

# Selection of r-K strategies by soil bacterial communities in response to grassland degradation

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## Abstract

Numerous studies have investigated bacterial community structure in grassland ecosystems and bacterial community responses to human management at various spatial and temporal scales; however, research on soil bacterial community assembly dynamics in the course of grassland degradation is limited. Here, the authors investigate the response and assembly processes of bacterial communities adopted in two grasslands with different degrees of degradation. Stochastic processes dominated bacterial community assembly processes in response to grassland degradation, with the bacterial diversity decreasing; however, functional gene diversity increased. Furthermore, different phyla exhibited distinct response strategies: Proteobacteria and Bacteroidetes, as r-strategists, exhibited positive responses, with increases in diversity, abundance, and niche width with an increase in grassland degradation, enhancing biodiversity and productivity; other phyla (mainly Acidobacteria) exhibited greater phylogenetic dispersion and functional redundancy, and less niche overlap, highlighting the role of K-strategy in improving community resource-use efficiency in response to resource loss in degraded grasslands. The transition from K- to r- strategy in bacterial communities following grassland degradation could help communities adapt to environmental disturbance in the form of nutrient loss. The results of the present study enhance our understanding of how nutrient loss in natural grassland ecosystems leads to shifts in bacterial community composition and assembly processes mediated by different response strategies of different phyla.

## 1. Introduction

Grasslands are rich natural resources and cover approximately 40% of the global land surface (Hu et al., 2016). Grassland host biodiversity, and participate in climate regulation, carbon (C) sequestration, water purification, and erosion control, among other functions, which are critical ecosystem services and are essential for ecological stability (Habel et al., 2013; Bengtsson et al., 2019; Lyu et al., 2020). However, due to climate change and human activity, almost a half of the grassland ecosystems are undergoing degradation (Gang et al., 2014), with subsequent biodiversity loss and ecosystem function impairment (Han et al., 2020).

Soil microbial communities regulate soil biogeochemistry and ecological function (Morin & McGrady-Steed, 2004; Yang et al., 2013). Researchers have previously attempted to elucidate the responses of microbial communities to grassland degradation, which could facilitate the restoration of degraded grassland ecosystems (Yao et al., 2018; Han et al., 2020; Raiesi & Salek-Gilani, 2020).

The diverse micro habitats and available nutrient resources in grasslands potentially influence soil microbial community structure (Maharning et al., 2009). In addition, shifts in vegetation composition and soil characteristics in the course of grassland degradation can influence soil microbial community structure and activity (Bardgett et al., 2001; Yao et al., 2018; Han et al., 2020). Plant growth and development are highly correlated with soil factors, so that plant activities can indirectly influence soil microbial community structure through their influence on soil factors (Ke et al., 2015; Chen et al., 2016; Yao et al., 2018; Widdig et al.,

2020). Furthermore, soil nutrient conditions directly influence soil microbial community structure (Yang et al., 2013; Wang, Wang, et al., 2018; Widdig et al., 2020). As elements essential for microbial metabolism, C and nitrogen (N) can regulate soil microbial community diversity and composition based on their contents in soil (Siciliano et al., 2014; Delgado-Baquerizo et al., 2016; Liu, Jiang, et al., 2020; Widdig et al., 2020). Soil C content can influence microbial biomass and indirectly influence microbial diversity, which is a major mediator of the relationship between soil microbial diversity and biomass across different biomes (Bastida et al., 2021). N accumulation in soil can cause bacterial diversity loss since some microorganisms are not adapted to nutrient-rich and acidic environments (Nie et al., 2018; Wang, Liu, et al., 2018; Liu, Jiang, et al., 2020). Additionally, soil pH could influence bacterial community diversity and composition (Zhalnina et al., 2015; Ren et al., 2018; Tan et al., 2020; Widdig et al., 2020), nutrient solubility and availability (Zhalnina et al., 2015), and bacterial interactions and biological activity (Rashid et al., 2014; Fan et al., 2018), in addition to regulating soil microbial community assembly processes (Fan et al., 2018; Tan et al., 2020; Widdig et al., 2020).

Previous studies that have investigated the influence of soil nutrient loss on soil microbial community structure under grassland degradation have concluded that soil nutrients regulate microbial biomass, diversity, and community composition in grasslands (Hu et al., 2014; Dong, Shi, et al., 2021); however, it is not clear how microbial community assembly in turn respond to grassland degradation. Some studies have suggested that the reason for the microbial community dynamics in degraded grasslands are associated with different responses of different phyla to soil nutrient loss and decrease in available substrate (Delgado-Baquerizo et al., 2016; Dong, Shi, et al., 2021); furthermore, biotic interactions such as facilitation, niche complementation, and competition, as major drivers of community assembly (Dong et al., 2019), influence the responses of microbial communities to modulate resource-use efficiency (Yu, Polz, et al., 2019).

Distinct life strategies also help microbial communities to better respond to changes in environmental conditions (Ho et al., 2017; Vadstein et al., 2018; Li et al., 2021; Wang, Zhang, Li, et al., 2021). The balance between  $r$ -selection and  $K$ -selection strategies in a community determines the productivity levels of communities and the survival of individuals (Pianka, 1970; Reznick et al., 2002; Ye et al., 2018). In addition, soil microorganisms in arid ecosystems tend to host oligotrophic communities rather than copiotrophic communities, highlighting the potential of oligotrophic microbial communities to serve as rich sources of novel functions under resource scarcity (Chen et al., 2021). In contrast, the accumulation of soil nutrients such as N promotes a more active copiotrophic community as a result of the shift in microbial phylogenetic, metabolic and catabolic responses (Fierer et al., 2012). Such studies highlight the importance of microbial life strategies in facilitating responses to environmental change. Therefore, considering the underlying mechanisms via which microbial communities respond to grassland degradation remain poorly understood (Luo et al., 2020; Dong, Shi, et al., 2021; Ren et al., 2021), more studies should explore the response strategies of communities and the dominant community assembly processes in the course of grassland degradation to elucidate the responses of microbial communities across environments with different levels of degradation (Yang et al., 2013). The results of such studies could facilitate further studies as well as grassland ecosystem management activities.

In the present study, we collected soil samples from two grasslands with different degrees of degradation and analyzed the variation in community diversity, composition, and function, in addition to niche breadth and phylogenetic turnover among different phyla across resource gradients under grassland degradation. Our specific objectives were to investigate (a) the dominant response dynamics and bacterial community assemblages in degraded grasslands and (b) the dominant response strategies among various soil bacterial taxa. The results of the present study could provide a theoretical basis for further studies on microbial dynamics in degraded grasslands and facilitate grassland management activities.

