Maximized photosynthetic capacity and decreased hydraulic failure risk during aging in the clump bamboo, Bambusa chungii

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Abstract

To assess the water use of a clumped bamboo species, we investigated water use, physiological responses, and structural changes related to culm aging in the clumped bamboo species Bambusa chungii. Anisohydric behavior was characterized by the changed leaf water potential (ΨL), constant stomatal conductance (gs), and the low stomatal sensitivity (-m) in the young (0.52) and mature groups as well as the aged group (0.41). Intercellular CO2 (Ci) was negatively related to gs, especially during the dry season (R2 = 0.62). Hydraulic conductivity (ks) decreased by 57.9% and 58.8% in the mature and aged groups. This was accompanied by a leaf area (AL) that decreased by 55.7% and 63.7% and water transport path (h) that shortened by 8.5% and 23.3% to maintain the hydraulic safety. The photosynthetic rate (An) was similar among the three age groups even during the dry season when water deficits occurred. This might be due to compensation by increased chlorophyll content (5.3% greater for the mature group) and stomata density (7.4% and 8.1% greater for the mature and aged groups). Physiological and structural regulation contributes to reproductive success for B. chungii.

Full Text

Preamble

Maximized Photosynthetic Capacity and Decreased Hydraulic Failure Risk During Aging in the Clump Bamboo, *Bambusa chungii*

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Highlights - We depicted the anisohydric behavior of *Bambusa chungii* stomata to maximize carbon assimilation - We found their structural compensation to maintain hydraulic safety

Abstract

To assess water use in a clumped bamboo species, we investigated water consumption, physiological responses, and structural changes related to culm aging in *Bambusa chungii*. Anisohydric behavior was characterized by declining leaf water potential (ΨL), constant stomatal conductance (gs), and low stomatal sensitivity (-m) in young (0.52), mature, and aged groups (0.41). Intercellular CO_2 (Ci) was negatively related to gs, especially during the dry season ($R^2 = 0.62$). Hydraulic conductivity (ks) decreased by 57.9% and 58.8% in mature and aged groups, respectively. This was accompanied by reductions in leaf area (AL) of 55.7% and 63.7% and shortening of water transport path (h) by 8.5% and 23.3% to maintain hydraulic safety. The photosynthetic rate (An) remained similar among the three age groups even during the dry season when water deficits occurred, likely due to compensatory increases in chlorophyll content (5.3% greater for the mature group) and stomatal density (7.4% and 8.1% greater for mature and aged groups). Physiological and structural regulation contributes to reproductive success for *B. chungii*.

Key words: Bamboo, TDP method, senescence, hydraulic balance, carbon assimilation

Introduction

Carbon and water cycle balances in forest ecosystems have been profoundly altered by direct and indirect effects of increased atmospheric temperature and changing precipitation patterns resulting from elevated greenhouse gas levels [?, ?, ?]. Understanding how physiological regulation and structural adaptations of plants are linked to environmental conditions provides the foundation for predicting their future development [?, ?]. Bamboo plantations are widely established in subtropical Asia, where they contribute to precipitation cycling, soil water conservation, and soil fertility improvement [?, ?, ?, ?, ?, ?, ?].

