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Leaf Anatomical Plasticity of *Phyllostachys glauca* McClure in Limestone Mountains Was Associated with Both Soil Water and Soil Nutrients

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Abstract: Little is known on how karst plants adapt to highly heterogeneous habitats via adjusting leaf anatomical structures. *Phyllostachys glauca* McClure is a dominant species that grow across different microhabitats in the limestone mountains of Jiangxi Province, China. We investigated the leaf anatomical structures, plant biomass, soil water content, soil total nitrogen (TN), and soil total phosphorus (TP) from three habitats characterized by different rock exposure, including high rock exposure (HRE), medium rock exposure (MRE) and low rock exposure (LRE), and aimed to discern the relationships between the leaf anatomical plasticity and edaphic factors. The leaves of *P. glauca* in different habitats showed significant anatomical plasticity in two aspects. First, the leaves adjusted cuticle thickness, papillae length, bulliform cell size and mesophyll thickness to lower water loss and then adapt to the water-deficient habitats (HRE). Second, the leaves enlarged vessels and vascular bundles (first-order and second-order parallel veins) to improve water and nutrient transportation and then enhance plant growth in nitrogen-rich habitats (HRE). Soil water and soil nutrients purely explained the total variation of leaf anatomical traits by 21.7% and 15.7%, respectively, and had a shared proportion of 15.8%. Our results indicated that the leaf anatomical variations in different habitats were associated with both soil water and soil nutrients. Moreover, we found that leaf anatomical structures were more affected by TN than TP. The present study advanced the current understanding of the strategies employed by karst plants to cope with highly heterogeneous habitats via leaf anatomical plasticity.

Keywords: phenotypic plasticity; edaphic factors; habitat heterogeneity; leaf epidermis; vascular bundle; mesophyll; redundancy analysis (RDA)

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1. Introduction

Currently, climate change and increasing anthropogenic activities have generated a more variable environment that plants need to survive [1–3]. Phenotypic plasticity is a key strategy for plants to adapt to heterogeneous habitats and changing environments [4–7]. Via adjusting morphological and physiological traits, plants respond to the ambient environmental changes and enhance their fitness [5,7–9]. This process is usually referred to as phenotypic plasticity. Exploring the mechanism of phenotypic plasticity will help us understand future changes in species distribution and community composition, and manage the forest and crop production [10–12]. At present, phenotypic plasticity is a central issue of plant eco-physiological studies [13].

Leaf phenotypic plasticity is crucial for plant growth and reproduction because the leaf is responsible for many important physiological activities such as gas exchange and

assimilate production. Previous studies show that leaves are more sensitive to environmental changes than other organs, and thus, their phenotypic plasticity properly reflects the adaptive strategies to environments [14–17]. Variations in leaf anatomical structure imply functional adaptations to changing environments, and it has received increasing attention in recent years [10,17–19]. Of the environmental factors, soil water is the most focused factor selected to explore how plants cope with water deficit or drought via anatomical adjustments.

The change in soil water causes leaf plasticity in the epidermis, mesophyll, and vascular structure. Previous studies show that soil water deficit results in increases in the thickness of leaf cuticle, epidermis, mesophyll, as well as the length and density of epidermal appendages, and decreases in the vessel size to reduce water loss and ensure hydraulic safety [10,16,18,20–22]. On the other hand, some experiments found that the responses of leaf anatomical plasticity were various, and the magnitude and direction of their response might depend on the extent of drought and specific species [18,23–26]. In some monocotyledons families such as Poaceae, Cyperaceae, and Juncaceae, bulliform cells are efficient structures and result in leaf curling and inhibiting water loss [18,27,28]. Under water stress, contrasting responses of bulliform cells were observed. The size of bulliform cells was enlarged in *Stipa lagascae* Roem. & Schult. [18] whereas it shrank in Sugarcane [28]. Therefore, discerning the variations in leaf anatomical features can interpret the adaptive strategies of plants to drought.

Besides soil water, previous findings show that soil nutrients also have a remarkable influence on anatomical structures [4,15,29,30]. Some studies confirmed that additions of nitrogen (N) or phosphorus (P) increase the size of midribs, vascular bundles and vessels, and the cross-sectional area of xylems [4,31–33]. In an experiment of N and P addition, researchers found that both N and P have effects on leaf anatomical traits, although the effect of N outweighs that of P [4]. However, we still know little about how leaf anatomical traits respond to changes in both soil water and soil nutrients.

