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Variations of soil metal content, soil enzyme activity and soil bacterial community in *Rhododendron delavayi* natural shrub forest at different elevations

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Abstract

Background *Rhododendron delavayi* is a natural shrub that is distributed at different elevations in the karst region of Bijie, China, and that has an important role in preventing land degradation in this region. In this study, we determined the soil mineral element contents and soil enzyme activities. The composition of the soil bacterial community of *R. delavayi* at three elevations (1448 m, 1643 m, and 1821 m) was analyzed by high-throughput sequencing, and the interrelationships among the soil bacterial communities, mineral elements, and enzyme activities were determined.

Results The Shannon index of the soil bacterial community increased and then decreased with increasing elevation and was highest at 1643 m. Elevations increased the number of total nodes and edges of the soil bacterial community network, and more positive correlations at 1821 m suggested stronger intraspecific cooperation. Acidobacteria, Actinobacteria and Proteobacteria were the dominant phyla at all three elevations. The Mantel test and correlation analysis showed that Fe and soil urease significantly affected bacterial communities at 1448 m; interestingly, Chloroflexi was positively related to soil urease at 1448 m, and Actinobacteria was positively correlated with Ni and Zn at 1821 m. Fe and soil urease significantly influenced the bacterial communities at lower elevations, and high elevation (1821 m) enhanced the positive interactions of the soil bacteria, which might be a strategy for *R. delavayi* to adapt to high elevation environments.

Conclusion Elevation significantly influenced the composition of soil bacterial communities by affecting the content of soil mineral elements and soil enzyme activity.

Keywords Karst rocky desertification, Elevation, Soil properties, Soil bacterial community

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Introduction

Karst rocky desertification (KRD) is a major type of karst, which is caused by a vulnerable natural environment and human activities. It is also an eco-environmental problem in Southwest China [1]. The provinces of Guizhou, Guangxi and Yunnan are the main distribution area of karst in China [2]. Among them, Guizhou is the most serious karst area, with an especially strong and typical karst landform and the largest region of exposed carbonate rocks worldwide [3, 4]. The bedrock in the karst area of Guizhou is mainly composed of limestone, dolomite, and dolomitic limestone, which are hard, highly soluble and cannot produce a large amount of soil [1, 5]. This leads to shallow soil layers, severe soil erosion, sparse vegetation and a low vegetation coverage rate [6, 7]. With the growth of the economy and population, the conflict between humans and land has become increasingly serious, leading to the large-scale destruction of karst forests, and exacerbating the vulnerability of karst environments [8]. To prevent the continuous expansion of rocky desertification, with the encouragement and support of the local government, people began to expand the vegetation coverage by planting trees, and grass and building ecological nature reserves to reduce soil erosion [9].

The Baili Rhododendron scenic spot is the largest natural rhododendron forest at the same latitude and low altitude on Earth [10]. It is also one of the few relatively complete high-altitude rhododendron forests preserved in China [11]. The Guizhou Baili Rhododendron Scenic Spot is rich in species of *Rhododendron*, such as *Rhododendron delavayi*, *Rhododendron agastum*, and *Rhododendron irroratum*, which form various monodominant populations [12]. Among them, *R. delavayi* is the main dominant species of the *Rhododendron* community in the Baili Rhododendron Nature Reserve [13]. This species has a wide ecological niche and is distributed in the altitude range of 1400–1900 m, but *R. agastum* and *R. irroratum* are limited to a range of 1600–1800 m [11, 14, 15]. Due to its high ornamental value, *R. delavayi* attracts tourists from across the nation and is also utilized to produce medicine [16]. It can not only promote local economic development as an ornamental plant but also prevent ecological degradation [17]. At present, research on *R. delavayi* has focused mainly on flower color formation [18], reproduction and breeding [19, 20], the composition and diversity of the plant community [10], and changes in the composition of the rhizosphere microbial community [21]. However, the pattern in which changes in soil bacterial communities facilitate the adaptation of *R. delavayi* to various elevation environments remains largely unexplored.

Elevation, as a comprehensive environmental factor, causes drastic changes in water, temperature, light, oxygen and ultraviolet radiation, which eventually affect the composition of the plant community, soil properties, soil heavy metal content and soil enzyme activity, resulting in differences in the soil microbial community structure [22–26]. The elevation dependence of soil enzyme activities is mainly influenced by climatic factors such as light and temperature, which can affect soil enzyme activities by regulating vegetation changes and plant root metabolic activities [27–29]. Soil enzymes play an important role in the transformation of nutrients such as C, N and P [30]. Soil invertase, an index of the C cycle, can hydrolyze carbohydrate polymers into simpler sugars. Soil urease plays a vital role in the nitrogen cycle and can hydrolyze urea and transform organic nitrogen into inorganic nitrogen [31]. Esters and anhydrides of phosphoric acid can be hydrolyzed by phosphatase [32]. Catalase can decompose hydrogen peroxide into molecular oxygen and water, thus preventing cells from being damaged by reactive oxygen species [33]. One study demonstrated that different elevations obviously affected plant types and heavy metal contents [34]. For example, the Zn content at 1250 m was significantly greater than that at 380 m and 820 m, while the Pb content at 380 m was greater than that at 820 m and 1250 m [35]. Microorganisms are also important indicators of soil health; they can predict changes in soil environmental quality because they are sensitive to external disturbances [36]. The soil microbial community is principally composed of bacteria and fungi, but bacteria are more resistant to heavy metals than are fungi [37]. The diversity and composition of soil bacterial communities change with elevation, and the response of soil C- and N-cycling microbial structures is complex as plants adapt to different elevations [38]. For example, the relative amount of psychrophilic heterotrophic bacteria, fungi and gram-negative bacteria increased with increasing elevation in the Austrian Central Alps [39].

