Calcium content and high calcium adaptation of plants in karst areas of southwestern Hunan, China

Xiaocong Wei[1], Xiangwen Deng[1,2*], Wenhua Xiang[1,2] Pifeng Lei[1,2], Shuai Ouyang[1,2], Hongfang Wen[1], Liang Chen[1,2]

[1] Faculty of Life Science and Technology, Central South University of Forestry and Technology, Changsha 410004, Hunan Province, China

[2] Huitong National Field Station for Scientific Observation and Research of Chinese Fir Plantation Ecosystem in Hunan Province, Huitong 438107, China

Correspondence to: Xiangwen Deng, Email: dxwfree@126.com, Tel.: +86 0731 85623483

Abstract. Rocky desertification is a major ecological problem of land degradation in karst areas. Its high soil calcium (Ca^{2+}) content has become an important environmental factor which can affect the restoration of vegetation in such rocky desertification areas. Consequently, the screening of plant species, which can adapt to soil high Ca^{2+} environments, is a critical step for vegetation restoration. In this study, three different grades of rocky desertification sample areas (LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification) were selected in karst areas of southwestern Hunan, China. Each grade of these sample areas had 3 sample plots in different slope positions, each of which had 4 small quadrats (1 in rocky side areas, 3 in non-rocky side areas). We measured the Ca^{2+} content of leaves, branches and roots from 41 plant species, as well as soil total Ca^{2+} (TCa) and exchange Ca^{2+} (ECa) at depths of 0–15, 15–30 and 30–45 cm under each small quadrat. The results showed that the soil Ca^{2+} content in rocky side areas was significantly higher than that in non-rocky side areas ($p<0.05$). The mean soil TCa and ECa content increased gradually along with the grade of rocky desertification, in the order IRD > MRD > LRD. For all plant functional groups, the plant Ca^{2+} content of aboveground parts was significantly higher than that of the belowground parts ($p<0.05$). The soil ECa content had significant effects on plant Ca^{2+} content of the belowground parts, but had no significant effects on plant Ca^{2+} content of the aboveground parts. According to the differences in Ca^{2+} content between the aboveground and belowground parts of 17 dominant species (important value, *IV*>1) and their correlations with soil ECa content, these 17 species can be divided into three categories:

Ca-indifferent plants, high-Ca plants and low-Ca plants. Our results provides a vital theoretical basis and practical guide for vegetation restoration and ecosystem reconstruction in rocky desertification areas.

Keywords: Rocky desertification; High Ca adaptation; Plant functional groups; Plant Ca content; Soil Ca content.

1 Introduction

Karst is a kind of typical calcium (Ca)-rich environment and a unique ecological environment system. This type of ecosystem is widely distributed, accounting for 12% of the world's total land area (Zeng et al., 2007; Zhou et al., 2009; Luo et al., 2012). Karst landforms in China are mainly distributed in southwestern areas. Rocky desertification is an extreme form of land degradation in karst areas, and has become a major social problem in terms of China's economic and social development (Sheng et al., 2015). The severity of rocky desertification was ranked in fourth in Hunan Province of China (Li et al., 2016). The restoration and reconstruction of rocky desertification ecosystems has become the urgent environment improvements, regional economic development by using agroforestry system and helping to support people out of poverty(Jing et al., 2016). Soil with high Ca content in rock desertification areas has become one of the most important environmental factors affecting the local plant physiological characteristics and distribution in these areas (Ji et al., 2009). From the origin of rocky desertification, its remediation should focus on vegetation restoration (Wang et al., 2004). Consequently, the screening of plant species which can grow successfully in high-Ca environments in rocky desertification areas is an extremely critical step.

Role of Ca^{2+} in plant physiology: Ca^{2+} is one of the most essential nutrients needed for the regulation of plant growth and is also plant signal sensor (second messenger) under conditions of environment stress (Poovaiah and Reddy, 1993; Hepler, 2005; Hong-Bo and Ming, 2008; Batistič and Kudla, 2012). In the absence of nutrients (such as phosphorus), plants will inhibit the activity of nitrate reductase, thereby inhibiting the absorption of nitrate nitrogen, and ultimately inhibiting the absorption of Ca^{2+} (Reuveni et al., 2000). Ca^{2+} combine with pectin in the cell walls of plants to form pectin Ca, which is a vital component of the cell wall (Kinzel, 1989). Ca also has the function of maintaining the structure and function of cell membranes, regulating the activity of biological enzymes, and maintaining the anion-cation balance in vacuoles (Marschner, 2011).

Mechanisms of plant defense to high soil Ca^{2+} concentrations: Over the past decades, the progress has been made in

identifying the cellular compartments (e.g., endoplasmic reticulum, chloroplasts and mitochondria) that regulate Ca balance and signal transduction in plants (M üller et al., 2015). Ca^{2+} is an essential macronutrient, but low Ca^{2+} concentrations must be maintained within the plant cytoplasm to avoid toxicity (Larkindale and Knight, 2002; Borer et al., 2012). Plants can be adapted to high salt, drought and high temperature environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998). The plant cell not only rapidly increase the free Ca^{2+} concentration of the cytoplasm to adapt to environmental changes, but also maintain a low Ca concentration to prevent harm caused by high Ca. This fine regulatory mechanism is mainly achieved by Ca^{2+} channels (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2005). The vacuoles may account for 95% of the plant cell volume and are able to store Ca within the cell. Thus, empty vacuoles represent an efficient means of Ca storage (Ranjev et al., 1993).

Specific variability in plant Ca^{2+} content and tolerance: The concentration of free Ca^{2+} in vacuoles varies with plant species, cell type and environment, which may also affect the release of Ca^{2+} in vacuoles (Peiter, 2011). Cytoplasmic Ca^{2+} is mainly combined with proteins and other macromolecules; the concentration of free Ca^{2+} is generally only 20–200 nmol L^{-1} and is stored in cell gaps and organelles such as vacuoles, endoplasmic reticulum, mitochondria and chloroplasts (Wu, 2008). However, excess free Ca^{2+} in cytoplasm combines with phosphate to form a precipitate, which interferes with the physiological processes associated with phosphorus metabolism, thus hindering normal signal transduction and causing significant detriment to plant growth (White and Broadley, 2003; Hirschi, 2004).

Plants adaptation to high Ca soil environment: Some plants fix excess Ca^{2+} by forming calcified deposits in root tissue in order to limit the upward transport of Ca^{2+} (Musetti and Favali, 2003). In addition, Ca oxalate crystals in the plant's crystal cells play a role in regulating plant Ca content (Ilarslan et al., 2001; Pennisi and McConnell, 2001; Volk et al., 2002). In a high Ca environments, some plants will form Ca oxalate crystal cells in order to fix excess Ca^{2+} (Moore et al., 2002). Furthermore, an active Ca efflux system plays an important role in the adaptation of plants to high Ca environments (Bose et al., 2011): Excess Ca^{2+} in plants is exported from mature leaves to the outside, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003). The regulation of internal Ca storage predominantly depends on plasma membrane Ca transport and intracellular Ca storage; collectively these processes can regulate the intracellular Ca^{2+} concentration to a lower level (Bowler and Fluhr,

2000). Plants that adapt to high Ca environments promote excess Ca^{2+} flow through the cytoplasm or store Ca^{2+} in vacuoles via the cytoplasmic Ca^{2+} outflow and influx system (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2006).

