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Hydraulic and Economical Traits in Short- and Long-Shoot Leaves of *Ginkgo biloba* Males and Females

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Abstract: *Ginkgo biloba* is a dioecious heterophyllous tree species with two leaf types. Short shoots always bear many leaves, which are undivided or slightly bilobed, but long-shoot leaves are smaller and are deeply divided into two or more lobes. To clarify the functional difference between the two leaf types, we investigated the morphology, anatomy, gas exchange, and hydraulic traits of the two leaf types in male and female trees. Our results showed that long-shoot leaves possessed a lower specific leaf area (*SLA*), higher leaf thickness, gas exchange rates, and average tracheid number than short-shoot leaves. When compared to short-shoot leaves, long-shoot leaves in male trees had higher leaf hydraulic conductance (K_{leaf}) and leaf water potential of 50% loss in K_{leaf} (leaf *P50*), while female trees exhibited relatively higher K_{leaf}. When comparing sexual differences, male trees possessed higher leaf thickness, leaf dissection index, vein density (*VD*), and predawn leaf water potential and lower *SLA*, tracheid number, and midday leaf water potential, while no significant difference in leaf *P50* was observed between the two sexes. Male trees exhibited greater leaf gas exchange rates, which were tightly associated with other functional traits, such as *VD* and K_{leaf}. We found strong shoot-based and sex-related heterogeneities in the leaf hydrology, anatomy, and economy of *Ginkgo biloba*. Moreover, male ginkgoes may perform better due to their greater leaf hydraulic efficiency and gas exchange rates.

Keywords: dioecious; heterophylly; leaf anatomy; hydraulic efficiency; leaf gas exchange

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1. Introduction

Many plant species have different leaf forms within a single plant, which is termed heterophylly. In some cases, heterophylly has been recognized as an adaptive mechanism that allows plants to respond optimally to micro-environmental heterogeneity [1]. The development of heteromorphic leaves may reflect an ecological adaptation of plants. For example, the floating and aerial leaves of *Nuphar lutea* (L.) Smith. should be considered sun-adapted, while submerged near-base leaves should be considered shade-adapted. This phenomenon can be an adaptive strategy for plants to ensure photosynthetic efficiency under different light intensities [2]. Another form of heterophylly is that some plants have distinct leaf forms developed from different shoot types, such as short shoots and long shoots. Short shoots are linked with reproductive organs, whereas long shoots are contributed to the rapid extension of branches and perform photosynthesis at high rates [3,4]. Although many heterophylly has been studied in angiosperm species [2,5], further research is needed on gymnosperms [4,6].

A notable gymnosperm with shoot-based heterophylly is *Gingko biloba* L. (Ginkgoaceae), which has two leaf types that differ in their morphology. The short-shoot varieties bear many leaves, which are undivided or slightly bilobed and appear in early spring from overwintering buds, while the leaves of long-shoot varieties are smaller and are deeply divided into two or more lobes [4,7]. Previous studies suggested that the morphological differences between sun and shade leaves (a form of heterophylly) might be mainly controlled through restricted hydraulic supply during cell expansion, which results in deeper lobes, higher vein density, and stomatal density in sun leaves [8,9]. Notably, short-shoot leaves in ginkgoes absorb water exclusively through mature stems, whereas long-shoot leaves rely on water provided by the xylem that is not yet fully developed [4]. In addition, Little et al. [10] found that the short shoots of *G. biloba* produce more radial tracheids per single ring than long shoots. Thus, the differences in developmental and morphological traits between long-shoot leaves and short-shoot leaves suggest that long-shoot leaves might have a greater hydraulic threshold, and consequently, the leaves developed from these two shoot types may differ in their carbon economy (e.g., gas exchange, specific leaf area, and leaf thickness) and hydrology.

The leaves are the bottleneck of the plant hydraulic system, and their venation network must be sufficient to provide enough water for a given stomatal pore area and gas exchange rate [11,12]. Previous studies have found strong positional variations in leaf anatomy, which proved that the structure could be heterogeneous even in a given leaf [12–15]. Leigh et al. [4] further confirmed this result by finding that the leaf vein density, stomatal density, and tracheid numbers in *G. biloba* were highly heterogeneous among positions. Unfortunately, many studies on the leaf anatomy of *G. biloba* still focus on a single region [16–18]. Notably, *G. biloba* has distinctive fan-shaped leaves with an open dichotomous venation pattern. By understanding the differences in the anatomical traits of fan-shaped leaves, we may be able to explore the adaptation mechanisms of these two leaf types when they are under abiotic stress.

Dioecy was found in almost 65% (8 of the 12 families) of contemporary gymnosperms [19], and dioecious plant species play an essential role in terrestrial ecosystems [20,21]. In nature, both structure and physiology could markedly differ between individual dioecious plants. According to a recent review of *Populus*, Melnikova et al. [22] concluded that the growth and photosynthesis of females were more susceptible to inhibition by drought stress than males. Olano et al. [23] found that the xylem anatomy traits of male *Juniperus thurifera* (L.) Spach, a dioecious conifer species, showed a more conservative strategy, while females sacrificed hydraulic safety for higher hydraulic conductivity. Such phenomena indicated sex-specific variation in the ability to resist environmental stress, and male individuals may perform better than female ones under abiotic or biotic stresses [24–26]. The morphological, proteomic, and/or physiological research of single-sex ginkgo responses to drought or salt stress has been undertaken [27,28]. However, the differences in adaptation to environmental conditions between sexes remain unclear.