Bamboo species are fast-growing with well-developed rhizomes and clonal reproduction, enabling them to rapidly colonize disturbed habitats and form bamboo-dominated forest canopies [?, ?, ?, ?] that significantly alter plant species composition compared to natural conditions [?, ?]. Bamboo has great potential for biomass production and may serve as a net sink for CO_2 carbon sequestration, particularly in native areas [?, ?, ?, ?]. Leaf stomata simultaneously regulate both CO_2 influx and water efflux between leaf and environment [?, ?], so substantial water loss would be expected. The Moso bamboo forest has an annual transpiration of 567 mm, accounting for ~32% of annual precipitation [?, ?]. This is quite similar to the 620.5-mm annual transpiration of *Abies amabilis* forest [?, ?], and greater than the 250-mm annual transpiration of *Fagus sylvatica* forest [?, ?]. However, shallow bamboo root systems distributed mostly within the upper 0–20 mm of soil may not be well adapted for water uptake during drought [?, ?, ?]. Additionally, gradually decreased xylem conductivity during secondary growth increases the possibility of hydraulic failure [?, ?, ?]. Hydraulic failure occurs when water in xylem conduits cavitates under high tension, with resulting embolisms restricting water transport to the canopy and ultimately causing tissue death through desiccation. Clarifying the relationship between large transpiration demand and a vulnerable conductive system enhances our understanding of plant responses to variable resource supplies [?, ?, ?]. Few studies have addressed how bamboos maximize carbon assimilation while maintaining hydraulic safety in their conductive systems.

Generally, stomata respond to increasing vapor pressure deficit between leaf and air by partially closing [?, ?], thereby avoiding high transpiration and water potential decline [?, ?]. This prevents excessive dehydration and physiological damage to the photosynthetic apparatus but decreases carbon assimilation [?, ?]. However, some species maintain low midday leaf water potential as soil water potential decreases during drought while sustaining high stomatal conductance with increasing VPD—an example of anisohydric behavior [?, ?]. It remains unknown whether such behavior exists in bamboo species during growth when they balance water consumption needs with hydraulic limitations in response to environmental conditions. Some anisohydric shrubs and herbs wilt in response to extreme drought stress to avoid sustained damage to photosynthetic functions [?, ?, ?, ?, ?]. Many studies have reported isohydric behavior (reduced stomatal conductance to limit transpiration) in bamboo, with adaptations to water stress such as leaf rolling by unique bulliform cells also found in Gramineae species (including bamboo) [?, ?]. In this manner, leaf area and boundary layer conductance are reduced [?, ?, ?]. We hypothesize that anisohydric behavior might also serve as a water stress regulation strategy for bamboo.

Age-related tree structure can significantly influence plant water regulation. Some researchers propose that plant structure tends to converge on adaptations most suitable to given conditions [?, ?, ?]. However, this conclusion has been validated only for dicotyledon species, which have active cambium for producing new xylem tissues to maintain water transport capacity and avoid hydraulic limitations caused by aging and allometric growth. In contrast, water use by monocotyledon species such as bamboo may not exhibit allometric change with age because secondary growth is absent. The hydraulic system of bamboo, formed during early growth stages, does not change with age. However, increased embolization can block conduits within the xylem, leading to declining hydraulic conductivity [?, ?]. Physical limitations on water flow through hydro-active tissue (sapwood in the xylem) influence stomatal behavior and transpiration in trees, which can be expressed based on Darcy' s law as:

$$J_S = k_s \frac{\Delta\Psi}{h} \quad (1)$$

where J_S is sap flux density, k_s is sapwood-specific hydraulic conductivity (whole-plant conductance per unit sapwood), $\Delta\Psi$ represents the water potential difference between rhizosphere and leaf, and h is tree height. This equation can quantify changes in whole-tree water conductivity. If isohydric behavior is present in bamboo water use regulation, based on this equation, the decreased k_s caused by physical limitations would be compensated by reduced leaf water status ($\Delta\Psi$) if J_S is related to carbon assimilation and h is assumed constant, which would place bamboo species in danger of hydraulic failure. Thus, other hydraulic (architectural or physiological) regulations are expected. However, it remains unclear whether bamboo species can maintain anisohydric behavior while optimizing carbon assimilation as plants age.