## 2. Materials and Methods

### 2.1 Sample collection

Soil samples were collected from Yudaokou prairie in Chengde city, Hebei province, China. The prairie has a

total area of 1,000 km<sup>2</sup>, with an altitude of 1200–1800 meters. It is located in a region with a cold temperate continental monsoon climate with an average annual temperature of 3°C. Our sampling was carried out in August, and the daytime temperature was 15–30 degrees. Some areas displayed obvious degradation due to overgrazing. To ensure the accuracy and repeatability of the research results, two grasslands in which vegetation cover was reduced significantly to different degrees were employed (**Figure S1**). Grassland 1 (G1) was dominated by a perennial herb species, *Bothriochloa ischaemum* (L.) Keng, whereas grassland 2 (G2) was dominated by an annual herb species, *Polygonum aviculare* L. Based on the different vegetation landforms, G1 (ca. 1400 m, 42°21' N; 117°7' E) was classified into six levels of degradation with each plot area being more than 200 m<sup>2</sup>, whereas G2 (~1200m, 42°7' N; 116°57' E) was classified into five levels of degradation (> 200 m<sup>2</sup> each) (**Figure S1**). Soil erosion and soil nutrient loss are major phenomena observed under grassland degradation (Dong, Wang, et al., 2021). Hence degradation levels were classified according to the levels of major soil nutrients (organic matter [OM], total N [TN], total phosphorus [TP]) in combination with physical appearance (**Table S1**). A lower index value indicated higher soil nutrient contents and lower degradation. The deserted regions had the highest degradation levels (“6” at G1 and “5” at G2) because of desertification and low vegetation cover. Six soil samples were collected from the top 20-cm soil layer in each degraded grassland region using the checkerboard sampling method. Each soil sample was sieved through a 2-mm mesh to remove plant debris and stones, and was then stored at -80°C for DNA extraction and at 4°C for chemical analyses. In addition, soil bulk density was determined by *in situ* sampling using the volumetric ring method, with a volume of 100 cm<sup>3</sup>.

## 2.2 High throughput sequencing and bioinformatics

Soil samples (0.5 g) were used in DNA extraction using Fast DNA Spin Kit (MP Biomedicals, Shanghai, China) according to the manufacturer’s instructions. The primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for amplification of the V4 region of the 16S rRNA gene. The amplified PCR products were sequenced on the Illumina Nova 6000 platform (Illumina, San Diego, CA, USA). Subsequently, the native sequences were quality controlled using QIIME2 v2020.11.0 (<https://qiime2.org/>). DADA2 (<https://github.com/benjjneb/dada2>) and Deblur (<https://github.com/biocore/deblur>) were used to clear low-quality sequences and to carry out noise cancellation. Afterward, the remaining sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence similarity and assigned to taxa to the species level with the SILVA Database v132 (Quast et al., 2013) as the reference database. After the deletion of the unassigned OTUs and singleton OTUs, the number of OTUs was counted and an OTU taxonomy information table was computed for further analysis.

## 2.3 Variation in bacterial community diversity and composition across grassland degradation gradients

Significant differences in soil variables and microbial alpha-diversity were calculated using one-way Analysis of Variance (ANOVA) in IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). The principal co-ordinates analysis (PCoA) and permutational multivariate ANOVA (PERMANOVA) analysis were conducted to analyze differences in microbial composition using the ‘vegan’ and ‘pairwiseAdonis’ packages in R 4.0.4 (R Core Team, Vienna, Austria). The Mantel test was carried out to determine the correlation between environmental variables and community composition using the ‘vegan’ package in R 4.0.4 (R Core Team), and the unique OTUs in each community were visualized using the ‘UpSetR’ package in R 4.0.4 (R Core Team). Furthermore, the ‘DESeq2’ and ‘ggplot2’ packages in R 4.0.4 (R Core Team) were used for the identification of OTUs with significant differences and visualization of the data, respectively.

## 2.4 Identification of bacterial biomarkers for grassland degradation

To identify the major biomarkers (in soil bacterial community) driving community assembly during the grassland degradation process, Random Forest (RF) analysis (using the ‘randomForest’ package in R) was used to calculate the correlation between OTU relative abundance (>0.1%) and soil nutrient contents (OM, TN, TP) following Z-score transformation. The top 30 OTUs with the greatest increases in mean square error (MSE), which were considered the major biomarkers, were selected to verify the role of predictors in

the process of grassland degradation.

## 2.5 Community assembly processes and habitat niche breadth

To evaluate the assembly processes of microbial communities at each sampling site, the standardized effect size measure of the mean nearest taxon distance (ses.MNTD) was calculated to estimate phylogenetic clustering degrees within each community using the ‘picante’ package in R (Purcell et al., 2007). Negative and positive ses.MNTD values denote shorter and longer nearest taxon distances within a community, respectively, than expected by chance, and the level of phylogenetic clustering or dispersion, respectively (Webb et al., 2002). We also explored differences in phylogenetic turnover between samples using the between-community version of the  $\beta$ -mean-nearest taxon distance ( $\beta$ MNTD) to investigate the underlying community assembly mechanisms (Stegen et al., 2013). Differences between observed  $\beta$ MNTD and the null expectation based on 999 randomization repeats are divided by the standard deviation (of the null expectation) to obtain the  $\beta$ -nearest taxon index ( $\beta$ NTI) (Stegen et al., 2013).  $\beta$ NTI values  $>+2$  and  $<-2$  indicated significantly greater or less phylogenetic turnover than expected by chance, respectively, as a result of the influence of deterministic selection (e.g., environmental filtering, biotic interactions, non-random diversification) in communities, while values between  $-2$  and  $+2$  indicated dominance of stochastic processes (e.g., weak selection, weak dispersal, and diversification) in community assembly (Stegen et al., 2012; Zhou & Ning, 2017).

Niche breadth was calculated using the Levin’s niche breadth index ( $B$ ) equation (Pandit et al., 2009):

$$B_j = \frac{1}{\sum_{i=1}^N P_{ij}^2},$$

where  $B_j$  represents habitat niche breadth of OTU  $j$  in a metacommunity;  $N$  is the total number of communities in each metacommunity;  $P_{ij}$  is the proportion of OTU  $j$  in metacommunity  $i$ . A high  $B$  indicates that the OTU occurs extensively and evenly along a wide range of locations, representing wide habitat niche breadth. The average  $B$ -values of all OTUs within each sampling community ( $B_{\text{com}}$ ) were calculated and indicated habit niche breadth at the community level. To evaluate the niche breadth of each phylum within a metacommunity, the average Z-scores for OTUs within each phylum were calculated on a common scale based on standard deviation units (for all taxa within the community). A microbial group with a wider niche breadth is considered more metabolically flexible in the metacommunity than other microbial groups (Luan et al., 2020). Differences in average Z-score values for each phylum in communities in different stages of grassland degradation were explored to investigate variation in niche breadth at the phylum level across different levels of degradation.