Urban trees were often exposed to more severe dry heat stress due to the urban heat island (UHI) effect. Increased UHI will lead to increased evapotranspiration of trees; thus, drought stress in urban trees may be more severe in the future [29]. *Ginkgo biloba* has been widely planted in China for centuries and has become a popular roadside tree due to its high economic and landscape values and drought-resistance ability [18,30]. Thus, the aims of this study were to (1) determine the differences in hydraulic, anatomical, and economic traits between long- and short-shoot leaves, and (2) test the drought resistance of male and female ginkgoes. We hypothesized that (1) long-shoot leaves tend to have higher hydraulic efficiency due to their initial hydraulic constraints, and (2) male plants exhibit superior drought resistance than females due to their greater leaf hydraulic efficiency.

2. Material and Methods

2.1. Study Site and Plant Material

The study site (31°86′19″ N, 117°25′70″ E) was located at Anhui Agricultural University Campus, Hefei, Central Anhui Province, China. It has a humid subtropical monsoon climate with an average temperature of 15.7 °C, and an average rainfall of 1000 mm. From July to September 2021, eight mature trees (four of each sex) growing in an exposed area were selected for the study. The diameter at breast height ranged between 30.0 and 32.6 cm and showed no significant difference between the two sexes. We used a 6 m long averruncator to collect the fully extended branches (height about 3 m) facing south. The sampled tree individuals in this study were transplanted from a nursery in Hefei city around 2010. The scheme of experimental design and the climate of the study area are shown in Figures 1 and S1, respectively.

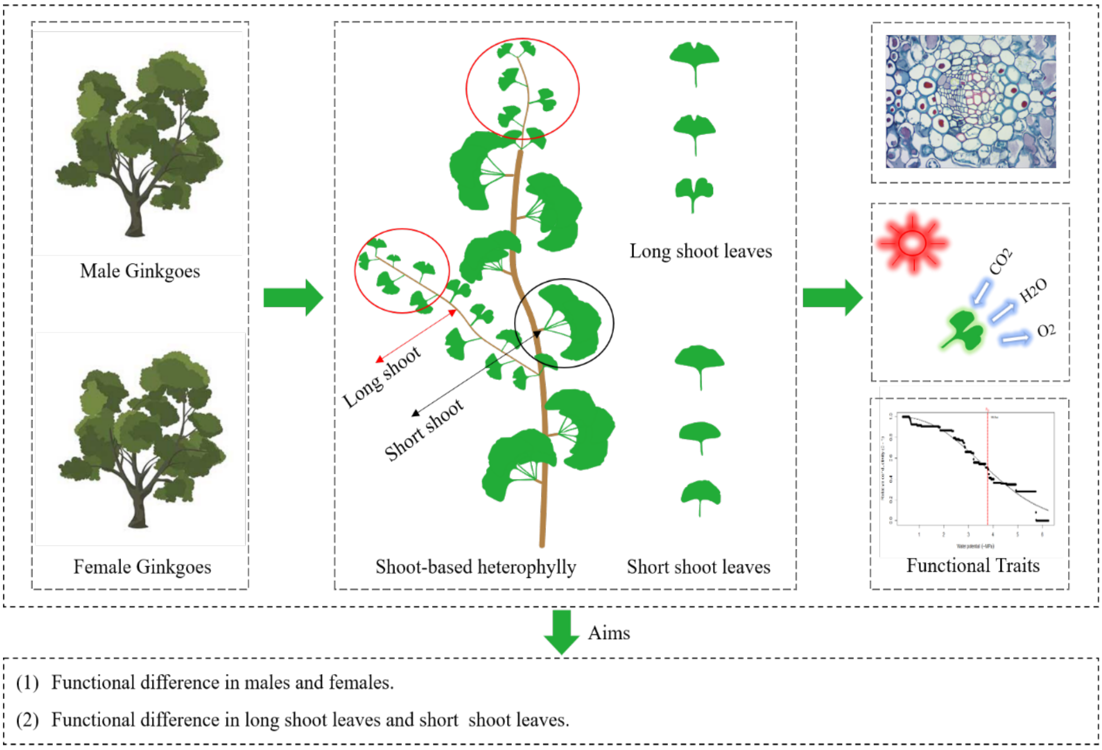

Figure 1. The scheme of experimental design.

2.2. Leaf Water Potential

The leaf water potentials were measured using two Scholander-type pressure chambers (PMS 1505D, PMS Instruments Co., Albany, OR, USA). On consecutive sunny days in July 2021, we collected three fully extended branches from each tree individual and six leaves (three leaves in each shoot type) from each branch. The measurements were performed between 05:30 and 07:00 for the predawn leaf water potential (Ψ_{pd}; MPa) and between 13:00 and 15:00 for the midday leaf water potential (Ψ_{md}; MPa).

2.3. Leaf Gas Exchange and Hydraulic Conductance

In August 2021, a total of 12 fully extended branches with expanded healthy leaves 2–3 m above the ground from 4 individuals per sex were collected. Three fresh and healthy leaves from each shoot type were randomly chosen for gas exchange measurements using a portable photosynthesis system (Li-6800, Li-Cor Inc., Lincoln, NE, USA) between 09:00 and 11:30 on sunny days. Depending on the weather condition of measurement, the chamber's CO_2 concentration was 400 μmol mol^{-1}, the light intensity was 800 μmol m^{-2} s^{-1}, the relative humidity was 60%, and the leaf temperature was 25 °C. During measurements, the branches were put into a large bucket full of water to avoid water loss. Leaf gas exchange parameters were monitored to ensure reaching a stable state before data were recorded. The instantaneous water use efficiency (WUE_{i}; mmol mol^{-1}) was calculated as the net photosynthesis rate (A; µmol m^{-2} s^{-1}) divided by the transpiration rate (Tr; mmol m^{-2} s^{-1}).