Karst habitats are characterized by rocky and shallow soil, with heterogeneous soil water and nutrients [34–37]. Plants frequently suffer from water stress due to the low water-holding capacity of rocky soils [35,36,38], and soil water is regarded as the primary factor limiting plant growth in karst. On the other hand, some studies confirmed that soil nutrients also play an important role in the growth of karst plants [39–42]. However, little is known on how karst plants adjust their leaf anatomical traits to adapt to the highly heterogeneous soil water and soil nutrients. In the present study, we aimed to discern the leaf anatomical plasticity and its connections with the soil water and soil nutrients by exploring a running bamboo (*Phyllostachys glauca* McClure), a species dominated in limestone mountains in Jiangxi province, China. The bamboo species can occupy the rocky habitats in limestone mountains where other species find it hard to live [43,44]. We hypothesized that: (1) the leaves of *P. glauca* show evident plasticity in anatomical traits in different habitats to adapt to the change in soil water and soil nutrients; and (2) the leaf anatomical variations were chiefly related to soil water rather than soil nutrients because the soil water is the most important factor determining plant distribution and growth in karst.

2. Materials and Methods

2.1. The Species and Study Site

Phyllostachys glauca McClure is a woody and evergreen species, 5–12 m in height and 20–50 mm in culm diameter [45], and exchanges leaves every year [46]. The bamboo is widely distributed from the Yellow River valley to the Yangtze River valley in China [45]. In our study site (Ruichang City, Jiangxi province, China; 29°23′ N~29°51′ N, 115°06′ E~115°44′ E), *P. glauca* dominates in the limestone mountains and forms pure bamboo forests with a total area of 9.9 km^2 [44]. *P. glauca* grows across various microhabitats with different rock exposures. Ruichang City has a subtropical humid monsoon climate,

a mean annual temperature of 16.6 °C, and a mean annual precipitation of 1394 mm, most of which falls during the growing season (from March to September).

2.2. Habitat Classification

Habitats in the limestone region are highly heterogeneous, characterized by shallow soil and high rock exposure [36,41,47]. Soils were developed from limestone, contained rock fragments, and were usually distributed discontinuously. According to previous habitat classification in karst [48–50], the habitats in our study site were classified into three types according to their rock exposure: high rock exposure (HRE), medium rock exposure (MRE), and low rock exposure (LRE), respectively. HRE was the habitat with a rock exposure ranging from 50% to 80%, which had shallow and discontinuously distributed soils; MRE was the habitat with a rock exposure ranging from 30% to 50%, which had a deeper soil layer than MRE; and LRE was the habitat with a rock exposure lower than 30%, which had a relatively deeper and continuously distributed soil.

2.3. Experiment Design and Field Sampling

To exclude the effect of topography on leaf anatomical traits, the randomized block design was employed in this study. In late August 2016, plots of three habitats (HRE, MRE, LRE) in the same block were established at sites with similar aspects, slopes, and elevations. The plot size was 10 m × 10 m, and there were three replicates for each habitat. In each plot, six plants of *Phyllostachys glauca* were randomly selected to investigate leaf anatomy and determine plant biomass.

For each plant, three healthy and mature leaves were sampled from the southern branches in the middle canopy. Leaves were cut into small pieces (about 10 mm × 5 mm) and fixed in FAA (45% alcohol, 0.25% acetic acid, and 1.85% formaldehyde). Then, leaf samples were vacuumized and taken back to laboratory for anatomical investigations. Rock exposure (%) was calculated by the proportion of the aboveground rocks to the ground surface in a plot [41]. After removing the litter layer, five soil cores (0–300 mm depth) were collected from each plot using a soil auger (46 mm internal diameter) and pooled as a single composite sample in the analyses for soil nutrients. Soil depth was assessed by coring from the soil surface to the rock matrix. In a plot, soil depth was calculated by the average of five soil corings.

2.4. Experimental Methods

2.4.1. Leaf Anatomical Investigations

After 48 h fixed in FAA, leaf specimens were put in 15% (v/v) hydrogen fluoride to de-siliconize for 38 h and dehydrated in a graded series of alcohol solutions (began at 50%). For anatomical investigations, the leaf specimens were cut into 7-µm-thick paraffin sections using a rotary microtome (Leica RM2235, Heidelberger, Germany) and stained with safranin and fast green. The sections were mounted in Canada balsam. The paraffin sections were observed and photographed using an optical microscope (Leica DM2500, Heidelberger, Germany) with a camera linked to a computer. The anatomical structures, including epidermis, cuticle, papillae, palisade and spongy tissue, bulliform cell, and vessel and vascular bundle, were investigated. QwinV3 software was used to measure those structure thickness and size, and at least 100 observations were recorded for each structure.

