Response to the reviews, list of all relevant changes, and marked-up manuscript

Part 1: Point-by-point response to Reviewers' Comments

We highly appreciate the valuable comments and suggestions by the editor and two anonymous reviewers on our manuscript. We have attempted to address each point raised by the editor and the reviewers. The following is our detail responses we have made, with reference to the order of the comments by the editor and the reviewers.

Reviewer 1:

1. Abstract:

P1 L13 removes the sentence “However, the Ca2+ dynamics of plants and soil are not well understood” into the P4 L15
P1 L14 deletes the s from the “samples”
P1 L16 “slop” should be slope
Reword all words “underground” into belowground in the entire manuscript

Re.1 Thanks for the positive suggestion. And sorry for the mistakes to write "slope" as "slop" and "belowground" is more suitable for our purposes. We have corrected all of them in the entire manuscript.

2. Introduction

P2 L2: use provides to replace “can provide”
P2 L8: delete “Of course”
Change the sentence “the severity of rocky desertification in Hunan Province was ranked fourth (Li et al., 2016) into； The severity of rocky desertification was ranked in fourth in Hunan Province of China (Li et al., 2016)
P2 L9: Insert the “Rocky desertification is an extreme form of land degradation in karst areas, and 10 has become a major social problem in terms of China's economic and social development (Sheng et al., 2015)” should be before ”The severity of rocky desertification was ranked in fourth in Hunan Province of China (Li et al., 2016)”

Re.2 Thanks, we agree to these advices and the manuscript was revised, accordingly.

P2 L110-12: Change the “The restoration and reconstruction of rocky desertification ecosystems has become the immediate focus of agro-forestry production environment improvements, regional economic development and helping to support people out of poverty (Jing et al., 2016)" into "The restoration and reconstruction of rocky desertification ecosystems have become the urgent environment improvements, regional economic development by using agroforestry system and helping to support people out of poverty (Jing et al., 2016).

P2L13 "soil with high Ca"

Re.3 Thanks, it is a good suggestion.

P2L14-15: rewrite "From the origin of rocky desertification, 15 the restoration of vegetation is key to the process of remediation (Wang et al., 2004). Consequently, the screening of plants which can grow successfully in high- calcium environments is an extremely critical step."

Re.4 Thanks, We have rewrote the two puzzling sentences to be:

"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."

P3L2: Change "Ca2+ and pectin in the cell walls of plants combine" into: "Ca2+ combine with pectin in the cell walls of plants"

P3L11: change" than cannot" into "not"

P4L3-L4 "Ji et al. (2009) revealed that 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." should be: "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).

P4L7-L19 "These results indicate that there are differences in soil Ca content between different areas and that there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. Collectively, these differences lead to different degrees of adaptability of plants to high Ca environments." Should be "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."

P4L10-L11 delete "there is a scarcity of extensive research into" should be" the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas and the Ca2+ dynamics of plants and soil are not well understood.

P4L14: delete "In order to", capitalize the "To"

P4L15 "we did the following:" should be "the following investigations were explored"

Re.5 Thanks for the constructive suggestions! And we have corrected all of them.

3. Materials and methods

P5: site description is too simple, should add more information regarding to the study, e.g. slope, soil pH, soil properties, and vegetation cover

Re.6 Thanks, We have corrected it. In order to make site description more detailed, we added a table.

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

P5L4 title “Data collection” should be “Experimental design and data collection”

P5L6 delete “period. These four main indices”

P5L11: “We conducted a detailed survey of the three sample areas and collected samples in October 2016.” Should be “The sample collection in these three sample areas were conducted in October 2016. “

Re.7 Thanks, Very good suggestions! Done.

P5L13: use “Within” to replace the “For”

P5L14: add were set up after “(upper, middle, and lower slope)”

P5L14-L15: add (3x4x3) were set up before the “for analysis”, delete “We chose to study” and “the common plant species of the region, 15 and gathered plants using the whole plant harvest method. In each small quadrat, every kind of shrubs and herbs are collected.” Should be “The common plant species of the region were gathered using the whole plant harvest method in each small quadrat as well as all shrubs and herbs are collected.”

P5L17: “heated” should be “oven tried’

Re.8 Thanks for your suggestions. We have corrected all of them.

P5L18: add “and after” after the de-enzyme”

P5L18: not clear, “constant weight at 80oC, L 17 you mentioned 105oC, why?

Rewrite it

P5L18: delete “and bagged” and add “chemical” before the “analysis”

Re.9 We add “and then” after the de-enzyme.

The 105°C is in order to de-enzyme, and the time does not need too long (only 15minutes). The 80°C is designed to make samples complete dehydration and it take a long time, but excessive temperature will carbonize the sample. And this sentence should be: Plant samples were taken back to the laboratory, rinsed with distilled water before being oven tried at 105°C for 15 min to de-enzyme, and then dried to a constant weight at 80°C for about 480 minutes, crushed and passed through a 0.149 mm sieve, for later chemical analysis.

P5L30: delete “Finally” add “were sampled”

Re.12 Thanks, “Finally” was deleted, but “were sampled” cannot be added.

This sentence should be: “Soil TCa, ECa content and plant Ca content were measured using an Atomic Absorption Spectrophotometer (3510, Shanghai, China).”

P6L5: “biennial herbs, while ‘deciduous shrubs’ included deciduous trees with a height less than 2 m or a ground diameter less than 3 cm.” not clear, rewrite

Re.13 In the small quadrats, there were very few biennial herbs, as a result, we gathered them to the ‘Annual herbs’. We rewrite this sentence as:

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’.

P6L8-L9: “One-way analysis of variance (ANOVA), Two-way ANOVA and Pearson correlation analysis ($\alpha = 0.05$) were used to analyze the Ca content of soil and plants within and between different grades of rocky desertification.” Not clear, rewrite it

Re.14 Sorry for the confusing, We have rewrote it as:

“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.”

4. Results

P6L13: add in soil after “The mean TCa content”

P6L14: Use “location” to replace “points”

P6L15: delete “Furthermore”, and add “The”, and to use “that” replacing “to be”

P6L17: Add “Ca content” after: ”average”, and use “the” to replace “with”

P6L21: Use “Total” to replace “The”

P7L1: use “.” To replace “,”, and then use “Compare to” to replace “while”

P7L3: delete “when compared across”, to use” throughout”

P7L4: “aboveground” add “and belowground”, and delete “or that of underground parts, there”

P7L5: Delete the whole sentence “Furthermore, the grades of rocky desertification had no obvious effect on the Ca content of the aboveground and underground parts of the plants generally (Fig. 2).”

P7L8: “The 41 plant species were identified in the 36 small quadrats; these plants were divided into different functional groups” should be “The 41 plant species were identified and were divided into different functional groups in the 36 small quadrats.”

P7L8-L9: delete “For each functional group,” add “The” before Ca

P7L9-L10: “Ca content between the aboveground and underground parts were significantly different ($p < 0.05$), and 10 the Ca content of the aboveground parts was higher than that of the underground parts ($p<0.05$)” should be " The Ca content of the aboveground parts significantly was higher than that of the belowground parts in each group ($p<0.05$)”

P7L15-L16: “In terms of life form functional groups, shrubs showed a significantly higher Ca content, both aboveground and underground than herbs ($p<0.05$)” should be “In life form functional groups, shrubs showed a significantly higher in Ca content than herbs in both aboveground and underground ($p<0.05$)”

P7L18: “The aboveground and underground Ca content of dicotyledons” should be”
The Ca content of dicotyledons in aboveground and belowground parts”

Re.15 Thank you for these suggestions. We have corrected all of them.

P7L21-23: “In terms of monocotyledons and dicotyledons, further analysis revealed no significant differences in the Ca content of the aboveground parts when compared between the different grades of rocky desertification; this was also true for the Ca content of the underground parts.” Its not clear, rewrite it

Re.16 Sorry for the confusing, We have rewrote it as:

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.

P7L23: Delete “The Ca content of both the aboveground and underground parts of monocotyledons was always low while those of dicotyledons were always high”

Re.17 Thanks. Done !.

P8L1-2 “The Ca content of dicotyledons was significantly higher than those of monocotyledons across” should be “The Ca content of dicotyledons in both of was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout”

Re.18 Yes. But we think this sentence should be: The Ca content of dicotyledons was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout.

P8L3: “For the 41 common plants collected, 17 plant species (which exist in each sample area) were widespread throughout the southwestern rocky desertification areas of Hunan.” Should be “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.”

P8L5: Delete “For each of t”, capitalize “T”

P8L3: use were calculated replace “we calculated”

P8L5: Delete “. These plants were common species in the local area”

Re.19 Thanks for the constructive suggestions! We have corrected them.

P8L5-“We carried out two-way ANOVA for both species and soil for these 17 plants to determine differences in plant Ca content” should be moved to the data analysis part, not in the results part

Re.20 Yes. We have moved it to the data analysis part. (See Re. 14).

P8L6: Delete “. The soil was graded into three categories: LRD, MRD and IRD.”

P8L7: Delete “df=16, F=11.277”

P8L8: Use “related among” to replace “significant among the different

P9L9: (df=2, F=2.299, p=0.117)

P8L9: “The” Ca not For “Ca”, delete “differences”

P8L10: Use “among the species”, delete “(df=16, F=8.543, p<0.01)”, and delete “but also among the different” and it throughout all the” , and delete “df=2, F=4.104,”

Re.21 Thanks. We have corrected them.

P8L12-13: “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" this sentence should not in results part, may be in discussion section.

Re.22 Yes. We have put it in the discussion section (4.2).

P8l13: Too many "For this" and "In terms of", delete them.

Re.23 Agree. And we have deleted them.

P8L15: "which indicated that Sanguisorba officinalis was affected greatly by soil ECa conten" should be not in the results section.

P8L17: "indicating that the underground parts of these species were also greatly affected by soil ECa content." should be not in the results section.

P8L19" which indicated that the aboveground parts of Themeda japonica was also greatly affected by soil ECa 20 content" should be not in the results section

Re.24 Thanks. We have put them in the discussion section (4.2).

P9L2: Delete "kinds of"

P9L3: "and were also the representative species that are able to adapt to a high Ca soil environment." How do you know it? Suggest to delete it

Re.25 Agree. And done!.

P9L6-9: "The capacity of these plants which are 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, we classified these plants into the following groups: Ca-indifferent plants, high-Ca plants and low-Ca plants (Ji et al., 2009)." This should be moved to the discussion section. Results just present your results, no explanation and citation.

Re.26 Thanks. we have put it in the discussion section (4.3).

P9L10: The definition "Ca-indifferent plants" is it correct?

Re.27 Yes. It is correct.

P9L12:" The Ca content of these plants increased or decreased correspondingly with increases or reductions in soil ECa content, but plant growth was not affected by such changes." Not clear, rewrite it

Re.28 Yes. This sentence was rewrote and was moved to 4.3. This sentence was:

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.

P9L17: “High-Ca plants”, refer it “high Ca demand plants

Re.29 Yes. But High-Ca plants, Low-Ca plants and Ca-indifferent plants were used as terminology. See Ji, F. T., 2009.

P9L20: “Moreover, the physiological activities of these plants had a higher demand for Ca and may have a strong ability to enrich soil Ca.” should be in the discussion part

Re.30 Yes. We have put it in the discussion section (4.3)

P9L21: “Low-Ca plants” should be Low Ca demand plants

Re.31 Yes. The same as Re. 29.

P9L23: Why do you use “19g/kg” as the boundary to determine the low or high Ca demand plants?

Re.32 Mainly according to the data of calcium content of plant (from 0.42 to 41.79 $g \cdot kg^{-1}$) and refer to the relevant references(See Ji, F. T., 2009).

P10L2-5: the whole paragraph should belong to the discussion, not in the results. Again, the results should just present your results, do not need any explanation in this part, any explanation and citation should be in the discussion section.

Re.33 Thanks. We have put it in the discussion section (4.3)

5. Discussion

P10L9: delete “The aboveground parts of plants had a higher Ca content than the underground 10 parts, although”

P10L10: Capitalize T (The), delete when compared

P10L17: period after the (2014), and then separate the paragraph

Re.34 Thanks .

For the first two questions, we deleted “The aboveground parts of plants had a higher Ca content than the underground parts, although there was no significant difference in plant Ca content when compared between

aboveground or underground 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 underground parts of the plants studied herein.

For the third question, we did not separate the paragraph

P10L18: What is the ABC soil?

Re.35 Thanks. the ABC is a shorthand (full name: acid buffering capacity).

P10L18: “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 pointed to differences in both organic layer thickness and soil chemistry as a reason for affecting Ca accumulation of low- and 20 high-ABC stands” is unclear, rewrite it

Re.36 We have rewrote it. This sentence should be: “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”.

P11L1: Add “compared to the aboveground and belowground Ca content in our study,” before the “The”, and then use lowercase of “t”

P11L3: “,” should be “.”

Re.37 Thanks . We did not added “compared to the aboveground and belowground Ca content in our study,”. The next sentence should be: 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 is lower than the Ca content of calcareous plants leaves with the maximum value of 85.13 g·kg^{-1} by Luo et al. (2014).

P11L1-4: “The maximum and minimum calcium content of plant aboveground parts were 41.79 g·kg-1 and 2.15 g·kg-1 respectively, and the maximum and minimum calcium content of plant underground parts were 40.14 g·kg-1 and 0.42 g·kg-1 respectively, Which is lower than the calcium content of calcareous plants leaves (maximum 85 .13 g·kg-1 ,minimum 6.26

g·kg-1) by Luo et al. (2014).” Aboveground includes leaves and branches, how do you compared with leaves only? Ca presents the Calcium, should keep the constant in the manuscript.

Re.37 Sorry for the confusing. This sentence should be:

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).

P11L9: The beginning of the paragraph should present your research results pattern first, and then discuss and explain it.