The leaf hydraulic conductance (K_{leaf}; mmol m^{-2} s^{-1} MPa^{-1}) was measured using the gas exchange method [31]. Briefly, a total of nine leaves in each shoot type were measured from each tree individual. The leaves were wrapped in black plastic bags and aluminum foil on the night before the measurement, and thus, the wrapped leaf water potential (Ψ_{w}; MPa) was assumed to be equivalent to the stem water potential. On the next day, the adjacent leaves of the wrapped leaves were selected for gas exchange measurements. When the leaf gas exchange was finished, the water potentials of the leaves used for gas exchange (Ψ_{gas}; MPa) and Ψ_{w} were immediately measured using two Scholander-type pressure chambers, respectively.

$$K_{\mathrm{leaf}} = \frac{Tr}{\Psi_{\mathrm{w}} - \Psi_{\mathrm{gas}}}$$

2.4. Leaf Vulnerability Curves

Before the leaf vulnerability curve was plotted, considering the fact that long-shoot leaves are relatively less common than short-shoot leaves, we collected three branches (about 60 cm long) from each sex and immediately recut them (about 5 cm long) underwater. In the lab, the cut branches were covered overnight with a black plastic bag until the leaf water potential was >-0.10 MPa.

The branches were removed from the water and covered with black plastic bags so that the leaf water potential of the whole branch was balanced between the upper (long shoot) and the lower (short shoot) values. The branches were dehydrated naturally for 72 h, and the leaf water potentials for long shoots and short shoots were measured every 1 h to ensure that there was no significant difference between the two leaf types. Therefore, unless otherwise stated, when measuring the values for the vulnerability curve of long-shoot leaves, the water potential of short-shoot leaves was used instead when the number of long-shoot leaves was insufficient.

A total of 48 leaves (24 leaves of each shoot type) from 8 individuals were selected to plot the leaf vulnerability curve, which was evaluated using the optical technique [32]. When the leaf water potential of the cut branches was close to 0 MPa, we began to dehydrate them for 72 h in a black plastic bag while imaging the healthy and fully expanded leaf. We cleaned the leaf surface with deionized water and clamped the leaf firmly in the middle with two transparent glass plates (the leaf was still attached to the shoot). This limited the leaf movement and shrinking during dehydration. The leaf was imaged every 5 min using a custom-built imaging clamp (http://www.opensourceov.org, accessed on 3 February 2023). The imaged area near the center of the leaf was at least 500 mm^2. The leaf water potential was measured every 1 to 2 h via a Scholander-type pressure chamber. Image sequences were analyzed as described in http://www.opensourceov.org/process/ (accessed on 3 February 2023) to measure the embolized area for each image and generate the vulnerability curve, expressed as a percentage of embolized pixels vs. leaf water potential; also, the leaf water potential of 50% loss in K_{leaf} (leaf $P50$) was calculated using the R package ‘fitplc’ [33].

2.5. Leaf Morphology and Anatomy

After the K_{leaf} measurement, the leaves were collected and cleaned with deionized and then scanned using a scanner. Leaf area and perimeter were measured using ImageJ (National Institute of Health, Bethesda, MD, USA). After leaf-area measurement, the leaf samples were oven-dried at 60 °C for 72 h to estimate their dry mass. The leaf dissection index (LDI) and specific leaf area (SLA; cm^2 g^{-1}) were calculated as follows:

$$LDI = \text{Leaf perimeter} / \sqrt[2]{\text{Leaf area}}$$

$$SLA = \text{Leaf dry mass}/\text{Leaf area}$$

A total of 80 leaves (40 leaves of each shoot type) from 4 individuals per sex were selected to measure the vein density (*VD*; mm mm^{-2}) at 3 locations on each leaf (at the apex, middle, and base). The pre-experiment found that the leaf veins of *G. biloba* could be clearly observed under the microscope even if the leaves were not treated. Thus, the leaf samples were saved in FAA (37% formalin + 70% alcohol + 100% acetic acid) fixative solution to consume most of the leaf pigments, making it possible that all the vein networks could be seen without further clearing or staining. The samples were then imaged using a light microscope, and the total vein length was analyzed using ImageJ. The *VD* was defined as the total vein length per unit of leaf area. The stomatal density (*SD*; no mm^{-2}) of the abaxial leaf surface was determined for the apex, middle, and base with clear nail varnish and measured under 10× magnification using a light microscope (DM2500, Leica Microsystems Ltd., Wetzlar, Germany). Leaf thickness and the parameters of tracheid measurements were performed on 18 leaves (9 leaves of each shoot type) in each tree. Leaf cross-sections at the three locations (at the apex, middle, and base) were obtained via a rotary microtome (RM2255, Leica Microsystems Ltd., Germany). The leaf thickness and the number of every tracheid lumen in a vein cross-section were photographed with a light microscope (DM2500, Leica Microsystems Ltd., Germany) at 10× magnification and 40× magnification, respectively, and then measured using ImageJ. At the same time, the tracheid was assumed to be oval. Thus, the average tracheid diameter was calculated by multiplying the length of the two axes together and taking the square root of the product.

2.6. Statistical Analysis

Two-way nested ANOVA was used to estimate the effects of leaf type and position, as well as of leaf type and sex. An independent-sample *t*-test was conducted to identify the anatomical differences between the two sexes. Multiple comparisons were performed using Ducan's test to evaluate the differences in variables between leaf types using SPSS 20.0 (SPSS, Inc., Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant. Leaf vulnerability curve fitting, principal component analyses (PCA), and correlation analysis were conducted using R 4.1.2 (The R Foundation for Statistical Computing, Austria).