2.4.2. Plant Biomass Determination

After leaf anatomy samples were collected, the plants of *Phyllostachys glauca* were coded and harvested into five components: leaves, branches, culm, rhizomes, and roots. With careful excavation, the rhizome was cut off at the middle location between the sampling plant and the next connected plant. Roots included culm roots (roots growing on culm base) and rhizome roots (roots growing on rhizome nodes). The rhizomes and roots were washed out. Then, all component samples were dried at 70 °C for 72 h and weighed. Plant biomass was the sum of the dry mass of five components.

2.4.3. Soil Water and Nutrient Analysis

Using a soil moisture meter (HH2, Delta-T Devices, Cambridge, UK), soil water content (SWC, % vol) in each plot was measured with five replicates in the field. All SWC measurements were finished within a sunny day after five days without rainfall. After being air-dried, soil samples were cleared of roots and organic debris and ground to pass through a 1-mm sieve for the subsequent chemical analysis. Soil total nitrogen (TN) was analyzed with the Kjeldahl method (K-370, Buchi Scientific Instruments, Flawil, Switzerland). Soil total phosphorus (TP) was measured spectrophotometrically after digestion with sulfuric acid (H_2SO_4). The P concentrations were determined by an ultraviolet spectrophotometer (TU-1901, Beijing General Analysis Instrument, Beijing, China).

2.5. Statistics

To check the differences in soil features and leaf anatomical traits among different habitats (HRE, MRE, LRE), one-way ANOVAs were applied with post hoc LSD tests. Pearson correlation analysis was performed to detect the relationships between leaf anatomical traits and plant biomass, and interrelationships between SWC, soil depth, and rock exposure. Redundancy analysis (RDA) was used to explore the effects of edaphic factors (SWC, soil depth, rock exposure, TN, and TP) on the leaf anatomical traits. Variation partitioning was conducted to identify the pure contributions of soil water and soil nutrients to the variance of anatomical traits. To simplify the interrelationships in the RDA, the traits from the same category were combined as follows: epidermis thickness was the sum of upper epidermis thickness and lower epidermis thickness; mean vessel diameter was the averaged diameter of vessels in three veins (midrib, first-order parallel vein, and second-order parallel vein); mean diameter of vascular bundle was the averaged diameter of vascular bundles in three veins. One-way ANOVAs and Person correlation analysis were conducted in SPSS 16.0 (SPSS Inc., Chicago, IL, USA), and RDA and variation partitioning were performed using CANOCO 5.0 (Microcomputer Power, Ithaca, NY, USA). Figures were graphed with OriginPro 9.0 (Origin Lab Corporation, Northampton, MA, USA).

3. Results

3.1. Soil Features in Three Habitats

The investigated three habitats, high rock exposure (HRE), medium rock exposure (MRE), and low rock exposure (LRE), were distinct from each other in rock exposure, water content, and soil nutrients (Table 1). HRE was characterized by the highest rock exposure, TN and TP, and the lowest soil depth and water content. By contrast, LRE owned the lowest rock exposure and TN, and the highest soil depth and water content. TP in MRE was significantly lower than that in HRE and LRE.

Table 1. Rock exposure and soil characteristics of three habitats in *Phyllostachys glauca* forest.

Habitats	Rock Exposure (%)	Soil Depth (m)	Water Content (%)	Total Nitrogen ($mg \cdot g^{-1}$)	Total Phosphorus ($mg \cdot g^{-1}$)
High rock exposure (HRE)	71.20 ± 9.66 a	0.18 ± 0.03 c	9.27 ± 0.56 c	3.22 ± 0.41 a	0.38 ± 0.05 a
Medium rock exposure (MRE)	42.53 ± 3.83 b	0.37 ± 0.03 b	14.32 ± 1.31 b	2.36 ± 0.31 b	0.29 ± 0.02 b
Low rock exposure (LRE)	4.83 ± 0.73 c	0.75 ± 0.04 a	23.55 ± 1.28 a	1.47 ± 0.19 c	0.38 ± 0.05 a

Values are mean ± S.E.; different lowercase letters indicate significant differences among different habitats ($p < 0.05$).

3.2. Leaf Anatomical Features in Three Habitats

The leaf anatomical features of *Phyllostachys glauca* were similar in the three habitats: HRE, MRE, and LRE (Figure 1). Both upper and lower epidermis were composed of a layer of cells, including epidemic cells, bulliform cells, and stomata. The outer wall of epidermic cells was suberized or silicified to form cuticles. The bulliform cells were transparent with thin cell walls and composed of 3–7 sectorial cells embedded into mesophyll. As to the

location, bulliform cells were located between parallel veins. Many stomata and papillae were distributed on the lower epidermis.