The mean soil ECa was 3.61 g kg^{-1} in the Puding, Huajing, Libo and Luodian Counties of Guizhou Province, which is several times that of non-limestone areas in China (Ji et al., 2009). Wang et al. (2011) found that plant rhizosphere soil TCa content in calcareous soil area ware above 14.0 mg g^{-1}. Zhang (2005) studied the growth habits of *Eurycorymbus caraleriel* and *Rhododendron decorum* under different concentrations of Ca^{2+} and found that a high Ca^{2+} concentration (50 mmol L^{-1}) could promote growth in *Eurycorymbus caraleriel*, but inhibit growth in *Rhododendron decorum*. Luo et al. (2013) showed that Ca^{2+} concentration affected plant photosynthesis. When the daily net photosynthetic rate of *Cyrtogonellum Ching* and *Diplazium pinfaense Ching* reached the highest value, the concentrations of Ca^{2+} were 30 mmol L^{-1} and 4 mmol L^{-1}, respectively. Qi et al. (2013) found that a significant difference in calcium content among *Primulina* species from different soil types, with high average calcium content (2,285.6 mg/kg) in *Primulina* from calcareous soil relative to low levels present in *Primulina* from both acid soil (1,379.3 mg/kg) and Danxia red soil (1,329.1 mg/kg). There are variations in soil Ca content among different areas and differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. These differences need to identify the variety of the plants to adapt with high Ca environments. However, to data, the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas and the Ca^{2+} dynamics of plants and soil are not well understood.

In this study, we investigated plant Ca content, soil exchangeable Ca (ECa) and total Ca (TCa) contents on the rocky and non-rocky sides of three different grades of rocky desertification areas in southwestern China. Specifically, we hypothesized that the dynamics of Ca content in plants and soil would be significantly affected by the grade of rocky desertification. To test this hypothesis, the following investigations were explored: (i) to measure the soil ECa and TCa contents in rocky and non-rocky side areas; (ii) to investigate and compare the Ca content of aboveground and belowground parts among of plants from different functional groups; and (iii) to reveal correlation between plant Ca content and soil ECa content.

2 Materials and methods

2.1 Site description

The study site is located in LijiaPing town of Shaoyang County, Hunan Province, China (latitude 27°0' N; longitude 113°36' E, elevation 400–585 m above sea level; see Fig. 1). This region experiences a humid mid-subtropical monsoon climate. Mean annual air temperature is 16.9 ℃, and maximum and minimum temperatures are 41.0 ℃ and −10.1°C, respectively. Mean annual precipitation is 1399 mm, mostly occurring between April and August, and the frost-free period is 288 days. The study site mainly consists of black and yellow lime soil, and vegetation is scarce. Groundwater level is low and groundwater storage is poor (see Table. 1).

2.2 Experimental design and data collection

Rocky desertification was graded by using the sum of four index scores: bedrock expose rate, vegetation type, vegetation coverage and soil thickness were quantified according to the State Forestry Administration of the People's Republic of China industrial standard 'LY/T 1840—2009' (China, 2009). Three 1 hm^2 sample areas were selected, which were each representative of the three different grades of rocky desertification: LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification. Within each sample area, we recorded environmental factors, including longitude, latitude, altitude, topography, vegetation type, degree of bare bedrock, and other conditions. The sample collection in these three sample areas were conducted in October 2016.

Within each of the three sample areas, four (2×2)small quadrats in different slope positions (upper, middle, and lower slope) were set up. In total, we assigned 36 small quadrats (3×4×3) for analysis. The common plant species of the region were gathered using the whole plant harvest method in each small quadrat as well as shrubs and herbs were collected. Shrubs were divided into three parts: branches, leaves and roots. Herbs were divided into two parts: aboveground and belowground. Plant samples were taken back to the laboratory, rinsed with distilled water before being oven tried at 105 ℃ for 15 min to de-enzyme, and then dried to a constant weight at 80 ℃ about 480 minutes, crushed and passed through a 0.149 mm sieve, for later chemical analysis. For the soil samples, we measured the TCa and ECa relating to the quadrat soil (top soil: 0-15 cm; middle soil: 15-30 cm; bottom soil, 30-45 cm). Soil TCa, ECa content and plant Ca content were measured using an Atomic

Absorption Spectrophotometer (3510, Shanghai, China).

2.3 Data analysis

All plant species were divided into different functional groups: (1) nitrogen-fixing plants and non-nitrogen-fixing plants groups according to nitrogen-fixing function, (2) dicotyledons and monocotyledons groups according to system development type, (3) C3 and C4 plants groups according to photosynthetic pathway, and (4) deciduous shrubs, evergreen shrubs, annual herbs and perennial herbs according to life form. The biennial herbs were gathered to the 'Annual herbs'. The deciduous trees with a height less than 2 m or a ground diameter less than 3 cm were gathered to the 'deciduous shrubs'. Branches and leaves were treated together as aboveground part, while the belowground part only included roots. We carried out two-way ANOVA for both species and soil for these 17 plants to determine differences in plant Ca content. One-way analysis of variance (ANOVA) was used to analyze the Ca content of soil and plants between different grades of rocky desertification. Pearson correlation analysis ($\alpha = 0.05$) was used to analyze the correlation between plant Ca and soil ECa content. All statistical analyses were performed using R 3.3.3 (R Development Core Team, 2017).

3 Results

3.1 The properties of soil in different grades of rocky desertification

The mean TCa content in soil was 2.40 g kg^{-1} (range: 0.10–8.09 g kg^{-1}) while mean ECa content was 1.46 g kg^{-1} (range: 0.02–3.92 g kg^{-1}). Differences between different samples location (non-rocky side and rocky side) were significant ($p<0.05$) for both TCa and ECa. The mean soil TCa and ECa content were found that the highest in areas of IRD, followed by MRD, followed by LRD. However, only the mean soil ECa content showed significant differences ($p<0.05$) across the three different grades of rocky desertification. Regarding the availability of Ca, the average Ca content was 59.75%, the MRD showing the highest content at 72.55%, followed by IRD at 58.98%, and LRD showing the lowest content at 47.72 % (Table. 2).

3.2 The Ca content of plants

3.2.1 The Ca content of plant in different grades of rocky desertification areas

Total 41 plant species were collected from the three different grades of rocky desertification. The mean Ca content of the aboveground parts of these plants was 19.67 g kg^{-1} (range: 4.34–40.24 g kg^{-1}). Compare to the mean Ca content of the belowground parts was 10.79 g kg^{-1} (range: 4.41–33.62 g kg^{-1}). The Ca content of the aboveground parts was significantly higher than that of the belowground parts ($p<0.05$) throughout the same grades of rocky desertification. Whether the Ca content of aboveground and belowground part of the plants were no significant differences ($p>0.05$) among the three different grades of rocky desertification. (Fig. 2).

