



Article Stochastic Processes Shape Bacterial Community Diversity Patterns along Plant Niche Gradients

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Abstract: The ecological niche gradient is an important determinant of microbial community structure. In this paper, we studied variation in rhizosphere bacterial diversity and community composition along an ecological niche gradient. We used the high-throughput sequencing of 16S rRNA genes to study changes in the rhizosphere soil microbial communities of six grass and four shrub species during the secondary succession of abandoned farmland on the Loess Plateau of China. A structural equation model (SEM) was employed to disentangle the relative contribution of ecological niche and soil properties to bacterial diversity and community composition. Proteobacteria, Acidobacteria, and Actinobacteria were the dominant phyla of rhizosphere bacteria in all samples. During the dynamics of the plant niche from low to high, bacterial community composition transitioned from Actinobacteria + Acidobacteria to Proteobacteria + Bacteroidetes higher abundance. Moreover, the bacterial diversity and species richness changed with an increasing niche gradient, showing a clear differentiation in the rhizosphere bacterial community of grassland and shrubland. Further, diversity and species richness decreased from the middle niche of *B. ischaemum* to the poles, indicating that the succession process had not yet reached the climax community stage. Community assembly analysis suggested that the stochastic process gradually strengthened along the increasing ecological niche gradient, especially the drift effect. Furthermore, SEM analysis showed that the ecological niche had significant negative effects on soil properties and bacterial richness, while the effects on bacterial diversity and the stochastic processes of community assembly were weakened and insignificant. Altogether, our findings suggest that the complex interaction of the ecological niche with bacterial diversity and composition was determined by soil properties. Further, bacterial diversity was not necessarily higher with increasing ecological niche gradients.

Keywords: bacterial community; rhizosphere; niche gradient; diversity; plants; succession

1. Introduction

The ecological niche theory is an important concept in ecological research, describing the mechanisms of species distribution. According to this theory, different species coexist in a homogeneous environment because they occupy various distinct ecological niches [1,2]. Species succession describes the process of niche division [3–5]. When different species occupy and utilize the resources of various ecological niches, fierce competition can be avoided, and coexistence can be achieved. The ecological niche of plants is influenced by a number of factors, such as light, water, mineral nutrition, rhizosphere microbial diversity, as well as other aboveground and underground parts [6–8]. The full utilization of these niche resources promotes the continuous succession and coexistence of plants. To date, many studies have confirmed that the succession process and coexistence of plants are strongly



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Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). related to the degree of occupation by different ecological niche resources [9–12]. However, the underlying mechanisms through which plants from different ecological niches maintain complex rhizosphere microbial diversity and composition are unclear.

Rhizosphere microbial diversity and composition play important roles in maintaining the ecological niche of plants [13,14]. Rhizosphere microbes provide different nutrients for plants to avoid the adverse effects caused by intense interspecific competition. However, studies on the rhizosphere microorganisms of plants in different ecological niches are mostly limited to describing microbial diversity and taxonomy [13,15,16]. Meanwhile, the mechanisms of diversity and composition remain elusive. The ecological niche resources of various dimensions can lead to plant differentiation, which further affects the diversity and composition of rhizosphere microorganisms. The aboveground parts of a plant also indirectly affect rhizosphere microbes through their effect on underground parts. These seemingly contradictory factors complicate the interpretation of findings from different reports. For example, it has been reported that variation in rhizosphere exudates can significantly affect differences in microbial diversity and composition [17]. Further, differences in the genotype or ecotype of the same plant can also affect microbial diversity and composition [18]. Both soil properties and plant species were suggested to lead to variations in microbial diversity and composition, yet to a different extent [19]. Taken together, studies have reported conflicting findings, albeit all supported by solid evidence. Scarce research on the mechanisms of diversity and composition in community assembly further limits our understanding of rhizosphere microbial diversity and the composition of plants in different ecological niches.

