12th World Bamboo Congress

Taiwan, 18-22 April, 2024

www.worldbamboo.net

How long do young bamboos receive carbohydrates from the society of Moso bamboo *(Phyllostachys edulis)*?

Shitephen Wang[1,2*], Keito Kobayashi[1,2], Satoru Takanashi [1,3], Daneil Epron [1,2], Yuji Kominami [4], Tsai-Huei Chen [5], Chiung-Pin Liu [6], and Masako Dannoura [1,2]

[1]*These authors contributed equally to this study and share the first authorship.*

[2]*Graduate School of Agriculture, Kyoto University (KU), Kyoto, 606-0096, Japan*

[3]*Kansai Research Centre, Forestry and Forest Products Research Institute (FFPRI), Kyoto, 612-0855 Japan*

[4]*Department of Disaster Prevention, Meteorology and Hydrology, Forestry and Forest Products Research Institute (FFPRI), Ibaraki, 305-8687, Japan*

[5]*Silviculture Division, Taiwan Forest Research Institute (TFRI), Taipei, 10066, Taiwan*

[6]*Department of Forestry, National Chung Hsing University (NCHU), Taichung, 40227, Taiwan*

Abstract

In bamboo forests, there exists a fascinating phenomenon where mature bamboo plants transfer vital nutrients and carbohydrates to young bamboos, conquering abiotic stress and aiding their growth. Previous studies have posited that mature bamboos provided nourishment and carbohydrates to juvenile bamboos through their rhizome systems. Recent studies, supported by evidence such as the analysis of stable isotope has proved this hypothesis. To further know how long young bamboos, rely on mature bamboos and rhizome systems in the society of *Phyllostachys edulis* (*P. edulis*) (also known as moso bamboo), we recorded the timing of bamboo shoots coming out and leaf formed, applied Richards and logistic models for estimating the duration of nursing behaviour, and employed ^{13}C isotopes to test the photosynthesis in developing culms. Our key findings revealed intriguing patterns: Moso bamboo shoots growing up from the ground to finish height growth and to form producing leaves took 50 days and 101 days in Kyoto, Japan. Even though some studies found the activation of C_4 key enzymes in developing culms, we rejected the possibility that C_4 photosynthesis mainly provided carbohydrates to developing culms itself which means the transfer of carbohydrates is mainly from mature bamboos to young bamboo shoots and developing culms through the rhizome systems. Our results support that the bamboo society ceases to provide carbohydrates to young bamboos in around 101 days until the producing leaf formed. However, even if bamboo leaves are formed, they still occasionally receive carbon from other matures in the society of *P. edulis* when they encounter photosystem damage caused by abiotic stresses in late autumn.

Keywords   Moso bamboo (*Phyllostachys edulis*); Richards function; Logistic function, Structural carbohydrates (SC); ^{13}C pulse labelling; bamboo shoots

*Corresponding Author: Shitephen Wang, Graduate School of Agriculture, Kyoto University (KU), Kyoto, 606-0096, Japan

1. Introduction

The society of *Phyllostachys edulis (P. edulis)*, also known as moso bamboo, as a living testament to the intricacies of interdependence and succession due to their connecting rhizome systems caused the nursing behaviour from mature bamboos to young bamboos (Wang et al. 2020). The plant grows incredibly fast from young shoots emerging from the ground to mature bamboos (10–15 m high) within 60 days without producing leaves, the photosynthesising organs (Yen and Lee 2011) due to around 40 internodes undergoing cell division (0.06 cells $cell^{-1}$ h^{-1}) and elongation (0.49 μm $μm^{-1}$ h^{-1}) at the same time during the fast-growing period (FGP) (Chen et al. 2022). The fast-growing developing culm is implicated in demanding large amounts of carbon sources.

