

Article

Dry Season Irrigation Promotes Leaf Growth in *Eucalyptus urophylla* × *E. grandis* under Fertilization

Fei Yu ¹, Thuy Van Truong ¹, Qian He ¹, Lei Hua ^{2,3}, Yan Su ¹ and Jiyue Li ^{1,*}

¹ Guangdong Key Laboratory for Innovative Development and Utilization of Forest Plant Germplasm, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou 510642, China; fishing_ok@163.com (F.Y.); truongthuyvan@huaf.edu.vn (T.V.T.); heqian69@126.com (Q.H.); suyan@scau.edu.cn (Y.S.)

² South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China; yeshualei@scbg.ac.cn

³ University of Chinese Academy of Sciences, Beijing 100049, China

* Correspondence: ljyue@scau.edu.cn; Tel.: +86-135-3511-1309

Received: 20 November 2018; Accepted: 11 January 2019; Published: 15 January 2019



Abstract: Leaves are essential for photosynthesis and gas exchange, and their growth characteristics are the key factors that influence the carbon budget. Eucalyptus is widely afforested in south China due to its fast-growing and high-yield features. Water and fertilizer are the main factors affecting plant growth. Studying the effects of different water and fertilizer treatments on the growth of Eucalyptus leaves under seasonal drought could further elucidate the optimal additions for Eucalyptus productivity. In this study, we investigated the leaf area, length, width, perimeter, and expansion rates of the commercial species *E. urophylla* × *E. grandis* under different treatments of dry season irrigation and fertilizer application to elucidate the growth dynamics of the leaves. The results indicated that both dry season irrigation and fertilizer could affect whole leaf expansion. Leaf area was largest when water and fertilizer were added at the same time. In this experiment, we found that fertilization had a significant effect on the leaf shape index of the Eucalyptus leaves. The leaf shape index was larger with the fertilizer treatment, which made the leaves slender. Dry season irrigation shorten the peak period of leaf growth and increase the leaf area. Our results help to further understand the mechanism of Eucalyptus productivity under seasonal drought and provide theoretical support for Eucalyptus production.

Keywords: dry season irrigation; *Eucalyptus urophylla* × *E. grandis*; leaf growth dynamics; productivity

1. Introduction

The leaf is the main site for photosynthesis, which is an important part of terrestrial ecosystem function [1]. An important factor that impacts terrestrial ecosystem productivity is the total leaf area [2]. Plants adapt to changing environments by changing their leaf morphologies and chemical contents, thus enabling them to obtain a reasonable carbon budget [1,3]. Leaves can gradually adapt to water and solar illumination, balancing photosynthesis and transpiration conditions. They are sensitive to changes in the environment during the evolution of organs, which produce a series of leaf characteristics due to the environmental changes that occur through long-term adaptability [4,5]. Therefore, leaf traits are useful ecological tools [6] that provide a link between environmental factors and leaf function [7].

Leaf phenotypes are influenced by many factors, both biotic (e.g., herbivory) and abiotic (e.g., temperature, soil, light environment) [8–11]. While soil factors mainly include water and fertilizer.

Studies have showed that water and fertilizer will promote the growth of both seedlings [12] and plants from natural forests [13]. Fertilizer could increase the leaf water use efficiency, water conductivity [13], leaf area index [14], and N content [15] under the appropriate soil water condition to promote the plant productivity. However, the leaf traits of different species may perform differently under these two treatments. Therefore, studying the effects of water and fertilizer on the leaves of *Eucalyptus urophylla* × *E. grandis* can be used as a reference for fertilization and water supply in the research process.

Eucalyptus occurs widely and is planted for its fast growing, high yield, and wood availability characteristics. Maire et al. [16] studied the method of measuring the leaf area index of *Eucalyptus* using medium-resolution remote sensing satellites. Macfarlane et al. [17] and Diao et al. [18] explored the indirect estimation of the total leaf area of *Eucalyptus* plantations using the plant canopy analyzer LAI-2000 and the leaf weighing method. Nouvellon et al. [19] found a significant negative correlation between *Eucalyptus*-specific leaf weight and breast diameter and height. Li et al. [20] found that the influence of leaf area and biomass were inconsistent in the dry and wet seasons. The leaf area was positively correlated with the biomass, and the leaf area in the wet season was larger than in the dry season. *Brachiaria brizantha* and *E. grandis* had a negative effect on the development of *E. urophylla* seedlings when they were grown simultaneously under three different water contents (20%, 23%, and 26% of the mass), which altered the leaf area and biomass [21]. Aspinwall et al. [22] pinpointed that warming reduced the photosynthetic and respiration rate of *Eucalyptus* leaves. Nevertheless, in the P addition experiment, it was found that the P content of the *Eucalyptus* leaves increased significantly but did not affect leaf photosynthesis and increased the stem growth rate [23]. However, dynamic changes in the development of fertilization and dry season irrigation have rarely been reported to measure the growth of *Eucalyptus* leaves. Exploring *Eucalyptus* leaf area growth and development dynamics and the relationship between water and fertilizer can further elucidate the resource utilization strategies and response mechanisms of water and fertilizer by *Eucalyptus* leaves to provide a theoretical basis for reasonable scientific fertilization and irrigation applications on plantations.

This experiment aims at the commercial production of *E. urophylla* × *E. grandis*, studying leaf growth under different treatments of dry season irrigation and fertilizer. Because water and fertilizer are important factors for leaf growth, the absorption of water can further promote the absorption of nutrients. Therefore, the following hypothesis is proposed in this paper: (1) dry season irrigation and fertilizer can affect leaf area; and (2) dry season irrigation shorten the peak period of leaf growth and increase the leaf area. The aim of this experiment is to reveal the response mechanisms of water and fertilizer factors on the growth dynamics of *E. urophylla* × *E. grandis*. This research has important guiding significance for the wild cultivation and tending of *Eucalyptus*, which provides theoretical support for the related research on the formation process of *Eucalyptus* productivity.