Community assembly is among the driving forces of microbial diversity and composition [20,21]. It includes deterministic as well as stochastic processes. The deterministic processes are based on the ecological niche theory [9,10]; that is, species have different habitat preferences, with the community being formed through the filtration selection of environmental factors such as soil pH, temperature, humidity, and mineral nutrient content. The stochastic processes are based on the neutral theory [9,10], which holds that the birth, death, and migration of species are random, resulting in random fluctuations of species abundance and diversity in the community via ecological drift and limited dispersal. Emerging evidence suggests that these two groups of processes work together to balance community assembly [20,22] and that their relative influence depends on the geographic scale, intensity of environmental gradients, and biological characteristics (body size and dispersal patterns) [8,23]. At smaller geographical scales, stochastic processes are more important than deterministic factors, because decreases in habitat heterogeneity often lead to reduced habitat preferences. In the study of microorganism community assembly, the evolutionary and ecological factors affecting diffusion may also decrease at a smaller geographical scale. Therefore, reducing the study size can control environmental filtering and diffusion limitations due to recruitment failure so as to minimize the confounding effect of unmeasured variables and identify the direct or indirect correlations between the rhizosphere microbial community assembly process and ecological niches of different plants. Growing evidence suggests that microbes exhibit distinct adaptations along niche gradients [24]. However, how rhizosphere bacterial diversity and abundance vary with the ecological niche and the underlying mechanisms are unclear. Although variations in diversity are often hypothesized to reflect the relative importance of different community assembly mechanisms [11,20,25], plant species may also drive variations in diversity at the ecological niche scale.

The natural succession of abandoned land is key to ecological restoration in the Loess Plateau. Once restored to a certain degree, a relatively stable grassland community is established, and a coexistence relationship may be formed between grasses and shrubs in different ecological niches within the community [26]. The theory of vegetation succession suggests that under suitable temperature, moisture, soil nutrients, and other conditions, stable grassland communities transform into shrub communities over time [27,28]. Thus, many studies have focused on rhizosphere microbial diversity and rich-

ness during succession [12,29,30]. In contrast, the role of rhizosphere microbial variation at the ecological niche level is usually overlooked, yet may play a central part in the process of succession from grassland to shrubland.

In this study, we assessed the rhizosphere microbial diversity and community composition, exploring the diversity patterns and community composition of bacteria along the ecological niche gradient. Further, we disentangled the effect of community assembly on diversity and richness based on changes in the rhizosphere of different niches, which plant species occupied within a typical stable grassland community on the Loess Plateau. We hypothesized that (1) based on the ecological niche status and dominance of species in the plant community, the bacterial diversity and richness of shrub species in high niches would be relatively higher than those of grasses in low niches; (2) bacterial community composition and assembly can be influenced by the ecological niche.

2. Materials and Methods

2.1. Study Site and Soil Sampling Collection

The research was carried out at the Ansai Research Station of the Chinese Academy of Sciences (109°19' E, 36°51' N). This area is a key demonstration area of China's "Grain for Green" project, and it is also a typical watershed for the comprehensive management of soil erosion on the Loess Plateau. The soil type is typical for Loess. This is a forested steppe area with widely distributed grasses and shrubs. Typical grasses are Artemisia sacrorum (AS, RIV = 0.17), Bothriochloa ischaemum (BI, RIV = 0.28), Cleistogenes squarrosa (CS, RIV = 0.063), Lespedeza bicolor (LB, RIV = 0.09), Stipa bungeana (SB, RIV = 0.065), and Stipa grandis (SG, RIV = 0.11). Typical early successional shrubs are Buddleja alternifolia (BA, RIV = 0.32), Clematis fruticose (CF, RIV = 0.277), Periploca sepium (PS, RIV = 0.28), and Sophora *viciifolia* (SV, RIV = 0.30) [26]. The ecological niche values of six grasses and four shrubs were assessed by Levins' niche breadth, which was used as a quantitative indicator of the ability of plant species to utilize environmental resources [26]. The low-to-high ranking order of ecological niche gradient is consistent with the recording time of plant emergence in abandoned land. In this stable grassland community, the ecological niche values of six grass plants were generally less than 0.2, while four shrubs' values were generally more than 0.2. Therefore, we regarded six grass plants as the low-ecological niche plants for RIV as less than 0.2, and four shrub plants as high-ecological niche plants for RIV as more than 0.2.