## 2.6 Bacterial community function

Phylogenetic investigation of communities by reconstructing unobserved states (PICRUSt) has been used extensively to infer approximate microbial community functions based on amplicon sequences, although it has some limitations (Douglas et al., 2020). The sequences corresponding to each OTU were compared in the Greengenes database (<http://greengenes.lbl.gov>) to obtain KEGG ortholog (KO) data (DeSantis et al., 2006). Subsequently, community function richness and diversity were calculated based on the KO prediction results, and the total number of KO genes was divided by the number of OTUs to obtain the average number of KO genes in an OTU.

## 3. Results

### 3.1. Soil properties and bacterial community composition changed with grassland degeneration

Soil nutrient contents were different among the fields in the two sampled grasslands (**Table S1**). The contents of organic matter (OM; 32.31–4.44 g/kg in G1 and 20.11– 3.04 g/kg in G2), TN; 1.79–0.22 g/kg in G1 and 1.10–0.19 g/kg in G2, TP; 0.46–0.22 g/kg in G1 and 0.46–0.22 g/kg in G2) decreased with an increase in level of degradation. The highly deserted regions had the lowest OM (2.13 g/kg in G1 and 5.02 g/kg at G2), TN (0.14 g/kg in G1 and 0.30 g/kg in G2) and TP (0.18 g/kg in G1 and 0.28 g/kg in G2) contents. In addition, as the degree of degradation increased, soil moisture content decreased; soil pH did not exhibit any variation.

Bacterial richness (3526–2930 in G1 and 3709–3021 in G2) and Shannon index (9.61–8.97 in G1 and 9.72–8.36 for G2) decreased with an increase in level of degradation; furthermore, bacterial communities in the deserted regions had the lowest levels of alpha diversity (**Table S2,  $p < 0.05$** ). Bacterial community composition varied significantly among regions with different degrees of degradation. Phylum Proteobacteria was the most dominant phylum in the communities in degraded grasslands (**Figure 1A and 1C**). In addition, the relative abundances of phyla Bacteroidetes and Firmicutes increased with an increase in the level of degradation, and had significantly negative correlations with soil nutrient concentrations (OM, TN, and TP); however, the relative abundances of other major phyla decreased with the reduction of soil nutrient concentrations in the course of grassland degradation (**Figure 1**). In addition, PERMANOVA analysis results showed significant variation in bacterial community composition among the regions with different levels of degradation (**Table S3,  $P < 0.05$** ). Bray-Curtis dissimilarity among the communities was positively correlated with OM ( $r = 0.398$  in G1 and  $0.545$  in G2), TN ( $r = 0.388$  in G1 and  $0.584$  in G2), TP ( $r = 0.460$  in G1 and  $0.482$  in G2), and TK ( $r = 0.342$  in G1 and  $0.431$  in G2) contents (**Figure S2,  $P < 0.05$** ), indicating that the soil nutrient significantly influenced bacterial community composition in the degraded grassland environments.

### 3.2 Responses of different phyla to grassland degradation

To decipher the responses of bacterial communities to grassland degradation, we explored the shifts in the abundance and species richness among the top eight phyla (relative abundance  $> 3\%$ ).

Proteobacteria, Bacteroidetes, and Firmicutes exhibited relatively high species richness values, whereas other phyla, such as Acidobacteria, Actinobacteria, Gemmatimonadetes, Planctomycetes and Chloroflexi, exhibited relatively low richness values as degradation level increased (1–5 in G1; 1–4 in G2) (**Table 1**). The decrease in species richness observed in the deserted regions (region 6 in G1; region 5 in G2) could be attributed to the substantial decrease in richness at the community level. The proportions of Proteobacteria (21.2–32.4% in G1 and 21.9–28.6% in G2) and Bacteroidetes (3.8–11.0% in G1 and 4.7–7.6% for G2) increased with an increase in grassland degradation level (**Figure 2A and 2B**), indicating that Proteobacteria and Bacteroidetes exhibited positive responses based on biodiversity in the course of grassland degradation.

Conversely, with advancement in degradation, bacterial communities had more significantly altered OTUs (**Figure S3**). After classifying the altered species into corresponding phyla, we observed that the numbers of the enriched OTUs in Proteobacteria and Bacteroidetes increased with an increase in the degradation level in the two grasslands (**Figure 2C and 2D**). On the contrary, Actinobacteria, Acidobacteria, Chloroflexi, and Planctomycetes had greater depleted OTUs than other phyla with an increase in degradation level. The results indicate that Proteobacteria and Bacteroidetes had more positive responses with regard to survival than other phyla under grassland degradation.

To determine the dominant bacterial biomarkers along the grassland degradation gradient, we conducted RF to selected the top 30 most important OTUs and classified them into phyla. Subsequently, their correlation with environmental factors was determined using Pearson correlation analysis. The major microbial biomarkers were classified into the phyla Proteobacteria, Acidobacteria, and Actinobacteria in the process of degradation (**Figure S4**). In the case of Acidobacteria, the biomarker OTUs were almost all classified into the Subgroup\_6, and were significantly and positively correlated with soil nutrient contents (OM, TN, and TP) in G2 (**Figure 2E and 2F,  $P < 0.05$** ). The Actinobacteria biomarker species in the two grassland types were largely classified into the Thermoleophilia and Actinobacteria classes, which were positively and negatively correlated with nutrient content, respectively (**Figure 2E and 2F**). Furthermore, the biomarker OTUs in Proteobacteria were mostly classified into Gammaproteobacteria in G1, and into Gammaproteobacteria and Alphaproteobacteria in G2. The abundance of biomarkers classified into Alphaproteobacteria were positively correlated with soil nutrient (OM, TN, and TP) contents in G2 (**Figure 2E and 2F**). However, Gammaproteobacteria biomarker species exhibited significant and negative correlations with nutrient contents in both grassland types (**Figure 2E and 2F**).

### 3.3 Niche breadths and niche overlap of phyla in degraded grasslands

To explain the responses of different phyla to grassland degradation, we further assessed the niche widths of

each phylum across the different degradation levels, as well as the relationships between taxon niche width and environmental variables.