2. Materials and Methods

2.1. Study Site and Plant Material

The experiment was planted in April 2017 in the South China Agricultural University Teaching & Research Base in Zengcheng District, Guangzhou, which is located at 23°14'48 N, 113°38'20 E, the transition zone between tropical and subtropical Asia. According to meteorological records of 1981–2010, the annual average temperature is 21.91 °C. The average annual rainfall is 2004.5 mm and sufficient, while the annual rainfall is uneven. A marked dry season between October and March (accounted for 17.31% of the whole year rainfall) results in a seasonal drought. Therefore, April to September is called the rainy season and October to March is the dry season. The study area was 0.536 ha in total. Using a completely random block design, each region was randomly arranged from 20–92 trees (Figure 1). The field water holding capacity is 20.41% and soil volumetric weight is 1.55 g/cm³. The effective soil chemistry was pH: 4.92, organic matter: 7.03 g/kg, total nitrogen: 0.35 g/kg, total phosphorus: 0.15 g/kg, and total potassium: 8.83 g/kg. The material was a 3-month tissue culture of *E. urophylla* × *E. grandis* clone DH32-29 from Guangdong Gaoyao Jiayao Forestry

Development Co., Ltd, China. The average seedling height was approximately 20–35 cm, and the ground diameter was 0.15–0.20 cm, and there was no disease or mechanical damage. The planting density was 3×2 m, 1650 plants/ha.

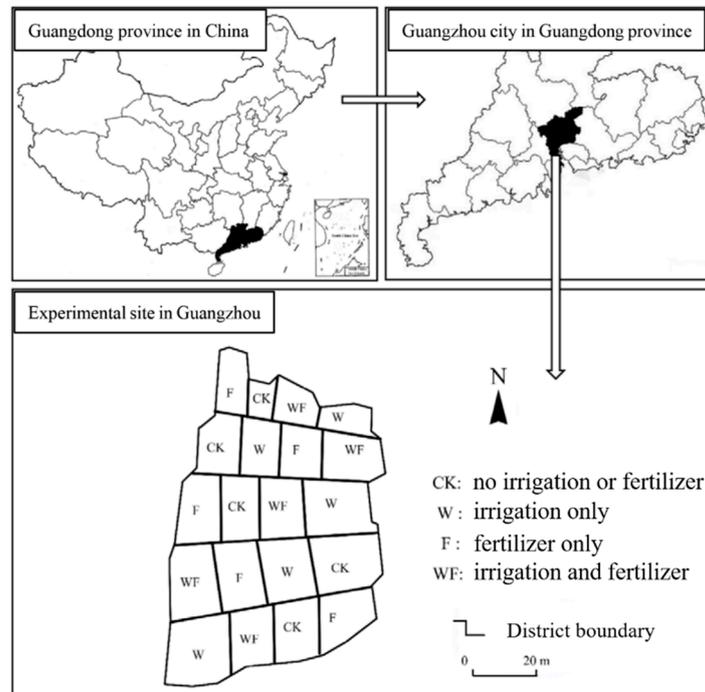


Figure 1. Study area with four treatments, was located in Guangzhou City in Guangdong Province, China.

2.2. Experimental Method

The experiment was constructed by the orthogonal design of water and fertilizer, and water supplementation was carried out only in the dry season from 17 October 2017 to 1 December 2017. The CK treatment was non-irrigated without fertilizer. The W treatment was treated with water and no fertilizer. The F treatment was treated with anhydrous fertilizer. The WF treatment was treated with water and fertilizer. The water and fertilizer treatment methods are shown in Table 1. The design of this experiment ensured that the place of water content (soil water content/field water holding capacity) in W and WF treatments, which was 40 cm depth and 40 cm away from the trees, was maintained at 90% for three days during dry season irrigation. Drip irrigation was applied 8 h/week at 4 L/h, a total of 32 L/week. In this experiment, the irrigation time was from 1 October to 1 December, a total of nine weeks. Each plant was given a total of 288 L for the experiment. The fertilizer additions for F and WF treatments were completely determined based on the amount of commercial Eucalyptus planted on the Chinese market. The special base fertilizer and the top dressing of Eucalyptus were purchased from Guangdong Dayi Agricultural and Forestry Ecological Technology Co., Ltd, China. The base fertilizer was applied at 400 g per hole on 25 March 2017. The effective amount of each element was N: 24 g, P: 72 g, and K: 24 g. On 29 July 2017, the top dressing was applied to the north and south sides of all the trees in the F and WF treatments, and a total of 300 g/plant was applied. The effective amount of each element was N: 45 g, P: 21 g, and K: 24 g. On 18 November 2017, all the Eucalyptus trees were measured and the following features were obtained (Table 2).

Table 1. The four combined treatments. Supplemental irrigation and fertilizer addition was as follows: CK, no irrigation or fertilizer; W—irrigation only; F—fertilizer only; WF—irrigation and fertilizer.

| Treatment | Fertilizer (Eucalyptus Fertilizer) | Irrigation Time(s) | Irrigation Time |
|-----------|------------------------------------|--------------------|--|
| CK | - | - | - |
| W | - | 8 h/week | October to March of the following year |
| F | base fertilizer and top dressing | - | - |
| WF | base fertilizer and top dressing | 8 h/week | October to March of the following year |

Note: Continuous drip irrigation can reach 90% of the field water holding capacity for 4 h, drip irrigation twice a week for 4 h each time.

Table 2. On 18 November 2017, all the Eucalyptus trees were investigated and the following growths were obtained.

| Treatment | Number | Height/cm | Clear Bole Height/cm | Crown Length/cm | Crown Width/m | Ground Diameter/mm | Diameter at Breast Height (DBH)/mm | Branching Quantity |
|-----------|--------|------------------|----------------------|------------------|---------------|--------------------|------------------------------------|--------------------|
| CK | 70 | 150.36 ± 8.38 b | 21.77 ± 1.08 b | 128.59 ± 8.50 b | 1.18 × 1.16 b | 22.72 ± 1.36 b | 14.73 ± 1.33 b | 31.56 ± 3.07 b |
| W | 84 | 168.37 ± 6.87 b | 26.65 ± 1.10 a | 141.71 ± 6.83 b | 1.23 × 1.24 b | 24.25 ± 1.15 b | 15.29 ± 0.90 b | 35.00 ± 2.53 b |
| F | 66 | 454.12 ± 10.78 a | 21.17 ± 1.10 b | 432.95 ± 10.73 a | 2.17 × 2.13 a | 58.10 ± 1.54 a | 41.10 ± 1.30 a | 67.08 ± 3.58 a |
| WF | 71 | 438.80 ± 8.67 a | 22.87 ± 1.15 b | 415.93 ± 9.04 a | 2.16 × 2.12 a | 55.72 ± 1.45 a | 39.38 ± 1.31 a | 64.92 ± 2.45 a |

Note: The mean ± standard error in the table, and the different letters represent the significant difference between different treatments under the same index ($p < 0.05$).

