

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 distributed at different elevations in the karst region of Bijie, China, and has an importance in prevention of land degradation in this region. The soil bacterial community structure of *R. delavayi* at three elevations (1448 m, 1643 m, 1821 m) was analyzed by high-throughput sequencing, and soil mineral elements and enzymes activities were determined.

Results In this study, our results showed that the soil Fe, Mg, Cr, Ni, Cu and Zn content decreased with increase in elevations, the soil K content varied in a V-shape, while the soil Cd and Pb showed an inverted V-shape. In addition, the variation of soil urease and phosphatase activity also showed an inverted V-shape. Invertase activity increased while catalase activity decreased with elevation increase. The Shannon index of soil bacterial community initially increased and then decreased with elevation, and was highest at 1643 m. At 1821 m, Fe was positively correlated with Acidobacteria; Ca, Ni, Cu, Zn and Pb were positively correlated with Actinobacteria. Urease and phosphatase significantly affected the soil bacterial community, while elevation affected the diversity of bacterial community by affecting Fe and urease content. The results of Linear discriminant effect size (LEfSe) showed that there were different biomarkers at different elevations. The bacterial co-occurrence network analysis showed that the dominant bacterial communities at different elevations in Baili *Rhododendron* scenic spot had a complex structure. Acidobacteria, Actinobacteria and Proteobacteria were the most dominant phyla at 1448 m, 1643 m and 1821 m. Mantel test and correlation analysis showed that Fe and urease significantly affected bacterial OUT at 1448m. Moreover, Cr, Ni and catalase had a negative impact on TM7 at 1448m but there was no significant correlation between soil variables and bacteria communities on bacterial OUT at 1643m and 1821m.

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

Introduction

Karst rocky desertification (KRD) is a major type in karst mountains which is caused by vulnerable natural environment and human activities. It is also a kind of eco-environmental problems in Southwest China¹. The landscape of karst accounts for about 12% of the total land area of the world and mainly distributed in European Mediterranean basin, Dinaric Karst and Southwest China². The provinces of Guizhou, Guangxi and Yunnan are the main distribution areas of karst in China³. 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 all over the world^{4,5}. The bedrock in the karst area of Guizhou is mainly composed of limestone, dolomite, and dolomitic limestone, which is hard, highly soluble and cannot produce a large amount of soil^{2,6}. It results in shallow soil layers, serious soil erosion, sparse vegetation and low vegetation coverage^{7,8}. With the growth of economy and population, the contradiction between human and land has become increasingly serious, leading to the destruction of large-scale karst forests, exacerbating the vulnerability of karst environment⁹. According to the second monitoring report of the

State Forestry Administration on rocky desertification, China had about 120,000 km² of KRD in 2012². To prevent the continuous expansion of rocky desertification, with the encouragement and support of the local government, people began to expand vegetation coverage by planting trees, grass and building ecological nature reserves to reduce soil erosion¹⁰.

Baili Rhododendron scenic spot is the largest natural rhododendron forest in the same latitude and low altitude of the earth¹¹. It is also one of the few relatively complete high-altitude rhododendron forests preserved in China¹². Guizhou Baili Rhododendron Scenic spot is rich in the species of rhododendron, such as *Rhododendron delavayi*, *Rhododendron agastum*, *Rhododendron irroratum* and so on, forming various monodominant populations¹³. Among them, *R. delavayi* is the main dominant species of rhododendron community in Baili Rhododendron Nature Reserve¹⁴. It has a wide ecological niche and is distributed in the area of 1400–1900 m, but *R. agastum* and *R. irroratum* are only distributed in the area of 1600–1800 m^{12, 15, 16}. With high ornamental value of *R. delavayi*, attracts visitors from around the country and is used to make medicine¹⁷. It can not only promote local economic development as an ornamental plant, but also prevent ecological degradation¹⁸. At present, the research on *R. delavayi* has mainly focused on flower color formation¹⁹, reproduction and breeding^{20, 21}, the community structure and diversity¹¹, and changes in the structure of rhizosphere microbial²² and so on. However, little research has been done on how changes in soil bacterial communities help *R. delavayi* adapt to different elevation environment.

Elevation, as a comprehensive environmental factor, causes drastic changes in water, temperature, light, oxygen and ultraviolet, which eventually affect the composition of plant community, soil properties, soil heavy metal content and soil enzyme activity, resulting in the difference of soil microbial community structure^{23–27}. Some studies have shown that the different elevations could significantly affect the activities of soil invertase, urease and acid phosphatase²⁸. Soil enzymes, as an important indicator of soil fertility, can accelerate the composition and transformation of nutrients, such as C, N and P cycles²⁹. Invertase, as an index of C cycle, can hydrolyze carbohydrate polymers into simpler sugars. Urease plays a vital role in the nitrogen cycle, which can hydrolyze urea and transform organic nitrogen into inorganic nitrogen³⁰. Esters and anhydrides of phosphoric acid can be hydrolyzed by phosphatase³¹. Catalase can decompose hydrogen peroxide into molecular oxygen and water, thus preventing cells from being damaged by reactive oxygen species³². A study demonstrated that different elevations gradients had different plant types and contents of heavy metal³³. For example, Zn content in 1250 m was significantly higher than that in 380 m and 820 m, while Pb content in 380 m was higher than that in 820 m and 1250 m³⁴. Microorganisms are also an important indicator of soil health, they can predict the change of soil environmental quality earlier, because it is sensitive to external disturbance³⁵. Soil microbial community is principal composed of bacteria and fungi, but bacteria are more resistant to heavy metals than fungi³⁶. The diversity and structure of soil bacterial communities changed along different elevations, and the response of soil C- and N-cycling microbial structure was complex as plants adapted to different

elevations³⁷. For example, the relative amount of psychrophilic heterotrophic bacteria, fungi and gram-negative bacteria increased with the increase of elevation in the Austrian Central Alps³⁸.

Currently, studies on rhizosphere microorganisms of *R. delavayi* mainly focuses on the soil microorganisms community structure at single elevation^{22, 39} or different elevations⁴⁰. However, it is unclear how the changes of soil bacterial community structure at different elevations, especially soil bacteria, help *R. delavayi* adapt to KRD environment. Therefore, it is of great significance to study the effects of soil enzyme activity and soil metal changes on soil bacteria structure along the change of elevation gradient for exploring the process of plant adaptation to different elevations in the karst areas. The purpose of our study was to understand (1) the effect of various elevations on soil mineral elements and soil enzyme activities, (2) the effect of different elevations on soil bacterial community structure of *R. delavayi*, (3) to explore the relationship between soil mineral elements, soil enzyme activities and soil bacterial community in *R. delavayi*.

Results

The content of soil K, Mg, Fe and Ca vary across elevations in *R. delavayi* natural shrub forest

We found that the content of K, Mg and Fe were significantly different between two different elevations (Fig. 1a, 1b, 1c), but the content of Ca was remained unchanged (Fig. 1d). Mg and Fe level were clearly decreased along increased elevation. The change of K content was V-shaped along the increase of elevation. Interestingly, the change trend of Ca content was opposite to K content.

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

The content of soil Cr, Ni, Cu, Pb and Zn were decreased along the elevations gradient increased (Fig. 2a, 2b, 2c, 2e, 2f). The content of soil Cr, Ni, Cu, Pb and Zn had no obvious change between 1448 m and 1643 m, but they had significant difference between 1448 m and 1821 m. The content of Ni, Cu, Pb and Zn were increased by 629%, 123%, 101% and 124% at 1448 m than 1821 m, respectively. However, soil Cd content was not significantly different between two elevations (Fig. 2d).

The differences of soil enzyme activity at different elevations

As Fig. 3 showed that with the elevation increase, the activity of invertase showed an increasing trend. The invertase activity showed no clear difference between 1448 m and 1643 m, 1643 m and 1821 m, but it was obviously different between 1448 m and 1821 m (Fig. 3a). The variety trend of urease and phosphatase activity was the same, increased first and then decreased as elevation increased. The activity of them showed the significant difference between two different elevations. Interestingly, there was no significant difference in urease activity between 1448 m and 1643 m (Fig. 3b, 3c). Soil catalase activity was reduced along elevation increased, and catalase activity at 1643 m and 1821 m decreased by 1.05 and 2.80 times compared to 1448 m (Fig. 3d).

Effect of elevations on Shannon index and structure of soil bacterial communities

As Figure S1 showed that Shannon index firstly increased and then decreased along the increase of elevations, but elevations were not able to influence the Shannon index change of soil bacterial communities. Furthermore, our results indicated that the number of Acidobacteria, Actinobacteria and Proteobacteria dominated regardless of the elevations (Fig. 4a). Acidobacteria (55.43%), Actinobacteria (9.95%), AD3 (9.08%), Gemmatimonadetes (1.15%), Proteobacteria (20.27%) and Verrucomicrobia (1.42%) were the main bacteria phyla with relative abundance more than 1% at 1448 m (Fig. 4a). Similarly, the relative abundance greater than 1% of dominant bacteria phyla were Acidobacteria (48.45%), Actinobacteria (11.56%), Bacteroidetes (1.37%), Proteobacteria (32.35%) and Verrucomicrobia (1.46%) at 1643 m (Fig. 4a). In addition, Acidobacteria (45.38%), Actinobacteria (13.15%), Bacteroidetes (1.7%), Chlamydiae (1.34%), Proteobacteria (31.96%), TM7 (1.26%) and Verrucomicrobia (1.56%) at 1821 m (Fig. 4a).