First, the mean niche width at community level in each degraded region was calculated, and then the corresponding OTUs in each phylum (top eight dominant phyla) were calculated based on the Z-score-transformed abundances of all OTUs. The niche widths of all phyla in each degraded region were obtained from the average values of corresponding Z-score-transformed OTUs, and were compared among different regions. Proteobacteria and Bacteroidetes could be distinguished from other phyla because of their broader niche widths that were significantly and negatively correlated with nutrient contents (**Figure 3A, 3C and S6,  $P < 0.05$** ). On the contrary, the other major phyla (Acidobacteria, Actinobacteria, Planctomycetes, Chloroflexi, and Gemmatimonadetes) exhibited decreasing niche widths with an increase in soil nutrient contents (**Figure 3A, 3C and S6,  $P < 0.05$** ). Species with broader niche breadths were distributed widely and evenly, suggesting greater stability in response to environmental disturbance (Pandit et al., 2009; Li et al., 2019). Therefore, the greater niche breadths of Proteobacteria and Bacteroidetes indicated their high adaptability and positive responses when compared to other phyla under grassland degradation.

To evaluate variation in niche overlap within each phylum across the different grassland degradation conditions, we Z-score transformed the niche overlaps of each comparison (OTU–OTU) and compared the mean values within each phylum with an increase in degree of degradation. In the two grasslands, as degradation intensified, the mean values (Z-score) of niche overlap within Acidobacteria, Actinobacteria, Planctomycetes, and Gemmatimonadetes decreased, while the average niche overlaps of Proteobacteria and Bacteroidetes increased (**Figure 3B and 3D**), which may be partly due to changes in their species diversity, which would lead to greater species competition (**Table 1**).

The results suggest that phyla with great survivability and others with weak survivability adopted different strategies under grassland degradation. With an increase in degree of degradation, Proteobacteria and Bacteroidetes exhibited higher alpha diversity, and niche width and niche overlap; on the contrary, other phyla (mainly Actinobacteria, Acidobacteria, Gemmatimonadetes, and Planctomycetes) exhibited decreased alpha diversity, and niche width and niche overlap (**Table 1; Figure 3**), which may be attributed to the elimination of certain species with functional redundancy, which can facilitate better adaptation to grassland degradation in bacterial communities.

### 3.4 Assembly processes and phylogenetic structuring under grassland degradation

The  $\beta$ NTI values of the two grasslands were mainly between -2 and +2 (**Figure 4A and 4B**), indicating the dominance of stochasticity and weak environmental selection during community assembly across different levels of grassland degradation.

According to the results of correlation analysis, Proteobacteria and Bacteroidetes relative abundance had no significant correlation with phylogenetic clustering; furthermore, there were no significant correlations between environmental variables and ses.MNTD (**Figure 4C and 4D,  $P > 0.05$** ). However, soil nutrient contents were negatively correlated with ses.MNTD values in other phyla (**Figure 4C and 4D,  $P < 0.05$** ), indicating that soil nutrient loss decreased phylogenetic clustering in bacterial communities. Considering the decrease in the niche overlap (**Figure 3B and 3D**) and the correlation between alpha diversity and ses.MNTD of the phyla across different levels of grassland degradation (**Table 2**), the phylogenetic dispersion could have resulted from the elimination of some close phylogenetic species with functional redundancy.

### 3.5 Functional responses of bacterial community to grassland degradation

PICRUSt analysis was conducted to investigate variation in bacterial community functional structure in the course of grassland degradation. Bacterial functional gene diversity in highly degraded grassland was higher than that in slightly degraded grasslands (**Table S4,  $P < 0.05$** ). In addition, the KO gene numbers of each OTU increased significantly with the progression of degradation, and were negatively correlated with soil nutrient (OM, TN, and TP) contents (**Table S5,  $P < 0.05$** ). The results indicated that bacterial communities in degraded grassland conditions and relatively poor nutrient conditions would harbor relatively

high amounts of genetic information. In addition, genetic information harbored by species increased with an increase in ses.MNTD (**Figure 5**,  $P < 0.05$ ), indicating that phylogenetic dispersion in community assembly under grassland degradation would lead to more functional genes, which could facilitate better adaptation by communities to resource scarcity.

## 4. Discussion

### 4.1 Stochastic processes dominated community assembly under natural grassland degradation

Both deterministic and stochastic community assembly processes play important roles in shaping community composition and structure in natural ecological systems (Chase, 2010; Chase & Myers, 2011; Zhou et al., 2013). Therefore, elucidating the assembly processes of microbial community could enhance the understanding of the responses of communities to grassland degradation. However, numerous studies that emphasized the importance of environmental filtration have focused on determining the dominant environmental factors driving and structuring bacterial community structure (Feng et al., 2017; Knelman et al., 2019; Yu, Wu, et al., 2019; Liu, Graham, et al., 2020), with the assembly processes in bacterial communities in the course of grassland degradation and the community response strategies remaining poorly understood. Therefore, in the present study, the authors investigated the turnover and assembly strategies of bacterial communities in response to shifts in resource conditions under grassland degradation. Overall, the authors observed that bacterial community assembly processes in degraded grassland systems were dominated by stochastic processes (**Figure 4A and 4B**).

Numerous researchers have reported that as environmental change is prolonged, deterministic processes may become more important due to the accumulation of environmental selection; however, the above often occurs after stochastic processes have dominated the initial phase (Ferrenberg et al., 2013; Zhang et al., 2016; Liu, Graham, et al., 2020). Therefore, the stochastic processes observed in the present study could be associated with the short term and limited spatial scales applied for the sampled grasslands under degradation, and continuous environmental change could alter the dominant community assembly processes (**Figure 4A and 4B**). In addition, degree of change in environmental variables is a key factor influencing community assembly process at the regional scale (Feng et al., 2018). In the present study on a degraded grassland with low human interference, the mild changes in environmental variables could be another major reason for the dominance of stochastic processes in community assembly processes (Liu, Graham, et al., 2020).

Stochastic processes represent undetermined ecological assembly processes that yield community diversity patterns that are random (Zhou & Ning, 2017). Stochastic processes (e.g., colonization and extinction) are as important as or more important than deterministic processes (e.g., species selection and species interactions) in structuring natural microbial community (Chase & Myers, 2011). Although numerous studies have highlighted the importance of stochastic factors in driving the structure and functions of communities (Zhou et al., 2008; Caruso et al., 2011; Chase & Myers, 2011; Stegen et al., 2012; Zhou et al., 2013; Zhou & Ning, 2017), the dominance of stochastic process in community assembly makes it extremely challenging to explore the underlying community dynamics because of the unpredictable compositional variation (Zhou et al., 2013; Zhang et al., 2016). Consequently, we investigated community composition dynamics in grasslands with different levels of degradation in an attempt to elucidate the corresponding mechanisms of bacterial community assembly.