P11L11: Use “had a” to replace “was extremely”. Use “.” and delete “and” to separate the sentence. The sentence “our results showed several plants (*Sanguisorba officinalis, Dendranthema indicum, Castanea henryi and Themeda japonica*) and soil Eca content was a positive correlation, but most plant calcium content and soil ECa content was not relevant.” Should be “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.”

Re.38 Thanks . We have corrected it.

P11L14: what are “species-related factors,”? Do you mean plant species physiological factors?

Re.39 No. ‘species-related factors’ refers to ‘species factors’.

P11L15-16: “was in accordance with data reported previously by Ji et al. (2009).” should be “was supported with data reported by Ji et al. (2009).”

Re.40 Yes and thanks. Done !

P11L17: “Since the transport of Ca was mainly one-way (upward), this result indicated that nitrogen-fixing plants were the most efficient in terms of the upward transport of Ca, and that Ca was mainly concentrated in the aboveground parts of the plant; these findings were not consistent with those of Ji et al. (2009).” Is not clear, rewrite it.

Re.41 Sorry. We have rewrote it.

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 ......

P11L19: delete “In the paper”

P11L20: “.” after Ca, The sentence” and studied only three types of plants (pteridophytes, dicotyledons, monocotyledons) that did not include nitrogen-fixing plants, which may be the reason for the inconsistency of this previous data with our current findings.” Should be” They used three types of plants (pteridophytes, dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings.”

P11L22-P12L1: delete “in terms of”, the sentence “In terms of the Ca content of monocotyledons, we found significant differences ($p<0.01$) between the aboveground and underground parts, but the study by Ji et al. (2009) revealed that these differences were not significant. This may be because most of the monocotyledons collected were low-Ca plants.” should be” 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 demand plants.”

Re.42 Thanks. We have corrected them.

P12L2-3: “Owing to the fact that the aboveground parts of low-Ca plants maintain a lower Ca content for different grades of rocky desertification, a significant difference was found between the aboveground and underground parts in monocotyledons. In addition, the Ca content of monocotyledons was lower than that reported for monocotyledons (Ji et al., 2009),” is not clear, rewrite it

Re.43 Thanks. We have rewrote it as: “A significant difference was found between the aboveground and 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)”.

P12L7-8: “Over the past decade, 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 (Müller et al., 2015). “ may move to the introduction section.

Re.44 Yes, We have put it in the introduction section.

P12L8-P13L14: again, authors should present the results pattern at the beginning of the discussion to explain your results.

This paragraph should be rewritten. Lots of “in terms of” showed in the manuscript, delete them. In this paragraph, I did not see any results at the beginning of the discussion. The discussion is used to explain the results

Re.45 Thanks. We have deleted it, and added some content.

P13L15-22: suggest deleting the paragraph because it does not make sense in your discussion, as well as so many times to cite the literature Ji et al (2009)

Re.46 Thanks . We have deleted it.

6. Conclusions

P13L5 “Conclusions”

P1323-P14L1: delete “followed by” add “and” before “LRD”

P14L1-2: delete the sentence” Significant differences were detected for both soil ECa and TCa content when compared between the rocky side and non-rocky side of each grade of rocky desertification areas. “

P14L3: add “sites” after “studied”, delete “Furthermore”

P14L5: Delete “($p<0.05$)

L14L6； Delete “the” Ca

Re.47 Thanks. We have deleted them.

P14L6: Ca-indifferent” is correct?

Re.48 Yes. Thanks.

P14L7: “,” after “Themeda japonica”, delete “For these plants,” and put had

P14L8: “High-Ca plants included Pyracantha fortuneana,” should be” High-Ca plants in our study were Pyracantha fortuneana,

P14L10: delete “In this case”， the sentence “the aboveground parts of these plant were able to maintain a higher Ca content under conditions of variable soil ECa content. ”should be” the aboveground parts of these plant were able to absorb a high Ca content from various of ECa content soils.

Re.49 Thanks. We have corrected them.

Reviewer 2:

1. General comments

The authors' manuscript "Calcium content and high calcium adaptation of plants in karst areas of southwestern Hunan, China" introduced an investigation and an analysis of the relationships between the degree of rocky desertification and calcium content in soil and plant. The author's results are interesting. These results can be seen as a valuable reference, which could be helpful in related research works to screen plant species for vegetation restoration in karst areas of China. As for the study itself, this paper is worthy to be published in the journal "Biogeosciences". However, this manuscript needs a major revision before publication, especially revisions in paper structure and writing quality.

Re.1 Thanks for the positive comments.

2. Specific comments

Because there is no line number in the manuscript that I downloaded, I suggest the authors put numbers for each line in the revised version if the manuscript goes to the next stage. Here I provide my comments for the sections.

(1) For "Introduction"

In the section of Introduction, I cannot find sufficient reviews or introductions of existing studies on the issue addressed by the authors' study. There are only two existing studies (Zhang 2005; Ji et al., 2009) mentioned in this section. The study background need be introduced more in this section. I wonder if the authors can provide a brief review for this issue including some important related studies reported for other countries. Readers may want to know whether the authors' hypothesis, "the dynamics of Ca content is significantly affected by the grade of rocky desertification", is supported by more studies or not.

The second, third, and fourth paragraphs are lengthy in describing the knowledge of plant physiology. I suggest shortening them to several sentences for outlining some key processes.

Re.2 Thanks for the suggestion. We have added 3 references in this section. To the second, third, and fourth paragraphs. We have restructured and shorted these paragraphs.

(2) For "Results"

The major problem is that there are lots of explanations and analyses in this section, especially in section 3.2.2 and 3.4. Of course, for a better understanding, it may be ok to arrange a few explanations in "Results". But any analysis should not appear in the "Results". Otherwise, this section can be "Results and discussions".

Re.3 Thanks, this is a very good suggestion. We have moved all sentences with explanations to the discussion section (4.3). In addition, we also modified the section 3.3.

(3) For “Discussions”

Overall, there are still too much knowledge descriptions of plant physiology. I think that only the necessary knowledge should be mentioned corresponding to the new findings, instead of a detailed introduction of knowledge. I would rather see that the discussions focus on the main points and your hypothesis, or analyze your main work, for example, the three parts of your work: (i) to measure the soil Ca contents; (ii) to compare between above- and below-ground parts of plants; and (iii) to analyze correlation between Ca in plants and soils.

Re.4 Thank you for your constructive suggestions. And done accordingly!

For section 4.1:

According to the text, the authors may discuss their works (i) and (ii) in section 4.1. However, section 4.1 seems not well organized. Actually, the results have already shown the dynamics of soil Ca, and the difference between above- and below-ground parts of plants. I hope the section 4.1 can summarize these two works clearly, and can indicate some new findings. In addition, for readability, readers may need to know percentages of your measurements to the values reported by other studies. I also suggest the authors consider changing the title from “Dynamics of Ca content in plants and soil” to “Dynamics and ranges of Ca content in plants and soil” since most parts in this section are talking about the “ranges”.

Re.5 Yes. We have changed the 4.1 title to “Dynamics and ranges of Ca content in plants and soil”. The contents have also been reorganized, and the content was divided into two paragraphs. The first and second paragraphs discussed soil Ca content and plant Ca content, respectively. Thanks.

For section 4.2:

The authors’ work (iii) was discussed in this section. It seems to me, the content needs to be reorganized very logically. Section 4.2 lists some results and other researchers’ conclusions, however, the logical relationships between those results and conclusions are not clear. For example, the first sentence states “The Ca2+ content in plant cells was proportional to soil Ca2+”. Then what parts of plants are you talking about? Aboveground, below-ground, or whole tree? The second sentence states “Calcium-rich soils caused cells to absorb more calcium than the 10 cells themselves require (White and Broadley, 2003)”. Then is this cited sentence for supporting the first sentence, just explaining the cause, or conducting the third sentence? A conjunction word seems necessary.

The third sentence states “Zou et al. (2010) showed that soil ECa content and leaf calcium content [were] extremely significant positive correlation”. Is this conclusion cited for comparing your results? If so, it would be better to indicate the plant name(s). Your next statement is “our results showed several plants (......) and soil Eca content was a positive correlation, but most plant calcium content and soil ECa content was not relevant”. Zou et al. focused on the leaf, but, do you focus on whole tree? The results and comparisons need to be explained more clearly and logically to avoid reader’s confusion.

These above writing issues are raised just for example. The whole section needs to be rewritten for better readability

Re.6 Thanks. We have rewrote the section 4.2.

(1) To the first sentence, we are sorry for the confusing sentences. And it was rewrote.

(2) The second sentence is in order to compare to our results (the next sentence). We have added a conjunction "but", and put the next sentence (our results) in front of this sentence. Furthermore, analyze the reasons that leading to different results.

(3) To the third sentence, this conclusion is for comparing our results, the plant is "Tobacco", and we have added the plant name in the manuscript. We analyzed the correlation between plant and soil calcium content, and our plant was divided into aboveground and underground parts (see Table. 3).

In addition to the three sentences mentioned above, other problems have also been revised.

For section 4.3:

This section discusses the most important scientific issue (High calcium adaptation of plants) addressed in the study. The solution of this issue may provide useful guidance on vegetation restoration. However, basic knowledge descriptions take up lot of space. I had liked to see the discussions on: (a) Based on study results for the 17 selected species, what are the primary characteristics for each of the three categories (Ca-indifferent plants, high-Ca plants and low-Ca plants)? (b) What should the screening of plant species notice in the vegetation restoration? (c) What are the application prospects in solving the problem of land degradation using the authors' results in karst areas? (d) Is there any unsolved issue, related with this study and remained for further research?

Again, results are interesting and helpful for associated studies. I suggest accepting this manuscript after a major revision. The writing quality should be improved, including a spelling check. As I am not a native English speaker, I will not suggest more regarding language. Good luck!

Re.7 Thanks you for your advice, and we have deleted lots of basic knowledge descriptions, only a small part reserved.

Re. to the question (a): We have added the primary characteristics of three categories in 3rd paragraph.

Re. to the question (b): We added the corresponding content in the fourth paragraph.

Re. to the question (c): 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 areas all over the world. And we have added this content in the forth paragraph.

Re. to the question (d): Thanks. It is necessary to further explore other nutrient element in soil during vegetation restoration, and long-term positioning observation is crucial for the study of this issue. We added this new content in the fourth paragraph.

Part 2: A list of all relevant changes made in the manuscript:

1. P1L13: Removes the sentence "However, the Ca2+ dynamics of plants and soil are not well understood" into the P5 L14.
2. P1L16: deleted the" s" from the "samples"
3. P1L16: changed "slop" into "slope"
4. P2L2: changed "can provide" into "provides"
5. P2L8-10: inserted the "Rocky desertification is an extreme form of land degradation in karst areas, and 10 has become a major social problem in terms of China's economic and social development (Sheng et al., 2015)" after "……in China are mainly distributed in southwestern areas.", and deleted P2 L11-102 this sentence "Rocky desertification is an extreme form of land degradation in karst areas, and 10 has become a major social problem in terms of China's economic and social development (Sheng et al., 2015)"
6. P2L10: deleted "Of course"
7. P2L10-11: changed the sentence "the severity of rocky desertification in Hunan Province was ranked fourth (Li et al., 2016)" into "The severity of rocky desertification was ranked in fourth in Hunan Province of China (Li et al., 2016)"
8. P2L12-14: changed the "The restoration and reconstruction of rocky desertification ecosystems has become the immediate focus of agro-forestry production environment improvements, regional economic development and helping to support people out of poverty (Jing et al., 2016)" into "The restoration and reconstruction of rocky desertification ecosystems have become the urgent environment improvements, regional economic development by using agroforestry system and helping to support people out of poverty (Jing et al., 2016)
9. P2L16: inserted "with" between "Soil" and "high Ca"
10. P2L17-20: "From the origin of rocky desertification, the restoration of vegetation is key to the process of remediation (Wang et al., 2004). Consequently, the screening of plants which can grow successfully in high- calcium environments is an extremely critical step." was modified to "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."
11. P2L21-23: "Ca^{2+} is one of the most essential nutrients needed for the regulation of plant growth and also plays a central role in helping plants overcome environmental stress (Hepler, 2005)" was modified to "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)"

12. P3L1-4: deleted "A low cytosolic Ca^{2+} concentration is crucial for appropriate cell signaling (Müller et al., 2015). At the same time, Ca^{2+} is a versatile plant signal sensor under conditions of soil water stress (Hong-Bo and Ming, 2008). In addition, Ca^{2+} as a second messenger in the process of cell signal transduction, which plays a key regulatory role in how plants respond to environmental changes (Poovaiah and Reddy, 1993; Batistič and Kudla, 2012)."
13. P3L6-7: deleted "However, high Cacalcium stress can exert influence over the photosynthetic and growth rate of plants (Ji et al., 2009; Hui et al., 2003)"
14. P3L7-8: changed "Ca^{2+} and pectin in the cell walls of plants combine to form pectin Calcium" into: "Ca^{2+} combine with pectin in the cell walls of plants to form pectin Ca"
15. P3L11: inserted "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 (Müller et al., 2015)." after "Mechanisms of plant defense to high soil Ca^{2+} concentrations:"
16. P3L14: inserted "; Larkindale and Knight, 2002" before "Borer et al., 2012"
17. P3L14-15: changed "Plants can be adapted to high salt environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998)" into "Plants can be adapted to high salt, drought and high temperature environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998)"
18. P3L16-19: deleted "Drought is a common environmental stress factor in rocky desertification areas, and high temperatures enhance the degree of heat damage, causing oxidative damage to the cell membrane. However, if the Ca2+ concentration of plants can be increased, this process can be effectively inhibited, thereby preventing or reducing heat damage (Larkindale and Knight, 2002). A fine regulatory mechanism exists in t"

    P3L19: changed "the plant" into "The plant", and deleted "that can"

    P3L21: deleted ", which play a key role in the Ca^{2+} transport system in plants"
19. P3L22-24: deleted "The Ca^{2+} transport system (Ca^{2+} channel, Ca^{2+} / H^+ reverse transport carrier and Ca^{2+}-ATPase) plays an important role in the uptake, transport and distribution of Ca in plants (White and Broadley, 2003)."
20. P4L3-5: deleted "The Ca content of plants usually lies between 0.1 % and 5.0 %, and mostly exists in cell walls and vacuoles - in the form of pectin combination morphology and insoluble organic and inorganic Ca salts (Kinzel, 1989)."
21. P4L11-21: inserted "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)."

