. korshinskii shrub area and effectively enhanced soil organic carbon (233.94%), available nitrogen (87.77%), and available phosphorus (53.67%) contents. As well, the Shannon indices of soil bacteria and fungi were greater in the fenced plot. Plant-soil changes profoundly affected the alpha- and beta-diversity of soil bacteria. Fence management also altered the soil microbial community structure, significantly increasing the relative abundances of Acidobacteriota (5.31%-8.99%), Chloroflexi (3.99%-5.58%), and Glomeromycota (1.37%-3.28%). The soil bacterial-fungal co-occurrence networks under fence management had higher complexity and connectivity. Based on functional predictions, fence management significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions and decreased the relative abundance of bacteria with nitrate and nitrite respiration functions. The relative abundances of ecologically functional fungi with arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and saprotrophs also significantly increased under fence management. In addition, the differential functional groups of bacteria and fungi were closely related to plant-soil changes. The results of this study have significant positive implications for the ecological restoration and reconstruction of dry desert steppe and similar areas.

Full Text

Preamble

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Effects of Long-Term Fencing on Soil Microbial Community Structure and Function in the Desert Steppe, China

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Abstract

One of the primary goals of grazing management in desert steppe ecosystems is to improve their overall ecological functioning. However, relatively little is known about how soil microbial communities respond to grazing management practices in these environments. This study investigated plant diversity and aboveground biomass of *Caragana korshinskii* Kom. shrub communities in long-term fenced and grazed areas, combined with analyses of soil physicochemical properties and microbial genomics, to understand how fencing management affects plant-soil-microbial interactions in China’ s desert steppe. Our results demonstrated that fencing (exclosure) significantly increased plant diversity and aboveground biomass in *C. korshinskii* shrublands, while substantially enhancing soil organic carbon (by 233.94%), available nitrogen (by 87.77%), and available phosphorus (by 53.67%) contents. Additionally, the Shannon diversity indices for both soil bacteria and fungi were greater in fenced plots. Changes in plant and soil properties profoundly influenced the alpha- and beta-diversity of soil bacteria. Fencing management also altered soil microbial community structure, significantly increasing the relative abundances of Acidobacteriota (from 5.31% to 8.99%), Chloroflexi (from 3.99% to 5.58%), and Glomeromycota (from 1.37% to 3.28%). The soil bacterial-fungal co-occurrence networks under fencing exhibited higher complexity and connectivity. Functional predictions revealed that fencing significantly increased the relative abundance of bacteria with nitrification and nitrate reduction functions while decreasing those with nitrate and nitrite respiration functions. The relative abundances of ecologically functional fungi, including arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and saprotrophs, also increased significantly under fencing. Furthermore, differential functional groups of bacteria and fungi were closely related to plant-soil changes. These findings have important positive implications for ecological restoration and reconstruction of arid desert steppe and similar ecosystems.

Keywords: desert steppe; fence management; *Caragana korshinskii*; soil physicochemical property; soil microorganism

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1 Introduction

Desert steppe—the transitional zone between typical steppe and desert—is an important ecosystem unique to Eurasia with a long history of livestock grazing [?, ?]. In desert steppe ecosystems characterized by relatively simple plant species richness and community structure, high-intensity grazing exacerbates environmental pressure on plant communities [?, ?]. To alleviate this threat and mitigate negative impacts on grassland ecosystems, the Chinese government has implemented a series of ecological protection policies and restoration projects since the late 1990s and early 2000s. For example, the Returning Pasture to Grassland project initiated in 2003 employs grazing bans and rotational grazing to reduce pressure on natural grasslands [?, ?]. Subsequently, the Grassland Ecological Conservation project launched in 2011 aimed to mitigate grassland degradation and increase vegetation biomass [?]. These ecological engineering projects have positively impacted plant community structure and soil physicochemical properties, contributing to grassland ecosystem restoration [?, ?].