### 4.2 Different phyla taxa showed varied responses to grassland degradation

According to the results of the present study, environmental factors (OM, TN, and TP) significantly influenced community composition in the degraded grassland (**Figure S2**). However, the low correlation between environmental factors and bacterial community composition means that environmental factors can hardly explain microbial community composition (**Figure S2**); as observed in previous studies, which highlighted the importance of stochastic processes in microbial community assembly (Ramette & Tiedje, 2007; Graham et al., 2016; Zhang et al., 2016; Zhou et al., 2017).

The relationships between microbial community assembly in the environment and ecosystem processes remain

unclear (Graham et al., 2016). In the present study, we investigated bacterial community dynamics at phylum level in degraded grasslands. Previous studies have reported that different soil bacterial phyla display different responses in abundance in grasslands following shifts in nutrient conditions (Zeng et al., 2015; Dong, Shi, et al., 2021).

In the present study, although the differences in dominant herbaceous species and soil characteristics in the two grasslands may lead to different belowground bacterial community composition, similar results were observed in soil bacterial community dynamics under grassland degradation. Proteobacteria and Bacteroidetes had more positive responses to grassland degradation than other phyla, in the form of richer diversity, higher abundance, and more unique and enriched species under relatively severe grassland degradation (**Figure 1 and 2; Table 1**). The increase in the abundance of unique species and species diversity (**Table 1; Figure 2A and 2B**) highlighted the importance of Proteobacteria and Bacteroidetes, whose taxa enhanced community function and stability under grassland degradation (Loreau & de Mazancourt, 2013; Delgado-Baquerizo et al., 2016; Wang et al., 2017). Moreover, Proteobacteria and Bacteroidetes exhibited greater abundance, richness, and broader niche-widths (**Figure 1, 2C, 2D, 3A and 3B**) with an increase in degree of degradation, indicating the proportions of resources available to the two phyla may increase under degraded conditions. Conversely, the abundance of other phyla, such as Acidobacteria, Actinobacteria, Chloroflexi, and Planctomycetes, decreased with an increase in degradation. In addition, although different biomarkers were observed between the two grasslands, the biomarkers negatively correlated with nutrients content (OM, TN, and TP) were mainly assigned to Gammaproteobacteria, indicating the importance of Gammaproteobacteria in the adaptation of the community to grassland degradation. The results demonstrated that nutrient limitation in degraded grasslands has contrasting effects on different taxa.

The responses of different taxa to their habitat environments are associated with their ecological characteristics (Ho et al., 2017). Proteobacteria and Bacteroidetes are considered copiotrophic taxa, and are enriched in soil with high resource availability (Leff et al., 2015; Wang, Zhang, Liu, et al., 2021). In comparison, oligotrophic bacterial phyla, such as Acidobacteria and Chloroflexi, would outcompete copiotrophs under low resource availability (Fierer et al., 2007; Ai et al., 2015; Ho et al., 2017). However, in the present study, inconsistent results were observed, with copiotrophic phyla (Proteobacteria, Bacteroidetes) exhibiting greater survival, with higher abundance with a decrease in soil nutrient contents (**Figure 1**). Previous studies have focused on stronger environmental selection associated with artificial disturbance and larger temporal and spatial scales (Cleveland et al., 2006; Fierer et al., 2012; Leff et al., 2015; Wang, Zhang, Liu, et al., 2021). However, the present study focused on the effect of grassland degradation on microbial community assembly over relatively small spatial and temporal scales, which are associated with rather mild shifts in resource availability. Moderate shifts in resource availability may offer bacterial communities with opportunities to adapt to environmental change, rather than exposing communities to strong selection by the environment. Consistently, in the present study, stochastic processes dominated community assembly, with weak selection observed, based on the observed  $\beta$ NTI values that ranged between -2 and +2 (**Figure 4A and 4B**). Therefore, community member life strategies may be more instructive than physiological characteristics in predicting bacterial community assembly dynamics under degraded grassland conditions.

### 4.3 Taxa strategies in response to grassland degradation

Considering the microbial strategies adopted in response to grassland degradation, the life strategies of bacterial phyla observed in the present study were classified into  $r$ - and  $K$ -selection strategies, based on the life-history evolution theory by Pianka (Pianka, 1970).  $r$ -selection strategy is associated with high productivity, and is adopted to improve survival chances under variable and unpredictable environmental conditions, in contrast to  $K$ -strategy that is associated with high efficiency, and is adopted to maintain survival ability in response environmental stress (Pianka, 1970; Reznick et al., 2002; Ye et al., 2018). In addition,  $r$ -extreme represents a quantitative extreme with no density effects and no competition, whereas  $K$ -extreme represents a status with active competition and is the optimal strategy for allocating limited resources to ensure resource efficiency, for example, by producing a few extremely fit offspring (Pianka, 1970; Ye et al., 2018). No organism adheres to either  $r$ -extreme or  $K$ -extreme completely, and communities



must achieve a balance between the two extremes in response to environmental disturbance (Pianka, 1970; de Vries & Shade, 2013).

In the present study, Proteobacteria and Bacteroidetes had greater species diversity, broader niche-widths, and random phylogenetic clustering in degraded grasslands (**Table 1; Figure 3A, 3B, 4C and 4D**), showing high adaptability to the resource limitation, which can be considered adoption of  $r$ -strategy. The finding is consistent with the results of a previous study, in which Proteobacteria could survive in diverse ecological niches due to their variable morphology and physiology, which facilitate an  $r$ -strategy (Shin et al., 2015). Conversely, other phyla (mainly Acidobacteria, Actinobacteria, Planctomycetes, and Gemmatimonadetes) had lower diversity, abundance, and niche-overlap (**Table 1 and 2; Figure 1 and 3C and 3D**) so that bacterial abundance was reduced following functional redundancy to enhance resource-use efficiency, which can be considered  $K$ -selection. Similar results have been reported in a study on the relationship between phyla-level responses and life strategies under drying-rewetting disturbance, in which Proteobacteria employed opportunistic strategies ( $r$ -selection) whereas Acidobacteria adopted tolerant strategies ( $K$ -selection) (Evans et al., 2014).

Although the  $r/K$  strategy theory is oversimplified, it provides researchers with a fundamental ecological perspective for understanding mechanisms of assembly of microbial communities in response to environmental change (de Vries & Shade, 2013; Li et al., 2021). The increases in the relative abundances of Proteobacteria and Bacteroidetes with grassland degradation indicate that the bacterial community adopted  $r$ -selection strategies. Generally,  $r$ -selection strategies are more adaptable to variable environmental conditions than  $K$ -selection strategies (Ye et al., 2018); furthermore, high variable productivity observed under  $r$ -selection potentially offers more opportunities for community evolution (Fierer et al., 2012) in conditions such as degraded grasslands with nutrient loss.