2.3. Index Measure

The experiment started on 17 October 2017, and five trees with relatively average growth in each treatment were selected. Three apical germinated leaves were selected at the top 1/3 of the tree height. The leaf length and width were recorded as zero and marked with red lines. Photographs of the leaves were taken every five days. A method of placing a flat blade between cardboard and graph paper was used, and a digital camera was used to vertically photograph the cardboard. The taking of photos and observations were stopped when the leaf length and width no longer increased before and after. The leaf length, leaf width (maximum width perpendicular to the main vein), leaf area, and leaf perimeter were measured using ImageJ software.

2.4. Statistical Analysis

Excel 2013 computer package program and SPSS 18.0 statistical analysis software was used for single factor and double factor variance analysis, bivariate correlation analysis, and regression analysis. Origin Pro 2016 statistical analysis software was used for performing the logistic regression equation fitting and saliency analysis.

The leaf shape index was the leaf width divided by the leaf length. Using the leaf area and the growth time fitted by a logistic curve model, the logistic growth curve was modeled with the following: $y = a/(1 + be^{-kx})$. In the equation, x , y , and a represent the growth time (day), leaf area (cm^{-2}) and growth ceiling (cm^{-2}), respectively. The growth time and leaf area were simulated by a logistic curve. The test was optimized by the four-point method and the least squares method. The maximum linear growth rate can be determined as $V_{\max} = 1/4ak$. The equation was further derivable to obtain $t_1 = (\ln b - 1.317)/k$, $t_2 = \ln b/k$, $t_3 = (\ln b + 1.317)/k$. In the equation, t_1 , t_2 , and t_3 represent the initial process (the beginning of the rapid growth of the leaves), the peak period (the peak period of leaf growth), and the end stage (the end period of the rapid growth of the leaves), respectively [24]. The leaf expansion rate ($\text{cm}^{-2} \cdot \text{day}^{-1}$) was for a single leaf area/peak period [25]. The leaf expansion rate in this paper was the relative leaf expansion rate in the peak period. It was the time from the initial process to the end stage calculated by the logistic regression equation. The leaf expansion rate was equal to the single peak area growth divided by the growth time. In this experiment, the peak period of CK was 15–30 days, and the leaf expansion rate was $R (\text{cm}^{-2} \cdot \text{day}^{-1}) = (y_{\text{day } 30} - y_{\text{day } 15})/(30 - 15)$. The peak periods of W, E, and WF were 15–30, 15–35, and 10–30 days, respectively.

The two-factor analysis of variance adopted a fixed mode [26]. The fixed effect linear model was $y_{ij} = \beta_0 + \beta_{1 \times 1ij} + \beta_{2 \times 2ij} + \dots + \beta_{k \times kij} + e_{ij}$.

The effect of k treatments with k was fixed within the experimental processing range. A_i and B_j are the effects of A_i , B_j ; $\alpha_i = \mu_i - \mu$, $\beta_j = \mu_j - \mu$. In the equation, μ_i and μ_j are the average of the observed values of A_i and B_j , respectively, and $\sum \alpha_i = 0$, $\sum \beta_j = 0$. The effect variance of each treatment in the fixed mode was represented by k_{α}^2 . The expression was $k_{\alpha}^2 = \sum (\mu_i - \mu)^2 / (k - 1) = \sum \mu_i^2 / (k - 1)$.

3. Results

3.1. Changes in Leaf Growth Trend

Bivariate correlations of leaf length, width, area, perimeter, expansion rate, and shape index were made in Table 3. Strong correlations were found between leaf length, width, area, perimeter, and expansion rate. The leaf area of Eucalyptus showed an “S” growth curve pattern (Figure 2). The initial growth was slow, and after a period of peak growth, the growth tended to be flat and was finally close to zero increase. The whole leaves grew gently after 40 days, basically reaching the maximum leaf area. After 45 days of treatment, the leaf area of the WF treatment (23.20 cm^2) was largest, which was 1.30–1.44 times the leaf area of the other three treatments. The WF treatment was significantly different ($p < 0.05$) from the other three treatments, and there was no significant difference between the three treatments. The leaf length (9.83 cm) and perimeter (21.99 cm) of the WF treatment were consistent with the leaf area, which was significantly different from the other three treatments. The leaf width of

the F treatment (2.81 cm) was the smallest, only 0.77 of that of the WF treatment, which was different from the other three groups. As the leaf growth decreased, the leaf shape index decreased by 30.7%, 28.1%, 23.0%, and 22.8% in the CK, W, F, and WF treatments, respectively. On the 45th day, there was a significant difference in the leaf shape index between the CK (2.26), W (2.28), F (2.93), and WF (2.89) treatments. The leaf expansion rate was a single peak curve growth. The leaf expansion rate was the highest from the 25th to the 30th day (0.87, 0.80, 0.60, and 0.89 cm²/day, respectively, in the CK, W, F, and WF treatments), and the rate on the 45th day tended to be 0.

Table 3. Bivariate correlations of leaf length, width, area, perimeter, expansion rate and shape index (Pearson correlation coefficient).

| Index | W | LA | LP | R | Leaf Shape Index (L/W) |
|-------------------------|----------|----------|----------|----------|------------------------|
| Leaf length (L) | 0.644 ** | 0.880 ** | 0.988 ** | 0.749 ** | 0.370 * |
| Leaf width (W) | | 0.916 ** | 0.751 ** | 0.918 ** | −0.452 ** |
| Leaf area (LA) | | | 0.940 ** | 0.933 ** | −0.086 |
| Leaf perimeter (LP) | | | | 0.831 ** | 0.23 |
| Leaf expansion rate (R) | | | | | −0.259 |