The huge carbon demand was always assumed supplied by mature bamboos vis rhizome systems (Song et al. 2016; Wang et al. 2020). Stable isotope pulse labelling provided direct evidence that mature bamboos supplied carbon and nitrogen to young bamboo shoots *ex situ* (Shi et al. 2022) and *in situ* (Kobayashi et al 2020; Wang et al. 2021a). However, some studies also indicated C_4 enzymes activating in developing bamboo culm might operate C_4 photosynthesis to contribute the main part of carbon sources. (Wang et al. 2012; Wang et al. 2021b). Later, the possibility has been rejected by the evidence of ^{13}C pulse labelling in developing culm (Wang et al., 2023), and these C_4 enzymes may activating for other synthetic and metabolic pathways (e. g. anaplerotic pathway). So, the huge demand of carbon sources must be supplied from mature bamboos via rhizome systems.

The maturation of bamboo individuals, characterised by its special growth patterns, beckons us to unravel the enigma of how mature bamboos nursing immature bamboos. Combining the latest research, the young bamboos grow extremely fast compared to other plants during the FGP because meristem divides and elongates cells in all internodes at the same time which means 25-40 times faster than other plant individuals. Moreover, each young bamboo receives an average of more than 3 mature bamboos supplying carbon based on the general structure of managed *P. edulis* stands (Chen et al. 2016) satisfying the demand for carbon in immature bamboos during the FGP.

While the nursing behaviour of mature bamboos in providing structural stability and resource reservoirs has been underscored, the temporal extent of this dependence has eluded thorough scrutiny. Hence, this conference paper embarks on a meticulous exploration to discern the

temporal boundaries within which the carbon of sustenance is passed from the mature to the young within the society of *P. edulis*. Our object is to clarify how long young bamboos receive carbohydrates from matures in the society of *P. edulis*. We (1) measure the diameter at breast high (DBH) and culm height growth in different developing culms in a *P. edulis* stand for building allometric models, (2) record the phenology of culm developing from bamboo shoots to matures, and (3) integrate the results of the series of studies including ^{13}C pulse labelling in developing culms and activity of photosystems in leaves for estimating the period of the nursing behaviour in the society of *P. edulis*. In the end, based on the physiological evidence and behaviour of *P. edulis* society, we answer the question of how long young bamboos receive carbon from matures until they get into the autotropic stage (Figure 1), and put forward suggested management methods based on plant physiology and growth patterns for practical problems (e.g. the expansion of abandoned *P. edulis* forests in Japan).
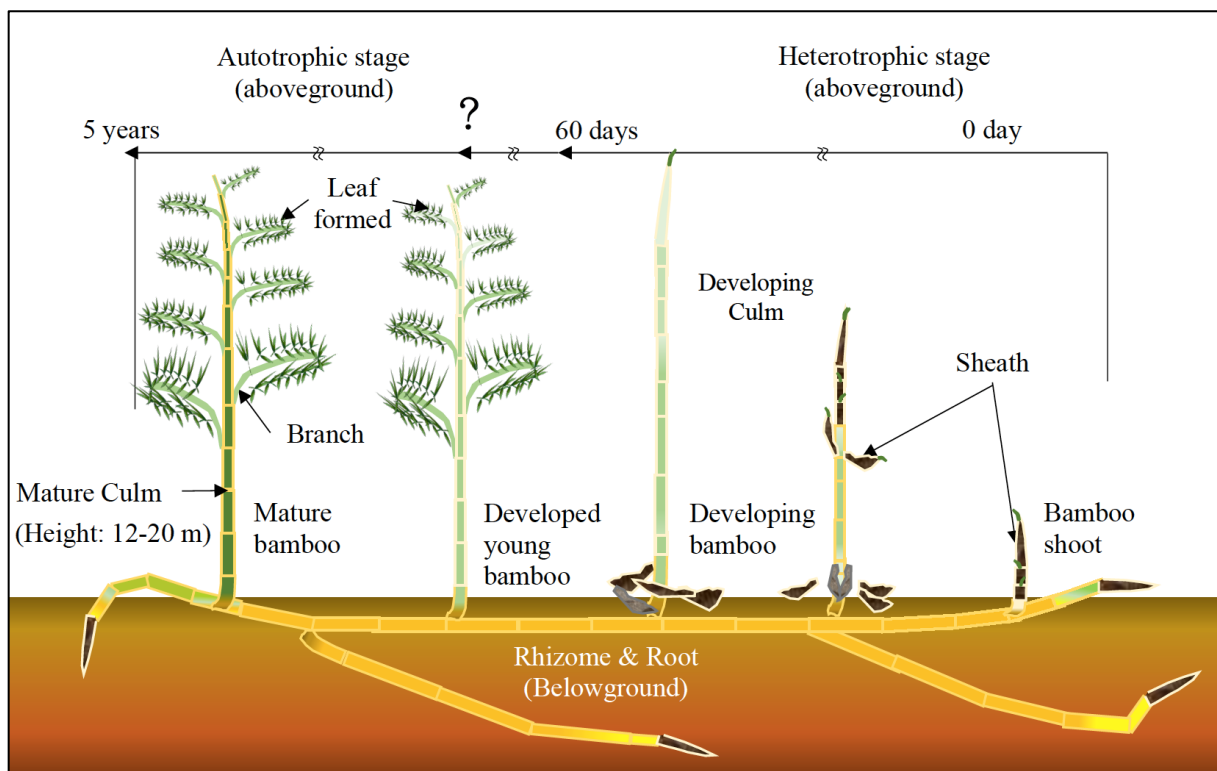
Figure 1. Illustrations of *P. edulis* at different growth stages. Mature bamboos (autotrophic stage) bear branches and leaves (aboveground) and rhizomes and roots in the soil (belowground). Young bamboo shoots emerge from the ground attached to mature bamboo rhizomes. Developing, immature culms grow rapidly in 2-3 months (heterotrophic stage). Young bamboo shoots are shoots covered by sheaths before the internodes undergo extensive elongation. Developing culms are the growing culms that undergo a period of rapid internode