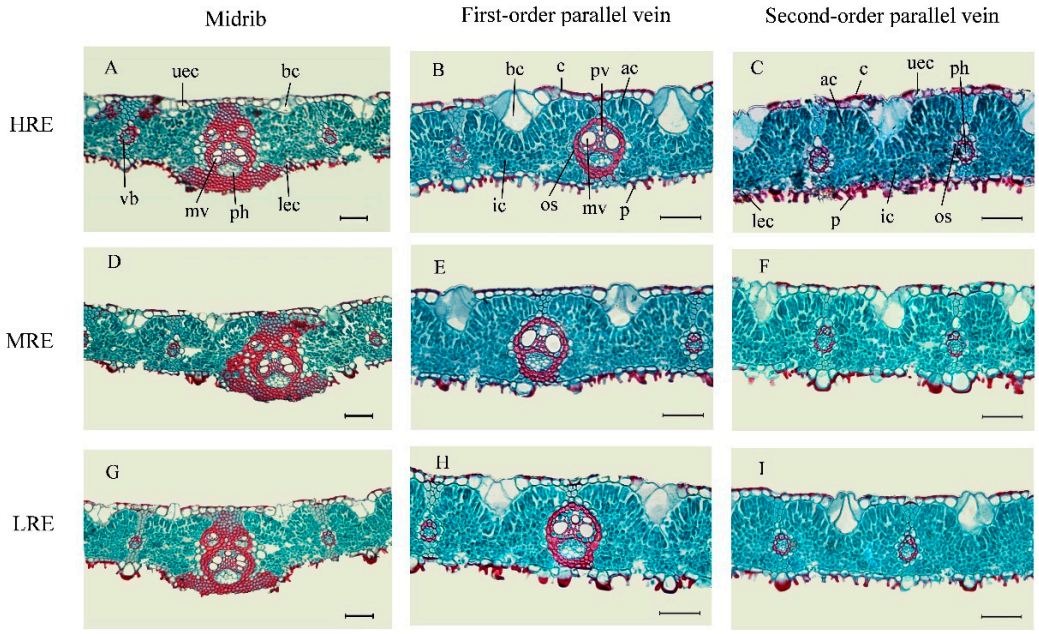


Figure 1. Leaf cross-sections of *Phyllostachys glauca* from three habitats in the limestone mountain. (**A**–**C**), the anatomical features of the midrib, first-order parallel vein, and second-order parallel vein in the habitat of high rock exposure (HRE), respectively; (**D**–**F**), the anatomical features of the midrib, first-order parallel vein, and second-order parallel vein in the habitat with medium rock exposure (MRE), respectively; and (**G**–**I**), the anatomical features of the midrib, first-order parallel vein, and second-order parallel vein in the habitat of low rock exposure (LRE), respectively. Scale bars are 50 μm. vb, vascular bundle; uec, upper epidermal cell; mv, meta-xylem vessel; ph, phloem; lec, lower epidermal cell; bc, bulliform cell; ic, irregular cell; c, cuticle; os, outer-bundle sheath; pv, proto-xylem vessel; ac, arm cell; and p, papillae.

Arm cells and irregular cells composed the palisade tissue and spongy tissue, respectively. Arm cells were below the upper epidermis, whereas irregular cells were close to the lower epidermis. The midrib was a complex vascular system composed of multiple vascular bundles, with well-developed sclerenchyma. The outer-bundle sheath of the midrib was composed of 2–3 layers of cells. The vascular bundles of first-order and second-order parallel veins were close to the lower epidermis. Evident meta-xylem vessels existed in the midribs and first-order parallel veins.

3.3. Leaf Anatomical Variations in Three Habitats

In three habitats, the thickness of the upper and lower epidermis was similar ($p > 0.05$) (Figure 2). Conversely, the cuticle thickness significantly declined with a decrease of habitat rock exposure ($p < 0.05$). The papillae length of HRE and MRE were 12.52 ± 0.18 μm and 12.08 ± 0.14 μm respectively, which were significantly higher than that of LRE ($p < 0.05$), with the value of 11.45 ± 0.15 μm.