Currently, research on the rhizosphere microorganisms of *R. delavayi* has focused mainly on the soil microbial community structure at a single elevation [21, 40] or at different elevations [41]. However, it is unclear how changes in the soil bacterial community composition at different elevations, especially changes in soil bacteria, help *R. delavayi* adapt to the KRD environment. Therefore, it is highly important to study the effects of soil enzyme activity and soil metal changes on the soil bacterial community structure along an elevation gradient to explore the process of plant adaptation to different elevations in karst areas. The aims of our study were to understand (1) the effects of various elevations on soil mineral elements and soil enzyme activities, (2) the effects of different elevations on the soil bacterial community structure of *R. delavayi*, and (3) the relationships among soil mineral elements, soil enzyme activities and the soil bacterial community in *R. delavayi*.

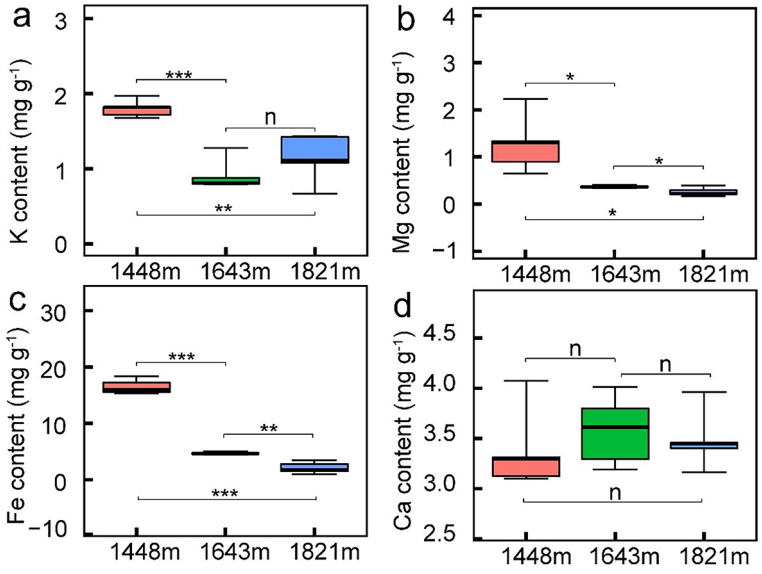

Fig. 1 The effect of the different elevations on the content of soil K, Mg, Fe, Ca. **a**: K content, **b**: Mg content, **c**: Fe content, **d**: Ca content. *, **, *** indicate that there are significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$ between two different elevations, respectively; n indicate that there is no significant difference between two different elevations

Materials and methods

Study sites and soil samples

The Baili Rhododendron Nature Reserve is located in Bijie city, Guizhou province. We selected three elevations representing the typical *R. delavayi* community, including 1448 m(E106°2', N27°13'), 1643 m (E105°51', N27°13') and 1821 m (E105°53', N27°15'). There were 15 plots at three different elevations, each elevation had 5 replicate plots, the size of each plot was 4 m × 4 m, and the distance between them was 10 m. Furthermore, three separate soil samples were collected from each plot of *R. delavayi*. The litter was removed, and three points were selected for collecting soil from each plot. The three individual soil samples were blended into a composite of soil from each plot. Before collecting the soil samples, the litter and humus layers were removed, and the 0–10 cm soil layer was collected. The visible stones and roots were removed from the soil samples with a 2 mm sieve before soil analysis. A portion of each soil sample was placed into a 50 mL aseptic tube, frozen in liquid nitrogen, and stored at -80 °C until the soil DNA was extracted and the soil enzymes were measured. Then, another part of each soil sample was stored in plastic bags on ice, transported to the laboratory, and air dried before determining the soil metal content.

Determination of soil metal content and soil enzyme activity

The content of heavy metals measured in this study was the total content in the soil. The contents of soil potassium (K), magnesium (Mg), iron (Fe), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), zinc (Zn), and nickel (Ni) were determined by atomic absorption spectroscopy according to the procedure of Qin et al. [42]. In addition, soil invertase, phosphatase and urease activities were analyzed according to the methods of Hou et al. [43], and soil catalase activity was analyzed according to the method of Zhang et al. [44].

DNA extraction, high throughput sequencing and raw data analysis

DNA was extracted from 350 mg of soil via the MP BIO FastDNA SPIN Kit (MP Bio, Santa Ana, CA). The V5V7 region of the 16S rRNA gene was amplified with the PCR primer pair 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3'). The PCR products were sent to Genesky Biotechnologies, Inc., Shanghai, 201, 315 (China), for to high throughput sequencing via the Illumina NovaSeq 6000 platform. We removed the primer sequences and adaptors with the cut adapt [45] plug-in of QIIME2 [46]. We used the DADA2 plug-in of QIIME2 to filter the data and denoised, merged and removed chimeras to obtain high-quality sequences [47]. The clean reads were processed into amplified sequence variants by using QIIME2.

Significant differences between the two different elevations in terms of the soil metal elements content, soil enzymes activity and Shannon index were determined via independent *t* tests, and the differences were considered significant at $P<0.05$. The Shannon index of the soil bacterial community, redundancy analysis (RDA), PCoA, heatmap, Mantel test, co-network analysis of the soil bacterial communities at the phylum level, and analysis of the relationships between the soil properties and the top

10 phyla were performed with R (version 4.1.3). LEfSe analysis (LDA > 3, $P < 0.05$) was performed using Omic-Studio tools (https://www.omicstudio.cn/tool).

Results

The contents of soil K, Mg, Fe and Ca varied awith elevation in the *R. delavayi* natural shrub forest

Mg and Fe levels clearly decreased with increasing elevation (Fig. 1b, c). The soil K content was highest at 1448 m and lowest at 1643 m (Fig. 1a). Interestingly, the change trend of the Ca content was opposite to that of the K content, but no significant difference was observed between elevations for either element (Fig. 1d).

Effect of elevation on the contents of soil Cr, Ni, Cu, Cd, Pb and Zn

The contents of soil Cr, Ni, Cu, Pb and Zn decreased with increasing elevation (Fig. 2a, b, c, e, f). The contents of Cr, Ni, Cu, Pb and Zn in the soil did not obviously change between 1448 m and 1643 m, but they had significantly differed between 1448 m and 1821 m. The contents of Ni, Cu, Pb and Zn increased by 629%, 123%, 101% and 124%, respectively, at 1448 m compared with those at 1821 m. However, the soil Cd content did not significantly differ between the two elevations (Fig. 2d).