In September 2017, we selected four $30 \text{ m} \times 30 \text{ m}$ sites from a typical stable grassland community that had been abandoned for more than 30 years as the experimental sites [26]. The two locations were more than 20 m apart. At each site, four shrubs and six grasses were selected, with a total of 16 shrublands and 24 grasslands. The selection method was as follows: (1) if there were shrubs in the diagonal direction of the site (the diagonal length was approximately 42 m), we chose a $2 \text{ m} \times 2 \text{ m}$ shrubland with the shrub as the center; (2) if there were no shrubs, we chose a $1 \text{ m} \times 1 \text{ m}$ grassland. The distance between any two pieces of land was approximately 2.5 m. This process provides an alternative for shrubland and grassland plots. First, residues and impurities were removed from the soil surface, and a shovel was used to remove the roots from the 0–30 cm soil layer. We collected the rhizosphere soil by lightly brushing the soil attached to the roots of each plant, and then transferring it to the laboratory to test its nutrient content and microbial diversity.

2.2. Analysis of Soil Physical and Chemical Properties

The soil samples were divided into two parts. The first part was used to determine soil organic carbon (SOC), soil total nitrogen (TN), ammonium nitrogen (NH_4^+ -N), nitrate nitrogen (NO_3^- -N), available phosphorus (AP), soil moisture (M), and pH. The second part was stored in a -80 °C ultra-low temperature freezer to preserve soil microbial samples. The physicochemical properties of the rhizosphere soil are listed in Table S1. The concentration of soil organic carbon and total nitrogen were determined using the H_2SO_4 - $K_2Cr_2O_7$ oxidation method [31] and the Kjeldahl method [32], respectively. Soil

nitrate-nitrogen was measured using a continuous-flow analyzer. The soil ammonium nitrogen content was determined using UV spectrophotometry. Available phosphorus was determined via the sodium bicarbonate extraction molybdenum antimony anti-colorimetric method. The percentage of moisture content was determined via the drying method. pH was determined using a pH meter (water:soil = 2.5:1).

2.3. DNA Extraction and Illumina HiSeq High-Throughput Sequencing

According to the instructions of the FastDNA Spin Kit for Soil (MP Biomedicals, Cleveland, OH, USA), total DNA was extracted from 0.5 g of soil. The quantity of soil DNA was assessed using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, CA, USA). The integrity of extracted soil DNA was determined using 1.5% agarose gel electrophoresis. DNA was amplified via PCR using primers (forward primer: GTGAATCATCGATC; reverse primer: TCCTCCGCTTATTGAT) targeting the V3-V4 region of the 16S rRNA gene [33]. The PCR primers contained barcodes that uniquely tagged each sample. The PCR amplification mix contained 10.75 µL sterile deionized water, 0.25 µL polymerase (5 U/mL, TransStart Fastpfu DNA Polymerase, TransGen, Beijing, China), 5.0 μ L dNTPs (1.0 mM each), 5.0 μ L 5 \times Ex Taq buffer, and 10 mM concentration of each primer in a volume of 1 μ L. Finally, 2 μ L of total DNA (50 ng/ μ L) was added to each PCR amplification mix. PCR program was as follows: initial denaturation at 98 °C, 3 min; 30 cycles of 98° C for 30 s, 56 °C for 30 s, and 72 °C for 40 s; and a final 5 min extension at 72 °C; thereafter kept at 4 °C [34]. The PCR amplicons were pooled and purified using 1.5% agarose gel electrophoresis. The PCR amplification products were gel-extracted, and quantified using PicoGreen (Invitrogen, Carlsbad, CA, USA). Finally, the amplicons of all samples were mixed in equimolar concentrations for high-throughput sequencing.