3.2.2 Ca content in different plant functional groups

The 41plant species were identified and were divided into different functional groups in the 36 small quadrats. The Ca content of the aboveground parts was significantly higher than that of the belowground parts in each group ($p<0.05$). Nitrogen-fixing plants (22.48 g kg^{-1}) showed a slightly higher Ca content in the aboveground parts than non-nitrogen-fixing plants (19.39 g kg^{-1}; $p>0.05$), although Ca content in the belowground parts of nitrogen-fixing plants (6.76 g kg^{-1}) was lower than that of non-nitrogen-fixing plants (11.12 g kg^{-1}; $p>0.05$). For C3 plants, Ca content in the aboveground and belowground parts were 21.08 g kg^{-1}, and 13.18 g kg^{-1}, respectively, and were both significantly higher than that of C4 plants (aboveground: 15.68 g kg^{-1}; belowground: 6.42 g kg^{-1}; $p<0.05$). In life form functional groups, shrubs showed a significantly higher in Ca content than herbs in both aboveground and belowground parts ($p<0.05$), although there were no significant differences ($p>0.05$) between deciduous and evergreen shrubs ($p>0.05$). There was no statistical difference with this respect between annual herbs and perennial herbs ($p>0.05$). The Ca content of dicotyledons in aboveground and belowground parts were 21.39 g kg^{-1} and 12.19 g kg^{-1}, respectively, and were significantly higher than that of monocotyledons (9.63 g kg^{-1} and 4.79 g kg^{-1}, respectively; $p<0.05$) (Fig. 3).

To monocotyledons and dicotyledons, there were no significant differences in the plant Ca content of the aboveground parts among the different grades of rocky desertification; this was also true for the plant Ca content of the belowground parts. The

Ca content of dicotyledons was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout the three grades of rocky desertification ($p<0.05$) (Fig. 4).

Within total 41 common plants species, 17 plant species were found in each sample plot and were widespread throughout the southwestern rocky desertification areas of Hunan. These 17 species were calculated their important values (*IV*) (Table. 3). Data showed that the Ca content in the aboveground parts of the 17 plant species were highly significant ($p<0.01$) among species, although these differences were not related among grades of rocky desertification. The Ca content in the belowground parts were highly significant difference not only   among the species, and it throughout all the grades of rocky desertification ($p<0.01$).

3.3 Correlation between plant Ca content and soil ECa content

These 17 plant species, the Ca content in the aboveground and belowground parts of *Sanguisorba officinalis* had a significant positive correlation ($p<0.01$) with soil ECa content. The Ca content in the belowground parts of *Dendranthema indicum* ($p<0.05$), and *Castanea henryi* ($p<0.01$), showed a significant positive correlation ($p<0.01$) with soil ECa content. The Ca content in the aboveground parts of *Themeda japonica* showed a significant positive correlation *($p<0.01$)* with soil ECa content. The other plants, the Ca content of the aboveground and belowground parts did not show a significant positive correlation ($p>0.05$) with soil ECa content (Table. 4).

3.4 Capacity of plants adapting to soil high Ca environments

The above 17 plants were dominant and common species in rocky desertification areas. These species appear to have a strong capacity to adapt to high Ca environments in rocky desertification areas. The aboveground parts of plants play an important role in physiological metabolism, and their elemental content reflects the physiological and ecological characteristics of plants.

The Ca-indifferent plants included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica.* For these plants, there was a significant positive correlation between Ca content in the aboveground or belowground parts and the soil ECa content. These plants did not strictly control the absorption and transport of Ca and may be insensitive to

the changes of their own Ca content, and their growth was less affected by soil Ca content. In addition, for other plants, the relationship between Ca content in the aboveground and belowground parts and soil ECa content did not show a positive correlation, then these plants were divided into high-Ca plants and low-Ca plants, based on the differences in Ca content in the aboveground parts of these plants. High-Ca plants included *Pyracantha fortuneana*, *Rhus chinensis*, *Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. The aboveground parts of these plants could maintain a high Ca content (more than 19 g kg^{-1}) under conditions of varying ECa content in the soil. Low-Ca plants included *Abelia chinensis*, *Vitex negundo*, *Smilax china*, *Miscanthus sinensis*, *Artemisia carvifolia* and *Digitaria sanguinalis*. The aboveground parts of these plants could maintain a low Ca content (less than 19 g kg^{-1}) under conditions of varying ECa content in the soil (Table. 5).

4 Discussion

4.1 Dynamics of Ca content in plants and soil

With the grades of rocky desertification increased, the Ca content of soil increased. This indicated that soil Ca content was affected by the grade of rocky desertification. The mean soil ECa content was 1.46 g kg^{-1} in three rocky desertification areas, which was lower than the average ECa content in tobacco-planting soil in Hunan (3.548 g kg^{-1}) (Xu et al., 2007). The average ECa content in IRD areas was 3.09 g kg^{-1}, which was several times higher than the previously-reported ECa for non-limestone regions in China (Xu et al., 2007). The range of soil ECa content in the study areas is from 0.02(LRD) to 3.92 g kg^{-1} (IRD), with the maximum and minimum being lower than that of soil on Barro Colorado Island, Panama by Messmer et al. (2014). Tanikawa et al. (2017) revealed that concentrations of TCa and ECa were also low at the deeper horizons in the low-acid buffering capacity (ABC) soils, and differences in both organic layer thickness and soil chemistry could be a reason affecting Ca accumulation of low- and high-ABC stands. Our research shown soil mean TCa and ECa content were the lowest in LRD areas,and the difference of soil TCa and ECa may be caused by bedrock expose rate (the main chemical composition: $CaCO_3$) (Ji et al., 2009).

There was no significant difference in plant Ca content between aboveground or belowground parts ($p>0.05$) across the different grades of rocky desertification. This indicated that the grade of rocky desertification had no obvious effect on the Ca content of the aboveground and belowground parts of the plants studied herein. But the average Ca content of aboveground parts of plants (19.67 g kg^{-1}) was lower than that of Hunan flue-cured tobacco (21.93 g kg^{-1}) (Xu et al., 2007). The maximum and minimum Ca content of plant aboveground parts were 41.79 g kg^{-1} and 2.15 g kg^{-1} respectively, and the maximum and minimum Ca content of plant belowground parts were 40.14 g kg^{-1} and 0.42 g kg^{-1} respectively. The maximum Ca content of plant (41.79 g kg^{-1}) was found in the leaves which was lower than the Ca content of calcareous plants leaves with the maximum value of 85.13 g kg^{-1} by Luo et al. (2014). To most plant Ca content, the aboveground part was larger than the belowground part, and for a few plants Ca content, the aboveground part was lower than the belowground part (such as *Sanguisorba officinalis*, *Pyracantha fortuneana* and *Castanea henryi*), which was consistent with the findings of Wang et al. (2014).