elongation, after which the senescence of the sheath occurs, and eventually mature into fully formed culms. The question mark is the core question of this study - How long do young bamboos receive carbohydrates from the society of *P. edulis* bamboo? (How long does young bamboo transfer from the heterotrophic stage to the autotrophic stage?) (The illustration is modified from Wang et al. 2023)

2. Materials & Methods

2.1. Experimental site

This main study was conducted in a *P. edulis* forest in Katsura campus, Kyoto University (Kyoto Prefecture, Japan; 34°59′06.2″N, 135°40′48.4″E, 110 m alt.). Mean annual temperature and mean annual precipitation in 2021 were 16.9 °C and 1552 mm. The stand management included rough selective cutting and bamboo shoot harvesting without fertilisation. The mean height of the culms of mature bamboos was 15.3 m and the average diameter at breast height (DBH) was 9.9 cm. The DBH distributions ranged mainly from 8 to 12 cm. Four DBH classes were classified (6≦8, 8≦10, 10≦12, and 12≦14 cm). The detail of the study site is described in Wang et al. 2023.

2.2. Growth modelling of developing culms

Richards function for estimating growth at different stages

The heights of developing culms, obtained from 7 targets distributed in different classes, were frequently recorded from April to August 2021 (20, 22, 23, 26, 28 in April, 4, 6, 8, 13, 19, 26, 28 in May, 2, 17 in June, 16, 27 in July, and 6 in August). The relationships between culm height and daily growth were used in non-linear models based on the Richards function (Richards 1959) to simulate culm development. This growth function is widely used in bamboo culm growth (Yen 2003; Yen 2016) as equation (1):

$$Y = A \times [1 - \exp(-k \times t)]^{1/(1-m)} \tag{1}$$

where Y is growth amount (e.g. culm height, volume, volume accumulated ratio, culm height accumulated ratio, or aboveground biomass), t is the day started to measure, and A, k, and m are parameters of the Richards function.