2.4. Processing of 16S rRNA Sequencing Data

Raw paired-end reads containing more than 5% unknown nucleotides, reads with low (more than 50% bases with Q-value of \leq 20) average quality scores, and barcodes were excluded to obtain high-quality sequences [35]. Then, clean sequence data were demultiplexed, processed, and analyzed using QIIME2 [14]. Chimeric sequences were detected using the UCHIME algorithm [22]. The remaining demultiplexed reads were clustered using the UPARSE software, and assigned to operational taxonomic units (OTUs) when the similarity reached 97%. Bacterial taxonomic properties were determined using the RDP classifier and the Silva reference database (http://www.arb-silva.de, accessed on 8 January 2021). PICRUSt was used to predict the functions of the bacterial communities [14]. All sample sequences were submitted to the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI) under accession number PRJNA 729890.

2.5. Statistical Analysis of Sequencing Data

In this study, OTU numbers, ACE index, Shannon index, and phylogenetic diversity index were used to calculate microbial alpha-diversity. Non-metric multidimensional scaling (NMDS) plots were drawn using weighted Bray–Curtis distances to illustrate clustering across samples, and taxonomic diversity was analyzed using ANOSIM. Random forest (RF) analysis was used to identify the main drivers of differences in bacterial community structure in the rhizospheres of grasses and shrubs. Bacterial beta-diversity was quantified using the axes of NMDS1 and NMDS2. In the RF model, alpha- (ACE index, Shannon index, and phylogenetic diversity) and beta-diversity indices were used as predictors of rhizosphere bacterial community composition. The A3 package was used to evaluate the significance of the model and the R² value for cross-validation [22]. The simple regression analysis was referred to assess the correlations between the relative important value (RIV) and the index of alpha- and beta-diversity, as well as the re-abundance of the community composition. Community assembly was performed using quantitative Inference of Community Assembly Mechanisms using the Phylogenetic Bin-based Null Model Analysis (iCAMP) to predict the relative importance of stochastic and deterministic processes [36].

All statistical analyses were performed using R software (version 4.0.4). p < 0.05 was considered statistically significant.

3. Results

3.1. Diversity Patterns of Bacterial Communities along the Ecological Niche Gradient

The relationship between rhizosphere bacterial (alpha and beta) diversity and niche was almost curvilinear, but varied greatly, except for the Simpson index (Figure 1). The Chao1 index, ACE index, phylogenetic diversity, and beta-dissimilarity were unimodal along the niche distribution, with the highest diversity at 0.181 (the relative importance value of the *A. sacrorum* niche). However, rhizosphere bacterial communities with higher niches (such as *S. viciifolia*, *P. sepium*, *B. alternifolia*, and *C. fruticosa*) did not exhibit greater diversity and abundance, including the rhizosphere of *B. ischaemum*, which was also lower than that of *A. sacrorum*. In contrast, the Shannon index distribution along the niche tended to decrease significantly; however, the correlation coefficient was weak.



Figure 1. Regression for the relative importance value (RIV) with bacteria α and β diversity index. Note: black point indicates significance, *p* < 0.05; grey point indicates non-significance.

Based on the trends in alpha- and beta-diversity along ecological niche gradients, which was indicated by the ranking order of relative importance value (RIV), there was a clear difference in diversity between high- (>0.2) and low-ecological niches (<0.2). This phenomenon was also confirmed via NMDS (Non-metric multidimensional scaling) analysis (stress = 0.1232, $R^2 = 0.427$, p = 0.001) (Figure 2A). NMDS analysis clearly showed that soil bacterial beta diversity was significantly different between high- and low-ecological niches. Further, RF (random forest) analysis indicated that the response of soil bacterial beta-diversity along the NMDS1-axis was divided into two parts ($R^2 = 0.434$, p < 0.01) (Figure 2B). These results strongly suggest that the rhizosphere microbial diversity and richness differ between the high- (>0.2) and low-ecological niches (<0.2).