Notes: Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

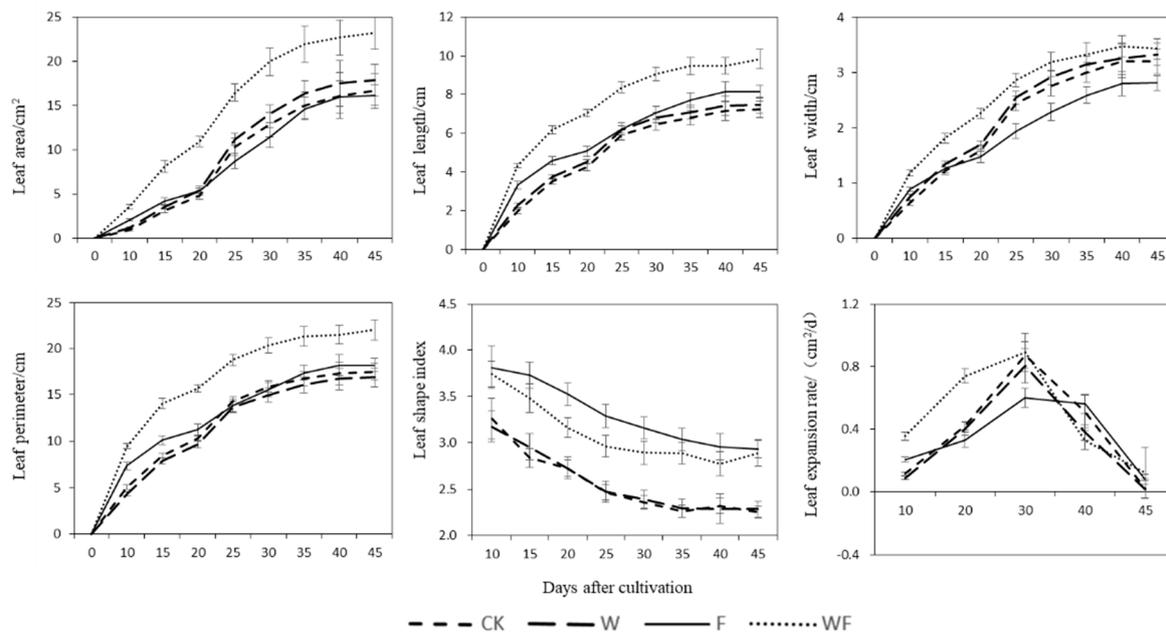


Figure 2. Dynamic changes of leaf area, leaf length, leaf width, leaf perimeter, leaf shape index, and leaf expansion rate of the four treatments.

3.2. Two-Factor Analysis of the Leaf Index in Dry Season Irrigation and Fertilization

The two-factor analysis of growth indicators showed that dry season irrigation affected the height under the branches, while fertilizer affected the remaining growth indicators (Table 4). After 45 days of treatment, dry season irrigation did not have a significant influence on the leaf characteristics of the Eucalyptus (Table 5). The fertilizer mainly controlled the leaf length ($F = 15.438$ **), perimeter ($F = 9.639$ **) and shape index ($F = 45.287$ **). Under the water–fertilizer combination (WF) treatment, the leaf length ($F = 5.124$ *), area ($F = 5.494$ *), perimeter ($F = 5.420$ *), expansion rate ($F = 6.409$ *), and length \times width ($F = 5.338$ *) had significant effects. The leaf area of each growth period was obtained by a double factor analysis of water and fertilizer (Figure 3). It can be seen from the effect variance analysis chart that a smaller effect is close to the horizontal axis, and the larger the value (ordinate), the greater the influence on the leaf characteristics. Both the water and fertilizer treatments had a more significant effect on early leaf expansion, but the effect gradually decreased later. As the

leaves developed, the influence of fertilizer on the leaf characteristics existed in the fluctuation mode. The effect of the dry season irrigation and the water–fertilizer combination on the leaf area, length, width, and perimeter showed a “single peak” growth pattern, but the effect on the leaf shape index was weak and fluctuated in its expansion rate. The water–fertilizer combination effect was more apparent in the leaf area and width, which was highlighted after the 15th day. The leaf shape index was affected only by fertilizer and not by time.

3.3. Leaf Logistic Model Fitting

Setting the expansion time and the area of the leaves at x (day) and y (cm^2), the growth regression curve was used to fit the logistic model (Table 6). We obtained four regression equations with R^2 values greater than 0.99. The maximum growth limit for the four treatments was the WF treatment (23.604 cm^2), which was 1.41 times that of the minimum treatment, the W treatment (16.706 cm^2). The maximum linear growth rates were 0.90, 0.84, 0.61, and $0.99 \text{ cm}^2 \cdot \text{day}^{-1}$ for the CK, W, F, and WF treatments, respectively, and the F treatment was the minimum. The initial process ranged from 12.14–16.85 days, while the end stage ranged from 27.91–34.57 days. The earliest initial process and end stage for the WF treatment were, respectively, the 12.14th and 27.91th days. The peak period of the F treatment was the longest (18.95 days), which was the ratio of the remaining three treatments by 1.43, 1.45, and 1.20 times for the CK, W, and WF treatment, respectively. The number of days from the initial process for the fertilizer treatments, F and WF, were advanced, and the number of days in the peak period was expanded. A significance analysis of the regression equation using OriginPro 2016 showed that the four curves were significantly different from each other ($p < 0.05$).

Table 4. Analysis of double factors of Eucalyptus growth after 45 days of treatment by *F*-value test (*F* value).

| Source of Difference | Height/cm | Clear Bole Height/cm | Crown Length/cm | SN Crown Width/m | EW Crown Width/m | Ground Diameter/mm | Diameter at Breast Height (DBH)/mm | Branching Quantity |
|----------------------|-------------|----------------------|-----------------|------------------|------------------|--------------------|------------------------------------|--------------------|
| Water | 0.013 | 8.548 ** | 0.233 | 0.007 | 0.251 | 0.179 | 0.105 | 0.046 |
| fertilizer | 1133.549 ** | 3.699 | 1127.112 ** | 566.200 ** | 560.166 ** | 614.645 ** | 196.417 ** | 121.021 ** |
| Water × Fertilizer | 2.787 | 1.889 | 2.186 | 0.204 | 0.434 | 1.721 | 0.399 | 0.886 |

Notes: Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

Table 5. Analysis of double factors of Eucalyptus leaf size after 45 days of treatment by *F*-value test.

| Source of Difference | Leaf Length (L) | Leaf Width (W) | Leaf Area (LA) | Leaf Perimeter (LP) | Leaf Expansion Rate (R) | Leaf Shape Index (L/W) | Leaf Length × Width (LW) |
|----------------------|-----------------|----------------|----------------|---------------------|-------------------------|------------------------|--------------------------|
| Water | 2.971 | 1.551 | 2.780 | 2.934 | 3.006 | 0.013 | 2.843 |
| Fertilizer | 15.438 ** | 0.556 | 1.920 | 9.639 ** | 0.016 | 45.287 ** | 2.181 |
| Water × Fertilizer | 5.124 * | 3.746 | 5.494 * | 5.420 * | 6.409 * | 0.144 | 5.338 * |

Notes: Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

Table 6. Leaf area logistic growth model and characteristic value of the Eucalyptus leaf growth process.