4.2 Correlation between plant Ca content and soil ECa content

Our results showed that most plants had no correlation relationship between soil ECa and plant Ca excepting several plant (*Sanguisorba officinalis*, *Dendranthema indicum*, *Castanea henryi* and *Themeda japonica*) had a positive correlation between soil Eca and plant Ca content (Table. 4). But some study showed that Ca-rich soils caused cells to absorb more Ca than the cells themselves require (White and Broadley, 2003), and soil ECa content and leaf Ca content (Flue-cured Tobacco) had a significant positive correlation in pot experiment (Zou et al., 2010), which may be caused by species factors for the difference between our finds and their finds. The correlation between plant Ca content and soil ECa content reflects what extent soil Ca content influences plant Ca content, and may also reflect how different plants respond to differences in soil ECa content (Ji et al., 2009). The Ca content of *Sanguisorba officinalis* in the aboveground and belowground parts had a significant positive correlation (*p<0.01*) with soil ECa content, which indicated that *Sanguisorba officinalis* was affected greatly by soil ECa content. The Ca content of *Dendranthema indicum* (*p<0.05*) and *Castanea henryi* (*p<0.01*) in the belowground parts, showed a significant positive correlation (*p<0.01*) with soil ECa content, indicating that the belowground parts of these

species were also greatly affected by soil ECa content. The Ca content of *Themeda japonica* in the aboveground parts showed a significant positive correlation *(p<0.01)* with soil ECa content, which indicated that the aboveground parts of *Themeda japonica* was also greatly affected by soil ECa content. Two-way ANOVA of species and soil showed that the Ca content of the aboveground parts of 17 plant species was mainly affected by species factors, while the Ca content of the

5   belowground parts was affected by both species factors and the grade of rocky desertification, which was supported with data reported by Ji et al. (2009). The Ca content in the aboveground parts of nitrogen-fixing plants was significantly higher than that of the belowground parts. And this result indicated that nitrogen-fixing plants were the most efficient in the Ca upward transport, since the transport of Ca was mainly upward; which was not the same with those of Ji et al. (2009). Ji et al. (2009) revealed that dicotyledons were the most efficient at the upward transport of Ca. They used three types of plants (pteridophytes,

10   dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings. We found significant differences ($p<0.01$) between the aboveground and belowground parts in Ca content of monocotyledons in our study. However, Ji et al. (2009) revealed that no significant differences between the aboveground and belowground parts in Ca content of monocotyledons. This phenomenon may contribute the most of the monocotyledons sample plants were low-Ca plants. A significant difference was found between the aboveground and

15   belowground parts in monocotyledons, which may be because low-Ca plants maintain a lower Ca content in different grades of rocky desertification. In addition, the Ca content of monocotyledons was lower than that reported for monocotyledons (Ji et al., 2009), indicating that different individual monocotyledons showed differing abilities to absorb soil Ca.

4.3 High Ca adaptation of plants

The different plant functional groups revealed the differences in Ca content (Fig. 3). In some cases, even within the same plant,

20   there was an inconsistent correlation between Ca content in the aboveground and belowground parts and the soil ECa content. Collectively, these findings showed that not all plants adapted to soil high Ca environments in the same way, and that they exhibited a variety of adaptive mechanisms.

The aboveground parts of a plant represent the main site of its physiological activity. Thus, the Ca content in the

aboveground part reflects the Ca demand of the plant's physiological activity (Grubb and Edwards, 1982). The capacity of these plants which was able to adapt to high Ca soil environments can be reflected by two indicators: (i) the correlation between Ca content in the aboveground parts of the plants and soil ECa content; (ii) the species differences in terms of the Ca content of the aboveground parts of plants. Thus, based on the above two indicators, the plants were classified into the following groups: Ca-indifferent plants, high-Ca plants and low-Ca plants (Ji et al., 2009). In the present paper, we used this classification method to categorize our plants species, which were widely distributed across our study environments, thus providing theoretical guidance for vegetation restoration in rocky desertification areas. In both high-Ca and low-Ca soil environments, the Ca-indifferent plants can survival normally. And the Ca content of them changes correspondingly with the change of soil ECa content. The physiological activities of high-Ca plants had a higher demand for Ca and may have a strong ability to enrich soil Ca. The physiological activities of low-Ca plants had a lower demand for Ca and could alleviate high Ca stress by inhibiting the absorption of Ca through the root system and its upward transport.

These results are of great significance to the vegetation restoration in karst areas. High-Ca plants should be preferentially selected (such as *Pyracantha fortuneana*, *Rhus chinensis*, and *Loropetalum chinense*, *Serissa japonica*), followed by Ca-indifferent plants (such as *Sanguisorba officinalis*, *Castanea henryi*, and *Dendranthema indicum*). Low-Ca plants also have a strong adaption ability on high calcium environments, and it can be used as an alternative species to increase species diversity during the process of ecological restoration. Our findings not only have important guiding significance for solving the problem of rocky desertification in China, but also provide species screening ideas for the rocky desertification ecosystem restoration in other parts of the world. Rocky desertification is a major ecological problem in karst areas. It is necessary to further explore other nutrient elements in soil during vegetation restoration, and long-term positioning observation is crucial for the study of this issue.

5 Conclusions

Our results indicated that the mean soil TCa and ECa content were highest in areas of IRD, followed by MRD, and LRD. The Ca content in the aboveground parts of plants was significantly higher than that in the belowground parts for the three grades

of rocky desertification studied sites. Significant differences in Ca content were found between the aboveground and belowground parts of each plant functional group. The soil ECa content had a significant effect on the Ca content of the belowground parts of plants, but had no significant effect on Ca content of the aboveground parts. Ca-indifferent plants included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica*, had a significant positive

5   correlation existed between the Ca content in the aboveground or belowground parts and the soil ECa content. High-Ca plants in our study were *Pyracantha fortuneana, Rhus chinensis, Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. The aboveground parts of these plants were able to absorb a high Ca content from various of ECa content soils. Finally, low-Ca plants included *Abelia chinensis*, *Vitex negundo*, *Smilax china*, *Miscanthus sinensis*, *Artemisia carvifolia* and *Digitaria sanguinalis*. The aboveground parts of low-Ca plants were able to maintain a lower

10   Ca content under conditions of variable soil ECa content.

Acknowledgements

This work was supported by the Forestry Science and Technology Promotion Project of State Forestry Administration of China ([2014]52), and the Desertification (Rocky Desertification) Monitoring Project of State Forestry Administration of China (20150618 and 20160603).

References

Batistič, O., and Kudla, J.: Analysis of calcium signaling pathways in plants, Biochim. Biophys. Acta (BBA)-General Subjects., 1820, 1283–1293, 2012.

Borer, C. H., Hamby, M. N., and Hutchinson, L. H.: Plant tolerance of a high calcium environment via foliar partitioning and sequestration, J. Arid Environ., 85, 128–131, 2012.

Bose, J., Pottosin, I. I., Shabala, S. S., Palmgren, M. G., and Shabala, S.: Calcium efflux systems in stress signaling and adaptation in plants, Front. Plant Sci., 2, 85, 2011.

Bowler, C., and Fluhr, R.: The role of calcium and activated oxygens as signals for controlling cross-tolerance, Trends Plant Sci., 5, 241–246, 2000.

Bressan, R. A., Hasegawa, P. M., and Pardo, J. M.: Plants use calcium to resolve salt stress, Trends Plant Sci., 3, 411–412, 1998.

China, S. F.: Technology Regulations of Vegetation Restoration in Karst Desertification Zone (LY /T 1840-2009) China Standards Press, Beijing, 2009. (in Chinese with English abstract)

Ji, F. T., Li, N., and Deng, X.: Calcium contents and high calcium adaptation of plants in karst areas of China, Chinese J. Plant Ecol., 33, 926–935, 2009. (in Chinese with English abstract)

Grubb, P. J., and Edwards, P. J.: Studies of mineral cycling in a montane rain forest in New Guinea. III. The distribution of mineral elements in the above-ground material, J. Ecol., 70, 623, 1982.

Hepler, P. K.: Calcium: a central regulator of plant growth and development, Plant Cell, 17, 2142, 2005.

Hetherington, A. M., and Brownlee, C.: The generation of Ca signals in plants, Annu. Rev. Plant Biol., 55, 401–427, 2004.