Differences in soil enzyme activity at different elevations

Fig. 3 shows no clear difference in invertase activity between 1448 m and 1643 m, or between 1643 m and 1821 m, but the invertase activity was significantly greater at 1821 m than at 1443 m. (Fig. 3a). The soil urease and catalase activities were significantly lower at 1821 m than at 1448 m and 1643 m. In addition, the soil urease activity was highest at 1448 m, while the catalase activity was highest at 1643 m (Fig. 3b, d). The soil phosphatase activity was highest at 1643 m and lowest at 1821 m, and its activity significantly differed between these two elevations (Fig. 3c)

Effects of elevation on the Shannon index and composition of soil bacterial communities

As shown in Fig. S1 the Shannon index tended to first increase and then decrease with elevation, but these changes did not reach statistical significance. Furthermore, our results indicated that Acidobacteria, Proteobacteria, and Actinobacteria were dominant at all three elevations (Fig. 4a). At 1448 m, Acidobacteria (55.43%), Proteobacteria (20.27%), and Actinobacteria (9.95%) were the main bacterial phyla (Fig. 4a). Similarly, at 1643 m, the dominant bacterial phyla were Acidobacteria (48.45%), Proteobacteria (32.35%), and Actinobacteria (11.56%) (Fig. 4a). At 1821 m, the abundances were Acidobacteria (45.38%), Proteobacteria (31.96%), Actinobacteria (13.15%), (Fig. 4a).

When we investigated the bacterial communities of *R. delavayi* at the genus-level, we found that there were more variation patterns of classified genera between different elevation gradients (Fig. 4b). Our results showed that *Rhodoplanes* (20.01%), *Candidatus Solibacter* (12.76%), and *Bradyrhizobium* (6.61%) were the main bacteria genera at 1448 m. Additionally, at 1643 m, the dominant bacteria genera were *Rhodoplanes* (15.34%), *Candidatus Solibacter* (7.65%), and *Burkholderia* (3.2%). At 1821 m, the abundances were *Rhodoplanes* (17.2%), *Candidatus Solibacter* (8.19%), and *Burkholderia* (7.77%),, (Fig. 4b).

The results of β-diversity showed that the first principal coordinate and the second principal coordinate explained 30.29% and 22.27% of the total variation of the soil bacterial community at all three elevations. The soil

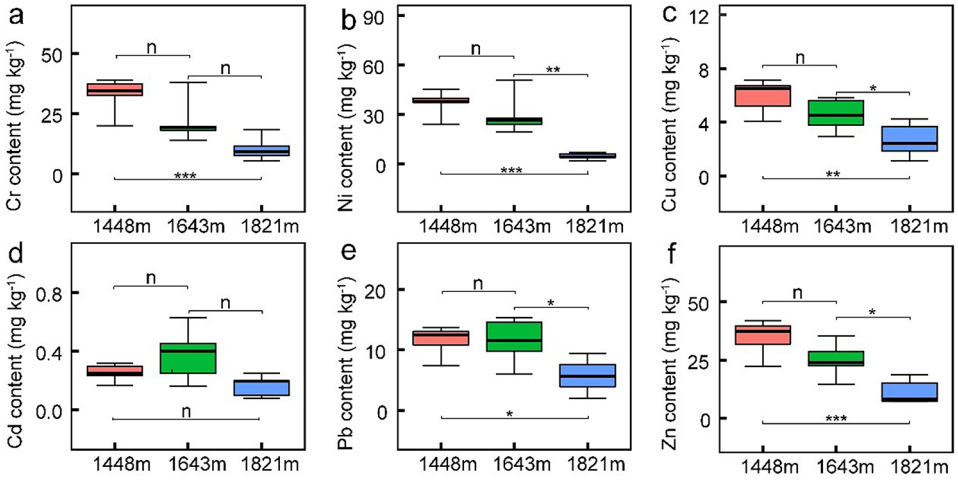

Fig. 2 Effect of the different elevations on the content of soil Cr, Ni, Cu, Cd, Pb, Zn. **a**: Cr content, **b**: Ni content, **c**: Cu content, **d**: Cd content, **e**: Pb content, **f**: Zn content. *, **, *** indicate that there are significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ between two different elevations, respectively; n indicate that there is no significant difference between two different elevations

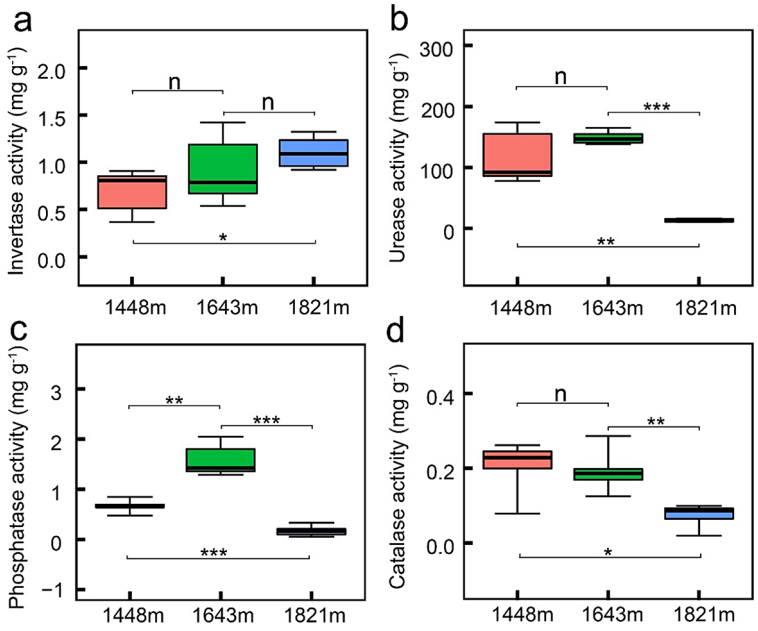

Fig. 3 Effect of the different elevations on the activity of soil enzyme. **a**: invertase activity, **b**: urease activity, **c**: phosphatase activity, **d**: catalase activity. *, **, *** indicate that there are significant difference at $P < 0.05$, $P < 0.01$ and $P < 0.001$ between two different elevations, respectively; n indicate that there is no significant difference between two different elevations