3. Results

3.1. Leaf Morphoanatomical Traits of Long Shoot and Short Shoot

Long-shoot leaves exhibited remarkably lower specific leaf area (*SLA*) and greater leaf dissection index (*LDI*) than short-shoot leaves in both sex individuals (Figure 2). Meanwhile, males showed significantly higher *LDI* and lower *SLA* than females (Figure 2). The nested ANOVA found that the leaf thickness and average tracheid number were significantly affected by position ($p < 0.001$) and leaf type (Figure 3), and these two traits in long-shoot leaves were almost higher than in short-shoot leaves in both sex individuals. Meanwhile, the tracheid of long-shoot leaves' basal region was narrower and relatively abundant than that of short-shoot leaves in male and female *G. biloba* (Figure 3c–f). However, the leaf tracheid diameter, stomatal density (*SD*), and vein density (*VD*) in male *G. biloba* were unaffected by leaf type, while the *SD* and *VD* in female *G. biloba* were affected by leaf type (Figures 3e,f and 4). The leaf thickness and *VD* were decreased from the base to the apex in both two leaf types (Figures 3a,b and 4c,d). Regardless of the leaf type and position effects, males possessed a lower average tracheid number but higher *VD* and thicker leaves than females (Table S2).

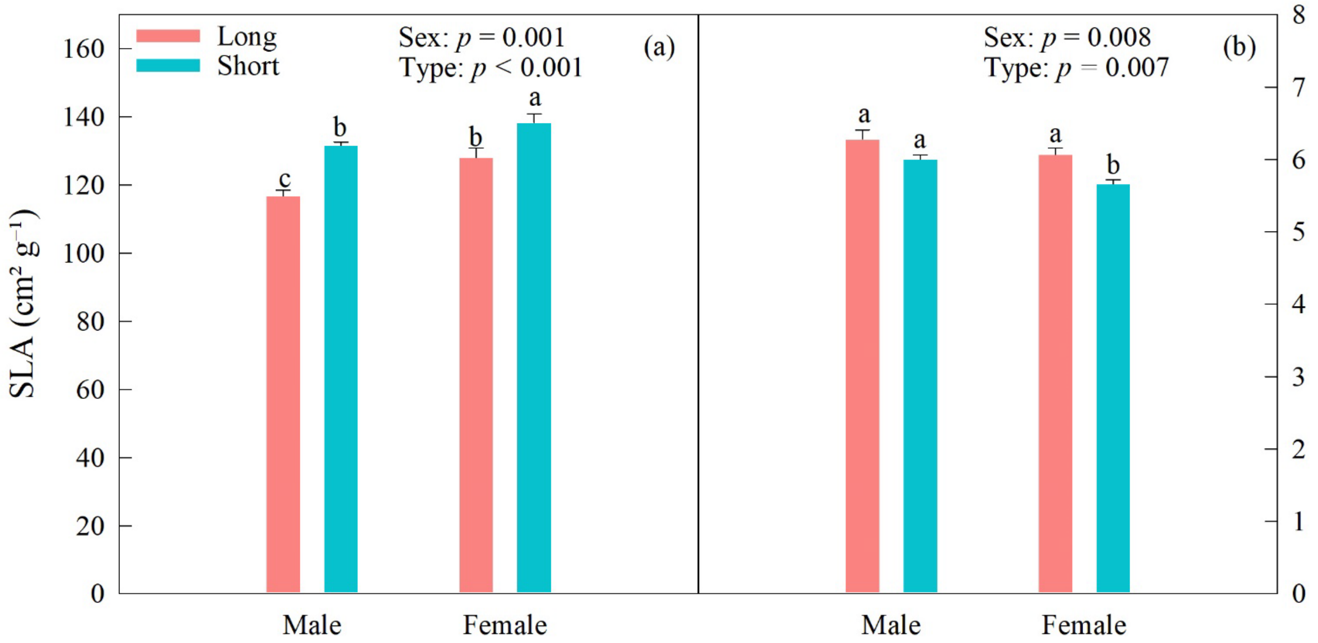

Figure 2. Specific leaf area (**a**) and leaf dissection index (**b**) differences between long- and short-shoot leaves in males and females *Ginkgo biloba*. Values are mean ± SE (n = 4). Lowercase letters indicate significant differences between long- and short-shoot leaves of males and females at the $p < 0.05$ level.

3.2. Leaf Gas Exchange of Long Shoot and Short Shoot

Long-shoot leaves exhibited significantly higher net photosynthesis rate and transpiration rate (*Tr*) than short-shoot leaves in both sexes (Figure 5a,c). The *Tr* of male long-shoot leaves was significantly higher than that of short-shoot leaves (p = 0.02). The net photosynthesis rate, *Tr*, and stomatal conductance (g_s) in both leaf types had similar trends. Moreover, the WUE_i of male long-shoot leaves and g_s of female long-shoot leaves were significantly higher than those of short-shoot leaves (Figure 5b,d). Without considering leaf-type effects, males exhibited markedly higher net photosynthesis rate, *Tr*, g_s, and a higher instantaneous water use efficiency (WUE_i) than females.

