Seasonal and Spatial Variation of Nitrogen Distribution for Leaves of *Phyllostachys Pubescens* and Its Response to Precipitation Exclusion

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Abstract. The leaf nitrogen content in different canopy position for different ages of individuals for *Phyllostachys pubescens* was studied systematically with the season change in the experiment of precipitation exclusion in the bamboo field. The results showed that under different treatments (natural growth and precipitation exclusion treatment), the leaf nitrogen content of bamboo varied with seasons and in the decreasing order of spring, autumn, summer and winter. The change of leaf nitrogen content with bamboo age was also affected by the growing season. Precipitation exclusion treatment changed the seasonal and age difference of leaf nitrogen content in bamboo. In spring, the age differences in leaf nitrogen content of bamboo were significant (p<0.05). The leaf nitrogen content of bamboo varied with the height of the canopy, and the vertical distribution gradient was affected by the growing season. It increased with the increase of canopy height in summer and autumn, which also proved the applicability of the "light-nitrogen" hypothesis to the bamboo. Under two treatments, the leaf nitrogen content was linearly positively correlated with bamboo age. It changed with the change of soil water content in different growing seasons under treatments. Under precipitation exclusion, it was more sensitive to soil water content. This study confirmed the existence of the vertical distribution gradient of nitrogen in the leaves of *P. pubescens* and its variation with the growing season.

1 Introduction

Nitrogen is an indispensable and important nutrient element for plant growth, and an important limiting factor for the productivity of forest ecosystems. Nitrogen is an important component of the photosynthetic system and enzymes necessary for photosynthesis. The 30-40% of nitrogen in leaves participates in the carboxylation reaction, and the proportion of leaf nitrogen allocated to the carboxylation system determines the final photosynthetic efficiency. It is undoubtedly an important guarantee for the increase of carboxylation rate and net photosynthetic rate [1-2].

Photosynthesis of leaves within canopy under different light conditions will help to improve carbon harvesting and growth adaptability and resistance of the whole plant [3-4]. Due to the light gradient distribution in the canopy and the need to utilize resources effectively for plants, the plant leaf traits also change significantly in the canopy. Compared with leaves in shaded environment in canopy, leaves under higher light conditions have larger leaf mass per area, nitrogen content and photosynthetic capacity per leaf area [5-6], which are explained as the optimal distribution theory of plant resources, especially nitrogen resources in the canopy layers [7]. The gradient distribution of nitrogen content in canopy leaves is one of the basic characteristics of tree canopy. With the increase of atmospheric CO2 concentration and the change of rainfall distribution pattern, the effect of nitrogen gradient pattern within canopy on leaf photosynthesis and the improvement of plant productivity are more important [6].

Moso bamboo (*Phyllostachys pubescens*) is an important forest resource in southern China with the advantages of fast growth, early maturity, high yield, wide range of uses and high income. Today, the bamboo forest is an increasing carbon sink. However, under the global climate change, the frequency and intensity of extreme climate (weather) events are increasing, and extreme drought events have posed unprecedented challenges to ecosystem structure and function. As an important condition for plant growth, water is closely related to the productivity of *P. pubescens*. Therefore, drought has an important potential impact on the dynamic change of the nitrogen gradient within the canopy of *P. pubescens* and its productivity. *P. pubescens* is rich in research content [8-11]. However, as an important growth trait, the temporal and spatial distribution of nitrogen in *P. pubescens* leaves, the vertical distribution gradient in canopy and its changes with drought have not been reported. In this paper, the field rain interception and drought experiment of *P. pubescens* was carried out to study the dynamic changes of nitrogen content in leaves of different ages of *P. pubescens* in different seasons and canopy layers. The response to drought stress was used to confirm the existence of a vertical gradient of nitrogen distribution in

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P. pubescens leaves and its response to drought with seasons.

2 Materials and methods

2.1 Study area

The research was carried out at the Miaoshanwu auxiliary station of the Qianjiangyuan Forest Ecosystem Positioning Research Station of the State Forestry and Grassland Administration, located in Fuyang District, Hangzhou city (119°56′～120°02′ E, 30°03′～30°06 'N). The terrain of this area belongs to the remnants of the Tianmu Mountains in the low hilly area of western Zhejiang, and belongs to the mid-subtropical monsoon climate zone. The annual average temperature is 16.1℃ (the highest temperature is 40.2℃, the lowest temperature is -14.4℃), and the annual average precipitation is 1441.9 mm. The soil is acidic red soil.

