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Branch Lignification of the Desert Plant *Nitraria tangutorum* Altered the Structure and Function of Endophytic Microorganisms

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Abstract: Xerophytes in desert improve their fitness under stress through the development of stems and branches. However, little is known about changes in the structure and function of endophytic microorganisms in response to interactions between desert plants and their environment. In this study, we analyzed the lignification indices of young and mature branches during their development in a typical desert xerophyte, *Nitraria tangutorum*, and combined 16S and ITS high-throughput sequencing techniques to draw the following conclusions. *Nitraria tangutorum* accumulated more lignin, cellulose, and hemicellulose content during lignification. In addition, the number of OTUs and diversity of endophytic bacteria and fungi were reduced. Both endophytic bacteria and fungi were governed by stochastic processes during the development of stems and branches of *Nitraria tangutorum* and were significantly affected by lignification indices. Meanwhile, the development of stems and branches increased the relative abundance of Cyanobacteria and Ascomycota, and the dominant bacterial genera were mostly positively correlated with the lignification indices. In addition, stem and branch lignification reduced endophytic microbial interactions in the relationship between the endophytic bacterial and fungal networks of *Nitraria tangutorum*. Functional prediction analysis further revealed that lignification of *Nitraria tangutorum* branches changed the metabolic function of endophytic bacteria. The results of this study indicate that plant endophytic microorganisms play an important role in resisting and adapting to adversity and provide support for related studies on microbial ecology in desert areas.

Keywords: *Nitraria tangutorum*; branch lignification; endophytic microorganisms; structure and function

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1. Introduction

In arid and semiarid regions, available soil water is the main factor limiting plant growth and distribution. Xerophytic shrubs in desert regions have gradually become the dominant species because of their unique stress resistance mechanisms [1–3]. To avoid the restriction of growth and development of xerophytic shrubs owing to water stress, leaf cuticle thickness, leaf size, and number of stomata were changed to reduce water loss under drought conditions [4]. Additionally, by improving root development and enhancing the ability of xylem to transport water to leaves, plants can compensate for the water

loss caused by transpiration and keep stomata open to maintain photosynthesis [5]. Furthermore, xerophytes accumulate and store absorbed carbon in secondary cell walls, mainly cellulose, hemicellulose, and lignin, thus enhancing secondary stem cell wall development and effectively avoiding and tolerating adversity and survival [6,7]. As plant growth and stress responses also depend on the complex interactions of endophytic microbial communities [8], the development and interactions of endophytic colonies during the stem lignification of xerophytes need to be further studied.

Desert plants are vulnerable to a variety of biotic and abiotic stresses throughout their life cycle, including bacterial, viral, and fungal infestations caused by infectious organisms as well as by drought, salinity, heavy metal pollution, and nutrient deficiencies [9]. Plant endophytes are known to have reciprocal relationships with plant tissues through colonization of plant cells or intercellular spaces. For example, plant endophytic bacteria can improve the biological utilization of mineral nutrients [10], produce plant hormones (IAA, ABA, and gibberellin) [11], and enhance the morphological development and water absorption of plant roots to resist drought stress [12,13]. Endophytic fungi also affect the genetic and phenotypic characteristics of the host to resist pathogens and herbivores, providing nutrients to the host plant by increasing nitrogen metabolism, which in turn activates host defense mechanisms by affecting phytohormone concentrations and secreting metabolites and lysozymes [14,15]. In conclusion, the changes and interactions of the endophytic microbial community structure are important indicators for adaptation to environmental changes, while the ecological process of endophytic microbial community interactions between plants and the environment still requires further research.

Generally, plant endophytic bacteria include Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, whereas endophytic fungi are mostly composed of Ascomycota and Basidiomycota [16,17]. It is difficult to understand the dynamic feedback of the plant microbial community in the environment because changes in the plant microbial community structure are often affected by many factors [9]. Recent studies have shown that plants respond to different physiological states at different developmental stages by regulating endophytic bacterial community structure. In particular, plant tissue type, growth stage, and nutrient uptake affect the composition of host endophytic communities [18–20]. Academic research related to plant lignification has mainly focused on reducing lignin content and increasing the rate of decomposition in herbivores by regulating the expression of genes related to lignin biosynthesis, increasing lignin content, and changing lodging resistance and disease resistance of plants to cope with various stresses [21–24]. Thus, plant growth and the ability to cope with stress are equally dependent on the complex roles of the internal microbial community [8,25]. However, the changes and interrelationships of endophytic microorganisms during the development of the stems and branches of xerophytes require further investigation.

In summary, stem lignification in xerophytes can be regulated to different degrees in response to environmental stress and is a dynamic physiological process [26]. However, the structure of the endophytic community and its response to lignification remain unknown. *Nitraria tangutorum* is a perennial woody plant found in the desert areas of Northwest China. It has a high degree of lignification in its stems and branches, which improves its ability to adapt to harsh habitats under drought and salinization [27,28]. However, studies on the changes in endophytic microorganisms during lignification of *N. tangutorum* have not received much attention. Through the determination of physiological indicators in the process of lignification and high-throughput sequencing analysis of endophytes, this study answered: (1) whether endophytic microorganisms change during the process of *N. tangutorum* lignification and (2) whether the key physiological indicators of lignification (lignin, cellulose, hemicellulose, and pectin) are associated with endophytic colonies.

2. Materials and Methods

2.1. Study Area and Sample Collection

The stems were sampled in August 2020 from *N. tangutorum* plants growing at the Laba Lake Forest Farm, Ningxia, China (106°52′–106°54′ E; 38°44′–38°47′ N) (Figure 1). The area has an altitude of approximately 1250 m above sea level, an average annual temperature of 10.0 °C, and a rainfall of approximately 100 mm, and it belongs to the desert steppe ecosystem. Eighteen *N. tangutorum* plants with the same growth were selected and each plant was spaced 200 m apart. In this study, the anterior and terminal segments of *N. tangutorum* branches were cut using sterile shears and defined as young branches (YB) and mature branches (MB), respectively. Each of these three branches was used as a single replicate. Six young and six mature branch samples were collected. Each branch was sampled to a length of approximately 10–12 cm and returned to the laboratory in sterile centrifuge tubes in a vehicle-mounted refrigerator. The classified *N. tangutorum* branches were washed with sterile water four to five times and then soaked in 70% ethanol for 5 min and in 5% sodium hypochlorite solution for another 5 min. After washing with sterile water, branches were dried under sterile conditions. The samples were divided into two parts: one was placed in a –80 °C refrigerator for endophytic microbial sequencing, and the other was used to determine the physiological parameters of lignification.