Researchers generally agree that improving grazing management systems is the principal strategy for revegetating and reconstructing arid and semi-arid grassland ecosystems [?]. Limiting selective foraging by herbivores directly alters light availability for subcanopy species, which in turn affects plant community structure and composition [?]. However, studies have shown inconsistent responses of species richness to fencing. For instance, long-term fencing of grasslands in the Mu Us Sandy Land, China, increased vegetation cover and Shannon-Wiener diversity index but decreased plant species richness [?]. Conversely, in shrub-dominated grasslands of Inner Mongolia Autonomous Region, China, long-term fencing increased both Shannon-Wiener diversity and species richness [?]. The responses of plant community diversity and richness to fencing in desert grassland ecosystems dominated by xerophytic shrubs warrant further investigation.

In desert steppe ecosystems, soil physicochemical properties have long been recognized as important factors influencing vegetation cover. Livestock trampling destroys soil surface crusts and aggregates while increasing soil erodibility [?]. Soil nutrient loss is further exacerbated by external forces such as wind [?]. Additionally, grazing-induced vegetation degradation reduces the quantity and quality of aboveground plant litter input, adversely affecting soil physicochemical properties [?]. Fencing typically increases vegetation cover in arid and semi-arid grasslands, reduces evapotranspiration, and facilitates soil water storage [?]. Removing animal feces and urine can also effectively reduce soil pH [?]. Furthermore, fencing protects both aboveground and belowground plant parts, enhancing plant-soil interactions that facilitate soil organic carbon accumulation and improve soil nutrient pools such as available nitrogen and phosphorus [?].

Soil microorganisms constitute a vital component of ecosystems and serve as an important medium for nutrient and information transfer between plants and the environment [?]. Studies have shown that fencing-induced changes in plant community structure and soil physicochemical properties also affect soil microbial community structure and function [?, ?]. For example, fencing significantly increases the abundance of soil bacteria and fungi in desert grasslands [?] and alters the relative abundances of Actinobacteria, Proteobacteria, and Ascomycota [?]. Additionally, fencing effects on soil moisture, pH, and nutrients create ecological niches for belowground microbial communities [?]. Microbial network relationships can further explain these changes, as more stable and connected networks facilitate niche occupation by bacteria and fungi and play crucial roles in maintaining microbial community stability [?, ?]. For instance, fencing increases soil microbial nitrogen utilization efficiency, reduces soil N_2O loss [?], and increases the relative abundance of functional fungal groups [?]. However, the responses of soil bacterial and fungal functional groups to fencing in desert grasslands remain insufficiently investigated.

Many grassland management studies have focused on the synergistic changes and responses of plant-soil-microorganisms under different grazing regimes. Given the importance of soil microbial communities in ecosystem restoration, we focused on *Caragana korshinkii* Kom. shrublands on the Ordos Plateau in northern China, located between the Helan Mountains to the west and the Mu Us Sandy Land to the east. *C. korshinkii* is the dominant species for ecological restoration of desert grasslands and possesses wind prevention and drought resistance characteristics [?]. Through investigation of plant communities, analysis of soil physicochemical properties, and high-throughput sequencing of bacterial 16S ribosomal RNA (rRNA) genes and fungal internal transcribed spacer (ITS) regions, we addressed two key questions: (1) whether changes in plant community structure and soil physicochemical properties under fencing management positively affect ecological niche competition among soil microorganisms; and (2) how the potential functions of soil bacteria and fungi respond to fencing management.