3.3. Leaf Hydraulics of Long Shoot and Short Shoot

In females, the predawn leaf water potential (Ψ_{pd}) of short-shoot leaves was more negative than that of long-shoot leaves (Figure 6a). Additionally, a significant difference in the midday leaf water potential (Ψ_{md}) between the two leaf types in male and female *G. biloba* was observed, with the water potential of short-shoot leaves being more negative (Figure 6b). Male long-shoot leaves exhibited remarkably greater leaf hydraulic conductance (K_{leaf}) and the water potential of 50% loss in leaf hydraulic conductance (leaf *P50*) compared with short-shoot leaves, whereas female long-shoot leaves displayed slightly higher K_{leaf} (Figure 6c,d). No significant difference was observed in leaf *P50* between males and females without considering the effects of leaf types. Females had a more negative Ψ_{pd}, while males had a more negative Ψ_{md} and higher K_{leaf} (Figure 6).

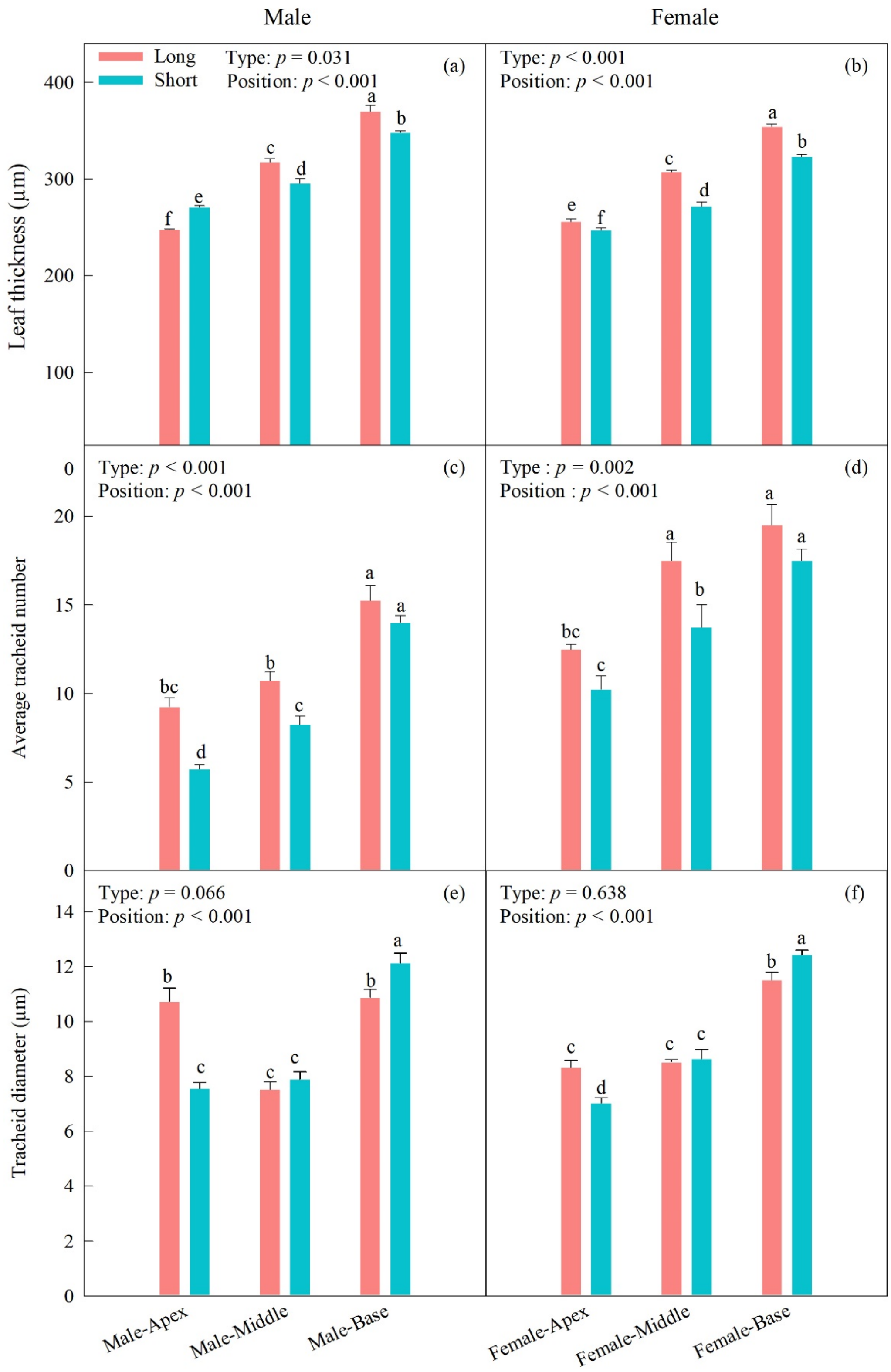

Figure 3. Leaf thickness (**a**,**b**), tracheid number (**c**,**d**), and tracheid diameter (**e**,**f**) variations at different positions between long- and short-shoot leaves in *Ginkgo biloba* males (left panels) and females (right panels). Values are mean ± SE (n = 4). Lowercase letters in each sex indicate significant differences between long- and short-shoot leaves at the $p < 0.05$ level.