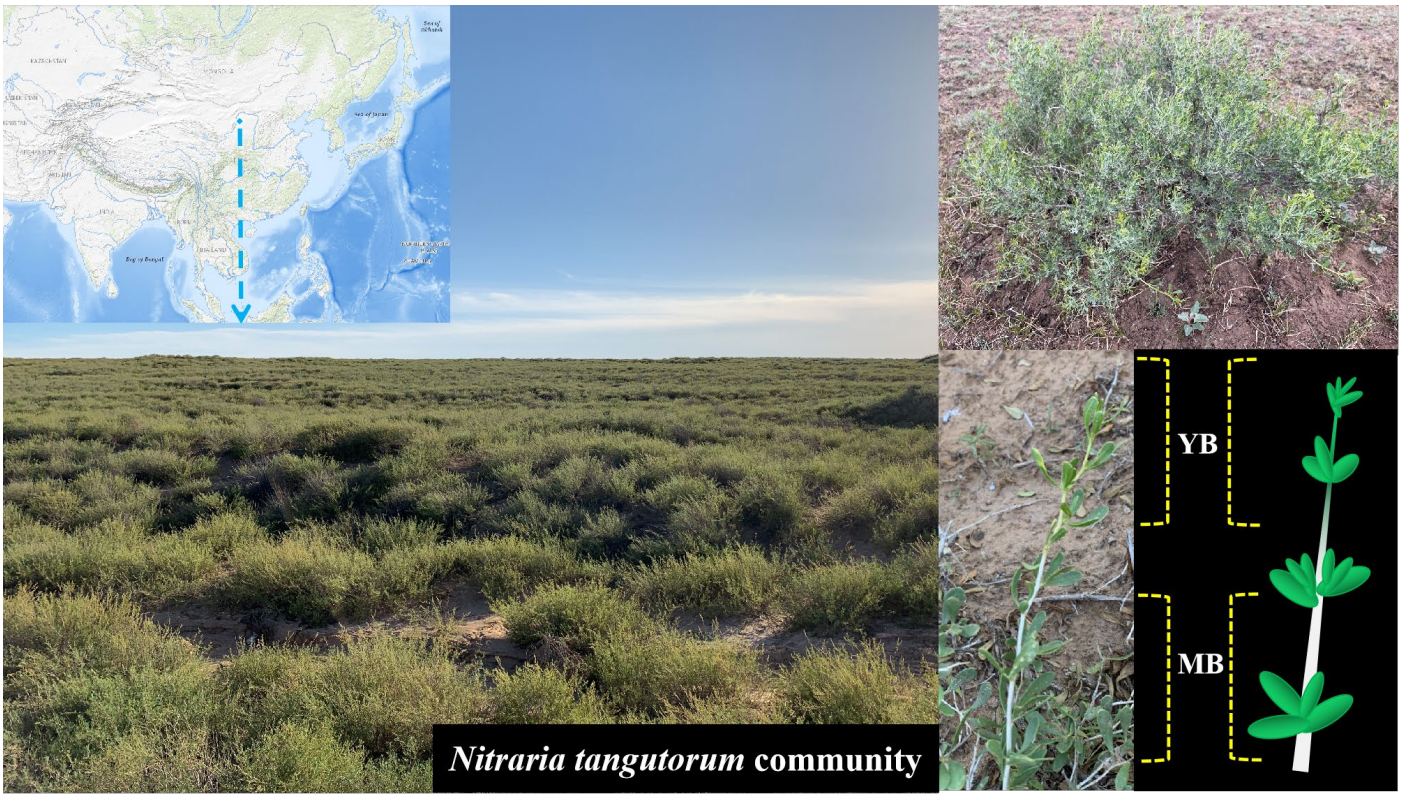

Figure 1. Sampling diagram of *Nitraria tangutorum* in Laba Lake Forest Farm in Northwestern China.

2.2. Determination of Lignification in Young and Mature Branches of N. tangutorum

Fresh tissues from the six young branches and six mature branches were weighed separately, and the thick cell walls of the different tissues were successively extracted using 80% ethanol and acetone. Thick cell walls were added to a 5 mg/mL neutral protease solution and soaked for 15 h, and the extracts were obtained as cell wall substances. The dried cell wall substances were weighed and added to distilled water and concentrated sulfuric acid, and the cellulose content of the plant stems and branches was determined by colorimetry using 2% anthrone reagent. The pectin content was determined by colorimetry. Fresh tissues of *N. tangutorum* were weighed and homogenized with a methanol:

chloroform (1:1) extract solution, and then 50 mmol/L Na_2CO_3 solution was added after extraction in a boiling water bath. After centrifugation to remove the supernatant, concentrated sulfuric acid was added to the boiling water bath and 0.15% carbazole reagent and distilled water were used for colorimetric analysis. Similarly, lignin content was determined using the colorimetric method. The stems and branches were dried in an oven at 80 °C to a constant weight, crushed, sieved, and then added into a water bath with 25% bromo–acetal–acetic acid solution and perchloric acid solution, respectively, followed by 2 mol/L NaOH and a 0.5 mol/L hydroxylamine hydrochloride mixture. After full oscillation, the supernatant was absorbed and acetic acid was added. Colorimetry measurements were performed at a wavelength of 260 nm. The dried and sieved plant stems and branches were weighed, a mixture of acetic and nitric acids was added, and the supernatant was discarded after boiling in a water bath. The precipitate was added with 0.01 mol/L potassium dichrodate solution, followed by 20% potassium iodide solution and 0.5% starch solution, and then mixed and centrifuged. 3.5-dinitrosalicylic acid and distilled water were added, and the content of hemicellulost in nitraria nitraria was determined by colorimetry [29,30].

2.3. DNA Extraction and PCR Amplification from Young and Mature Branches of N. tangutorum

Total genomic DNA was extracted from young and mature branches of *N. tangutorum* using the cetyltrimethylammonium bromide (CTAB) method [31]. The V5-V7 region of 16S rRNA was used to amplify the DNA of endophytic bacteria, and the ITS1-ITS2 region of ITS was used to amplify the DNA of endophytic fungi. The endophytic bacteria were amplified using the primers 799F (5′-AACM GGAT TAGA TACC CKG–3′) and 1193R (5′-ACGT CATC CCCA CCTT CC–3′). The endophytic fungi were amplified using the primers ITS1-F (5′-CTTGGTCATTTA GAGG AAGT AA–3′) and ITS2 (5′-GCTG CGTT CTTC ATCG ATGC–3′) [32,33]. Endophytic bacterial and fungal amplification results from 12 branches (six young and six mature branches) of *N. tangutorum* were sequenced using the NovaSeq PE250 platform of Novogene Bioinformatics Technology Co., Ltd. The raw data were deposited in the NCBI Sequence Read Archive database (PRJNA719708 and PRJNA719773).

