

Response of *Lamiophlomis rotata* (Benth.) *Kudo* to degradation of alpine grasslands in the western Sichuan plateau, China

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Research

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Abstract

Background: *Lamiophlomis rotata* (Benth.)Kudo, also known as Duiyiwei in China, is considered to be one of the most common Tibetan medicine. It is famous for the significant effects on improving blood circulation and relieving pain. Due to the environmental deterioration and excessive consumption, the wild resources of *L.rotata* is decreasing year by year. However, its artificial cultivation has not been realized. This study aimed to initially explore the adaptation mechanism of *L.rotata* to degraded alpine grassland, and provide reference for its wild tending and artificial cultivation.

Methods: In this study, we used a sample method to conduct a population ecological survey of plants and collect plant leaf samples from mildly, moderately, and extremely degraded grassland. The content of SOD, POD, CAT, PRO, MDA, SS, SP, carotene, chlorophyll A and chlorophyll B in different sample leaves was measured by colorimetry. Microscopic characteristics of the cross-section, epidermis and submicroscopic structure of the leaves were observed and compared.

Results: The density of *L. rotata* in moderately degraded grassland was the highest where *L. rotata* had the strongest resistance. And the analysis of seven physiological indicators combined with stress resistance showed that the average content of MDA, SOD, POD, CAT and SS is the highest in *L. rotata* of the moderately degraded grassland. The microscopic observation showed that, by increasing the thickness of the epidermis, the density of the glandular scales, the proportion of palisade tissue and the number of gland scales, *L. rotata* was able to adapt to the degraded grassland. Meanwhile, morphological studies have shown that *L. rotata* could enhance root growth in the degraded grassland. Furthermore, in response to the degradation of alpine grassland on the Qinghai-Tibet Plateau, *L. rotata* population and biomass distribution in the underground parts have increased. However, *L. rotata* does not have a competitive advantage in mildly degraded grassland. Compare with the mildly and moderately habitat, the population of *L. rotata* is small in the extremely degraded habitat.

Conclusion: The results showed that among the 3 levels of degraded grassland, *L. rotata* in the moderately degraded grassland shows the strongest resistance. This study can provide a reference for breeding and ecological planting of *L. rotata*, suggests that in the nursery stage, the medium degraded grassland should be chosen for ecological breeding to enhance the survival rate of seedlings. While after large-scale planting, reasonable community interventions, like the *P. fruticosa*+*F. rubra* community, should be built to simulate the yield of medicinal materials.

Background

Lamiophlomis rotata (*L.rotata*), also known as Duiyiwei in China, is considered to be one of the most common Tibetan medicine and is also often used in Mongolia, Naxi and other minority[1, 2]. It was firstly recorded in the classic Tibetan medicine "Yuewang Pharmacy" and also recorded in the famous Tibetan medical books "Four Tantra" and "Jing Zhu Ben Cao" more than 1,000 years ago. Tibetan Medicines use it for the treatment of osteomyelitis, huangshui pathologic (rheumatic), fractures, falls and gunshot wounds[3]. It has been recorded in Chinese Pharmacopoeia since 2005 and has the effects of invigorating circulation, hemostasis, dispelling wind and relieving pain and commonly used to treat bruises and bone pain[4]. It has attracted attention of researchers by its good analgesic effects without any addiction from clinical practice.

In view of the significant clinical efficacy and little side effects of *L.rotata*, there are more and more preparations and products that using *L.rotata* as the main raw material, which means the demand for *L.rotata* is increasing. According to statistics, the annual demand of a pharmaceutical factory in Tianjin has increased its demand for *L.rotata* from

50t in 2003 to 100t in 2009. The annual average consumption of this herbal medicines in China is about 2500t[5]. However, due to its harsh growth environment and long population renewal cycle, the maximum annual yield is only 1700t and the medicinal resource is obviously in short supply. As a result, *L.rotata* has been listed as a first-class endangered Tibetan medicine in 2000. Several years later, the medicinal parts of it were restricted from whole herbal to ground parts from the 2010 edition of the Chinese Pharmacopoeia, which can leave the main root to germinate next year[6]. In summary, it is urgent to realize the large-scale planting of *L.rotata* to reduce its resource pressure.

The field investigation shows that *L.rotata* mainly distributed in Qinghai, Sichuan, Tibet, Gansu and Yunnan province in China and grows in the stone-beaches on the high mountains or on the meadows and river shoal which locate in 2700 m ~ 4500 m altitude of Qinghai-Tibet Plateau[7]. Now, Due to the plateau climate change like temperature rising and freeze thaw cycle change, together with other factor like overgrazing, the degradation of plateau grassland increased. Furthermore, series of problems appeared such as serious desertification, soil and water loss, the species of grassland communities decrease, solar radiation increases and other ecological problems[8, 9]. Faced with the decrease in the amount of available resources (water, light, nutrients, etc.), plateau plants can adopt different response strategies and adaptation methods[10]. Morphologically, it can maximize the absorption of resources by adjusting the biomass allocation pattern of each organ (root system, leaves, etc) or strengthening the development of mechanical tissues (glandular hairs, palisade tissues, etc.)[11]. Physiologically, it activates the enzymatic defense system and regulates the content of physiological indexes related to the osmotic adjustment system, photosynthesis and respiration to maintain the normal growth state[12]. As an important medicinal plant in the Qinghai-Tibet Plateau, it is urgent to explore the response strategies of *L.rotata* to the degraded grassland to accumulate necessary knowledge for its large-scale cultivation in the future.

The degradation of plateau grasslands has a great negative impact on the growth of plants. Which is usually the main reason of large-scale reduction of plant populations in traditional view. However, in our field investigations, we found that the main producing areas of *L.rotata* are concentrated in degraded meadows at an altitude of 4000m~5000 m in the northeast of the Tibetan Plateau which is the border area of Sichuan, Qinghai and Gansu Province. There are also distributions on bare land formed by strongly degrading, building roads, heavy grazing and other causes. It fully shows that *L.rotata* has its own adaptation strategy to the change of climate and environment in the Qinghai-Tibet Plateau, and has the characteristics of pioneer species in the degraded grassland. But now, study on plant ecology of *L.rotata* mainly focuses on the breeding system and the development of seed seedlings[13]. There are few reports on the adaptability of wild *L.rotata* to the environment except some research on genetic diversity, ecologically suitable regional studies and initial exploration of its blind cultivation[14, 15, 16]. Therefore, the population ecology survey, leaf microscopic and submicroscopic structure observation, related physiological index and photosynthetic pigment content determination of *L.rotata* from 3 degradation degree grasslands were studied in this research. The differences initially explain two main issues: (1) The population distribution, morphological and physiological differences of *L.rotata* from degraded grasslands of different grades. (2)Its adaptation mechanism to degraded grassland. The results of the study can provide reference for wild tending and artificial cultivation of *L.rotata*, and it has great significance to solve the problem of medicinal resources.

Materials And Methods

Site description

The research area is located in Ruohehe Ranch of Aba County, Aba Tibetan and Qiang Autonomous Prefecture, Sichuan Province (N33°13'04.82", E101°28'02.01"; Altitude 3774 m). The surveyed area belongs to the plateau, with a

cold temperate semi humid monsoon climate, which does not have 4 obvious seasons, has a large temperature difference between day and night, a short frost-free period, and strong solar radiation. The elevation map of the survey site is shown in Fig 1.

This study took grassland degradation as a primary consideration when plotting, and the samples were distributed within 1000m². The habitats of *L. rotata* were divided into 3 types: the *P. fruticosa*+*F. rubra* community is the mildly degraded plot, the *F. rubra*+*L. rotata* community is the moderately degraded plot and the bare land after slope excavation is the extremely degraded plot[17].

The soil bulk density was determined by the ring knife method. The soil organic matter was prepared by the potassium dichromate volumetric method. The soil available nitrogen (N) was alkali-distilled and the available phosphorus (P₂O₅) was extracted by the NH₄F-HCl-molybdenum antimony colorimetric method. The melting time is measured by a button-type temperature measuring instrument, and the ecological environment factors of the different habitats are shown in Table 1.

The survey area belongs to the grazing area where the grazing stress is obvious, and the thickness of herbaceous layer is less than 20cm. Different habitats reflect different stages of grassland degradation. The *P. fruticosa*+*F. rubra* community has lower stress due to lower terrain and nearby water, and the germination stress is lower in *P. fruticosa* shrub. The grazing stress of the *F. rubra*+*L. rotata* community is relatively large, the colony of the herbaceous *F. rubra* is short and the degree of degradation is increased. The gravel content of 0~30cm soil in the bare land of the slope is 85%, no turf layer is present, and the pressure of drought and freezing stress is high, so its degradation degree is the highest.