| Treatment | Regressive Equation | R ² (COD) | F | Vmax/(cm ² · Day ⁻¹) | t ₁ /Day | t ₂ /Day | t ₃ /Day | Peak Days/Day |
|-----------|--|----------------------|------------|---|---------------------|---------------------|---------------------|---------------|
| CK | $Y = 18.064 / (1 + 101.652 \times \exp(-0.199 \cdot x))$ | 0.996 | 1694.95 ** | 0.90 | 16.61 | 23.22 | 29.84 | 13.24 |
| W | $Y = 16.706 / (1 + 110.307 \times \exp(-0.201 \cdot x))$ | 0.996 | 1389.47 ** | 0.84 | 16.85 | 23.40 | 29.95 | 13.10 |
| F | $Y = 17.608 / (1 + 32.718 \times \exp(-0.139 \cdot x))$ | 0.994 | 1166.41 ** | 0.61 | 15.62 | 25.09 | 34.57 | 18.95 |
| WF | $Y = 23.604 / (1 + 28.321 \times \exp(-0.167 \cdot x))$ | 0.996 | 1976.4 ** | 0.99 | 12.14 | 20.02 | 27.91 | 15.77 |

Notes: Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

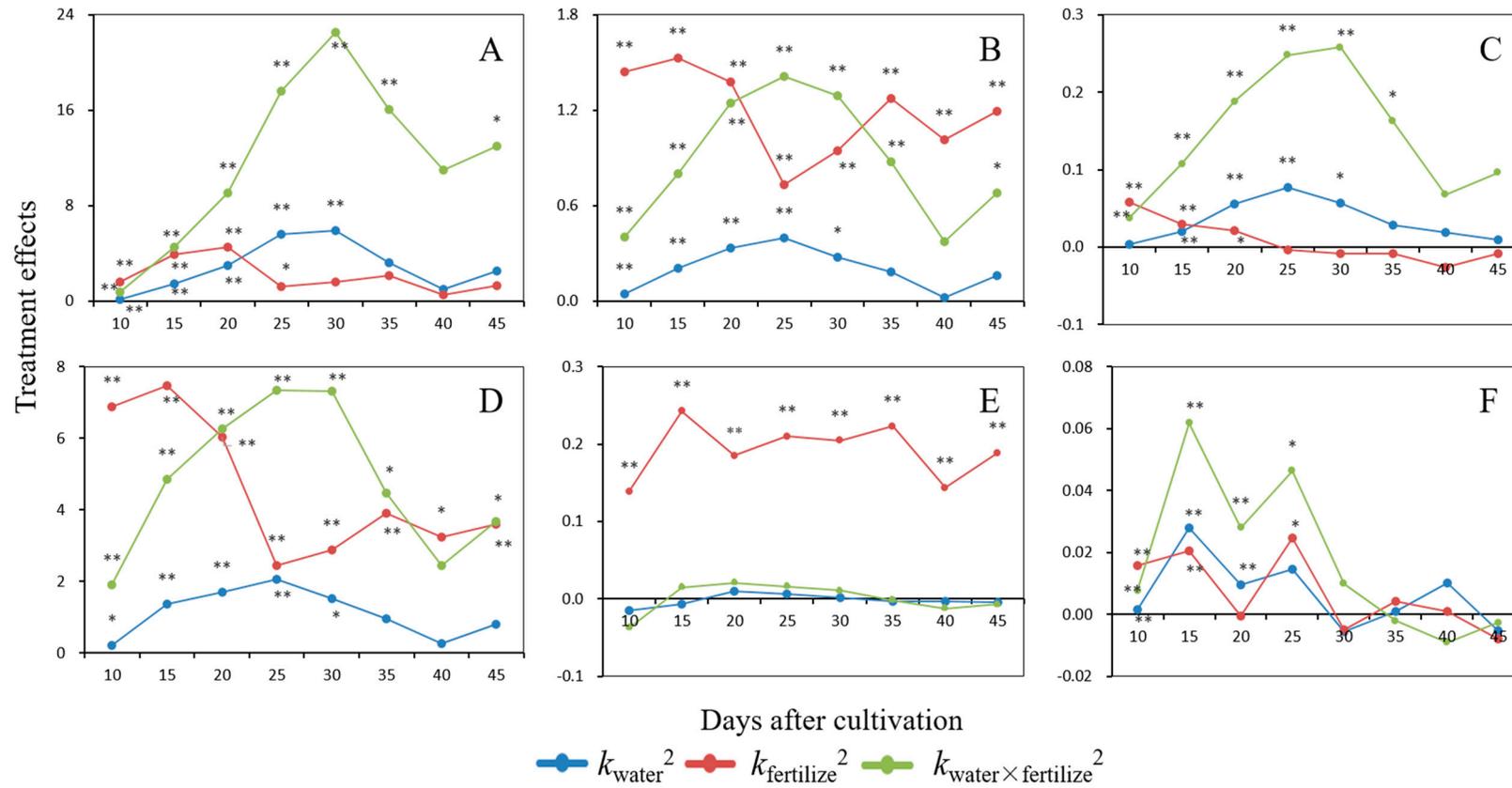


Figure 3. The effect of variance changes of water, fertilizer, and their combination during the leaf expansion. Note: (A) to (F) respectively represent the treatment effects of leaf area, leaf length, leaf width, leaf perimeter, leaf shape index, and leaf expansion rate. Notes: Levels of significance are indicated by asterisks: * $p < 0.05$; ** $p < 0.01$.

4. Discussion

4.1. Effects of Water and Fertilizer on Leaf Growth

The leaf growth dynamics of *E. urophylla* × *E. grandis* exhibited a logistic growth curve pattern, which was consistent with popular perceptions of leaf growth model [27]. The primordium shows a slow expansion due to the cell proliferation in the early leaf development, and the following rapid expansion mainly because of the cell expansion and cell divisions at the same time [28]. As a “fast growing” species, *E. urophylla* × *E. grandis* showed its resource acquisition abilities. During the leaf expansion, the effects of dry season irrigation, fertilizer, and their combination were all positive on leaf area, leaf length, and leaf perimeter. Both dry season irrigation and fertilizer can affect whole leaf expansion. Irrigation can facilitate the transport of fertilizers in the soil to various parts of the tree. Especially, the first post-planting irrigation was the most important control for production [29]. Therefore, we can find that the leaf area of WF (dry season irrigation and fertilizer) treatment was the largest. This may mean that leaf area growth is not promoted when the soil is poor or dry. The impacts of water, fertilizer, and the water–fertilizer combination were significant in the early stage of leaf expansion and weakened in the later stage because of many factors, such as species genes [30]. In this experiment, dry season irrigation had no significant effect on each leaf characteristic due to short-time treatment (nine weeks, a total of only 288 L/tree). The Eucalyptus was still in the early stage of plantation, and the response to water was slowly accumulating. Dry season irrigation is conducive to fertility and has a significant impact on leaf area. The combined effect of water and fertilizer is similar to the study by Albaugh et al. [14], which indicated that the volume, total biomass and maximum leaf area index of *Pinus taeda* increased after four years of water and fertilizer experiments.

