Responses to the Comments

We highly appreciate the valuable comments and suggestions by the editor and anonymous reviewer 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. We have asked an English editor from '*The Charlesworth Author Services Team (http://www.charlesworthauthorservices.com/)*' to improve the language. Hope this version will meet the requirements of BG.

Part 1: Point-by-point response to Comments by the Editor (from attachment file)

Note 1: suppress in

Re.1 Thanks for the positive suggestion. Done!

Note 2: This sentence is vague and seems a repetition of sentence of line 9. Could you explain more specifically the socioeconomic impact of this degradation

Re.2 Thanks for your suggestion. We rewrote this sentence as: "Rocky desertification could lead to frequent natural disasters, reduce human survival and development space, threaten local people's production, life and life safety, cause ecological deterioration, reduce arable land resources, aggravate poverty, and affect sustainable economic and social development (Jing et al., 2016). In other words, Rocky desertification is an extreme form of land degradation in karst areas, and it has become a major social problem in terms of China's economic and social development (Sheng et al., 2015).".

Note 3: what do you want to say here with "From the origin of desertification". It could be nice if you better explain the origin of this degradation. Is it due to an intensive cultivation of soils? or due to an urbanisation of this area? Climatic origin?

Re.3 Sorry for the puzzle. We added new content to explain the origin of rock desertification degradation. They are "Given the origin of rocky desertification, the main factors that lead to rocky desertification are unreasonable human activities (reclamation on steep slope), causing damage to vegetation and exacerbating rocky desertification; its remediation should focus on vegetation restoration (Wang et al., 2004)."

Note 4: this part of sentence can be more synthetic: Ca^{2+} is an essential nutrients for plant growth and participate to....

Re.4 Thanks. Done ! .

Note 5: I do not understand the link between the different ideas of your sentence. I think you are talking about coupling between nutrient cycles, but this is not understandable. Develop your ideas

Re.5 Sorry for this unclearly expression. We added content in order to make it easier to understand. They are "And Ca^{2+} is a very important signal component in plants responsive to environmental stresses. Ca^{2+} signal takes the influential role as a second messenger in hormone signal transduction, particularly in the abscisic acid signal transduction process (Hetherington, et al, 2004). Plants can adapt to high salt, drought and high temperature environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998). Ca^{2+} is also involved in nutrient cycling coupling process, for example, 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)."

Note 6: we imagine that the association of pectin and Ca gives pectin-Ca...no need to write that. However, it might be important to specifiy which cell functions is controlled by this compound.

Re.6 Thanks for your suggestion. We changed them as: "Ca^{2+} combines with pectin in the cell walls of plants to form pectin Ca, which is a vital component of the intercellular layer in cell wall, and can buffer the compression between cells without hindering the expansion of cell surface area (Kinzel, 1989)."

Note 7: suppress "the"

Re.7 Thanks. Done. And this sentence was moved ahead.

Note 8: This looks like the description of Ca function in cell physiology. This should maybe transferred in the previous paragraph. This reference to signal transduction is not enough supported by explanation. Why Ca is important for rignal transduction?

Re.8 Thanks for the positive suggestion. Changed and moved to the "Role of Ca^{2+} in plant physiology:" part.

Note 9: it seems this is an important process. Please take time to present it

Re.9 Yes. But moved to the paragraph of "Role of Ca^{2+} in plant physiology:"

Note 10: the release in (Ca goes into the vacuole) or from (Ca flows out)? Please take your time to well explain

Re.10 Yes. There are Ca^{2+} channels Ca^{2+} pump and Ca^{2+}/H^{+} reverse conveyor on tonoplast. The former controls Ca^{2+} outflow, and the latter two pump cytoplasmic Ca^{2+} into vacuole (Wu, 2008).

Note 11: There are not much informations in the different specific variability in plant Ca concentration and tolerance. Nothing is known about which species are tolerant? and by which mechanisms!? I do not believe that

Re.11 Thanks for the positive suggestion. The following are information we added:

"Plants maintain low calcium content in aboveground part by reducing calcium uptake and transporting from underground part to aboveground part. This type of plant has *Nephrolepis auriculata, Parathelypteris glanduligera, Cyrtomium fortunei*, *Pteris vittata*, and so on. In contrast, other plants have a higher demand for calcium. For example, *Cayratia japonica* and *Corchoropsis tomentosa*. these plants maintain high calcium content by enhancing calcium uptake and transporting from underground to aboveground (Ji et al., 2009)."

Note 12: But this title is a repetition of the same idea developped in the paragraph "Mechanism of plant defense to high soil Ca concentration". You should merge the redondant paragraphs (or find another way to present your ideas if you really think it's different ideas).

Re.12 Thanks, very good suggestions! We merged them.

Note 13: cristals in cristals? this seems weird

Re.13 Sorry for the puzzling expression, and we have corrected it .

Note 14: this is a repetition of the previous sentence, merge these two sentences

Re.14 Thanks. Done.

Note 15: exported where? Do you mean that plant concentrate ca in mature leave?

Re.15 Sorry. Our means are: "When the leaves matured, excess Ca^{2+} in plants is excreted via stomata on the back of the leaves, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003)."

Note 16: what do you mean by growth habits? growth rate?

Re.16 Sorry for the ambiguous word. It was growth conditions.

Note 17: I am not sure to understand your use of word species. Are they different species between these two soils (calcareous and acidic soils). If yes why do you not insert the name of these species. Maybe you mean different ecotypes of the same species?

Re.17 Sorry for the confusing. And those sentences are:

"Qi et al. (2013) found that a significant difference in calcium content among *Primulina* species (*P. linearifolia, P. medica, P. swinglei, P. verecunda, P. obtusidentata, P. heterotricha,* and so on) from different soil types. and the average Ca content (2,285.6 mg/kg) in *Primulina* from calcareous soil was higher than the average calcium content of *Primulina* from both acid soil (1,379.3 mg/kg) and Danxia red soil (1,329.1 mg/kg)."

Note 18: I do not really understand the message of your sentence: do you mean that differences among plant species in term of strategy to regulate Ca concentration in cell and capability to adapt to high soil Ca offer opportunity to identify the most relevant plants for the restoration of karst area? If yes you should better explain this idea.

Re.18 I am sorry for the puzzling expression. This sentence should be

"There are variations in soil Ca content among different areas. And there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport, storage and other physiological processes. These differences need to be taken into account in order to identify the variety of plants able to adapt to high-Ca environments. However, to date, 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."

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

Abstract:

1. P1L10: changed "Its" into "In these areas, the".
2. P1L11-12: changed "which" into "that", and deleted "in such rocky desertification areas".
3. P1L12-13: changed "Consequently, the screening of plant species, which can adapt to soil high Ca^{2+} environments, is a critical step for vegetation restoration." into "Consequently, the screening of plant species that can adapt to high Ca^{2+} soil environments is a critical step in vegetation restoration.".
4. P1L13-15: changed "In this study, three different grades of rocky desertification sample areas (LRD, light rocky desertification; MRD, moderate rocky desertification; IRD, intense rocky desertification) were selected in karst areas of southwestern Hunan, China." into "In this study, three grades of rocky desertification sample areas were selected in karst areas of southwestern Hunan, China (LRD: light rocky desertification; MRD: moderate rocky desertification; and IRD: intense rocky desertification)." .
5. P1L16-17: changed "(1 in rocky side areas, 3 in non-rocky side areas)" into " (1 in rocky-side areas, 3 in non-rocky-side areas)".