Hirschi, K. D.: The calcium conundrum. Both versatile nutrient and specific signal, Plant Physiol., 136, 2438–2442, 2004.

Hong-Bo, S., Li-Ye, C., and Ming-An, S.: Calcium as a versatile plant signal transducer under soil water stress, BioEssays., 30, 634–641, 2008.

Ilarslan, H., Palmer, R. G., and Horner, H. T.: Calcium oxalate crystals in developing seeds of soybean, Ann. Bot., 88, 243–257, 2001.

Kinzel, H.: Calcium in the vacuoles and cell walls of plant tissue, Flora, 182, 99–125, 1989.

Larkindale, J., and Knight, M. R.: Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene, and salicylic acid, Plant Physiol., 128, 682–695, 2002.

5 Luo, X. Q., Wang, S. J., Zhang, G. L., Wang, C. Y., Yang, H. Y., and Liao, X. R.: Effects of calcium concentration on photosynthesis characteristics of two fern plants, Ecology and Environmental Science., 22, 258-262, 2013. (in Chinese with English abstract)

Sheng, M. Y., Xiong, K. N., Cui, G. Y., and Liu, Y.: Plant diversity and soil physical-chemical properties in karst rocky desertification ecosystem of Guizhou, China, Acta Ecol. Sin, 35, 434–448, 2015. (in Chinese with English abstract)

Marschner, H.: Marschner's Mineral Nutrition of Higher Plants, Academic Press, 2011.

10 Moore, C. A., Bowen, H. C., Scrasefield, S., Knight, M. R., and White, P. J.: The deposition of suberin lamellae determines the magnitude of cytosolic Ca elevations in root endodermal cells subjected to cooling, Plant J., 30, 457, 2002.

Müller, M. N., Ramos, J. B. E., Kai, G. S., Riebesell, U., Kaźmierczak, J., Gallo, F., Mackinder, L., Li, Y., Nesterenko, P. N., and Trull, T. W.: Phytoplankton calcification as an effective mechanism to alleviate cellular calcium poisoning, Biogeosciences., 12, 6493–6501, 2015.

15 Musetti, R., and Favali, M. A.: Cytochemical localization of calcium and X-ray microanalysis of Catharanthus roseus L. infected with phytoplasmas, Micron, 34, 387–393, 2003.

Messmer, T., Elsenbeer, H., and Wilcke, W.: High exchangeable calcium concentrations in soils on Barro Colorado Island, Panama, Geoderma., 212-224, 217-218(3), 2014.

Peiter, E.: The plant vacuole: emitter and receiver of calcium signals, Cell Calcium, 50, 120–128, 2011.

20 Pennisi, S. V., and McConnell, D. B.: Inducible calcium sinks and preferential calcium allocation in leaf primordia of Dracaena sanderiana Hort. Sander ex MT Mast.(Dracaenaceae), HortScience, 36, 1187–1191, 2001.

Poovaiah, B. W., and Reddy, A. S.: Calcium and signal transduction in plants, CRC Crit. Rev. Plant Sci., 12, 185–211, 1993.

Qi, Q. W., Hao, Z., Tao, J. J., and Kang, M.: Diversity of calcium speciation in leaves of *Primulina* species (Gesneriaceae), Biodiversity Science., 21, 715-722, 2013. (in Chinese with English abstract)

Ranjev, R., Thuleaua, P., and Schroederb, J. I.: Signal transduction and calcium channels in higher plants, Curr. Opin. Biotechnol., 4, 172–176, 1993.

Reuveni, R., Dor, G., Raviv, M., Reuveni, M., and Tuzun, S.: Systemic resistance against Sphaerotheca fuliginea in cucumber plants exposed to phosphate in hydroponics system, and its control by foliar spray of mono-potassium phosphate, Crop Prot., 19, 355–361, 2000.

Volk, G. M., Lynch-Holm, V. J., Kostman, T. A., Goss, L. J., and Franceschi, V. R.: The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in Pistia stratiotes L. leaves, Plant Biol., 4, 34–45, 2002.

Wang, C. Y., Wang, S. J., Rong. L., and Luo, X. Q.: Analyzing about characteristics of calcium content and mechanisms of high calcium adaptation of common pteridophyte in Maolan karst area of China, Chinese J. Plant Ecol., 35, 1061-1069, 2011. (in Chinese with English abstract)

Wang, S. J., Liu, Q. M., and Zhang, D. F.: Karst rocky desertification in southwestern China: geomorphology, landuse, impact and rehabilitation, Land Degrad. Dev., 15, 115–121, 2004.

Wang, Y. J., Yu, J. N., Chen, T., Zhang, Z. G., Hao, Y. J., Zhang, J. S., and Chen, S. Y.: Functional analysis of a putative Ca channel gene TaTPC1 from wheat, J. Exp. Bot., 56, 3051–3060, 2005.

Wang, H., Inukai, Y., and Yamauchi, A.: Root development and nutrient uptake, CRC Crit. Rev. Plant Sci., 25, 279–301, 2006.

Wu, W. H.: Plant Physiology, second edition, Science Press., 2008. (in Chinese with English abstract)

White, P. J., and Broadley, M. R.: Calcium in plants, Ann. Bot., 92, 487–511, 2003.

Wang, C. M., and Yi, Y.: Physiological activity and calcium content of calciphile, ubiquists and calcifuge under nature environment, Hubei Agricultural Sciences., 3840-3844, 53(16), 2014. (in Chinese with English abstract)

Zhang, X. Q.: Ecophysiological Characteristics of Calcicole and Calcifuge Responding to External Ca2+ Concentration, Guizhou Normal University., 2005. (in Chinese with English abstract)

Luo, X. Q., Wang, C. Y., Yang, H. Y., and Liao, X. R.: Studies on adaptive mechanisms of karst dominant plant species to drought and high calcium stress, Chinese Agricultural Science Bulletin., 28, 1–5, 2012. (in Chinese with English abstract)

Luo, X. Q., Zhang, G. L., Du, X. L., Wang, S. J., Yang, H. y., and Huang, T. Z.: Characteristics of element contents and

ecological stoichiometry in leaves of common calcicole species in Maolan Karst Forest, Ecology and Environmental Sciences., 1121-1129, 23(7), 2014. (in Chinese with English abstract)

Li, Y. Q, Deng, X. W., Yi, C. Y., Deng, D. H., Huang, Z. H., Xiang, W. H., Xi, F., and Jing, Y. R.: Plant and soil nutrient characteristics in the karst shrub ecosystem of southwest Hunan, China, Chinese Journal of Applied Ecology, 27, 1015–1023, 2016. (in Chinese with English abstract)

Jing, Y. R., Deng, X. W., Deng, D. H., Xiang, W. H., Wenhua, X., Fang, X., Li, Y. Q., and Zhang, S. L.: Soil properties and their correlations under different grades of rocky desertification ecosystems in Southwest Hunan, China, Journal of Soil and Water Conservation, 30, 189–195, 2016. (in Chinese with English abstract)

Tanikawa, T., Ito, Y., Fukushima, S., Yamashita, M., Sugiyama, A., Mizoguchi, T., Okamoto, T., and Hirano, Y.: Calcium is cycled tightly in *Cryptomeria japonica* stands on soils with low acid buffering capacity, Forest Ecology and Management., 399, 64-73, 2017.