2.1 Study Area and Experimental Design

The study area was located in Otog Front Banner, Inner Mongolia Autonomous Region, China (37°43 -37°55 N, 107°21 -107°38 E; elevation 1300–1350 m a.s.l.; Fig. 1 [Figure 1: see original paper]), characterized by a typical temperate continental climate. Sampling was conducted in late June 2021. *C. korshinskii* was planted around 2004, and fences were installed in 2014. Nine study plots (5 m × 5 m) were established inside and outside the fenced enclosures, with samples spaced more than 50 m apart. Plant diversity and richness were assessed using the per-tree survey method. The studied plant species included *C. korshinskii*, *Achnatherum splendens* (Trin.) Nevski, *Stipa glareosa* P.A. Smirn., *Lespedeza potaninii* Vass., *Cynanchum komarovii* Al., and *Sophora alopecuroides* L.

2.2 Soil Sample Collection

Soil samples were collected in August 2022. Within the study area, nine grazing plots (5 m × 5 m) and nine fencing plots (5 m × 5 m) were delineated. Each quadrat was further divided into five sub-samples using the five-point sampling method, which were then mixed to form one composite soil sample (Fig. S1). A total of 18 soil samples were obtained. The sampling depth was 0–20 cm, with litter removed from the surface and plant roots avoided during collection [**?**]. Samples were immediately placed in an icebox and transported to the laboratory [**?**]. Fresh soil samples were sent to Novogene Bioinformatics Technology Co., Ltd., Beijing, China, and stored at low temperature for subsequent bacterial and fungal analyses.

2.3 Analysis of Soil Physicochemical Properties

Air-dried soil was used to determine physicochemical properties. Soil samples were sieved and weighed to measure soil water content (SWC). Ten grams of soil were placed in a beaker with 50 mL deionized water, and a conductivity meter (DDS-307A, LEICI Scientific Instruments Co., Ltd., Shanghai, China) was used to determine soil electrical conductivity (EC), while a pH meter (PHS-3C, LEICI Scientific Instruments Co., Ltd., Shanghai, China) measured soil pH [**?**]. Air-dried soil samples were analyzed using an Elementar Vario Macro cube to determine total carbon (TC) and total nitrogen (TN) contents. Total phosphorus (TP) was measured using the alkaline potassium persulfate digestion method [**?**]. Soil organic carbon (SOC) content was determined by dichromate oxidation, available nitrogen (AN) by alkali hydrolysis, and available phosphorus (AP) by ammonium molybdate colorimetry [**?**, **?**].

2.4 Polymerase Chain Reaction (PCR) Amplification and Sequence Processing

Soil DNA was extracted from 18 soil samples using the hexadecyl trimethyl ammonium bromide (CTAB) method. The bacterial V3-V4 gene region and fungal internal transcribed spacer-1 (ITS-1) were amplified using primers (341F, 806R, ITS1F, and ITS2) [?, ?]. After PCR amplification, products were extracted from 2% agarose gel and purified for quantification [?, ?]. The NEXTflex™ Rapid DNA-Seq Kit (Bioo Scientific, Austin, USA) was used to construct the paired-end (PE) library, which was sequenced on a NovaSeq PE250 platform. The Trimmomatic method was used for quality control of raw MiSeq data, which were then merged using FLASH v.1.2.11 [?]. UPARSE v.7.0.1090 performed operational taxonomic unit (OTU) clustering at 97% similarity [?]. Representative OTU sequences were obtained by removing chimeras during clustering. Taxonomic classification was performed using the Ribosomal Database Project (RDP) classifier compared against the Silva database (SSU128) with a confidence threshold of 0.7 [?]. The 16S rRNA and ITS gene sequences obtained in this study were submitted to the NCBI Sequence Read Archive database under accession numbers PRJNA1026058 and PRJNA1026303, respectively.

2.5 Data Analysis

Plant community indices, including Shannon-Wiener (H), Simpson (H), Pielou (E), and Patrick (R) indices [?, ?], were calculated using the following equations:

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

where P_i is the relative importance value of the ith species and S is the number of species.

Aboveground biomass (AGB; kg) was estimated using the method described by Conti et al. [?]:

$$\mathrm{AGB} = 2.281 \times \mathrm{BD}^{1.525} \times \mathrm{CD}^{0.831} \times \mathrm{HP}^{0.523}$$

where BD is ground diameter (cm), CD is crown breadth (cm), and HP is plant height (cm).