6. P1L18: changed “exchange” into “exchangeable”, changed “under” into “in”.
7. P1L24-P2L3: changed “According to the differences in Ca^{2+} content between the aboveground and belowground parts of 17 dominant species (important value , *IV*>1) and their correlations with soil ECa content, these 17 species can be divided into three categories: Ca-indifferent plants, high-Ca plants and low-Ca plants.” into “Of the 41 plant species that were sampled, 17 were found to be dominant(important value >1). The differences in Ca^{2+} content between the aboveground and belowground parts of the 17 dominant species were calculated, and their correlations with soil ECa content were analyzed. The results showed that these 17 species can be divided into three categories: Ca-indifferent plants, high-Ca plants and low-Ca plants.”.
8. P2L3: changed “Our results” into “These findings”; changed “provides” into “provide”.

1 Introduction

9. P2L7: changed “Karst is a kind of typical calcium (Ca)-rich environment and a unique ecological environment system” into “Karst is a calcium-rich environment and a unique ecological system”
10. P2L8: changed “world” into “world’s”.
11. P2L9-11: deleted “Rocky desertification is an extreme form of land degradation in karst areas, and it has become a major social problem in terms of China’s economic and social development (Sheng et al., 2015).”.
12. P2L11-12: Changed “The severity of rocky desertification was ranked in fourth in Hunan Province of China” into “The Hunan Province of China has been ranked fourth for the severity of rocky desertification”.
13. P2L12-16: add this sentence “Rocky desertification could lead to frequent natural disasters, reduce human survival and development space, threaten local people's production, life and life safety, cause ecological deterioration, reduce arable land resources, aggravate poverty, and affect sustainable economic and social development”, and deleted “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”
14. P2L17-18: added “In other words, Rocky desertification is an extreme form of land degradation in karst areas, and it has become a major social problem in terms of China’s economic and social development (Sheng et al., 2015).”, after “(Jing et al., 2016).”.
15. P2L18: Changed “high calcium Ca” into “high calcium (Ca)”.
16. P2L20: change “From” into “Given”.
17. P2L20-22: add “the main factors that lead to rocky desertification are unreasonable human activities (reclamation on steep slope), causing damage to vegetation and exacerbating rocky desertification;” after “desertification,”.
18. P2L23: changed “which” into “that”.
19. P3L2-4: added “Over recent decades, 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 “in plant physiology.”.
20. P3L4-5: deleted “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”; and add “Ca^{2+} is an essential nutrients for plant growth and also participate to a transduction” before “(Poovaiah and Reddy, 1993; Hepler, 2005; Hong-Bo and Ming, 2008; Batistič and Kudla, 2012)”.
21. P3L6-10: added “And Ca^{2+} is a very important signal component in plants responsive to

environmental stresses. Ca^{2+} signal takes the influential role as a second messenger in hormone signal transduction, particularly in the abscisic acid signal transduction process (Hetherington, et al, 2004). Plants can adapt to high salt, drought and high temperature environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998). Ca^{2+} is also involved in nutrient cycling coupling process, for example," after "(Poovaiah and Reddy, 1993; Hepler, 2005; Hong-Bo and Ming, 2008; Batistič and Kudla, 2012)."

22. P3L10: "changed ". In' into "in".
23. P3L13-14: Added "the intercellular layer in cell wall, and can buffer the compression between cells without hindering the expansion of cell surface area" after "of"; deleted "the cell wall".
24. P3L17-19: deleted "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).".
25. P3L20-22: deleted "Plants can be adapted to high salt, drought and high temperature environments by activating the Ca2+ signal transduction pathway (Bressan et al., 1998).".
26. P4L2-12: added ". Some plants fix excess Ca2+ by forming calcified deposits in root tissue in order to limit the upward transport of Ca2+ (Musetti and Favali, 2003). In addition, Ca oxalate crystals in the plant cells play a role in regulating plant Ca content (Ilarslan et al., 2001; Pennisi and McConnell, 2001; Volk et al., 2002), and some plants will form Ca oxalate crystal cells in order to fix excess Ca2+ (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). For example, when the leaves matured, excess Ca2+ in plants is excreted via stomata on the back of the leaves, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003). The regulation of internal Ca storage depends predominantly on plasma membrane Ca transport and intracellular Ca storage; collectively these processes can regulate the intracellular Ca2+ concentration to a lower level (Bowler and Fluhr, 2000). Plants that adapt to high-Ca environments promote excess Ca2+ flow through the cytoplasm or store Ca2+ in vacuoles via the cytoplasmic Ca2+ outflow and influx system (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2006)." after "means of Ca storage (Ranjev et al., 1993).".
27. P4L14-16: Added "There are Ca^{2+} channels Ca2+ pump and Ca^{2+}/H^{+} reverse conveyor on tonoplast. The former controls Ca^{2+} outflow, and the latter two pump cytoplasmic Ca^{2+} into vacuole (Wu, 2008)." After "……the release of Ca^{2+} in vacuoles (Peiter, 2011)."
28. P4L18: added "the" before "cytoplasm combines".
29. P4L19: added "the" before "phosphorus metabolism".
30. P4L20-P5L1: added "Plants maintain low calcium content in aboveground part by reducing calcium uptake and transporting from underground part to aboveground part. This type of plant has *ephrolepis auriculat*, *Parathelypteris glanduligera*, *Cyrtomium fortunei*，*Pteris vittata*，and so on. In contrast, other plants have a higher demand for calcium. For example, *Cayratia japonica* and *Corchoropsis tomentosa*. these plants maintain high calcium content by enhacing calcium uptake and transporting from underground to aboveground." after "…. growth (White and Broadley, 2003; Hirschi, 2004).".
31. P5L2-12: deleted "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 depends 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).".

32. P5L13: changed "ECa" into "exchangeable Ca^{2+} (ECa)".
33. P5L15: changed "TCa" into "total Ca^{2+} (ECa)".
34. P5L15: changed "area were" into "areas was".
35. P5L16: changed "habits" into "conditions".
36. P5L20-24: deleted "that". Changed "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 was *Primulina* from both acid soil (1,379.3 mg/kg) and Danxia red soil (1,329.1 mg/kg)." into "Qi et al. (2013) found that a significant difference in calcium content among *Primulina* species (*P. linearifolia, P. medica, P. swinglei, P. verecunda, P. obtusidentata, P. heterotricha,* and so on) from different soil types. and the average Ca content (2,285.6 mg/kg) in *Primulina* from calcareous soil was higher than the average calcium content of *Primulina* from both acid soil (1,379.3 mg/kg) and Danxia red soil (1,329.1 mg/kg).".
37. P5L24-P6L4: changed "There are variations in soil Ca content among different areas and differences between calcareous and non-calcareous plants in terms of Ca absorption, transport and storage and other physiological processes. These differences need to identify the variety of the plants to adapt with high Ca environments. However, to data, the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas and the Ca^{2+} dynamics of plants and soil are not well understood." into 'There are variations in soil Ca content among different areas. And there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport, storage and other physiological processes. These differences need to be taken into account in order to identify the variety of plants able to adapt to high-Ca environments. However, to date, 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.".
38. P6L8: changed "rocky and non-rocky side areas" into "rocky-side and non-rocky-side areas"

2 Materials and methods

39. P6L13: changed "is" into "was". And deleted "of" added "in".
40. P6L14: deleted "; see Fig. 1", and added ", as shown in Fig.1" after bracket ")".
41. P6L20: changed "expose" into "exposure".
42. P6L21: added ". These index" before "were quantified".
43. P7L1-2: deleted "LRD,", and added "(LRD)" after "light rocky desertification".
    deleted "MRD,", and added "(MRD)" after "moderate rocky desertification".
    changed "LRD," into "and", and added "(IRD)" after "intense rocky desertification".
44. P7L3: changed "," into "which", changed "including" into "included".