Zeng, F. P., Peng, W. X., Song, T. Q., Wang, K. L., Wu, H. Y., Song, X. J., and Zeng, Z. X.: Changes in vegetation after 22 years' natural restoration in the Karst disturbed area in northwestern Guangxi, China, Acta Ecologica Sinica., 27, 5110–5119, 2007.

Zou, W. T., and Xiong, D. Z.: Effects of soil available calcium on some physiological metabolism of flue cured tobacco, Journal of Anhui Agricultural University., 369-373, 37(2), 2010. (in Chinese with English abstract)

Shang, Z. L., Mao, G. H., A., and Sun, D. Y.: The specificity of calcium signaling in plant cells, Plant Physiology Communications., 39, 93–100, 2003. (in Chinese with English abstract)

Zhou, J., Huang, Y., and Mo, M.: Phylogenetic analysis on the soil bacteria distributed in karst forest, Braz. J. Microbiol., 40, 827–837, 2009.

Xu, Z. C., Li, Y. Y., Xiao, H. Q., Li,H. W., and Liu, C. K.: The contents of ECalcium and magnesium in Hunan tobacco growing soils and their effects on tobacco quality, Acta Ecol. Sin., 27, 4425–4433, 2007. (in Chinese with English abstract)

Table 1. Basic description for different grades of rocky desertification sites

Sample areas	Score of rocky desertification	Aspect	soil pH	Gradient (°)	Altitude (m)	Bedrock expose rate	Vegetation coverage	Disturbance regimes
LRD	34(≤45)	South	5.56	20 °	500	35%	80%	Slight human disturbance, rarely grazing
MRD	48(46~60)	Northeast	5.57	18 °	500	57%	75%	Abandoned farmland, no disturbance after abandoning cultivation
IRD	67(61~75)	Southwest	5.59	17 °	480	73%	40%	Slight human disturbance, rarely grazing

LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification.

Table 2. Soil TCa and ECa content from different grades of rocky desertification.

Ca typical (g kg^{-1})	Sample location	LRD	MRD	IRD
TCa	Non-rocky side	1.19±0.45Aa	2.33±0.53Ba	2.62±0.97Ba
	Rocky side	1.68±0.53Ab	2.97±0.29Bb	5.66±1.37Cb
	Average	1.31±0.51A	2.53±0.56B	3.38±1.71B
ECa	Non-rocky side	0.51±0.26Aa	1.68±0.37Ba	1.63±0.88Ba
	Rocky side	0.97±0.39Ab	2.20±0.39Bb	3.09±0.58Cb
	Average	0.63±0.36A	1.83±0.44B	2.00±1.03C
Ca effectiveness	ECa/TCa (%)	47.72	72.55	58.98

Data represent mean ± standard deviation. Different lower-case letters in each column represent significant differences in different sample points within the same grade of rocky desertification. Different upper-case letters in each row represent significant differences between different grades of rocky desertification ($p < 0.05$).

Table 3. The main species of plant identified during this study and their important value (*IV*) in different grades of rocky desertification.

Vegetable layer	Species	Important Value (*IV*)		
		LRD(%)	MRD(%)	IRD(%)
Shrubs	*Abelia chinensis*	18.56	6.91	21.65
	Castanea henryi	22.33	1.35	5.32
	Indigofera tinctoria	5.10	16.64	4.30
	Pyracantha fortuneana	5.26	4.83	1.63
	Loropetalum chinense	-	1.00	10.45
	Serissa japonica	4.13	5.80	7.45
	Vitex negundo	4.85	11.38	19.07
	Rhus chinensis	0.84	7.11	2.24
	Smilax china	-	1.23	1.02
	Glochidion puberum	11.36	4.81	4.19
	Ilex chinensis	2.25	-	-
	Ilex cornuta	-	-	1.32
	Elaeagnus pungens	-	1.70	-
	Lespedeza bicolor	3.01	0.58	-
	Symplocos chinensis	2.07	-	1.57
	Broussonetia kaempferi	-	0.79	-
	Populus adenopoda	1.06	-	-
Herbs	*Miscanthus sinensis*	36.54	5.82	36.36
	Artemisia carvifolia	17.38	9.04	14.02
	Sanguisorba officinalis	1.41	1.01	2.14
	Themeda japonica	1.85	18.23	5.03
	Dendranthema indicum	3.82	16.94	6.55
	Digitaria sanguinalis	6.83	3.95	10.57
	Aster baccharoides	2.40	-	4.30
	Imperata cylindrica	-	3.30	-
	Salvia plebeia	-	-	0.81
	Patrinia scabiosaefolia	0.29	-	-
	Sonchus arvensis	-	-	0.51

"-" indicates that the important value (*IV*) of these species are less than 1.

Table 4. Correlations between the Ca content of 17 plant species and the soil ECa content of different rocky desertification areas.

Species	Ca content in aboveground parts			Ca content in belowground parts		
	Range (g kg^{-1})	Mean±SE (g kg^{-1})	Correlation coefficient	Range (g kg^{-1})	Mean±SE (g kg^{-1})	Correlation coefficient
Smilax china	5.77~36.35	18.5±12.24	0.302	3.11~8.61	5.89±2.75	0.931
Aster baccharoides	16.16~24.03	20.00±3.6	0.418	6.20~12.02	8.91±2.58	0.315
Vitex negundo	5.53~26.31	18.03±7.44	0.198	2.83~8.17	5.59±2.02	−0.116
Sanguisorba officinalis	17.68~27.77	24.01±4.47	0.995**	13.41~40.14	32.25±12.71	0.996**
Themeda japonica	2.15~9.23	5.51±2.45	0.963**	0.42~7.91	3.88±2.7	0.488
Pyracantha fortuneana	9.16~29.84	19.61±8.46	0.240	17.08~31.86	21.43±7.02	−0.189
Loropetalum chinense	10.33~33.44	27.25±7.29	−0.203	13.62~27.69	19.69±7.09	0.542
Serissa japonica	9.69~33.66	23.26±9.9	−0.027	4.27~20.51	12.01±7.81	0.838
Indigofera tinctoria	10.18~40.24	24.17±11.49	0.215	3.39~9.83	5.98±2.33	−0.289
Digitaria sanguinalis	4.75~9.8	6.67±2.73	0.257	1.36~5.33	3.37±1.98	−0.915
Abelia chinensis	5.07~29.64	18.08±10.12	−0.163	0.87~7.12	4.10±2.16	0.070
Artemisia carvifolia	15.34~19.39	17.37±1.42	0.400	6.39~14.07	9.18±3.07	0.028
Glochidion puberum	11.13~26.99	20.49±7.04	0.357	5.33~13.64	10.45±4.48	0.775
Miscanthus sinensis	4.34~7.6	5.61±1.44	0.000	2.88~13.1	5.82±4.87	0.118
Rhus chinensis	10.52~28.16	19.93±6.43	0.076	8.92~20.38	14.13±4.13	0.336
Dendranthema indicum	20.97~24.96	22.54±1.86	0.666	2.97~7.39	5.39±1.7	0.877*
Castanea henryi	12.99~38.74	22.4±8.17	0.151	20.52~31.37	25.28±3.92	0.963**

Coefficients are significant at $p < 0.05$ (*) and < 0.01 (**).

Table 5. Adaptation of plants to high Ca environments in rocky desertification areas.