2.2 Materials and sample plot settings

The bamboo forest in the experimental area was planted in the 1960s and is a natural regeneration forest under extensive management. The slope of the sample plot is about 20° and due south, and the altitude is 169 m. Three standard plots of 20m × 20m were established, and the diameter at breast height (DBH) and height of all bamboo plants in the plots were measured. The bamboo forest density was 3 875 plants•hm-2, the canopy density was 0.95, the DBH of bamboo ranged from 4.0 to 13.6 cm, the average DBH was 9.8 cm, and the average bamboo height was 13.2 m. There are almost no shrubs and herbs under the forest, with an average thickness of about 2.0 cm of litter. The mountain is split once every 2 years, no fertilization, no ploughing, only spring bamboo shoots are harvested.

2.3 Rain interception drought experiment

In late July 2014, 2 plots of 10m × 10m were selected respectively within each of the 3 standard plots of 20m × 20m in the moso bamboo forest for the control and the rain interception drought experiment plots respectively, and so there are 3 natural growth (control) plots and 3 rain interception drought treatment plots. The sparse shrubs in the rain-intercepting drought plot were removed, and the canopy method was used to simulate the rain interception. Specific methods: In the rain interception drought plot, in order to remove the edge effect and ensure that the dry area is 10m × 10m, a rain interception greenhouse (with an area of 11m × 11m) of PVC waterproof sheet is built at a height of 1.5m above the ground to intercept the rain. Use glue to glue the gap between the plastic sheet and the bamboo. In order to ensure that the slope, topography and stand conditions are as consistent as possible with the control plot, one side of the greenhouse is parallel to the contour line. A trench with a depth of about 50cm and a width of about 20cm was excavated around the sample plot, and a 50cm-deep iron sheet was buried along the trench. The inside of the trench was also covered with a plastic film to prevent the infiltration of water from the side and to allow better drainage. For the control plot, only similar trenches were dug around, and no other treatment was performed.

2.4 Nitrogen content determination

After 2 years of growth under two treatment experiments, in clear and cloudless weather in the middle months of each month in March (spring), August (summer), October (autumn) and December (winter) of 2016, and March of the following year, samples were selected for continuous determination of leaf nitrogen content, leaf water content, soil water content and other indicators. In each plot, 2 Moso bamboos of different ages with the similar DBH were selected. With the help of the ladders temporarily built in the field, the sunny branches in the upper, middle and lower canopy of Moso bamboo with different age were hooked by using a sickle, and all the leaves were quickly picked and brought back to the laboratory for the determination of nutrient content and water content of the leaves. The 30 leaves were taken from each treatment (respectively for age and canopy) for nitrogen content determination as three replicates, and the average value was taken at last.

Determination of leaf nitrogen content: The remaining leaf samples were dried in an oven at 102℃ for 2h and at 65℃ for 24h and ground through a 100-mesh sieve for the determination of leaf nitrogen content. Nitrogen content of leaves was determined by Foss Kjeldahl analyzer, and the digested liquid of the test sample was digested with concentrated sulfuric acid-hydrogen peroxide solution for the determination of total nitrogen (*N*mass).

2.5 Soil moisture content determination

During the experiment, soil samples were taken synchronously from the field in the plots to measure soil water content. Four soil profiles were collected in the rain interception plot and the control plot as replicates, respectively, and the sampling depth was 0-20 cm, the soil was packed in an aluminum box and brought back, and 20g of fresh soil was weighed with an electronic balance (0.01g) and dried at 80℃ to constant weight. The calculation formula is: soil water content = [(fresh weight - dry weight)/fresh weight] × 100%. In this experiment, there were seasonal differences in soil water content at a depth of 0-20 cm in the control and the rain interception plots. The soil water content in the control plot was the highest in winter and spring (average of 29.82% and 30.15%, respectively), followed by summer (The average soil water content was 27.12% in autumn (October), and it was the lowest in autumn (23.42% on average). The seasonal differences of soil water content in the rain interception plots were not significant (the average soil water content was in the range of 4.44%-4.92%). The soil water content in the control plots of moso bamboo in each growing season was significantly higher than that in the rain interception plots.

2.6 Data analysis

The differences of nitrogen content in leaves of different ages among seasons and canopy layers were processed by Spss10.0 and Excel 2007, respectively, and each parameter was expressed as mean ± SE.