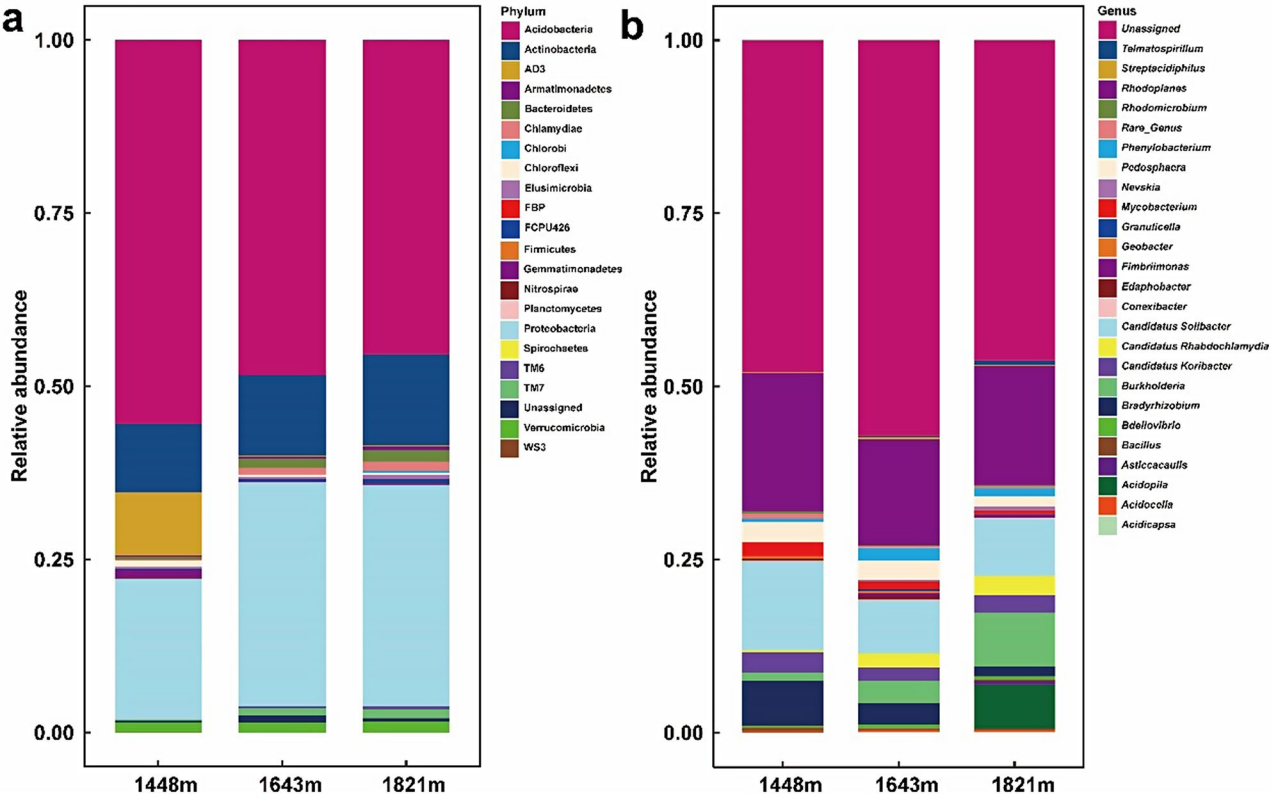

Fig. 4 Relative abundances of the bacteria community. **a**: Phylum level, **b**: Genus level

bacterial communities of the three elevations were obviously dispersed, but the points at the same elevation were aggregated. This indicated that the structures of the soil bacterial communities were clearly separated at 1448 m, 1643 m and 1821 m (Fig. 5). In addition, the heatmaps at the phylum and genus level also suggested patterns of bacteria communities at different elevations similar to the grouping pattern observed in PCoA (Fig. S2, S3). The relative abundance of the top 22 phyla at 1643 m and 1821 m was higher compared with that at 1448 m (Fig. S2). However, the relative abundance of the top 45 genera at 1448 m, 1643 m and 1821 m was similar (Fig. S3).

Redundancy analysis of the correlation between soil metals and soil enzymes and soil bacterial communities at the phylum and genus levels

The first and second axes of the RDA explained 65.15% and 16.50% of the total variance at the phylum level, respectively, for the soil mineral elements and soil bacterial phyla at the three elevations (Fig. S4a). The RDA revealed that Mg, Fe, Zn, Cr, Cu, Ni and Pb had strong positive effects on Gemmatimonadetes; K was positively correlated with Acidobacteria, AD3 and Chloroflexi; Ca had a positive effect on Chlamydiae, Bacteroidetes, TM7 and Verrucomicrobia; and Cd had a positive effect on Actinobacteria (Fig. S4a). In particular, Fe ($P=0.001$) was the factor most strongly correlated with the differences in the composition of the soil bacterial communities, explaining 22.3% of the variance in the observed variation among the elevations (Fig. S4a). For soil mineral elements and soil bacterial genera at the three elevations, the first and second axes of the RDA explained 43.54% and 21.84%, respectively, of the total variance (Fig. S4b). Cd, Pb, Ni and Cr were positively correlated with the genera *Pedosphaera* and *Candidatus. Koribacter*; K, Fe and Mg were positively correlated with *Mycobacterium*, *Bradyrhizobium*, *Rhodoplanes* and *Candidatus. Solibacter*, while Ca had a positive relationship with *Phenylobacterium* and *Candidatus*. Furthermore, Fe ($P=0.001$) was also the factor most strongly correlated with the differences in the composition of the soil bacterial communities, explaining 18.7% of the variation (Fig. S4b). The first and second axes of the RDA explained 78.53% and 15.18% of the total variance at the phylum level, respectively, for soil enzyme activities and soil bacterial phyla at the three elevations (Fig. S4c). The RDA revealed that the urease and catalase activities were positively correlated with Gemmatimonadetes and Acidobateria; phosphatase activity was positively correlated with Actinobacteria, Proteobacteria and TM7; invertase activity was positively correlated with Chlamydiae, Bacteroidetes and Verrucomicrobia. Moreover, invertase ($P=0.13$) explained the most variation, with 50.8% of the variation explained (Fig. S4c). The first and second axes of the RDA explained 62.35% and 21.15% of the total variance at the genus level, respectively, for soil enzyme activities and soil bacterial genera at the three elevations (Fig. S4d). Invertase was positively correlated with *Phenylobacterium* and *Candidatus*, while phosphatase, urease and catalase were only positively correlated with *Bradyrhizobium*. In particular, urease ($P=0.029$) explained the most variation, with 29.5% of the variation explained (Fig. S4d).