Mantel’ s test was used to describe correlations between plant community indices and soil physicochemical properties [?]. Quantitative Insights Into Microbial Ecology (QIIME) v.1.9.1 software was used to analyze microbiome alpha-diversity (OTUs, Shannon, and richness) [?]. Non-metric multidimensional scaling (NMDS) analyzed beta-diversity of bacterial and fungal communities

[?]. Phyla with relative abundance >1% were analyzed using the "ggalluvial" package in R [?]. Relative abundances of bacterial and fungal phyla were analyzed using Spearman' s test ($P<0.05$) [?]. Spearman' s coefficients were calculated for bacterial and fungal OTUs under different plots ($|r|>0.9$, $P<0.001$), and co-occurrence networks were visualized using Cytoscape v.3.7.1, with data on nodes and edges obtained for dominant groups (e.g., Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, Ascomycota) [?]. Network parameters were calculated for each OTU for subsequent analyses. Redundancy analysis (RDA) assessed fencing effects on soil physicochemical properties and microbial communities. Biogeochemical functions of bacterial communities were predicted using Functional Annotation of Prokaryotic Taxa (FAPROTAX) [?], while Fungi+Functional+Guild (FUNGuild) predicted fungal ecological functions [?]. Bacterial and fungal functional groups between plots were analyzed using one-way ANOVA and Duncan' s multiple range tests ($P<0.050$). Finally, Spearman's analysis examined correlations among plant community indices, soil physicochemical properties, and differential functional taxa [?].

3.1 Effects of Fencing on Plant Community Structure and Soil Physicochemical Properties

Fencing increased the Shannon-Wiener, Simpson, and Pielou indices of *C. korshinskii* communities in the desert steppe and significantly increased plant AGB, but had no significant effect on the Patrick index (Fig. 2a [Figure 2: see original paper]). Fencing also decreased soil pH and EC but had minimal effect on SWC. Further analysis revealed that fencing significantly increased soil TC, TN, and TP contents, with more pronounced increases in SOC, AN, and AP contents (Table 1). Additionally, fencing significantly increased SOC/TN and SOC/TP ratios. Mantel' s test showed that Shannon-Wiener, Simpson, and Pielou indices were significantly correlated with SOC/TP ratio in the grazing plot, while AGB was significantly related to EC ($P<0.050$; Fig. 2b). In the fencing plot, Shannon-Wiener, Simpson, and Pielou indices were significantly correlated with both SOC/TN and SOC/TP ratios ($P<0.050$; Fig. 2c).

3.2 Effects of Fencing on Soil Microbial Diversity

The number of OTUs and Shannon indices for bacteria and fungi increased under fencing, though the richness index showed no significant difference ($P<0.050$; Fig. 3a and b [Figure 3: see original paper]). NMDS analyses showed stress values for bacterial and fungal communities were less than 0.2000, indicating between-group differences in both communities across grazing and fencing plots, with more pronounced differences in soil fungal communities between the two treatments (Fig. 3c and d).

3.3 Effects of Fencing on Soil Microbial Community Structure

In both grazing and fencing plots, 84.36% of OTUs were annotated at the phylum level, with Actinobacteriota, Proteobacteria, Acidobacteriota, and Firmicutes as dominant bacterial phyla across all samples. Among fungi, 70.33% of OTUs were annotated at the phylum level, with Ascomycota, Basidiomycota, Mortierellomycota, and Glomeromycota as dominant phyla (Fig. 4a and b [Figure 4: see original paper]). Fencing significantly increased the relative abundances of Acidobacteriota (5.31%-8.99%) and Chloroflexi (3.99%-5.58%) while decreasing Proteobacteria (25.34%-15.24%). Fencing also significantly increased Glomeromycota abundance (1.37%-3.28%) (Fig. 4c and d).