Figure 2. Influence of bacterial β -diversity within the rhizospheres of grasses and shrubs on niche division. (**A**) Non-metric multidimensional scaling (NMDS) analysis of β -diversity among the soil samples, with 95% confidence ellipses shown around each group. Community similarity values of the samples in bacterial communities between grassland and shrubland soils were examined using the ANOSIM (analysis of similarities) test. Dots represent six grass plant species; triangles represent four shrub plant species. (**B**) RF means predictor importance of bacterial α - and β -diversity indices as predictors for niche division. The accuracy of the importance measure was computed for each tree and averaged over the forest (5000 trees). Significance levels are as follows: ** *p* < 0.01. Alpha-Shannon, Shannon index; Alpha ACE, ACE index; Alpha PD, phylogenetic diversity index; Beta-NMDS1, the first axis of non-metric multidimensional scaling analysis. Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of the predictors, and higher MSE values imply more important predictors.

3.2. Bacterial Community Composition of Plants in Different Ecological Niches

In terms of bacterial community composition, the most abundant phyla were Proteobacteria (20.8–34.9%), Actinobacteria (20.5–46.0%), Acidobacteria (12.1–19.8%), Chlorella (4.5–14.3%), Gemomonas (7.6–1.3%), Nitrospirae (1.1–3.0%), Bacteroidetes (1.1–4.1%), and Verrucomicrobia (0.1–2.8%) (Figure 3). Further, Proteobacteria, Nitrospirillum, and Geomonas increased from low to high niches, whereas Actinomycetes, Campylobacter, and Exomicrobes showed the opposite trends at the phylum level (Figure 4). Other phyla responded differently, but the differences were not statistically significant (p > 0.05). For the first nine orders, except for *Rhizobacteria*, which was not significant (p = 0.3261), three orders (*Sphin*gomonadales, Xanthomonadales, Rhizobiales) of Proteobacteria showed an obvious increasing trend along the low-to-high niche gradient, except for Rhizobiales, which was not significant (p = 0.3261) (Figure 5). However, two orders of Actinomycetes (Acidimicrobiales and Solirubrobacterales) showed a significant downward trend from the low-to-high niche, and another order (*Gaiellales*) showed the opposite trend (p = 0.5564) (Figure 5). Blastocatellales exhibited a significant decreasing trend from low to high niches, with Sphingobacteriales also showing a similar trend, yet without a significant difference (p = 0.257). Gemmatimonadales showed a significant decreasing trend (p < 0.05) (Figure 5). Taken together, these results further suggest that the niche has an effect on the composition of rhizosphere microorganisms at the phylum and order levels, with different effects observed for different bacteria.







Figure 4. Regression for the relative importance value with bacteria reabundance at the phylum level. Note: black point indicates significance, p < 0.05; grey point indicates non-significance.





3.3. The Relationship between Ecological Niche and Predicted Function

The function was predicted from operational taxonomic units (OTUs) using PICRUSt [14], and the correlation between ecological niche and bacterial community functions was analyzed. Several predicted pathways were enriched in genes associated with global and overview maps, carbohydrate metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, membrane transport, nucleotide metabolism, translation, replication and repair, as well as lipid metabolism. Genes involved in the global and overview maps exhibited the highest relative abundance in the bacterial community, followed by those involved in carbohydrate metabolism. Genes associated with global and overview maps (p < 0.05), carbohydrate metabolism (p < 0.01), and lipid metabolism (p < 0.01) pathways were significantly decreased along the low-to-high niche gradients, whereas the other pathways were not significantly related to the ecological niche (Figure 6). However, genes associated with environmental adaptation (p < 0.05) pathways significantly increased along with the low-to-high niche gradient.



Figure 6. Regression for the relative importance value with bacterial 16S predicted function. Note: black point indicates significance, p < 0.05; grey point indicates non-significance.