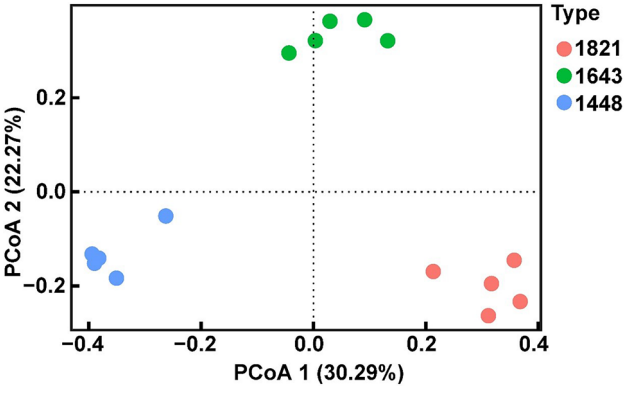

Fig. 5 Effect of different elevations on bacteria β-diversity. Principal coordinate analysis (PCoA) of all bacteria communities based on Bray-Curtis

Correlations between soil mineral elements and soil enzyme levels with soil bacterial phyla

The Mantel test and correlation analysis showed that Fe and urease significantly affected the bacterial community at 1448 m. However, there was no significant correlation between soil variables and bacterial communities at 1643 m and 1821 m (Fig. 6). The response of bacterial phyla at different elevations to changes in soil mineral elements differed (Fig. S5a, b, c). At 1448 m, we found that the K content was negatively related to Gemmatimonadetes, the Fe content was negatively related to Chloroflexi; and the Cr and Ni contents were negatively related to TM7. However, the contents of Mg, Ca, Cu, Zn, Cd and Pb had no significant relationships with the bacteria. At 1643 m, the Mg content had negative relationships with Bacteroidetes and Chlamydiae, the Ca content had a negative relation with TM7, the Ni content had a negative relation with Chloroflexi, and the Cu and Pb contents had a negative relation with Chlamydiae. Interestingly, the contents of K, Fe, Cr, Zn and Cd had no significant relationship with the type of bacteria. At 1821 m, the K content also had a negative relation with Gemmatimonadetes; the Fe content had a positive relation with Acidobacteria, and the contents of Cr, Cu, Zn, and Pb had a negative relation with Acidobacteria but a positive relation with Actinobacteria. The Ni content had a positive relationship with Actinobacteria, while the contents of Mg, Ca and Cd had no significant correlation with the type of bacteria. (Fig. S5a, b, c). The RDA also revealed that at the different elevations, the contents of

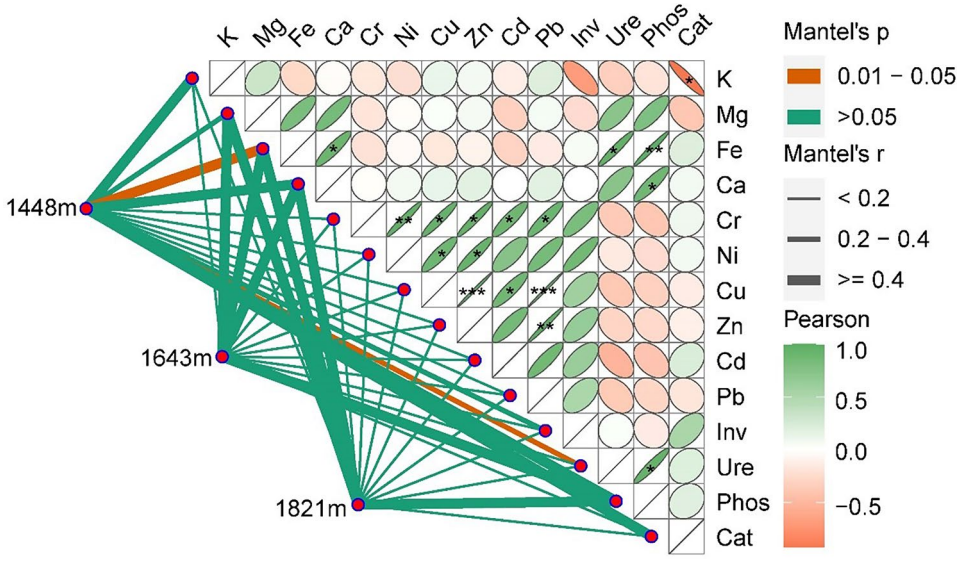

Fig. 6 Correlation analysis among soil mineral elements, soil enzyme and soil bacteria communities (Bray-Curtis distances) in the three elevation gradients on the Mantal Mantel test. Inv: invertase, Ure: urease, Phos: phosphatase, Cat: catalase