Selection strategies can also be reflected in community functional structure, which is correlated with community response to environmental change (Schimel & Bennett, 2004; Fierer et al., 2012; de Vries & Shade, 2013). In the present study, bacterial community functional diversity and functional information were enriched with nutrient loss (**Table S4 and S5**). Moreover, phylogenetic dispersion was significantly and positively correlated with the number of KO genes carried (**Figure 5**), highlighting the potential importance of stochastic assembly processes in the regulation of community functional structure. The community assembly and niche analysis results in the present study jointly indicate bacterial communities could directionally regulate their functional structure based on diversity and composition under grassland degradation, which could facilitate their adaptation to changes in resource availability.

We do not exclude the influence of species-competition and/or environmental filters on the community composition, because microbial response are likely affected by multiple mechanisms simultaneously (Ho et al., 2017). Environmental selection was potentially weak due to the rather mild changes in resources in the course of grassland degradation over the relatively small spatial and temporal scales. Nevertheless, environmental factors can indirectly influence community response strategies in the form of community assembly (Fierer et al., 2007; Li et al., 2021). The balance between  $r$ - and  $K$ -strategies mediated by community members of different phyla facilitates adaptation to resource loss under grassland degradation (**Figure 6**). However, following long degradation periods, shifts in environmental nutrient conditions could induce shifts from  $r$ - to  $K$ -selection strategies, considering the accumulation of environmental selection and the strengths of oligotrophic bacteria following environmental filtering (Pianka, 1970). Simultaneously, the relative importance of stochastic process may decrease, with the deterministic processes playing more dominant roles in structuring communities (Ferrenberg et al., 2013; Zhang et al., 2016).

## 5. Conclusion

Stochastic process with weak selection dominated bacterial community assembly process during grassland degradation, with mild and continuous environmental changes on a small spatial scale. Different bacterial phyla exhibited different response strategies in the form of diversity, abundance, niche-width, and phylogenetic turnover under grassland degradation (**Figure 6**). Proteobacteria and Bacteroidetes, the most

abundant phyla, exhibited positive responses, adopting  $r$ -strategies, which facilitated the enhancement of biodiversity and in turn community productivity. In contrast, the relative abundances of other dominant phyla were restricted following grassland degradation, with  $K$ -strategies being adopted to facilitate efficient resource utilization. The shift from  $K$ - to  $r$ -strategies by communities under grassland degradation brought increased functional diversity, which could facilitate adaptation by communities to resource limitation. A key limitation of our study is that not all phyla can be classified as  $r$  or  $K$  strategists (Li Hui, 2021); nevertheless, the system can still be employed to partly explain the response mechanisms of microbial communities to environmental changes. In addition, although we determined the influence of community response strategies on functional structure, the specific functional structure dynamics involved in community adaptation to grassland degradation require further research. In summary, our study clarified the assembly processes of soil bacterial communities in response to grassland degradation, and the results offer insights into the ecological dynamics involved in the responses of various soil bacterial communities and taxa to grassland degradation. The findings of the present study could facilitate not only the prediction of soil microbial community structure in grassland ecosystem under degradation but also their sustainable management.

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## Conflict of Interest

The authors declare no conflicts of interest.

## Author Contributions

Yuanhua Dong and Jiangang Li: Conceived the study; Yang Hu and Yang Sun: Collected the data; Junwei Peng and Hong Liu: analyzed the data; Junwei Peng, Jiangang Li, and Qin Liu: Led the writing and revision of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## Data Availability Statement

The sequences were deposited in NCBI SRA database (BioProject accession no. PRJNA778017).

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**Figure 1** . Bacterial community composition at phylum level across different degradation levels in grassland 1 (**A** ) and grassland 2 (**C** ); Pearson correlation between relative abundance of dominant phyla and soil nutrient contents in grassland 1 (**B** ) and grassland 2 (**D** ).

There were six levels of degradation in grassland 1 and five levels of degradation in grassland 2, which are represented by numerical indices. The magnitude of the number represents the level of degradation. A smaller numerical index indicates a lower degradation level.

The red blocks in heatmap represent positive correlation and the blue blocks represent negative correlation. “\*”, “\*\*\*”, “\*\*\*\*” represent significant correlations with *P* values less than 0.05, 0.01, and 0.001, respectively. TP, total phosphorous; TN, total nitrogen; TK, total potassium; OM, organic matter.

**Figure 2** . Bacterial community dynamics in response to grassland degradation. The proportions of unique species of different phyla in communities across the degradation levels in grassland 1 (**A** ) and grassland 2 (**B** ); The proportions of enriched and deleted species of different phyla in communities across different degradation levels in grassland 1 (**C** ) and grassland 2 (**D** ); Heatmap showing correlations between the relative abundances of the top 30 predictive operational taxonomic units (OTUs) and nutrient contents in grassland 1 (**E** ) and grassland 2 (**F** ).

The magnitude of the number represents level of degradation. A smaller numerical index indicates lower degradation level.

The enriched (depleted) OTUs indicated that the relative abundance of OTUs in degraded grassland regions was significantly higher (lower) than that in the region with the least degradation level.

The red blocks in heatmap represent positive correlation and the blue blocks represent negative correlation. “\*”, “\*\*\*”, “\*\*\*\*” represent significant correlations with *P* values less than 0.05, 0.01, and 0.001, respectively.

**Figure 3** . Variation in niche breadth and niche overlap of phyla under grassland degradation. The mean niche breadths (Z-score-transformed) of members in each phyla across the degradation levels in grassland 1

(**A**) and grassland 2 (**B**); The mean niche overlaps (Z-score-transformed) of members in each phyla across the different degradation levels in grassland 1 (**C**) and grassland 2 (**D**)

**Figure 4** . Assembly patterns of bacterial communities under grassland degradation. Patterns of between-community nearest taxon index ( $\beta$ NTI) across different degradation levels in grassland 1 (**A**) and grassland 2 (**B**). Horizontal dashed lines indicate upper and lower significant thresholds at +2 and -2, respectively; The correlations between ses.MNTD values of phyla and synergic soil nutrient content (Z-score-transformed) in grassland 1 (**C**) and grassland 2 (**D**), respectively. PB indicates community members belonging to Proteobacteria and Bacteroidetes, OUT-PB indicates all community members excluding Proteobacteria and Bacteroidetes.

**Figure 5** . Correction between the average amounts of KO genes within each species and ses.MNTD values of communities.