45. P7L4: changed "The sample collection of samples in these three sample areas were conducted in October 2016." into "The collection of samples in these three sample areas was conducted in October 2016.".
46. P7L11: changed "we measured the TCa and ECa relating to the quadrat soil" into "we measured the soil TCa and ECa content of each quadrat".
47. P7L18: changed "The biennial herbs were gathered to the 'Annual herbs'. The deciduous" into "Biennial herbs were gathered to the 'annual herbs'. Deciduous".
48. P7L20-21: added "analysis of variance" after "two-way".
49. P7L21: Deleted "these"; and added "widespread" after "17".
50. P7L22: deleted "analysis of variance"; and changed "(ANOVA)" into "ANOVA".

3 Results

51. P8L6: changed "that the" into "to be".
52. P8L7: changed "followed by" into "then".
53. P8L8-10: changed "Regarding the availability of Ca, the average Ca content was 59.75%, the MRD showing the highest content at 72.55%, followed by IRD at 58.98%, and LRD showing the lowest content at 47.72 % (Table. 2)." into "Regarding the availability of Ca, the average availability of Ca was 59.75%, with the MRD showing the highest value at 72.55%, followed by IRD at 58.98%, and LRD showing the lowest value at 47.72 % (Table. 2)."
54. P8L13: changed "Total of" into "A total of".
55. P8L14: deleted "Compare to t"; and added "T".
56. P8L17: deleted "of the plants were", and added "s showed".
57. P9L4: deleted "than" into "compared to".
58. P9L17: Changed "Within total 41 common plants species" into "Within the total of 41 common plant species".
59. P9L19: added "differences of" after "Data showed that the".
60. P9L21-22: changed "The Ca content in the belowground parts were highly significant difference not only among the species, and it throughout all the grades of rocky desertification ($p<0.01$)." into "Differences in the Ca content of the belowground parts were highly significant not only among species, but throughout all the grades of rocky desertification ($p<0.01$).".
61. P10L2: deleted "T", and added "Of t".
62. P10L6: deleted "T", added "With regard to t"; and changed "of" into "in".
63. P10L8: deleted "soil'; and added "soil" before "environments".
64. P10L14-15: Changed "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." into "These plants did not exercise a strict control over the absorption and transport of Ca and may be insensitive to changes in their own Ca content. Moreover, their growth was less affected by soil Ca content.".
65. P10L15-17: changed "In addition," into "As", and added "the" after "for"; deleted ", then T", and added "T", and added "then" after "were".

4 Discussion

66. P11L3: added "also" after "the Ca content of soil". And changed "indicated" into "indicates".
67. P11L11-13: changed "affecting" into "for the different of";
deleted "mean", and added "ed that the mean" after "Our research show";
changed "of" into "in"; changed "expose" into "exposure".

68. P11L16: changed “But” into “However”; added “the” before “aboveground parts of plants (19.67 $g·kg^{-1}$)”.
69. P11L21: added “detected” after “value of 85.13 $g·kg^{-1}$”.
70. P11L21-P12L1: Changed “To most plant Ca content, the aboveground part was larger than the belowground part, and for a few plants Ca content, the aboveground part was lower than the belowground part (such as *Sanguisorba officinalis*, *Pyracantha fortuneana* and *Castanea henryi*), which was consistent with the findings of Wang et al. (2014).” into “For most plants, the Ca content in the aboveground part was higher than in the belowground part, but for a few plants the Ca content in the aboveground part was lower than in the belowground part (such as *Sanguisorba officinalis*, *Pyracantha fortuneana* and *Castanea henryi*), which was consistent with the findings of Wang et al. (2014).
71. P12L4: changed “had” into “which showed”.
72. P12L6: changed “, and” into “. Additionally,”.
73. P12L7-8: Deleted “for the difference between our finds and their finds” and “, which”, and added “. The difference between the findings of these studies and ours” before “may be caused by species factors”.
74. P12L13: added “also” before “showed a significant positive”.
75. P12L20: changed “, which was supported with data reported by Ji et al. (2009).” into “. This finding is supported by data reported by Ji et al. (2009).”
76. P12L21-P13L3: changed “. And this result indicated that nitrogen-fixing plants were the most efficient in the Ca upward transport, since the transport of Ca was mainly upward; which was not the same with those of Ji et al. (2009). Ji et al. (2009) revealed that dicotyledons were the most efficient at the upward transport of Ca. They used three types of plants (pteridophytes, dicotyledons, monocotyledons) exclude nitrogen-fixing plants in their study, which may have a conflicting result compared with our current findings.” into “, and this result indicates that nitrogen-fixing plants were the most efficient in Ca upward transport. In contrast, Ji et al. (2009) found that dicotyledons were the most efficient in the upward transport of Ca. They used only three types of plants (pteridophytes, dicotyledons, and monocotyledons) without researching nitrogen-fixing plants in their study, which may have produced a conflicting result compared with our current findings.”.
77. P13L4: added “the” before “Ca content of monocotyledons in our study.”.
78. P13L4: added “there are” after “Ji et al. (2009) revealed that”.
79. P13L6: changed “ may contribute” into “could be due to”.
80. P13L16: changed “could be due to” into “may contribute”.
81. P13L9: deleted “individual”.
82. P13L14-15: changed “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.” into “these findings show that not all plants adapted to high Ca soil environments in the same way, but rather exhibited a variety of adaptive mechanisms.”.
83. P13L17-18: changed “which was” into “that are”.
84. P13L20-21: changed “ 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).” into “Thus, based on these two indicators, plants can be placed into the following groups: Ca-indifferent plants, high-Ca plants, and low-Ca plants (Ji et al., 2009).”.

85. P13L22: Changed “our plants species” into “the 17 plant species”; changed “, which” into “that”.
86. P14L1-2: Changed “survival” into “survive”.
    Changed “. And the Ca content of them changes correspondingly with the change of soil ECa content.” into “, and their Ca content changes correspondingly with changes in soil ECa content.”
87. P145-L6: Deleted “selected”, and added “selected” before “preferentially”.
88. P14L7-8: changed “Low-Ca plants also have a strong adaption ability on high calcium environments,” into “Low-Ca plants also have a strong ability to adapt to high calcium environments,”.
89. P14L7-8: Changed “Low-Ca plants also have a strong adaption ability on high calcium environments,” “Low-Ca plants also have a strong ability to adapt on high calcium environments,”. And deleted “it”.
90. P14L9-10: deleted “guiding”, and changed “solving” into “guiding solutions to”.
91. P14L11: added “ecosystem restoration in” after “but also provide species screening ideas for”, deleted “the”, changed “ecosystem restoration” into “areas”.
92. P14L12: added “, and further explorations are required to solve this problem.” after “…a major ecological problem in karst areas”.
93. P14L14: changed “the study of” into “understanding”.

5 Conclusions

94. P14L16: changed “indicated” into “indicate”.
95. P14L16: changed “and” into “then”.
96. P14L18: deleted “studied sites”, and added “sites in our study “.
97. P14L19: added “plants in” before “each plant functional group.”.
98. P14L20: Deleted “,” and “had”, and added “the” before “Ca content of the aboveground parts.”
99. P14L21: Added “, which” after “Ca-indifferent plants”.
100. P14L22: changed “had” into “showed”, and deleted “existed”.
101. P15L1-2: changed “The aboveground parts of these plants were able to absorb a high Ca content from various of ECa content soils.” into “The aboveground parts of these plants were able to absorb a lot of Ca from soils with varying ECa content.”.
102. P15L3-4: Changed “The aboveground parts of low-Ca plants were able to maintain a lower Ca content under conditions of variable soil ECa content.” into “The aboveground parts of low-Ca plants were able to maintain a lower Ca content from soils with varying ECa content.”.
103. Table 1, changed “expose” into “exposure”, and changed “rarely grazing” into “rarely grazed”
104. Table 2, deleted “D”, added “The d”.
105. Table 3, deleted all “(IV), and changed “are’ into “is”.
106. References were adjusted in order.