The thickness of mesophyll tissue varied in different habitats (Figure 3). With an increase of rock exposure, the thickness of palisade tissue significantly increased ($p < 0.05$). The thickness of spongy tissue in habitats of HRE and MRE was remarkably higher than that of LRE ($p < 0.05$). In the three habitats, the leaf of HRE had the greatest bulliform cells, with a mean cross-sectional area of 1986 ± 35.32 μm^2.

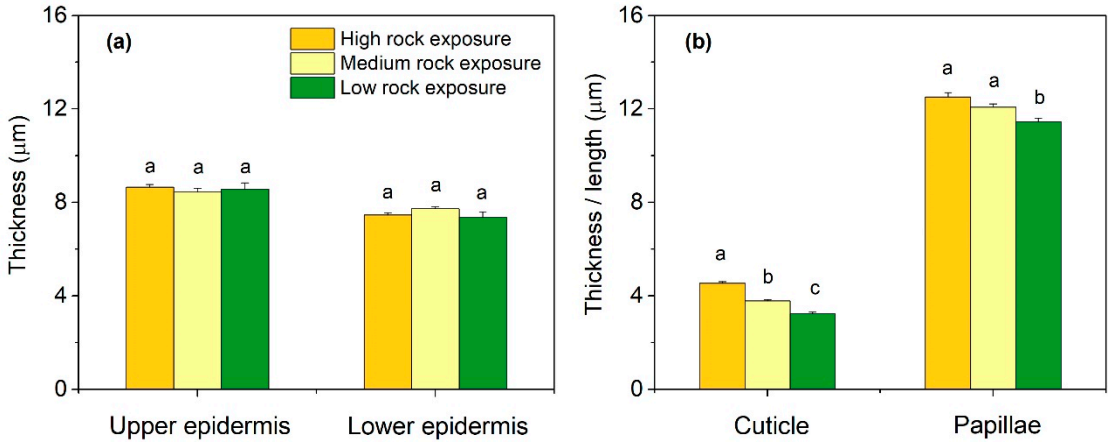


Figure 2. Leaf epidermal traits of *Phyllostachys glauca* from three habitats (high rock exposure, medium rock exposure, and low rock exposure) in the limestone mountain. (**a**), the thickness of upper epidermis and lower epidermis; (**b**), the thickness of cuticle and the length of papillae. Values are means ± S.E. (n = 100). Lowercase letters that differ within an anatomical trait indicate significant ($p < 0.05$) differences among different habitats.

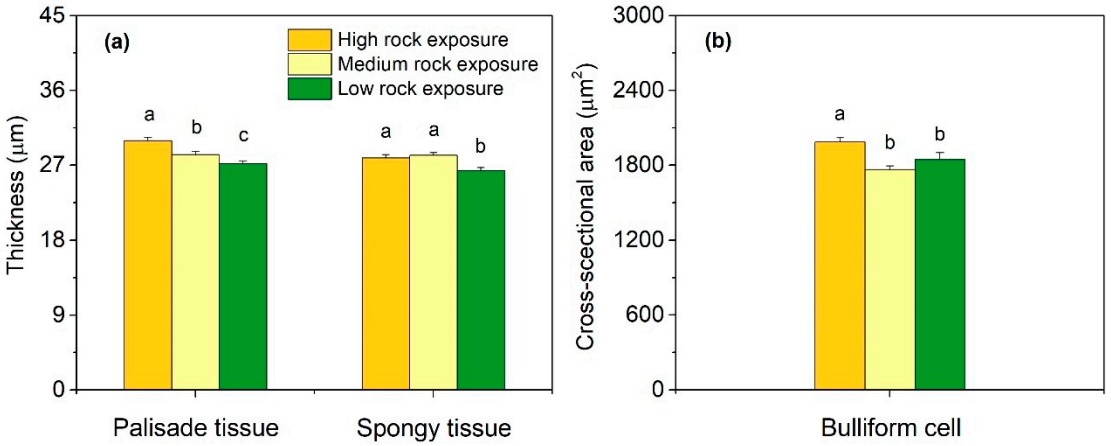


Figure 3. Mesophyll anatomical traits of *Phyllostachys glauca* from three habitats (high rock exposure, medium rock exposure, and low rock exposure) in the limestone mountain. (**a**), the thickness of palisade and spongy tissue; (**b**), the cross-sectional area of bulliform cell. Values are means ± S.E. (n = 100). Lowercase letters that differ within an anatomical trait indicate significant ($p < 0.05$) differences among different habitats.