Logistic function for estimating growth at different stages

However, in the case of developing culms, it was observed that the function (1) did not fit well during the initial growth phase and when culms were close to completing their height development. And not all developing culms start growing from the ground on the same day. Therefore, we provided another logistic function to describe it as follows:

$$Y = \frac{c}{1 + p \times \exp(-v \times t)} + d \tag{2}$$

where c is the estimated maximum equilibrium cumulative growth after the initial measurement, p and v are parameters of growth curvature and velocity, d is the estimated cumulative growth at the initial measurement, and then $c+d$ is the estimated maximum equilibrium cumulative growth.

Allometric function for estimating biomass of developing culms before producing leaves formed

To estimate biomass at different growth stages, each stage collected 3 to 6 samples and divided in 2 m sections to obtained the density of bamboo shoots and developing bamboos. Then, the relationship between aboveground biomass and DBH were predicted by allometric function which has been commonly used:

$$Y = a \times DBH^b \tag{3}$$

(Y is aboveground biomass, and a and b are parameter of the simple allometric function.)

2.3. Natural sample collection and $^{13}CO_2$ labelling experiment

(for rejecting the possibility of C_4 photosynthesis in developing culms)

For measuring the natural abundance of ^{13}C, we collected various organs (culms, branches, leaves, rhizomes, roots, bamboo shoots, and developing culms) from individuals between April and May 2021. Samples were quickly frozen in liquid nitrogen in the field and then stored at -20°C in the lab. We also conducted a $^{13}CO_2$ labelling experiment on developing culms. For the labelling experiment, we chose six similar-sized, leafless, and vigorous immature culms. Three

chambers were attached to three culms, and 75 mL of 99% $^{13}CO_2$ was injected into each chamber. In three other culms, 50 mL of 99% $^{13}CO_2$ was injected directly into the 7th internode. Two hours later, we sampled internodes to analyse the labelled ^{13}C, preserving them at -20°C after initial freezing in the field. Details of method and materials are described in Wang et al. 2023.

Statistical analysis

Richards function, logistic function, and allometric models were determined by the smallest root mean squared error (RMSE). One-way ANOVA followed by the Tukey HSD post hoc test was performed to test the between-organ differences in $\delta^{13}C$ in bulk organic matter. Univariate student's t-test was used to test whether the excess ^{13}C in the polar fraction was significantly higher than 0 after pulse-labelling developing culms with $^{13}CO_2$. All statistical analyses were performed using R. Details are described in Wang et al. 2023.