3.4 Effects of Fencing on Soil Bacterial-Fungal Network Relationships

In both plots, OTUs from Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexi, and Ascomycota occupied large ecological niches in the network (>5% of nodes). In the grazing plot, Actinobacteria and Proteobacteria had more network nodes than in the fencing plot (Fig. 5a and b [Figure 5: see original paper]). The grazing plot contained 1,311 nodes and 2,926 edges (2,216 positive, 710 negative), while the fencing plot had 1,715 nodes and 3,706 edges (1,965 positive, 1,741 negative). Additionally, average path length and graph diameter parameters of the bacterial-fungal co-occurrence network were higher in the fencing plot (Table 2).

3.5 Effects of Fencing on Soil Bacterial and Fungal Functions

RDA of plant community indices and soil physicochemical properties revealed that RDA1 explained 82.96% of variation in soil properties, with AGB having the greatest effect ($P=0.005$; Fig. 6a [Figure 6: see original paper]). Subsequent RDA of soil microbial communities constrained by soil physicochemical properties showed RDA1 explained 45.34% of microbial community variation, with all factors except SWC and TN/TP ratio strongly correlating with microbial communities ($P<0.050$; Fig. 6b).

Functional predictions using FAPROTAX (bacteria) and FUNGuild (fungi) showed significant differences in nitrogen cycle functional taxa (e.g., aerobic ammonia oxidation, nitrification, denitrification, nitrite respiration, fermentation) between grazing and fencing treatments, while saprotrophic and ectomycorrhizal fungi dominated fungal functional groups (Table S1). FAPROTAX analysis revealed fencing significantly increased bacteria with nitrification and nitrate reduction functions while decreasing those with nitrate and nitrite respiration functions. FUNGuild predictions showed increased relative abundances of functionally important fungi such as arbuscular mycorrhizal and ectomycorrhizal fungi under fencing. Correlation analysis between differential bacterial and fungal functions with plant-soil properties showed that in the grazing plot, SOC, SOC/TN, and SOC/TP ratios were negatively correlated with pathogen-saprotroph taxa (Fig. 7a [Figure 7: see original paper]). In the fencing plot, TN/TP ratio showed negative correlations with aerobic chemoheterotrophy and chemoheterotrophy ($P<0.050$; Fig. 7b).

4.1 Soil Nutrient Accumulation in the Desert Steppe Under Fencing

Fencing represents a major driver of plant community structure and function in desert steppe ecosystems [?]. Our results showed fencing significantly increased Shannon-Wiener, Simpson, and Pielou indices and AGB compared to grazing (Fig. 2). Previous research demonstrated that fencing increases plant Shannon-Wiener and Simpson indices, effectively improving biodiversity in desert regions [?]. Zhang et al. [?] reported that grazing intensity was negatively correlated with AGB and helped maintain higher species diversity and productivity in northern China’ s desert steppe. Our findings confirm these previous results and additionally show that pH and EC were significantly lower in fenced plots (Table 1). Li et al. [?] noted that reduced AGB from grazing increased surface water evaporation, causing surface salt accumulation and increased soil pH and EC. Additionally, accumulation of herbivore urine and feces has been reported to increase soil pH [?]. Notably, Shannon-Wiener, Simpson, and Pielou indices were significantly correlated with SOC/TP ratio in both grazing and fencing plots (Fig. 2b and c). Under conditions of limited soil phosphorus, plant litter input increases SOC content [?, ?]. In this study, fencing effects on plant biomass represented one of the most important factors affecting soil physical properties.