22. P4L22-23: changed "Ji et al. (2009) revealed that 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." into "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)."
23. P4L23-24: inserted "Wang et al. (2011) found that plant rhizosphere soil TCa content in calcareous soil area ware above 14.0 mg·g-1." before "Zhang (2005)….."
24. P5L3-7: inserted "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)." after " ……,but inhibit growth in *Rhododendron decorum*."
25. P5L7-13: changed "These results indicate that there are differences in soil Ca content between different areas and that there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. Collectively, these differences lead to different degrees of adaptability of plants to high Ca environments." into "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."
26. P5L13-15: changed "However, to date, there is a scarcity of extensive research into the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas." into "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."
27. P5L18-19: deleted "In order to", capitalize the "To". And changed "we did the following:" into "the following investigations were explored"
28. P6L2: changed "was" into "is"
29. P6L7: inserted "(see Table. 1)" after "…….is poor", and add a "Table 1. Basic description for different grades of rocky desertification sites" in P24.
30. P6L8: changed title "Data collection" into "Experimental design and data collection"
31. P6L10: deleted ". These four main indices"
32. P6L13-14: changed "range of characteristics and data relating to the surrounding environment" into "environmental factors"
33. P6L15-16: changed "We conducted a detailed survey of the three sample areas and collected samples in October 2016." into "The sample collection in these three sample areas were conducted in October 2016.
34. P6L18: use "Within" to replace the "For", deleted "we assigned", and changed "2×2" into "（2×2)"
35. P6L19: add "were set up" after "(upper, middle, and lower slope)"

36. P6L19-L15: add “(3x4x3)” before the “for analysis”, deleted “We chose to study”
37. P6L19-L21: changed “the common plant species of the region, and gathered plants using the whole plant harvest method. In each small quadrat, every kind of shrubs and herbs are collected.” into “The common plant species of the region were gathered using the whole plant harvest method in each small quadrat as well as all shrubs and herbs were collected.”
38. P6L22: deleted “parts” before “.”
39. P7L1: changed “heated” into “oven tried’, and add “and then” after “the de-enzyme,”
    P7L1: inserted “about 480 minutes” after “at 80°C”, and deleted “for”. Deleted “and bagged” and add “chemical” before the “analysis”
40. P7L3: deleted “Finally”, and changed “soil” into “Soil”
41. P7L9-13: changed “‘Annual herbs’ included both annual herbs and biennial herbs, while ‘deciduous shrubs’ included deciduous trees with a height less than 2 m or a ground diameter less than 3 cm. The aboveground part of plants included branches and leaves, while the underground part included roots.” into “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.”
42. P7L13-17: changed “One-way analysis of variance (ANOVA), Two-way ANOVA and Pearson correlation analysis (α = 0.05) were used to analyze the Ca content of soil and plants within and between different grades of rocky desertification.” into “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 (α = 0.05) was used to analyze the correlation between plant Ca and soil ECa content.”
43. P7L21: add “in soil” after “The mean TCa content”
44. P7L22: changed “points” into “location”
45. P8L1: deleted “Furthermore”, add “The”, and changed “to be” into “that”
46. P8L3-4: add “Ca content” after “average”, and use “the” to replace “with”
47. P8L8: changed “The 41” into “Total 41”
48. P8L9: use “.” To replace “,”, and then use “Compare to” to replace “while”
49. P8L11: deleted “when compared across”, changed into “throughout”
50. P8L12-13: add “and belowground” after “aboveground”, and deleted “or that of underground parts, there”
51. P8L14-15: deleted “Furthermore, the grades of rocky desertification had no obvious effect on the Ca content of the aboveground and underground parts of the plants generally.”
52. P8L17-18: changed “The 41plant species were identified in the 36 small quadrats; these plants were divided into different functional groups” into “The 41 plant species were identified and were divided into different functional groups in the 36 small quadrats.”
53. P8L18: deleted “For each functional group,”

54. P8L18-20: changed "Ca content between the aboveground and underground parts were significantly different (p< 0.05), and the Ca content of the aboveground parts was higher than that of the underground parts (p<0.05) (Fig.3)." into "The Ca content of the aboveground parts was significantly higher than that of the belowground parts in each group (p<0.05)."
55. P9L3-5: changed "In terms of life form functional groups, shrubs showed a significantly higher Ca content, both aboveground and underground than herbs (p<0.05)" into "In life form functional groups, shrubs showed a significantly higher in Ca content than herbs in both aboveground and underground (p<0.05)"
56. P9L7- 8: changed "The aboveground and underground Ca content of dicotyledons" into "The Ca content of dicotyledons in aboveground and belowground parts"
57. P9L9: add "(Fig. 3)" after "(9.63 $g\cdot kg^{-1}$ and 4.79 $g\cdot kg^{-1}$, respectively; p<0.05)"
58. P9L10-12: changed "In terms of monocotyledons and dicotyledons, further analysis revealed no significant differences in the Ca content of the aboveground parts when compared between the different grades of rocky desertification; this was also true for the Ca content of the underground parts." into "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."
59. P9L12-13: deleted "The Ca content of both the aboveground and underground parts of monocotyledons was always low while those of dicotyledons were always high"
60. P9L13-15: changed "The Ca content of dicotyledons was significantly higher than those of monocotyledons across" into "The Ca content of dicotyledons was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout"
61. P9L16-17: changed "For the 41 common plants collected, 17 plant species (which exist in each sample area) were widespread throughout the southwestern rocky desertification areas of Hunan." into "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."
62. P9L17: deleted "For each of t", and add capitalize "T"
63. P9L18: use "were calculated" replace ",we calculated", and changed "Table.2" into "Table.3"
64. P9L18: deleted ". These plants were common species in the local area"
65. P9L18-20: deleted "We carried out two-way ANOVA for both species and soil for these 17 plants to determine differences in plant Ca content. The soil was graded into three categories: LRD, MRD and IRD."
66. P9L21: deleted "df=16, F=11.277"
67. P9L21-22: Use "related among" to replace "significant among the different"
68. P9L22: deleted (df=2, F=2.299, p=0.117)
69. P9L22: changed "For Ca" into "The Ca"
70. P9L23: deleted ", differences", and add "difference" before "not only"
71. P9L23-P10L2: changed "in terms of plant species" into "among the species", deleted "(df=16, F=8.543, p<0.01)", and deleted "but also among the different" and add "it throughout all the" before "grades of rocky desertification", and

deleted “df=2, F=4.104,”

72. P10L4-5: deleted “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”, and changed “For these17” into “These 17”
73. P10L7: deleted “, which indicated that *Sanguisorba officinalis* was affected greatly by soil ECa conten”
74. P10L8: add “($p<0.01$)” after “*Castanea henryi*”
75. P10L19-10: deleted “, indicating that the underground parts of these species were also greatly affected by soil ECa content”
76. P10L11-12” deleted “, which indicated that the aboveground parts of *Themeda japonica* was also greatly affected by soil ECa 20 content”, and changed “For the other” into “The other”
77. P10L13: changed “(Table. 3)” into “(Table. 4)”
78. P10L15: deleted “kinds of”
79. P10L15-16: deleted “, and were also the representative species that are able to adapt to a high Ca soil environment.”
80. P10L18-22: deleted “(Grubb and Edwards, 1982). The capacity of these plants which are 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, we classified these plants into the following groups: Ca-indifferent plants, high-Ca plants and low-Ca plants (Ji et al., 2009).”
81. P11L3-4: deleted “The Ca content of these plants increased or decreased correspondingly with increases or reductions in soil ECa content, but plant growth was not affected by such changes.”
82. P11L11-12: deleted “Moreover, the physiological activities of these plants had a higher demand for Ca and may have a strong ability to enrich soil Ca.”
83. P11L14-16: deleted “. In addition, the physiological activities of these 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”
84. P11L16: changed “(Table. 4)” Into “(Table. 5)”
85. P11L17-20: deleted “Finally, the different plant functional groups revealed the differences in Ca content (Fig. 2). In some cases, even within the same plant, 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.”
86. P12L3-7: deleted “The aboveground parts of plants had a higher Ca content than the…… the plants studied herein.”
87. P12L14: deleted “pointed to”, and add “could be” after “and soil chemistry”, deleted “as”
88. P12L18-20: add “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” after “(the main chemical composition: CaCO3) (Ji et al., 2009).”
89. P12L20-21: changed “The average calcium content of aboveground parts of plants was 19.67 $g\cdot k^{g-1}$, which was lower

than that of Hunan flue-cured tobacco (21.93 $g\cdot kg^{-1}$) (Xu et al., 2007).” into “the average Ca content of aboveground parts of plants (19.67 $g\cdot kg^{-1}$) was lower than that of Hunan flue-cured tobacco (21.93 $g\cdot kg^{-1}$) (Xu et al., 2007).”

90. P12L22-P13L2: changed “The maximum and minimum calcium content of plant aboveground parts were 41.79 $g\cdot kg^{-1}$ and 2.15 $g\cdot kg^{-1}$ respectively, and the maximum and minimum calcium content of plant underground parts were 40.14 g·kg-1 and 0.42 $g\cdot kg^{-1}$ respectively, which is lower than the calcium content of calcareous plants leaves (maximum 85.13 $g\cdot kg^{-1}$，minimum 6.26 $g\cdot kg^{-1}$) by Luo et al. (2014).” into “The maximum and minimum Ca content of plant aboveground parts were 41.79 $g\cdot kg^{-1}$ and 2.15 $g\cdot kg^{-1}$ respectively, and the maximum and minimum Ca content of plant belowground parts were 40.14 $g\cdot kg^{-1}$ and 0.42 $g\cdot kg^{-1}$ respectively. The maximum Ca content of plant (41.79 $g\cdot kg^{-1}$) was found in the leaves which was lower than the Ca content of calcareous plants leaves with the maximum value of 85.13 $g\cdot kg^{-1}$ by Luo et al. (2014).”
91. P13L7-11: Changed “and our results showed several plants (*Sanguisorba officinalis, Dendranthema indicum, Castanea henryi and Themeda japonica*) and soil Eca content was a positive correlation, but most plant calcium content and soil ECa content was not relevant.” into “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).”
92. P13L11: deleted “The Ca^{2+} content in plant cells was proportional to soil Ca^{2+}.”
93. P13L11-15: changed “Calcium-rich soils caused cells to absorb more calcium than the cells themselves require (White and Broadley, 2003). Zou et al. (2010) showed that soil ECa content and leaf calcium content are extremely significant positive correlation in pot experiment.” into “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.”
94. P13L15-23: inserted “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.” Before “Two-way ANOVA of species and soil……”
95. P14L1-2: deleted two “-related”
96. P14L3: changed “in accordance with data reported previously” into “supported with data reported”
97. P14L5-8: changed “Since the transport of Ca was mainly one-way (upward), this result indicated that nitrogen-fixing plants were the most efficient in terms of the upward transport of Ca, and that Ca was mainly concentrated in the

aboveground parts of the plant; these findings were not consistent with those of Ji et al. (2009).” into “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).”

98. P14L8: deleted “In their paper,”
99. P14L9-10: changed “,” into “.”, and add “They used three types of plants (pteridophytes, dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings.” after “the upward transport of Ca.” after “……at the upward transport of Ca”
100. P14L10-18: deleted “and studied only three types of plants (pteridophytes, dicotyledons, monocotyledons) that did not include nitrogen-fixing plants, which may be the reason for the inconsistency of this previous data with our current findings. In terms of the Ca content of monocotyledons, we found significant differences ($p<0.01$) between the aboveground and underground parts, but the study by Ji et al. (2009) revealed that these differences were not significant. This may be because most of the monocotyledons collected were low-Ca plants.”, and changed into “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.”
101. P14L18-21: deleted “Owing to the fact that the aboveground parts of low-Ca plants maintain a lower Ca content for different grades of rocky desertification,” and changed “a significant difference was found between the aboveground and underground parts in monocotyledons.” into “A significant difference was found between the aboveground and belowground parts in monocotyledons, which may be because low-Ca plants maintain a lower Ca content in different grades of rocky desertification.”
102. P15L2- P16L6: deleted “P15L2- P16L6 “content
103. P16L7-10: inserted “The different plant functional groups revealed the differences in Ca content (Fig. 3). In some cases, even within the same plant, 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.”
104. P16L12-16: inserted “(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).” After “……the Ca demand of the plant’s physiological activity”
105. P16L16-19: deleted “The research conducted by Ji et al. (2009) was based on the differences in correlation between the Ca content of the aboveground parts of plants and its soil Ca content; these authors analyzed the capacity of plants to adapt to high Ca environments, and divided the dominated species into Ca-indifferent plants, high-Ca plants and low-Ca plants.”
106. P16L21-P17L1: inserted “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." after "……. vegetation restoration in rocky desertification areas."