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. In these areas, the ~~Its~~ high soil calcium (Ca) content has become an important environmental factor ~~which~~ that can affect the restoration of vegetation ~~in such rocky desertification areas~~. Consequently, the screening of plant species~~,~~ that~~which~~ can adapt to ~~soil~~ high Ca soil environments~~,~~ is a critical step in~~for~~ vegetation restoration. In this study, three ~~different~~ grades of rocky desertification sample areas were selected in karst areas of southwestern Hunan, China (LRD:~~,~~ light rocky desertification; MRD:~~,~~ moderate rocky desertification; and IRD:~~,~~ intense rocky desertification) ~~were selected in karst areas of southwestern Hunan, China~~. Each grade of these sample areas had 3 sample plots in different slope positions, each of which had 4 small quadrats (1 in rocky-side areas, 3 in non-rocky-~~–~~side areas). We measured the Ca content of leaves, branches and roots from 41 plant species, as well as soil total Ca (TCa) and exchangeable Ca (ECa) at depths of 0–15, 15–30 and 30–45 cm in~~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 content of aboveground parts was significantly higher than that of the belowground parts (p<0.05). The soil ECa content had significant effects on plant Ca content of the belowground parts~~,~~ but had no significant effects on plant Ca content of the aboveground parts. Of the 41 plant species that were sampled ~~in total~~, 17 were found to be dominant (important value >1). T~~According to t~~he differences in Ca^{2+} content between the

aboveground and belowground parts of the 17 dominant species were calculated~~(important value, *IV*>1)~~, and their co~~ and their co~~rrelations with soil ECa content were analyzed. The results showed that~~,~~ these 17 species can be divided into three categories: Ca-indifferent plants, high-Ca plants, and low-Ca plants. These findings~~Our results~~ provide~~s~~ a vital theoretical basis and practical guide for vegetation restoration and ecosystem reconstruction in rocky desertification areas.

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

1 Introduction

Karst is a~~ kind of typical~~ calcium~~ (Ca)~~-rich environment and a unique ecological~~ environment~~ system. This type of ecosystem is widely distributed, accounting for 12% of the world's total land area (Zeng et al., 2007; Zhou et al., 2009; Luo et al., 2012). Karst landforms in China are mainly distributed in southwestern areas.~~ Rocky desertification is an extreme form of land degradation in karst areas, and it has become a major social problem in terms of China's economic and social development (Sheng et al., 2015).~~ The Hunan Province of China has been~~was~~ ranked fourth ~~for~~in t~~T~~he severity degree of rocky desertification ~~was ranked in fourth in Hunan Province of China~~ (Li et al., 2016). Rocky desertification could lead to frequent natural disasters, reduce human survival and development space, threaten local people's production, life and life safety, cause ecological deterioration, reduce arable land resources, aggravate poverty, and affect sustainable economic and social development ~~The restoration and reconstruction of rocky desertification ecosystems has become the urgent environment improvements, regional economic development by using agroforestry system and helping to support people out of poverty~~(Jing et al., 2016). In other words, Rocky desertification is an extreme form of land degradation in karst areas, and it has become a major social problem in terms of China's economic and social development (Sheng et al., 2015). Soil with high calcium (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). Given~~From~~ the origin of rocky desertification, the main factors that lead to rocky desertification are unreasonable human activities (reclamation on steep slope), causing damage to vegetation and exacerbating rocky desertification; its remediation should focus on vegetation restoration (Wang et al., 2004). Consequently, the screening of plant species that~~which~~ can grow successfully in high-Ca environments in rocky desertification areas is an extremely critical step.

Role of Ca^{2+} in plant physiology: Over recent decades, 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 one of the most essential nutrients needed for the regulation of plant growth and is also plant signal sensor (second messengunder conditions of environmentCa^{2+} is an essential nutrients for plant growth and also participate to signal transduction (Poovaiah and Reddy, 1993; Hepler, 2005; Hong-Bo and Ming, 2008; Batistič and Kudla, 2012). And Ca^{2+} is a very important signal component in plants responsive to environmental stresses. Ca^{2+} signal takes the influential role as a second messenger in hormone signal transduction, particularly in the abscisic acid signal transduction process (Hetherington, et al, 2004). Plants can adapt to high salt, drought and high temperature environments by activating the Ca^{2+} signal transduction pathway (Bressan et al., 1998). Ca^{2+} is also involved in nutrient cycling coupling process, for example, .Iin the absence of nutrients (such as phosphorus), plants will inhibit the activity of nitrate reductase, thereby inhibiting the absorption of nitrate nitrogen, and ultimately inhibiting the absorption of Ca^{2+} (Reuveni et al., 2000). Ca^{2+} combines with pectin in the cell walls of plants to form pectin Ca, which is a vital component of the intercellular layer in cell wall, and can buffer the compression between cells without hindering the expansion of cell surface area 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). The plant cell not only rapidly increases the free Ca^{2+} concentration of the cytoplasm to adapt to environmental changes, but also maintains a low Ca concentration to prevent harm caused by high Ca. This fine regulatory mechanism is mainly achieved by Ca^{2+} channels (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2005). The vacuoles may account for 95% of the plant cell volume and are able to store Ca^{2+} within the cell. Thus, empty vacuoles represent an efficient means of Ca storage (Ranjev et al., 1993). 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 cells play a role in regulating plant Ca content (Ilarslan et al., 2001; Pennisi and McConnell, 2001; Volk et al., 2002), and 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). For example, when the leaves matured, eExcess Ca^{2+} in plants is excreted exported from mature leaves to the outsidevia stomata on the back of the leaves, thereby maintaining a lower concentration of leaf Ca (Musetti and Favali, 2003). The regulation of internal Ca storage depends predominantly 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).

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). There are Ca^{2+} channels Ca^{2+} pump and Ca^{2+}/H^{+} reverse conveyor on tonoplast. The former controls Ca^{2+} outflow, and the latter two pump cytoplasmic Ca^{2+} into vacuole (Wu, 2008). Cytoplasmic Ca^{2+} is mainly combined with proteins and other macromolecules. ; Tthe 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 the cytoplasm combines with phosphate to form a precipitate, which interferes with the physiological processes associated with the phosphorus metabolism, thus hindering normal signal transduction and causing significant detriment to plant growth (White and Broadley, 2003; Hirschi, 2004). Plants maintain low calcium content in aboveground part by reducing calcium uptake and transporting from underground part to aboveground part. This type of plant has *Nephrolepis auriculata*, *Parathelypteris glanduligera*, *Cyrtomium fortunei*， *Pteris vittata*， and so on. In contrast, other plants have a higher demand for calcium. For example, *Cayratia japonica* and *Corchoropsis tomentosa*. these plants maintain high calcium content by enhancing calcium uptake and transporting from underground to aboveground (Ji et al., 2009).

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 depends predominantly depends on plasma membrane Ca transport and intracellular Ca storage; collectively these processes can regulate the intracellular Ca^{2+} concentration to a lower level (Bowler and Fluhr, 2000). Plants that adapt to high-Ca environments promote excess Ca^{2+} flow through the cytoplasm or store Ca^{2+} in vacuoles via the cytoplasmic Ca^{2+} outflow and influx system (Shang et al., 2003; Hetherington and Brownlee, 2004; Wang et al., 2006).

The mean soil exchangeable Ca (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 total Ca (TCa) content in calcareous soil areas wasre above 14.0 mg g^{-1}. Zhang (2005) studied the growth habitsconditions 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+} concentrations 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 (*P. linearifolia*, *P. medica*, *P. swinglei*, *P. verecunda*, *P. obtusidentata*, *P. heterotricha*, and so on) from different soil types, with highand the average calciumCa content (2,285.6 mg/kg) in *Primulina* from calcareous soil relative to low levels present in was higher than the average Ca content of *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 And there are differences between calcareous and non-calcareous plants in terms of Ca absorption, transport~~ and~~, storage and other physiological processes. These differences need to be taken into account in order to identify the variety of~~ the~~ plants able to adapt to~~with~~ high Ca environments. However, to date~~a~~, the mechanisms by which plants adapt to high Ca conditions, particularly in karst areas, and the Ca dynamics of plants and soil are not well understood.