Sequencing of endophytic bacteria and fungi in YB and MB of *N. tangutorum* yielded an average of 90,439 and 100,3930 tags and an average of 87,748 and 95,815 valid tags, respectively, after quality control. Effective quality control data were spliced using FLASH (1.2.11) and UPARSE (7.0.1090) and clustered into operational taxonomic units (OTUs) based on a similarity of 97% [34]. A total of 506 bacterial OTUs and 1463 fungal OTUs were identified. A total of 501 (99.01%) bacterial OTUs were annotated to the database after species annotation of bacteria in the SILVA 138 database [35]. A total of 1027 (70.20%) OTUs were annotated in the database after species annotation of fungal OTUs sequences using the UNITE database [36].

2.4. Data Analysis

Alpha diversity indices (OTUs, Shannon, and ACE) of endophytic bacteria and fungi from the YB and MB of *N. tangutorum* were calculated using QIIME (V1.9.1) [37]. The beta diversity of endophytic bacterial and fungal communities was calculated and described using the Bray–Curtis distance matrix in young and mature branches of *N. tangutorum* [38]. To quantify the ecological processes of endophytic bacteria and fungi during lignification of *N. tangutorum* in young and mature branches, the beta-nearest taxon index (βNTI) and Raup–Crick (RCbray) were calculated for 12 samples of young and mature branches [39,40].

The endophytic bacterial and fungal community structures of young and mature branches during the development of stems and branches of *N. tangutorum* were presented by chordal plots of phylum-level interaction frequencies, and changes in the top 20 genera with the highest relative abundance were described. Spearman's correlations between the dominant genera and lignification indices were calculated and described previously [41].

To further clarify the effect of stem and branch development on the ecological niches of endophytic bacterial and fungal communities in YB and MB, we calculated all possible

Spearman correlation coefficients ($R > |0.9|$ and $p < 0.01$) between endophytic bacterial and fungal OTUs in six young and six mature branches of *N. tangutorum* and visualized them using the Cytoscape software (3.7.1) [42]. The above analysis was performed using R software, and all indicators in this study were described using one-way analysis of variance and Duncan's multiple range test for significant differences between groups.

3. Results

3.1. Difference of Lignification in Young and Mature Branches of N. tangutorum

The lignin, cellulose, and hemicellulose contents of YB and MB of *N. tangutorum* differed significantly; the lignin content of MB was 65.85% higher than that of YB, and the cellulose and hemicellulose contents of MB were 4.95% and 41.87% higher than that of YB, respectively ($p < 0.05$). However, the pectin content did not differ significantly between YB and MB (Figure 2A).

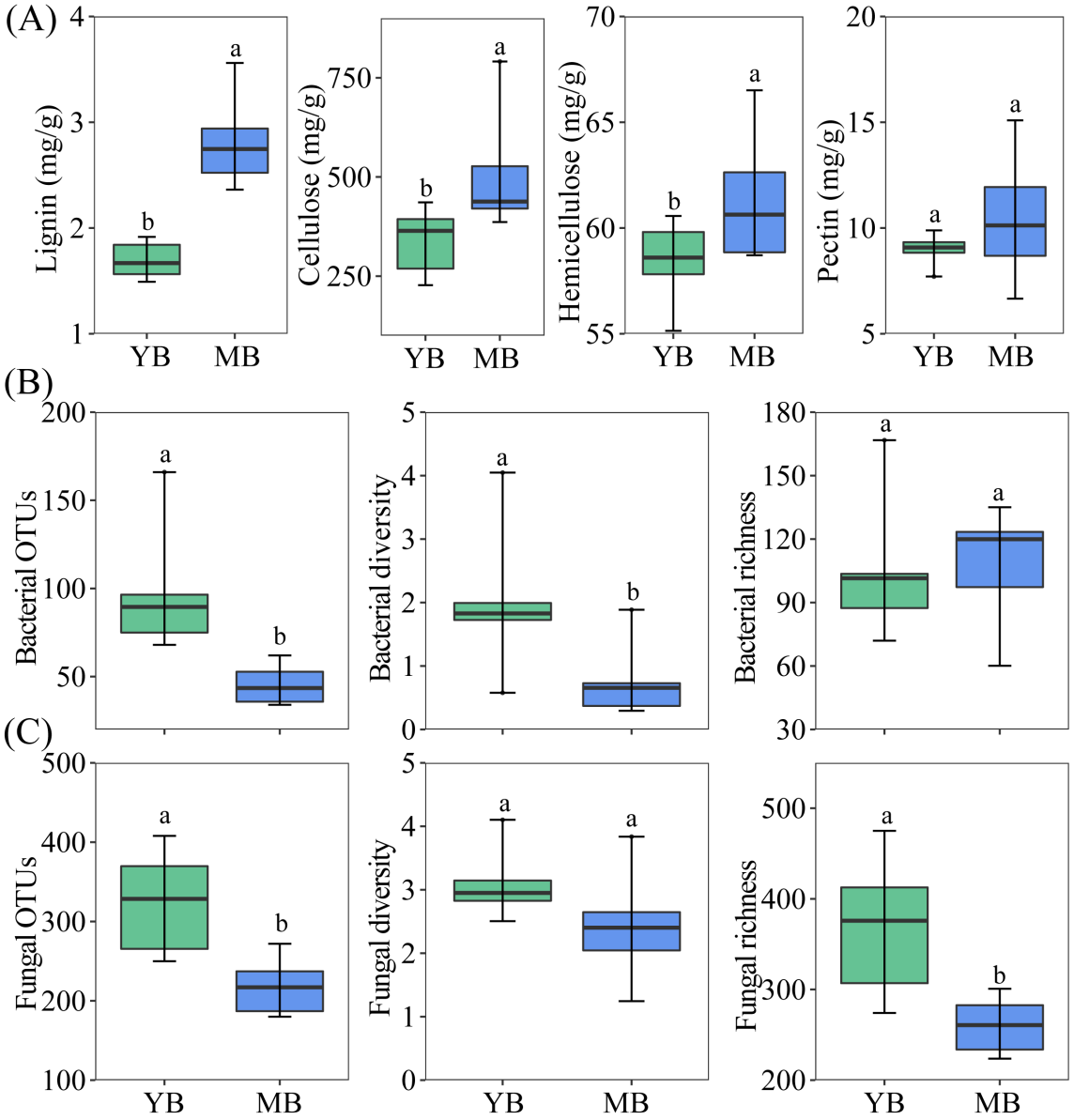

Figure 2. Lignification indices (**A**) and diversity of endophytic bacterial (**B**) and fungal (**C**) communities in young and mature branches of *Nitraria tangutorum*. YB: young branches; MB: mature branches. The letters a and b indicate significant differences.