**3. Results**

The culm height of bamboos increases with increasing DBH

A linear regression equation of Y = 1.2293x + 3.0378 (R^2=0.88, F=877, P=0.0000) (Figure 2). We found a strong positive correlation between these two variables using a correlation analysis to examine the relationships between DBH and culm height. Our study is the same as previous studies, which pointed out that DBH and culm height are highly correlated (Yen et al. 2010; Yen and Lee 2011; Yen 2016).
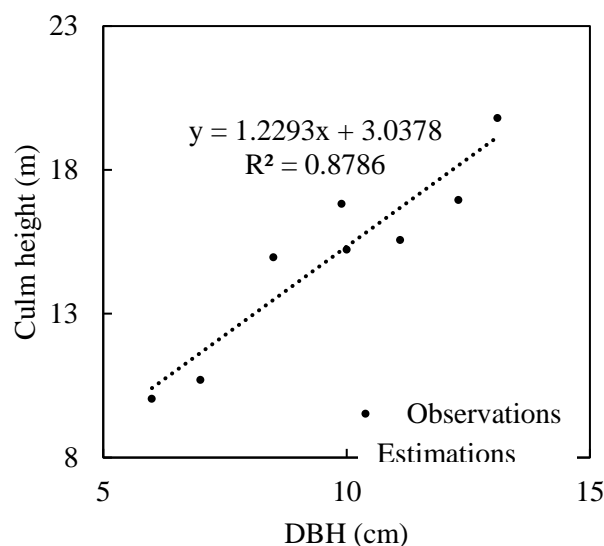
Figure 2. Relationships between DBH (x) and culm height (y) for moso bamboo. A linear regression of Y = 1.2293x + 3.0378 (R = 0.8786) is used to predict culm height by DBH.
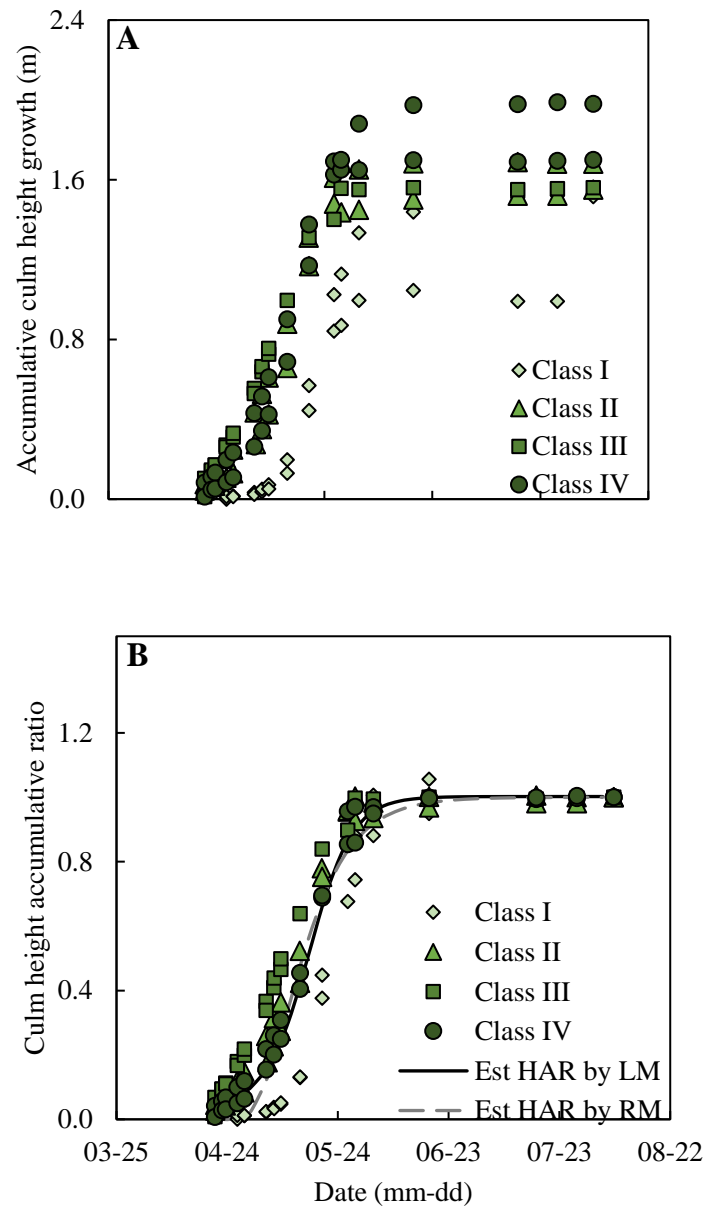
Figure 3. The actual A. culm height growth and B. height accumulative ratio (HAR) of the different DBH for *P. edulis* and the estimation of the Logistic model (LM) and Richards model (RM) used to fit the observations of each DBH class. Mist green diamond means 6<DBH≦8 (class I), lime triangle means 8<DBH≦10 (class II), green square means 10<DBH≦12 (class III), and dark green circle means 12<DBH≦14 (class IV). Black line and grey dotted line represent the values of the culm HAR estimated by LM and RM.