When we investigated the bacteria communities of *R. delavayi* at genus-level, we found that there were more variation patterns of classified genera between different elevation gradients (Fig. 4b). Our results showed that *Bradyrhizobium* (6.61%), *Burkholderia* (1.13%), *Candidatus Koribacter* (2.95%), *Candidatus Solibacter* (12.76%), *Mycobacterium* (1.94%), *Pedospaera* (2.92%) and *Rhodoplanes* (20.01%) were the main bacteria genera with relative abundance more than 1% at 1448 m. Additionally, the relative abundance greater than 1% of dominant bacteria genera was *Bradyrhizobium* (3.09%), *Burkholderia* (3.2%), *Candidatus Koribacter* (1.96%), *Candidatus Rhabdochlamydia* (2.0%), *Candidatus Solibacter* (7.65%), *Mycobacterium* (1.07%), *Pedospaera* (2.74%), *Phenylobacterium* (1.83%) and *Rhodoplanes* (15.34%) at 1643 m. Moreover, we found that the relative abundance greater than 1% of dominant bacteria genera were *Acidopila* (6.49%), *Bradyrhizobium* (1.37%), *Burkholderia* (7.77%), *Candidatus Koribacter* (2.54%), *Candidatus Rhabdochlamydia* (2.82%), *Candidatus Solibacter* (8.19%), *Pedospaera* (1.41%), *Phenylobacterium* (1.22%), *Rhodoplanes* (17.2%) at 1821 m (Fig. 4b).

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

Redundancy analysis of the correlation between soil metal and soil enzymes with soil bacterial communities at the phylum-level and genus-level

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

Correlation between soil mineral elements and soil enzymes with soil bacterial phyla

The Mantel test and correlation analysis showed that Fe and urease significantly affected bacterial OUT at 1448m. However, there no significant correlation between soil variables and bacteria communities on bacterial OUT at 1643m and 1821m (Fig. 6). The response of bacteria phylum level of different elevations to the change of soil mineral elements was different (Figure S5a, b, c). At 1448 m, we found that K content had a negative effect on Gemmatimonadetes; Fe content had a negative impact on Chloroflexi; the content of Cr and Ni had a negative effect on TM7. However, the content of Mg, Ca, Cu, Zn, Cd and Pb had no significant impacts on the type of bacteria. At 1643 m, Mg content had a negative impact on Bacteroidetes and Chlamydiae; Ca content had a negative impact on TM7; Ni content had a negative on Chloroflexi; the content of Cu and Pb had a negative impact on Chlamydiae. Interestingly, the content of K, Fe, Cr, Zn and Cd had no significant impact on the type of bacteria. At 1821 m, K content also had a negative effect on Gemmatimonadetes; Fe content had a positive effect on Acidobacteria; the content of Cr, Cu, Zn, Pb had a negative effect on Acidobacteria, but positive effect on Actinobacteria; Ni content had a positive effect on Actinobacteria; the content of Mg, Ca and Cd had no significant impact

on the type of bacteria. (Figure S5a, b, c). RDA also showed that at different elevation gradients, the content of Fe, Cr and K were the main factors affecting soil bacteria communities at 1448 m; Ca content was the main factor affecting the bacteria communities at 1643 m (Figure S4a). The response of bacteria phylum level of different elevations to the change of soil enzyme activities was different (Figure S5d, e, f). At 1448 m, Invertase activity had a positive effect on Chlamydiae; Urease activity had a negative effect on Chloroflexi; Phosphatase activity had a positive effect on Actinobacteria; Catalase had a negative effect on TM7. At 1643 m, there were no significant effect between all soil bacteria communities and soil enzyme activity, except catalase activity had a positive effect on Acidobacteria. At 1821 m, Invertase activity had a positive effect on Chloroflexi; Phosphatase activity had a positive effect on Chloroflexi, but negative on Proteobacteria. Only urease and phosphatase activities were no significant effect on the type of bacteria (Figure S5d, e, f). RDA also showed that urease and catalase activity were the main factors affecting soil bacteria communities at 1448 m, and phosphatase activity was the main factor affecting soil bacteria communities at 1821 m (Figure S4c). These variables were considered as potential factors affecting these communities.

Different biomarkers of phyla and genus level are present at different three elevations

LEfSe analysis revealed that there were different biomarkers in 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; the abundance of soil bacteria of *R. devalayi* at 1821 m, enrichment of *Burkholderia*, *Acidopila*, Bacteroidetes, Chlamydiae, TM7, *Candidatus Rhabdochlamydia*, *Desulfovibrio*, TM6 and *Telmatospirillum* were significant (Fig. 7).

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

In the co-occurrence network analysis, a node in network indicated a phylum, while a link indicated a relationship between two nodes (Fig. 8). The total nodes in the network were 1448 m (291), 1643 m (317) and 1821 m (345). At the bacterial phyla level, the total edges were 2153, 317 and 345 at 1448 m, 1643 m and 181 m, respectively. And the positive (99.91% edges) and negative (0.09% edges) were identified at 1448 m and 1643 m. Interestingly, the positive edges were identified with 100% at 1821 m (Table 1). The abundance of Proteobacteria, Acidobacteria and Actinobacteria were high at three elevations was 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.

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

Degree	1448m	1643m	1821m
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

Discussion

The variations of elevations gradients could cause different ecosystem functions by changing the soil type, vegetation type, climate, temperature, humidity et al.^{47–50}, and soil mineral elements can be affected by soil parent material^{51, 52}. Previous reports demonstrated that elevations decreased the content of Fe, Mg, Cu, Zn and Ni in soil^{53–55}. Our results were consistent with them that the content of Mg, Fe, Cr, Ni, Cu, Pb and Zn in soil gradually decreased while elevation increased. Interestingly, K content was the lowest at 1643m, while Ca and Mg content were the highest. The reason for this phenomenon might be that Baili Rhododendron Nature Reserve contains a large number of mineral resources, such as coal mines, pyrite and so on. It makes the high content of Fe, Ni, Cu and other mineral elements in the original soil parent material⁵⁶. In addition, the soil parent material in this area is mainly soluble carbonatite, which is easily weathered and dissolved⁵⁷. Similarly, elevation gradients affect plant litter, thus altering the microbial activity and composition, leading to changes soil nutrients⁵⁸. This is consistent with our reference to the experimental soil nutrient study by Liu et al.⁴⁰. In our study, K content from high to low was followed 1448 m > 1821 m > 1643 m at three different elevations. Feng et al.⁵⁹ also showed that K content in low elevation areas was higher than that in high elevation areas. This phenomenon probably caused by the different degree of soil differentiation in different elevation areas, because the sufficient light and good ventilation are greatly affected by rainfall in high elevations⁶⁰. As a results, the content of soil organic matter and humus is different, and it has functional groups and chelating properties, which reduce the bioavailability of heavy metals and increase the content of heavy metals^{61, 62}.

Elevations correspond to soil type and microbial activity. These changes have an important impact on migration and transformation of soil heavy metals⁶³. It is reported that heavy metals affect the soil C and N cycle mediated by soil microorganisms and enzymes⁶⁴. Our results showed that between two different elevations, there were significant effects on the content of Fe and Mg in soil, which indicated that Fe and Mg were the dominant factors controlling soil quality. Fe and Mg content were the highest at 1448 m and the lowest at 1821 m, implying that soil parent material aggravated the accumulation of soil mineral

elements, which was consistent with previous studies^{54, 55}. Moreover, in our study, we found that there was significant difference in K, Cr, Ni, Cu, Zn and Pb between 1448 m and 1821 m, but there was no significant difference of those mineral elements between 1448 m and 1643 m. Li et al.⁶⁵ research showed that the increase of elevation decrease temperature, which lead to the decomposition degree of litter in low elevation areas being higher than that in high elevation areas, resulting in the release of K. Besides, 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 compounds⁶³.

In karst ecosystem, plant type⁶⁶, heavy metal content⁶⁷, temperature⁶⁸, humidity⁶⁹, soil properties⁷⁰ affect the growth of soil microorganisms and soil enzyme activity. Our study showed that invertase activity increased with elevation increased, which was consistent with the research of Ma et al.⁷¹. Probably, the content of soil heavy metals decreased along elevation increased, and the inhibition of heavy metals on soil invertase activity weakened⁷². In addition, the decomposition of organic matter was inhibited by the decrease of soil temperature when elevation increased, so it was accumulated⁷¹. Invertase was directly involved in the metabolic process of soil organic matter. Generally, the soil organic matter content is directly proportional to the invertase activity⁷³. Interestingly, our current research also suggested that catalase activity decreased when the elevation increased. The reason for this phenomenon could be that the content of heavy metals was high at 1448 m, and a large amount of H₂O₂ produced by plants when they are stressed by heavy metal stress. The increase of catalase activity can effectively decompose H₂O₂ into H₂O and O₂, and reduce the toxic effect to plants⁷⁴. In addition, our study showed that urease and phosphatase content increased first and then decreased with elevation increase, the enzyme activities were the lowest at 1821m, and there was significant difference between 1643 m and 1821 m, 1448 m and 1821 m. The contents of urease and phosphatase were also positively correlated with Fe. The possible reason might be that elevation affects soil types and organic matter content, (1) as the substrate of enzymatic reaction, the content of soil organic matter affects the activity of soil enzymes⁷¹; (2) soil enzyme activity is directly related to climate change at different elevations⁷⁵, and the better water and heat conditions at 1643 m may be the reason for the higher soil enzyme activity; (3) weak alkaline calcareous soil in karst mountainous can promote the enzymatic reaction of urease⁷⁶. Most studies have shown that heavy metal pollution can reduce soil enzyme activity²⁷. However, Ciarkowska⁷⁷ pointed out that heavy metals do not have toxic inhibition on soil enzymes in all cases. For example, Fang⁷⁸ pointed out that urease contains trace transition metal atoms or ions, which plays the role of auxiliary group, coenzyme or active center. 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 current study showed that the Shannon index of soil bacteria was 1643 m > 1448 m > 1821 m, and the α -diversity of bacterial communities was no significant difference between two elevation gradients, but only reflected β diversity, which was consisted with Yao et al⁷⁹. Our results were the same as

Zhang⁸⁰ that Acidobacteria, Actinobacteria and Proteobacteria were the dominant species in soil bacteria communities at different elevations. Rhododendron forests in Baili Rhododendron scenic spot are heavily foliated and have a large thickness of litter, and those bacteria have good adaptability in different elevations, mainly because they can decompose organic matter in soil⁸¹. In addition, *Rhododendron* likes to grow in acidic soil, and Acidobacteria belongs to acidophilic bacteria, so its relative abundance is high in this study⁸². *Burkholderia*, which belongs to Proteobacteria and *Bradyrhizobium*, can decompose lignin to promote soil carbon cycling and improve the solubility of soil fixed phosphorus and applied phosphorus resulting plant yield increase^{9, 83, 84}. Actinobacteria is very important for soil carbon and nitrogen cycling, and their relative abundance can better adapt to various extreme environments, such as low temperature, anaerobic and nutrient-deficient ecological environment⁸⁵. The relative abundance of AD3, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Chlamydiae and TM7 are low, and the main functions of them are nitrogen fixation and carbon fixation. Chloroflexi is the main carbon-fixing bacteria in soil. Its growth and increase can provide carbon source for heterotrophic bacteria, and then help them grow and reproduce⁸⁶.