3 Results

3.1 Response of seasonal variation of leaf nitrogen content to drought

The research showed that the nitrogen content of leaves under natural growth (control treatment) was 23.24±2.24g·kg-1, which was slightly lower than that of leaves under rain interception drought treatment (24.39±1.14g·kg-1). However, the difference between the two treatments was not significant (P>0.05) (Fig. 1(a)). There were seasonal differences in the nitrogen content of leaves under different treatments, but they were all insignificant (P>0.05). The nitrogen content of leaves under the two treatments all varied with seasons as spring>autumn>summer>winter (Fig. 1(b)). In the same season, there was no significant difference in nitrogen content among different treatments (P>0.05). It can be seen that the drought did not significantly change the nitrogen content of bamboo leaves and its seasonal variation trend.

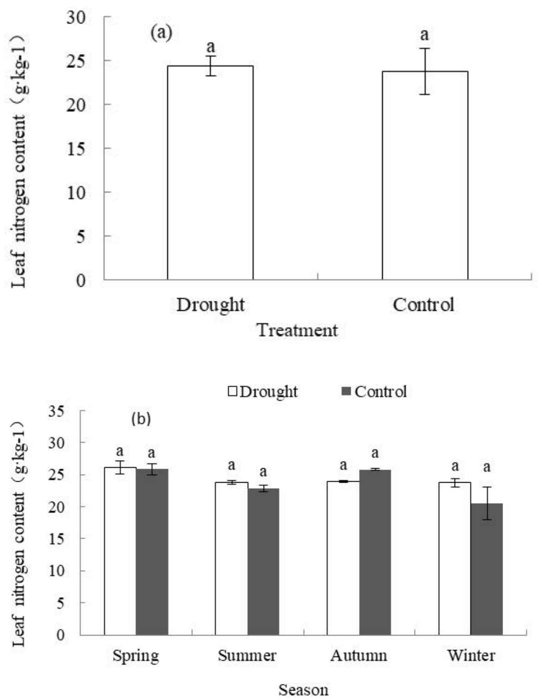
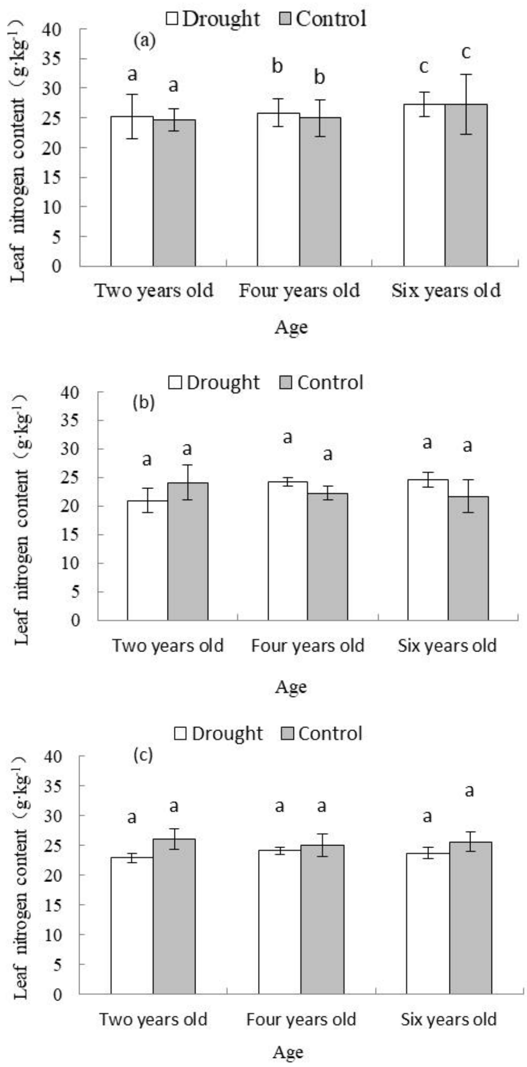


Fig. 1. The seasonal change of leaf nitrogen content for different ages of *P. pubescens* between control and drought stress (Note: Different lowercases in the same treatment denote significant differences at p<0.05 among the seasons and different uppercases in the same season denote significant differences at p<0.05 among the treatments. The same below.）