Population survey of *L. rotata*

In July 2017, *L. rotata* plots were selected based on the degradation degree of grassland. In view of the large area of the *F. rubra*+*L. rotata* community, 3 equilateral triangles were randomly arranged, each contained 10 m × 10 m quadrats at intervals of 50m; in view of the area of bare land and the *P. fruticosa*+*F. rubra* community is small, so only one 10m×10 m quadrat was set. Then, each of the 10 m × 10 m quadrats was divided into 25 sample quadrats (2 m × 2 m). The number of total *L. rotata* and the number of flowering plants were accurately counted in each 10 m × 10 m quadrat, and the leaf spread of *L. rotata* (length and width) in the 2 m × 2 m sample in the lower right corner of the *F. rubra*+*L. rotata* community was calculated. The quantity of *L. rotata* on bare land and the *P. fruticosa*+*F. rubra* community is really small, so the leaf spread (length and width) of all *L. rotata* in the samples was determined. The square setting is shown in Fig.2.

Morphological characteristics of *L. rotata*

Observation of leaf anatomical features: 10 *L. rotata* plants of similar size were selected separately from each habitat. 2 large leaves were removed from the top, placed in the FAA solution and brought back to the laboratory. The transverse section and lower epidermal section of the leaves from 30 *L. rotata* plants were prepared by hand-sliced method; then, dilute glycerol was added, and the leaf material was observed under a fluorescence microscope (Olympus, BX41). Furthermore, we used the transverse section of the leaves to observe the thickness of the leaf and the stratum corneum, the morphology of the upper epidermal cells, the number of columns of palisade tissue, and the looseness of the sponge tissue, and then, the thickness rate of palisade and sponge tissue was calculated. The type and number of stomata and the density of glandular phosphorus were observed on lower epidermis section. The amount of the glandular phosphorus was counted under low power lens(10×), and the number of stomata and

epidermal cells was counted under high power lens(40×). All the measurements were recorded using a C4 type 0.05 ulnar eyepiece and calculated as follows[18]:

$$\text{Stomatal index(SI)} = \frac{s}{(e+s)} \times 100$$

The leaf tissue compactness (CTR) = the palisade tissue thickness /the leaf thickness, and the leaf tissue porosity = sponge tissue thickness / leaf thickness[19].

Observation of chloroplast submicroscopic structure: In 3 different habitats, *L. rotata* plants of similar size were selected separately. The 2 large leaves on the top were taken, the leaves were cut in approximately 0.5 cm×1.0 cm and quickly placed into an EP tube(ependorf) that was pre-filled with 4°C,3% glutaraldehyde pre-fixation solution, and clean gauze was inserted to ensure the leaf tissue was fully immersed in the fixing solution. Then all the samples were placed in the cooler and returned to laboratory. The pre-fixed samples were fixed by 1% osmium tetroxide, and dehydrated by acetone step by step. The samples were embedded by Epon812. Followed by semi-thin section optical positioning, then ultra-thin sections were cut and double stained by uranium acetate and lead citrate. All the sections were observed with a Hitachi H-600IV transmission electron microscope. Photos were taken at 5000x, 15,000x and 20,000 x magnification.

Physiological index content of *L. rotata*

Collection of *L. rotata* leaves: 30 plants of similar size were selected separately from each habitat. Picked the 2 large leaves on the top, withered leaves and thick leaf veins were not used. The samples were weighed quickly and accurately, placed into 5ml cryotubes and putted into a liquid nitrogen tank immediately then carry back to laboratory. Finally, the samples were transferred to the ultralow freezer for storage.

Determination of physiological indicators of stress resistance: Each sample was ground with liquid nitrogen, extracted in PBS buffer which pH value was 7.4, then centrifuged at 4°C (Refrigerated centrifuge: Thermo Electron LED GmbHD-37520 Osterode). The supernatant from the above process was the sample solution used in the tests. The centrifugal condition for superoxide dismutase(SOD), peroxidase(POD), malondialdehyde(MDA), proline(PRO) and soluble protein(SP) is as follow:3500r/min, 10min; that for catalase is 10000r/min,20min; and for soluble sugar(SS) is 4000r/min,10min. All other indicators were measured using a physiological indicator kit (developed and produced by Nanjing Jiancheng Bioengineering Research Institute) except for CAT, and the indicators were determined according to the instructions. The CAT was determined by UV spectrophotometry and enzyme label method (enzyme mark instrument: Gene Company Limited PXS2)[20].

Data analysis

This test used Excel 2013 software and SPSS19.0 software for analysis. The one-way analysis of variance was used to analyze the results of seven physiological indicators and anatomical characteristics of *L. rotata* from the different degraded grasslands. The correlation analysis of the seven stress resistance indicators was carried out by Pearson test.

The fuzzy membership function method[21] and the standard deviation coefficient weighting method were used to comprehensively evaluate the stress resistance of *L. rotata* from the 3 levels of degraded grasslands. According to the fuzzy membership function calculation formula, the specific membership value μ of each physiological index in the different habitats was calculated. The physiological index content is positively correlated with the stress resistance according to formula μ . The negative correlation is calculated according to formula μ . And on

accumulation of the resistance value of each indicator, the greater the average value sought is, the stronger the resistance. The standard deviation coefficient V of each index is calculated according to formula (3); the weight coefficient W is calculated according to formula (4); and the comprehensive evaluation D value is calculated according to formula (5). According to the value of D , the resistance of *L.rotata* in the 3 degrees of degraded grasslands was ranked. The larger the D value is, the stronger the resistance.

$$\mu = \frac{(X - X_{\min})}{(X_{\max} - X_{\min})} \quad (1)$$

$$\mu = 1 - \frac{(X - X_{\min})}{(X_{\max} - X_{\min})} \quad (2)$$

$$V = \frac{1}{\bar{X}_j} \sqrt{\sum_{i=1}^n (X_{ij} - \bar{X}_j)^2} \quad (3)$$

$$W = V_j / \sum_{i=1}^n V_j \quad (4)$$

$$D = \sum_{i=1}^n [\mu(X_j) * W_j] \quad (5)$$

Results

Community characteristics of *L.rotata*

According to the statistics of *L. rotata* in the different habitats, the plant density of the *F. rubra*+*L. rotata* community is significantly higher than that of the *P. fruticosa*+*F. rubra* community and bare land (Fig 3; Table 2); the proportion of flowering plants in the *P. fruticosa*+*F. rubra* community is significantly higher than that in the other grades. The plant spread of the *P. fruticosa*+*F. rubra* community is significantly greater than that of the moderately degraded and the bare land. The standard deviation of the spread of *L. rotata* in bare land is large (Table 2), and from our field observation, some large plants were scattered on the bare land, and their spread is even greater than that in the *P. fruticosa*+*F. rubra* community. Analysis of the differences among the groups of the different habitats (length and width) shows that the differences between *L. rotata* in the *P. fruticosa*+*F. rubra* community and the other three *F. rubra*+*L. rotata* community, the *F. rubra*+*L. rotata* community 1 and the bare land are significant (Table 2).

Leaf microscopic characteristics

The palisade tissue of leaves in the *P. fruticosa*+*F. rubra* community is obviously irregularly curved, arranged loosely with obvious cell space. The palisade tissue of leaves in the *F. rubra*+*L. rotata* community and bare land is arranged regularly, and the cells are organized in 2 to 3 columns, forming a regular strip (Fig 4). Analysis showed that the thickness of the sponge tissue in the *P. fruticosa*+*F. rubra* community had a significant difference to that from the *F. rubra*+*L. rotata* community and the bare land (Table 3). We found the ratio of palisade tissue to sponge tissue, and the leaf porosity of the *P. fruticosa*+*F. rubra* community were significantly different from those of the bare land (Table 3).

With the increase in the degree of degradation, the thickness of the leaves of *L. rotata* gradually decreases meanwhile the thickness of stratum corneum increases, the morphology of upper epidermal cells tends to be regular, and the arrangement tends to be neat and tight. The amount of glandular phosphorus in the same field of view increases, and the stomata are mostly infinite or unequal types (Fig 5). The leaf thickness and thickness of stratum corneum on upper epidermis of the *P. fruticosa*+*F. rubra* community have significant differences to those samples from the *F. rubra*+*L. rotata* community and the bare land. In addition, the density of glandular phosphorus in all the different habitats is significantly different and increases with the aggravation of degradation; the stomata index of leaves from the bare land is the highest, which just has a significant difference with the samples from the *P. fruticosa*+*F. rubra* community (Table 4).