3.2. Diversity and Community Assembly of Endophytic Bacterial and Fungal Community in YB and MB of N. tangutorum

The alpha-diversity results showed that the number of OTUs and diversity index of endophytic bacteria were higher in YB than in MB, whereas the richness index was lower in YB than in MB, but the difference was not significant (Figure 2B). The number of OTUs, diversity, and richness index of endophytic fungi in YB were higher than those in MB, and the number of OTUs and richness index were significantly different ($p < 0.05$) (Figure 2C).

Non-metric multidimensional scaling (NMDS) analysis showed that the composition of endophytic bacterial communities differed between YB and MB, whereas the composition of endophytic fungal communities was similar (bacteria: $R = 0.8241$, $p = 0.0024$; fungi: $R = 0.5093$, $p = 0.0023$). βNTI and null model analyses further indicated that the endophytic bacterial and fungal communities in YB and MB of *N. tangutorum* were dominated by stochastic processes. Interestingly, the homogenizing dispersal process of the endophytic bacterial community was replaced by ecological drift with the development of stems and branches of *N. tangutorum*. Further analysis of the correlation between Bray–Curit dissimilarity and lignification index showed that the endophytic bacterial community was significantly correlated with the contents of lignin, cellulose, and hemicellulose. The endophytic fungal community was related to lignin, hemicellulose, and pectin content ($p < 0.05$) (Figure 3).

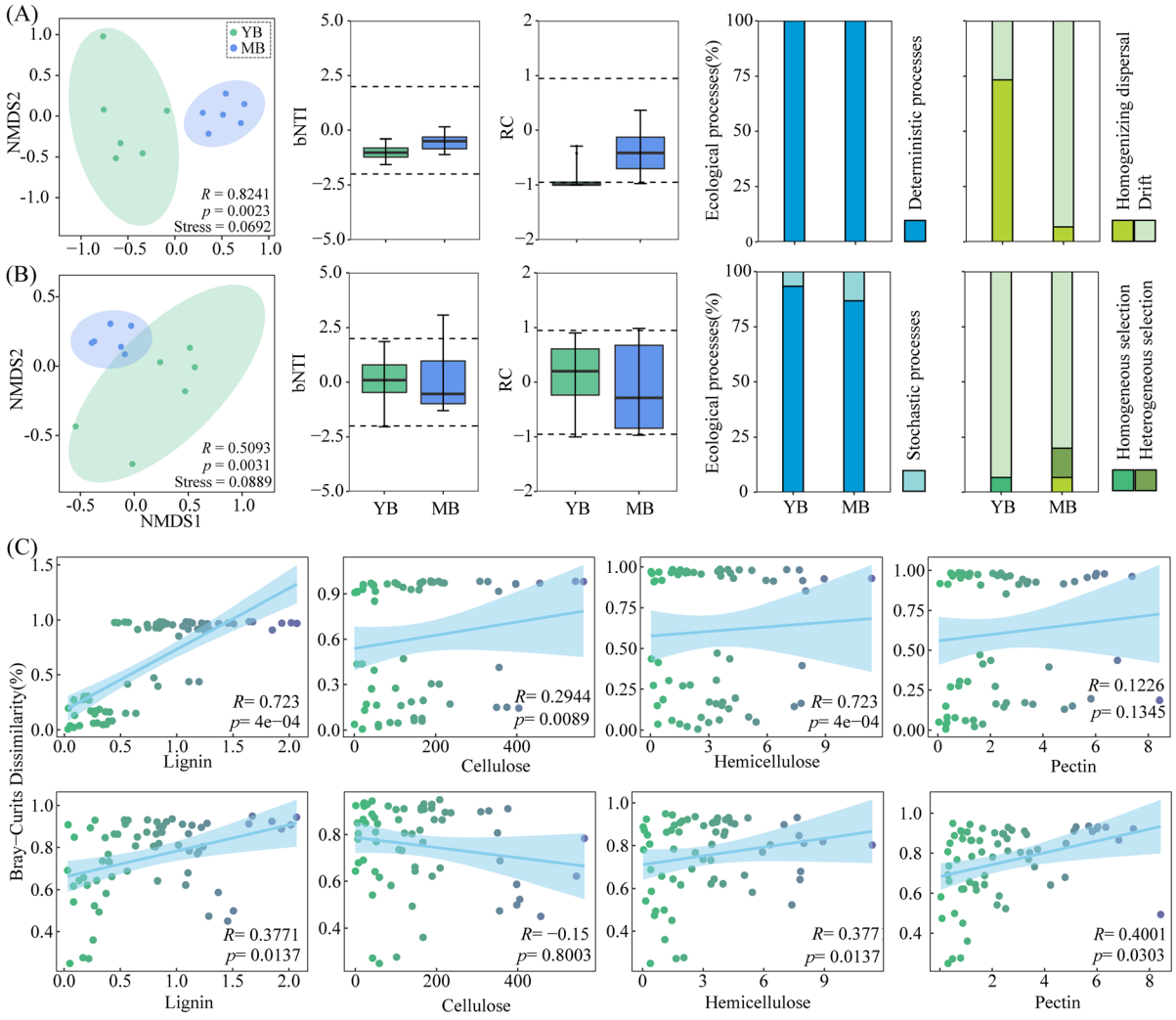

Figure 3. NMDS analysis and non−metric multidimensional scale ordering of endophytic bacterial (**A**) and fungal (**B**) communities, and the relationship between Bray–Curtis dissimilarity with lignification indices (**C**) in young and mature branches of *Nitraria tangutorum*. YB: young branches; MB: mature branches.