Multiple studies demonstrate that increased plant AGB after fencing, along with enhanced litter and root exudates, contributes to soil carbon and nitrogen accumulation [?]. Similarly, our study found higher TC and TN contents in fenced plots (Table 1). Wang et al. [?] found that soil carbon and nitrogen stocks changed most significantly in the surface layer (0–30 cm) after fencing, noting that fencing effectively improves carbon and nitrogen pools in desert steppe ecosystems. Grassland productivity in northern China is known to be constrained by soil phosphorus content [?]. Our study found fencing increased soil TP content (Table 1), consistent with a meta-analysis indicating fencing can increase TP content in grasslands of arid and semi-arid northern China [?]. Fencing can thus improve carbon, nitrogen, and phosphorus contents in desert steppe surface soils.

Soil organic carbon is a major source of soil nutrients, and fencing directly affects SOC content by influencing AGB accumulation [?]. Our findings support this, showing SOC content was more significantly associated with AGB and that fencing substantially increased SOC content (Table 1). Fencing also increased AN and AP contents. Previous studies noted that soil TN and AN contents were significantly correlated after long-term fencing and that fencing effectively increased these nutrients [?]. While some reports indicate short-term fencing has less effect on soil AN, our study presents results from a long-term (20-year) exclosure experiment, which may explain the more pronounced effects. Additionally, *C. korshinskii* belongs to the legume family and thus has stronger biological nitrogen fixation capacity than typical forage species. Consequently, plant growth was further promoted in the absence of grazing, improving soil AN content [?]. Overall, fencing is an effective measure for increasing grassland productivity and improving soil fertility, particularly in the arid and semi-arid desert steppes of northern China.

4.2 Soil Microbial Community Structure in the Desert Steppe Under Fencing

Our study demonstrated that fencing significantly increased diversity of soil bacterial and fungal communities (Fig. 3). Research has shown that grassland restoration through fencing increases microbial diversity alongside plant diversity and biomass [?]. Differences between soil bacterial and fungal communities were observed in both plots, consistent with other studies [?]. Further analysis revealed that dominant soil microorganisms in desert steppe include Proteobacteria, Acidobacteria, Bacteroidetes, Firmicutes, Ascomycota, and Basidiomycota [?, ?]. Our study not only revealed changes in these dominant phyla but also found that Acidobacteriota had high explanatory power in desert steppe ecosystems. Zhang et al. [?] and Li et al. [?] noted that fencing could increase Acidobacteria relative abundance, which positively correlated with SOC, TN, available potassium, and AP.

Co-occurrence networks have been used to visualize soil microbial community responses to environmental changes [?]. We constructed bacterial-fungal co-occurrence networks for grazing and fencing plots, finding more complex network relationships under fencing. The fencing plot had more nodes and edges than the grazing plot, with greater average path length and graph diameter. Previous studies found that fencing increases soil bacterial and fungal network complexity [?], which plays a crucial role in maintaining microbial community stability [?]. Our analysis further revealed that bacterial-fungal co-occurrence networks under fencing had higher complexity and connectivity than under grazing. Research indicates that increased grazing intensity reduces network complexity, while more stable and connected networks favor improved plant production performance [?]. Therefore, soil bacterial-fungal network relationships can serve as an important basis for evaluating fencing management in desert steppe ecosystems.

4.3 Function of Soil Microbial Community in the Desert Steppe Under Fencing

Soil microorganisms play key roles in energy flow and nutrient cycling in ecosystems [?]. Our FAPROTAX predictions showed fencing significantly increased bacteria with nitrification and nitrate reduction functions while decreasing those with nitrate and nitrite respiration functions. Similarly, Zhang et al. [?] found grazing increased bacteria with denitrification functions. Notably, SOC/TN and SOC/TP ratios correlated with nitrogen cycle taxa in the fencing plot, possibly due to increased SOC content affecting denitrification processes [?]. Other studies have highlighted grazing effects on nitrogen-cycling bacteria, which may lead to soil nutrient loss [?, ?]. Combined with plant community and soil physicochemical properties, our results clearly demonstrate that fencing positively affects soil nitrogen cycling and accumulation.