Chloroplast submicroscopic structure

The sub-micrographs of the leaves from the 3 degraded habitats were compared (Fig 6); the individual chloroplasts of the leaves were attached to the cell wall with clear outline and perfect structure. No rupture or vacuoles were observed, and no significant difference between the number of inner layer folds. Starch granules were observed around the chloroplast layer of the leaves from the *P. fruticosa*+*F. rubra* community and the bare land, and the former is more than the latter. While in the leaves from the *F. rubra*+*L. rotata* community, there was no starch granules was found around the chloroplast. The chloroplast lamellae was distributed along the chloroplast membrane. The chloroplasts were strip-shaped, and the eosinophilic granules were the largest in all the samples. Meanwhile, the chloroplasts were fusiform, arranged irregularly, and the eosinophilic granules have similar size as the samples from the other 2 communities.

Content of Physiological indexes

Among the seven physiological indicators, the contents of SOD and POD in the three habitats were significantly different, the MDA, PRO and SS contents in the samples from the *F. rubra*+*L. rotata* community were significantly different from those from the other 2 habitats (Table 5; Fig 7). No significant differences were noted in the other physiological indicators.

Correlation between physiological indicator content

A certain correlation existed between the stress resistance indexes in the leaves of *L. rotata*. There are significant positive correlations between SOD and POD, SOD and MDA, POD and MDA, POD and SS. Meanwhile, the correlations between POD and PRO, PRO and SS, MDA and SP, SS and SP are significant negative (Table 6).

In this study, no significant differences existed in the indicators of osmotic adjustment in the leaves from the three degraded grasslands, and no significant accumulation of PRO, which fully indicated that the osmotic adjustment

system was not influenced greatly by the adverse conditions. In summary, the leaves in the *F. rubra*+*L. rotata* community resisted adversity mainly through enhancing the protective enzyme content of the enzymatic defense system. However, for the *P. fruticosa*+*F. rubra* community, both the soil moisture content, and the fertility were higher than that of the *F. rubra*+*L. rotata* community and the bare land, so the content of each stress resistance index is low as a result of the low stress intensity of environment. While in the bare land, except for the enzymatic defense system, the palisade structure of the leaves is more developed, to guarantee the photosynthesis demand for water, and the cell morphology becomes a regular strip shape; these cells are more closely arranged and evenly distributed. A large number of chloroplasts improve the utilization efficiency of light energy and improve growth under adverse conditions.

Stress resistance evaluation

The result showed, the greater the membership value, the stronger the stress resistance. Compared the membership functions of *L. rotata* in three levels of degraded grasslands as follows: the *F. rubra*+*L. rotata* community > the *P. fruticosa*+*F. rubra* community > the bare land. The membership function values of each physiological index were weighted to obtain the comprehensive evaluation D value. As shown in Table 7, to compare the stress resistance, the *F. rubra*+*L. rotata* community > the *P. fruticosa*+*F. rubra* community > the bare land, shows the same analytical results as the fuzzy membership function method.

Chlorophyll content

In the different degraded grasslands, the chlorophyll content is different by comparing the mean values (Table 8). Compared the ChlA and ChlB content from different habitats as follows: the *P. fruticosa*+*F. rubra* community > the bare land > the *F. rubra*+*L. rotata* community. Compared the carotene content as follows: the bare land > the *F. rubra*+*L. rotata* community > the *P. fruticosa*+*F. rubra* community. LSD multiple comparative analysis showed that the content of ChlA was significantly different between the *P. fruticosa*+*F. rubra* community and the *F. rubra*+*L. rotata* community. There was significant difference in the content of ChlB and carotene between the *P. fruticosa*+*F. rubra* community and the *F. rubra*+*L. rotata* community, while it showed no significant difference compared with the bare land.

Discussion

Population structure of *L. rotata*

Relative to the mild, moderate and extreme levels for qualitative grading standards [22], this study considers the *P. fruticosa*+*F. rubra* community is a mildly degraded grassland, which is in line with the classification criteria of "more than 95% coverage of grasses and fewer annual plants". The *F. rubra*+*L. rotata* community is a moderately degraded grassland, where the gramineous plants are low due to the high grazing intensity.

It is consistent with the classification standard of "grass plant coverage 80-94%, soil aridification". Because of the high grazing intensity, gramineous plants are scarce. The bare land is similar to the extremely degraded grassland, which is in line with the classification criteria of "black soil beach, bare land or gravel beach".

As shown in the Table 2, the *L. rotata* population structure has different adaptation strategies for grassland degradation. In the *F. rubra*+*L. rotata* community, the population density of *L. rotata* is large and it is one of the grouped species. Its leaf spreading distance is small, and the proportion of plants entering breeding period is small, showed obvious diffusion ability and characteristics of pioneer plants. In the mildly degraded grassland, which is

represented by the *P. fruticosa*+*F. rubra* community, although the community competition, water, and fertilizer conditions improved, there's still no competition advantage of *L. rotata* for its short plant height. The growth of the young plants were inhibited by community competition, plants are expanding towards large-scale development for the ecological position. In the bare land, the seedling growth is inhibited due to the poor habitat of the soil parent layer (lack of fertilizer, severe temperature change, and the long melting period) [23]. This paper speculates that the population depends on the ultralong root system to achieve population reclamation, and through the plant's large-scale increases in its investment in sexual reproduction.

Morphological structure of leaf

There are several characteristics of morphological structure for the plant of *L. rotata* to adapt degraded grassland. Firstly, although as a low-growing plant, the mature plants of *L. rotata* always have a root more than 50cm long, which can penetrate the meadow surface soil and reach the deep earth to overcome the adversity stress in the surface layer of degradation grassland caused by the drought, freezing and thawing, and persistent loss of nutrient[24]. On the other hand, *L. rotata* seedling can grow root more than 15cm long in the first year in the planting stage, which also showed that *L. rotata* adapts to the bad habitat through this strategy.

Furthermore, the leaves of *L. rotata* have thick cuticle layer, developed palisade tissue, regular strip-shape sponge cells, closely arranged, developing gland scales, with functions of heat insulation, water retention, damage resistance, etc. These all are adaptation of plants to severe environment such as strong light, strong ultraviolet radiation, strong airflow changes at high altitude, and physiological drought[25,26]. Meanwhile, leaf stomatal index is closely related to the net photosynthetic rate of plant[27]. Moderate drought will increase the stomatal index, while excessive drought will decrease it[28,29]. In this study, the stomatal index increased with increasing grassland degradation, which means the environment of degraded grassland has not exerted excessive stress on growth of *L. rotata* and this is the common characteristics of xerophytes like *Caragana stenophylla* Pojark[30]. *L. rotata* of the *F. rubra*+*L. rotata* community and the bare land can adapt to the harsh habitat by increasing the thickness of the epidermis, the density of glandular scales, the rate of palisade to spongy. At the same time, reduce the thickness of the sponge tissue and the looseness of the leaf tissue structure to adapt to the harsh habitat. However, its structure in the *F. rubra*+*L. rotata* community and the bare land did not change in accord with the degree of grassland degradation.

Physiological adaptability of *L. rotata*

L. rotata showed physiological strategies to adapt degraded grassland as follows: Firstly, the response of enzymatic defense system of *L. rotata* in the *F. rubra*+*L. rotata* community increased significantly, while the PRO and SS content of osmotic adjustment system showed significant decrease and increase respectively, there was a significant negative correlation between them, this regulation mechanism needs further study. The analysis of 7 physiological indicators of stress resistance showed that the average content of MDA, SOD, POD, CAT and SS was the highest in *L. rotata* of the moderately degraded grassland, and the MDA, SOD, POD and SS were significantly different in leaves from the other habitats. An increase in environmental stress is usually accompanied with per-oxidation action of membrane lipid, produced MDA as final product[31,32]. Which could impair the normal function and structure of the cell membrane[33]. Therefore, the accumulation of MDA can reflect to the degree of membrane lipid per-oxidation and the resistance of plants under stress in some degree[34]. Meanwhile, to prevent damage to the cell membrane system, plants activate the enzymatic defense system to produce protective enzymes to scavenge free radicals generated in the cells[35], and reduce the degree of membrane lipid per-oxidation, thus resist the effects of stress. SOD, POD and CAT are the 3 most common protective enzymes, and their content can reflect the sensitivity

of plants to stress[36,37,38]. SS such as glucose, galactose, fructose, etc., also accumulated under stress. PRO is one of the most effective osmotic adjustment substances. Under stress conditions such as drought, high temperature, saline, and freezing, plant cells may have osmotic stress due to a lack of sufficient water support, and the plants will increase the PRO content to enhance the osmotic adjustment ability of cells[39].