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

2 Materials and methods

2.1 Site description

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

2.2 Experimental design and data collection

Rocky desertification was graded by using the sum of four index scores: bedrock exposure~~e~~ rate, vegetation type, vegetation coverage and soil thickness. These index 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 (LRD); ~~MRD,~~ moderate rocky desertification (MRD); and~~IRD,~~ intense rocky desertification (IRD). Within each sample area, we recorded environmental factors~~,~~ which included~~ing~~ longitude, latitude, altitude, topography, vegetation type, degree of bare bedrock, and other conditions. The~~ sample ~~collection of samples in these three sample areas w~~e~~as~~re~~ conducted in October 2016.

Within each of the three sample areas, four (2×2) small quadrats in different slope positions (upper, middle, and lower slope) were set up. In total, we assigned 36 small quadrats (3×4×3) for analysis. The common plant species of the region were gathered using the whole plant harvest method in each small quadrat, as well as shrubs and herbs were collected. Shrubs were divided into three parts: branches, leaves and roots. Herbs were divided into two parts: aboveground and belowground. Plant samples were taken back to the laboratory, rinsed with distilled water before being oven dried at 105 ℃ for 15 min to de-enzyme, and then dried to a constant weight at 80 ℃ about 480 minutes, crushed and passed through a 0.149 mm sieve for later chemical analysis. For the soil samples, we measured the soil TCa and ECa content of each~~relating to the~~ quadrat~~ soil~~ (top soil: 0-15 cm; middle soil: 15-30 cm; bottom soil, 30-45 cm). Soil TCa, ECa content and plant Ca content were measured using an Atomic Absorption Spectrophotometer (3510, Shanghai, China).

2.3 Data analysis

All plant species were divided into different functional groups: (1) nitrogen-fixing plant~~s~~ and non-nitrogen-fixing plant~~s~~ groups according to nitrogen-fixing function; (2) dicotyledons and monocotyledons groups according to system development type; (3) C3 and C4 plant~~s~~ groups according to photosynthetic pathway; and (4) deciduous shrubs, evergreen shrubs, annual herbs and perennial herbs according to life form. B~~The b~~iennial herbs were gathered to the 'a~~A~~nnual herbs'. D~~The d~~eciduous 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 the aboveground part, while the belowground part only included roots. We carried out two-way analysis of variance (ANOVA) for both species and soil for~~ these~~ 17 widespread 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. 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 the mean ECa content was 1.46 g kg^{-1} (range: 0.02–3.92 g kg^{-1}). Differences between different sample~~s~~ locations (non-rocky side and rocky side) were significant (p<0.05) for both TCa and ECa. The mean soil TCa and ECa content were found to be~~that the~~ highest in areas of IRD, followed by MRD, then~~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 availability of Ca ~~content~~ was 59.75%, with the MRD showing the highest value~~content~~ at 72.55%, followed by IRD at 58.98%, and LRD showing the lowest value~~content~~ at 47.72 % (Table. 2).

3.2 The Ca content of plants

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

A t~~T~~otal of 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}). T~~Compare to t~~he mean Ca content of the belowground parts was 10.79 g kg^{-1} (range: 4.41–33.62 g kg^{-1}). The Ca content of the aboveground parts was significantly higher than that of the belowground parts (p<0.05) throughout the same grades of rocky desertification~~. Whether~~, but the Ca content of aboveground and belowground parts showed~~ of the plants~~ ~~were~~ no significant differences (p>0.05) among the three different grades of rocky desertification (Fig. 2).

3.2.2 Ca content in different plant functional groups

The 41 plant species were identified and~~ were~~ divided into different functional groups in the 36 small quadrats. The Ca content of the aboveground parts was significantly higher than that of the belowground parts in each group (p<0.05). Nitrogen-fixing plants (22.48 g kg^{-1}) showed a slightly higher Ca content in the aboveground parts compared to~~than~~ non-nitrogen-fixing plants (19.39 g kg^{-1}; p>0.05), although Ca content in the belowground parts of nitrogen-fixing plants (6.76 g kg^{-1}) was lower than that of non-nitrogen-fixing plants (11.12 g kg^{-1}; p>0.05). For C3 plants, Ca content in the aboveground and belowground parts were 21.08 g kg^{-1} and 13.18 g kg^{-1}, respectively, and were both significantly higher than that of C4 plants (aboveground: 15.68 g kg^{-1}; belowground: 6.42 g kg^{-1}; p<0.05). In the life form functional groups, shrubs showed a significantly higher in Ca content than herbs in both aboveground and belowground parts (p<0.05), although there were no significant differences (p>0.05) between deciduous and evergreen shrubs (p>0.05). There was no statistical difference in~~with~~ this respect between annual herbs and perennial herbs (p>0.05). ~~–~~The Ca content of dicotyledons in aboveground and belowground parts were 21.39 g kg^{-1} and 12.19 g kg^{-1}, respectively, and were significantly higher than that of monocotyledons (9.63 g kg^{-1} and 4.79 g kg^{-1}, respectively; p<0.05) (Fig. 3). To monocotyledons and dicotyledons, there were no significant differences in the plant Ca content of the aboveground parts among the different grades of rocky desertification; this was also true for the plant Ca content of the belowground parts. The Ca content of dicotyledons was significantly higher than that~~ose~~ of monocotyledons in both aboveground and belowground parts throughout the three grades of rocky desertification (p<0.05) (Fig. 4).

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

3.3 Correlation between plant Ca content and soil ECa content

Of t~~T~~hese 17 plant species, the Ca content in the aboveground and belowground parts of *Sanguisorba officinalis* had a significant positive correlation (p<0.01) with soil ECa content. The Ca content in the belowground parts of *Dendranthema indicum* (p<0.05)~~,~~ and *Castanea henryi* (p<0.01)~~,~~ also showed a significant positive correlation (p<0.01) with soil ECa content. The Ca content in the aboveground parts of *Themeda japonica* also showed a significant positive correlation (p<0.01) with soil ECa content. With regard to t~~T~~he other plants, the Ca content in~~of~~ the aboveground and belowground parts did not show a significant positive correlation (p>0.05) with soil ECa content (Table. 4).

3.4 Capacity of plants adapting to ~~soil~~ high Ca soil environments

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

The Ca-indifferent plants included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica*. For these plants, there was a significant positive correlation between Ca content in the aboveground or belowground parts and the soil ECa content. These plants did not exercise a strict~~ly~~ control over the absorption and transport of Ca and may be insensitive to ~~the~~ changes in ~~of~~ their own Ca content. Moreover,~~, and~~ their growth was less affected by soil Ca content. As~~In addition,~~ for the other plants, the relationship between Ca content in the aboveground and belowground parts and soil ECa content did not show a positive correlation.~~, then~~ T~~t~~hese plants were then divided into high-Ca plants and low-Ca plants, based on the differences in Ca content in the aboveground parts of these plants. High-Ca plants included *Pyracantha fortuneana*, *Rhus chinensis*, *Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. The aboveground parts of these plants could maintain a high Ca content (more than 19 g·kg^{-1}) under conditions of varying ECa content in the soil. Low-Ca plants included *Abelia chinensis*, *Vitex negundo*, *Smilax china*, *Miscanthus sinensis*, *Artemisia carvifolia* and *Digitaria sanguinalis*. The aboveground parts of these plants could maintain a low Ca content (less than 19 g·kg^{-1}) under conditions of varying ECa content in the soil (Table. 5).