Daily height growth estimation of young bamboo culm

The observations of accumulative culm height growth in different diameter classes were shown in Figure 3A. Except for Class I bamboo culms with relatively small DBH, the bamboo growth rate and cumulative height are similar in different DBH classes. We also fit a general Richard function and logistic function using the min-max normalised observations as shown in Figure 3B. We found that the observation of the growth patterns showed a sigmoid shape for bamboo height growth. The observations closely match the estimation by the logistic function, revealing that the function accurately simulated bamboo height development, even though the Richard function is not so fitted to observations at the initial stage of bamboo shoots (Figure 3B.). Detail parameters of Richards function and logistic function are shown in supplementary materials. The developing culms arrived at their average maximum heights (over 99%) in about 50 days (Figure 3B). And the young developed bamboo finished the branch and leaf forming in about 100 days (supplementary materials).

Allometric models for estimating biomass in *P. edulis* individuals

The initial stage of growth generally ended after around 50 days when the developing culms reached their maximum height (99%), and the aboveground biomass of 0-year-old before leaf formed is 2.12-19.96 kg culm^{-1} (details in supplementary materials). The parameters of allometric models were used to estimate the relationship between aboveground biomass and DBH for *P. edulis* in different growth stages (Figure 4).
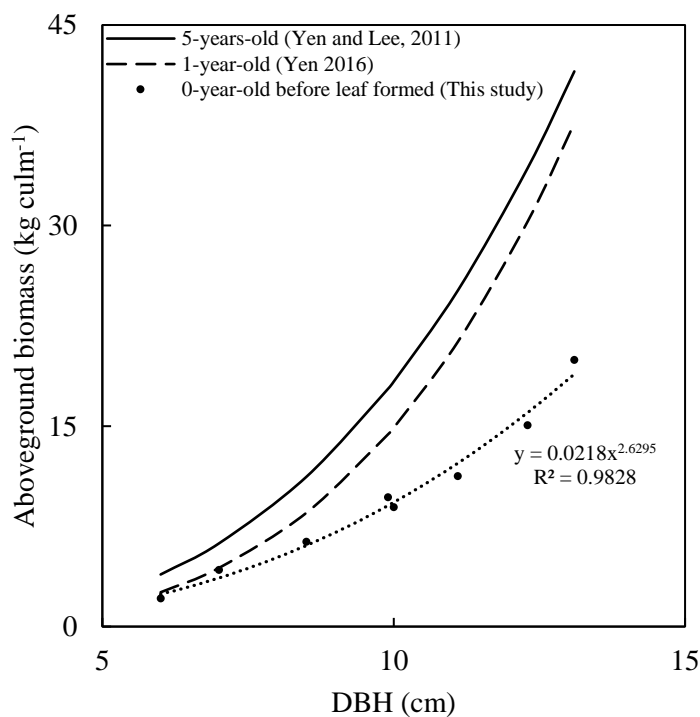
Figure 4. Relationships between aboveground biomass of observed data and DBH of moso bamboo and the allometric model were used to predict the biomass. The allometric model is y = 0.0218DBH 2.6295 (RMSE = 0.711882 m, R^2 = 0.9823) for this study. The allometric model of a 5-year-old (y = 0.0171 DBH 3.03) and a 1-year-old (y=0.0054 DBH 3.44) were cited from Yen and Lee (2011) and Yen (2016), and was used for comparison in this study.

Discussion

This study delves into the intriguing growth patterns and resource allocation strategies within the community of *P. edulis*. By employing Richards and logistic models, we seek to understand the growth dynamics of young bamboo shoots through on-site observations. Our investigation reveals that developing culms receive carbohydrates from their society for an average duration of 101.9 days, despite completing their height growth in an average of 50.8 days (Figure 3B) in Kyoto, Japan. Additionally, the average duration required for branch and leaf growth stands at 51.1 days. The rate of growth exhibited by *P. edulis* when compared to tree species, both at the individual and stand levels, is undeniably remarkable.