Among Asia-Pacific region countries, China has the highest bamboo diversity (626 species) [?, ?], with the greatest species richness occurring in south China forests. Morphologically, all bamboo species can be categorized as either monopodial or sympodial [?, ?, ?]. Monopodial bamboos are native to temperate climates with cool, wet winters, while sympodial bamboos are adapted to tropical climates with pronounced dry seasons [?, ?]. The tight clumping habit of tropical species exposes less rhizome surface to dehydration during extended dry seasons [?, ?]. In this study, we characterize the hydraulic regulation at different ages of a sympodial species, *Bambusa chungii.* Specifically, we determine whether anisohydric behavior is maintained with aging, test hydraulic compensation for decreased xylem water conductance in *B. chungii* culms, and assess whether carbon assimilation is preserved.

Materials and Methods

1. Experimental Site and Sap Flow Measurements

Field observations were conducted from January to December 2014 at the South China Botanical Garden in Guangzhou (23°10' 39.9" N, 113°21' 17.6" E), located in a transitional zone between tropical and subtropical regions. The year is divided by hydrothermal conditions into a wet season extending from April to September and a dry season from October through the following March [?, ?]. The site altitude is above 41 m, with annual precipitation of 1,612–1,909 mm and mean annual temperature of 21.4–21.9°C. Minimum and maximum temperatures typically occur in February and July, respectively.

Three clumps of *B. chungii* with an average density of 180 individuals were selected for sap flow measurements. Fifteen individuals per clump were chosen and classified by age (1-2 years, 3-4 years, $ $5 years, corresponding to young, mature, and aged individuals) based on morphological differences characterizing different growth stages [?, ?]. Sap flux densities were calculated using Granier' s equation [?, ?] with newly derived parameters from unpublished work where we shortened the original 20 mm TDP probes to 5 mm and performed calibration through both cut culm segment experiments under laboratory conditions and pot experiments under field conditions. Detailed mounting procedures for in situ measurements followed those for woody species [?, ?, ?], except that the two probes were installed across bamboo knots, namely on both sides of a knot.

2. Stand Parameters

Diameter at breast height (DBH) of all sample bamboo culms was measured with a diameter tape. The cross-sectional area (A_C) of the culm wall was determined by inserting a hook to cover the wall depth through a 2-mm diameter hole drilled into the wall (Figure S1). The depth covered by the hook corresponded to wall thickness. Depth measurements from four orientations were averaged to improve A_C accuracy. After field measurements, twelve individuals (four per age group) were harvested to collect all leaves and measure height (H) and knot numbers (n_k). Leaf subsamples (five per individual) were used to measure leaf area with a portable leaf area meter (Licor-3000, USA) and total fresh weight with an electronic balance. Total leaf area (A_L) per culm was estimated from the relationship between fresh mass and leaf area derived from subsamples for each individual. We fitted A_L to DBH to estimate A_L for sample culms used in sap flow measurements. H was unrelated to DBH but positively related ($R^2 = 0.68$) to n_k.

3. Canopy Stomatal Conductance

In forests where transpiration is well-coupled with atmospheric conditions, mean stomatal conductance can be calculated based on a simplified equation [?, ?]. Assuming that J_S scaled by the ratio of culm wall cross-sectional area to leaf area (A_C/A_L) equals transpiration rate per unit leaf area (E_L), the mean stomatal conductance for individual culms (G_S) can be calculated as:

$$G_S = \frac{E_L \cdot G_V \cdot T_a}{\rho \cdot VPD} \quad (2)$$

where E_L is whole-culm transpiration per unit leaf area (g m^{-2} s^{-1}), G_V is the universal gas constant adjusted for water vapor (0.462 m^3 kPa K^{-1} kg^{-1}), T_a is air temperature (K), ρ is water density (998 kg m^{-3}), and VPD is in kPa. G_S is expressed in units of mmol m^{-2} s^{-1} [?, ?]. In this study, the bamboo canopy was open and met the application requirements of equation (2).

Leaf area (A_L) was assumed equal between dry and wet seasons, so G_S calculation was not subject to seasonal leaf area dynamics. Estimation involved (1) performing cross-correlation analysis between VPD and F_d, using the time lag to infer time-corrected F_d, and (2) filtering data for VPD $<$ 0.6 kPa during early morning and late afternoon hours [**?**, **?**].