The structure of soil bacteria communities was influenced by elevations⁷⁹ and also related with soil heavy metal and soil enzyme activity^{28, 87}, which confirmed our research. Numerous studies had confirmed that soil pH, N, P, K are the main factors to alter soil bacteria communities^{88–90}, but only few studies examined how soil mineral elements influence soil bacterial community structure. In our study, we found that Cr, Ni, Cu and Pb were negatively correlated with soil bacterial communities. Possibly due to that Cr, Cu, as essential elements for microbial growth, can promote microbial growth at low levels of metal contamination, but toxic to microbial growth and inhibit their life activities when they exceed the amount required for microbial growth⁹¹. In addition, we found that Fe showed similar correlation with core bacteria (Actinobacteria). Fe, as an essential trace element for plant growth, 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^{92, 93}. However, Fe deficiency limits the absorption of nitrogen and phosphorus by plants. Therefore, in the case of Fe deficiency, plants cannot effectively utilize nitrogen and phosphorus^{94, 95}. Soil enzymes are mainly secreted by microorganisms, plant roots and animals, and will be affected by soil metals²⁷. In our study, Urease and invertase were main factors that effected soil bacterial communities at different elevations. Acidobacteria, Actinobacteria, Proteobacteria and Chloroflexi are mostly involved in the decomposition of organic matter, and the soil microbial communities formed by these groups are related to soil enzymes at different elevation gradients^{86, 96, 97}.

Microorganisms in soil are not isolated, but 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^{98, 99}. Our results were consisted with He¹⁰⁰ that the OTU co-network of bacteria differed with increasing elevation, the number of total nodes and total edges gradually increased, indicating that the structure of the co-network became more complex, and the bacterial communities were more closely connected to each other. The research of Santolini 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 change¹⁰¹. In addition, the most abundant phyla studied in network were Proteobacteria, Acidobacteria, Actinobacteria, which was consistent with the findings of Xue et al.¹⁰². They were able to live in acidic soil, balance plant hormones, regulate the growth of root, promote nutrient absorption and prevent pathogens from invading¹⁰³, and present a crucial role in the structure of bacterial communities at different elevations.

Conclusions

In our study, different elevations showed significant effects on soil mineral element content, soil enzyme activity and bacterial community. Especially, elevation gradients affected soil K, Fe, Cr, Cu, Zn, urease and phosphatase between 1448 m and 1821 m. Acidobacteria, Actinobacteria and Proteobacteria were the dominant phyla at three different elevations. At 1448m, Fe and urease had significant effects on bacterial OUT, and TM7 was negatively affected by Cr, Ni and catalase. In addition, elevation gradients affected the complexity of the co-occurrence network of soil bacterial structure. Probably due to the elevation change in karst region, which made the mineral element content and enzyme activity change while soil environmental changed, and the microbial community helped plants to adapt the different soil environments by changing their structure and diversity. In conclusion, soil mineral element content, enzyme activity and soil bacterial communities in karst areas were strongly influenced by elevations. The current study provides a theoretical basis for vegetation restoration in karst fragile ecosystems.

Materials and Methods

Study sites and soil samples

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

The determination of soil metal content and soil enzymes

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.⁴¹. The activity of soil invertase, phosphatase and urease was analyzed by the procedure of Hou et al.⁴², and the activity of soil catalase was analyzed by potassium permanganate titration according to the procedure of Zhang et al.⁴³.

DNA extraction, high throughput sequencing and raw data analysis

350 mg soil were extracted DNA by the procedure of MP BIO FASTDNA SPIN Kit (MP Bio, Santa Ana, CA). The V5V7 region of 16S rRNA gene was amplified with primers [799F (5'-AACMGGATTAGATACCCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3')] by PCR. The PCR products were sent to Genesky Biotechnologies Inc., Shanghai, 201, 315 (China) to high throughput sequence by Illumina NovaSeq 6000 platform. We removed the primer sequences and adaptor with the cutadapt⁴⁴ plug-in of QIIME2⁴⁵. We used DADA2 plug-in of QIIME2 to filter the data, denoise, merge and remove chimera to obtain high-quality sequencing⁴⁶. The clean reads were processed into amplified sequence variant by the QIIME2.

The significant difference between two different elevations, for soil metal elements content, soil enzymes activity and Shannon index was performed by independent *T*-tests, and the significant differences were at $P < 0.05$. The Shannon index of soil bacterial community, redundancy analysis (RDA), PCoA, heatmap, mantel test, co-network of soil bacterial communities at phyla level, relationship between soil properties and top 10 with phyla level with R (4.1.3 version). The LEfSe analysis was used the OmicStudio tools (<https://www.omicstudio.cn/tool>).

Declarations

Author contributions

Designed research: Jianfeng Wang, Yin Yi. Performed research: Xin Kong, Jie Liu, Jianfeng Wang. Analyzed data: Xin Kong, Jiyi Gong, Li Wang, Xianlei Chen, Lanlan Chen, Jianfeng Wang. Wrote the paper: Ming Tang, Xin Kong, Kamran Malik, Jianfeng Wang, Yin Yi.

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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.

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 that they have no competing interests

References

1. Yonghui Y. Progress and prospect of Karst rocky desertification research in Southwest China. *Progress in Geography*. 2014;33(1):76–84.
2. Jiang Z, Lian Y, Qin X. Rocky desertification in Southwest China: Impacts, causes, and restoration. *Earth Sci Rev*. 2014;132:1–12.
3. Wang SJ, Liu QM, Zhang DF. Karst rocky desertification in southwestern China: geomorphology, landuse, impact and rehabilitation. *Land Degrad Dev*. 2004;15(2):115–21.
4. Li S, Zhang S, Yao Xiaohua, Huadong R. Effect of different land use modes on soil environment in karst rocky desertification zone, middle Guizhou province of China. *Resour Environ Yangtze Basin*. 2008;17(3):384.
5. Luo G, Li Y, Wang S, Chen A, Wenli D. Comparison of ecological significance of landscape diversity changes in karst mountains: a case study of 4 typical karst area in Guizhou Province. *Acta Ecol Sinica*. 2011;31(14):3882–9.
6. Lan Anjun; Xiong Kangning, Yulun A. Analysis on driving factors of karst rock-desertification — With a special reference to Guizhou province. *Bulletin of Soil and Water Conservation* 2001, 21 (6), 5.
7. Zhu S, Jiang B, Tao L. Discussion on influence of soil and water loss in Guizhou province and methods of governance and efficiency. *J Green Sci Technol* 2020, (22), 2.
8. Gu Yangyang; Zou Changxin; Qiao Xuning; Huang Xianfeng; Ye Xin; Xu Mengjia; Yang Zhongqing, Shipeng D. Spatio-temporal variations of fractional vegetation coverage and influencing factors in Qianxi’nan prefecture from 2000 to 2015. *Journal of Ecology and Rural Environment* 2021, 37 (11), 1413–1422.
9. Li S, Kelin W. Effect of Human disturbance on soil nutrients and plant diversity of grassland in karst mountain. *Res Soil Water Conserv*. 2016;23(5):8.
10. Xiao Hua; Xiong Kangning, Zhang H, Qianzhu Z. Research progress for karst rocky desertification control models. *China Popul Resour Environ*. 2014;24(3):5.

11. Wang J, Li W, Zhang H, Wu D, Zhang J, Zhenzhen J. Study on the diverdity and stability of plant communities in Baili Rhododendron Scenic Area in Guizhou province. For Resour Managemen 2020, (2), 6.
12. Chen X, Huang J, Xie H, Chen X, Guilong G. A study on plant resource of Rhododendron (Ericaceae) in Nature Reserve of Azalea Forest. Guizhou Sci. 2010;28(4):26–34.
13. Yang C, Li G, Deng Lunxiu, Chen J, Yunli J. A study on Rhododendron species and ornamental characteristics in Baili Dujuan Nature Reserve of Guizhou. J West China Forestry Sci. 2006;35(4):6.
14. Li W, Xun C. A preliminary study on structure and regeneration of Rhododendron delavayi population in Baili Azalea Forest Park. Guizhou Sci. 2005;23(3):4.
15. Zhang C, Huang C, Huang J, Wang L, Zhang J, Yongpeng M. Investigation of germplasm resources of the genus Rhododendron in baili Nature Reserve in Guizhou. Plant Divers Resour. 2015;37(3):357–64.
16. Wu Q; Dong Jianwen; Zhen Yu; Fu Weicong; Li Hui; Zhu Zhipeng; Chen Zirui; Guochang D. Niches of the main plant species in Baili Rhododendron National Forest Park. *Journal of Nanjing Forestry University (Natural Sciences Edition)* 2017, 41 (2), 6.
17. Zhang Lu, Xu P; Cai Yanfei; Ma Lulin; Li Shifeng; Li Shufa; Xie Weijia; Song Jie; Peng Lvchun; Yan Huijun; Zou Ling; Ma Yongpeng; Zhang Chengjun; Gao Qiang; Jihua W. The draft genome assembly of Rhododendron delavayi Franch. var. delavayi. *GigaScience* 2017, 6 (10), 1–11.
18. Basnett S, Ganesan R. A Comprehensive Review on the Taxonomy, Ecology, Reproductive Biology, Economic Importance and Conservation Status of Indian Himalayan Rhododendrons. *Bot Rev* 2022.
19. Sun W, Zhou N, Wang Y, Sun S, Zhang Y, Ju Z, Yi Y. Characterization and functional analysis of RdDFR1 regulation on flower color formation in Rhododendron delavayi. *Plant Physiol Biochem.* 2021;169:203–10.
20. Xiong Han; Zhuang Yufang; Tang Xiaoxin; Yi Yin, Jie L. Method of inducing callus of hybrid species of Rhododendron agastum and Rhododendron delavayi. *Molecular Plant Breeding* 2021, 19 (3), 923–928.
21. Peng L, Li H, Song J, Xie W, Zhang L, Li S, Cai Y, Zhao Z. Morphological and transcriptome analyses reveal mechanism for efficient regeneration of adventitious buds from in vitro leaves of Rhododendron delavayi regulated by exogenous TDZ. *In Vitro Cellular & Developmental Biology - Plant* 2022.
22. Fang Min, Xu X, Tang M, Jing T. Structure and composition variation of the root-microbiota of Rhododendron delavayi. *Acta Microbiol Sinica.* 2019;59(8):1522–34.
23. Tang CQ. Forest vegetation as related to climate and soil conditions at varying altitudes on a humid subtropical mountain, Mount Emei, Sichuan, China. *Ecol Res.* 2005;21(2):174–80.
24. Djukic I, Zehetner F, Mentler A, Gerzabek MH. Microbial community composition and activity in different Alpine vegetation zones. *Soil Biol Biochem.* 2010;42(2):155–61.
25. Li L, Wang L, Wang X, Zhiyuan M. Analysis of the effect of altitude gradient on plant growth and development. *Prot For Sci Technol* 2016, (5), 2.