3.2 Response of leaf nitrogen content for different age of bamboo to drought

The variation of leaf nitrogen content with bamboo age was also affected by the growing season (Fig. 2). Under natural growth, the leaf nitrogen content of Moso bamboo with different bamboo ages in spring showed a trend of three-degree bamboo > two-degree bamboo > one-degree bamboo, that is, the leaf nitrogen content increased with the increase of bamboo age. The change of leaf nitrogen content in summer was just the opposite and it decreased with the increase of bamboo age. In autumn and winter, it showed "first decrease and then increase". Under the rain interception, the nitrogen content of leaves increased with the increase of bamboo age in spring and summer, and the opposite was in winter, and it decreased with the increase of bamboo age. In autumn, it showed the changing trend of the second degree > the third degree > the first-degree of bamboo.

Compared with the natural growth, the interception of rain changed the seasonal and age differences of nitrogen content in leaves for different age of Moso bamboo. The analysis of variance showed that in spring, the difference of bamboo age in leaf nitrogen content was significant (P<0.05), but there was no significant difference between treatments (P>0.05).



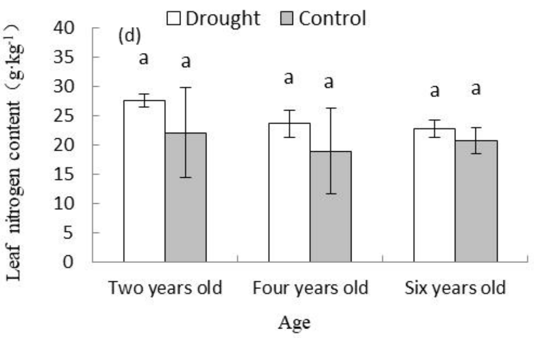


Fig. 2. Seasonal variation of leaf nitrogen content for different ages of *P. pubescens* under different treatments (Note: Figure (a), (b), (c) and (d) denotes spring, summer, autumn and winter, respectively.)

3.3 Response of leaf nitrogen content variance within canopy height to drought

The result showed that the nitrogen content of leaves changed with the canopy height for moso bamboo, and the gradient distribution characteristics were affected by the growing season (Fig. 3). Under natural growth, it increases with the canopy height in summer and autumn, while that of leaves in spring and winter tends to decrease with the increase of canopy height. Under the drought, that of leaves in summer was the highest in the upper part of the canopy, followed by the lower part. In spring and autumn, it decreased with the increase of canopy height, and was the highest in the middle of the canopy and the smallest in the upper part in winter. It can be seen that under natural growth and rain interception, the nitrogen content of leaves varies with the canopy height in spring as the upper part of the canopy < middle part < lower part, and the upper part of the canopy is the highest in summer.

The study found that the difference in the canopy gradient variation pattern of nitrogen content in Moso bamboo leaves between treatments was also affected by the growing season (Fig. 3). Except in autumn, it is lower under natural growth than that under drought in other seasons, indicating that the rain interception increases the nitrogen content of bamboo leaves in each canopy layer.

Analysis of variance showed that in spring, it was significantly different among canopies (P=0.008<0.01), however, in the same canopy location it was not significant between the control and the rain interception treatment (P>0.05). In autumn, there was significant differences between treatments (P<0.05 and P<0.01, respectively).