The leaf vessels in HRE were significantly greater in diameter than those in MRE and LRE ($p < 0.05$), irrespective of vessels from the midrib, first-order parallel vein or second-order parallel vein (Figure 4a). Likewise, the vascular bundle diameter of leaf first-order parallel vein and second-order parallel vein in HRE were greatest in the three habitats. As to the vascular bundle diameter of the midrib, the values of HRE and LRE were significantly higher than that of MRE ($p < 0.05$) (Figure 4b).

3.4. Relationships between Anatomical Traits and Edaphic Factors

The first four axes of RDA explained 53.2% of the total variance of leaf anatomical traits in three habitats. Approximately 47% of the total variance was not explained by edaphic factors, including soil water content (SWC), soil depth, rock exposure, soil total nitrogen (TN), and soil total phosphorus (TP). The first two axes represented the most explained variance (35.43% for the first axis and 9.48% for the second axis), whereas the third and fourth axes only explained 5.59% and 2.70%, respectively. Based on the RDA of the first two axes (Figure 5), enhanced SWC explicitly increased the mean vascular bundle

diameter and cross-sectional area of bulliform cell, and remarkably decreased the cuticle thickness, mean vessel diameter, and papillae length. Compared with other leaf anatomical traits, mean vessel diameter, cross-sectional area of bulliform cell, palisade tissue thickness, and epidermis thickness were more closely associated with soil nutrients (TN and TP).

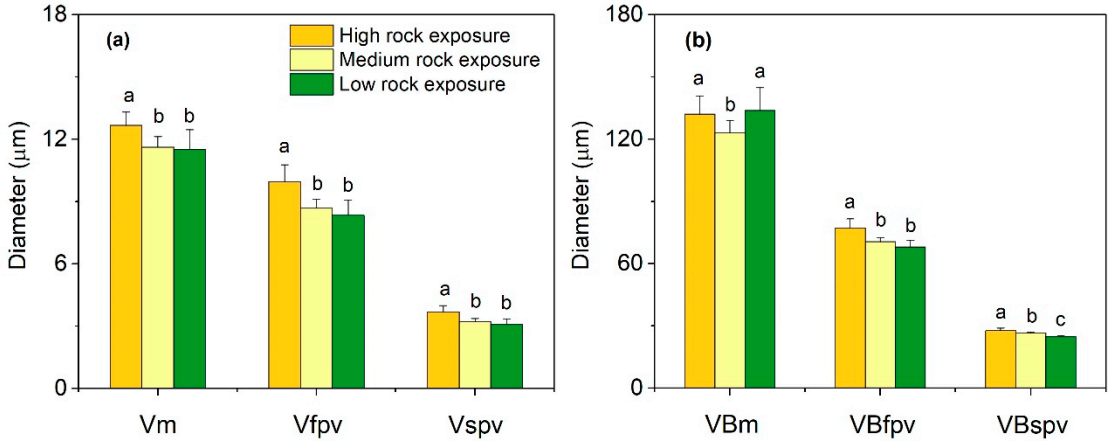


Figure 4. Vascular anatomical traits of *Phyllostachys glauca* from three habitats (high rock exposure, medium rock exposure, and low rock exposure) in the limestone mountain. (**a**), the vessel diameters of different veins; (**b**), the vascular bundle diameters of different veins. Vm, vessels of midrib; Vfpv, vessels of first-order parallel vein; Vspv, vessels of second-order parallel vein; VBm, vascular bundles of midrib; VBfpv, vascular bundles of first-order parallel vein; and VBspv, vascular bundles of second-order parallel vein. Values are means ± S.E. (n = 100). Lowercase letters that differ within an anatomical trait indicate significant ($p < 0.05$) differences among different habitats.

Of those edaphic factors, SWC was positively and significantly correlated with soil depth ($r = 0.977$, $p < 0.001$), and negatively and significantly correlated with rock exposure ($r = -0.964$, $p < 0.001$). Therefore, SWC, soil depth, and rock exposure were classified as soil water factors, and TN and TP were considered as soil nutrients. According to the variation partitioning results, soil water and soil nutrients purely explained the variation of anatomical traits by 21.7% and 15.7%, respectively, and they shared 15.8% of the total variation.