26. Zhou L; Yang Feng; Tan Yulan; Dong Yibo; Chao C. Effects and evaluation of grassland reclamation on heavy metals in soil at different altitudes. *Environmental Engineering* 2019, 37 (9), 5.
27. Tang B, Xu H, Song F, Ge H, Yue S. Effects of heavy metals on microorganisms and enzymes in soils of lead-zinc tailing ponds. *Environ Res.* 2022;207:112174.
28. Cao R; Wu Fuzhong; Yang Wanqin; Xu Zhenfeng; Tan Bo; Wang Bin; Li Jun; Chenhui C. Effects of altitudes on soil microbial biomass and enzyme activity in alpine-gorge regions. *Chinese Journal of Applied Ecology* 2016, 27 (4), 1257–1264.
29. Fan Z, Lu S, Liu S, Li Z, Hong J, Zhou J, Peng X. The effects of vegetation restoration strategies and seasons on soil enzyme activities in the Karst landscapes of Yunnan, southwest China. *J Forestry Res.* 2019;31(5):1949–57.
30. Bai Y, Li F, Yang G, Shi S, Dong F, Liu M, Nie X, Hai J. Meta-analysis of experimental warming on soil invertase and urease activities. *Acta Agriculturae Scandinavica Section B – Soil & Plant Science.* 2017;68(2):104–9.
31. Khadem A, Raiesi F. Response of soil alkaline phosphatase to biochar amendments: Changes in kinetic and thermodynamic characteristics. *Geoderma.* 2019;337:44–54.
32. Stpniewska Z, Wolińska A, Ziomek J. Response of soil catalase activity to chromium contamination. *J Environ Sci.* 2009;21(8):1142–7.
33. Wei Y, Yang Yongsheng, He H, Zhu J, Li H, Yingnian L. The characteristics of soil physicochemical properties and the roles of metallic elements in organic livestock production under three grassland types in middle and east of Qinghai-Tibet Plateau. *J Glaciology Geocryology.* 2018;40(6):1265–74.
34. Wan J; Nie Ming; Zou Qin; Hu Shaochang; Jiakuan C. Distribution characteristics of heavy metals along an elevation gradient of montane forest. *Spectroscopy and Spectral Analysis* 2011, 31 (12), 3371–3374.
35. Ren G; Zhang Qi; Qu Juanjuan; Yan Lilong; Xinhbin S. Bacterial community diversity in mining area polluted by lead and zinc. *Journal of Northeast Forestry University* 2012, 40 (10), 58–61.
36. Jin Z, Li Z, Li Q, Hu Q, Yang R, Tang H, Li M, Huang B, Zhang J, Li G. Canonical correspondence analysis of soil heavy metal pollution, microflora and enzyme activities in the Pb–Zn mine tailing dam collapse area of Sidi village, SW China. *Environ Earth Sci.* 2014;73(1):267–74.
37. Tang M, Li L, Wang X, You J, Li J, Chen X. Elevational is the main factor controlling the soil microbial community structure in alpine tundra of the Changbai Mountain. *Sci Rep.* 2020;10(1):12442.
38. Margesin R, Jud M, Tscherko D, Schinner F. Microbial communities and activities in alpine and subalpine soils. *FEMS Microbiol Ecol.* 2009;67(2):208–18.
39. Ren Weiheng; Tang Ming, Wang L, Yin Y. The characteristics of the soil fungus community of *Rhododendron delavayi* Franch in the Baili *Rhododendron* Nature Reserve. *Genomics and Applied Biology.* 2020;39(3):1172–7.
40. Liu J, Gong J, Hou W, Malik K, Jin J, Liu Y, Su J, Cheng C, Kong X, Xiong H, Tang X, Tang M, Wang J, Yi Y. Elevations change fungal communities of the bulk soil, rhizosphere and root of *Rhododendron*

- delavayi Franch (Ericaceae) by affecting soil properties in a karst area, southwest China. *Arch Agron Soil Sci* 2022, 1–16.
41. Qin FX, Yi Y, Gong JY, Zhang YB, Hong K, Li YK. Accumulation Characteristics and Risk Assessment of Potentially Toxic Elements for Major Crops and Farmland Around A High-arsenic Coal Mine in Xingren, Guizhou, Southwest China. *Nat Environ Pollution Technol.* 2020;19(3):909–21.
 42. Hou W, Wang J, Nan Z, Christensen MJ, Xia C, Chen T, Zhang Z, Niu X. *Epichloë gansuensis* endophyte-infection alters soil enzymes activity and soil nutrients at different growth stages of *Achnatherum inebrians*. *Plant Soil.* 2020;455(1–2):227–40.
 43. Zhang C, Liu G, Xue S, Song Z. Rhizosphere soil microbial activity under different vegetation types on the Loess Plateau, China. *Geoderma.* 2011;161(3–4):115–25.
 44. Marcel M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 2011;17(1):10–2.
 45. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodriguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolk T, Kreps J, Langille MGI, Lee J, Ley R, Liu YX, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Priesse E, Rasmussen LB, Rivers A, Robeson MS, 2nd, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vazquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson AD, Xu Y, Zhu. Q.; Knight, R.; Caporaso, J. G., Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019, 37(8), 852–857.
 46. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods.* 2016;13(7):581–3.
 47. Chen B-X, Sun Y-F, Zhang H-B, Han Z-H, Wang J-S, Li Y-K, Yang X-L. Temperature change along elevation and its effect on the alpine timberline tree growth in the southeast of the Tibetan Plateau. *Adv Clim Change Res.* 2018;9(3):185–91.
 48. Chen Q, Lu X, Zhao W, Liu Y, Yeguo M. Spatial differentiation of main soil types in Guizhou Province under different topographic conditions. *Jiangsu Agricultural Sciences.* 2022, 50(7):205–213 2022, 50(7), 205–213.
 49. Gebrehiwot K, Demissew S, Woldu Z, Fekadu M, Desalegn T, Teferi E. Elevational changes in vascular plants richness, diversity, and distribution pattern in Abune Yosef mountain range, Northern Ethiopia. *Plant Divers.* 2019;41(4):220–8.

50. Zhou Y, Yi Y, Liu H, Song J, Jia W, Zhang S. Altitudinal trends in climate change result in radial growth variation of *Pinus yunnanensis* at an arid-hot valley of southwest China. *Dendrochronologia* 2022, 71.
51. Qu F; Wen Linlong; Fan Chengwu; Liu Guihua,; Song Q. Heavy metal contents in soil developed from parent materials and their ecological risk assessment. *Acta Mineralogica Sinica* 2020, 40 (6), 8.
52. Li H. ; Lei Jing; Youdong., H., Roles and prospects of humus in remediation of heavy metal contaminated soil. *Agricultural Research and Application* 2017, (5), 6.
53. Moyse WD, Fernandez IJ. Trace metals in the forest floor at saddleback mountain, maine in relation to aspect, elevation, and cover type. *Water Air & Soil Pollution*. 1987;34(4):385–97.
54. Li W, Xie Y; Zhou Jiheng; Zhang Yiyang; Yang Rongsheng; Yang Shuyuan; Tang Langtao,; Hongqi Y. Distributions of available Ca and Mg contents in Tobacco at different altitudes – A case study of tobacco-growing area of Qujing in Yunnan. *Soil* 2010, 42 (6), 946–951.
55. Liqin L. The vertical distribution of iron oxide in Wuyi Mountain and its causes analysis. *Hunan Agricultural Sciences* 2010, (17), 70–1.
56. Tong X, Yubi J. Heavy metal pollution assessment and suggestions on soil nearby mining area in Baili Rhododendron National Forest Park. *Guizhou Agricultural Sciences*. 2011;39(4):114–6.
57. Ma Y, Li L; Zhang Meixue; Mo Chunhu,; Wei M. Discussion on soil forming rock type and its relation with cultivated land soil in Guizhou. *Guizhou Geology* 2020, 37 (4), 5.
58. Ma Huanfei; Xie Mengyi; Hu Han; Guo Yaoxin; Ren Chengjie, Fazhu Z. Effects of stoichiometric characteristics of soil-plant-litter on soil nitrogen components in different forests along an elevational gradient of Qinling Mountains. *Chinese Journal of Ecology* 2020, 39 (3), 749–757.
59. Feng Y; Liang Wenjun; Wei xi,; Weiwen Z. Analysis of soil nutrient characteristics of *Larix principis-rupprechtii* forests with different altitude gradients in Guandi Mountain. *Journal of West China Forestry Science* 2020, 49 (4), 7.
60. Bao Y, Zhuoma D. Different elevation of temperature humidity effects on soil nutrients. *Qinghai Prataculture*. 2014;23(4):3.
61. Wu Z-Y, Lin W-X, Chen Z-F, Fang C-X, Zhang Z-X, Wu L-K, Zhou M-M, Chen T. Variations of soil microbial community diversity along an elevational gradient in mid-subtropical forest. *Chin J Plant Ecol*. 2013;37(5):397–406.
62. Pukalchik M, Panova M, Karpukhin M, Yakimenko O, Kydralieva K, Terekhova V. Using humic products as amendments to restore Zn and Pb polluted soil: a case study using rapid screening phytotest endpoint. *J Soils Sediments*. 2017;18(3):750–61.
63. Han Zhangxiong; Wan Dejun; Hu Jianping, Liu L, Liu Q, Tianyang N. Migration and transformation of heavy metals in soil and its influencing factors. *Multipurp Utilization Mineral Resour* 2017, (6), 5.
64. Chowdhury N, Rasid MM. Heavy metal concentrations and its impact on soil microbial and enzyme activities in agricultural lands around ship yards in Chattogram, Bangladesh. *Soil Sci Annual*. 2021;72(2):1–21.