Secondly, the *L. rotata* also showed adaptation strategies of photosynthesis in the different degraded grasslands. Compared with the *P. fruticosa*+*F. rubra* community, the content of ChIA and ChIB in the *F. rubra*+*L. rotata* community was significantly lower, which was consistent with the observation of the leaf color of each sampling point in the field. And DAVID J. BURRITT and SUSAN MACKENZIE's study on a shade-loving plant named *Begonia erythrophylla* also showed the same result, that the content of ChIA and ChIB decreased in plant leaves when the plant was placed under conditions of full light[40]. It has been found that the activities of different hormones can be influenced to varying degrees by light[41]. The results indicate that strong illumination is a major stress disorder faced by *L. rotata* in the *F. rubra*+*L. rotata* community. However, in *P. fruticosa*+*F. rubra* community, *L. rotata* showed a significant increase in ChIB. This is an important indicator to judge plant shade tolerance for its advantage of use the blue-violet light in low light environment to enhance the ability of supplemental lighting. Such indicate the *L. rotata* has adapted the depression of the *P. fruticosa* bush[42]. At the same time, carotenoids are significantly reduced in mildly degraded plots. Carotenoids have the function of protecting chlorophyll under adverse conditions, further indicating that photosynthesis of *L. rotata* in the *P. fruticosa*+*F. rubra* community has not been greatly affected, and the plant has adapted to a low-light environment[43]. In the bare land, the content of three photosynthetic pigments in *L. rotata* was higher than that in the moderately degraded grassland, while the content of ChIA and ChIB was lower than that of the *P. fruticosa*+*F. rubra* community, and the carotenoid content was obviously increased. The result indicates that in the bare land, the *L. rotata* mainly protects the chlorophyll by increasing the carotenoid content, then adapts to the degraded grassland environment.

In addition, in the submicroscopic structure, the chloroplasts in the leaves of *L. rotata* in the *F. rubra*+*L. rotata* community are more closely arranged and distributed regularly, with no starch. Usually regular chloroplast arrangements could receive more light energy and produce more starch granules around the granules[44,45]. In this study, the unexpected lack of starch granules in the sub-microscopic structure of leaves in the *F. rubra*+*L. rotata* community was possibly a result of resisting habitat stress. *L. rotata* in this community fully utilizes the light energy by adjusting the position of the chloroplast. At the same time, to resist the grassland degradation stress, the energy consumption is higher than in the other 2 degraded grasslands.

Response of *L. rotata* to climate change

There's deep impact of climate warming and changes in dryness on grassland ecology, especially in the Sanjiangyuan region. Where large area of swampy meadow and wetland were greatly degraded and reduced, the surrounding grassland was seriously degraded, and desertification is serious. As a result, there is sustained soil erosion, and animal husbandry recession in that region[46,47,48]. These phenomena are bound to have a negative impact on fragile ecosystems. For example, land freezing and thawing is common in highland areas, and water frozen in soil is one of the main sources of water for highland plants[49]. The dry climate will cause the area of frozen soil to expand, the melting layer of the season will be thickened, and even the permafrost under the soil surface will disappear completely, which will directly lead to the reduction of soil moisture in the root layer of the plant, the drying of the topsoil and the reduction of vegetation coverage[50,51,52]. Previous studies have shown that with the increase of grassland degradation, soil fertility (organic matter, nitrogen, phosphorus)[53], water holding capacity, and total porosity continue to decrease, while the soil bulk density, freeze-thaw days[54], and freeze-thaw cycles continue to increase. This is consistent with the study of Anyuan, Xu Zhu and others[55]. Therefore, with the

development of climate warming and drying and the increase in grassland degradation, the stress of drought, fertilizer loss and freezing and thawing stress in grassland communities are expected continue to increase.

The analysis results indicated that due to the special morphological and physiological structure, especially the long root system, which could resist dry and warm stress. The pioneer plant characteristics of *L. rotata* in degraded grassland indicate that this plant can quickly occupy the bare land after degradation. The author speculates that the trend of dry and warm climate in the main producing areas will result in less competition in the *L. rotata* community and with no effect on its growth. Thus possibly promote expansion of the *L. rotata* population in some degree. On the other hand, the rapid expansion of *L. rotata* is also positively alleviating the changes in soil structure caused by dry and warming. The investigation showed that *L. rotata* is a good species for soil conservation in the context of dry warming.

Conclusions

Interestingly, among the three gradients of degraded grassland, *L. rotata* in the moderately degraded grassland shows the strongest resistance. Compared with the mildly degraded grassland, there are stronger stress of the moderately degraded grassland, the result mentioned above may be closely related to the little competition of communities in this habitat. On the other hand, this study considers that the differences in leaf microstructure of *L. rotata* among the three degraded grades are due to differences in light intensity and transpiration. At the same time, the long main roots and the large root area of *L. rotata* help it overcome stress caused by grassland degradation, so that the stress factors to mature plant of *L. rotata* do not increase positively with the degradation degree. For the key stress factors of *L. rotata* growth in the moderately degraded grassland, further research is needed for analysis.

The study showed that the soil stress may plays a key role in the colonization stage of *L. rotata* seedlings, resulting in a significant decrease in the density of the *L. rotata* population in the bare land habitat. In summary, the adaptation mechanism of *L. rotata* to degraded grassland is complex, which is a result of a combination of various factors. This study can provide a reference for breeding and ecological planting of *L. rotata*, suggests that in the nursery stage, the moderately degraded grassland should be chosen for ecological breeding to enhance the survival rate of seedlings. While after large-scale planting, reasonable community interventions should be used to simulate the *P. fruticosa* + *F. rubra* community to increase the yield of medicinal materials.

Declarations

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Authors' contributions

Tong Niu, Shihong Zhong, Hui Sun and Rui Gu were responsible for the design of the experiment and the writing of the manuscript. Shihong Zhong, Lin Liu, Jing Xie and Chaoqiong Chen were responsible for the revision of the manuscript. Tong Niu, Can Zhao and Rong Ding were responsible for the advancement of the experiment and the recording and statistics of the experimental data.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files or available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

CAT: catalase; MDA: malondialdehyde; POD: peroxidase; SOD: superoxide dismutase; PRO: Proline; SS: Soluble sugar; SP: Soluble protein

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Tables

Table 1 Eco-environment factors of different degraded grasslands

	Community type		
	P. fruticosa & F. rubra community (Mildly degraded habitats)	F. rubra & L. rotata community (Moderately degraded habitats)	Bare land (Extremely degraded habitats)
Altitude(m)	3765	3774	3767
Slope	5	5	10
Aspect	E	E	E
Plant coverage(%)	95	85	2
Colony species height(cm)	30 P. fruticosa 20 F. rubra	10 F. rubra	3 L. rotata
Soil organic matter (%)	12.5	8.97	3.44
Soil available nitrogen(mg·kg ⁻¹)	160.34	131.74	39.18
Soil available phosphorus(mg·kg ⁻¹)	8.76	5.98	3.21
Soil available potassium(mg·kg ⁻¹)	153.00	130.50	94.00
Soil field water holding capacity(%)	63.67	53.34	19.85
5cm soil freezing and thawing time(d)	—	11	62
15cm soil freezing and thawing time(d)	—	6	35
20cm soil freezing and thawing time(d)	—	10	27
25cm soil freezing and thawing time(d)	—	2	24

Table 2 Plant characteristics comparison of *L.rotata* from different degraded grasslands

Number of plants	Leaf spread (length) [cm]	Leaf spread (width) [cm]	Number of flowering plants	Flowering plant proportion	
<i>P. fruticosa</i> + <i>F. rubra</i> community	122	14.28±4.85a	11.36±4.40a	62	51%
<i>F. rubra</i> + <i>L. rotata</i> community 1	1071	9.06±2.71bc	7.29±2.67bc	164	15%
<i>F. rubra</i> + <i>L. rotata</i> community 2	860	10.29±2.93b	8.59±3.02b	212	25%
<i>F. rubra</i> + <i>L. rotata</i> community 3	716	10.09±3.96b	8.73±4.12 b	183	26%
Bare land	39	11.69±7.53ab	9.64±6.68ab	12	31%

Duncan test method was used to compare the length and width of the leaf of *L. rotata*. Numbers of Leaf spread in the table represent means ± standard deviation (P<0.05). Use letter-marking to indicate differences in plant size between different habitats.