4. Stomatal Sensitivity to Vapor Pressure Deficit

Granier et al. [**?**, **?**] proposed that stomatal sensitivity is proportional to G_S magnitude at low VPD (VPD = 1 kPa) when soil moisture is not limiting, expressed as:

$$-m = \frac{dG_S}{d \ln VPD} \quad (3)$$

where G_{Sref} is the intercept (i.e., G_S value at VPD = 1 kPa in a log-linear relationship), and $-m$ is the regression slope representing stomatal sensitivity to VPD (i.e., $dG_S/d\ln VPD$). Oren et al. [**?**, **?**] demonstrated that m is approximately 0.6. In this study, $-m$ and G_{Sref} in wet and dry seasons were used to delineate stomatal control of transpiration. A boundary line analysis of the VPD-G_S relationship was performed following Schäfer et al. [**?**, **?**] after excluding nighttime data ($Q_0 = 0$). The relationship between $\ln VPD$ and G_S for each small subset was linearly fitted, with intercept and slope corresponding to G_{Sref} (G_S at VPD = 1 kPa) and sensitivity in response to VPD ($dG_S/d\ln VPD$, mmol m^{-2} s^{-1} kPa^{-1}), respectively [**?**, **?**]. The relationship between G_{Sref} and $-d\ln VPD/dG_S$ for both seasons was then linearly fitted to estimate $-m$.

5. Leaf Gas Exchange and Physiological Characters

Daily courses of leaf net photosynthetic rate (A_n), stomatal conductance (g_s), and transpiration rate (E_t) were measured at 2-hour intervals from 5:00 am to 7:00 pm using a portable photosynthesis measurement system (LI-6400, Li-Cor, Lincoln, NE, USA) under ambient conditions on typical clear days during the dry season (January 15-17, 2014) and wet season (July 19-21, 2014). Ambient CO_2 concentration was ~390 mmol mol^{-1}, air humidity ranged 55-65%, leaf temperature varied between 12°C and 28°C, and VPD ranged 1.2-1.5 kPa. For each measurement, leaves were exposed to these conditions for 2-5 minutes to allow stabilization of photosynthetic parameters. Three sun-exposed mature shoots from each culm in each age group were selected for measurement. Leaf water potential (Ψ_L) was measured with a pressure chamber (PMS, Albany, OR, USA) on detached shoots after gas exchange measurements. Chlorophyll content and stomatal density were determined on four leaves collected from culms selected for gas exchange measurement. For chlorophyll content determination, fresh leaves (0.1-0.2 g) were cut into ~1-mm strips and soaked in an acetone:ethyl alcohol mixture (1:2, 30 ml) for 24 hours. The extract solution was used to measure chlorophyll content spectrophotometrically according to Arnon’ s equation [**?**, **?**]. Stomatal number was counted in a fixed field of view under an optical microscope to calculate leaf stomatal density, with stomatal diameter serving as a proxy for stomatal size.

6. Statistical Analysis

ANOVA was used to compare parameter values among different age groups and seasons. Statistical analyses were performed with SAS 9.2 (Statistical Analysis System, NC, USA). Origin 9.0 (Origin Lab, Northampton, MA, USA) was used for graphing.

Results

1. Sap Flow Response to PAR and VPD

On a daily basis, PAR and VPD induced leaf transpiration changes and leaf water status that drive sap movement [**?**, **?**]. Sap flow increased in the morning with increasing daylight intensity, reached a maximum around 12:00 p.m., and gradually decreased until PAR = 0 in both dry and wet seasons (Figure 1 [Figure 1: see original paper]). Sap flow data for all culms in the wet season were used to quantify indirect regulation by PAR and VPD through partial correlation analysis. This showed PAR-dominated sap flow variances ($0.63 < r < 0.96$ with a mean of 0.88, $p < 0.001$) without VPD dominance (mean $r = 0.13$, $p > 0.05$). We continually fitted exponential relationships between PAR and J_S for each culm ($0.68 < R^2 < 0.89$) to obtain predicted J_S (J_{S-p}) from fitted curves. The J_{S-p} for each culm was then normalized by its maximum (at max PAR) and averaged across all culms within each group.