65. Li Y; Ren Shaohua; Huang Zongsheng; Long Shilin; Fan Chuanhua; Haiyan L. Characteristics of litter decomposition and nutrient release of common tree species at different altitudes in Guizhou karst region. *Chinese Journal of Ecology* 2022.
66. Mou Ling; Wu Honglei; Gu Guojun; Lin Yu, Zhenfeng X. Soil microbes and enzyme activities in four vegetation types in Yunnan karst faulted basin. *Chinese Journal of Applied Environmental Biology* 2020, 26 (5), 1081–1086.
67. Li Q, Hu Q, Zhang C, Jin Z. Effects of Pb, Cd, Zn, and Cu on Soil Enzyme Activity and Soil Properties Related to Agricultural Land-Use Practices in Karst Area Contaminated by Pb-Zn Tailings. *Pol J Environ Stud.* 2018;27(6):2623–32.
68. Gao Yanna; Qi Zhiwei, Zhong Q, Li Y, Jiang N, Wang K, Li S, Tong F. Responses of soil enzymatic activities to long-term simulated warming in Yangtze estuarine wetlands with *Phragmites australis*. *Chinese Journal of Applied Environmental Biology.* Chin J Appl Environ Biology. 2017;23(3):0535–41.
69. Chao R; Zhang Dong; Chen Yalin; Wan Zhiqiang; Gao Qingzhu; Bao Tiejun; Jie Y. Effects of simulated temperature and precipitation increase on soil enzyme activity in typical steppe. *Arid Zone Research* 2018, 35 (5), 1068–1074.
70. Wang WY Bing, Dai Wei, Wang D, Xiaojuan A. Path analysis of soil enzyme activity and soil properties of Chinese fir plantations. *J Beijing Forestry Univ.* 2012;34(2):78–83.
71. Ma J; Liu Xiande; Chen Caixia; Jin Ming; Meng Haojun; Zhao Weijun; Xiurong W. Soil physicochemical properties and enzyme activities along the altitudinal gradients in *Picea Crassifolia* of Qilian Mountains. *Journal of Soil and Water Conservation* 2019, 33 (2), 7.
72. Karaca A, Cetin SC, Turgay OC, Kizilkaya R. Effects of Heavy Metals on Soil Enzyme Activities. In *Soil Heavy Metals*, 2010; pp 237–62.
73. Li H, Zhang J; Yao Tuo; Yang Xiaomei; Gao Yamin; Li Changning; Li Qi; Ying F. Soil nutrients, enzyme activities and ecological stoichiometric characteristics in degraded alpine grasslands. *Journal of Soil and Water Conservation* 2018, 32 (5), 287–295.
74. Zeng CQ, Liu WX, Hao JY, Fan DN, Chen LM, Xu HN, Li KZ. Measuring the expression and activity of the CAT enzyme to determine Al resistance in soybean. *Plant Physiol Biochem.* 2019;144:254–63.
75. Zuccarini P, Asensio D, Ogaya R, Sardans J, Penuelas J. Effects of seasonal and decadal warming on soil enzymatic activity in a P-deficient Mediterranean shrubland. *Glob Chang Biol.* 2020;26(6):3698–714.
76. Chen S, Guo Z, Qingping Y. Soil enzyme activities in Moso bamboo forests along an altitude gradient. *Chin J Ecol.* 2010;29(3):529–33.
77. Ciarkowska K, Solek-Podwika K, Wieczorek J. Enzyme activity as an indicator of soil-rehabilitation processes at a zinc and lead ore mining and processing area. *J Environ Manage.* 2014;132:250–6.
78. Youbin FG. Effects of Nanoscale Fe₃O₄ on microbial communities, enzyme activities and 2,4-D degradation in red soil. *Scientia Agric Sinica.* 2011;44(6):1165–72.

79. Genmei YL. W., Elevational distribution characteristics of soil bacterial community and enzyme activities in Mount Huangshan. *Environmental Science* 2019, 40 (2), 10.
80. Zhang Y, Cong J, Lu H, Li G, Xue Y, Deng Y, Li H, Zhou J, Li D. Soil bacterial diversity patterns and drivers along an elevational gradient on Shennongjia Mountain, China. *Microb Biotechnol*. 2015;8(4):739–46.
81. Qiao S; Zhou Yongna; Liu Jinxian; Jing Juhui; Jia Tong; Li Cui; Yang Xin,; Baofeng C. Characteristics of soil bacterial community structure in coniferous forests of Guandi Mountains, Shanxi Province. *Scientia Silvae Sinicae* 2017, 53 (2), 89–99.
82. Deng Jiaojiao; Zhou Yongbin; Yin You; Wei Yawei; Qin Shengjin, Wenxu Z. Soil bacterial community structure characteristics in coniferous forests of Montane Regions of eastern Liaoning Province, China. *Acta Ecologica Sinica* 2019, 39 (3), 997–1008.
83. Tao X, Feng J, Yang Y, Wang G, Tian R, Fan F, Ning D, Bates CT, Hale L, Yuan MM, Wu L, Gao Q, Lei J, Schuur EAG, Yu J, Bracho R, Luo Y, Konstantinidis KT, Johnston ER, Cole JR, Penton CR, Tiedje JM, Zhou J. Winter warming in Alaska accelerates lignin decomposition contributed by Proteobacteria. *Microbiome*. 2020;8(1):84.
84. Stursova M, Zifcakova L, Leigh MB, Burgess R, Baldrian P. Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiol Ecol*. 2012;80(3):735–46.
85. Wu J; Zhi Xiaoyang; Li Yan; Guan Tongwei; Tang Shukun; Xu Lihua,; Wenjun L. Comparison of Actinobacterial diversity in Jiangcheng and Heijing saline mines in Yunnan by using culture-independent approach. *Microbiology* 2008, (10), 1550–1555.
86. Zhang B, Kong W, Wu N, Zhang Y. Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. *J Basic Microbiol*. 2016;56(6):670–9.
87. Yang L. Analysis on bacterial community of soil in farmland contaminated by heavy metal in Shizishan mining area. *Anhui Agricultural Science Bulletin*. 2022;28:11.
88. Kim HM, Jung JY, Yergeau E, Hwang CY, Hinzman L, Nam S, Hong SG, Kim OS, Chun J, Lee YK. Bacterial community structure and soil properties of a subarctic tundra soil in Council, Alaska. *FEMS Microbiol Ecol*. 2014;89(2):465–75.
89. Liu H, Wang J, Zhao W, Chen Y, Lv N, Yi Z, Huang Z, Yang R, Lv X. Soil chemical properties drive the structure of bacterial communities in the cotton soil of arid Northwest China. *Ecol Res*. 2021;36(4):663–72.
90. Peralta RM, Ahn C, Gillevet PM. Characterization of soil bacterial community structure and physicochemical properties in created and natural wetlands. *Sci Total Environ*. 2013;443:725–32.
91. Xing Yi; Si Yanxiao, Yang HC. Impact of long-term heavy metal pollution on microbial community in iron mine soil. *Res Environ Sci*. 2013;26(11):1201–11.
92. Zancan S, Suglia I, La Rocca N, Ghisi R. Effects of UV-B radiation on antioxidant parameters of iron-deficient barley plants. *Environ Exp Bot*. 2008;63(1–3):71–9.

93. Hasegawa H, Rahman MA, Saitou K, Kobayashi M, Okumura C. Influence of chelating ligands on bioavailability and mobility of iron in plant growth media and their effect on radish growth. *Environ Exp Bot.* 2011;71(3):345–51.
94. Borlotti Andrea; Vigani Gianpiero, Graziano Z. Iron deficiency affects nitrogen metabolism in cucumber (*Cucumis sativus*L.) plants. *BMC Plant Biology* 2012, 12 (1), 1–15.
95. Lv S, Zhang X, Zhang F, Wenju L. The effect of ferric and manganese hydroxide cutan on root surface in different phosphorus concentrations on phosphorus uptake by rice plants. *Southwest China Journal of Agricultural Sciences* 1999, (S1), 7–12.
96. Dastager SG, Damare S. Marine actinobacteria showing phosphate-solubilizing efficiency in Chorao Island, Goa, India. *Curr Microbiol.* 2013;66(5):421–7.
97. Xian W, Zhang X, Wenjun L. Research status and prospect on bacterial phylum Chloroflexi. *Acta Microbiol Sinica.* 2020;60(9):1801–20.
98. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. Plant-microbiome interactions: from community assembly to plant health. *Nat Rev Microbiol.* 2020;18(11):607–21.
99. Zhang J; Wang Jianyu; Meng Zexin; He Jia; Dong Zhenghong; Liu Kaiqian; Aiqin C. Soil microbial richness predicts ecosystem multifunctionality through co-occurrence network complexity in alpine meadow. *Acta Ecologica Sinica* 2022, 42 (7), 2542–2558.
100. He Zhongsheng; Gu Xinguang, Jiang L, Xu D, Liu J, Li W, Wenwei C. Characteristics and its influencing factors of forest soil dominant bacterial community in different elevations on the southern slope of Daiyun Mountain, Fujian Province of eastern China. *J Beijing Forestry Univ.* 2022;4(7):107–16.
101. Santolini M, Barabasi AL. Predicting perturbation patterns from the topology of biological networks. *Proc Natl Acad Sci U S A.* 2018;115(27):E6375–83.
102. Xue L, Ren H, Li S, Leng X, Yao X. Soil Bacterial Community Structure and Co-occurrence Pattern during Vegetation Restoration in Karst Rocky Desertification Area. *Front Microbiol.* 2017;8:2377.
103. Chen P, He W, Shen Y, Zhu L, Yao X, Sun R, Dai C, Sun B, Chen Y. Interspecific Neighbor Stimulates Peanut Growth Through Modulating Root Endophytic Microbial Community Construction. *Front Plant Sci.* 2022;13:830666.