Table 3 Leaf tissue structure parameters of *L. rotata* from different degraded grasslands.

	Fence tissue thickness (mm)	Sponge tissue thickness (mm)	Thickness rate of palisade and sponge tissue	CTR	SR
<i>P. fruticosa</i> + <i>F. rubra</i> community	3.55±0.91a	3.28±0.72ab	1.11±0.30b	1.72±0.34a	1.59±0.20ac
<i>F. rubra</i> + <i>L. rotata</i> community	3.31±0.38a	2.40±0.59b	1.46±0.37a	1.85±0.39a	1.35±0.46ab
bare land	3.23±0.59a	2.06±0.66b	1.72±0.59ac	1.90±0.44a	1.19±0.33b

Numbers in the table represent means ± standard deviation (P<0.05). Use letter-marking to indicate differences in plant size between different habitats.

Table 4 Epidermal tissue structure parameters of *L. rotata* leaves from different habitats.

	Leaf thickness (mm)	Cuticle thickness (µm)		Glandular phosphorus density	Stomatal index
		Upper epidermis	Lower epidermis		
<i>P. fruticosa</i> + <i>F. rubra</i> community	2.09±0.34ac	123.33±40.28b	148.21±22.09b	7.40±0.92c	29.50±4.75b
<i>F. rubra</i> + <i>L. rotata</i> community	1.81±0.24b	161.67±36.36ac	151.79±29.07ab	9.00±1.18b	30.97±2.59a
bare land	1.74±0.23b	173.33±23.21a	169.64±23.49a	10.80±1.40a	33.44±2.34a

Numbers in the table represent means \pm standard deviation ($P < 0.05$). Use letter-marking to indicate differences in plant size between different habitats.

Table 5 Physiological indexes of stress resistance of *L. rotata* from different habitats

	<i>P. fruticosa</i> + <i>F. rubra</i> community	<i>F. rubra</i> + <i>L. rotata</i> community	bare land
SOD($\mu\text{g}\cdot\text{mgprot}\cdot\text{ml}^{-1}\cdot\text{min}^{-1}$)	2.069 \pm 0.613b	2.830 \pm 0.415a	1.181 \pm 0.543c
POD($\text{U}\cdot\text{mgprot}^{-1}$)	27.351 \pm 1.323b	42.629 \pm 3.947a	22.031 \pm 1.011c
CAT($\text{U}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$)	0.753 \pm 0.272a	0.851 \pm 0.344a	0.793 \pm 0.312a
MDA($\text{nmol}\cdot\text{mgprot}^{-1}$)	2.234 \pm 0.428b	2.920 \pm 0.767a	1.600 \pm 0.268b
PRO($\mu\text{g}\cdot\text{g}^{-1}$ Tissue wet weight)	114.947 \pm 9.459a	87.581 \pm 7.451b	104.498 \pm 14.307a
SP($\mu\text{g}\cdot\text{L}^{-1}$)	1.576 \pm 0.895a	1.126 \pm 0.627a	2.038 \pm 0.716a
SS($\mu\text{g}\cdot\text{mgprot}^{-1}$)	1.201 \pm 0.201b	1.838 \pm 0.234a	0.943 \pm 0.184b

Table 6 Pearson correlation analysis for the correlation between the contents of stress resistance indicators

	SOD	POD	CAT	MDA	PRO	SS
POD	0.758**					
CAT	-0.055	0.049				
MDA	0.477**	0.659**	0.281			
PRO	-0.314	-0.539**	-0.234	-0.357		
SS	0.573	0.847**	0.170	0.299	-0.512**	
SP	-0.247	-0.363*	-0.236	-0.720**	0.303	-0.391*

* $P < 0.05$, ** $P < 0.01$.

Table 7 Comprehensive evaluation of *L. rotata* leaves stress resistance in different habitats.

	Membership function value							Average membership value	Sort	D	Sort
	SOD	POD	CAT	PRO	MDA	SS	SP				
<i>P. fruticosa</i> + <i>F. rubra</i> community	0.575	0.242	0.479	0.686	0.686	0.398	0.473	0.506	2	0.467	2
<i>F. rubra</i> + <i>L. rotata</i> community	0.823	0.796	0.851	0.149	0.489	0.780	0.326	0.602	1	0.667	1
bare land	0.286	0.050	0.793	0.527	0.869	0.242	0.624	0.484	3	0.423	3

The mean values of the physiological indicators of *L. rotata* of different habitats and the comprehensive evaluation D values are recorded.

Table 8 Chlorophyll content in leaves of *L. rotata* from different habitats

	ChIA mg·L ⁻¹	ChIB mg·L ⁻¹	Carotene mg·L ⁻¹
<i>P. fruticosa</i> + <i>F. rubra</i> community	8.327±1.294a	3.680±0.424a	1.302±0.330b
<i>F. rubra</i> + <i>L. rotata</i> community	5.950±0.555b	2.774±0.242b	1.511±0.184a
bare land	7.613±1.685a	2.927±0.436b	1.593±0.208a

Numbers in the table represent means ± standard deviation (P<0.05). Use letter-marking to indicate differences in chlorophyll content between different habitats.

Figures

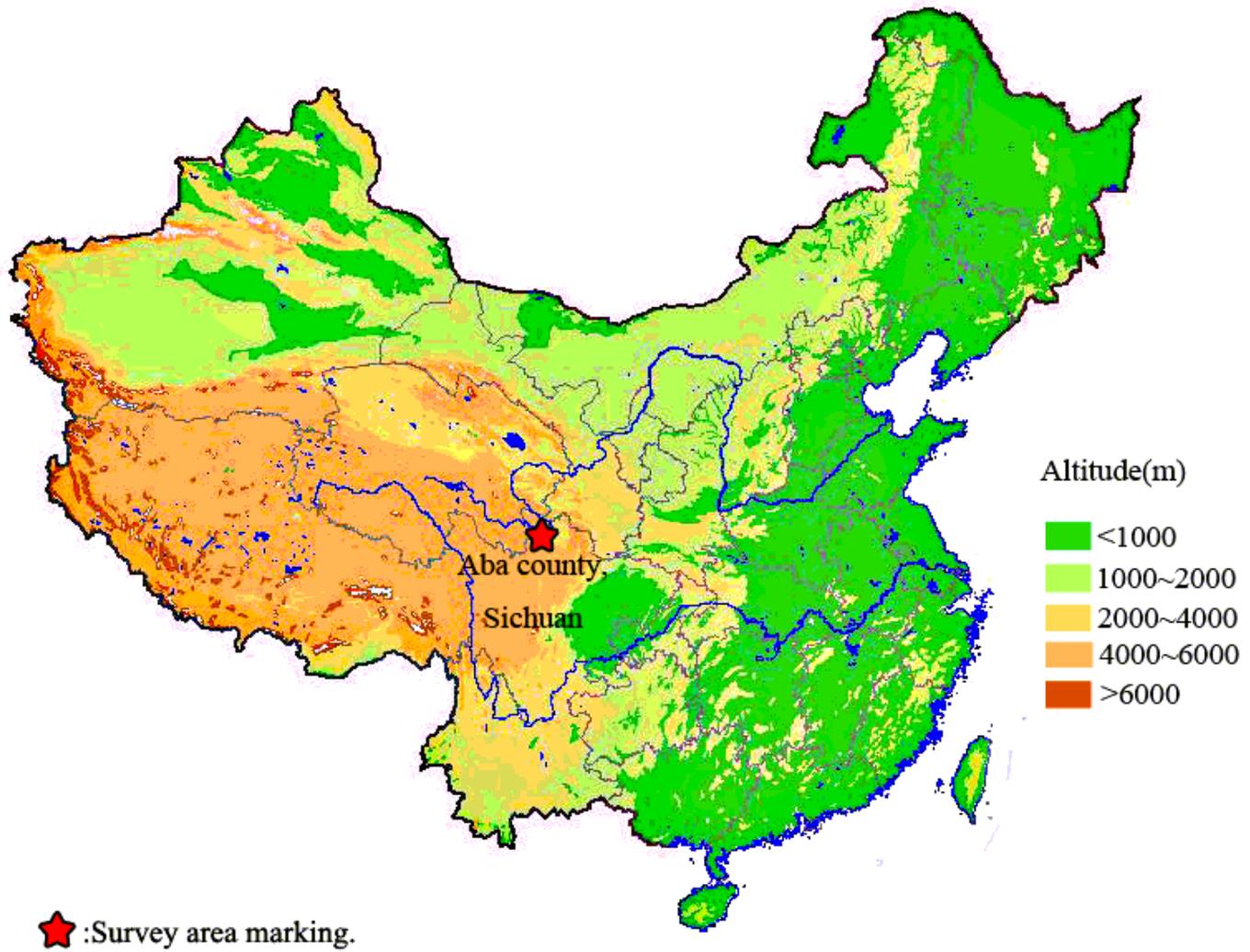


Figure 1

The elevation map of the survey location. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