3.4. Effects of Plants in Different Ecological Niches on Bacterial Community Assembly

The bacterial community assembly process was analyzed using iCAMP (Inference of Community Assembly Mechanisms using the Phylogenetic Bin-based Null Model Analysis) with a null model. Based on the iCAMP analysis, the stochastic process was more important than the deterministic process in bacterial community assembly, with an average relative importance of 71.1–83.5% and 16.5–28.7%, respectively. The stochastic process mainly consisted of the dispersal limit and drift, with an average relative importance of 23.6–49.0% and 25.6–48.3%, respectively (Figure 7). Regression analysis showed that the stochastic process of bacterial community assembly significantly increased along the low-to-high niche (p < 0.05), while the deterministic process exhibited the opposite trend (Figure 8). However, dispersal limit and drift did not exhibit significant increasing trends along the ecological niche gradient, similar to the stochastic process. Only the drift showed the same trend as the stochastic process, which was not significant. The dispersal limit showed a decreasing trend along the ecological niche gradient, which was also not significant.



Figure 7. Relative importance of different ecological processes in response to the niche of six grasses and four shrubs. DL, dispersal limit; DR, drift (and others); HD, homogenizing dispersal; HeS, heterogeneous select; HoS, homogeneous select. SV, *Sophora viciifolia*; BA, *Buddleja alternifolia*; PS, *Periploca sepium*; CF, *Clematis fruticose*; BI, *Bothriochloa ischaemum*; AS, *Artemisia sacrorum*; CS, *Cleistogenes squarrosa*; SG, *Stipa grandis*; SB, *Stipa bungeana*; LB, *Lespedeza bicolor*.



Figure 8. Regression for the relative importance value with bacterial community assembly. Note: black point indicates significance, p < 0.05; grey point indicates non-significance.

3.5. Ecological Niche as a Function of Interactions between Biotic and Abiotic Drivers

A structural equation model (SEM) was used to determine the degree to which abiotic factors (ecological niche and soil properties) influenced biological factors (bacterial richness, diversity, and stochastic processes) (Figure 9). SEM results indicated that the plant niche

contributed to significant negative effects on soil properties (standardized path coefficient, r = -0.66) and bacterial richness (r = -0.97), which explained the differences of 0.44 and 0.61, respectively. Conversely, the plant niche contributed to weakened effects on bacterial diversity (r = -0.40) and the stochastic process (r = 0.38) of community assembly, which was also not significant. Soil properties had a significant positive effect on bacterial diversity (r = 0.74) and an insignificant negative effect on bacterial richness (r = -0.56). The stochastic process of community assembly had positive effects on microbial diversity (r = 0.20) and negative effects on microbial richness (r = -0.11). However, the relative contribution was largely weakened and not significant. It is important to note that the substantial effects of the plant niche on community assembly as well as on bacterial richness and diversity remain unexplained in the model, which means that other relevant factors are not considered here. Altogether, the current results emphasize that plant niches can indirectly influence the richness and diversity of rhizosphere bacteria based on soil properties, in addition to directly affecting the stochastic process. Similarly, the stochastic process of community assembly had little influence on the richness and diversity of rhizosphere bacteria.



Figure 9. Structural equation model showing the relative influence of ecological niche and soil properties on bacterial composition and community assembly. Significant paths are shown in a solid line, and insignificant paths are shown in a dashed line. The amount of variance explained by the model (R^2) is shown for each response variable. Ecological niche: relative importance value of plant; diversity: axis 1 of NMDS; richness: Shannon index; soil properties: all properties were treated with PCA. *p* < 0.05.