Figures

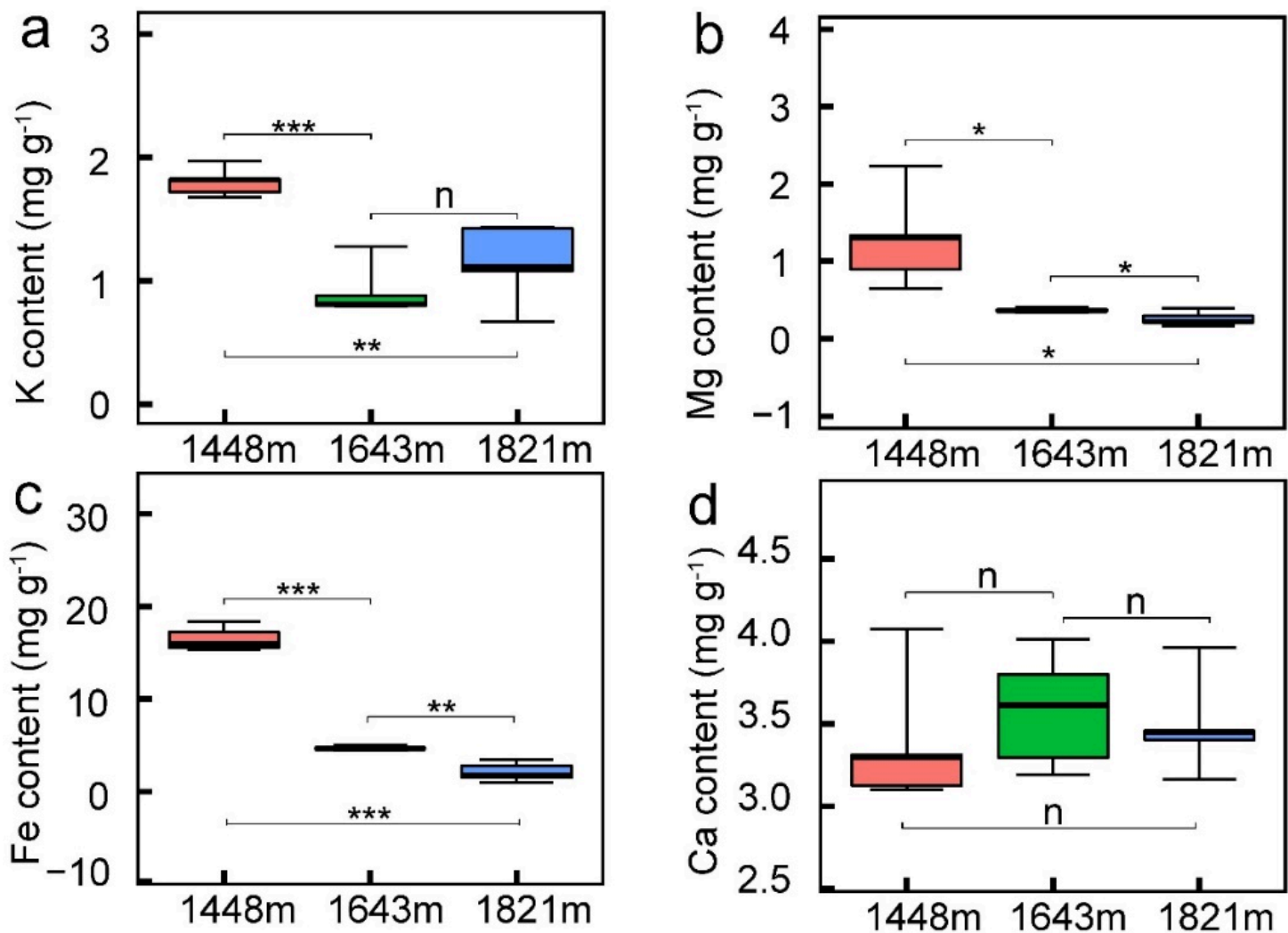


Figure 1

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 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.

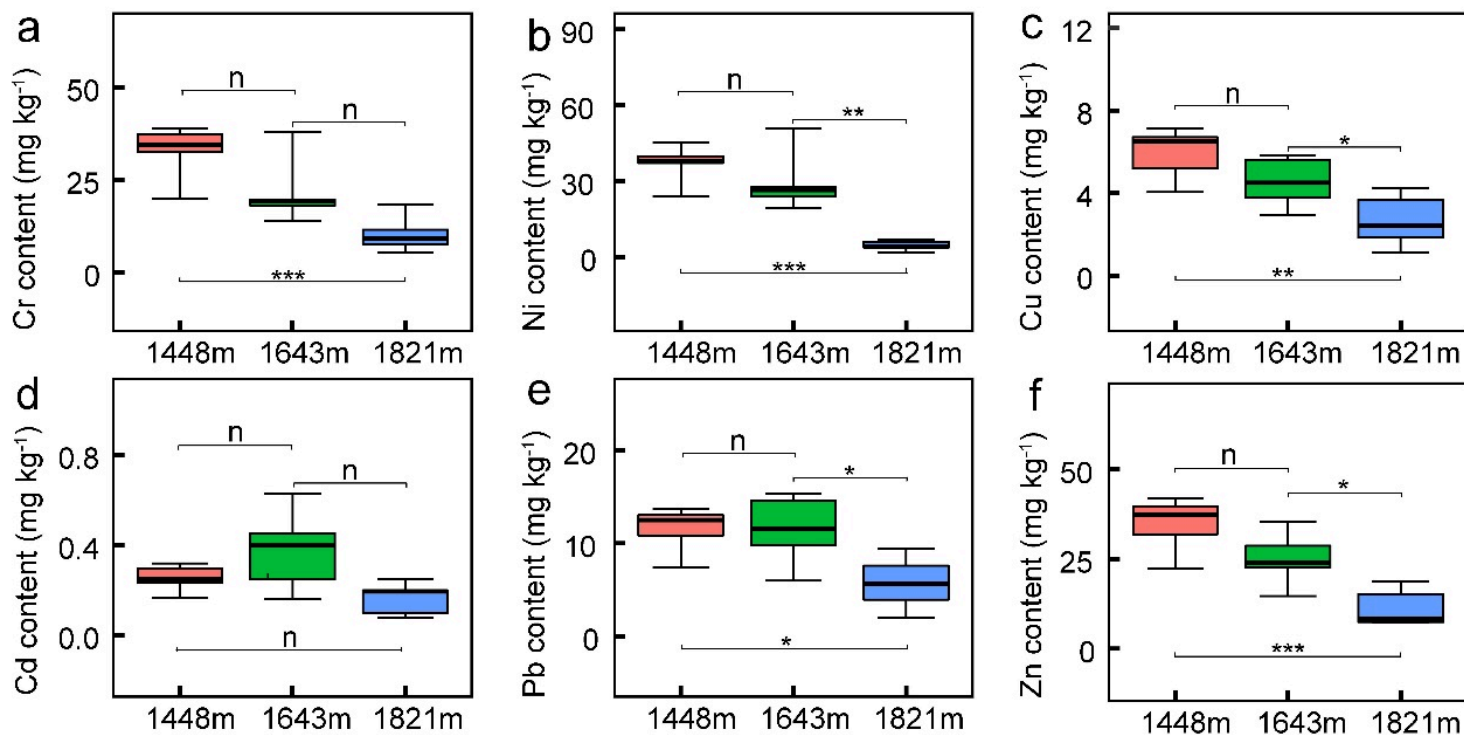


Figure 2

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.