3.3. Structural Changes in the Endophytic Bacterial and Fungal Communities in YB and MB of N. tangutorum

After species annotation, YB was dominated by Proteobacteria (84.9%), whereas MB was dominated by Cyanobacteria (92.7%). At the genus level, the relative abundance of Ralstonia was high in YB (Figure 4A). At the fungal phylum level, YB was dominated by Ascomycota (45.1%) and Basidiomycota (19.3%), with Ascomycetes (45.1~94.2%) gradually dominating as young branches developed into mature branches. At the fungal genus level, the relative abundance of Alternaria (13.7~49.8%), Coniothrum (10.8~22.2%), and Microidium (0.03~9.1%) increased with the development of stems and branches, while the relative abundance of Lysurus subsequently decreased (17.6~0.02%) (Figure 4B).

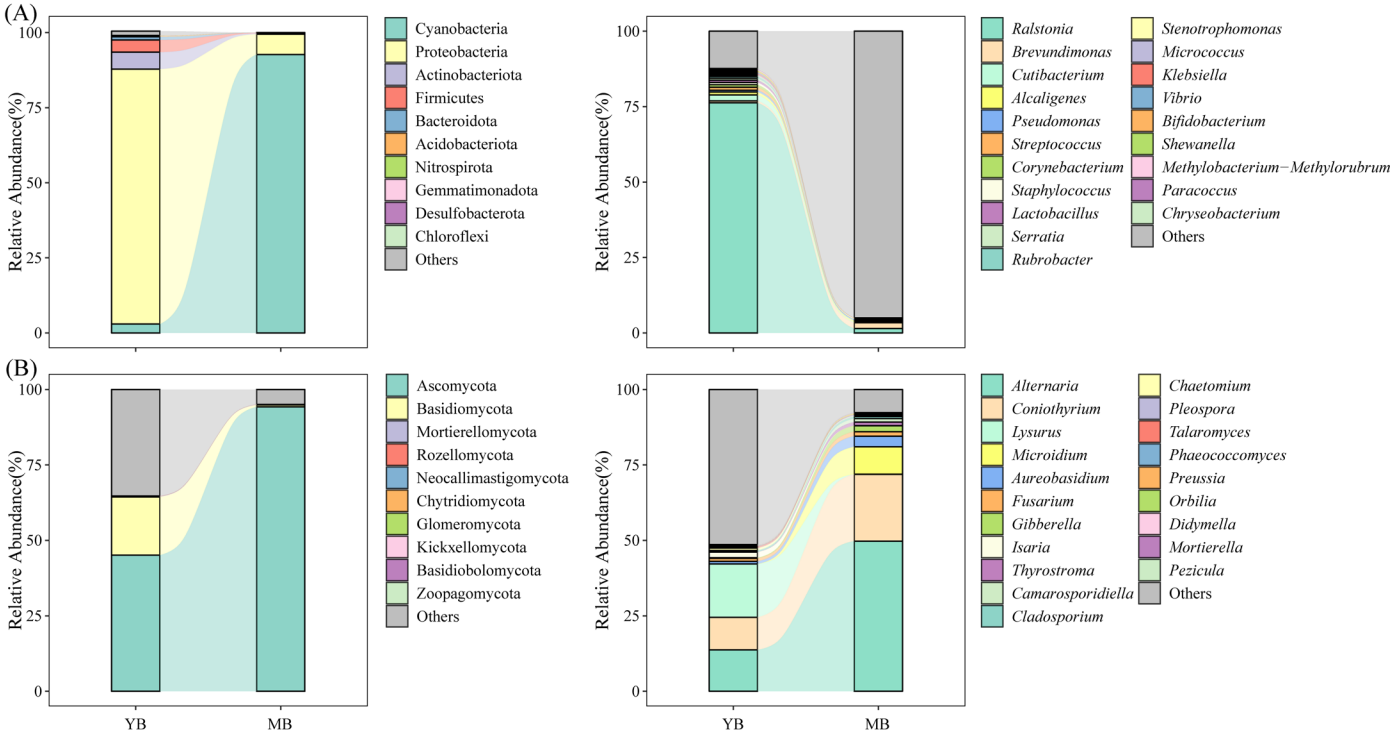

Figure 4. Microbial composition at the phylum and genus level of endophytic bacteria (**A**) and fungi (**B**) in young and mature branches of *Nitraria tangutorum*. YB: young branches; MB: mature branches.

A heat map of the correlation between the top 20 bacterial and fungal genera in terms of relative abundance and lignification indicators revealed that most of the bacterial genera were positively correlated with lignification-related indicators. For example, Brevundimonas, Cutibacterium, Alcaligenes, Serratia, and Stenotrophomonas were significantly positively correlated with lignin content ($p < 0.01$) (Figure 5A). Interestingly, the endophytic fungi of *N. tangutorum* branches, such as Alternaria, Microidium, Pleospora, Preussia, and Camarosporidiella, were negatively correlated with lignin and pectin. However, Lysurus showed a positive correlation with lignin and pectin contents ($p < 0.05$) (Figure 5B).