Types of adaptation	Species	Characteristics of Ca content in plants	Strategies of plant adaptation to high Ca environments
Ca-indifferent plants	*Sanguisorba officinalis* *Castanea henryi* *Dendranthema indicum* *Themeda japonica*	There is significant positive correlation between the Ca content in the aboveground/belowground parts of plants and the soil ECa content. The coefficient of variation for Ca content in plants has a wide range.	Plants adapt to different Ca contents in soil through high Ca^{2+} buffering capacity. By regulating Ca^{2+} binding in Ca stores, the Ca^{2+} concentration in cytoplasm is maintained at a stable level.
High-Ca plants	*Loropetalum chinense* *Serissa japonica* *Indigofera tinctoria* *Glochidion puberum* *Aster baccharoides* *Pyracantha fortuneana* *Rhus chinensis*	There is no significant positive correlation between the Ca content in the aboveground parts of plants and the soil ECa content. The aboveground part has a high level of Ca content and the coefficient of variation falls within a narrow range.	Plants maintain high Ca content by enhancing Ca uptake and transporting it from belowground to aboveground parts. High Ca is needed or tolerated in these plants.
Low-Ca plants	*Vitex negundo* *Abelia chinensis* *Smilax china* *Miscanthus sinensis* *Artemisia carvifolia* *Digitaria sanguinalis*	There is no significant positive correlation between the Ca content in the aboveground parts of plants and the soil ECa content. The aboveground part has a low level of Ca content and the coefficient of variation falls within a narrow range.	Plants maintain low Ca content in the aboveground parts by reducing Ca uptake and transporting it from belowground to aboveground parts.

Figure captions

Fig. 1 Geographical locations of the study sites.

Fig. 2 Characteristics of plants Ca content in different grades of rocky desertification. LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification. Different lower-case letters represent significant differences in the Ca content between the aboveground and belowground parts of the plants in the same grade of rocky desertification; different upper-case letters represent significant differences in the Ca content of the plants among the different grades of rocky desertification ($p<0.05$).

Fig. 3 Ca content in the aboveground and belowground parts of plants in different functional groups of plans. Different lower-case letters represent significant differences between the Ca content of the aboveground and belowground parts for the same functional groups ($p<0.05$); different upper-case letters represent significant differences among different functional groups, ($p<0.05$).

Fig. 4 Ca content in the aboveground and belowground parts of different plant types from three different rocky desertification sample areas. LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification. Values with the same letters were not significantly different ($p>0.05$).

Fig. 1

Different grades of rocky desertification

Fig. 3

Ca content of different plants functational groups (g kg^{-1})