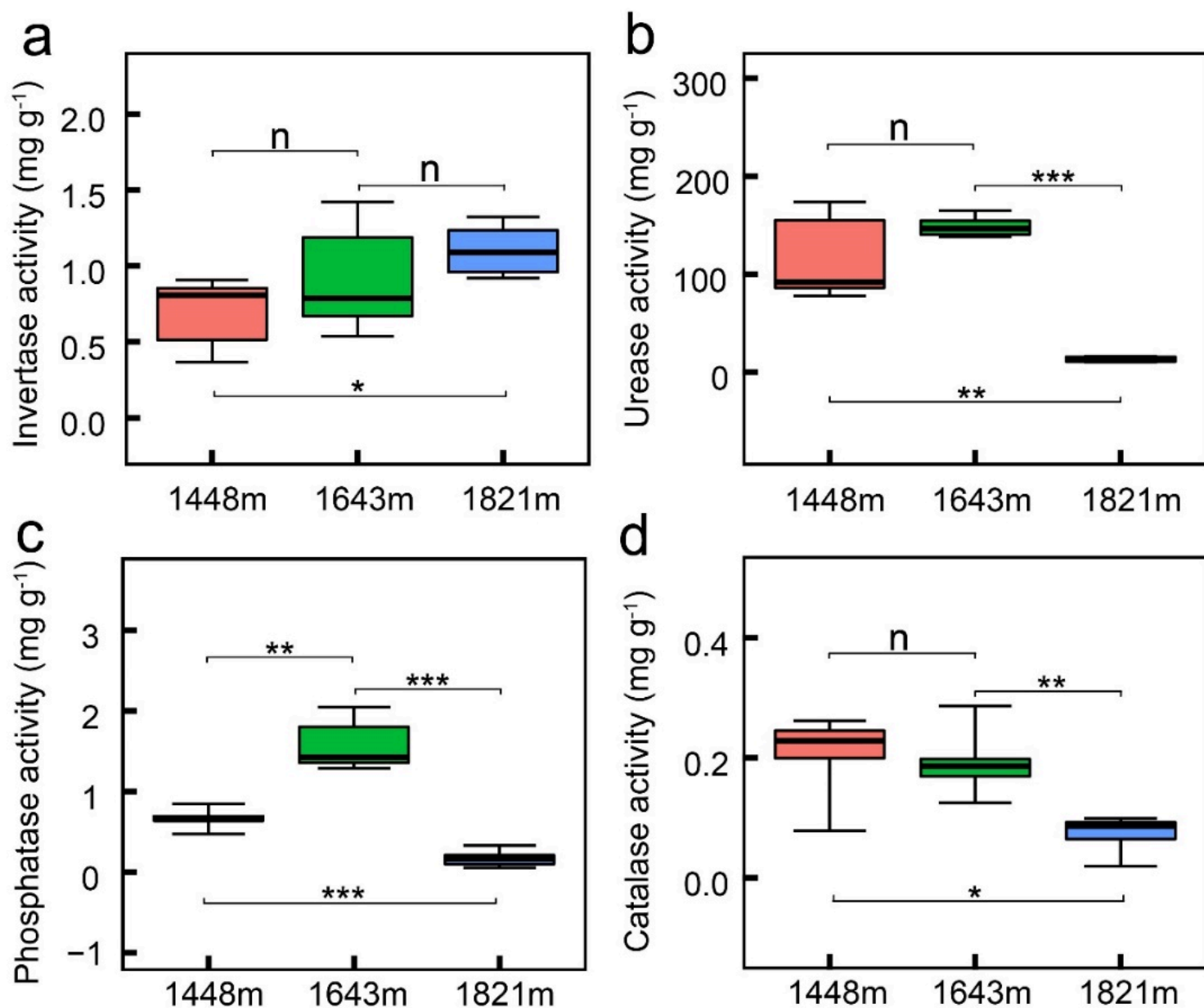


Figure 3

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.