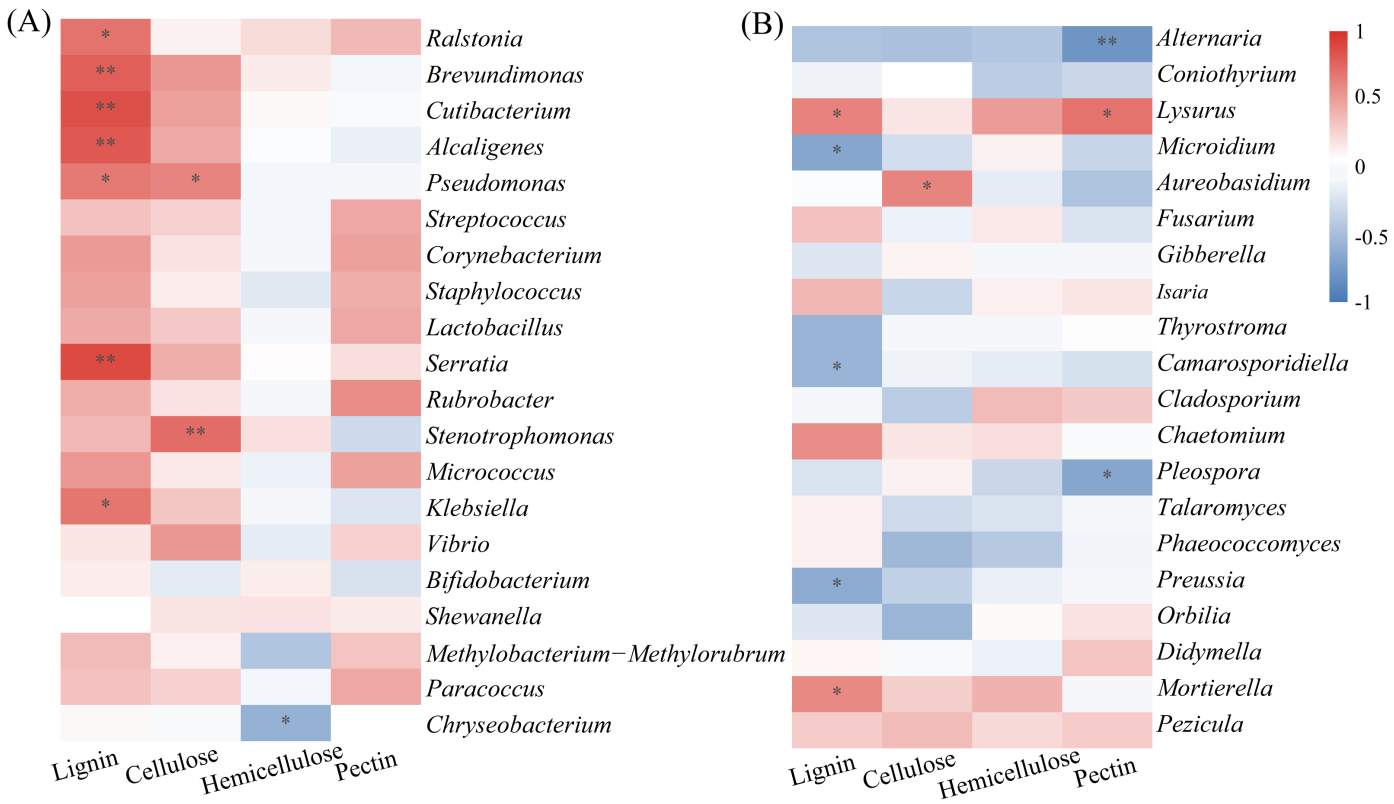

Figure 5. Spearman correlation between the top 20 genera of endophytic bacteria (**A**) and fungi (**B**) with lignification indices in young and mature branches of *Nitraria tangutorum*. (* $p < 0.05$, ** $p < 0.01$).

3.4. Co-Occurrence Network of the Endophytic Microorganism in YB and MB of N. tangutorum

The relationship between endophytic bacteria and fungi in YB and MB was further explored using the same module correlation network analysis, and it was found that there were fifteen modules in young branches and six modules in mature branches. There were more positive correlations in young branches. Compared to mature branches, fungi played a more important role in young branches, and the number of fungi increased from 27 (mature branches) to 54 (young branches), whereas the number of bacteria increased from 12 mature branches to 19 (young branches) (Figure 6, Table 1).

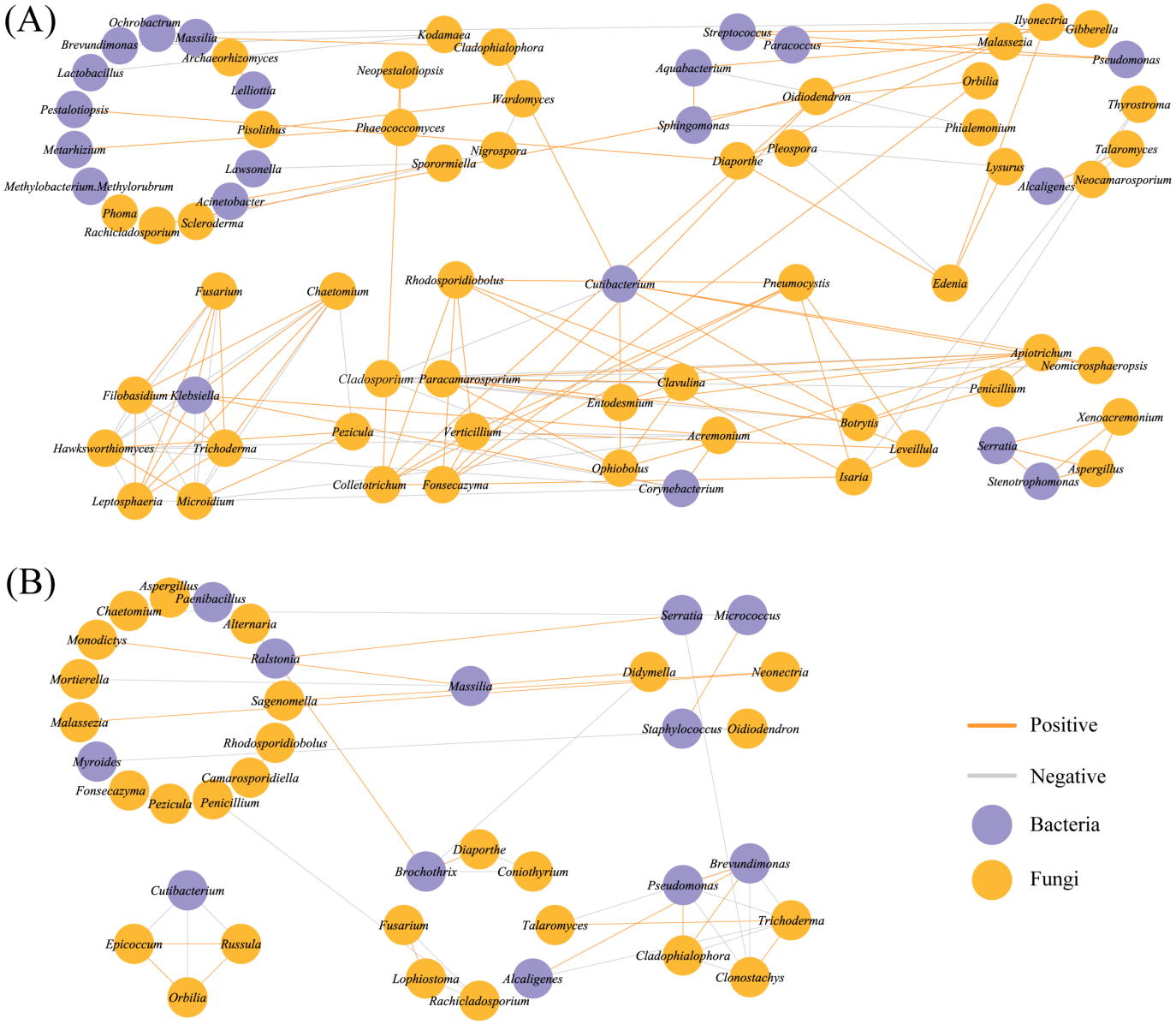

Figure 6. Correlation network of endophytic bacteria and fungi in young (**A**) and mature (**B**) branches of *Nitraria Tangutorum*. The orange lines represent positive links, the gray lines represent negative links, the purple circles represent bacteria, and the yellow circles represent fungi.