Figure 2a [Figure 2: see original paper] shows the response of normalized J_S (J_{S-N}) to PAR. Different response sensitivities to PAR were found among the three age groups. The PAR at $J_{S-N} = 0.8$ was 557.7, 1074.3, and 856 mol m^{-2} s^{-1} for young, mature, and aged groups, respectively. We selected J_S during 11:00-15:00 to fit with VPD and found constant transpiration across a VPD range of 1.7-3.0 kPa for all age groups (Figure 2b). Accordingly, mean J_S was 21.9, 13.4, and 17.4 g m^{-2} s^{-1} with significant differences among all age groups ($p < 0.01$, ANOVA).

1.1 Leaf Physiological Characters

A_n reached maxima of 6.9, 4.7, and 5.2 mol m^{-2} s^{-1} for young, mature, and aged individuals before "midday depression" under high radiation (PAR > 910.7 mol m^{-2} s^{-1}) in the wet season; however, this depression was not observed in the dry season (Figure 3a [Figure 3: see original paper]). A_n differences among groups were not significant except at 9:00 ($p = 0.02$) in the wet season. Daily mean A_n was much higher in the wet season (3.4 ± 1.6 mol m^{-2} s^{-1}) than in the dry season (1.8 ± 0.9 mol m^{-2} s^{-1}) ($p < 0.001$). g_s showed daily variation similar to A_n, with no significant difference between dry and wet seasons (0.018 ± 0.005 mol m^{-2} s^{-1} and 0.02 ± 0.01 mol m^{-2} s^{-1}, respectively, $p = 0.49$). E_t values for young and aged individuals were significantly higher than for mature ones (Figure 3c, p = 0.036) in the wet season, especially under high light intensity, consistent with J_S (Figure 1c, d). However, no differences were observed among age groups in the dry season. Leaf water potential (Ψ_L) decreased rapidly after 7:00 and recovered after 11:00 when stomata partially closed at 9:00 (Figure 3d). A rapid transpiration increase occurred after 13:00 (Figure 3c) when stomata reopened. This pattern mirrored dramatic water consumption from the stem in the morning (before 11:00 a.m.). Additionally, we found a Ψ_L sequence of young > aged > mature that was significant in the dry season ($p < 0.05$) and positively related to their J_S and transpiration (Figure 2b).

We also found seasonal differences in intercellular CO_2 (C_i) dynamics (Figure 4a [Figure 4: see original paper]). C_i began decreasing gradually at 6:00 in response to increasing A_n with rising PAR until 9:00, then increased again after 15:00 in the dry season. We did not conduct measurements at 5:00 in the wet season and predicted that C_i had the same value as in the dry season before sunrise (6:00). We observed that C_i decreased to a very low value (mean of 70.5 mol CO_2 mol^{-1} for all culms) at 7:00 in the morning, caused by rapidly increasing PAR (Figure 4a) before being promoted by increased g_s at 9:00, then decreasing again at 11:00 until 17:00 when it recovered. No significant differences were observed among age groups. C_i appears to have a dynamic trade-off with g_s. Thus, we fitted C_i to g_s for all individuals and found they were negatively related, especially during the dry season (Figure 4b).