4 Discussion

4.1 Dynamics of Ca content in plants and soil

With the grades of rocky desertification increased, the Ca content of soil also increased. This indicates~~d~~ that soil Ca content was affected by the grade of rocky desertification. The mean soil ECa content was 1.46 g kg^{-1} in three rocky desertification areas, which was lower than the average ECa content in tobacco-planting soil in Hunan (3.548 g kg^{-1}) (Xu et al., 2007). The average ECa content in IRD areas was 3.09 g kg^{-1}, which was several times higher than the previously reported ECa for non-limestone regions in China (Xu et al., 2007). The range of soil ECa content in the study area is from (LRD) 0.02 to (IRD) 3.92 g kg^{-1}, with the maximum and minimum being lower than that of soil on Barro Colorado Island, Panama by Messmer et al. (2014). Tanikawa et al. (2017) revealed that concentrations of TCa and ECa were also low at the deeper horizons in~~ the~~ low-acid buffering capacity (ABC) soils, and differences in both organic layer thickness and soil chemistry could be a reason ~~affecting~~ for the different of Ca accumulation in~~of~~ low- and high-ABC stands. Our research showed that the mean soil~~ mean~~ TCa and ECa contents were the lowest in LRD areas, and the difference in~~of~~ soil TCa and ECa may be caused by bedrock exposuree 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 indicates~~d~~ 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. However,~~But~~ the average Ca content of the aboveground parts of plants (19.67 g kg^{-1}) was lower than that of Hunan flue-cured tobacco (21.93 g kg^{-1}) (Xu et al., 2007). The maximum and minimum Ca content of plant aboveground parts were 41.79 g kg^{-1} and 2.15 g kg^{-1} respectively, and the maximum and minimum Ca content of plant belowground parts were 40.14 g kg^{-1} and 0.42 g kg^{-1} respectively. The maximum Ca content of plants (41.79 g kg^{-1}) was found in the leaves, which was lower than the Ca content of calcareous plant leaves with the maximum value of 85.13 g kg^{-1} detected by Luo et al. (2014). For~~To~~ most plant~~s Ca content~~, the Ca content in the aboveground part was higher~~larger~~ than in the belowground part, but~~and~~ for a few plants the Ca content in~~,~~ the aboveground part was lower than in the belowground part (such as *Sanguisorba officinalis*, *Pyracantha fortuneana* and *Castanea henryi*), which was consistent with the findings of Wang et al. (2014).

4.2 Correlation between plant Ca content and soil ECa content

Our results showed that most plants had no correlation relationship between soil ECa and plant Ca except for~~ing~~ several plant~~s~~ (*Sanguisorba officinalis*, *Dendranthema indicum*, *Castanea henryi* and *Themeda japonica*) which showed~~had~~ a positive correlation between soil ECa and plant Ca content (Table. 4). But some study showed that Ca-rich soils caused cells to absorb more Ca than the cells themselves require (White and Broadley, 2003). Additionally,~~, and~~ soil ECa content and leaf Ca content (Flue-cured Tobacco) had a significant positive correlation in a pot experiment (Zou et al., 2010). The difference between the findings of these studies and ours~~, 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 indicates~~d~~ 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 also 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 indicates~~d~~ that the aboveground parts of *Themeda japonica* were~~as~~ also greatly affected by soil ECa content.

Two-way ANOVA of species and soil showed that the Ca content of the aboveground parts of the 17 plant species was mainly affected by species factors, while the Ca content of the belowground parts was affected by both species factors and the grade of rocky desertification. This finding~~, which~~ is~~was~~ supported by~~with~~ data reported by Ji et al. (2009). The Ca content in the aboveground parts of nitrogen-fixing plants was significantly higher than that of the belowground parts, a~~. A~~nd this result indicates~~d~~ that nitrogen-fixing plants were the most efficient in~~ the~~ Ca upward transport.~~, since the transport of Ca was mainly upward;~~ In contrast,~~which was not the same with those of~~ Ji et al. (2009) found ~~. Ji et al. (2009) revealed~~ that dicotyledons were the most efficient in~~at~~ the upward transport of Ca. They used only three types of plants (pteridophytes, dicotyledons, and monocotyledons) ~~exclude~~without researching nitrogen-fixing plants in their study, which may have produced a conflicting result compared with our current findings. We found significant differences (p<0.01) between the aboveground and belowground parts in the Ca content of monocotyledons in our study. However, Ji et al. (2009) revealed that there were no significant differences between the aboveground and belowground parts in the Ca content of monocotyledons. This phenomenon could be due to~~may contribute~~ the most of the monocotyledons sample plants were low-Ca plants. In our study, a~~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 by ~~(~~Ji et al.~~,~~ (2009), indicating that different ~~individual~~ monocotyledons showed differing abilities to absorb soil Ca.

4.3 High Ca adaptation of plants

The different plant functional groups revealed~~ the~~ differences in Ca content (Fig. 3). In some cases, even within the same plant, there was an inconsistent correlation between Ca content in the aboveground and belowground parts and the soil ECa content. Collectively, these findings show~~ed~~ that not all plants adapted to~~ soil~~ high Ca soil environments in the same way, but rather~~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 those plants that are~~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; and (ii) the species differences in terms of the Ca content of the aboveground parts of plants. Thus, based on these~~ above~~ two indicators,~~ the~~ plants can be placed~~were classified~~ into the following groups: Ca-indifferent plants, high-Ca plants, and low-Ca plants (Ji et al., 2009). In the present paper, we used this classification method to categorize the 17~~our~~ plant~~s~~ species~~,~~ that~~which~~ were widely distributed across our study environment~~s~~, 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 surviv~~e~~~~al~~ normally, a~~. A~~nd their Ca content ~~of them~~ changes correspondingly with ~~the~~ changes in ~~of~~ soil ECa content. The physiological activities of high-Ca plants have~~d~~ a higher demand for Ca and may have a strong ability to enrich soil Ca. The physiological activities of low-Ca plants have~~d~~ a lower demand for Ca and can~~ould~~ alleviate high Ca stress by inhibiting the absorption of Ca through the root system and its upward transport.

These results are of great significance for~~to the~~ vegetation restoration in karst areas. High-Ca plants should be selected 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 ability to adapt~~ion ability~~ to~~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 guiding solutions to~~solving~~ the problem of rocky desertification in China, but also provide species screening ideas for ecosystem restoration in~~the~~ rocky desertification areas~~ecosystem restoration~~ in other parts of the world. Rocky desertification is a major ecological problem in karst areas, and further explorations are required to solve this problem. It is necessary to further explore other nutrient elements in soil during vegetation restoration, and long-term positioning observation is crucial for understanding~~the study of~~ this issue.

5 Conclusions

Our results indicate~~d~~ that the mean soil TCa and ECa content were highest in areas of IRD, followed by MRD, then~~and~~ LRD. The Ca content in the aboveground parts of plants was significantly higher than that in the belowground parts for the three grades of rocky desertification sites in our study~~studied sites~~. Significant differences in Ca content were found between the aboveground and belowground parts of plants in each plant functional group. The soil ECa content had a significant effect on the Ca content of the belowground parts of plants~~,~~ but ~~had~~ no significant effect on the Ca content of the aboveground parts. Ca-indifferent plants, which included *Sanguisorba officinalis*, *Castanea henryi*, *Dendranthema indicum* and *Themeda japonica*, showed~~had~~ a significant positive correlation~~ existed~~ between the Ca content in the aboveground or belowground parts and the soil ECa content. High-Ca plants~~ in our study~~ were *Pyracantha fortuneana*, *Rhus chinensis*, *Loropetalum chinense*, *Serissa japonica*, *Glochidion puberum*, *Indigofera tinctoria* and *Aster baccharoides*. The aboveground parts of these plants were able to absorb a lot of ~~high~~ Ca ~~content~~ from soils with a ~~a~~ varying~~ious of~~ ECa content~~ soils~~. Finally, low-Ca plants included *Abelia chinensis*, *Vitex negundo*, *Smilax china*, *Miscanthus sinensis*, *Artemisia carvifolia* and *Digitaria sanguinalis*. The aboveground parts of low-Ca plants were able to maintain a lower Ca content from soils with~~under conditions of~~ varying~~iable soil~~ ECa content.