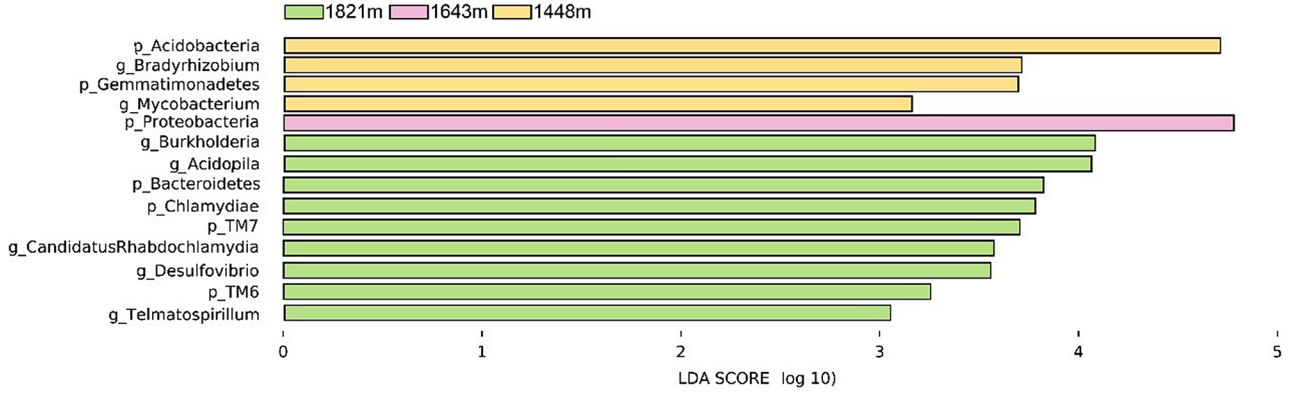

Fig. 7 Linear discriminant analysis (LDA) effect size (LEfSe) showing the differential abundance of phyla and genus level at three elevations. Phyla and Genera listed were both significantly different (LDA > 3, $P < 0.05$)

Fe, Cr and K were the main factors affecting the soil bacterial communities at 1448 m, and the Ca content was the main factor affecting the bacterial communities at 1643 m (Fig. S4a).

The bacterial phyla at different elevations to changes in soil enzyme activities differed (Fig. S5d, e, f). At 1448 m, invertase had a positive relationship with Chlamydiae, urease had a negative effect on Chloroflexi; phosphatase had a positive effect on Actinobacteria; and catalase had a negative relationship with TM7. At 1643 m, there was no significant correlation between any of the soil bacterial communities and soil enzyme activity, except for catalase, which had a positive relationship with Acidobacteria. At 1821 m, invertase was positively related to Chloroflexi, phosphatase was positively related to Chloroflexi, and was negatively related to Proteobacteria. Only urease and phosphatase were not significantly related to the bacteria (Fig. S5d, e, f). The RDA also revealed that urease and catalase activity were the main factors correlated with the soil bacterial communities at 1448 m, and phosphatase activity was the main factor correlated with the soil bacterial communities at 1821 m (Fig. S4c). These variables were considered potential factors affecting these communities.

Different biomarkers at the phylum and genus levels are present at three different elevations

LEfSe analysis revealed that there were different biomarkers in the soil at different elevations. For example, at 1448 m, enrichment of Acidobacteria, *Bradyrhizobium*, Gemmatimonadetes and *Mycobacterium* were significant; at 1643 m, only Proteobacteria was significance enriched; while at 1821 m, the enrichment of *Burkholderia*, *Acidopila*, Bacteroidetes, Chlamydiae, TM7, *Candidatus Rhabdochlamydia*, *Desulfovlbrio*, TM6 and *Telmatospirillum* were significant (Fig. 7).

Co-occurrence network characteristics of soil bacterial communities at three elevations

Co-occurrence network analysis revealed that the total number of nodes in the network was 291, 317 and 345 at 1448 m, 1643 m and 1821 m, respectively. At the bacterial phylum level, the total number of edges was 2153, 317 and 345 at 1448 m, 1643 m and 181 m, respectively. Positive (99.91%) and negative (0.09%) edges were identified at 1448 m and 1643 m, respectively. In addition, the positive edges were 100% at 1821 m (Table 1). The abundances of Proteobacteria, Acidobacteria and Actinobacteria were high at all three elevations; 1448 m (40.55%, 23.71%, 19.24%), 1643 m (41.64%, 22.71%, 17.98%) and 1821 m (39.71%, 22.32%, 16.81%), respectively (Fig. 8).

Discussion

Soil mineral elements can be affected by soil parent material, climate and topography [48–51]. Previous studies have demonstrated that the contents of Fe, Mg, Cu, Zn, and Ni in soil decrease with increasing elevation [52–54]. Our results were consistent with these results in that the contents of Mg, Fe, Cr, Ni, Cu, Pb, and Zn in the soil gradually decreased as the elevation increased. The reason could be that large amounts of surface water carry soil heavy metals from high to low elevations [55]. In addition, elevation causes changes in soil organic carbon content, and a large amount of organic matter strongly adsorbs metal elements, thus contributing strongly to the vertical gradient of metal elements [50]. Similarly, elevation affects plant litter, altering the microbial activity and composition; resulting in variations in soil nutrients [56, 57]. Our findings are consistent with the study of soil nutrients by Liu et al. [41]. This phenomenon is probably caused by the different degrees of soil differentiation at different elevations, because light and ventilation are greatly affected by rainfall at high elevations [58, 59]. As a result, the contents of soil organic matter and humus differ, and they contain functional groups and have chelating properties that limit the bioavailability of heavy metals and increase their concentrations [60, 61].

Elevations correspond to soil type and microbial activity. These changes have important impacts on the migration and transformation of heavy metals in soil [62]. It has been reported that heavy metals affect the soil C and N cycles via soil microorganisms and enzymes [63, 64].

Table 1 The co-occurrence network properties of soil bacterial communities

Degree	1448 m	1643 m	1821 m
Total nodes	291	317	345
Total edges	2153	2236	2815
Average degree	14.80	14.11	16.32
Positive edges	99.91%	99.91%	100.00%
Negative edges	0.09%	0.09%	0

Our results showed that between two different elevations, there were significant differences in the contents of Fe and Mg in the soil, which indicated that Fe and Mg were the dominant factors controlling soil quality. The Fe and Mg contents were the highest at 1448 m and the lowest at 1821 m, implying that the soil parent material aggravated the accumulation of soil mineral elements, which was consistent with previous studies [53, 54]. Moreover, we observed a significant difference in K, Cr, Ni, Cu, Zn and Pb between 1448 m and 1821 m, but there was no significant difference in these mineral elements between 1448 m and 1643 m. Li et al. [65] demonstrated that as elevation increases, temperature decreases, causing a greater degree of decomposition of litter in low elevation areas than in high elevation areas, resulting in the release of K. In addition, all heavy metals are positively correlated with soil organic matter, and the organic compounds produced by organic matter decomposition and the organic functional groups of humic acid adsorb heavy metals to form stable compounds [62].