1.2 Stomatal Response to VPD

We fitted the relationship between G_{Sref} and $-d \ln VPD/dG_S$ for the three age groups in both dry and wet seasons (Figure 5 [Figure 5: see original paper]). The young group had the highest slope (i.e., $-m$) of 0.52 ($p < 0.01$) without seasonal differences. The slopes of mature and aged groups were similar ($p > 0.05$), with values of 0.40 and 0.41 in wet and dry seasons; $-d \ln VPD/dG_S$ in the wet season tended to be higher than in the dry season. All $-m$ values were significantly lower than the standard value of 0.6 proposed by Oren et al. [?, ?], indicating that stomata were partially decoupled from VPD-induced stomatal regulation (decreased g_s) when VPD was very high.

1.3 Age Effects on Culm Form Features and Leaf Morphology

At the whole-culm level, both H and A_L significantly decreased with age (Figure 6a [Figure 6: see original paper], b). Meanwhile, A_C unexpectedly decreased (by 13.1% and 8.1%) for mature and aged groups (data not shown). Climatic variations among adjacent years may have induced growth differences in bamboo shoots responsible for such changes among age groups. We found a gradual increase (decrease) in annual mean relative humidity (temperature) from 2009 to 2014 (corresponding to aged to young age groups), which was favorable for younger bamboo growth [?, ?]. We estimated individual height of nine culms used for Ψ_L measurement based on equation (3) and calculated their k_s combining Ψ_L and J_S in the dry season using equation (1). The k_s of mature and aged individuals decreased by 57.9% and 58.8% compared to young groups, revealing significant conduit blockage with age. At the leaf level, mature groups had the highest chlorophyll content (Chl) of 1.02 mg g^{-1}, while young and aged groups had 0.97 and 0.75 mg g^{-1}, respectively (Figure 6c). In contrast, young groups had lower leaf stomatal density than the other two age groups, with stomatal density 6.9% and 7.5% lower than mature and aged groups, respectively (Figure 6d).

Discussion

Stomatal control in woody species under high light conditions is an adaptation for maintaining leaf water potential during excessive transpiration [?, ?, ?]. We found a rapid g_s decrease beginning at 9:00 when PAR = 910.7 mol m^{-2} s^{-1} with mean Ψ_L of -1.25 MPa in the wet season. However, this did not occur in the dry season with significantly lower mean Ψ_L (-2.53 MPa) when PAR reached a maximum of 792.8 mol m^{-2} s^{-1} (Figure 1a). Additionally, we compared g_s of all culms at 11:00 (Figure 3b) and found no differences among age groups or between seasons ($p > 0.05$), implying that stomatal control might not be a complete response to leaf water status in bamboo.

The constant J_S with increased VPD (Figure 2b) also reflected weak stomatal control of transpiration in the dry season as Ψ_L was quite low (Figure 3d). However, the decreased g_s shown in Figure 4 indicated C_i-induced stomatal regulation, especially during the dry season. Stomatal conductance regulation responds to leaf abscisic acid (ABA) release that tends to be activated when pH is low (for example when C_i is high) [?, ?, ?], eventually leading to stomatal closure. Thus, the rapidly increased C_i between 9:00-11:00 in the wet season could be responsible for stomatal closure at 9:00 and for PAR-dominating J_S (Figure 2a). This has been demonstrated in the bamboo *Indocalamus barbatus*, which showed more profound "midday depression"(decreased g_s) when exposed to simulated acid deposition [?, ?]. The traditional view of leaf water potential-induced stomatal control [?, ?] may not hold for *B. chungii*. ABA-induced stomatal regulation is an acclimatory response to drought in some herbs, shrubs, and tree species [?, ?, ?], resulting in increased water use efficiency under moderate stress due to maintained photosynthesis [?, ?]. These results imply reduced effects of leaf water status on photosynthesis and maintenance of high carbon assimilation in bamboo even under desiccating conditions (high VPD).