4.2. Leaf Shape Variation

Plants have similar strategies for coping with stress, which include enhancing their water retention, reducing their transpiration rate, and improving their photosynthetic efficiency [31,32]. In this experiment, we found that fertilization had a significant effect on the leaf shape index of the Eucalyptus leaves. The leaf shape index was larger with the fertilizer treatment, which made the leaves slender, but the leaf shape index was smaller without the fertilizer treatment. As planting in the bare ground, all trees would suffer the mechanical hazards from wind. Therefore, all leaves were supposed to have narrow laminas for wind damage [33]. For the trees under fertilization without water treatment, the leaf area, on the one hand, was considered to be promoted with enough fertilizer, while on the other hand, would be narrowed to counteract the negative effects of overheating and high transpiration rates under drought condition [34,35]. Thus, the leaves tend to have longer and narrower laminas, which causes the higher leaf shape index. Another possible reason is that the narrower shape may help absorb humidity directly from fog [36]. While for the trees with both fertilization and irrigation, they enlarged their leaf areas (Figure 2) to get more illumination for photosynthesis. The lengthening rate was faster than that of widening to avoid the overlaps of adjacent blades and physical hazards caused by wind, which also make the leaf shape index larger. In addition, leaf shape index decreased with the leaf growth, which was similar to the findings of Zheng [37]; as the tree age increased, the leaf shape index decreased.

4.3. Leaf Expansion Rate and Model Fitting

The leaf expansion rate is significantly different in dry and humid environments ($p < 0.001$) [38] and is lower with reduced water resources [39,40]. The WF treatment had the highest leaf spreading rate ($0.99 \text{ cm}^2 \cdot \text{day}^{-1}$) and the earliest initial period (12.14 days) and end stage (27.91 days). This pattern of leaf expansion rate is in accordance with the research of Zhu et al. [41], that is, when water and fertilizer are sufficiently available, the larger the leaf and the earlier the peak period end stage. Interestingly, the time at the initial process of the F and WF treatments was earlier than that of the CK and W treatments, and the peak days were longer (2–5 days more). This shows that fertilizer can advance the

initial process and promote leaf growth in the peak period. As the fertilizer availability increases, the growth rate and resistance to cavitation increases [42]. Differential fertilization and irrigation showed that *Quercus rubra* and *Quercus prinus* had a greater effect on tissue water relations on fertilization than irrigation [43]. Fertilizers can affect the structure of the leaves more than irrigation. Since the effect of fertilizer on leaf area begins from the leaf, the effect of irrigation is slowly enhanced. Therefore, in the early stage of expansion, fertilizer advanced the effect in the beginning, which was consistent with the study above. The peak period of the F treatment was 3 days longer than that of the WF treatment, but the area of the WF treatment was significantly larger; hence, dry season irrigation shortened the peak period of the WF treatment and increased the leaf area [44], conforming to the hypothesis.

5. Conclusions

In this experiment, the areas of Eucalyptus leaves treated with dry season irrigation and fertilizer were measured, showing that both dry season irrigation and fertilizer can affect whole leaf expansion. Leaf area was the largest when water and fertilizer were added at the same time. Dry season irrigation shortened the peak period of leaf growth and increase the leaf area. However, the experiment had a short water replenishment time. If these measures are adopted for the long-term dry season, the productivity of the plantation should be significantly improved. There are still many factors affecting leaf area, which are directly proportional to the soil nutrient utilization rate [45] and habitat productivity [46] and inversely proportional to height [47]. Therefore, there are still many doubts. In fact, most leaves are mostly shaded and are limited only by photosynthesis [48,49], which still strengthened the respiration and tree construction inputs [50].

Biomass and net primary productivity are directly proportional to leaf area [51]. General biomass and leaf area equations are used to assess and simulate forest productivity [52]. The innovation of this research was to study the response mechanism of leaf growth with water and fertilizer treatment in a Eucalyptus plantation during dry season irrigation, which further provided theoretical support for the study of commercial Eucalyptus production.

Author Contributions: F.Y. and J.L. contributed equally to the conceptualization and design of the work; writing—original draft preparation, F.Y., T.V.T. and L.H.; writing—review & editing, F.Y.; software, F.Y. and L.H.; visualization, F.Y.; validation, Q.H.; methodology and formal analysis, F.Y., T.V.T. and L.H.; investigation, F.Y. and T.V.T.; supervision and critical revision of the manuscript, J.L. and X.H.; project administration, Q.H. and Y.S.; and funding acquisition, J.L. All the authors approved the version to be published.

Funding: This research was supported by the National Key Research and Development Program of China (2016YFD0600201; 2016YFD060020102) and National Natural Science Foundation of China (31800527).

Acknowledgments: The authors wish to thank Xinsheng Hu for the technical support and analysis and Shili Liu for the composing.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z.; Bongers, F.; Cavenderbares, J.; Chapin, T.; Cornelissen, J.H.; Diemer, M.; et al. The worldwide leaf economics spectrum. *Nature* **2004**, *428*, 821. [[CrossRef](#)]
2. Grier, C.G.; Running, S.W. Leaf area of mature northwestern coniferous forests: Relation to site water balance. *Ecology* **1977**, *58*, 893–899. [[CrossRef](#)]
3. Wellstein, C.; Poschlod, P.; Gohlke, A.; Chelli, S.; Campetella, G.; Rosbakh, S.; Canullo, R.; Kreyling, J.; Jentsch, A.; Beierkuhnlein, C. Effects of extreme drought on specific leaf area of grassland species: A meta-analysis of experimental studies in temperate and sub-Mediterranean systems. *Glob. Chang. Biol.* **2017**, *541*, 516. [[CrossRef](#)] [[PubMed](#)]
4. Sinha, N. Shaping up: The genetic control of leaf shape. *Curr. Opin. Plant Biol.* **2004**, *7*, 65–72.
5. Barkoulas, M.; Galinha, C.; Grigg, S.P.; Tsiantis, M. From genes to shape: Regulatory interactions in leaf development. *Curr. Opin. Plant Biol.* **2007**, *10*, 660–666. [[CrossRef](#)] [[PubMed](#)]