In karst ecosystems, plant type [66], heavy metal content [67], temperature [68], humidity [69], and soil properties [70] affect the growth of soil microorganisms and soil enzyme activity. Our study showed that invertase activity increased with increasing elevation, which was consistent with the findings of Ma et al. [71]. Most likely, the content of soil heavy metals decreased with increasing elevation, and the inhibitory effect of heavy metals on soil invertase activity decreased [72]. In addition, the reduction in soil temperature with increasing elevation hindered the breakdown of organic materials, resulting in their accumulation [71]. Invertase is directly involved in the metabolic process of soil organic matter [73]. Generally, the soil organic matter content is directly proportional to invertase activity [74]. Interestingly, the findings of the current study suggested that catalase activity decreased when the elevation increased. This may be due to the high concentration of heavy metals at 1448 m, and plants also produce a substantial amount of H_2O_2 when subjected to heavy metal stress. An increase in catalase activity can effectively decompose H_2O_2 into H_2O and O_2 and reduce its toxic effect on plants [75, 76]. In addition, our study revealed that urease and phosphatase activity first increased and then decreased with increasing elevation. These enzyme activities were the lowest at 1821 m, and there was a significant difference between 1643 m and 1821 m between and 1448 m and 1821 m. The activities of urease and phosphatase were also positively correlated with Fe. The possible reasons might be that elevation affects soil type and organic matter content, (1) as a substrate for enzymatic reactions, the content of soil organic matter affects the activity of soil enzymes [71]; (2) soil enzyme activity is directly related to climate change at different elevations [77], and the

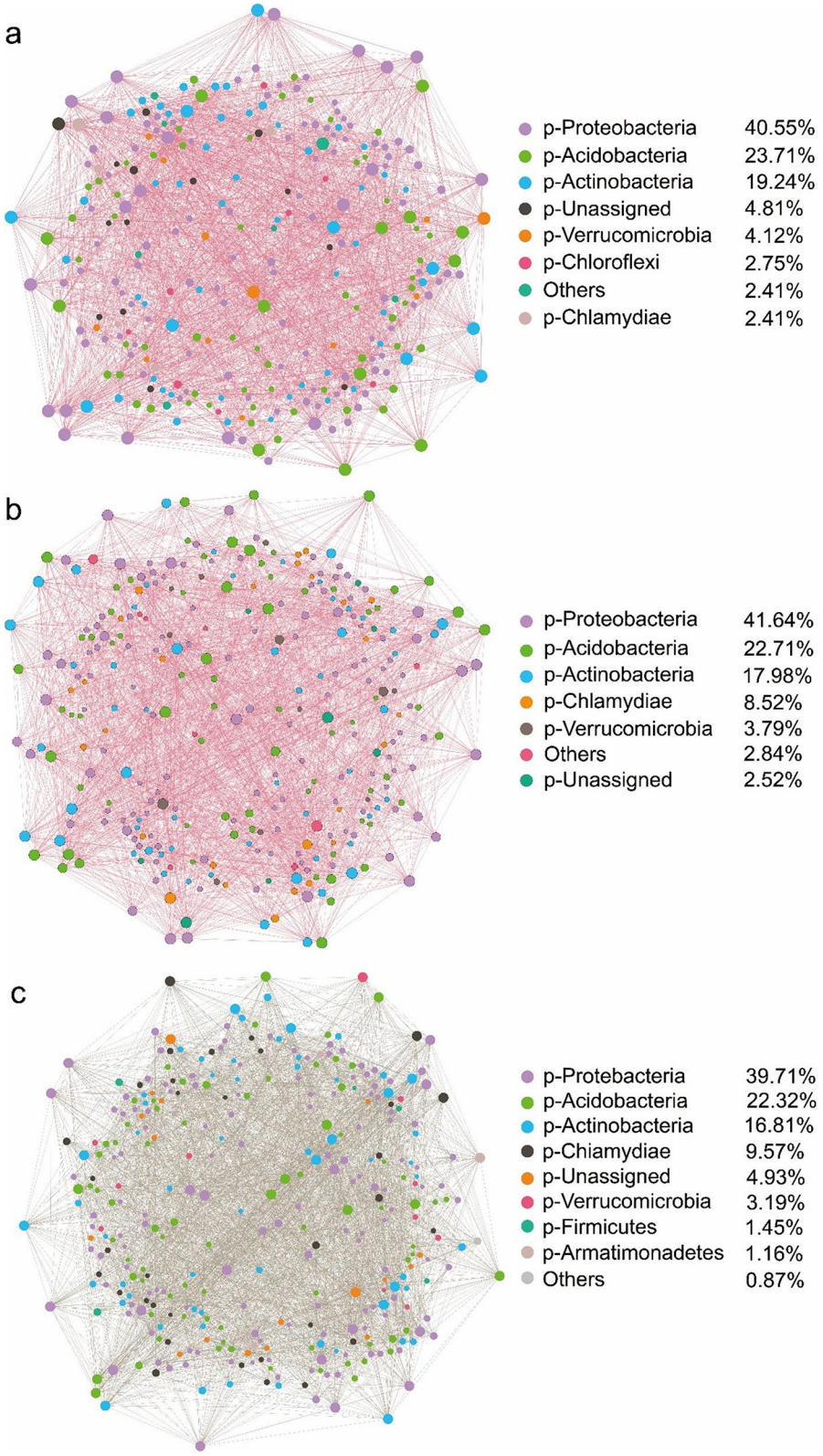

Fig. 8 Characteristics of co-occurrence network of soil bacteria at 1448 m (**a**), 1643 m (**b**) and 1821 m (**c**)

better water and temperature conditions at 1643 m may be the reason for the higher soil enzyme activity [78]; and (3) the weakly alkaline calcareous soil in karst mountainous areas can promote the enzymatic reaction of urease [79].