107. P17L2-10: inserted "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.
108. P17L11: changed "Conclusion" into "Conclusions"
109. P17L12-13: deleted "followed by" add "and" before "LRD"
110. P17L13-14: deleted the sentence" Significant differences were detected for both soil ECa and TCa content when compared between the rocky side and non-rocky side of each grade of rocky desertification areas."
111. P17L15-16: add "sites" after "studied", deleted "Furthermore", and changed "significant" into "Significant"
112. P17L17: deleted "($p<0.05$)
113. L17L18: deleted "the" after "but had no significant effect on"
114. P17L19-20: put "," after "*Themeda japonica*", deleted "For these plants," and put "had"
115. P17L21: changed "High-Ca plants included *Pyracantha fortuneana*," into "High-Ca plants in our study were *Pyracantha fortuneana*,"
116. P17L22- P18L1: deleted "In this case", and changed "the aboveground parts of these plant were able to maintain a higher Ca content under conditions of variable soil ECa content." into "the aboveground parts of these plants were able to absorb a high Ca content from various of ECa content soils.
117. P19L23-24: deleted reference "Xiang, H., Zhang, L., and Chen, J.: Effects of calcium concentration in solution on calcium content in the seedlings of five fig plants, Guihaia, 23, 165–168, 2003. (in Chinese with English abstract)"
118. P20L6-8: add reference "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)"
119. P20L9: deleted reference "Yin, L. P.: Plant Nutrition Molecular Biology and Signal Transduction, Science Press, 2006. (in Chinese with English abstract)"
120. P21L2-3: add reference "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)"

121. P21L4-5: deleted reference “Li, Q. Y., Ge, H. B., Hu, S. M., and Wang, H. Y.: Effects of sodium and calcium salt stresses on strawberry photosynthesis, Acta Bot. Boreali-Occidential Sinica., 26, 1713–1717, 2006. (in Chinese with English abstract)”
122. P21L11: deleted reference “Simpson, J. F. H.: A chalk flora on the Lower Greensand: its use in interpreting the calcicole habit, J. Ecol., 26, 218–235, 1938.”
123. P21L14-16: add reference “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)”
124. P22L4-5: deleted reference “Feng, X. Y., Hu, Z. P., and Yi, Y.: Variation of proline and soluble protein content in leaves of Eurycorymbus cavalerieian and Pinus armandii under Ca2+ stress, Guizhou Agricultural Sciences., 38, 169–170, 2010. (in Chinese with English abstract)”
125. P22L17-18: deleted reference “Tu, Y. L.: Floristic and ecological characteristics in the karst shrub of Guizhou, Journal of Guizhou Normal University: Natural Science, 13, 1–8, 1995. (in Chinese with English abstract)”
126. P22L22-23: deleted reference “Zhou, Y. C.: A study on the part plants’ main nutrient elements content of Guizhou karst region, Journal of Guizhou Agric., 16, 11–16, 1997. (in Chinese with English abstract)”
127. P24: added “Table 1. Basic description for different grades of rocky desertification sites”
128. P25: changed “Table 1” into ‘Table 2”, and change “point” into “location
129. P26: changed “Table 2” into ‘Table 3”
130. P29L11-12: deleted “Annual herbs include annual and biennial herbs, while deciduous shrubs include deciduous trees with a height < 2 m or a ground diameter < 3 cm.”
131. We changed all “underground” into “belowground” in the entire manuscript. And reworded all words “calcium” into “Ca” in the entire manuscript.

Part 3: **marked-up manuscript version**

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

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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. ~~However, the Ca^{2+} dynamics of plants and soil are not well understood.~~ In this study, three different grades of rocky desertification sample~~s~~ 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~~s~~ 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 ~~underground~~belowground parts (p<0.05). The soil ECa content had significant effects on plant Ca^{2+} content of the ~~underground~~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 ~~underground~~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 ~~can~~ provides a vital theoretical basis and practical guide for vegetation restoration and ecosystem reconstruction in rocky desertification areas.

Keywords: Rocky desertification; High Ca~~calcium~~ 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). ~~Of these, t~~The severity of rocky desertification ~~in Hunan Province~~ was ranked in fourth in Hunan Province of China (Li et al., 2016). ~~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 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. ~~the immediate focus of agro-forestry production environment improvements, regional economic development 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 ~~the restoration of vegetation is key to the process of remediation~~ (Wang et al., 2004). Consequently, the screening of plant~~s~~ species which can grow successfully in high-Ca~~calcium~~ 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 ~~also plays a central role in helping plants overcome environmental stress~~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). ~~A low cytosolic Ca^{2+} concentration is crucial for appropriate cell signaling (Müller et al., 2015). At the same time, Ca^{2+} is a versatile plant signal sensor under conditions of soil water stress (Hong-Bo and Ming, 2008). In addition, Ca^{2+} as a second messenger in the process of cell signal transduction, which plays a key regulatory role in how plants respond to environmental changes (Poovaiah and Reddy, 1993; 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). ~~However, high~~ Ca~~calcium stress can exert influence over the photosynthetic and growth rate of plants . (Ji et al., 2009; Hui et al., 2003).~~ Ca^{2+} combine with ~~and~~ pectin in the cell walls of plants ~~combine~~ to form pectin Ca~~calcium~~, 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 (Müller 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). ~~Drought is a common environmental stress factor in rocky desertification areas, and high temperatures enhance the degree of heat damage, causing oxidative damage to the cell membrane. However, if the Ca^{2+} concentration of plants can be increased, this process can be effectively inhibited, thereby preventing or reducing heat damage (Larkindale and Knight, 2002). A fine regulatory mechanism exists in t~~The plant cell ~~that can~~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~~, which play a key role in the Ca^{2+} transport system in plants~~ (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2005). ~~The Ca^{2+} transport system (Ca^{2+} channel, Ca^{2+} / H^{+} reverse transport carrier and Ca^{2+}-ATPase) plays an important role in the uptake, transport and distribution of Ca in plants (White and Broadley, 2003).~~ 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). The Ca content of plants usually lies between 0.1 % and 5.0 %, and mostly exists in cell walls and vacuoles - in the form of pectin combination morphology and insoluble organic and inorganic Ca salts (Kinzel, 1989). Cytoplasmic Ca^{2+} is mainly combined with proteins and other macromolecules; the concentration of free Ca^{2+} is generally only 20–200 $nmol \cdot L^{-1}$ 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 Cacalcium 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).

Ji et al. (2009) revealed that tThe mean soil ECa was 3.61 $g \cdot kg^{-1}$ 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 $mg \cdot g^{-1}$. 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. ~~These results indicate that there are differences in soil Ca content between different areas and that there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. Collectively, these differences lead to different degrees of adaptability of plants to 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. ~~However, to date, there is a scarcity of extensive research into the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas.~~

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. ~~In order t~~To test this hypothesis, ~~we did the~~the following investigations were explored: (i) to measure the soil ECa and TCa contents in rocky and non-rocky side areas~~s~~; (ii) to investigate and compare the Ca content of aboveground and ~~underground~~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 ~~was~~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°C, and maximum and minimum temperatures are 41.0°C 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~~Data collection~~

Rocky desertification was graded by using the sum of four index scores: bedrock expose rate, vegetation type, vegetation coverage and soil thickness~~. These four main indices~~ 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~~a range of characteristics and data relating to the surrounding environment~~, 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.~~We conducted a detailed survey of the three sample areas and collected samples in October 2016.~~

~~For~~Within each of the three sample areas, ~~we assigned~~ 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. ~~We chose to study t~~The common plant species of the region~~, and~~ were gathered~~ plants~~ using the whole plant harvest method~~. I~~in each small quadrat~~, every kind of~~ 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 ~~underground~~belowground~~ parts~~. Plant samples were taken back to the laboratory, rinsed with distilled water before being oven tried~~heated~~ at 105°C for 15 min to de-enzyme, and then dried to a constant weight at 80°C~~ for~~ about 480 minutes, crushed and passed through a 0.149 mm sieve, ~~and bagged~~ 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). ~~Finally, s~~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'.~~'Annual herbs' included both annual herbs and biennial herbs, while 'deciduous shrubs' included deciduous trees with a height less than 2 m or a ground diameter less than 3 cm.~~ ~~The aboveground part of plants included b~~Branches and leaves were treated together as aboveground part, while the ~~underground~~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.~~ The soil was graded into three categories: LRD, MRD and IRD.~~ One-way analysis of variance (ANOVA)~~, Two-way ANOVA and Pearson correlation analysis (α = 0.05)~~ was used to analyze the Ca content of soil and plants ~~within and~~ between different grades of rocky desertification. Pearson correlation analysis (α = 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 $g\cdot kg^{-1}$ (range: 0.10–8.09 $g\cdot kg^{-1}$) while mean ECa content was 1.46 $g\cdot kg^{-1}$ (range: 0.02–3.92 $g\cdot kg^{-1}$). Differences between different samples location ~~points~~ (non-rocky side and rocky side) were significant ($p<0.05$)

for both TCa and ECa. ~~Furthermore, ~~The mean soil TCa and ECa content were found that ~~to be~~ 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%, ~~with~~ 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~~he~~ 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 $g\cdot kg^{-1}$ (range: 4.34–40.24 $g\cdot kg^{-1}$).~~,~~ ~~While~~Compare to the mean Ca content of the ~~underground~~belowground parts was 10.79 $g\cdot kg^{-1}$ (range: 4.41–33.62 $g\cdot kg^{-1}$). The Ca content of the aboveground parts was significantly higher than that of the ~~underground~~belowground parts ($p<0.05$) ~~when compared across~~ throughout the same grades of rocky desertification. Whether the Ca content of aboveground and belowground part of the plants ~~or that of underground~~belowground ~~parts, there~~ were no significant differences ($p>0.05$) among the three different grades of rocky desertification. ~~Furthermore, the grades of rocky desertification had no obvious effect on the Ca content of the aboveground and underground~~belowground ~~parts of the plants generally~~ (Fig. 2).

3.2.2 Ca content in different plant functional groups

The 41-plant species were identified and ~~in the 36 small quadrats; these plants~~ were divided into different functional groups in the 36 small quadrats. ~~For each functional group, Ca content between the aboveground and underground~~belowground ~~parts were significantly different ($p<0.05$), and t~~The Ca content of the aboveground parts was significantly higher than that of the ~~underground~~belowground parts in each group ($p<0.05$) ~~(Fig. 3)~~.

Nitrogen-fixing plants (22.48 $g\cdot kg^{-1}$) showed a slightly higher Ca content in the aboveground parts than non-nitrogen-fixing plants (19.39 $g\cdot kg^{-1}$; $p>0.05$), although Ca content in the belowground parts of nitrogen-fixing plants (6.76 $g\cdot kg^{-1}$) 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 ~~underground~~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}; ~~underground~~belowground: 6.42 g·kg^{-1}; p<0.05). In ~~terms of~~ life form functional groups, shrubs showed a significantly higher in Ca content~~, both aboveground and underground~~~~belowground~~ 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 ~~aboveground and underground~~~~belowground~~ 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).

~~In terms of~~ To monocotyledons and dicotyledons, ~~further analysis revealed~~there were no significant differences in the plant Ca content of the aboveground parts ~~when compared~~ among~~between~~ the different grades of rocky desertification; this was also true for the plant Ca content of the ~~underground~~belowground parts. ~~The Ca content of both the aboveground and underground~~~~belowground parts of monocotyledons was always low while those of dicotyledons were always high.~~ The Ca content of dicotyledons was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout ~~across~~ the three grades of rocky desertification (p<0.05) (Fig. 4).

~~For the~~Within total 41 common plants species~~collected~~, 17 plant species were found ~~(which exist~~ in each sample plot and ~~area)~~ were widespread throughout the southwestern rocky desertification areas of Hunan. ~~For each of t~~These 17 species~~,~~ ~~we~~were calculated their important values (*IV*) (Table. 3~~2~~). ~~These plants were common species in the local area. We carried out two-way ANOVA for both species and soil for these 17 plants to determine differences in plant Ca content. The soil was graded into three categories: LRD, MRD and IRD.~~ Data showed that the Ca content in the aboveground parts of the 17 plant species were highly significant (~~df=16, F=11.277,~~ p<0.01) among species, although these differences were not related among ~~significant among the different~~ grades of rocky desertification ~~(df=2, F=2.299, p=0.117)~~. ~~For~~ The Ca content in the ~~underground~~belowground parts~~, differences~~ were highly significant difference not only ~~in terms of plant species~~ among the species (df=16, F=8.543, $p<0.01$), but also among the different and it throughout all the grades of rocky desertification (df=2, F=4.104, $p<0.01$).

3.3 Correlation between plant Ca content and soil ECa content

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. For tThese 17 plant species, the Ca content in the aboveground and undergroundbelowground parts of *Sanguisorba officinalis* 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 in the undergroundbelowground 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, indicating that the undergroundbelowground parts of these species were also greatly affected by 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, which indicated that the aboveground parts of *Themeda japonica* was also greatly affected by soil ECa content. For tThe other plants, the Ca content of the aboveground and undergroundbelowground parts did not show a significant positive correlation ($p>0.05$) with soil ECa content (Table. 43).

3.4 Capacity of plants adapting to soil high Ca environments

The above 17 kinds of plants were dominant and common species in rocky desertification areas, and were also the representative species that are able to adapt to a high Ca soil environment. 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 (Grubb and Edwards, 1982). The capacity of these plants which are 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, we classified these plants into the following groups: Ca-indifferent plants, high-Ca plants and low-Ca plants (Ji et al., 2009).

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 undergroundbelowground parts and the soil ECa content. The Ca content of these plants increased or decreased correspondingly with increases or reductions in soil ECa content, but plant growth was not affected by such changes. 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 undergroundbelowground 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 $g \cdot kg^{-1}$) under conditions of varying ECa content in the soil. Moreover, the physiological activities of these plants had a higher demand for Ca and may have a strong ability to enrich soil Ca. 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 $g \cdot kg^{-1}$) under conditions of varying ECa content in the soil. In addition, the physiological activities of these 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 (Table. 54).

Finally, the different plant functional groups revealed the differences in Ca content (Fig. 2). In some cases, even within the same plant, there was an inconsistent correlation between Ca content in the aboveground and undergroundbelowground 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.