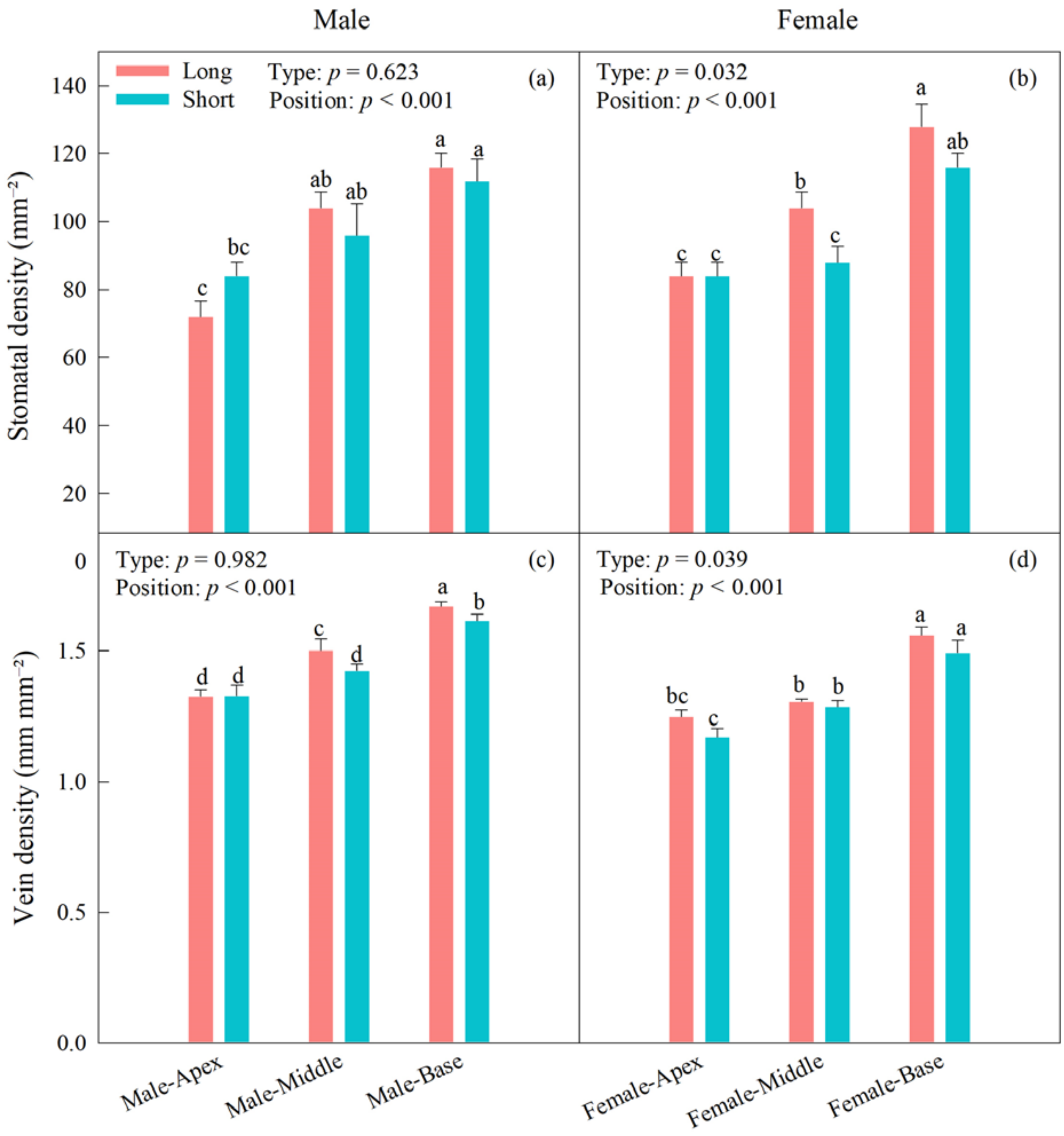

Figure 4. Leaf stomatal density (**a**,**b**) and vein density (**c**,**d**) variations at different locations on the leaf between long- and short-shoot leaves in *Ginkgo biloba* males (left panels) and females (right panels). Values are mean ± SE (n = 4). Lowercase letters in each sex indicate significant differences between long- and short-shoot leaves at the $p < 0.05$ level.