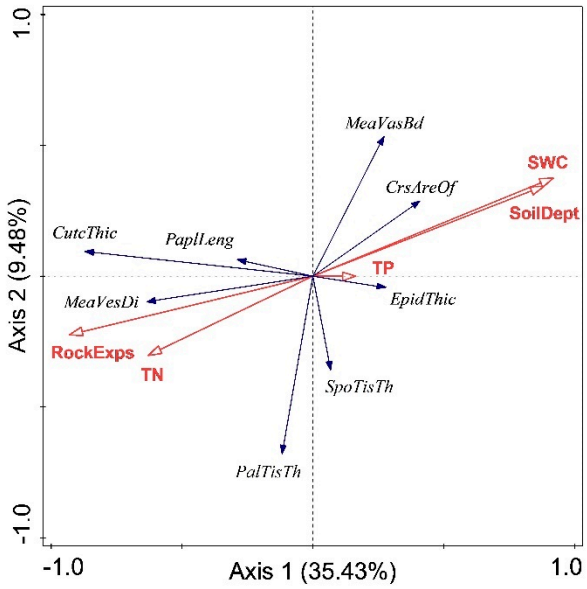


Figure 5. Redundancy analysis (RDA) of relationships among leaf anatomical traits and edaphic factors for *Phyllostachys glauca* in the limestone mountain. *CutcThic*, cuticle thickness; *PaplLeng*, papillae

length; *MeavasBd*, mean vascular bundle diameter; *CrsAreOf*, cross-sectional area of bulliform cell; *EpidThic*, epidermis thickness; *SpoTisTh*, spongy tissue thickness; *PalTisTh*, palisade tissue thickness; *MeaVesDi*, mean vessel diameter; SWC, soil water content; SoilDept, soil depth; TP, soil total phosphorus; TN, soil total nitrogen; and RockExps, rock exposure. Blue lines represent leaf anatomical traits, and red lines represent edaphic factors.

3.5. Relationships between Anatomical Traits and Plant Biomass

The relationships between thirteen anatomical traits and plant biomass were checked (Table 2). The vessel diameters of first-order and second-order parallel veins closely correlated with plant biomass at the 0.05 significance level (r = 0.761 and r = 0.762, respectively), whereas the vascular bundle diameters of first-order and second-order parallel veins tightly correlated with plant biomass at the 0.01 significance level, with correlation coefficients of 0.842 and 0.900, respectively. Moreover, the correlation between the cross-sectional area of bulliform cell and plant biomass was marginally significant (r = 0.761, p = 0.053). Other anatomical traits did not show significant relationships with plant biomass ($p > 0.05$).

Table 2. Correlations between plant biomass and anatomical traits of *Phyllostachys glauca* in the limestone mountain.

Anatomical Traits	*r*	*p*
Cuticle thickness	0.588	0.096
Upper epidermal thickness	0.226	0.559
Lower epidermal thickness	0.562	0.116
Papillae length	0.332	0.382
Palisade tissue thickness	0.601	0.087
Spongy tissue thickness	0.414	0.268
Cross-sectional area of bulliform cell	0.660	0.053
Vessel diameter of midrib	0.309	0.419
Vessel diameter of first-order parallel vein	0.761	0.017
Vessel diameter of second-order parallel vein	0.762	0.017
Vascular bundle diameter of midrib	0.001	0.998
Vascular bundle diameter of first-order parallel vein	0.842	0.004
Vascular bundle diameter of second-order parallel vein	0.900	0.001

Significant correlations ($p < 0.05$) are marked in bold; n = 9.

4. Discussion

4.1. The Response of Leaf Anatomical Plasticity to Habitat Heterogeneity

Our first hypothesis that the leaves of *Phyllostachys glauca* show evident plasticity in anatomical structure in different habitats to adapt to the change in soil water and soil nutrients was supported. In the present study, the leaves in a high rock exposure habitat had significantly higher values than those in low rock exposure habitats in the following anatomical traits: the thickness of cuticle, palisade tissue, spongy tissue, length of papillae, cross-sectional area of bulliform cell, vessel sizes, and vascular bundle sizes of first-order parallel veins and second-order parallel veins (Figures 1–4). Increases in the thickness of the cuticle and mesophyll reduce mesophyll water loss, and the long epidermal appendage enhances leaf boundary layer resistance [15,16,18,21]. Those structural adjustments are acknowledged as adaptive features to aridity. HRE was the driest habitat of the three habitats (Table 1), and the changes in the sizes of the cuticle, palisade tissue and papillae to allow the leaves to adapt to water deficit are consistent with those found in previous studies [15,18,21].

Bulliform cells function in curling leaves, which is acknowledged as a protective mechanism against water loss under severe drought stress [18,27,28]. In this work, the size of bulliform cells in HRE was significantly greater than that in MRE and LRE (Figure 3b). Our results are in agreement with the bulliform cell plasticity of *Stipa lagascae* Roem. & Schult. under drought stress [18]. HRE is a severer water-shortage habitat than MRE and

LRE, and the greater bulliform cells in HRE confer a more efficient leaf curling by losing their turgor [27,51,52].