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 aboveground parts of plants had a higher Ca content than the underground~~belowground ~~parts, although t~~There ~~was no significant difference in plant Ca content when compared between aboveground or underground~~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 underground~~belowground ~~parts of the plants studied herein.~~ 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 ~~pointed to~~ differences in both organic layer thickness and soil chemistry could be ~~as~~ a reason ~~for~~ 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 ~~T~~the average Ca~~calcium~~ content of aboveground parts of plants ~~was (~~19.67 g·kg^{-1}~~,) which~~ was lower than that of Hunan flue-cured tobacco (21.93 g·kg^{-1}) (Xu et al., 2007). ~~Compared to the aboveground and belowground Ca content in our study, T~~The maximum and minimum Ca~~calcium~~ content of plant aboveground parts were 41.79 g·kg^{-1} and 2.15 g·kg^{-1} respectively, and the maximum and minimum Ca~~calcium~~ content of plant ~~underground~~belowground parts were 40.14 g·kg^{-1} and 0.42 g·kg^{-1} respectively. The maximum~~,~~

Ca~~calcium~~ content of plant (41.79 g·kg^{-1}) was found in the leaves which was lower than the Ca~~calcium~~ content of calcareous plants leaves ~~(~~with the maximum value of 85.13 g·kg^{-1}~~, minimum 6.26 g·kg^{-1})~~ by Luo et al. (2014). To most plant Ca~~calcium~~ content, the aboveground part was larger than the ~~underground~~belowground part, and for a few plants Ca~~calcium~~ content, the aboveground part was lower than the ~~underground~~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). ~~and our results showed several plants（*Sanguisorba officinalis*, *Dendranthema indicum*, *Castanea henryi* and *Themeda jap onica*）and soil Eca content was a positive correlation, but most plant calcium content and soil ECa content was not relevant. The Ca^{2+} content in plant cells was proportional to soil Ca^{2+}.~~ But some study showed that Ca~~lcium~~-rich soils caused cells to absorb more Ca~~calcium~~ than the cells themselves require (White and Broadley, 2003), ~~.~~ and ~~Zou et al. (2010) showed that~~ soil ECa content and leaf Ca~~calcium~~ content (Flue-cured Tobacco) ~~— are extremely~~ 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~~-related~~ factors, while the Ca content of the ~~underground~~belowground parts was affected by both species~~-related~~ factors and the grade of rocky desertification, which was supported with data reported ~~in accordance with data reported previously~~ by Ji et al. (2009). The Ca content in the aboveground parts of nitrogen-fixing plants was significantly higher than that of the ~~underground~~belowground parts. And t~~Since the transport of Ca was mainly one-way (upward), t~~his result indicated that nitrogen-fixing plants were the most efficient in~~ terms of~~ the Ca upward transport~~ of Ca~~, since the transport of Ca was mainly upward~~and that Ca was mainly concentrated in the aboveground parts of the plant~~; ~~these findings~~which w~~ere~~as not ~~consistent~~the same with those of Ji et al. (2009). ~~In their paper,~~ Ji et al. (2009) revealed that dicotyledons were the most efficient at the upward transport of Ca.~~,~~ They used three types of plants (pteridophytes, dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings. ~~and studied only three types of plants (pteridophytes, dicotyledons, monocotyledons) that did not include nitrogen-fixing plants, which may be the reason for the inconsistency of this previous data with our current findings. In terms of the Ca content of monocotyledons, we found significant differences ($p<0.01$) between the aboveground and underground~~belowground ~~parts, but the study by Ji et al. (2009) revealed that these differences were not significant. This may be because most of the monocotyledons collected were low-Ca plants.~~ 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 ~~demand~~ plants. ~~Owing to the fact that the aboveground parts of low-Ca plants maintain a lower Ca content for different grades of rocky desertificattion, a~~A significant difference was found between the aboveground and ~~underground~~belowground parts in monocotyledons, which may be because ~~the aboveground parts of~~ 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 Cacalcium adaptation of plants

In high Ca environments, the photosynthetic and growth rate of plants may be affected, and a high Ca concentration within the cytoplasm may lead to death of the plant (Xiang et al., 2003; Li et al., 2006; Ji et al., 2009; Feng et al., 2010). The capacity of plants to adapt to high Ca environments is mainly reflected in two ways: adaptations of physiological structures and adaptations of physiological processes (Luo et al., 2012). In terms of adaptations to physiological structures, the most direct way in which plants can adapt to high Ca environments is to inhibit the plant roots from absorbing Ca and transporting it to the plant's aboveground parts (Luo et al., 2012). Some plants fix excess Ca by forming calcified deposits in root tissue in order to limit the upward transport of Ca (Musetti and Favali, 2003). In addition, calcium 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 environment, some plants will form calcium oxalate crystal cells in order to fix excess Ca (Moore et al., 2002; Feng et al., 2010). Furthermore, an active calcium efflux system plays an important role in the adaptation of plants to high Ca environments (Bose et al., 2011): Excess Ca in plants is exported from mature leaves to the outside, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003). The adaptations relating to physiological processes mainly involve two aspects: the regulation of internal Ca storage and the control of Ca absorption and transport (Luo et al., 2012). 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 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), in order to regulate the concentration of cytoplasmic Ca^{2+} to a normal level. The normal growth of plants is maintained by the photosynthetic process and by respiration of the aboveground parts. Therefore, the regulation and control of the concentration of Ca^{2+} in the plant's aboveground parts is also key in adapting to a high Ca environment (Yin, 2006).

When the Ca content of soil is high, different plants adopt different adaptation strategies. By considering the survival differences of plant species in high Ca environments such as lime soil, Simpson (1938) and Tu (1995) divided plants into non-calcium plants and calcicole. The former is only distributed in acidic soils and other areas with low concentrations of Ca^{2+};

these plants are also known as calcifugous plants or calcifuges (Simpson, 1938; Tu, 1995). According to the level of calcicole dependence on high Ca environments, these plants can be further divided into non-specific calcicole and specific calcicole. Of these, specific calcicole are only found within a carbonate matrix and calcareous soil; furthermore, these plants are specific to the soil environment (Tu, 1995). Depending on their Ca content, non-specific calcicoles can be divided into calciphiles, calciphilous plants, Ca-indifferent plants (Tu, 1995; Zhou, 1997). The adaptability of plants to high Ca soil environments is related to their ability to absorb, transport and accumulate Ca (Ji et al., 2009).

The different plant functional groups revealed the differences in Ca content (Fig. 3). In some cases, even within the same plant, 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). The research conducted by Ji et al. (2009) was based on the differences in correlation between the Ca content of the aboveground parts of plants and its soil Ca content; these authors analyzed the capacity of plants to adapt to high Ca environments, and divided the dominated species into Ca-indifferent plants, high-Ca plants and low-Ca plants. 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, ~~followed by~~ and LRD. ~~Significant differences were detected for both soil ECa and TCa content when compared between the rocky side and non-rocky side of each grade of rocky desertification areas.~~ The Ca content in the aboveground parts of plants was significantly higher than that in the ~~underground~~belowground parts for the three grades of rocky desertification studied sites. ~~Furthermore, s~~Significant differences in Ca content were found between the aboveground and ~~underground~~belowground parts of each plant functional group~~($p<0.05$)~~. The soil ECa content had a significant effect on the Ca content of the ~~underground~~belowground parts of plants, but had no significant effect on ~~the~~ Ca content of the aboveground parts. Ca-indifferent plants included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica*, ~~For these plants,~~ had a significant positive correlation existed between the Ca content in the aboveground or ~~underground~~belowground parts and the soil ECa content. High-Ca plants in our study were~~included~~ *Pyracantha fortuneana, Rhus chinensis*, *Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. ~~In this case,~~ The aboveground parts of these plants were able to absorb a high Ca content from various of ECa content soils. ~~the~~

~~aboveground parts of these plant were able to maintain a higher Ca content under conditions of variable soil ECa content.~~ 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 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).

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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~~1~~. Soil TCa and ECa content from different grades of rocky desertification.

Ca typical ($g \cdot kg^{-1}$)	Sample location~~point~~	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 32. 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 43. 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 undergroundbelowground 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 54. Adaptation of plants to high Ca environments in rocky desertification areas.

Types of adaptation	Species	Characteristics of Ca~~calcium~~ content in plants	Strategies of plant adaptation to high Ca~~calcium~~ environments
Ca-indifferent plants	*Sanguisorba officinalis* *Castanea henryi* *Dendranthema indicum* *Themeda japonica*	There is significant positive correlation between the Ca~~calcium~~ content in the aboveground/~~underground~~belowground parts of plants and the soil ECa content. The coefficient of variation for Ca~~calcium~~ content in plants has a wide range.	Plants adapt to different Ca~~calcium~~ contents in soil through high Ca^{2+} buffering capacity. By regulating Ca^{2+} binding in Ca~~calcium~~ 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~~calcium~~ content in the aboveground parts of plants and the soil ECa content. The aboveground part has a high level of Ca~~calcium~~ content and the coefficient of variation falls within a narrow range.	Plants maintain high Ca~~calcium~~ content by enhancing Ca~~calcium~~ uptake and transporting it from ~~underground~~belowground to aboveground parts. High Ca~~calcium~~ 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~~calcium~~ content in the aboveground parts of plants and the soil ECa content. The aboveground part has a low level of Ca~~calcium~~ content and the coefficient of variation falls within a narrow range.	Plants maintain low Ca~~calcium~~ content in the aboveground parts by reducing Ca~~calcium~~ uptake and transporting it from ~~underground~~belowground to aboveground parts.

Figure captions

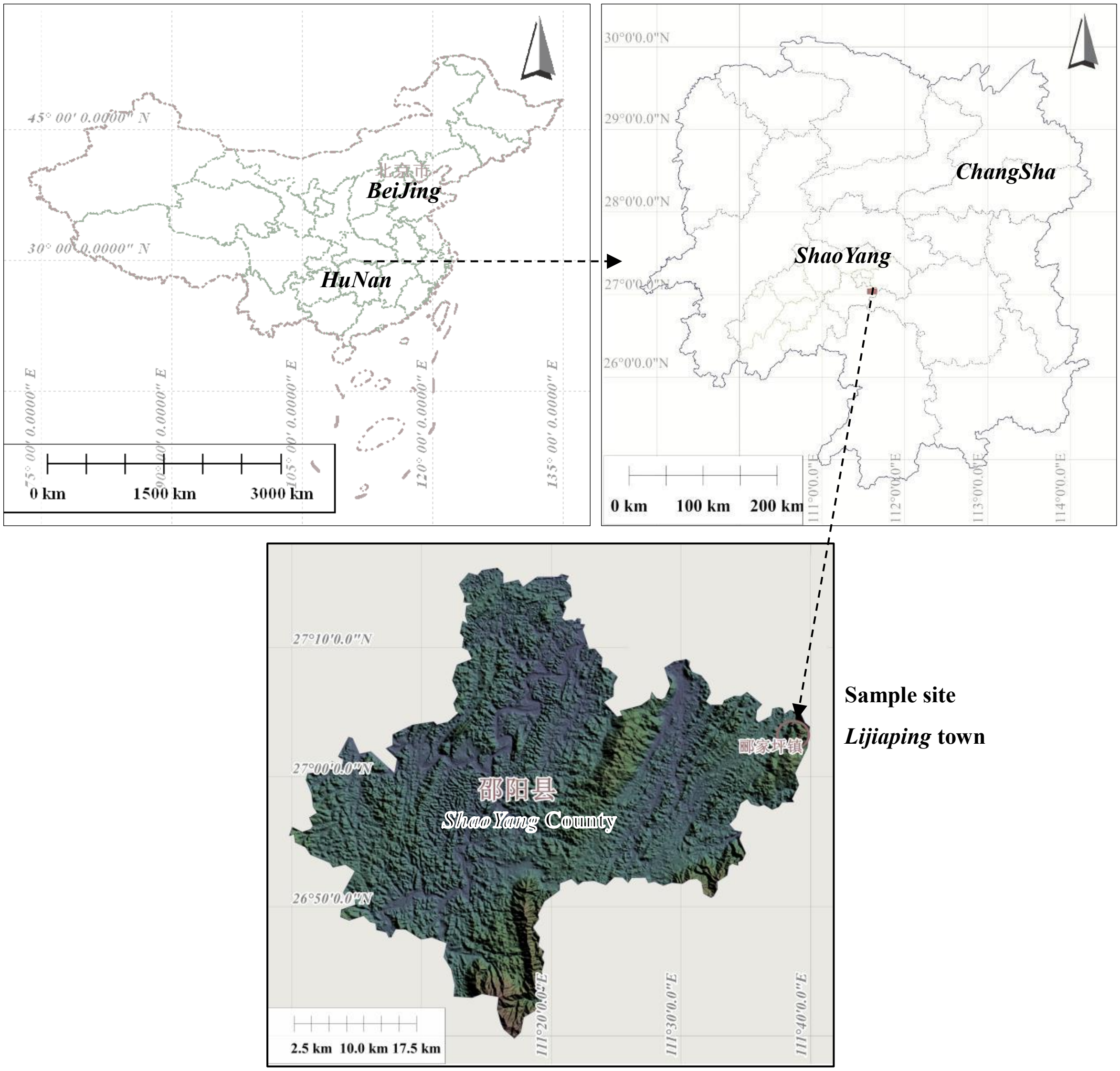
Fig. 1 Geographical locations of the study sites.