6. Traiser, C.; Klotz, S.; Uhl, D.; Mosbrugger, V. Environmental signals from leaves—A physiognomic analysis of European vegetation. *New Phytol.* **2005**, *166*, 465–484. [[CrossRef](#)] [[PubMed](#)]
7. Roche, P.; Díaz-Burlinson, N.; Gachet, S. Congruency analysis of species ranking based on leaf traits: Which traits are the more reliable? *Plant Ecol.* **2004**, *174*, 37–48. [[CrossRef](#)]
8. Yang, H.Y.; Wei, H.Y.; Sang, M.J.; Shang, Z.H.; Mao, Y.J.; Wang, X.R.; Liu, F.; Gu, W. Phenotypic Plasticity of *Schisandra sphenanthera* Leaf and the Effect of Environmental Factors on Leaf Phenotype. *Chin. Bull. Bot.* **2016**, *51*, 322–334.
9. Nicotra, A.B.; Cosgrove, M.J.; Cowling, A.; Schlichting, C.D.; Jones, C.S. Leaf Shape Linked to Photosynthetic Rates and Temperature Optima in South African Pelargonium Species. *Oecologia* **2008**, *154*, 625–635. [[CrossRef](#)]
10. Wang, C.; Liu, J.; Xiao, H.; Du, D. Response of Leaf Functional Traits of *Cerasus yedoensis* (Mats.) Yü Li to Serious Insect Attack. *Pol. J. Environm. Stud.* **2016**, *25*, 333–339. [[CrossRef](#)]
11. Martinez, K.A.; Fridley, J.D. Acclimation of leaf traits in seasonal light environments: Are non-native species more plastic? *J. Ecol.* **2018**. [[CrossRef](#)]
12. Villar-Salvador, P.; Penuelas, J.L.; Jacobs, D.F. Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. *Tree Physiol.* **2013**, *33*, 221–232. [[CrossRef](#)] [[PubMed](#)]
13. Wang, A.Y.; Wang, M.; Yang, D.; Song, J.; Zhang, W.W.; Han, S.J.; Hao, G.Y. Responses of hydraulics at the whole-plant level to simulated nitrogen deposition of different levels in *Fraxinus mandshurica*. *Tree Physiol.* **2016**, *36*, 1045–1055. [[CrossRef](#)] [[PubMed](#)]
14. Albaugh, T.J.; Allen, H.L.; Dougherty, P.M.; Kress, L.W.; King, J.S. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. *For. Sci.* **1998**, *44*, 317–328.
15. Lower, S.S.; Orians, C.M. Soil nutrients and water availability interact to influence willow growth and chemistry but not leaf beetle performance. *Entomol. Exp. Appl.* **2003**, *107*, 69–79. [[CrossRef](#)]
16. Maire, G.L.; Marsden, C.; Verhoef, W.; Ponzoni, F.J.; Seen, D.L.; Bégué, A.; Stape, J.; Nouvellon, Y. Leaf area index estimation with MODIS reflectance time series and model inversion during full rotations of Eucalyptus, plantations. *Remote Sens. Environ.* **2011**, *115*, 586–599. [[CrossRef](#)]
17. Macfarlane, C.; Coote, M.; White, D.A.; Adams, M.A. Photographic exposure affects indirect estimation of leaf area in plantations of *Eucalyptus globulus* Labill. *Agric. For. Meteorol.* **2000**, *100*, 155–168. [[CrossRef](#)]
18. Diao, J.; Lei, X.D.; Hong, L.X.; Kong, J.T.; Qiang, S. Single leaf area estimation models based on the leaf weight of Eucalyptus in southern china. *J. For. Res.* **2010**, *21*, 73–76. [[CrossRef](#)]
19. Nouvellon, Y.; Laclau, J.P.; Epron, D.; Kinana, A.; Mabiala, A.; Roupsard, O.; Bonnefond, J.M.; Maire, G.; Marsden, C.; Bontemps, D.; et al. Within-stand and seasonal variations of specific leaf area in a clonal Eucalyptus plantation in the Republic of Congo. *For. Ecol. Manag.* **2010**, *259*, 1796–1807. [[CrossRef](#)]
20. Li, L.L.; Li, M.; Zheng, R.M.; Cheng, X.P. Response of Eucalyptus Leaf Area and Biomass to Wet and Dry Season. *Sci. Technol. Eng.* **2016**, *16*, 167–170.
21. Silva, W.D.; Silva, A.A.D.; Sedyama, T.; Cardoso, A.A. Biomass and leaf area of *Eucalyptus citriodora* and *Eucalyptus grandis* seedlings as affected by water content in soil and association with *Brachiaria brizantha*. In *Pesquisa Agropecuaria Gaucha*; 1999. Available online: www.fepagro.rs.gov.br/upload/1398909545_art_04.pdf (accessed on 20 November 2018).
22. Aspinwall, M.J.; Drake, J.E.; Company, C.; Varhammar, A.; Ghannoum, O.; Tissue, D.T.; Reich, P.B.; Tjoelker, M.G. Convergent acclimation of leaf photosynthesis and respiration to prevailing ambient temperatures under current and warmer climates in *Eucalyptus tereticornis*. *New Phytol.* **2016**, *212*, 354–367. [[CrossRef](#)] [[PubMed](#)]
23. Crous, K.Y.; Ósvaldsson, A.; Ellsworth, D.S. Is phosphorus limiting in a mature Eucalyptus woodland? Phosphorus fertilisation stimulates stem growth. *Plant Soil* **2015**, *391*, 293–305. [[CrossRef](#)]
24. Wu, J.W.; He, Q.; Li, J.Y.; Wang, J.H.; Su, Y.; Wang, L.P.; Dong, J.L.; Bai, J.J. Dynamic changes of foliage growth of *Catalpa bungei* clones under different nitrogen exponential fertilizations. *J. Beijing For. Univ.* **2015**, *37*, 19–28.
25. Qiu, Q. Interactive Effects of Light with Water and Fertilizer on the Growth and Physiological Characteristics of *Catalpa bungei* Clones Seedlings. Master's Thesis, South China Agriculture University, Guangzhou, China, 2016.
26. Lei, X.D.; Li, Y.C.; Xiang, W. Individual basal area growth model using multi-level linear mixed model with repeated measures. *Sci. Silva. Sin.* **2009**, *45*, 74–80.