4. Discussion

4.1. Impact of Plant Ecological Niche on Bacterial Richness and Diversity

In the present study, bacterial alpha- and beta-diversity exhibited a unimodal distribution (Figure 1), where bacterial species richness peaked in the intermediate niche (RIV = 0.18) and declined toward the poles. Contrary to our initial expectations, soil bacterial diversity and richness did not show a niche gradient pattern (Figure 1). The reasons for the lack of a clear niche gradient in bacterial diversity and richness in the rhizosphere are multifaceted. The diversity and richness of bacterial communities were mainly limited by soil factors (Table S1), especially total nitrogen and moisture (Table S2). These results show that changes in diversity along niche gradients were significantly associated with heterogeneity in soil parameters, which is similar to other results indicating that the soil bacterial community structure is shaped by changes in soil conditions [26,33,37]. These findings suggest that niches, as complex gradients in which many environmental variables change, are insufficient for inferring the distribution patterns of bacterial diversity and richness.

Microbes can quickly respond to local environmental changes, and differences in root exudates during the succession of grasses in a low-niche to shrubs in a high-niche greatly affect the microbial community structure [38]. However, we observed small differences in bacterial alpha-diversity between plant rhizosphere soils of different niches. This may be because the experimental area was dominated by *B. ischaemum*, and the shrubland was just emerging and had not yet taken the lead in the succession stage. The root exudates of *B. ischaemum* reduce the surrounding soil heterogeneity, leading to the enrichment of some bacterial taxa and the loss of others [39], which is basically consistent with previous findings [7,40]. Plants recruit specific microbial communities because of their physiological and ecological characteristics at the later stages of succession.

Dominant soil microbial phyla in the rhizosphere of plants changed between ecological niches. The concept of eutrophic versus oligotrophic categories might explain why some specific bacterial phyla respond differently. Previous studies demonstrated that both Proteobacteria and Bacteroidetes are fast-growing eutrophic organisms that thrive in carbon-efficient environments [36,41]. Thus, increasing SOC (soil organic carbon) content in low-niche grasslands can accelerate the growth of both phyla. In contrast, the richness of Proteobacteria increased with the niche gradient, whereas the richness of Bacteroidetes remained largely unchanged. Significant reductions in soil structure may significantly increase soil nutrient availability and soil aeration, thereby increasing the richness of eutrophic populations [41]. Furthermore, within the class Proteobacteria, the branching Rhizobia order of α -Proteobacteria are considered rhizosphere plant-growth-promoting bacteria with the N_2 -fixing ability. However, the rhizobia showed a decreasing trend along the niche gradient, yet without significance. This implies that, in addition to C:N in rhizosphere soil, there may be other undetermined environmental factors or other related rhizosphere growth-promoting bacteria that play a role in the process of niche gradient formation. In contrast, Actinomycetes and Acidobacteria are generally considered oligotrophs, adapting to resource-limited conditions [41,42]. The reduction in Actinomycetes and Acidobacteria indicated an improvement in the contents of soil SOC, TN (soil total nitrogen), and AP (soil available phosphorus), especially in the low-niche grassland (Figure 3). Furthermore, this phenomenon is also accountable to an increasing tendency in the ratio of Proteobacteria + Bacteroidetes to Actinobacteria + Acidobacteria, which correspond to the eutrophic and oligotrophic groups, respectively (Figure S1). This suggests a shift in bacterial communities from oligotrophic to eutrophic groups. Collectively, the current results indicate that compared to high-niche shrubland, the low-niche grassland has a more important effect on soil structure and improves soil nutrient availability by an order of magnitude, which affects bacterial species composition and function. Combined with 16S function prediction analysis, the results also showed that, with the transition from low-niche grassland to high-niche shrubland, the rhizosphere soil environment became more complex, and the corresponding microbial functions showed an increase in genes associated with environmental adaptation.