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 ~~underground~~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 ~~underground~~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 ~~underground~~belowground parts for the same functional groups (p<0.05); different upper-case letters represent significant differences among different functional groups, (p<0.05). ~~Annual herbs include annual and biennial herbs, while deciduous shrubs include deciduous trees with a height < 2 m or a ground diameter < 3 cm.~~

Fig. 4 Ca content in the aboveground and ~~underground~~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



BeiJing
HuNan
0 km
1500 km
3000 km
30°0'0.0"N
29°0'0.0"N
28°0'0.0"N
26°0'0.0"N
ChangSha
ShaoYang
0 km
100 km
200 km
27°10'0.0"N
27°00'0.0"N
26°50'0.0"N
邵阳县
ShaoYang County
2.5 km 10.0 km 17.5 km
Sample site
Lijiaping town

Fig. 2

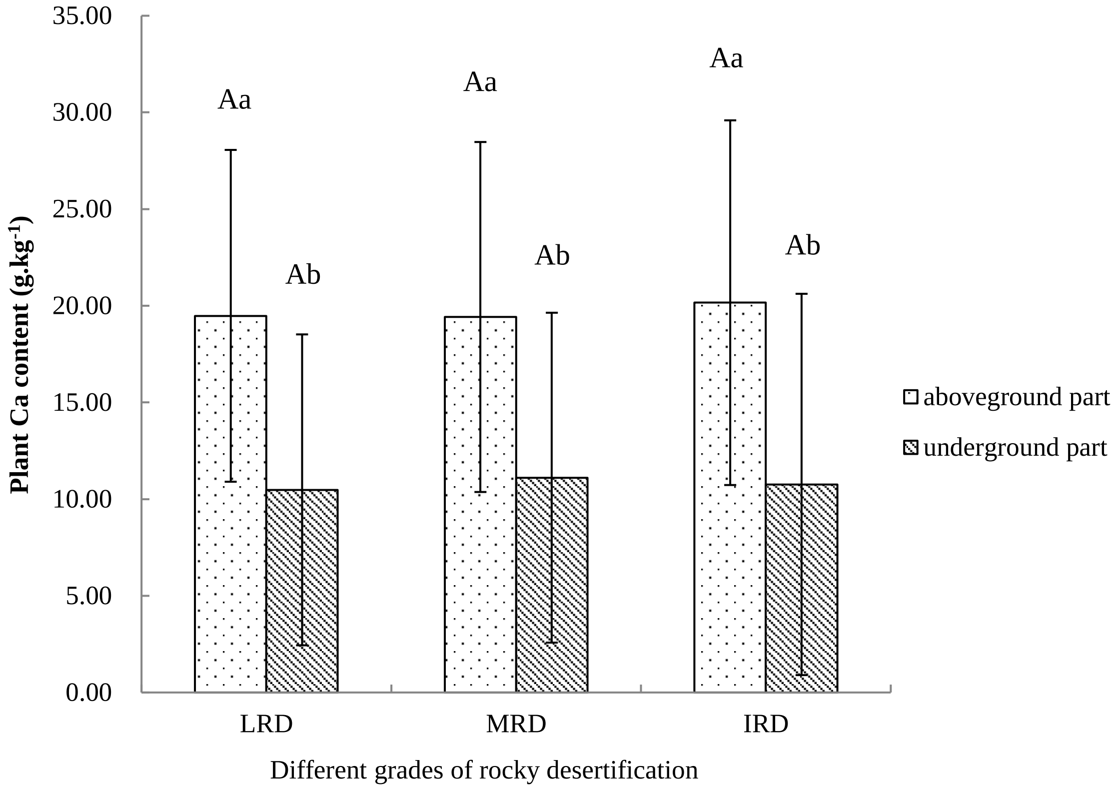
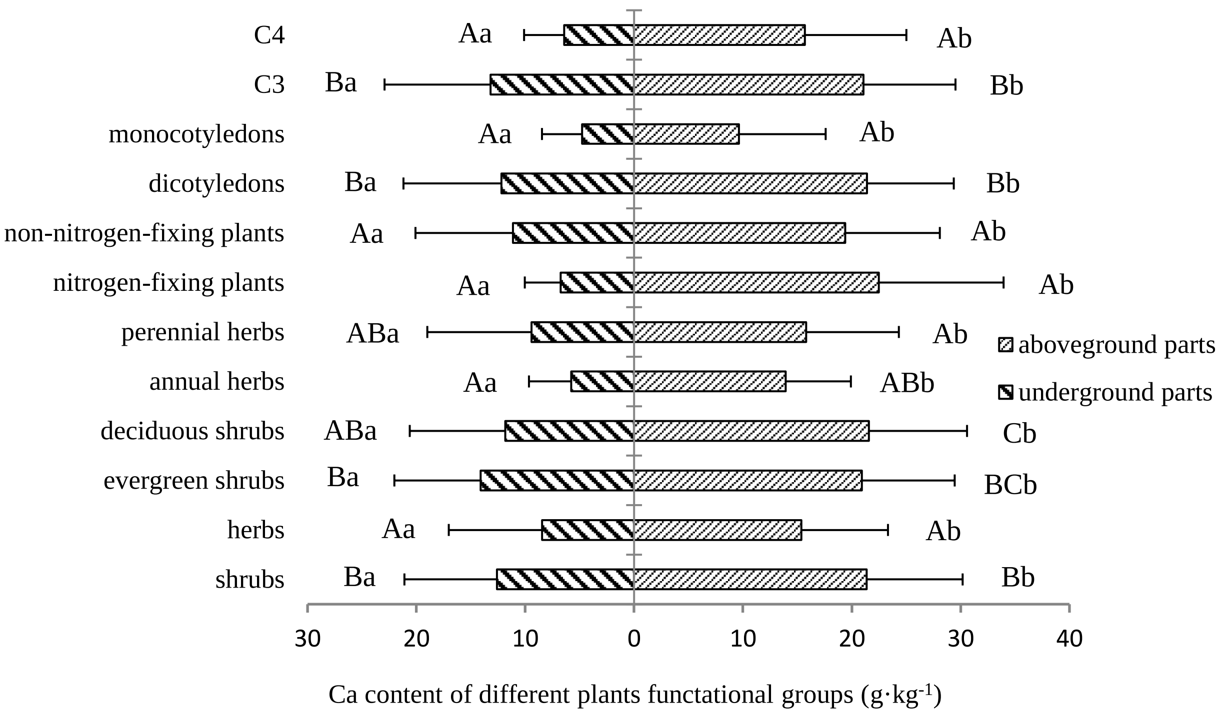
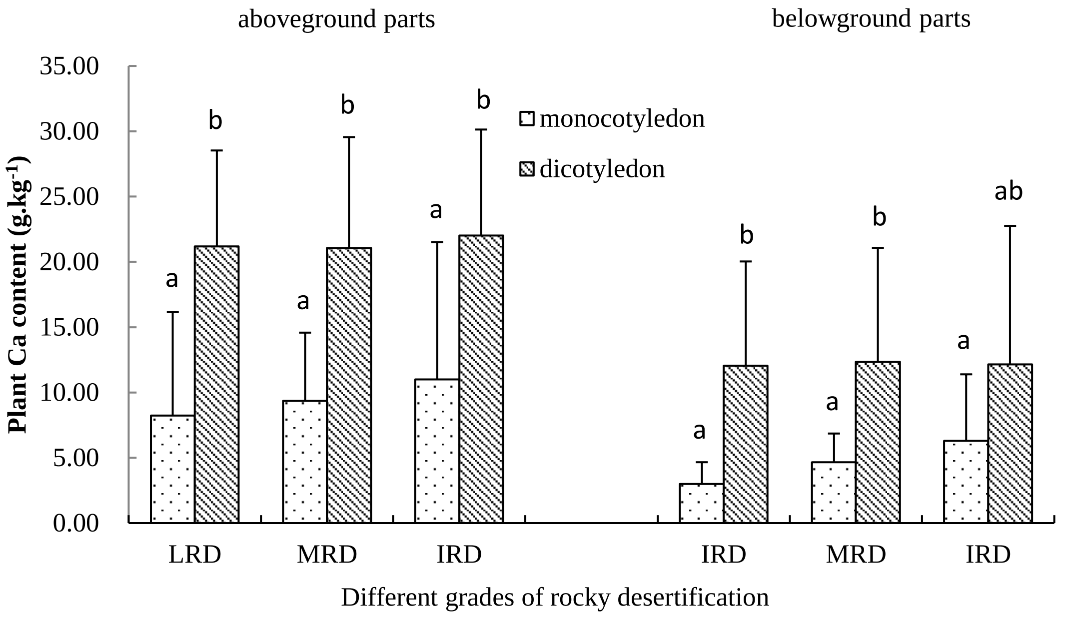

35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Plant Ca content (g.kg-1)
Aa
Ab
Aa
Ab
Aa
Ab
LRD
MRD
IRD
Different grades of rocky desertification
aboveground part
underground part

Fig. 3



C4
C3
monocotyledons
dicotyledons
non-nitrogen-fixing plants
nitrogen-fixing plants
perennial herbs
annual herbs
deciduous shrubs
evergreen shrubs
herbs
shrubs
Aa
Ab
Ba
Bb
Aa
Ab
Ba
Bb
Aa
Ab
Aa
Ab
ABa
Ab
Aa
ABb
ABa
Cb
Ba
BCb
Aa
Ab
Ba
Bb
aboveground parts
underground parts
30
20
10
0
10
20
30
40
Ca content of different plants functational groups (g·kg-1)

Fig. 4



aboveground parts
belowground parts
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Plant Ca content (g.kg-1)
monocotyledon
dicotyledon
a
b
a
b
a
b
a
b
a
b
a
ab
LRD
MRD
IRD
IRD
MRD
IRD
Different grades of rocky desertification

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.

Competing interests

The authors declare that they have no conflict of interest.

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

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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. ~~However, the Ca^{2+} dynamics of plants and soil are not well understood.~~ In this study, three different grades of rocky desertification sample~~s~~ 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~~s~~ 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 ~~underground~~belowground parts ($p<0.05$). The soil ECa content had significant effects on plant Ca^{2+} content of the ~~underground~~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 ~~underground~~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 can provides a vital theoretical basis and practical guide for vegetation restoration and ecosystem reconstruction in rocky desertification areas.

Keywords: Rocky desertification; High Cacalcium 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). Of these, tThe severity of rocky desertification in Hunan Province was ranked in fourth in Hunan Province of China (Li et al., 2016). 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 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. the immediate focus of agro-forestry production environment improvements, regional economic development 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 the restoration of vegetation is key to the process of remediation (Wang et al., 2004). Consequently, the screening of plants species which can grow successfully in high-Cacalcium 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 also plays a central role in helping plants overcome environmental stressis 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). ~~A low cytosolic Ca^{2+} concentration is crucial for appropriate cell signaling (Müller et al., 2015). At the same time, Ca^{2+} is a versatile plant signal sensor under conditions of soil water stress (Hong-Bo and Ming, 2008). In addition, Ca^{2+} as a second messenger in the process of cell signal transduction, which plays a key regulatory role in how plants respond to environmental changes (Poovaiah and Reddy, 1993; 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). ~~However, high~~ Ca~~calcium stress can exert influence over the photosynthetic and growth rate of plants (Ji et al., 2009; Hui et al., 2003).~~ Ca^{2+} combine with ~~and~~ pectin in the cell walls of plants ~~combine~~ to form pectin Ca~~calcium~~, 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 (Müller 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). ~~Drought is a common environmental stress factor in rocky desertification areas, and high temperatures enhance the degree of heat damage, causing oxidative damage to the cell membrane. However, if the Ca^{2+} concentration of plants can be increased, this process can be effectively inhibited, thereby preventing or reducing heat damage (Larkindale and Knight, 2002). A fine regulatory mechanism exists in t~~The plant cell ~~that can~~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~~, which play a key role in the Ca^{2+} transport system in plants~~ (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2005). ~~The Ca^{2+} transport system (Ca^{2+} channel, Ca^{2+}/ H^{+} reverse transport carrier and Ca^{2+}-ATPase) plays an important role in the uptake, transport and distribution of Ca in plants (White and Broadley, 2003).~~ 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).~~ The Ca content of plants usually lies between 0.1 % and 5.0 %, and mostly exists in cell walls and vacuoles – in the form of pectin combination morphology and insoluble organic and inorganic Ca salts (Kinzel, 1989).~~ 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~~calcium~~ 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).

~~Ji et al. (2009) revealed that t~~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. ~~These results indicate that there are differences in soil Ca content between different areas and that there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. Collectively, these differences lead to different degrees of adaptability of plants to 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. ~~However, to date, there is a scarcity of extensive research into the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas.~~

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. ~~In order t~~To test this hypothesis, ~~we did the~~the following investigations were explored: (i) to measure the soil ECa and TCa contents in rocky and non-rocky side areass; (ii) to investigate and compare the Ca content of aboveground and ~~underground~~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 wasis 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 collectionData collection

Rocky desertification was graded by using the sum of four index scores: bedrock expose rate, vegetation type, vegetation coverage and soil thickness. These four main indices 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 factorsa range of characteristics and data relating to the surrounding environment, 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.We conducted a detailed survey of the three sample areas and collected samples in October 2016.

ForWithin each of the three sample areas, we assigned 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. We chose to study tThe common plant species of the region, and were gathered plants using the whole plant harvest method . Iin each small quadrat, every kind of 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 undergroundbelowground parts. Plant samples were taken back to the laboratory, rinsed with distilled water before being oven triedheated at 105 ℃ for 15 min to de-enzyme, and then dried to a constant weight at 80 ℃~~ for~~ about 480 minutes, crushed and passed through a 0.149 mm sieve, ~~and bagged ~~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). ~~Finally, s~~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’.~~ ‘Annual herbs’ included both annual herbs and biennial herbs, while ‘deciduous shrubs’ included deciduous trees with a height less than 2 m or a ground diameter less than 3 cm.~~ ~~The aboveground part of plants included b~~Branches and leaves were treated together as aboveground part, while the ~~underground~~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.~~ The soil was graded into three categories: LRD, MRD and IRD.~~ One-way analysis of variance (ANOVA)~~, Two-way ANOVA and Pearson correlation analysis (α = 0.05)~~ was used to analyze the Ca content of soil and plants ~~within and ~~between different grades of rocky desertification. Pearson correlation analysis (α = 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 ~~points ~~(non-rocky side and rocky side) were significant (p<0.05) for both TCa and ECa. ~~Furthermore,~~ The mean soil TCa and ECa content were found that ~~to be~~ 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%, ~~with~~ 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~~he~~ 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}).~~,~~ ~~While~~Compare to the mean Ca content of the ~~underground~~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 ~~underground~~belowground parts (p<0.05) ~~when compared across~~ throughout the same grades of rocky desertification. Whether the Ca content of aboveground and belowground part of the plants ~~or that of underground~~belowground ~~parts, there~~ were no significant differences (p>0.05) among the three different grades of rocky desertification. ~~Furthermore, the grades of rocky desertification had no obvious effect on the Ca content of the aboveground and underground~~belowground ~~parts of the plants generally~~ (Fig. 2).