27. Sack, L.; Scoffoni, C.; Mckown, A.D.; Frole, K.; Rawls, M.; Havran, J.C.; Tran, H.; Tran, H. Developmentally based scaling of leaf venation architecture explains global ecological patterns. *Nat. Commun.* **2012**, *3*, 837. [[CrossRef](#)] [[PubMed](#)]
28. Donnelly, P.M.; Bonetta, D.; Tsukaya, H.; Dengler, R.E.; Dengler, N.G. Cell Cycling and Cell Enlargement in Developing Leaves of Arabidopsis. *Dev. Biol.* **1999**, *215*, 0–419. [[CrossRef](#)] [[PubMed](#)]
29. Lobell, D.B.; Ortiz-Monasterio, J.I.; Asner, G.P.; Naylor, R.L.; Falcon, W.P. Combining field surveys, remote sensing, and regression trees to understand yield variations in an irrigated wheat landscape. *Agron. J.* **2005**, *97*, 241–249.
30. Monclus, R.; Dreyer, E.; Villar, M.; Delmotte, F.M.; Delay, D.; Petit, J.M.; Barbaroux, C.; Thiec, D.L.; Bréchet, C.; Brignolas, F. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. *New Phytol.* **2006**, *169*, 765–777. [[CrossRef](#)]
31. Xu, F.; Guo, W.; Xu, W.; Wei, Y.; Wang, R. Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves? *Prog. Nat. Sci. Mater. Int.* **2009**, *19*, 1789–1798. [[CrossRef](#)]
32. Zhu, G.L.; Wei, X.Z. Leaf morphological plasticity of *Ziziphus jujuba* var. *spinosa* in response to natural drought gradient ecotopes. *Acta Phytoecol. Sin.* **2016**, *36*, 6178–6187.
33. Klich, M.G. Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. *Environ. Exp. Bot.* **2000**, *44*, 171–183. [[CrossRef](#)]
34. Gates, D.M. *Biophysical Ecology*; Springer: New York, NY, USA, 1980; 611p.
35. Yates, M.J.; Verboom, G.A.; Rebelo, A.G.; Cramer, M.D. Ecophysiological significance of leaf size variation in Proteaceae from the Cape Floristic Region. *Funct. Ecol.* **2010**, *24*, 485–492. [[CrossRef](#)]
36. Martorell, C.; Ezcurra, E. The narrow-leaf syndrome: A functional and evolutionary approach to the form of fog-harvesting rosette plants. *Oecologia* **2007**, *151*, 561–573. [[CrossRef](#)] [[PubMed](#)]
37. Zheng, Y.Q.; Feng, M.; Li, Z.J. Investigation of bud burst, shoot growth and leaf expansion in *Populus euphratica* of different ages. *Acta Ecol. Sin.* **2015**, *35*, 1198–1207.
38. Sadras, V.O.; Villalobos, F.J.; Fereres, E.; Wolfe, D.W. Leaf responses to soil water deficits: Comparative sensitivity of leaf expansion rate and leaf conductance in field-grown sunflower (*Helianthus annuus*, L.). *Plant Soil* **1993**, *153*, 189–194. [[CrossRef](#)]
39. Poorter, H.; Niinemets, U.; Poorter, L.; Wright, I.J.; Villar, R. Causes and consequences of, variation in leaf mass per area (LMA): A meta-analysis. *New Phytol.* **2010**, *182*, 565–588. [[CrossRef](#)]
40. Tardieu, F. Modelling leaf expansion in a fluctuating environment: Are changes in specific leaf area a consequence of changes in expansion rate? *New Phytol.* **2010**, *143*, 33–43. [[CrossRef](#)]
41. Zhu, X.B.; Liu, Y.M.; Sun, S.C. Leaf expansion of the dominant woody species of three deciduous oak forests in Nanjing, east China. *Acta Phytoecol. Sin.* **2005**, *29*, 128–136.
42. Goldstein, G.; Bucci, S.J.; Scholz, F.G. Why do trees adjust water relations and hydraulic architecture in response to nutrient availability? *Tree Physiol.* **2013**, *33*, 238–240. [[CrossRef](#)]
43. Kleiner, K.W.; Abrams, M.D.; Schultz, J.C. The impact of water and nutrient deficiencies on the growth, gas exchange and water relations of red oak and chestnut oak. *Tree Physiol.* **1992**, *11*, 271. [[CrossRef](#)]
44. Shen, A.; Liu, C. Effects of different application rate of urea on the growth of rice and N fertilizer utilization ratio under water leakage and non-leakage conditions. *Chin. J. Riceence* **1997**, *11*, 231–237.
45. Mcdonald, P.G.; Fonseca, C.R.; Westoby, M. Leaf-Size divergence along rainfall and soil-nutrient gradients: Is the method of size reduction common among clades? *Funct. Ecol.* **2010**, *17*, 50–57. [[CrossRef](#)]
46. Santini, B.A.; Hodgson, J.G.; Thompson, K.; Wilson, P.J.; Band, S.R.; Jones, G.; Charles, M.; Bogaard, A.; Palmer, C.; Rees, M. The triangular seed mass-leaf area relationship holds for annual plants and is determined by habitat productivity. *Funct. Ecol.* **2017**, *31*, 1770–1779. [[CrossRef](#)]
47. Milla, R.; Reich, P.B. Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. *Ann. Bot.* **2011**, *107*, 455. [[CrossRef](#)] [[PubMed](#)]
48. Drewry, D.T.; Kumar, P.; Long, S.; Bernacchi, C.; Liang, X.Z.; Sivapalan, M. Ecohydrological responses of dense canopies to environmental variability: 1. interplay between vertical structure and photosynthetic pathway. *J. Geophys. Res. Biogeosci.* **2015**, *115*, 985–989. [[CrossRef](#)]
49. Drewry, D.T.; Kumar, P.; Long, S.; Bernacchi, C.; Liang, X.Z.; Sivapalan, M. Ecohydrological responses of dense canopies to environmental variability: 2. Role of acclimation under elevated CO₂. *J. Geophys. Res. Biogeosci.* **2015**, *115*, 6291–6297. [[CrossRef](#)]

50. Srinivasan, V.; Kumar, P.; Long, S.P. Decreasing, not increasing, leaf area will raise crop yields under global atmospheric change. *Glob. Chang. Biol.* **2017**, *23*, 1626–1635. [[CrossRef](#)]
51. Gholz, H.L. Environmental limits on aboveground net primary production, leaf area, and biomass in vegetation zones of the Pacific Northwest. *Ecology* **1982**, *63*, 469–481. [[CrossRef](#)]
52. Forrester, D.I.; Tachauer, I.H.H.; Annighoefer, P.; Barbeito, A.; Pretzsch, H.; Ruiz-peinado, R.; Stark, H.; Vacchiano, G.; Zlatanov, T.; Chakraborty, T.; et al. Generalized biomass and leaf area allometric equations for European tree species incorporating stand structure, tree age and climate. *For. Ecol. Manag.* **2017**, *396*, 160–175. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).