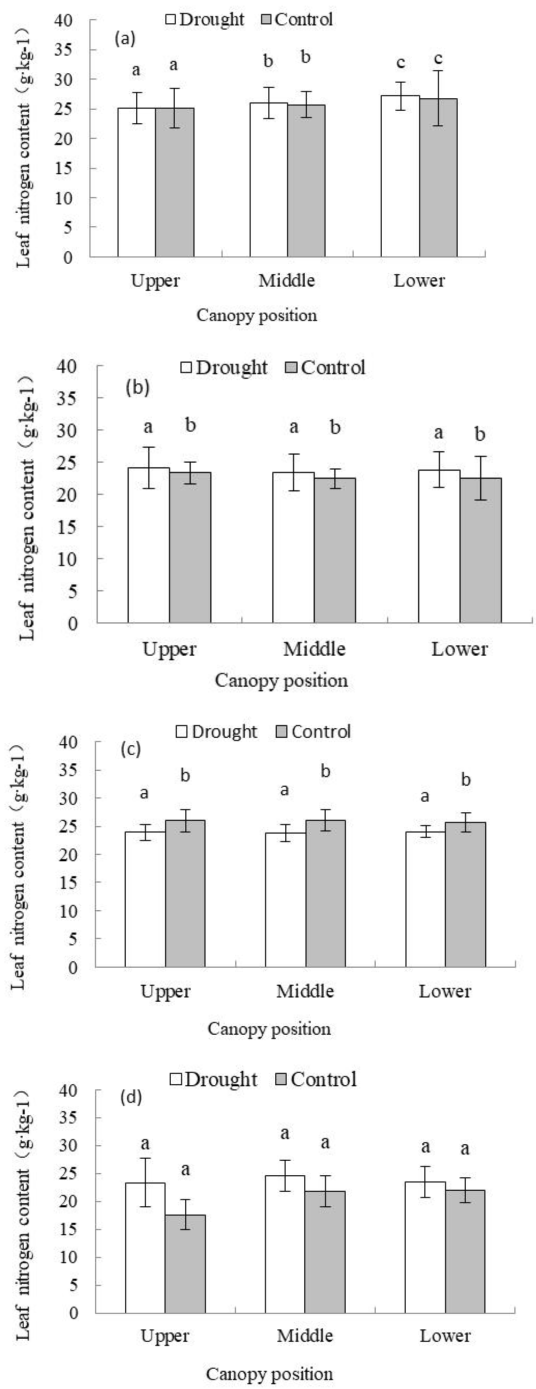


Fig. 3. Canopy variation of leaf nitrogen content for different ages of *P. pubescens* under different treatments (Note: Figure (a), (b), (c) and (d) denotes spring, summer, autumn and winter, respectively.)

3.4 Correlation of leaf nitrogen content with bamboo age, soil and leaf water content, and maximum net photosynthetic rate

It was linearly correlated with the increase of bamboo age under two treatments (R2=0.055 and R2=0.313, Figure 4), but not significant (P>0.05).

The nitrogen content of leaves changed with the change of soil water content in each growing season (Fig. 5). Under natural growth, it decreased with the increase of soil water content, but the correlation was not significant (*R*2=0.174). After the rain interception, there was a significant linear positive correlation with soil water content, indicating that the nitrogen content of bamboo leaves was more sensitive to the soil water content after the rain interception. Under natural growth, there was a small linear positive correlation between nitrogen content and leaf water content in *P. pubescens*, and a weak negative correlation existed under rain interception (Fig. 6).

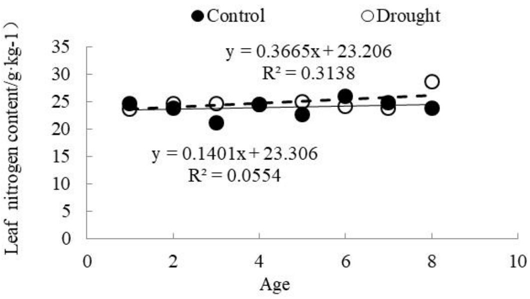


Fig.4. The correlation between leaf nitrogen content and age of *P. pubescens* under control and drought stress

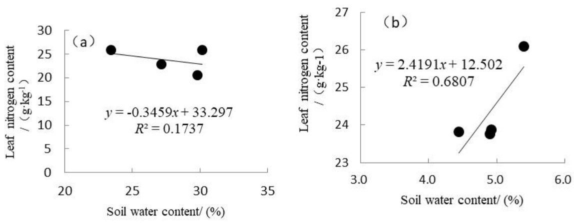


Fig.5. The correlation between leaf nitrogen content and soil water content for different growth stage of *P. pubescens* under control (a) and drought stress (b)

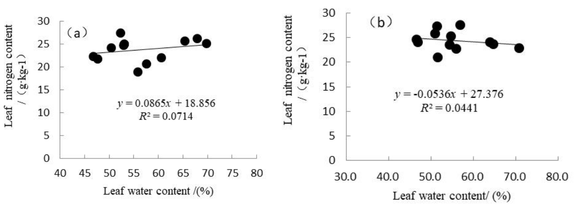


Fig.6. The correlation between leaf nitrogen content and leaf water content for different growth stage of *P. pubescens* under control (a) and drought stress (b)

4 Discussion

Nitrogen is one of the main limiting factors for plant growth [12]. The vertical gradient distribution of nitrogen content in canopy leaves is one of the important and common characteristics of tree canopy [13-14]. The maximum photosynthetic rate of trees is closely related to leaf nitrogen content and light radiation [2,15], the change of vertical distribution gradient of leaf nitrogen is regarded as a plastic response of plants to optimize nitrogen use during carbon assimilation [16]. Based on the basic physiological relationship between light radiation and nitrogen, the case studies of most scholars showed that when the leaves of trees in higher light contain higher nitrogen content than the leaves in shade, the photosynthesis of tree will reach the maximum, which is the "light-nitrogen" hypothesis [4,17-18].