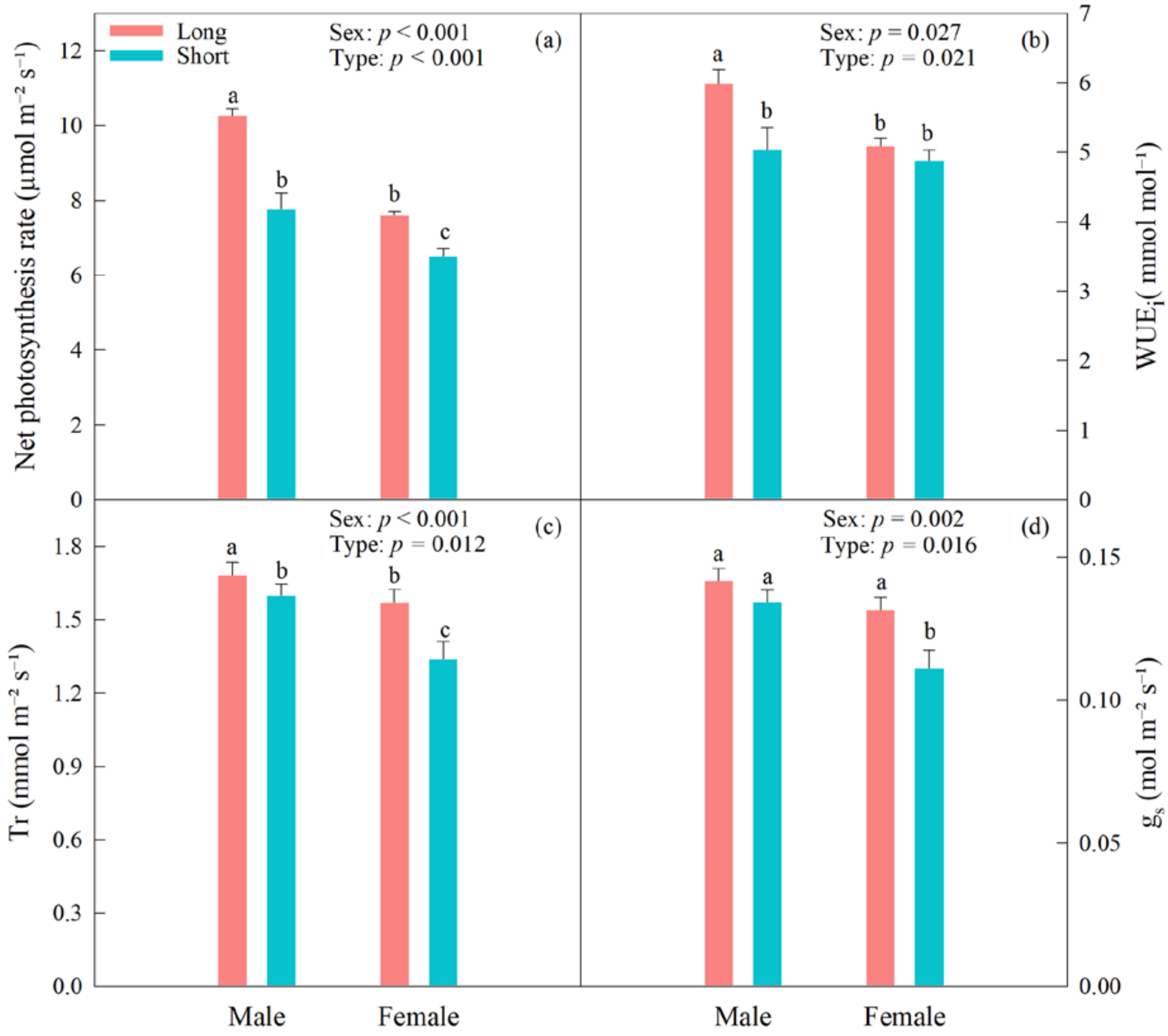

Figure 5. Leaf net photosynthesis rate (**a**), instantaneous water use efficiency (**b**), transpiration rate (**c**), and stomatal conductance (**d**) differences between long- and short-shoot leaves in male and female *Ginkgo biloba*. Values are mean ± SE (n = 4). Lowercase letters indicate significant differences between long- and short-shoot leaves of males and females at the $p < 0.05$ level.

3.4. Trait Coordination

We found that *VD* was greatly correlated with the leaf thickness and negatively correlated with *SLA* and tracheid number. In addition, the leaf net photosynthesis rate was positively correlated with *LDI*, leaf thickness, *VD*, *Tr*, g_s, and WUE_i, while it was negatively correlated with *SLA*. K_{leaf} was strongly related to Ψ_{pd}, the net photosynthesis rate, *Tr*, g_s, *LDI*, *SLA*, *VD*, and leaf thickness, while it was independent of tracheid number and the traits associated with leaf *P50* (Figure 7).

Overall, leaf types and sexes significantly affected the morphoanatomical and physiological responses for *Gingko biloba* (Figure S2). *LDI*, the net photosynthesis rate, g_s, WUE_i, *VD*, K_{leaf}, and Ψ_{pd} were located at the positive end, whereas *SLA* was located at the negative end on the PC1 axis. The PC2 axis was mostly influenced by Ψ_{md}, tracheid number (*TN*), *SD*, and the leaf drought tolerance trait (leaf *P50*) on the positive side.