Our results indicate that *B. chungii* may not maintain minimum leaf water potential during the dry season. It maintained g_s when leaf water potential was low, showing anisohydric behavior. The relationship between stomatal conductance and VPD showed that $-m$ in all three age groups was significantly less than the standard value of 0.6 ($p < 0.01$), especially for mature and aged groups (mean = 0.41). Oren et al. [?, ?] reported that if stomata do not regulate leaf potential near a constant value (anisohydric behavior), a lower slope is expected. Anisohydric behavior has been observed in many plant groups including species such as juniper (*Juniperus formosana*), sugar maple (*Acer saccharum*), sunflower (*Helianthus annuus*), and eucalyptus (*Eucalyptus gomphocephala*). Anisohydric behavior allows a greater Ψ_L range than occurs in isohydric species [?, ?, ?], enabling higher gas exchange rates during drought but at greater risk of hydraulic failure [?, ?]. These plants are more likely to die from cavitation than carbon starvation [?, ?]. As illustrated in Figures 3a and b, the significant reduction in leaf water potential during the dry season did not lead to decreased g_s and A_n at noon, and no g_s differences were found between wet and dry seasons. Ψ_L reached a minimum of -3.1 MPa for the mature group in the dry season. The wilting point leaf water potential (π_{tlp}) across 71 tropical forest tree species ranged from -1.4 to -3.1 MPa, with only 1.5% of species having π_{tlp} lower than -3.0 MPa [?, ?]. Despite maintained g_s in the dry season, A_n still suffered significant decrease, possibly indicating damage to the photosynthetic apparatus induced by drought stress [?, ?, ?]. Further studies on photosynthetic characteristics, such as chlorophyll fluorescence, are needed to clarify these results.

Our results also showed non-significant differences in A_n among the three age groups in both seasons (Figure 3a) even when leaves experienced significant water deficit during the dry season (Figure 3d). Meanwhile, mature and aged groups tended to be more anisohydric than the young group with lower stomatal sensitivity ($-m$ = 0.40 and 0.41) but more negative Ψ_L values in both seasons (Figure 5). ABA biosynthesis can be triggered by reduced leaf turgor in angiosperms—higher sensitivity of ABA synthesis to leaf turgor corresponds with higher stomatal sensitivity (higher $-m$) to VPD [?, ?]. Thus, decreasing $-m$ with increased plant age may relate to partial loss of enzyme activity for ABA synthesis, though this requires further verification. Our results suggest a stronger tendency to maximize carbon assimilation in mature and aged groups, consistent with findings by Emanuel et al. [?, ?]. The carbon-sequestration ability of five-year-old bamboo (*Phyllostachys pubescens*) was significantly higher than three- and one-year-old bamboos during peak growth periods [?, ?]. Increased chlorophyll content (Figure 6c) for mature groups and higher stomatal density for mature and aged groups (Figure 6d) might be associated with this tendency [?, ?]. Mature and aged bamboos transfer ~80% of all nutrient storage to bamboo shoots, significantly contributing to their reproductive and dispersal success [?, ?].

However, weak stomatal control would expose bamboo to hydraulic failure risk since shallow roots and decreased conductivity cannot provide sustained water availability for transpiration. Water content in bamboo stems decreased significantly during maturation phases up to three years [?, ?]. In *B. chungii*, significantly decreased J_S and its sensitivity to PAR were observed in mature and aged groups (Figure 2). Thus, decreased stomatal conductance and transpiration rate would be expected, as found previously in woody tree species [?, ?]. However, such changes in E_t and g_s were not observed for *B. chungii*, especially under high light conditions (Figure 1b, c). Instead, the young group had minimum E_t and g_s with means of 0.77 mmol m^{-2} s^{-1} and 0.0187 mol m^{-2} s^{-1}

during the wet season. Hydraulic architectures are reported to be convergent in plant water regulation [?, ?]. Whitehead et al. [?, ?] proposed an identity equation showing how g_s and Ψ_l are linked to structural features such as the leaf area to sapwood area ratio (A_L/A_C), axial flow path length (h), and xylem permeability (k_s):

$$g_s = \frac{k_s \cdot A_C}{A_L \cdot h} \cdot \frac{\Psi_s - \Psi_l - \rho g h}{\rho_w \eta} \quad (4)$$