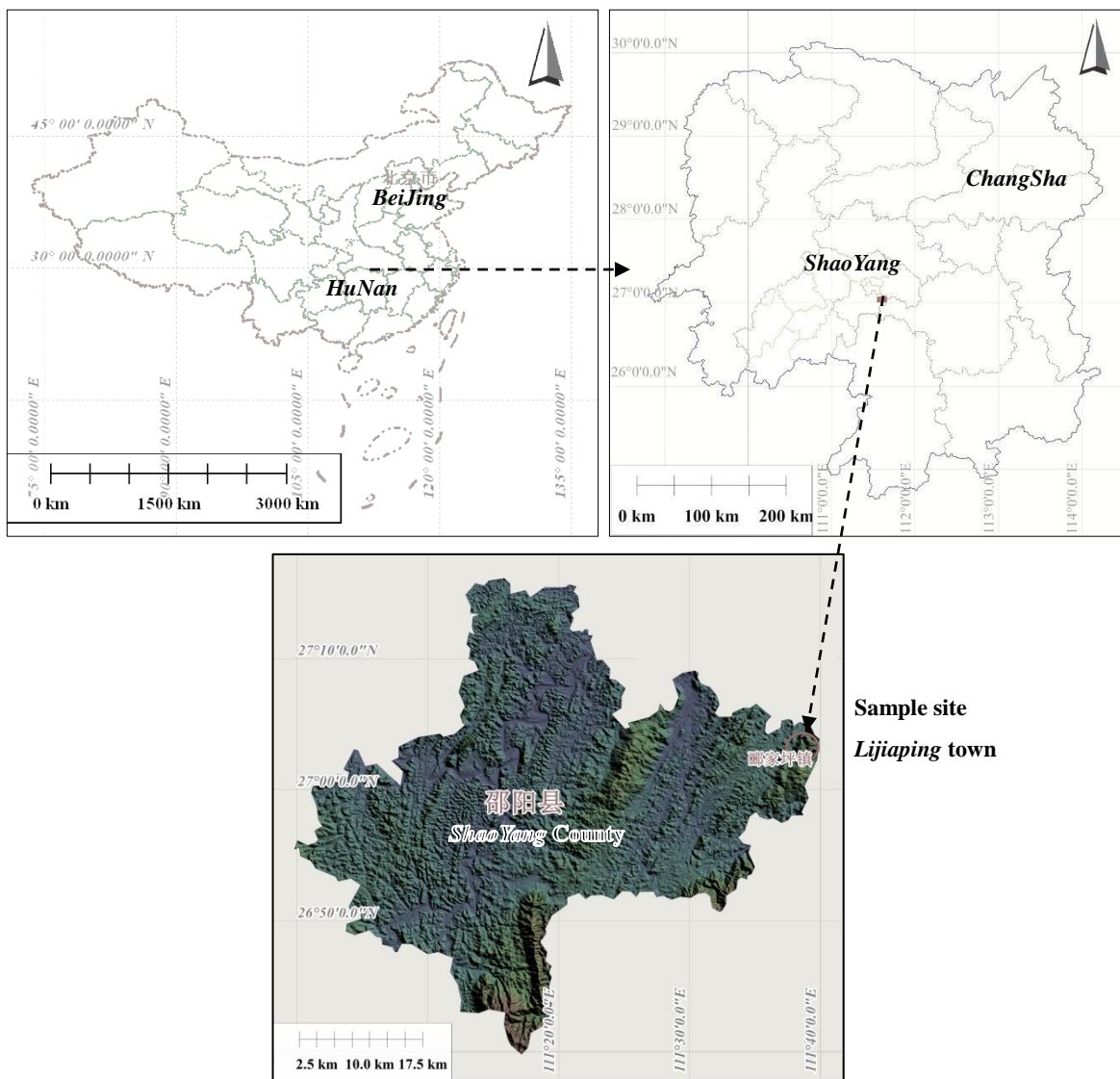
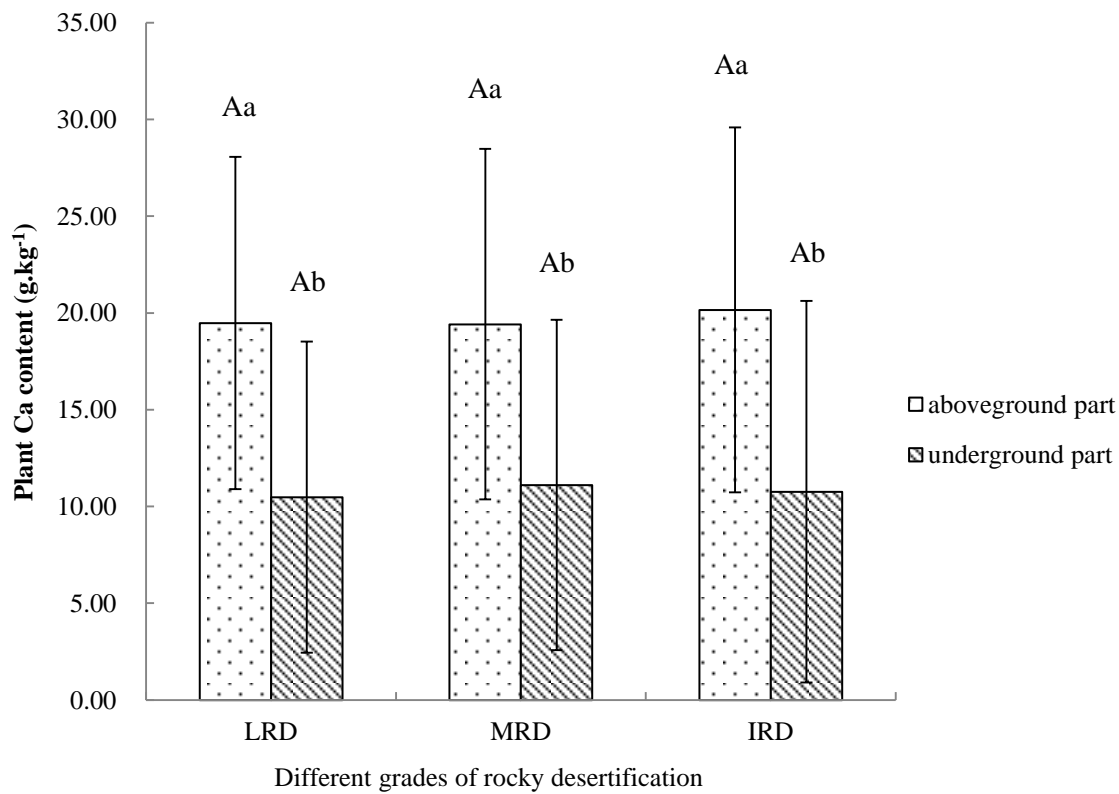
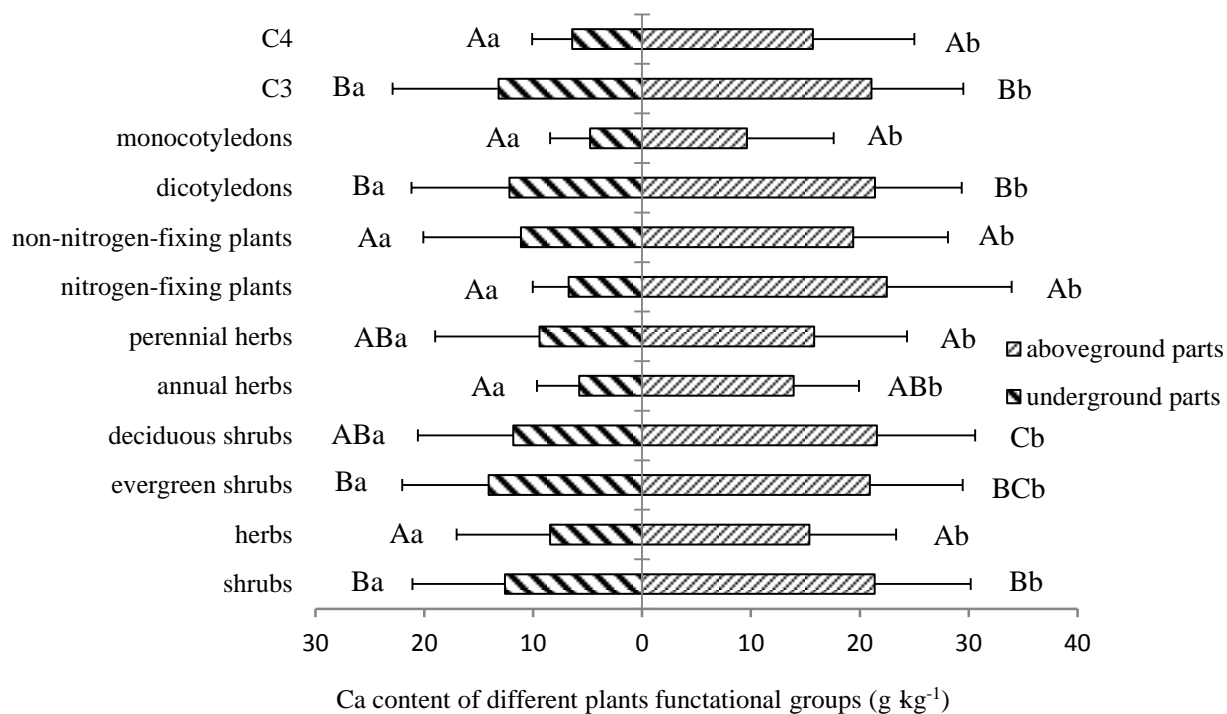
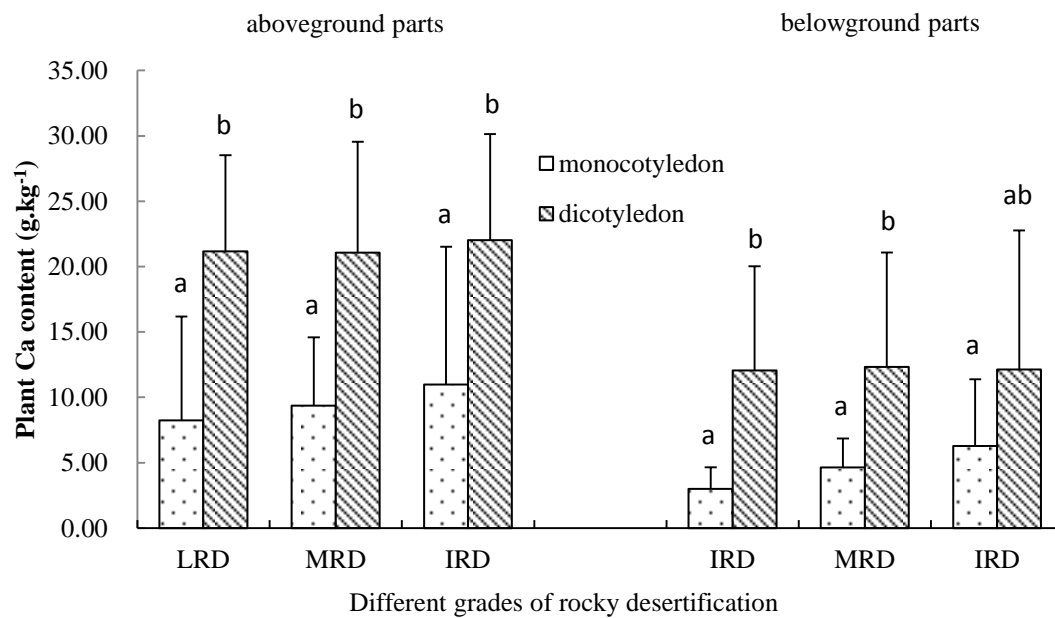



Fig. 4

Author contributions

Idea and study design: Deng X. W., Wei X. C.; Experiments and statistical analysis: Deng X. W., Wei X. C., and Wen H. F.; Manuscript writing: Wei X. C.; Discussion and revision: Xiang W. H., Ouyang S., Lei P. F., Chen L. All authors have read and approved the content of the manuscript.

5 Competing interests

The authors declare that they have no conflict of interest.