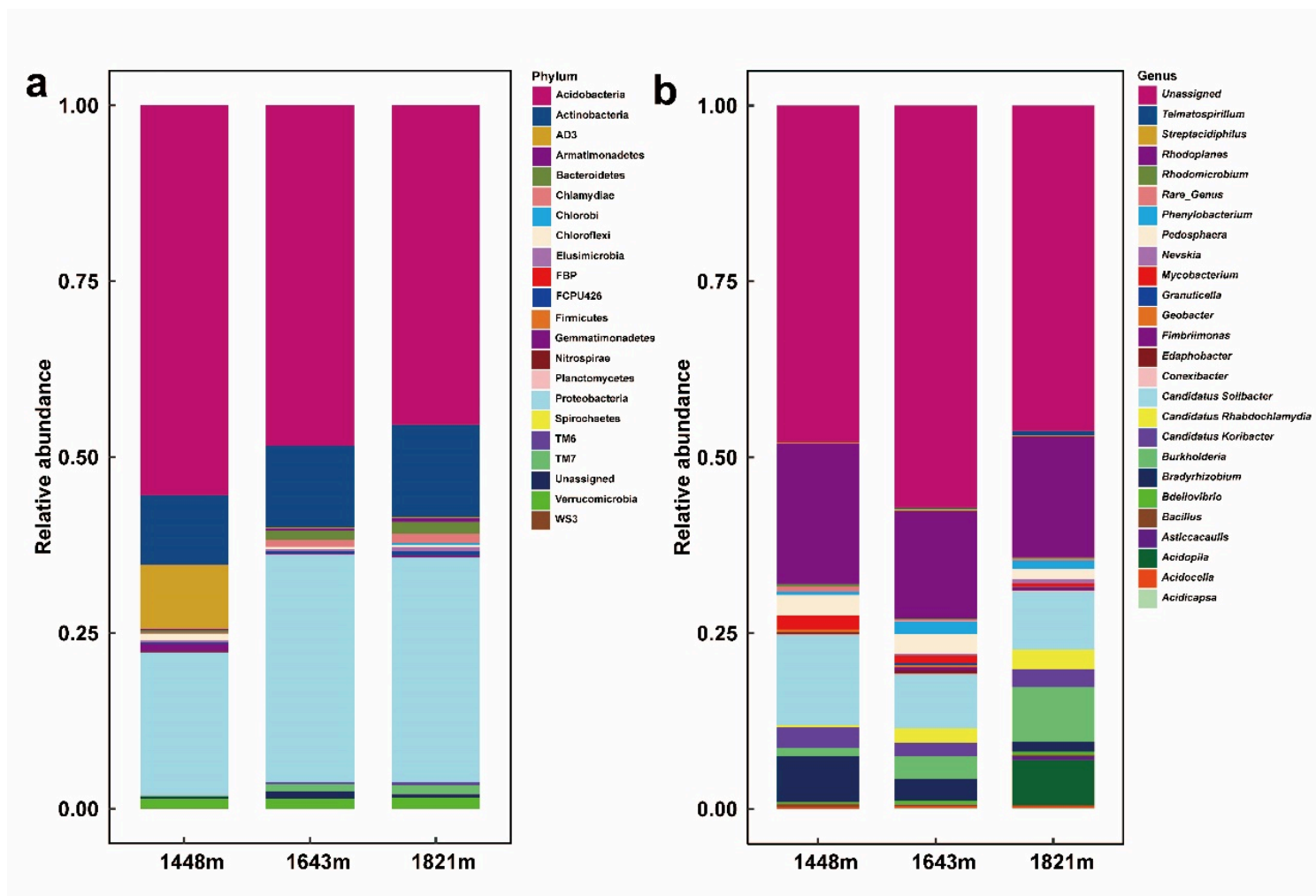


Figure 4

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

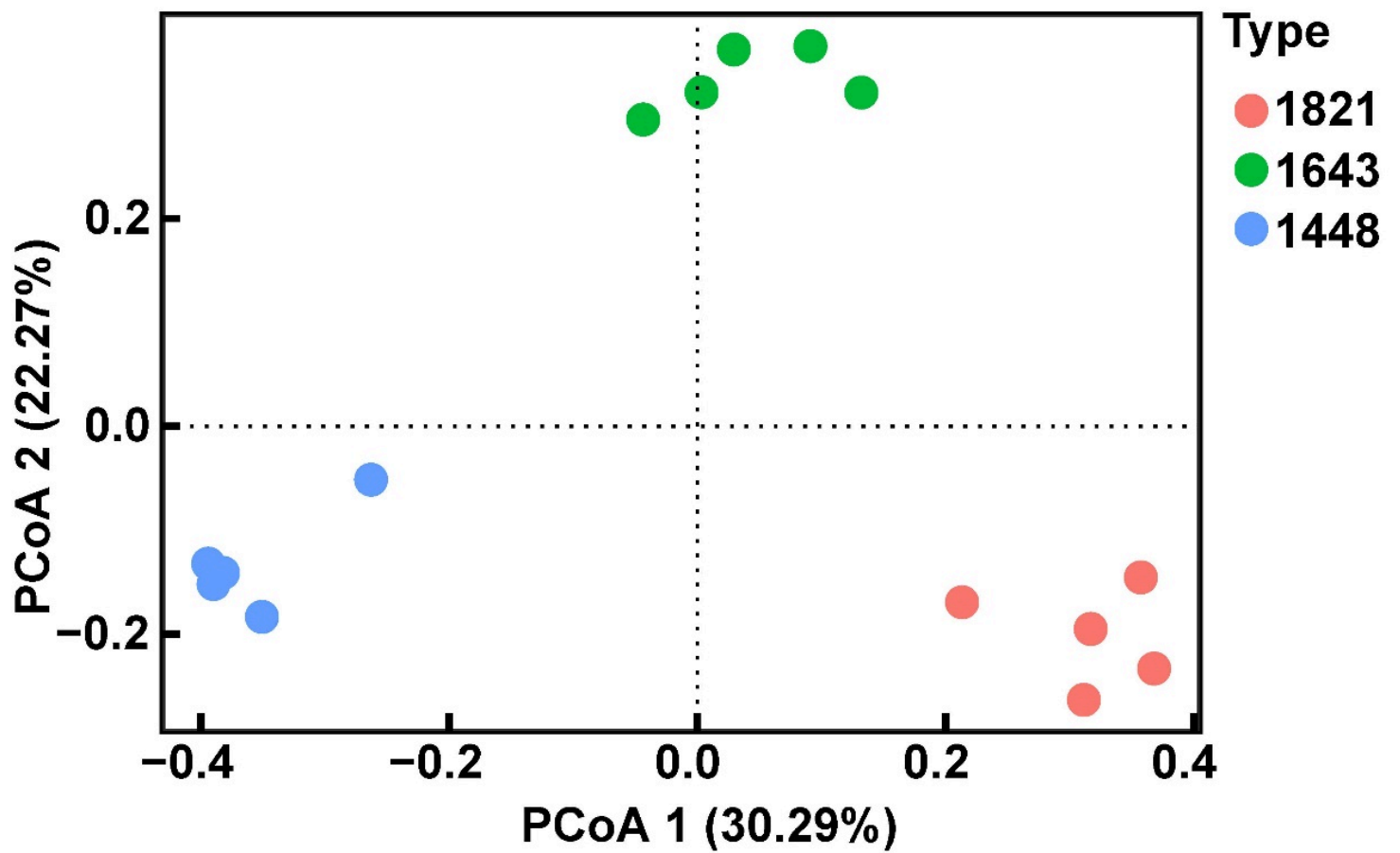


Figure 5

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

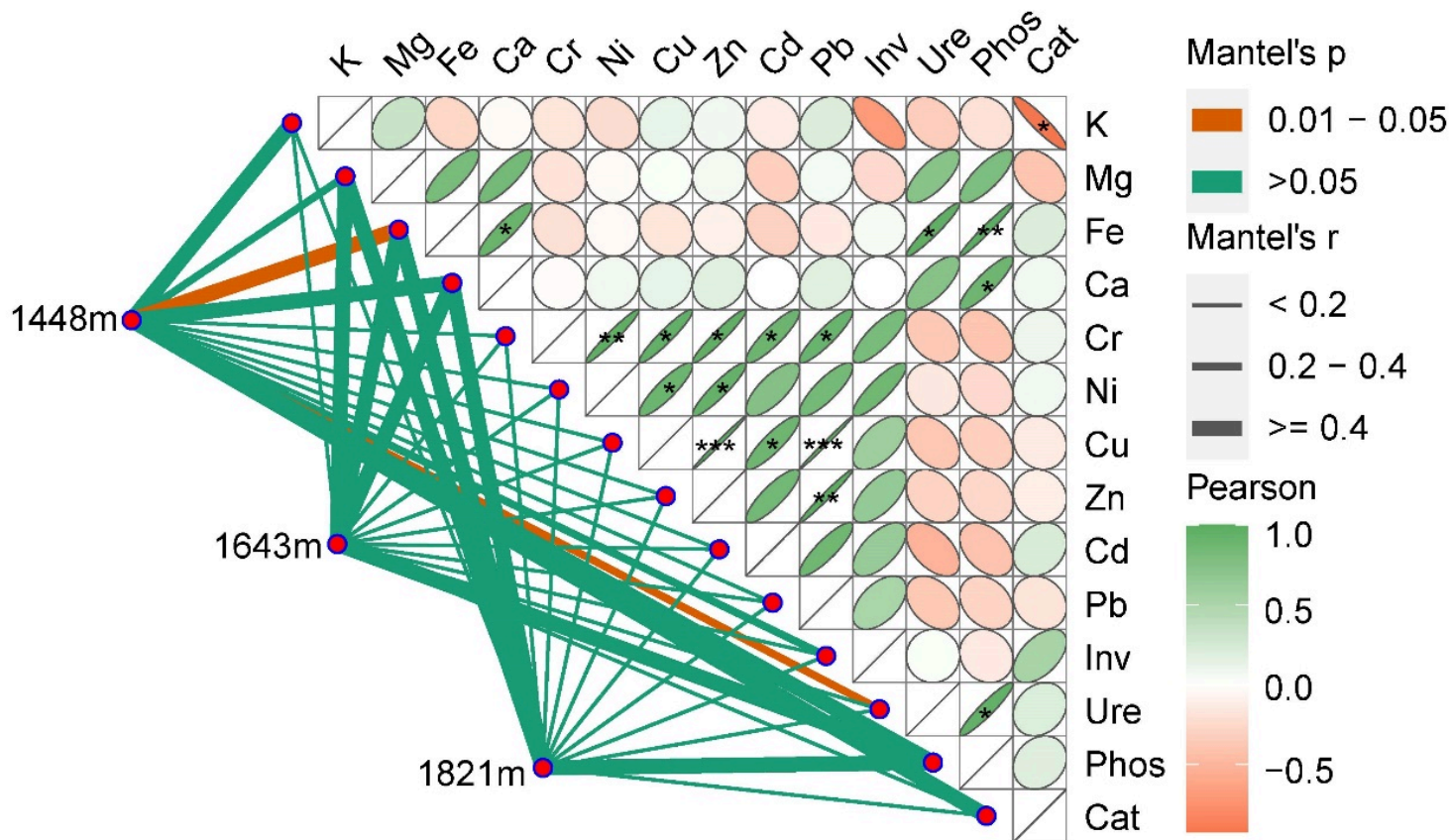


Figure 6

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.

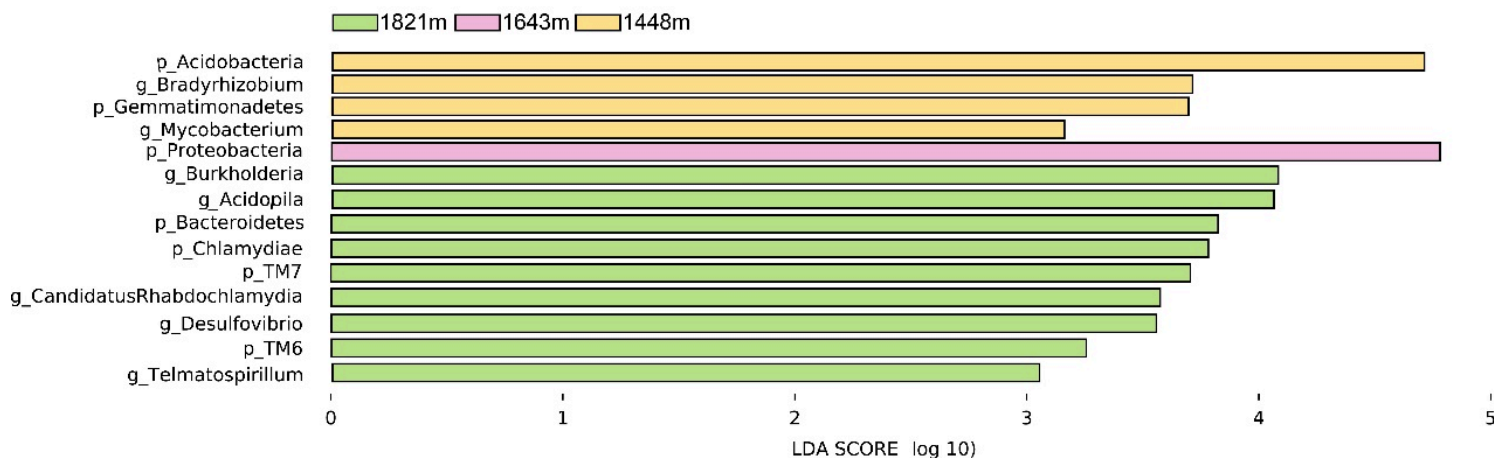


Figure 7

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 ($\alpha = 0.05$) and had a LEfSe score of > 3.0 .

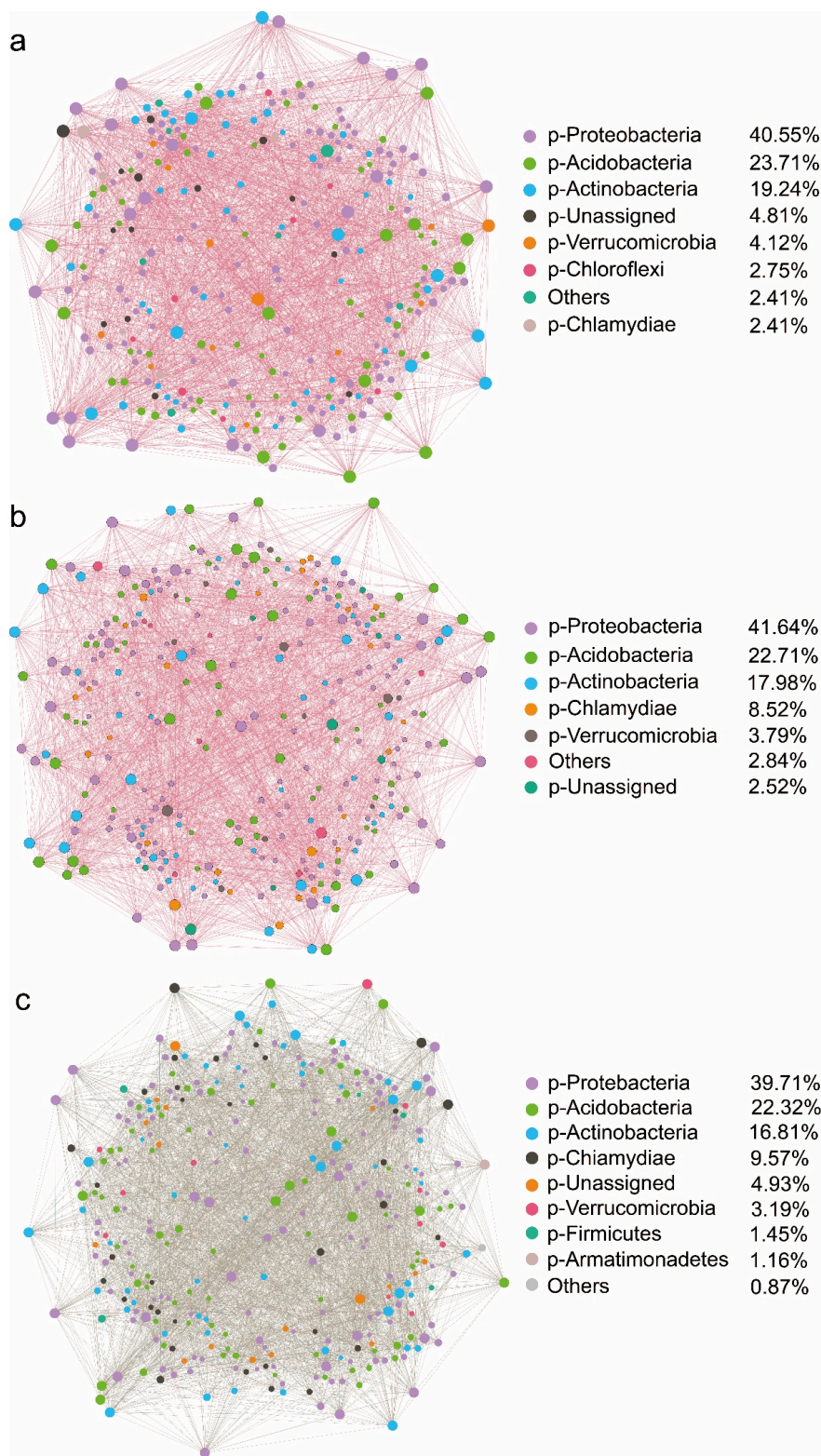


Figure 8

Characteristics of co-occurrence network of soil bacteria at a: 1448 m, b: 1643 m and c: 1821 m.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementary.docx](#)