FUNGuild predictions in this study indicated that fencing enhanced the relative abundance of fungi with ecological functions such as arbuscular mycorrhizal and ectomycorrhizal fungi (Table S1). Arbuscular mycorrhizal fungi are ubiquitous, specialized symbionts belonging to Glomeromycota [?]. Our results showed Glomeromycota relative abundance was significantly higher in fenced plots (Fig. 4), which may explain the increased arbuscular mycorrhizal and ectomycorrhizal fungal groups. Given the important role of mycorrhizal fungi in above- and belowground multi-nutrient cycling, we can infer that fencing increases mycorrhizal fungal abundance, thereby indirectly enhancing soil nutrient cycling [?, ?]. Additionally, fencing significantly increased saprotrophic fungal abundance. Previous studies reported that increased SOC enhances competition among saprophytic fungal groups [?]. Our study further demonstrated fencing effects on the potential relationships between saprotrophic fungi and soil physicochemical properties.

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5 Conclusions

In the context of increasing grazing disturbance, excluding livestock through fencing can restore and protect plant community structure and soil physicochemical properties in desert steppe ecosystems. Our results demonstrate that soil microbial community structure and function respond positively to fencing. The study showed that fencing improved soil conditions by increasing AGB levels in *C. korshinskii* soils. Additionally, long-term fencing markedly enhanced SOC, AN, and AP contents while increasing Shannon diversity indices for soil

bacteria and fungi. Plant-soil changes profoundly affected bacterial alpha- and beta-diversity. Grazing exclusion also altered soil microbial community structure, significantly increasing relative abundances of Acidobacteriota, Chloroflexi, and Glomeromycota. Soil bacterial-fungal co-occurrence networks under fencing exhibited higher complexity and connectivity. RDA demonstrated AGB’ s role in influencing desert steppe soil environments and the importance of SOC, AN, and AP contents for soil microbial communities. Functional predictions indicated that fencing significantly increased bacteria with nitrification and nitrate reduction functions while decreasing those with nitrate and nitrite respiration functions. The relative abundances of ecologically functional fungi, including arbuscular mycorrhizal, ectomycorrhizal, and saprotrophic fungi, also increased significantly under fencing. Furthermore, differential functional groups of bacteria and fungi were closely related to plant-soil changes. These comprehensive long-term results provide a solid basis for ecological restoration and reconstruction of arid desert steppe ecosystems.

Conflict of Interest

LI Xinrong is an editorial board member of *Journal of Arid Land* and was not involved in the editorial review or decision to publish this article. All authors declare no competing interests.

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Appendix

Fig. S1 Sampling diagram of *Caragana korshinskii* Kom. shrub in the desert steppe

Table S1 Differential functional taxa of bacterial and fungal communities of the GRA and FEN plots in the desert steppe

Functional taxa	P-value	Q-value	Interval lower limit	Interval upper limit
Aerobic ammonia oxidation Nitrification				

Functional taxa	P-value	Q-value	Interval lower limit	Interval upper limit
Denitrification				
Nitrite respiration				
Fermentation				
Aerobic chemo-heterotrophy				
Human pathogens septicemia				
Animal parasites or symbionts				
Plant pathogen				
Aromatic hydrocarbon degradation				
Aromatic compound degradation				
Aliphatic non-methane hydrocarbon degradation				
Hydrocarbon degradation				
Nitrate respiration				
Nitrate reduction				
Nitrogen respiration				
Ureolysis				
Chemoheterotrophy				
Animal pathogen-Plant pathogen-Soil saprotroph-Undefined saprotroph				

Functional taxa	P-value	Q-value	Interval lower limit	Interval upper limit
Arbuscular mycorrhizal				
Ectomycorrhizal				
Endophyte-Plant pathogen-Wood saprotroph				
Plant pathogen				

Note: SD, standard deviation; GRA, grazing; FEN, fencing.

Note: Figure translations are in progress. See original paper for figures.

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