Table 1. Network topological features of young and mature branches of *Nitraria tangutorum*.

	YB	MB
Nodes	73	39
Bacteria nodes	54	12
Fungi nodes	19	27
Links	132	43
Positive links	89	19
Negative links	43	24

YB: young branches; MB: mature branches.

3.5. Function Prediction of Endophytic Bacteria and Fungi in YB and MB of N. tangutorum

Functional prediction analysis of endophytic bacteria and fungi in YB and MB of *N. tangutorum* using PICRUSt (Level k) and FunGuild software showed significant changes

in the function of endophytic bacteria in YB and MB. The functional prediction in YB of *N. tangutorum* mainly includes putative transposase and sulfonate/nitrate/taurine transport system.. However, the functional prediction in YB mainly included the ABC-2 type transport system permease protein and phosphate transport system substrate-binding protein. In contrast, the functional prediction of fungi in YB and MB was mostly focused on dung saprotroph-Undefined saprotroph-wood saprotroph, animal pathogen-endophyte-fungal, parasite-plant pathogen-wood saprotroph, plant pathogen-soil saprotroph-wood saprotroph, animal pathogen-dung saprotroph-endophyte-plant pathogen-undefined saprotroph, and plant pathogen (Figure 7).

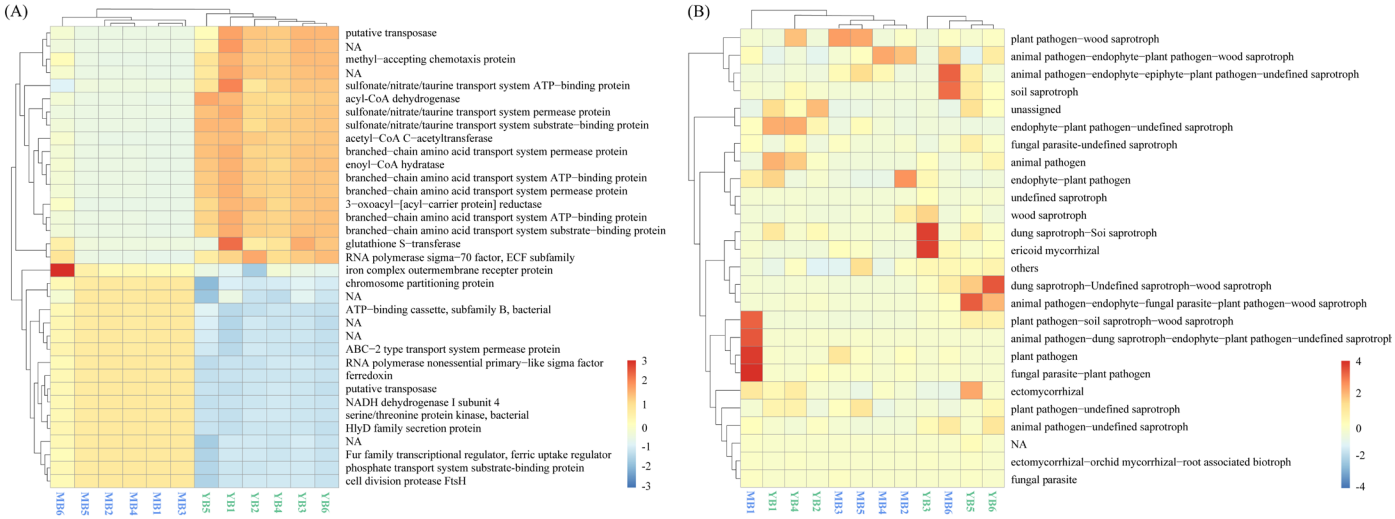

Figure 7. Heat map of clustering of differential metabolites of endophytic bacterial (**A**) and fungal (**B**) communities in young and mature branches of *Nitraria tangutorum*.

4. Discussion

4.1. The Development of Stems and Branches of N. tangutorum Changed the Diversity of Endophytic Microorganisms

Higher plants adapt to drought stress through physiological changes [4,42,43]. Drought stress causes a reduction in plant cell wall thickness and affects cell wall formation by reducing the efficiency of carbon distribution, thereby impeding water and nutrient transport [44]. In the present study, *N. tangutorum*, a shrub with strong resistance to stress, accelerated the development of stems and branches by accumulating lignin to adapt to adverse conditions (Figure 2A). Bray et al. (2004) reported that water deficits affect plant cellulose biosynthesis [45]. Our study found that the cellulose content in mature branches of *N. tangutorum* was significantly higher than that in young branches. Lignification of mature branches maintains the integrity of the cell wall structure and cell turgor pressure to improve cell development under low water potential conditions [46,47]. However, it can enhance cell wall thickness and effectively protect the transport of plant nutrients from the roots to the shoots [48]. In addition, in our results, pectin content was not significantly different between YB and MB, but was slightly higher in MB. It has been speculated that pectin plays an important role in plant cell wall structure and in coping with drought stress [49].

The tolerance of plant functional traits to biotic and abiotic stresses alters the structure of associated microbial communities [50]. Yu et al. (2015) indicated that endophyte OTUs are shared during the growth and developmental stages of plants, but some rare and highly diverse OTUs show specificity at different plant growth stages [51]. We found that the OTUs of both bacteria and fungi were higher in young branches than in mature *N. tangutorum*, indicating that endophyte diversity in plant stems and branches is higher at the developmental stage. In addition, Manirajan et al. (2016) confirmed that the

diversity and abundance of endophytes in plant tissues might be related to the environment and colonization capacity of different ecological niches [52]. In our study, there were differences in endophytic bacterial and fungal community structures between young and mature branches, suggesting that significant changes in the microbial community were closely related to plant growth and development.