where Ψ_s is soil water potential, g is gravitational acceleration, h is vertical distance, and ρ, η, and ρ_w are water density, dynamic viscosity, and molar volume, respectively. To maintain hydraulic balance for a given g_s, decreased k_s (57.9% and 58.8% lower in mature and aged groups) in bamboo must be offset by reductions in h and A_L/A_C or in Ψ_l. Culm height is expected to be constant after one year of growth [?, ?], but we found decreases of 8.5% and 23.3% in culm height for mature and aged groups compared to young culm height. The decreased h is associated with dead tissues at the stem top due to hydraulic failure related to the growth sequence of bamboo shoots (top-down, [?, ?]). A_L also declined by 55.7% and 63.7% (Figure 6a), with significant A_L decline also occurring in *Phyllostachys heterocycle* as it ages [?, ?]. Thus, compensation through decreased A_L and h allows for decreased Ψ_l that maintains hydraulic safety. These results suggest adaptive adjustment of hydraulic architecture to cope with conduits blocked by tyloses in stems [?, ?]. Although decreased individual carbon assimilation caused by reduced A_L may not be completely compensated, the increased lifespan eventually contributes more to the carbon sink for each individual.

Conclusion

B. chungii is a fast-growing species with significant eco-hydraulic effects. It maximized carbon assimilation through anisohydric behavior from the beginning of growth, while xylem cavitation and embolization triggered structural changes such as decreased leaf area and water path length, possibly by reducing tissues required to maintain hydraulic safety. To compensate for reduced carbon assimilation caused by decreased A_L, mature and aged groups improved photosynthetic capacity through increased stomatal density, higher chlorophyll content, and enhanced anisohydric behavior in response to water deficit. This helped older plants maintain A_n similar to young groups while maximizing fitness.

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Figure Legends

Figure 1. Daily course of PAR, VPD (a, b) and sap flux density (J_S, c, d) in dry and wet seasons.

Figure 2. (a) Normalized sap flow density (J_{S-N}) of each age group (by their maximum) plotted against photosynthetically active radiation (PAR) and (b) sap flow density (J_S) during 11:00–13:00 in response to VPD in the dry season (2014.01.01-2014.01.10). Bars indicate ± standard error of all culms within the age group.

Figure 3. Daily dynamics of net photosynthetic rate (A_n), stomatal conductance (g_s), leaf-level transpiration (E_t), and leaf water potential (Ψ_L) of three *B. chungii* individuals in each age group for three clear days in the dry season (January 16–18, 2014, open symbols and dashed lines) and wet season (July 19-21, 2014, solid symbols and lines). For b, c, d, e, and f, different symbols represent young (square), mature (circle), and aged (triangle) groups. Error bars correspond to ± standard error of three individuals in each group.

Figure 4. (a) Daily dynamics of intercellular CO_2 (C_i) in the dry season (January 16–18, 2014, open symbols and dashed lines) and wet season (July 19-21, 2014, solid symbols and lines) and (b) C_i in relationship with leaf stomatal conductance (g_s). Bars refer to standard error of culms within each age group (n = 15), dashed and solid lines are least squares fits for dry and wet seasons, and the asterisk is predicted C_i data in the wet season at 5:00 am.

Figure 5. Sensitivity of mean stomatal conductance (G_S) of bamboo culms in each age group in response to increasing vapor pressure deficit ($-dG_S/d\ln D$) as a function of canopy stomatal conductance at D = 1 kPa (G_{Sref}) in dry and wet seasons.

Figure 6. (a) Leaf area (A_L), conductive area (A_C) and (b) tree height (H), (c) chlorophyll content (Chl), and (d) stomatal density of *B. chungii* for each age group. Vertical bars represent one standard deviation. Different letters indicate significant differences at the 0.05 level.

Note: Figure translations are in progress. See original paper for figures.

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