**Figure 6** . Conceptual model of response strategies of bacterial communities under grassland degradation. In the conceptual model, stochasticity dominates the assembly processes of bacterial communities in response to grassland degradation with mild/continuous resource changes. Along the degradation gradient, the members (mainly Proteobacteria and Bacteroidetes) with  $r$  - strategy gradually increase to improve community productivity and opportunity, while other members with  $K$  - strategy (mainly Acidobacteria, Actinobacteria, and Chloroflexi) decrease due to adjustment due functional redundancy for enhanced community efficiency. A balance in community between  $r$  - and  $K$  - strategies facilitates adaptation to resource disturbance under grassland degradation.

Grassland regions	Proteobacteria	Proteobacteria	Bacteroidetes	Actinobacteria	Acidobacteria	Gemmatimonadetes
Grassland 1	Grassland 1					
1	777±66 ab	777±66 ab	327±49 ab	504±36 c	478±19 c	137±19 d
2	721±58 a	721±58 a	286±42 a	534±39 c	462±26 bc	120±14 c
3	815±48 b	815±48 b	311±39 ab	418±50 b	446±34 bc	103±6 b
4	825±76 b	825±76 b	348±44 abc	367±37 a	426±48 b	97±8 b
5	842±53 b	842±53 b	359±57 bc	344±24 a	437±28 bc	102±7 b
6	811±24 b	811±24 b	396±65 c	326±34 a	307±44 a	84±6 a
Grassland 2	Grassland 2					
1	824±29 a	824±29 a	348±40 a	467±49 b	476±37 b	148±7 b
2	845±65 ab	845±65 ab	350±63 a	473±34 b	494±25 b	141±14 b
3	838±46 ab	838±46 ab	344±51 a	504±51 b	469±15 b	142±5 b
4	895±38 b	895±38 b	410±64 a	352±21 a	391±44 a	111±20 a
5	817±73 ab	817±73 ab	400±27 a	319±38 a	343±69 a	105±14 a

**Table 1** . Species number (richness) within different phyla in the grassland regions with different degradation levels.

**Note:** Different lowercase letters in the same column represent a significant ( $P < 0.05$ ) difference in species richness within each phylum in different regions of degraded grasslands in grassland 1 and grassland 2. The magnitude of the number represents the level of degradation. A smaller numerical index indicates a lower degradation level.

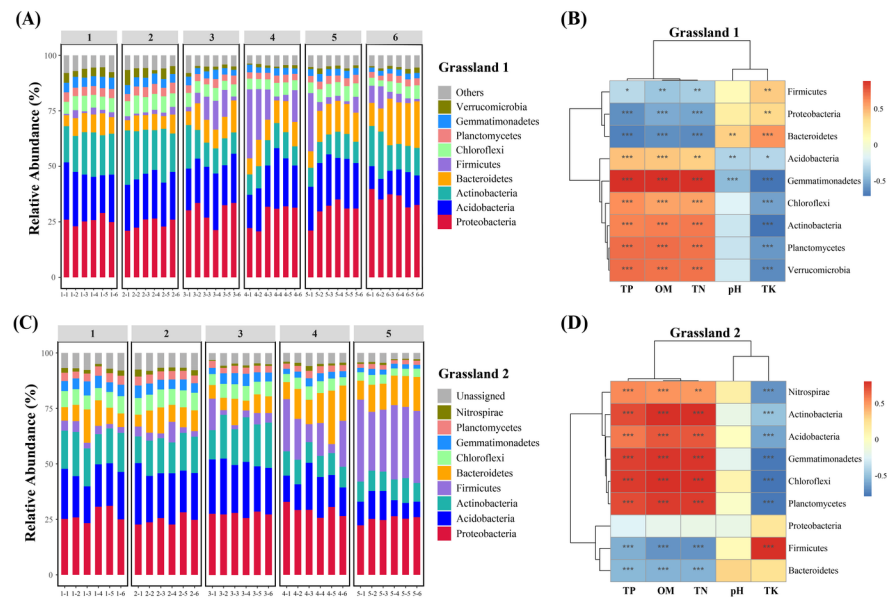
**Table 2** . Correlation between alpha diversity and mean nearest taxon distances (ses.MNTD) of different phyla.

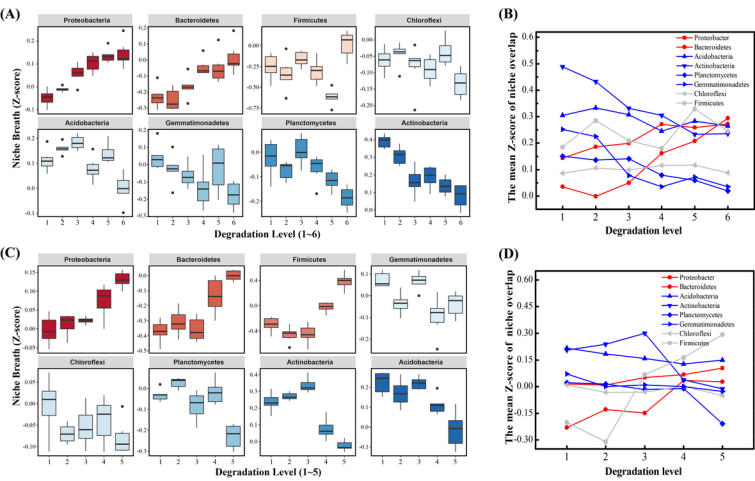
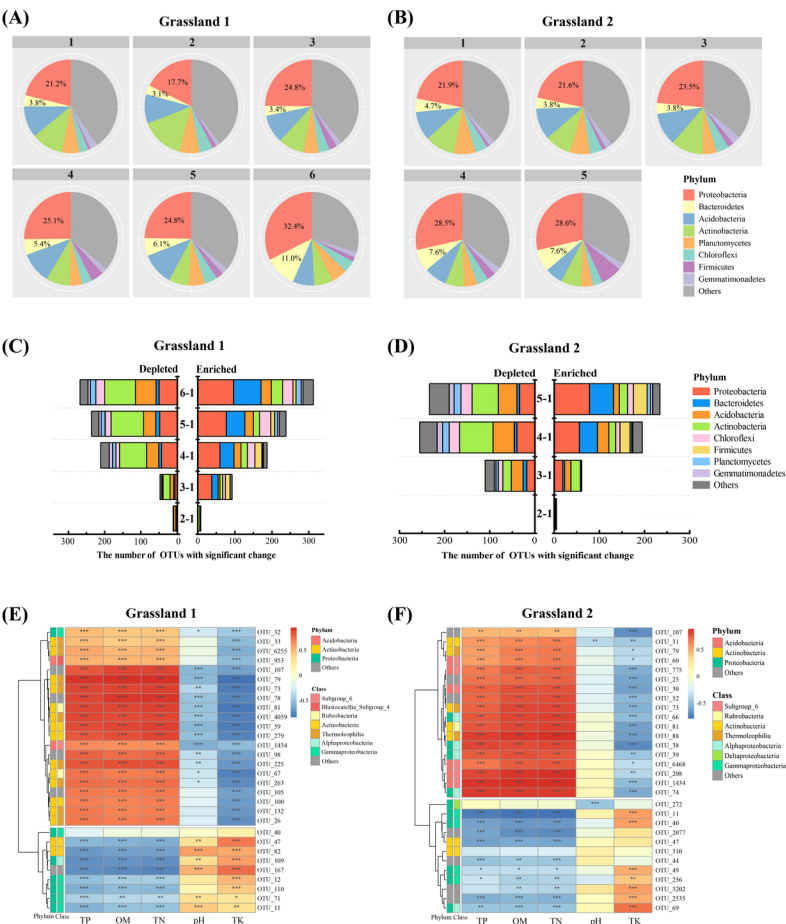
Members in different groups	All members	All members	All members	All members excluding Proteobacteria/Bacteroidetes
	Grassland 1	Grassland 1	Grassland 2	Grassland 1
Richness	-0.423*	-0.717*	-0.717*	-0.587*