There are many factors that affect the nitrogen gradient distribution in the canopy. There is a redistribution of nitrogen in canopy leaves at different growth stages of plants [6,18-19]. Therefore, at different growth stages of plants, the distribution of nitrogen in leaves determines the dynamic gradient distribution of nitrogen and its impact on canopy photosynthetic carbon fixation [19]. However, the effects of drought on the distribution of nitrogen gradient in the canopy are less studied.

At present, more studies have focused on traditional cultivation management techniques on bamboo forests [20-22], and there are recent reports on the limiting factors for the maximum height growth of bamboo plants [23]. Systematic research has also been carried out on its canopy photosynthesis [8,24-25]. However, there is still a lack of understanding of the distribution pattern of nitrogen in the canopy of *P. pubescens* and its correlation with soil water content and leaf water content, especially in the south of china where seasonal drought is becoming more and more obvious under global climate change.

Nitrogen is an important factor limiting the growth of *P. pubescens* [20,26-28]. Some scholars believed that different productivity levels affect the nitrogen distribution ratio of various organs of *P. pubescens*, and has a greater impact on the nitrogen distribution ratio of leaves and bamboo branches [29]. There are seasonal changes in the nitrogen content of bamboo leaves [20]. This study also found that the nitrogen content of leaves under two treatments varied with seasons as spring>autumn>summer>winter. In this study, the change of nitrogen content in leaves with bamboo age was affected by the growing season. Under natural growth, that of leaves increases with the increase of bamboo age in spring, but decreases with the increase of bamboo age in summer, which may be related to the consumption of nutrients by bamboo shoots in spring.

Scholars believed that the effect of canopy position on leaf nitrogen content during tree growth is staged [13]. The upper leaves of the canopy are in a high radiation environment and are prone to water shortage, which will affect the absorption, utilization and redistribution of nitrogen by plant leaves [14]. There is a significant linear correlation between the nitrogen content of manchurian ash leaves and the vertical height of the canopy, and it increases linearly with the increase of the vertical height of the canopy [14]. In this study, under natural growth, the nitrogen content of *P. pubescens* leaves basically increased with the canopy height in summer and autumn, which is consistent with the research results that the photosynthesis of *P. pubescens* in the upper part of the canopy is greater than that of the leaves in the lower part of the canopy[11] , and it indicated that the distribution pattern of nitrogen content in Moso bamboo leaves along the canopy gradient in summer and autumn is conducive to the demand for nitrogen in leaf photosynthesis, which also confirms the applicability of the "light-nitrogen" hypothesis to Moso bamboo in this growing season.

Effective leaf water supply can maintain stomatal opening to the maximum extent [30], thereby promoting photosynthesis. In this study, the nitrogen content of *P. pubescens* leaves decreased with the increase of canopy height in spring and autumn under drought. It can be seen that the rain interception has changed the canopy distribution pattern of nitrogen in Moso bamboo leaves to a certain extent. The canopy difference of nitrogen content becomes smaller. After rain interception, the response of leaf nitrogen content to soil water content is more sensitive, and it tends to increase with the increase of soil water content, and it is consistent with the result of Gao et al. (2019) that soil water content and leaf nitrogen are positively correlated in the process of vegetation restoration on the Loess Plateau [31]. Under natural growth, it is less affected by soil water content. The rain interception made the nitrogen content of leaves slightly higher than that of the naturally grown ones, which is in agreement with Fang Xuan et al. (2019) who believed that the leaf nitrogen of Chinese fir is more sensitive to water, and appropriate water deficit can increase the nitrogen concentration in the leaves [32]. A study on the relationship between leaf water content and photosynthesis at different leaf positions in the three-leaf stage of summer maize under continuous drought showed that leaf photosynthesis is closely related to its water content [33]. The results of this study provide a theoretical basis for sustainable management techniques based on nitrogen distribution in bamboo leaves under the background of climate change.

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