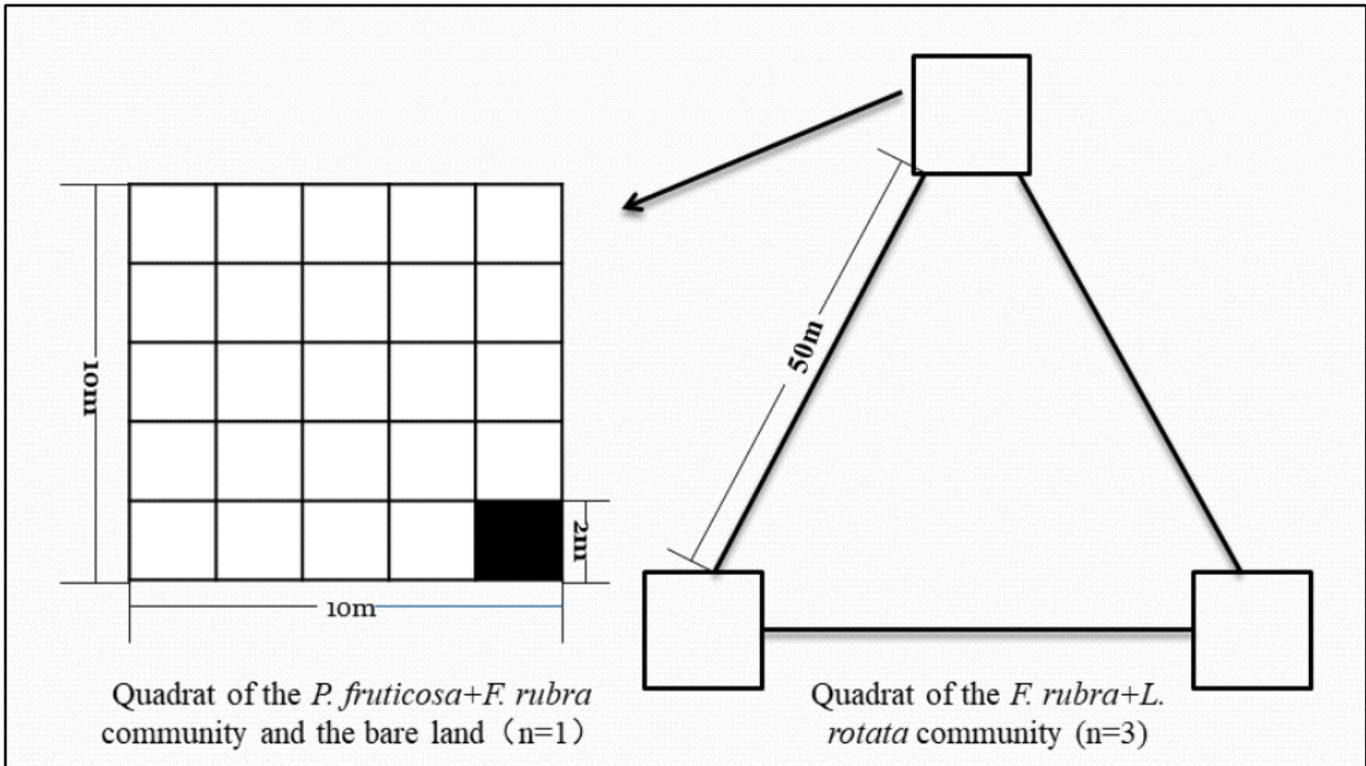


Figure 2

Schematic diagram of quadrats Note: The *F. rubra*+*L. rotata* community only measures the plant size in the 2m * 2m area in the lower right corner (the black filled area in the figure).

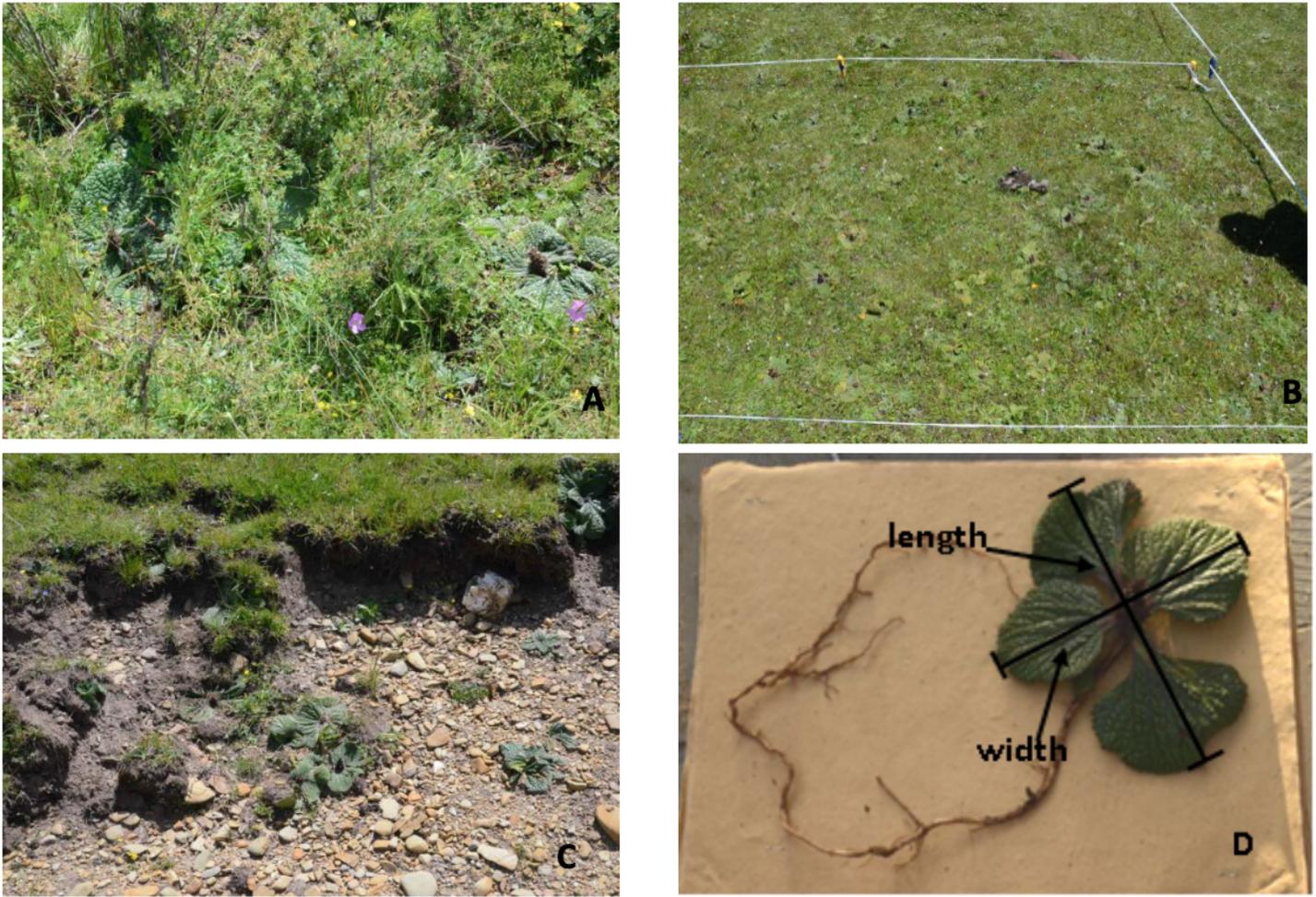


Figure 3

Different degradation habitats and plant of *L.rotata* A: *P. fruticosa*+*F. rubra* community; B: *F. rubra*+*L. rotata* community; C: bare land; D: plant with whole root. Leaf spread (length) represents the tip distance of two large leaves. Leaf spread (width) represents the tip distance of the two small leaflets.