To shed light on this phenomenon, we have synthesized insights from previous research, attributing the primary causes to three main factors: (1) immature baby bamboo receiving carbohydrates and nutrients from mature bamboos during their FGP (Song et al. 2016), (2) *P. edulis* engaging in C_4 photosynthesis and carbon fixation during its FGP (Wang et al., 2012; Wang et al. 2021b), and (3) the exceptionally high number of meristems (25-40 positions) within each culm, surpassing that of typical tree species (Chen et al. 2022). The first insight, was a hypothesis, though initially speculative, has been substantiated by $^{13}CO_2$ labelling experiments conducted both *ex situ* (Zhai et al. 2022) and *in situ* (Kobayashi et al., 2020; Wang et al., 2021a). The second insight, championed by Wang et al (2021b), observed the activation of key C_4 carbon fixation enzymes in developing culms during the FGP. However, further experimentation with $^{13}CO_2$ injection demonstrated limited evidence of C_4 photosynthesis, suggesting that anaplerotic fixation of respired CO_2 may play a more prominent role in partially sustaining fast growth (Figure 2, 6 in Wang et al. 2023). Nonetheless, the presence of these enzymes may indirectly contribute to the rapid growth observed.

The third perspective compares the growth rates of *P. edulis* with other plants (Chen et al. 2022). For instance, *P. edulis* had a maximum internode elongation rate of 0.08 cm cm^{-1} h^{-1} during culm development, which was lower than deepwater rice (around 0.11 cm cm^{-1} h^{-1}) (Métraux and Kende 1984) and maize root (0.4 cm cm^{-1} h^{-1}) (Erickson and Sax 1956). In terms of cell division, *P. edulis* had a maximum rate of around 0.06 cells cell^{-1} h^{-1}, in internodes during culm development, which was higher than most plants but still not the fastest. Oats, for instance, had a rate of 0.29 cells cell^{-1} h^{-1}, which is five times higher than *P. edulis* (Kaufman et al. 1965). Thus, contrary to intuitions, the rapid growth of the internode of developing culms is not due to particularly rapid cell growth or a particularly high division rate, but rather to the large number of culm internodes (each internode has its meristems). The complex interplay of these

factors, particularly evident in the initial growth stages, defies a simple fit to the Richards function, highlighting the chaotic situation of multiple external carbohydrate sources and internal carbon recycling during the FGP at the heterotrophic stage (Figure 3).

Our observations reveal disparities in growth timelines, velocities, and densities among developing culms of different sizes within the same study site (Figure 3, 4). Larger developing culms commence rapid growth before the onset of spring, nursing from the allocation of carbohydrates from matures and stocks in rhizome systems. In contrast, smaller developing culms begin their rapid growth towards the end of the growing season, as resources have already been invested in larger developing culms. This discrepancy underscores the intricate carbon source dynamics within the society of *P. edulis*.

Conclusions

The duration of nursing behaviour in the society of *P. edulis* is 101 days (around 3 months). The rapid growth of *P. edulis* is a multifaceted outcome resulting from various factors, including the transfer of resources from matures to immatures, the potential presence of C_4 enzymes for a limited anaplerotic fixation of respired CO_2, and the abundance of dividing and elongating cells in each internode. To prevent rapid expansion of *P. edulis* forests, we suggest a strategy based on our results and plant ecophysiology as follows. Limiting shoot harvesting during the shoot emergence period to prevent accelerated expansion of the belowground rhizome systems. Since resources are limited in the belowground rhizomes and bamboo shoots arise from limited node numbers, reducing bamboo shoot harvesting can decrease carbons and nutrients transfer to the tip of rhizomes for supplying expansion. Additionally, cutting developing culms (0-year-old) before leaf formed, and preserving older culms (>5 years old) can exhaust a lot of carbohydrates and resources in the society of *P. edulis*, curbing the expansion of bamboo forests and reducing their long-term vitality.