3.2.2 Ca content in different plant functional groups

The 41 plant species were identified and ~~in the 36 small quadrats; these plants~~ were divided into different functional groups in the 36 small quadrats. ~~For each functional group, Ca content between the aboveground and underground~~belowground ~~parts were significantly different (p<0.05), and t~~The Ca content of the aboveground parts was significantly higher than that of the ~~underground~~belowground parts in each group (p<0.05) ~~(Fig. 3)~~.

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 undergroundbelowground 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}; undergroundbelowground: 6.42 g kg^{-1}; p<0.05). In terms of life form functional groups, shrubs showed a significantly higher in Ca content, both aboveground and undergroundbelowground 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 aboveground and undergroundbelowground 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).

In terms of To monocotyledons and dicotyledons, further analysis revealedthere were no significant differences in the plant Ca content of the aboveground parts when compared amongbetween the different grades of rocky desertification; this was also true for the plant Ca content of the undergroundbelowground parts. The Ca content of both the aboveground and undergroundbelowground parts of monocotyledons was always low while those of dicotyledons were always high. The Ca content of dicotyledons was significantly higher than those of monocotyledons in both aboveground and belowground parts throughout across the three grades of rocky desertification (p<0.05) (Fig. 4).

For theWithin total 41 common plants speciescollected, 17 plant species were found (which exist in each sample plot and area) were widespread throughout the southwestern rocky desertification areas of Hunan. For each of tThese 17 species , wewere calculated their important values (IV) (Table. 32). These plants were common species in the local area. We carried out two-way ANOVA for both species and soil for these 17 plants to determine differences in plant Ca content. The soil was graded into three categories: LRD, MRD and IRD. Data showed that the Ca content in the aboveground parts of the 17 plant species were highly significant (df=16, F=11.277, p<0.01) among species, although these differences were not related among significant among the different grades of rocky desertification (df=2, F=2.299, p=0.117). For The Ca content in the undergroundbelowground parts , differences were highly significant difference not only in terms of plant species among the species~~ (df=16, F=8.543, *p*<0.01)~~, ~~but also among the different~~ and it throughout all the grades of rocky desertification (~~df=2, F=4.104,~~ *p*<0.01).

3.3 Correlation between plant Ca content and soil ECa content

~~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. For t~~These 17 plant species, the Ca content in the aboveground and ~~underground~~belowground parts of *Sanguisorba officinalis* 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 in the ~~underground~~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~~, indicating that the underground~~~~belowground~~~~ parts of these species were also greatly affected by 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~~, which indicated that the aboveground parts of *Themeda japonica* was also greatly affected by soil ECa content~~. ~~For t~~The other plants, the Ca content of the aboveground and ~~underground~~belowground parts did not show a significant positive correlation (*p*>0.05) with soil ECa content (Table. 4~~3~~).

3.4 Capacity of plants adapting to soil high Ca environments

The above 17 ~~kinds of~~ plants were dominant and common species in rocky desertification areas~~, and were also the representative species that are able to adapt to a high Ca soil environment~~. 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~~ (Grubb and Edwards, 1982)~~. ~~The capacity of these plants which are 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, we classified these plants into the following groups: Ca-indifferent plants, high-Ca plants and low-Ca plants (Ji et al., 2009).~~

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 ~~underground~~belowground parts and the soil ECa content. ~~The Ca content of these plants increased or decreased correspondingly with increases or reductions in soil ECa content, but plant growth was not affected by such changes.~~ 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 ~~underground~~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. ~~Moreover, the physiological activities of these plants had a higher demand for Ca and may have a strong ability to enrich soil Ca.~~ 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~~. In addition, the physiological activities of these 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~~ (Table. 54).

~~Finally, the different plant functional groups revealed the differences in Ca content (Fig. 2). In some cases, even within the same plant, there was an inconsistent correlation between Ca content in the aboveground and underground~~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.~~

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 aboveground parts of plants had a higher Ca content than the underground~~belowground ~~parts, although t~~There ~~was no significant difference in plant Ca content when compared between aboveground or underground~~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 underground~~belowground ~~parts of the plants studied herein.~~ 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 ~~pointed to~~ differences in both organic layer thickness and soil chemistry could be ~~as~~ a reason ~~for~~ 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 ~~T~~the average Ca~~calcium~~ content of aboveground parts of plants ~~was~~ (19.67 g kg^{-1}~~,~~) ~~which~~ was lower than that of Hunan flue-cured tobacco (21.93 g kg^{-1}) (Xu et al., 2007). ~~Compared to the aboveground and belowground Ca content in our study, T~~The maximum and minimum Ca~~calcium~~ content of plant aboveground parts were 41.79 g kg^{-1} and 2.15 g kg^{-1} respectively, and the maximum and minimum Ca~~calcium~~ content of plant ~~underground~~belowground parts were 40.14 g kg^{-1} and 0.42 g kg^{-1} respectively. The maximum~~,~~

Ca~~calcium~~ content of plant (41.79 g ~~l~~kg^{-1}) was found in the leaves which was lower than the Ca~~calcium~~ content of calcareous plants leaves ~~(~~with the maximum value of 85.13 g ~~l~~kg^{-1}~~, minimum 6.26 g kg^{-1})~~ by Luo et al. (2014). To most plant Ca~~calcium~~ content, the aboveground part was larger than the ~~underground~~belowground part, and for a few plants Ca~~calcium~~ content, the aboveground part was lower than the ~~underground~~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).~~ and our results showed several plants （*Sanguisorba officinalis*, *Dendranthema indicum*, *Castanea henryi* and *Themeda jap onica*) and soil Eca content was a positive correlation, but most plant calcium content and soil ECa content was not relevant. The Ca^{2+} content in plant cells was proportional to soil Ca^{2+}.~~ But some study showed that Ca~~lcium~~-rich soils caused cells to absorb more Ca~~calcium~~ than the cells themselves require (White and Broadley, 2003),~~.~~ and ~~Zou et al. (2010) showed that~~ soil ECa content and leaf Ca~~calcium~~ content (Flue-cured Tobacco) ~~— are extremely~~ 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-related factors, while the Ca content of the undergroundbelowground parts was affected by both species-related factors and the grade of rocky desertification, which was supported with data reported in accordance with data reported previously by Ji et al. (2009). The Ca content in the aboveground parts of nitrogen-fixing plants was significantly higher than that of the undergroundbelowground parts. And tSince the transport of Ca was mainly one-way (upward), this result indicated that nitrogen-fixing plants were the most efficient in terms of the Ca upward transport of Ca, since the transport of Ca was mainly upwardand that Ca was mainly concentrated in the aboveground parts of the plant; these findingswhich wereas not consistentthe same with those of Ji et al. (2009). In their paper, Ji et al. (2009) revealed that dicotyledons were the most efficient at the upward transport of Ca., They used three types of plants (pteridophytes, dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings. and studied only three types of plants (pteridophytes, dicotyledons, monocotyledons) that did not include nitrogen-fixing plants, which may be the reason for the inconsistency of this previous data with our current findings. In terms of the Ca content of monocotyledons, we found significant differences (p<0.01) between the aboveground and undergroundbelowground parts, but the study by Ji et al. (2009) revealed that these differences were not significant. This may be because most of the monocotyledons collected were low-Ca plants. 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 demand plants. Owing to the fact that the aboveground parts of low-Ca plants maintain a lower Ca content for different grades of rocky desertificattion, aA significant difference was found between the aboveground and undergroundbelowground parts in monocotyledons, which may be because the aboveground parts of 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 Cacalcium adaptation of plants

In high Ca environments, the photosynthetic and growth rate of plants may be affected, and a high Ca concentration within the cytoplasm may lead to death of the plant (Xiang et al., 2003; Li et al., 2006; Ji et al., 2009; Feng et al., 2010). The capacity of plants to adapt to high Ca environments is mainly reflected in two ways: adaptations of physiological structures and adaptations of physiological processes (Luo et al., 2012). In terms of adaptations to physiological structures, the most direct way in which plants can adapt to high Ca environments is to inhibit the plant roots from absorbing Ca and transporting it to the plant's aboveground parts (Luo et al., 2012). Some plants fix excess Ca by forming calcified deposits in root tissue in order to limit the upward transport of Ca (Musetti and Favali, 2003). In addition, calcium 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 environment, some plants will form calcium oxalate crystal cells in order to fix excess Ca (Moore et al., 2002; Feng et al., 2010). Furthermore, an active calcium efflux system plays an important role in the adaptation of plants to high Ca environments (Bose et al., 2011): Excess Ca in plants is exported from mature leaves to the outside, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003). The adaptations relating to physiological processes mainly involve two aspects: the regulation of internal Ca storage and the control of Ca absorption and transport (Luo et al., 2012). 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 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), in order to regulate the concentration of cytoplasmic Ca^{2+} to a normal level. The normal growth of plants is maintained by the photosynthetic process and by respiration of the aboveground parts. Therefore, the regulation and control of the concentration of Ca^{2+} in the plant's aboveground parts is also key in adapting to a high Ca environment (Yin, 2006).

When the Ca content of soil is high, different plants adopt different adaptation strategies. By considering the survival differences of plant species in high Ca environments such as lime soil, Simpson (1938) and Tu (1995) divided plants into non-calcium plants and calcicole. The former is only distributed in acidic soils and other areas with low concentrations of Ca^{2+};

these plants are also known as calcifugous plants or calcifuges (Simpson, 1938; Tu, 1995). According to the level of calcicole dependence on high Ca environments, these plants can be further divided into non-specific calcicole and specific calcicole. Of these, specific calcicole are only found within a carbonate matrix and calcareous soil; furthermore, these plants are specific to the soil environment (Tu, 1995). Depending on their Ca content, non-specific calcicoles can be divided into calciphiles, calciphilous plants, Ca-indifferent plants (Tu, 1995; Zhou, 1997). The adaptability of plants to high Ca soil environments is related to their ability to absorb, transport and accumulate Ca (Ji et al., 2009).

The different plant functional groups revealed the differences in Ca content (Fig. 3). In some cases, even within the same plant, 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). The research conducted by Ji et al. (2009) was based on the differences in correlation between the Ca content of the aboveground parts of plants and its soil Ca content; these authors analyzed the capacity of plants to adapt to high Ca environments, and divided the dominated species into Ca-indifferent plants, high-Ca plants and low-Ca plants. 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, ~~followed by~~ and LRD. ~~Significant differences were detected for both soil ECa and TCa content when compared between the rocky side and non-rocky side of each grade of rocky desertification areas.~~ The Ca content in the aboveground parts of plants was significantly higher than that in the ~~underground~~belowground parts for the three grades of rocky desertification studied sites. ~~Furthermore, s~~Significant differences in Ca content were found between the aboveground and ~~underground~~belowground parts of each plant functional group~~(*p*<0.05)~~. The soil ECa content had a significant effect on the Ca content of the ~~underground~~belowground parts of plants, but had no significant effect on ~~the~~ Ca content of the aboveground parts. Ca-indifferent plants included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica*, ~~For these plants,~~ had a significant positive correlation existed between the Ca content in the aboveground or ~~underground~~belowground parts and the soil ECa content. High-Ca plants in our study were~~included~~ *Pyracantha fortuneana*, *Rhus chinensis*, *Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. ~~In this case,~~ The aboveground parts of these plants were able to absorb a high Ca content from various of ECa content soils. ~~the~~

~~aboveground parts of these plant were able to maintain a higher Ca content under conditions of variable soil ECa content.~~ 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 Ca content under conditions of variable soil ECa content.

5 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).

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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~~1~~. Soil TCa and ECa content from different grades of rocky desertification.

Ca typical (g kg^{-1})	Sample location~~point~~	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 32. 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~~3~~. 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 ~~underground~~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 54. Adaptation of plants to high Ca environments in rocky desertification areas.