In arid environments, plants tend to reduce their vessel size to avoid wall collapse and cavitation and then acclimate to drought [10,17,20,53]. By contrast, some studies reported that the vessel and vascular bundle size were increased under the additions of N or P, and inferred that the plastic responses were adjusted to enhance hydraulic conductivity and nutrient transportation efficiency [4,19,31,32]. In the present study, the soil water content (SWC) decreased with an increase in rock exposure, whereas TN increased with an increase in rock exposure (Figure 5). From the perspective of arid acclimation, the leaf vessels in HRE were supposed to be smaller than those in MRE and LRE because the SWC in HRE was the lowest in the three habitats. Contrary to expectation, the vessels were enlarged from LRE to MRE, and to HRE (Figure 4). The variation trend of vessels size in the three habitats is in accordance with the anatomical response to increasing soil nutrient contents [4,19,31]. Likewise, the increasing vascular bundle size of first-order and second-order parallel veins was likely a plastic response to the TN increase from LRE to MRE, and to HRE. Furthermore, the positive and strong correlation between the sizes of vessels and vascular bundles and plant biomass implied that the anatomical structures were adjusted to improve water and nutrient transportation and then meet the need for plant growth (Table 2).

4.2. The Effect of Soil Water and Soil Nutrients on Leaf Anatomical Traits

Our second hypothesis that the leaf anatomical variations were chiefly related to soil water rather than soil nutrients because soil water is the most important factor determining plant distribution and growth in karst was partly supported. Although the rainfall is abundant in karst regions of China, the shallow and highly permeable soils cannot afford sufficient water for plant persistent utilizing, and plants in karst habitats are often subject to water deficit [35,54,55]. Thus soil water, the limiting factor for plant growth, plays a vital role in leaf anatomical acclimation [10,20,22]. Consistent with those studies, soil water imposed a remarkable influence on the leaf anatomical traits of *Phyllostachys glauca*, especially the mean vascular bundle diameter, mean vessel diameter, cross-sectional area of bulliform cell, cuticle thickness and papillae length (Figure 5). According to the results of variation partitioning, soil water occupied the highest proportion of purely explained variance with the value of 21.7%.

Contrary to our expectation, soil nutrients also have a prominent effect on the mean vessel diameter, cross-sectional area of bulliform cell, palisade tissue thickness, and epidermis thickness, and have a purely explained variance of 15.7% plus a part of 15.8% shared with soil water (Figure 5). Our findings are consistent with the previous studies, which confirmed that TN and TP significantly affected the sizes of phloem, vessels, leaf midribs, and their vascular bundles [4,31,32]. On the other hand, the effects of soil water and nutrients on plants are often coupled [56–60]. We also found that the interactions of soil water and nutrients determine the responses of leaf anatomical structure, with a shared effect of 15.8%. Therefore, the effect of soil nutrients on the leaf anatomy of *Phyllostachys glauca* in limestone mountains was remarkable and significant.

In a recent experiment that combined N and P addition, Cai et al. (2017) found that the leaf and stem anatomical traits of *Arabidopsis thaliana* were affected by N addition, whereas fewer by P addition, and claimed that the anatomical responses induced by N probably satisfied hydraulic conductivity and plant growth [4]. Similarly, other than TN, TP had a limited effect on the leaf anatomical traits of *P. glauca* (Figure 5). In our work, the different limestone habitats had similar concentrations of TP (Table 1). Thus, the relatively homogeneous TP might be responsible for a negligible influence on leaf anatomical plasticity.

5. Conclusions

The present study has advanced the current understanding of the strategies employed by karst plants to cope with highly heterogeneous habitats via leaf anatomical plasticity. Our results show that the leaves of *Phyllostachys glauca* in different limestone habitats exhibited evident anatomical plasticity. The leaves adjusted their cuticle thickness, papillae length, bulliform cell size, and mesophyll thickness to lower water loss and then adapt to the water-deficient habitats. Moreover, the leaves enlarged their vessels and vascular bundles to improve water and nutrient transportation and then enhance plant growth in nitrogen-rich habitats. Although soil water is the primary limiting factor for the growth of karst plants, the leaf anatomical variations in different habitats were associated with both soil water and soil nutrients in our case. Furthermore, we found that TP imposed far less influence on leaf anatomical plasticity than TN. Our findings shed light on an adaptive mechanism of bamboo species to acclimate heterogeneous habitats from the perspective of anatomy.

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