Funding

Declaration

We declare that we have no financial and personal relationships with other people or organisations that can inappropriately influence our work. There is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Conflict of Interest

The authors declare there is no conflict of interest

References

Chen, M., Guo, L., Ramakrishnan, M., Fei, Z., Vinod, K.K., Ding, Y., Jiao, C., Gao, Z., Zha, R., Wang, C. and Gao, Z., 2022. Rapid growth of Moso bamboo (*Phyllostachys edulis*): Cellular roadmaps, transcriptome dynamics, and environmental factors. *The Plant Cell*, *34*(10), 3577-3610.

Erickson, R.O. and Sax, K.B., 1956. Rates of cell division and cell elongation in the growth of the primary root of *Zea mays*. *Proceedings of the American Philosophical Society*, *100*(5), 499-514. https://www.jstor.org/stable/3143683.

Kaufman, P.B., Cassell, S.J. and Adams, P.A., 1965. On nature of intercalary growth and cellular differentiation in internodes of *Avena sativa*. *Botanical Gazette*, *126*(1), 1-13.https://www.jstor.org/stable/2473428.

Métraux, J.P. and Kende, H., 1984. The cellular basis of the elongation response in submerged deep-water rice. *Planta*, *160*, 73-77.

Song, X., Peng, C., Zhou, G., Gu, H., Li, Q. and Zhang, C., 2016. Dynamic allocation and transfer of non-structural carbohydrates, a possible mechanism for the explosive growth of Moso bamboo (Phyllostachys heterocycla). *Scientific reports*, *6*(1), 25908.

Wang L., Li Q., Gao P., Wei S., Lu J., Gao Y. and Zhang R., 2021b. Activities of key enzymes involved in photosynthesis and expression patterns of corresponding genes during rapid growth of *Phyllostachys edulis*. *Journal of Zhejiang A&F University 38*, 84–92.

Wang, S., Chen, T.H., Liu, E.U. and Liu, C.P., 2020. Accessing the nursing behaviour of Moso bamboo (Phyllostachys edilus) on carbohydrates dynamics and photosystems. *Scientific Reports*, *10*(1), 1015.

Wang, S., Epron, D., Kobayashi, K., Takanashi, S. and Dannoura, M., 2023. Sources of carbon supporting the fast growth of developing immature moso bamboo (Phyllostachys edulis) culms: inference from carbon isotopes and anatomy. *AoB Plants*, *15*(4), plad046.

Wang, S., Kobayashi, K., Dannoura, M., Takanashi, S., Fukushima, K., Onishi, Y., Kitayama, K., Onoda, Y. and Kominami, Y., 2021. Seasonal dynamics of carbon allocation between different organs in *Phyllostachys edulis* (Moso bamboo) forests. *The Japanese Forest Society Congress 132*(0), 473.

Wang, X., Liu, L., Zhang, J., Wang, Y., Wen, G., Gao, R., Gao, Y. and Zhang, R., 2012. Changes of photosynthetic pigment and photosynthetic enzyme activity in stems of *Phyllostachys pubescens* during rapid growth stage after shooting. *Chinese Journal of Plant Ecology 36*, 456–462.

Yen, T.M., 2016. Culm height development, biomass accumulation and carbon storage in an initial growth stage for a fast growing Moso bamboo (*Phyllostachy pubescens*). *Botanical Studies* 57, 10.

Yen, T.M., Ji, Y.J., Lee, J.S., 2010. Estimating biomass production and carbon storage for a fast-growing makino bamboo (*Phyllostachys makinoi*) plant based on the diameter distribution model. *Forest Ecology and Management 260*, 339–344.

Yen, T.M., Lee, J.S., 2011. Comparing aboveground carbon sequestration between Moso bamboo (*Phyllostachys heterocycla*) and China fir (*Cunninghamia lanceolata*) forests based on the allometric model. *Forest Ecology and Management 261*, 995–1002.

Zhai, W., Wang, Y., Luan, J., Liu, S., 2022. Effects of nitrogen addition on clonal integration between mother and daughter ramets of Moso bamboo: a ^{13}C-CO_2 pulse labeling study Zhang W-H (ed). *Journal of Plant Ecology 15*, 756–770.