Types of adaptation	Species	Characteristics of Ca~~calcium~~ content in plants	Strategies of plant adaptation to high Ca~~calcium~~ environments
Ca-indifferent plants	*Sanguisorba officinalis* *Castanea henryi* *Dendranthema indicum* *Themeda japonica*	There is significant positive correlation between the Ca~~calcium~~ content in the aboveground/~~underground~~belowground parts of plants and the soil ECa content. The coefficient of variation for Ca~~calcium~~ content in plants has a wide range.	Plants adapt to different Ca~~calcium~~ contents in soil through high Ca^{2+} buffering capacity. By regulating Ca^{2+} binding in Ca~~calcium~~ 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~~calcium~~ content in the aboveground parts of plants and the soil ECa content. The aboveground part has a high level of Ca~~calcium~~ content and the coefficient of variation falls within a narrow range.	Plants maintain high Ca~~calcium~~ content by enhancing Ca~~calcium~~ uptake and transporting it from ~~underground~~belowground to aboveground parts. High Ca~~calcium~~ 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~~calcium~~ content in the aboveground parts of plants and the soil ECa content. The aboveground part has a low level of Ca~~calcium~~ content and the coefficient of variation falls within a narrow range.	Plants maintain low Ca~~calcium~~ content in the aboveground parts by reducing Ca~~calcium~~ uptake and transporting it from ~~underground~~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 ~~underground~~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 ~~underground~~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 ~~underground~~belowground parts for the same functional groups ($p<0.05$); different upper-case letters represent significant differences among different functional groups, ($p<0.05$).~~ Annual herbs include annual and biennial herbs, while deciduous shrubs include deciduous trees with a height < 2 m or a ground diameter < 3 cm.~~

Fig. 4 Ca content in the aboveground and ~~underground~~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

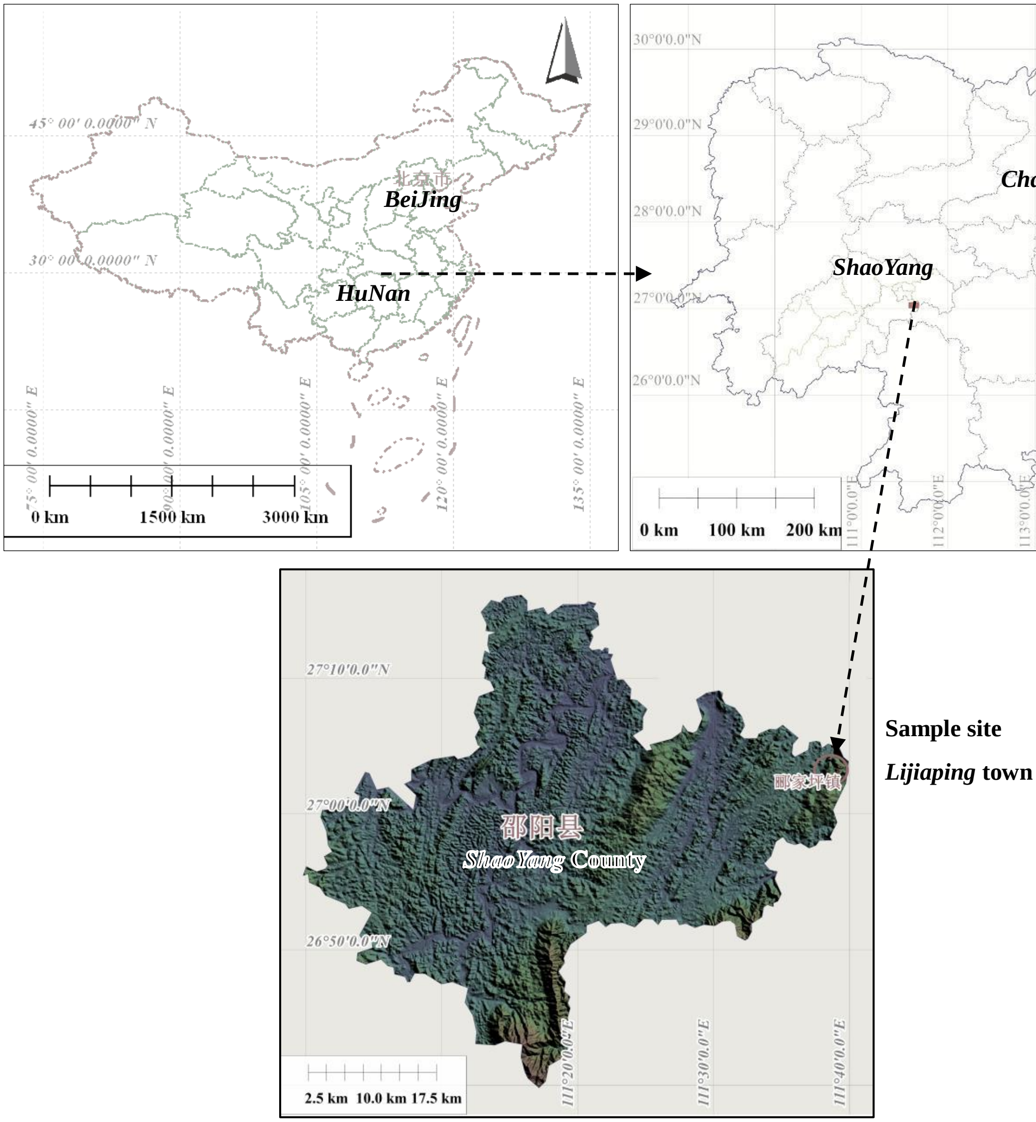

BeiJing
HuNan
45° 00′ 0.0000″ N
30° 00′ 0.0000″ N
0 km
1500 km
3000 km
ChangSha
ShaoYang
30°0′0.0″N
29°0′0.0″N
28°0′0.0″N
27°0′0.0″N
26°0′0.0″N
0 km
100 km
200 km
111°0′0.0″E
112°0′0.0″E
113°0′0.0″E
114°0′0.0″E
27°10′0.0″N
27°00′0.0″N
26°50′0.0″N
邵阳县
ShaoYang County
邮家坪镇
111°20′0.0″E
111°30′0.0″E
111°40′0.0″E
2.5 km 10.0 km 17.5 km
Sample site
Lijiaping town

Fig. 2

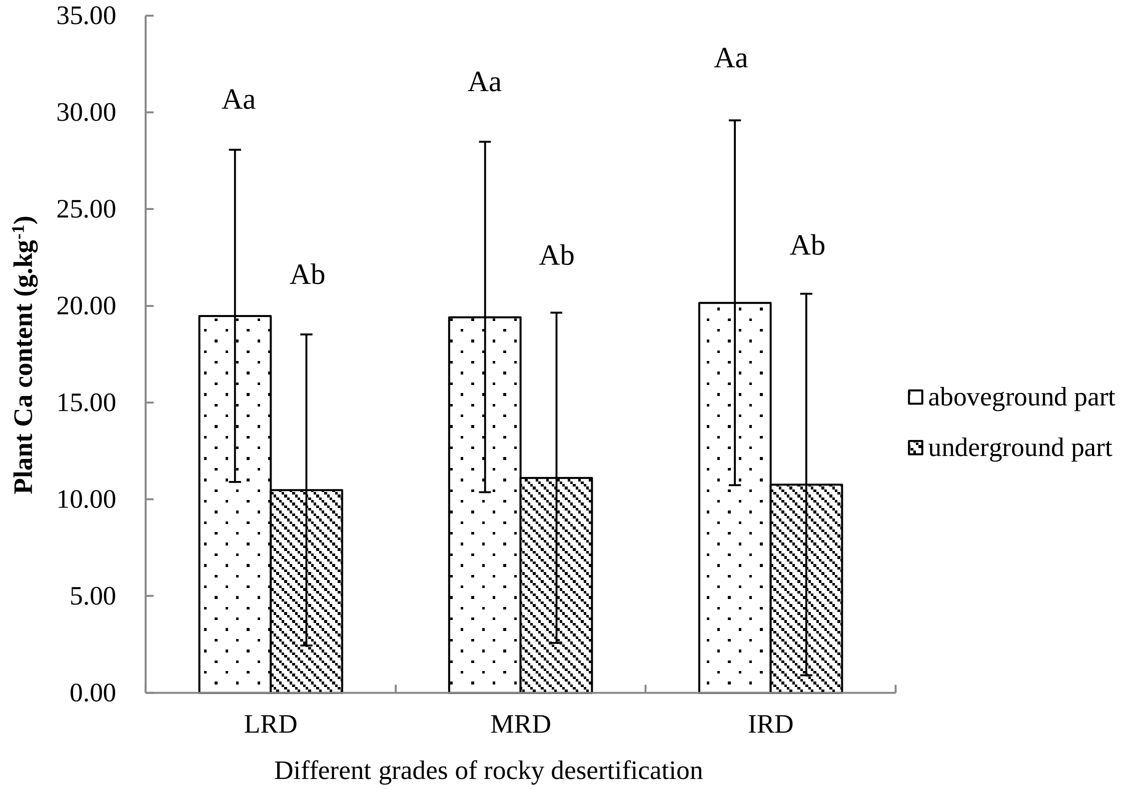

35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
Plant Ca content (g.kg-1)
Aa
Ab
Aa
Ab
Aa
Ab
LRD
MRD
IRD
Different grades of rocky desertification
aboveground part
underground part

Fig. 3

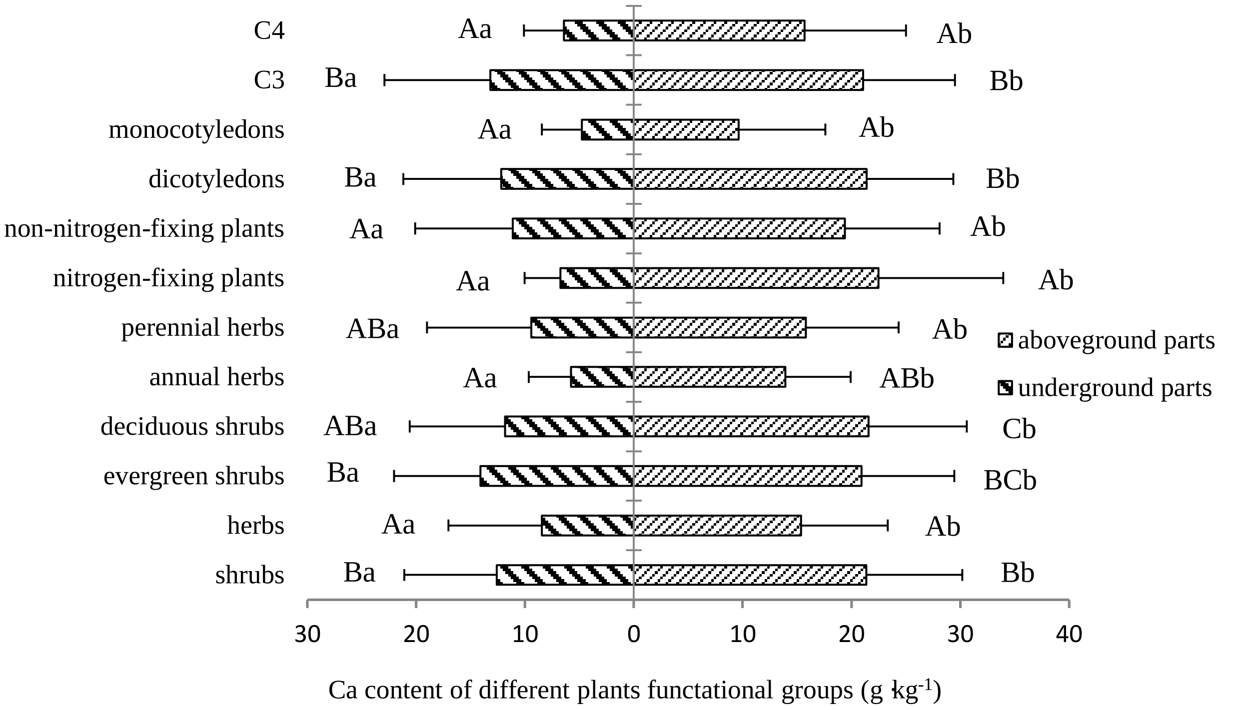
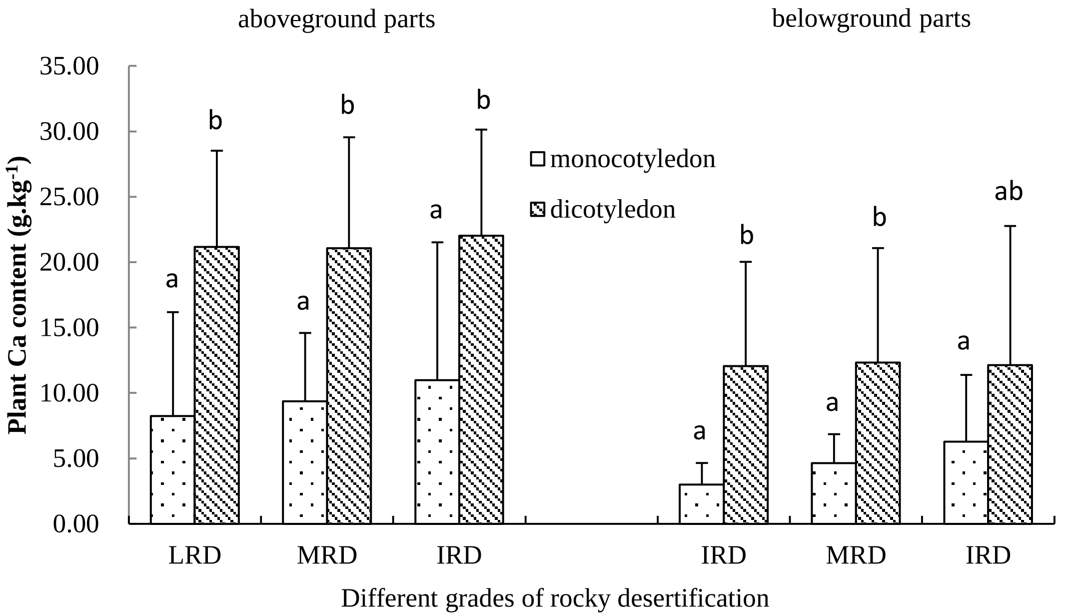

C4
C3
monocotyledons
dicotyledons
non-nitrogen-fixing plants
nitrogen-fixing plants
perennial herbs
annual herbs
deciduous shrubs
evergreen shrubs
herbs
shrubs
Aa
Ab
Ba
Bb
Aa
Ab
Ba
Bb
Aa
Ab
Aa
Ab
ABa
Ab
Aa
ABb
ABa
Cb
Ba
BCb
Aa
Ab
Ba
Bb
aboveground parts
underground parts
30
20
10
0
10
20
30
40
Ca content of different plants functational groups (g ·kg-1)

Fig. 4


aboveground parts
belowground parts
Plant Ca content (g.kg-1)
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
b
b
b
a
a
a
b
b
ab
a
a
a
monocotyledon
dicotyledon
LRD
MRD
IRD
IRD
MRD
IRD
Different grades of rocky desertification

Author contributions

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Competing interests

The authors declare that they have no conflict of interest.