One study showed that heavy metal pollution can reduce soil enzyme activity [26]. However, Ciarkowska et al. [80] noted that heavy metals do not have toxic effects on soil enzymes in all cases. For example, Fang et al. [81] noted that urease contains trace transition metal atoms or ions, which act as auxiliary groups, coenzymes or active centers. The morphology and structure of urease changed under the action of Fe, which activated its enzyme activity. Our results were also consistent with these findings. The present study revealed that the Shannon index of the soil bacterial community decreased in the order of 1643 m > 1448 m > 1821 m, and the Shannon index did not significantly differ between the two elevation gradients, which was consistent with the findings of Yao et al. [82]. In line with the findings of Zhang et al. [83], the results showed that Acidobacteria, Actinobacteria and Proteobacteria were the dominant phyla in the soil bacterial communities at different elevations. *Rhododendron* forests in the Baili *Rhododendron* scenic spot are heavily foliated and have large amounts of litter, and these bacteria have good adaptability at different elevations, mainly because they can decompose organic matter in soil [84]. In addition, *Rhododendron* prefers to grow in acidic soil, and Acidobacteria are acidophilic bacteria, so their relative abundance was high in this study [85]. *Burkholderia*, which are Proteobacteria and *Bradyrhizobium*, can decompose lignin to promote soil carbon cycling and improve the solubility of soil fixed phosphorus and applied phosphorus, resulting in an increased plant yield [8, 86, 87]. Actinobacteria are very important for soil carbon and nitrogen cycling, and their relative abundance allows them to adapt well to various extreme environments, such as low-temperature, anaerobic and nutrient-deficient ecological environments [88]. The relative abundances of AD3, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Chlamydiae and TM7 were low. Their main functions are nitrogen and carbon fixation. Chloroflexi are the main carbon-fixing bacteria in soil, providing carbon sources for heterotrophic bacteria, and helping them grow and reproduce [89].

The composition of soil bacterial communities is influenced by elevation [82] and is also related to soil heavy metals and soil enzyme activity [27, 90], which confirms our results. Some studies have reported that soil nutrient differences at different elevations can significantly affect soil bacterial communities [90–92]. A few studies have investigated the effects of different elevations on soil bacterial communities through differences in the content and composition of mineral elements. In this study, we found that the Fe, Cr and K contents were significantly related to Chloroflexi, Gemmatimonadetes and TM7, respectively, at 1448 m, and the contents of Mg, Ca, Ni, Cu and Pb were significantly correlated with the top 10 phyla at 1643 m. Zn and Pb contents were also found to be important driving factors influencing soil bacterial communities at 4000 m [93]. In addition, we found that Fe had a similar correlation with the core bacteria (Actinobacteria). As an essential trace element for plant growth, Fe participates in the electron transfer process in plant cells, and can promote the formation of chlorophyll and the reduction of nitric acid in plant roots [94, 95]. However, Fe deficiency limits the absorption of nitrogen and phosphorus by plants [96, 97]. Soil enzymes are mainly secreted by microorganisms and plant roots, and can be affected by soil metals [26]. In our study, soil urease was the main factor affecting soil bacterial communities at 1448 m, however, invertase, urease phosphatase and catalase were strongly related to Chlamydiae, Chloroflexi, Actinobacteria and TM7, respectively, at 1448 m. Invertase and phosphatase were significantly positively related to Chloroflexi at 1821 m, and phosphatase was also significantly correlated with Proteobacteria at 1821 m. Acidobacteria, Actinobacteria, Proteobacteria and Chloroflexi were mostly involved in the decomposition of organic matter, and the soil bacterial communities formed by these groups are related to soil enzymes at different elevation gradients [89, 98, 99].

Soil microorganisms form a complex network of ecological interactions through synergy, competition or antagonism to achieve material cycling, energy flow and information transfer in the network system [100, 101]. Our results were consistent with those of He et al., who reported that the co-network of the bacterial community differed with increasing elevation and that the number of total nodes and total edges gradually increased, indicating that the structure of the co-network became more complex and that the bacterial communities became more closely connected to each other [102]. One study suggested that complex networks with greater connectivity were more resilient to environmental perturbations than simple networks with lower connectivity, and more resilient in response to environmental change [103]. In addition, the most abundant phyla studied in the network in this study were Proteobacteria, Acidobacteria, and Actinobacteria, which was consistent with the findings of Xue et al. [104]. They are able to live in acidic soil, balance plant hormones, regulate root growth, promote nutrient absorption and prevent pathogens from invading, and play a crucial role in the structure of bacterial communities at different elevations [105].

Conclusions

In our study, different elevations had significant effects on the soil mineral element content, soil enzyme activity and bacterial community. In particular, the elevation gradient affected the soil K, Fe, Cr, Cu, and Zn contents and urease and phosphatase activities between 1448 m and 1821 m. Acidobacteria, Actinobacteria and Proteobacteria were the dominant phyla at all three elevations. Fe and urease had significant effects on the bacterial community at 1448 m, and Cr, Ni and catalase were negatively related to TM7. In addition, the elevation affected the complexity of the co-occurrence network of the soil bacterial community. This is likely due to changes in the karst region related to changes in the elevation, which caused the mineral element content and enzyme activity to change when the soil environmental conditions changed, and the microbial community assisted plants in adapting to different soil environments by modifying their structure and diversity. In addition, the number of nodes and edges in the soil bacterial community increased significantly with increasing elevation. The positive interaction observed at 1821 m compared to 1448 m suggested that elevation promoted intraspecific cooperation among bacteria as a strategy for *R. delavayi* to adapt to high altitudes. Elevation strongly influenced the soil mineral element content, enzyme activity, and soil bacterial communities in karst areas. The present study provides a theoretical basis for vegetation restoration in fragile karst ecosystems.

Supplementary Information

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Supplementary Material 1

Author contributions

Designed research: Wang J.F. and Yi Y.; Performed research: Wang L., Liu J. and Wang J.F. ; Analyzed data: Wang L.,Gong J.Y.,Kong X., Chen X.L., Chen L.L.,Tang R., Zheng R., Wang J.F. ; Wrote the paper: Wang L., Tang M., Kamran M., Wang J.F., Yi Y.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The DNA sequencing datasets generated and/or analyzed during the current study are available in the [NCBI] repository, [BioProject PRJNA1142549].

Declarations

Ethics approval

All authors have reviewed the final version of the manuscript and agree to its submission to this journal.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Statement

The sampling was conducted on public land and permission has been obtained from the relevant governing body to sample at this land.

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