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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 exposuree rate	Vegetation coverage	Disturbance regimes
LRD	34(≤45)	South	5.56	20°	500	35%	80%	Slight human disturbance, rarely grazed~~ing~~
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 grazed~~ing~~

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

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

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

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

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

Vegetable layer	Species	Important Value (IV)		
		LRD（%）	MRD（%）	IRD（%）
	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
Shrubs	*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	-	-
	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
Herbs	*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 isare less than 1.

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

Species	Ca content in aboveground parts			Ca content in belowground parts		
	Range ($g\ kg^{-1}$)	Mean ±SE ($g\ kg^{-1}$)	Correlation coefficient	Range ($g\ kg^{-1}$)	Mean ±SE ($g\ kg^{-1}$)	Correlation coefficient
Smilax china	5.77~36.35	18.5±12.24	0.302	3.11~8.61	5.89±2.75	0.931
Aster baccharoides	16.16~24.03	20.00±3.60	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±2.70	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±9.90	−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	4.75~9.80	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	4.34~7.60	5.61±1.44	0.000	2.88~13.10	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±1.70	0.877*
Castanea henryi	12.99~38.74	22.4±8.17	0.151	20.52~31.37	25.28±3.92	0.963**

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

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

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

Figure captions

Fig. 1 Geographical locations of the study sites

Fig. 2 Characteristics of plants Ca content in different grades of rocky desertification

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

Fig. 3 Ca content in the aboveground and belowground parts of plants in different functional groups ~~of plans~~.

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

Fig. 4 Ca content in the aboveground and belowground parts of different plant types from three different rocky desertification sample areas

LRD: light rocky desertification; MRD: moderate rocky desertification; IRD: intense rocky desertification. Values with the same letters were not significantly different ($p>0.05$).

Fig. 1

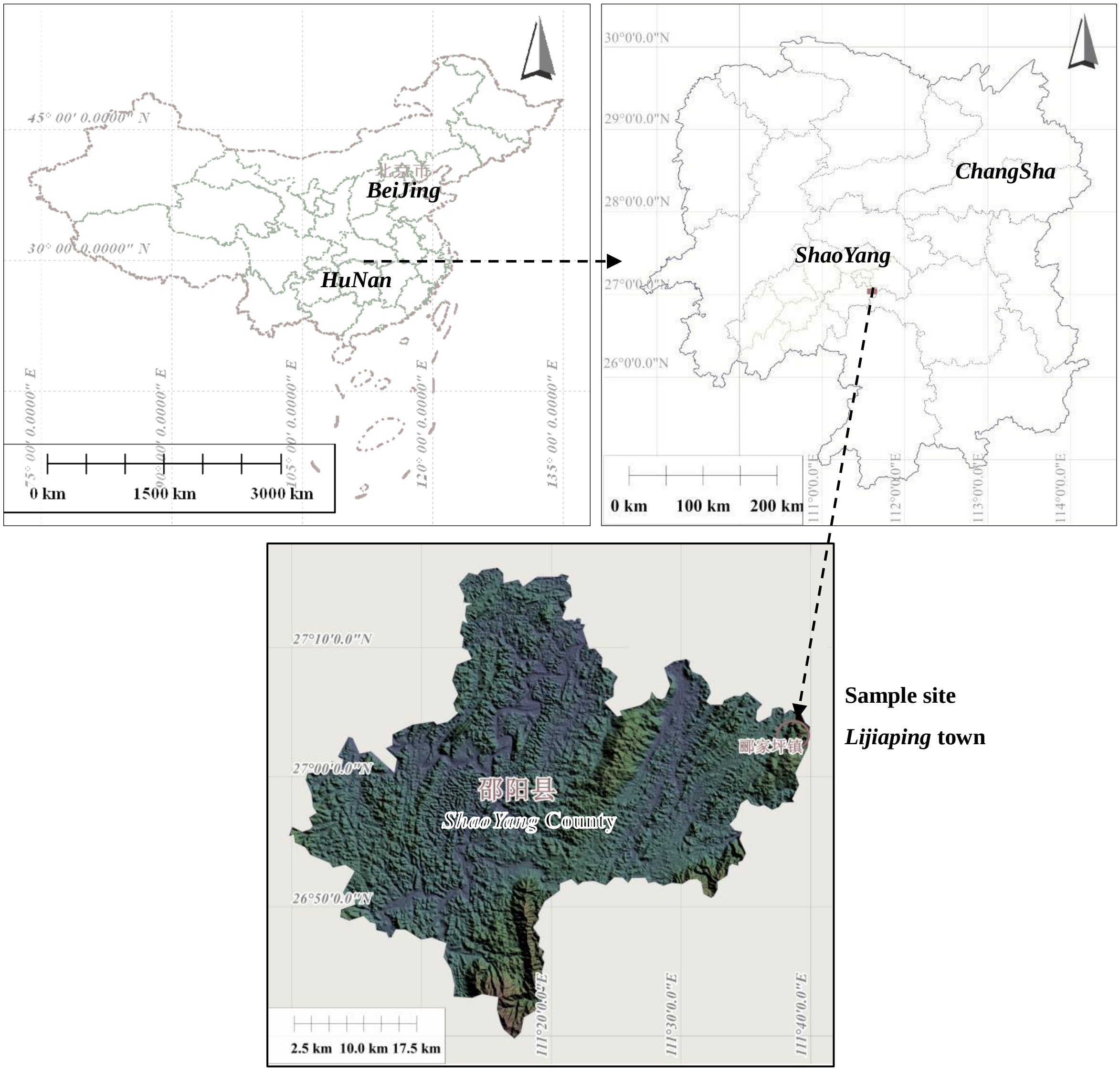
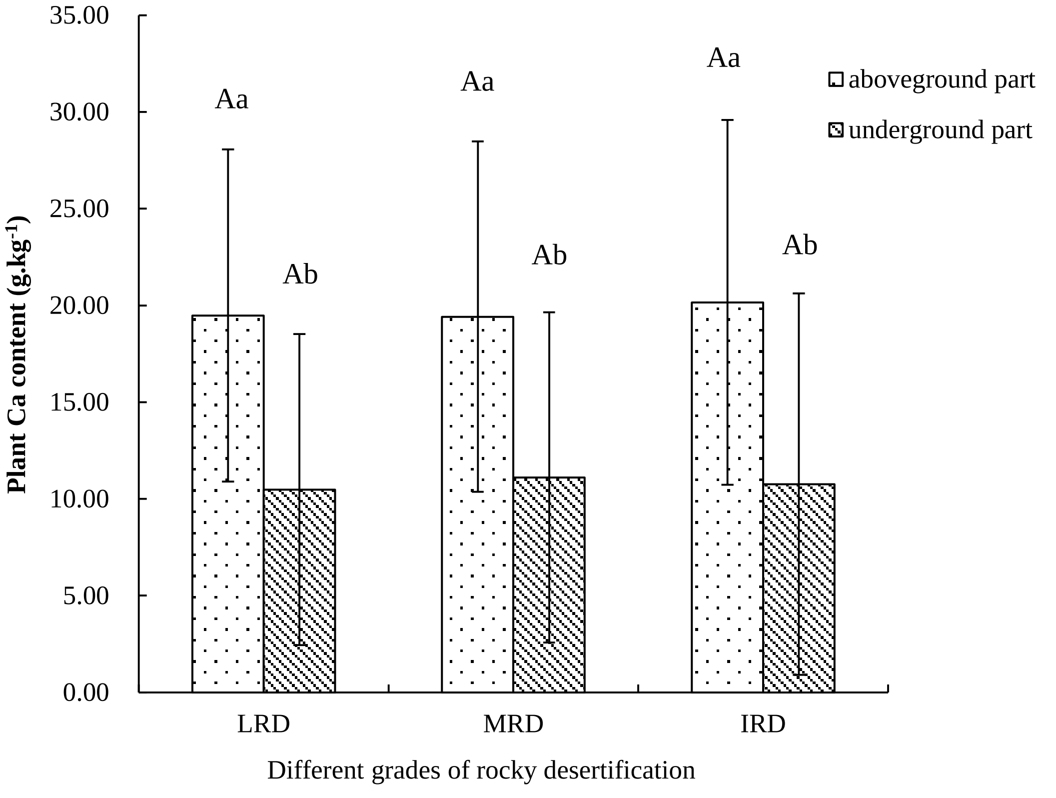