4.2. Impact of Plant Ecological Niche on Bacterial Community Assembly

In our study, changes in community assembly were influenced by the strength of stochastic processes rather than by deterministic processes (Figures 7 and 8). This contradicts the results of a previous study, in which community assembly across ecological niche gradients was strongly influenced by the degree of deterministic processes [43]. One possible explanation is that under extreme soil pH conditions, changes in soil nutrients do not dominate the community assembly process [44–47]. The high soil pH (>8) observed in all samples narrowed the niche of soil bacterial communities, leading to a reduction in

diversity by selecting relatively similar species (Figure 1 and Table S1). With an increase in the niche gradient, the contribution of the stochastic process is enhanced, while that of the deterministic process decreases, which is contrary to the general assumption. In the small-scale study area of ecological restoration, stochastic processes mainly include drift and diffusion. Niche differentiation occurs during succession, and physiological changes favoring plant growth occur. Plants with different ecological niches recruit specific beneficial microorganisms in the rhizosphere, as shown in Figure 9. Along the ecological niche gradient, the ratio of Proteobacteria + Bacteroidetes and Actinobacteria + Acidobacteria increased gradually. Therefore, an increase in the abundance of specific microorganisms leads to an increase in the abundance of microorganisms that synergize with *Proteobacteria* + Bacteroidetes. However, this results in a decrease in the abundance of microorganisms that antagonize Proteobacteria + Bacteroidetes. These changes affect the birth and death rates of microorganisms in a random manner, further altering the microbial community assembly process. However, according to the general theory of ecology, the assembly process of rhizosphere microorganisms in different ecological niches cannot be entirely determined by the increase in birth rate or decrease in the death rate of certain microorganisms, with other factors such as limited spread and drift also being implicated. Therefore, the final composition of the community is a complex interaction process. However, relevant studies in similar small-scale regions have also shown that random processes dominate and that the importance of deterministic processes is decreasing for different ecological niches. Therefore, our study not only suggests differences in the size and scale of microbial community assembly by ecological niche but also highlights the importance of small-scale research when studying the community assembly process of large-ecological niches. Given that our study explored only a snapshot, our findings may vary over time and season. Therefore, it is necessary to investigate multiple processes using theoretical ecological models, particularly under unusual conditions.

5. Conclusions

In this study, we provide comprehensive and empirical evidence for the relative contributions of niche and rhizosphere soil physicochemical properties to the richness and diversity of different plant rhizosphere bacterial communities and their community assembly processes. Our results showed that there was no significant correlation between the richness and diversity of rhizosphere bacterial communities and the plant ecological niche. The community composition changed from Acidobacteria and Actinomycetes to Proteobacteria and Bacteroidetes, with genes related to environmental adaptation being gradually enriched. The stochastic process of community assembly was significantly enhanced. Combined with the SEM analysis of the potential mechanism through which niches affect the rhizosphere microbial community, our results showed that the plant niche had significant negative effects on soil physicochemical properties and bacterial richness, directly influencing bacterial community diversity and the stochastic process of community assembly. However, none of these effects were significant. Ecological niches can indirectly affect the diversity of bacterial communities through their physicochemical properties. Our research advances the current understanding of factors that determine the diversity and composition of rhizosphere microbial communities in different ecological niches, highlighting the significance of niche theory in this context. However, due to the small scale of the study, the response mechanism of microbial communities to different ecological niches on a spatiotemporal scale requires further study.

Supplementary Materials: The following supporting information can be downloaded at: https://www. mdpi.com/article/10.3390/agronomy14010204/s1, Figure S1: Changes of *Proteobacteria* + *Bacteroidetes* to *Actinobacteria* + *Acidobacteria* ratio in six grasses and four shrubs plants; Table S1: Soil rhizosphere characteristics of different ecological niche plant species; Table S2: Taxonomic and phylogenetic α -diversity indices of bacterial communities in rhizosphere of six grasses and four shrubs plants. **Author Contributions:** Conceptualization, Z.Y., S.X. and Y.C.; Formal analysis, Z.Y.; Funding acquisition, S.X.; Investigation, Z.Y., J.X., J.L. and L.H.; Methodology, Z.Y., J.X., J.L., L.H., H.X. and X.G.; Resources, L.H. and H.X.; Supervision, S.X. and Y.C.; Validation, J.X. and J.L.; Visualization, Z.Y.; Writing—original draft, Z.Y.; Writing—review and editing, S.X. and Y.C. All authors have read and agreed to the published version of the manuscript.

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