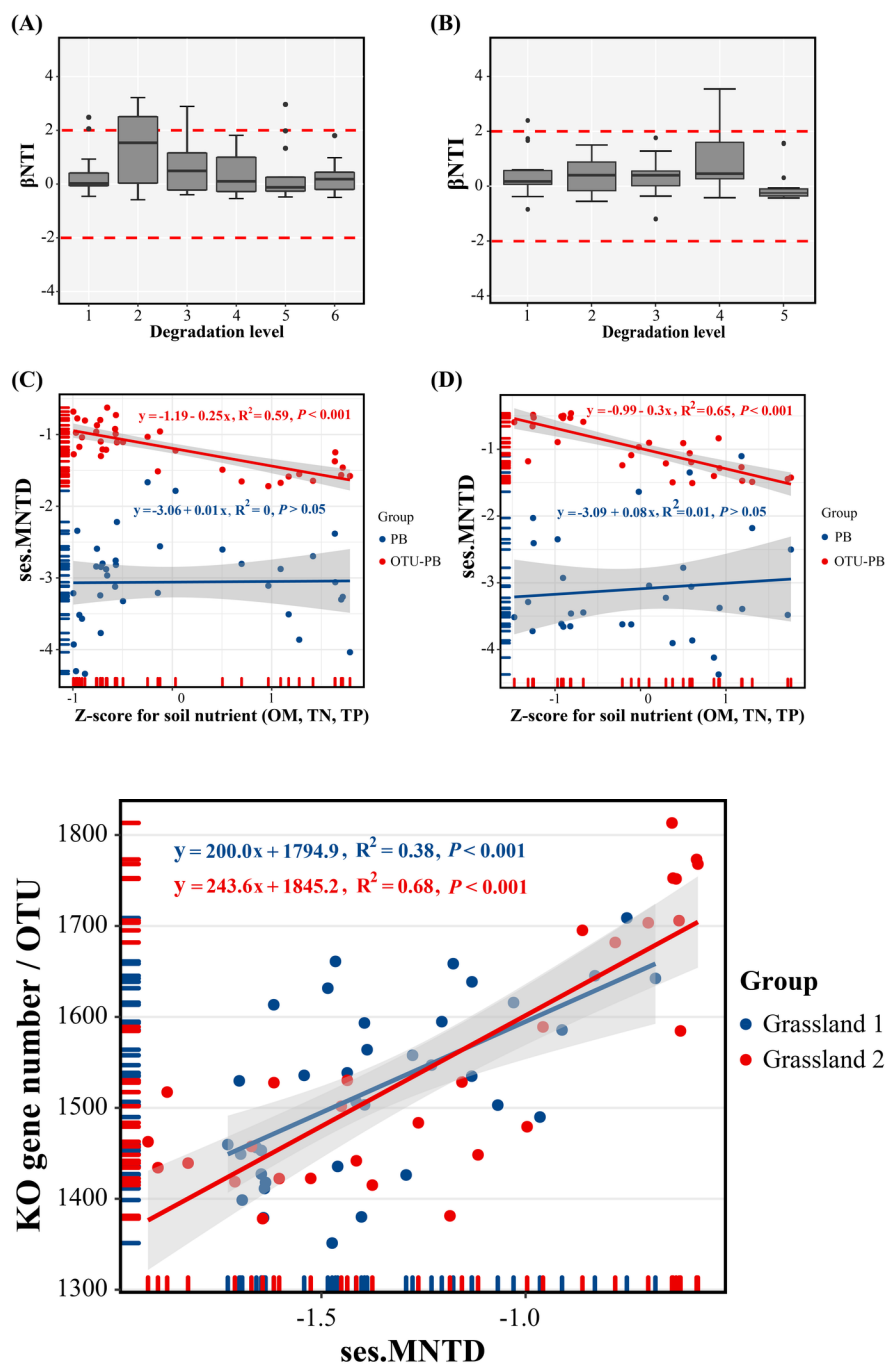


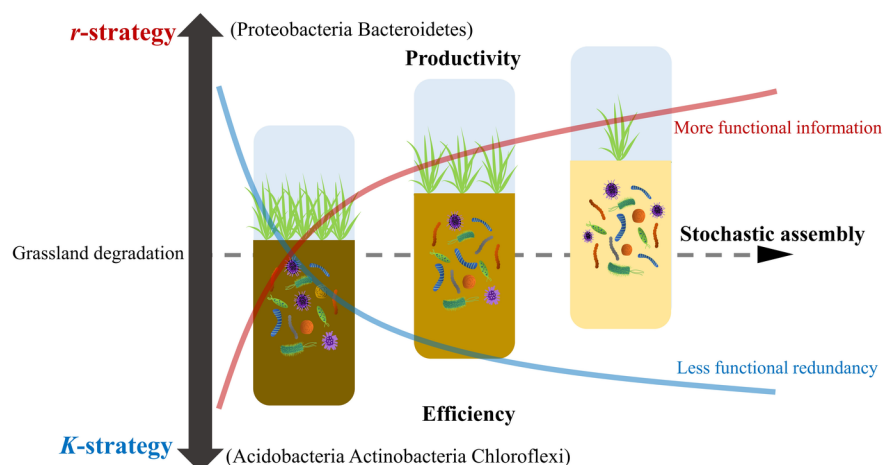
Members in different groups	All members	All members	All members	All members excluding Proteobacteria/Bacteroidetes
Shannon	-0.721*	-0.931*	-0.931*	-0.833*

**Note:** “\*” represents a significant ( $P < 0.05$ ) correlation between alpha diversity (richness/Shannon index) and ses.MNTD values of different groups in grassland 1 and 2, respectively.









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