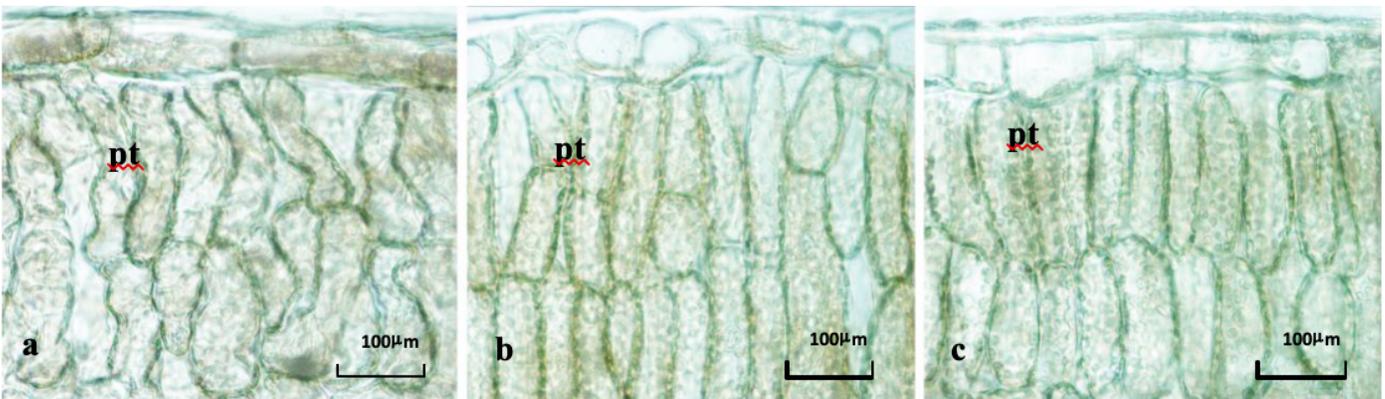


Figure 4

Micrograph of *L. rotata* leaf palisade tissue(40×). a: *L. rotata* leaf from the *P. fruticosa*+*F. rubra* community b: *L. rotata* leaf from the *F. rubra*+*L. rotata* community c: *L. rotata* from the bare land

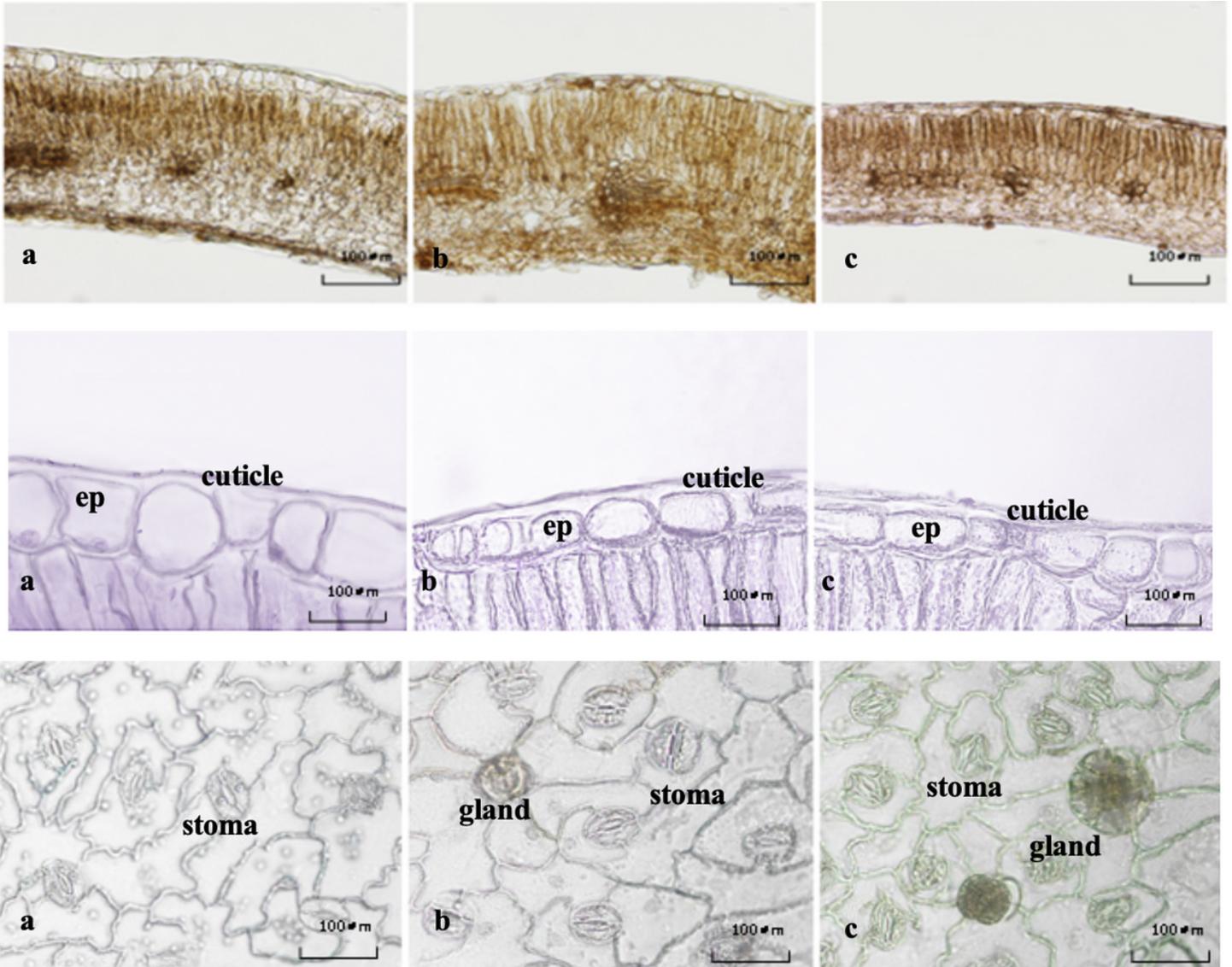
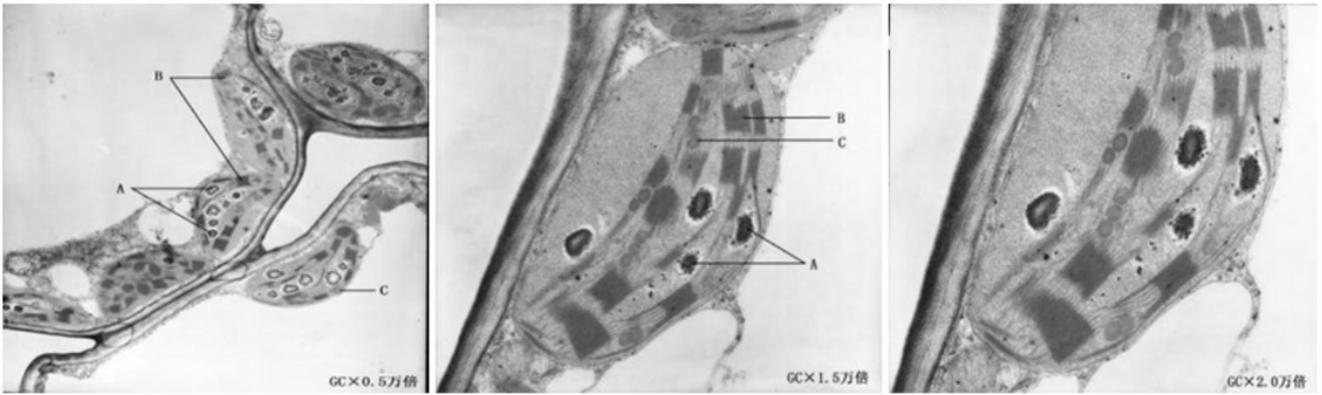
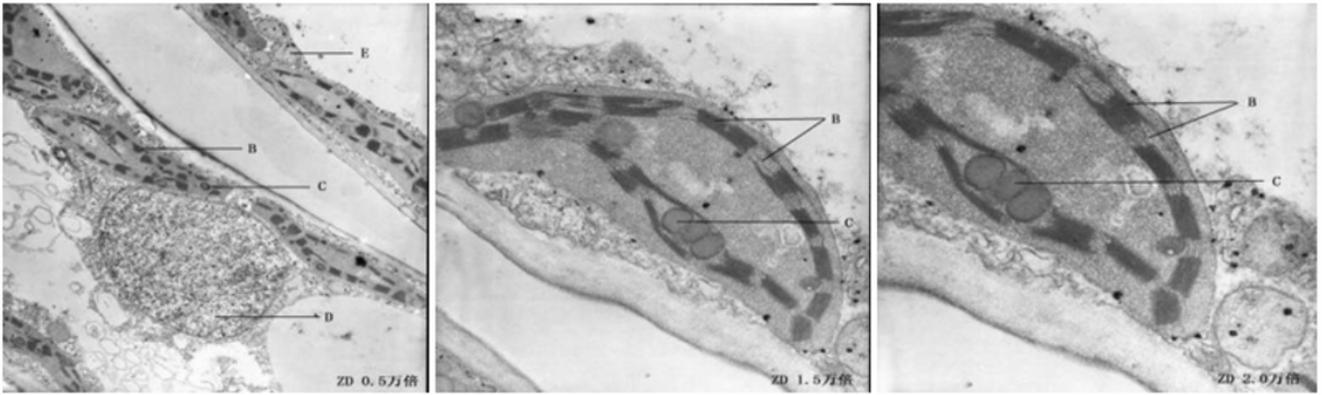