NMDS analysis showed that the fungal community structure of *N. tangutorum* branches had a certain overlap, which is similar to previous research [53]. However, in the present study, the endophytic bacterial community did not overlap, and we speculated that the endophytic bacterial community of *N. tangutorum* may play a more critical role in the development of stems and branches. Our study also showed that the ecological processes of endophytic bacterial and fungal community composition of *N. tangutorum* were dominated by stochastic processes, which has also been confirmed in studies of different plant endophytic communities [54,55]. Notably, ecological drift was the dominant factor in the ecological processes of the endophytic bacteria of *N. tangutorum*. It has been pointed out that changes in environmental stressful can induce dispersal limitation of microbial communities, ecological drift can change and shape microbial community structure over time, and environmental changes drive microbial community assembly processes. Our results further confirmed the above conclusions that the composition of both endophytic bacterial and fungal communities in *N. tangutorum* branches was affected by lignification, as well as by demonstrating the response characteristics of endophytic microbial communities to plant growth under stress.

4.2. The Development of Stems and Branches of N. tangutorum Changed the Composition of Endophytic Microorganisms

Previous studies have shown that environmental and temporal changes can alter the composition of plant endophytic microbial communities [56]. Our results indicated that the development of stems and branches of *N. tangutorum* changed the community structure of the endophytic microbial community. Plant endophytes can effectively promote plant growth by directly contributing to the production of plant growth hormones, cytokinins, and gibberellin [57,58]. In this study, the relative abundance of Proteobacteria, Firmicutes, and Bacteroidetes in young branches of *N. tangutorum* was higher, which could secrete 1-Aminocyclopropane-1-Carboxylate deaminase (ACC) and reduce ethylene content (adversity hormone), thus improving plant fitness under stress [59]. Ascomycota and Basidiomycota were the dominant phyla of endophytic fungi in *N. tangutorum* branches, which has been confirmed in previous studies [60].

Differences in endophyte community structure between young and mature branches were also caused by the exposure time of the stem tissue to the environment. Mature stems and branches usually face more environmental pressures than young stems and branches, such as light, radiation, temperature changes, and herbivorous animal invasions [61]. In the present study, this feature was better reflected by differences in endophytic bacteria in branches, and the relative abundance of Ralstonia, which is a typical pathogenic bacterium, was higher in *N. tangutorum*. This may be due to the low lignification of young branches of *N. tangutorum* and its susceptibility to the invasion of pathogenic bacteria [62]. Furthermore, Brevundimonas, Stenotrophomonas, and Alcaligenes all showed potential for biological control [63,64], whereas Cutibacterium was tolerant to arsenic stress [65]. In this study, the above bacteria were positively correlated with lignification indices, which showed that the development of stems and branches of desert plant branches increased the enrichment of beneficial bacteria and thus enhanced the ability of microbial-plant collaboration to resist stress.

In the fungal community, the development of stems and branches also changes the relative abundance of endophytic fungi. It has been reported that the biodiversity and bioactivity of Alternaria can be used to identify host and biogeographic characteristics. The relative abundance of Alternaria in mature branches of *N. tangutorum* in this study was higher than that in young branches, and this genus may be used as a characteristic

genus for the developmental stages of *N. tangutorum* [66,67]. In addition, the relative abundance of Coniothyrium and Aureobasidium increased with the development of stems and branches. It has been noted that Coniothyrium and Aureobasidium also have biocontrol potential [68,69], and it can be seen that lignification of *N. tangutorum* further improves the ability to resist biological stress. It is worth mentioning that Lysurus has a significant positive correlation with lignin and can increase cell membrane permeability and disrupt plant cell wall integrity after infesting plants [70]. We speculate that the prostrate growth characteristics of *N. tangutorum* may increase the chance of contact with pathogenic fungi [71]. The relationship between the properties of these genera and plants needs to be studied further.

4.3. The Development of Stems and Branches of N. tangutorum Changed Relationship and Function of Endophytic Microorganisms

The co-occurrence network among microorganisms can further reveal the social relationships among the microbial communities. In our study, the YB of *N. tangutorum* had a more complex network relationship, and it was speculated that the branch endophytic microbial interrelationships were influenced by biotic and abiotic stresses. Recent studies have pointed out that the influence of plant growth and development on the interrelationship between endophytic microorganisms is crucial, especially in terms of resistance to pathogenic bacterial invasion [72]. In addition, the number of modules and complexity of the network in MB of *N. tangutorum* were lower than in YB, and endophytic fungi occupied more ecological niches, which not only highlighted the importance of endophytic fungi but also indicated the reduction of functional redundancy of endophytic flora during stem and branch development [73]. Many studies have concluded that microbial interactions can be driven by environmental factors, as environmental changes can affect the environment in which microorganisms live [74]. Our study further suggests that the interrelationship between endophytic microbes is equally influenced by the composition of plant tissues, perhaps because the accumulation of carbohydrates in newborn branches is often higher than that in mature branches [75]. Additionally, endophytes can enhance plant resistance to pathogenic bacterial infections by producing antibiotics, inhibiting pathogenic volatile compounds, and inducing systemic resistance [76,77]. Our research further showed that the interrelationship between endophytes is a key factor in the adaptation of *N. tangutorum* branches to adversity during growth and development. In the prediction of endophytic bacterial function, young branches of *N. tangutorum* were significantly correlated with nutrition and energy transport, which not only indicated the involvement of endophytic microorganisms in plant growth and development [78] but also demonstrated that differences in the diversity and composition of endophytic bacterial communities at different developmental stages of plant tissues have different effects on metabolic functions [79]. It is worth noting that the function of endophytic bacteria in mature branches of *N. tangutorum* had a strong correlation with functional proteins such as ABC-2 type transport system permease protein and Fur family transcriptional regulator, ferric uptake regulator, further responding to the adaptability of the development of stems and branches to stress in this species [27,28,80].

5. Conclusions

In conclusion, although current studies have focused more on the differences in endophytes among different plant tissues, our analysis of the differences in endophytes between young and mature *N. tangutorum* branches by high-throughput sequencing revealed that young branches had higher numbers of OTUs and aggregated more bacterial and fungal communities, whereas mature branches had higher environmental adaptability. This study provides a scientific basis for the future responses of plant endophytes to stress, natural senescence, and pathogen infestation. Future studies should conduct a comprehensive analysis of plant rhizosphere habitats and endophytic bacterial and fungal community changes in the plant tissues. At the same time, the scope of this study should

be expanded to provide a detailed analysis of the environmental responses of plant endophytes at the spatial scale.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/. Figure S1: Fit of the neutral community model of community assembly and non-metric multidimensional scale ordering of endophytic bacterial (A) and fungal (B) communities in young and mature branches of *Nitraria tangutorum*. YB: young branches; MB: mature branches.

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