45° 00' 0.0000" N
BeiJing
30° 00' 0.0000" N
HuNan
120° 00' 0.0000" E
135° 00' 0.0000" E
0 km
1500 km
3000 km
30°0'0.0"N
29°0'0.0"N
28°0'0.0"N
26°0'0.0"N
ChangSha
ShaoYang
0 km
100 km
200 km
112°0'0.0"E
113°0'0.0"E
114°0'0.0"E
27°10'0.0"N
27°00'0.0"N
26°50'0.0"N
邵阳县
ShaoYang County
111°20'0.0"E
111°30'0.0"E
111°40'0.0"E
2.5 km 10.0 km 17.5 km
Sample site
Lijiaping town

Fig. 2



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

Fig. 3

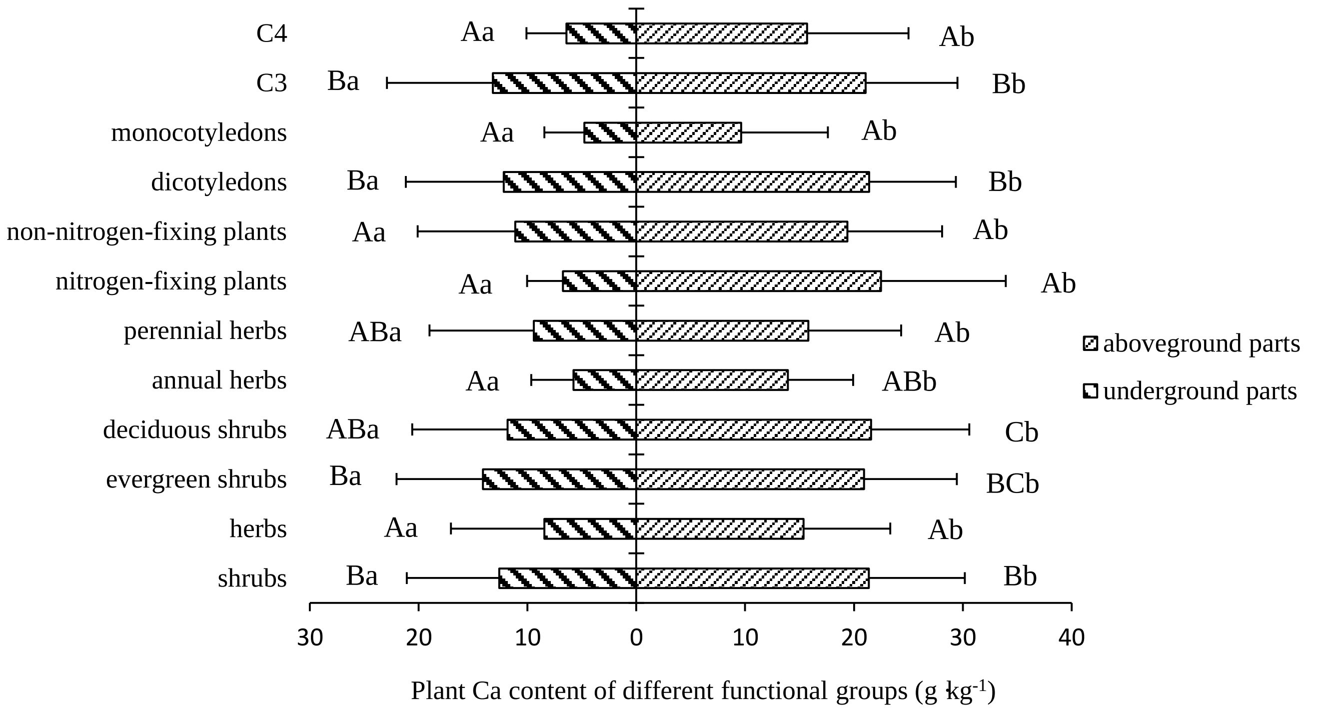
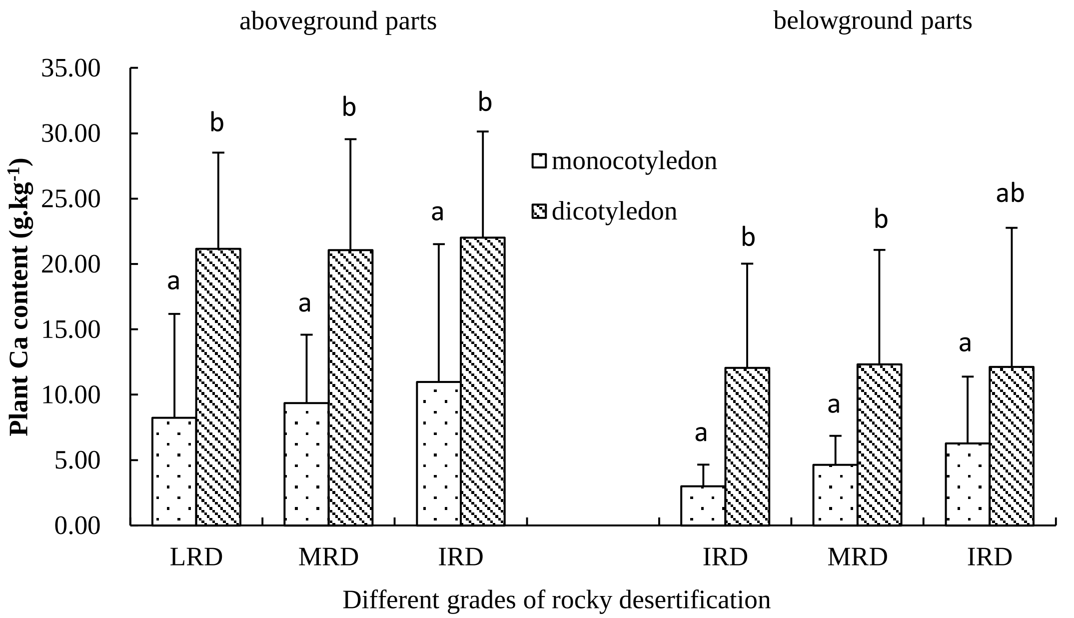

C4
C3
monocotyledons
dicotyledons
non-nitrogen-fixing plants
nitrogen-fixing plants
perennial herbs
annual herbs
deciduous shrubs
evergreen shrubs
herbs
shrubs
Aa
Ab
Ba
Bb
Aa
Ab
Ba
Bb
Aa
Ab
Aa
Ab
ABa
Ab
Aa
ABb
ABa
Cb
Ba
BCb
Aa
Ab
Ba
Bb
aboveground parts
underground parts
30
20
10
0
10
20
30
40
Plant Ca content of different functional 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.