Figure 5

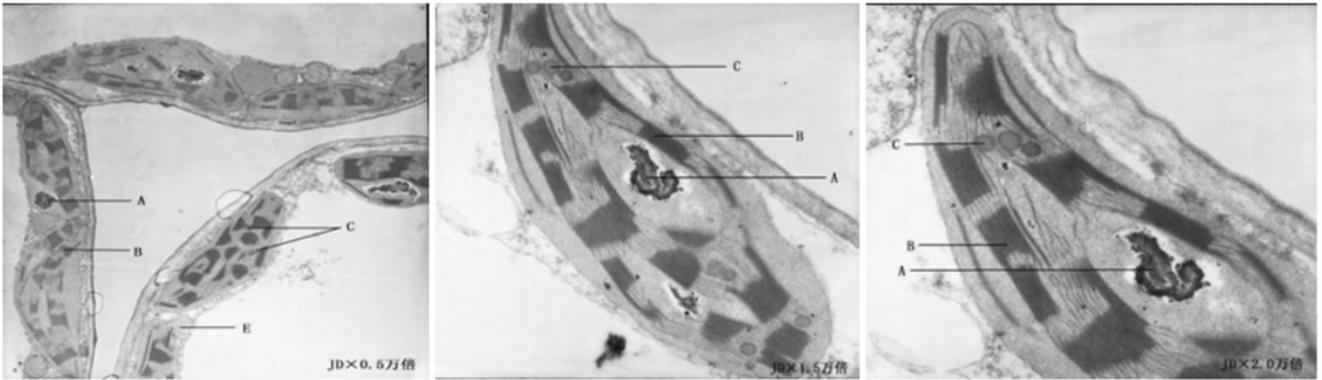
Micrograph of leaf epidermis structure a: *L. rotata* from the *P. fruticosa*+*F. rubra* community b: *L. rotata* from the *F. rubra*+*L. rotata* community c: *L. rotata* from the bare land



I



II



III

Figure 6

The chloroplast submicroscopic structure of *L. rotata* leaf. From left to right: the magnifications are 5000, 15000, 20000 times I: leaf from the *P. fruticosa*+*F. rubra* community II: leaf from the *F. rubra*+*L. rotata* community III: leaf from the bare land ; A:starch granules B:lamellar granules (in a superimposed, linear form) C:osmiophilic granules D:nucleus E:mitochondria

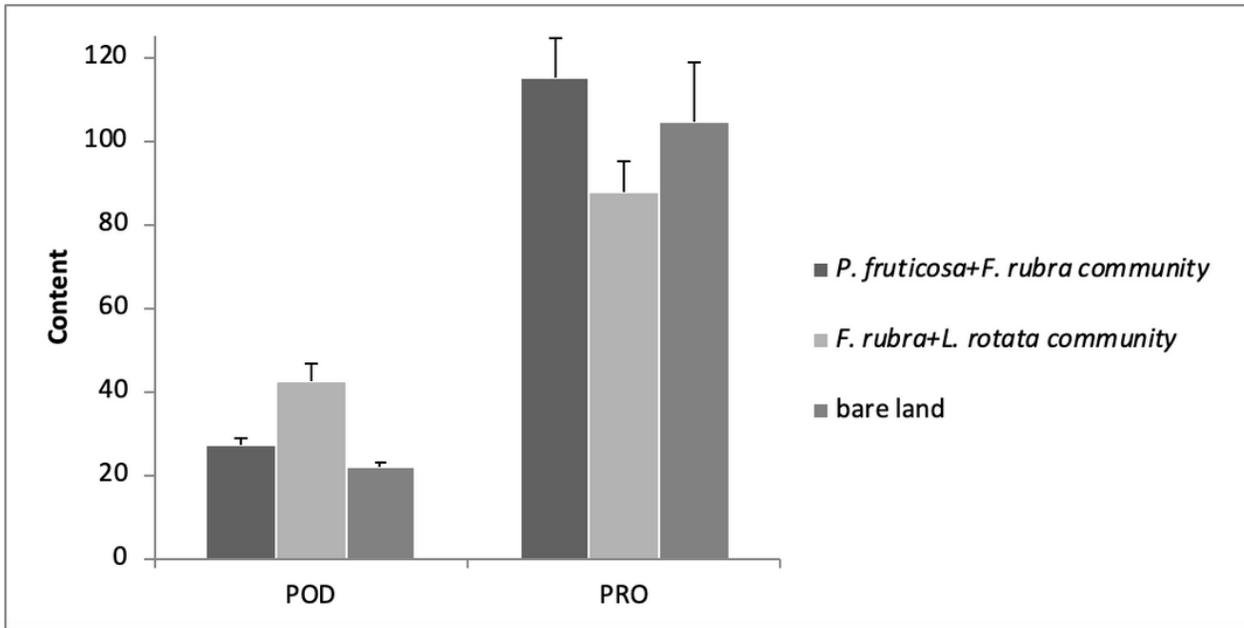
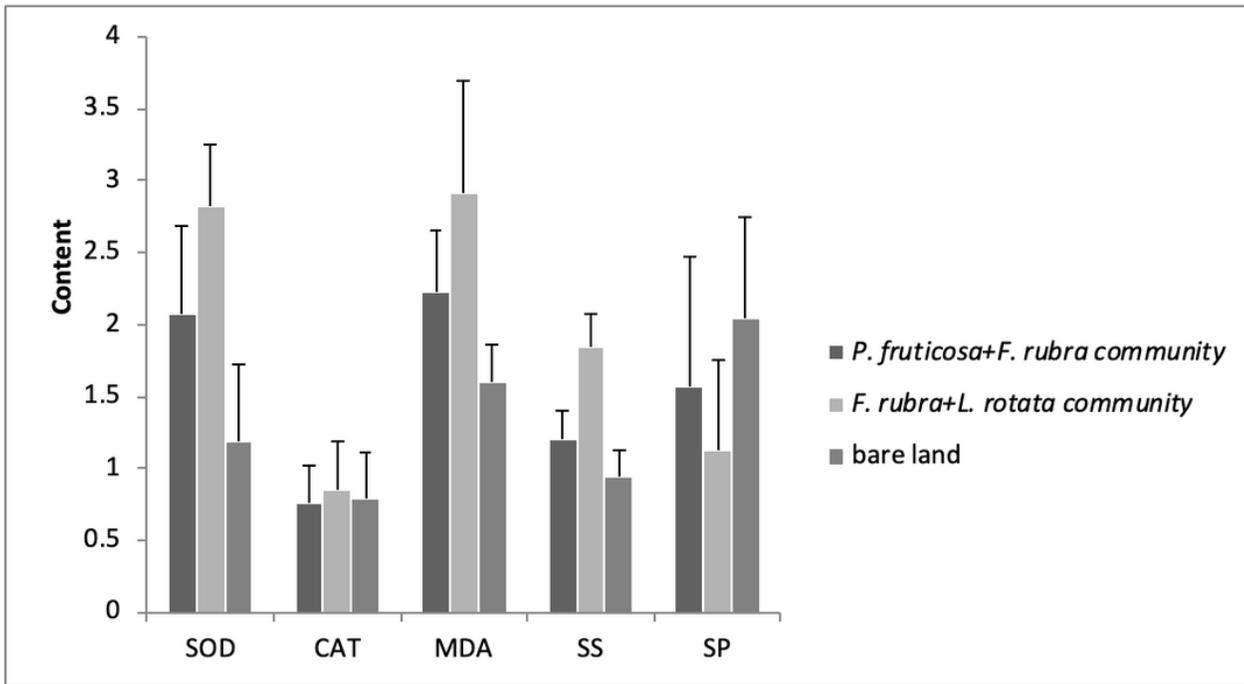


Figure 7

Physiological index of stress resistance of *L. rotata* from different habitats