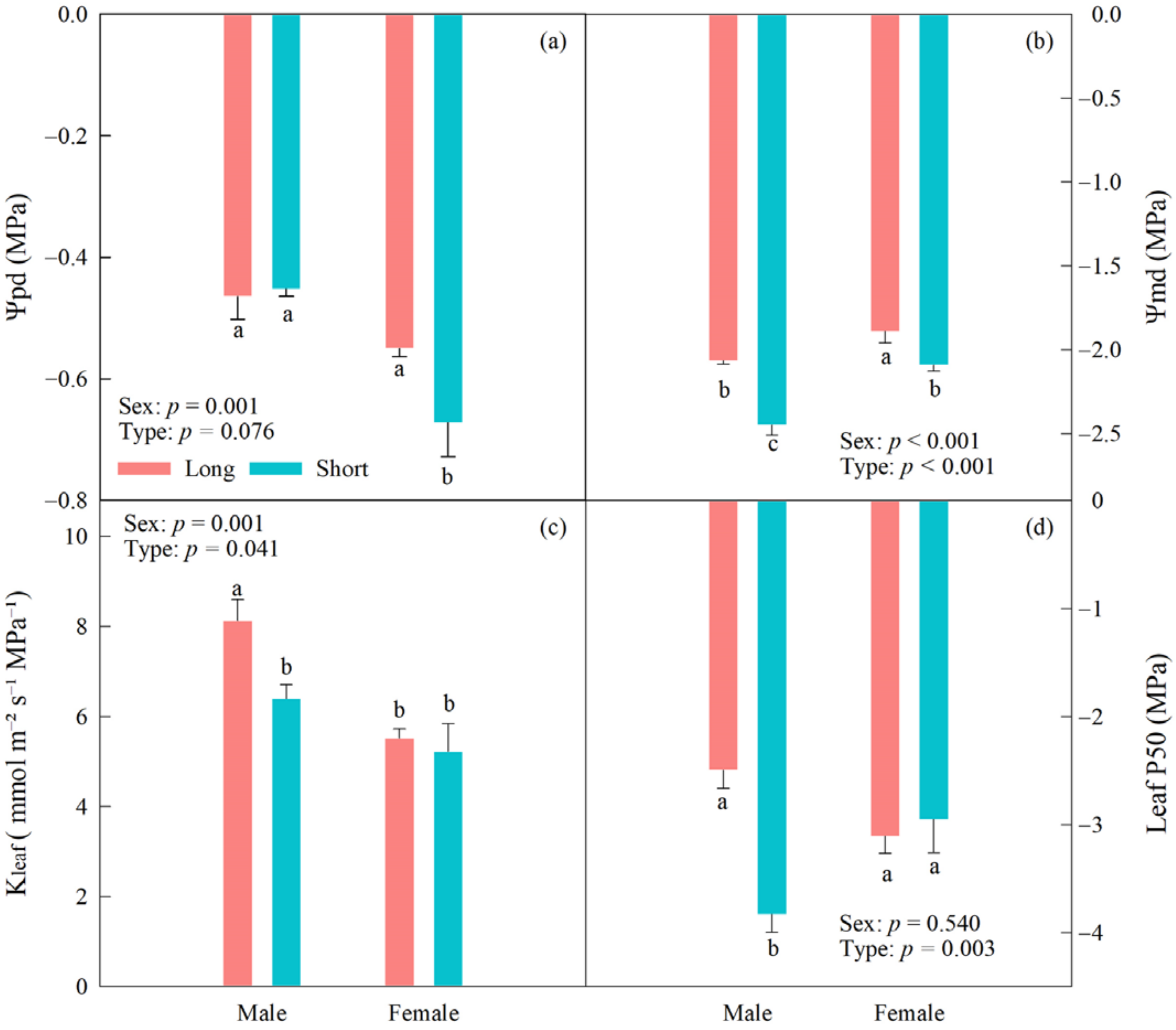

Figure 6. Predawn leaf water potential (**a**), midday leaf water potential (**b**), leaf hydraulic conductance (**c**), and water potential of 50% loss in leaf hydraulic conductance (**d**) differences between long- and short-shoot leaves in male and female *Ginkgo biloba*. Values are mean ± SE (n = 4). Lowercase letters indicate significant differences between long- and short-shoot leaves of males and females at the $p < 0.05$ level.

	A	gs	Kleaf	LDI	LT	P50	SD	SLA	TD	TN	Tr	VD	WUEi	Ψmd	Ψpd
Ψpd	0.58	0.61	0.6	0.62	0.76	−0.22	0.02	−0.64	−0.17	−0.46	0.58	0.77	0.41	−0.43	1
Ψmd	0.06	−0.02	−0.11	0.08	−0.14	0.5	0.25	−0.1	0.16	0.82	−0.01	−0.22	0.09	1	−0.43
WUEi	0.85	0.23	0.54	0.43	0.47	0.57	0.17	−0.73	−0.06	−0.17	0.4	0.66	1	0.09	0.41
VD	0.8	0.61	0.66	0.6	0.84	0.03	−0.18	−0.71	−0.03	−0.53	0.71	1	0.66	−0.22	0.77
Tr	0.82	0.92	0.74	0.77	0.81	0.01	−0.05	−0.7	−0.24	−0.34	1	0.71	0.4	−0.01	0.58
TN	−0.29	−0.27	−0.45	−0.17	−0.4	0.28	0.41	0.11	0.14	1	−0.34	−0.53	−0.17	0.82	−0.46
TD	−0.16	−0.29	0.11	−0.34	−0.27	−0.12	−0.19	−0.04	1	0.14	−0.24	−0.03	−0.06	0.16	−0.17
SLA	−0.86	−0.62	−0.81	−0.71	−0.71	−0.35	−0.1	1	−0.04	0.11	−0.7	−0.71	−0.73	−0.1	−0.64
SD	0.07	0.07	−0.16	−0.02	0.06	0.17	1	−0.1	−0.19	0.41	−0.05	−0.18	0.17	0.25	0.02
P50	0.38	−0.06	0.17	0.1	−0.01	1	0.17	−0.35	−0.12	0.28	0.01	0.03	0.57	0.5	−0.22
LT	0.74	0.84	0.59	0.8	1	−0.01	0.06	−0.71	−0.27	−0.4	0.81	0.84	0.47	−0.14	0.76
LDI	0.7	0.77	0.65	1	0.8	0.1	−0.02	−0.71	−0.34	−0.17	0.77	0.6	0.43	0.08	0.62
Kleaf	0.78	0.64	1	0.65	0.59	0.17	−0.16	−0.81	0.11	−0.45	0.74	0.66	0.54	−0.11	0.6
gs	0.67	1	0.64	0.77	0.84	−0.06	0.07	−0.62	−0.29	−0.27	0.92	0.61	0.23	−0.02	0.61
A	1	0.67	0.78	0.7	0.74	0.38	0.07	−0.86	−0.16	−0.29	0.82	0.8	0.85	0.06	0.58

Figure 7. Correlation analysis among leaf morphology and hydraulic and gas exchange traits. *SD*: stomatal density; *LT*: leaf thickness; *VD*: vein density; *WUE*$_i$: instantaneous water use efficiency; g_s: stomatal conductance; *A*: net photosynthesis rate; *Tr*: transpiration rate; *SLA*: specific leaf area; *LDI*: leaf dissection index; K_{leaf}: leaf hydraulic conductance; Ψ_{md}: midday leaf water potential; Ψ_{pd}: predawn leaf water potential; *P50*: water potential of 50% loss in leaf hydraulic conductance; *TN*: average tracheid number; *TD*: average tracheid diameter.

4. Discussion

4.1. Variation in Leaf Functional Traits between Long- and Short-Shoot Leaves

In the present study, we found a strong heterogeneity in leaf functional traits between the two leaf types in *Ginkgo biloba*. We found that the net photosynthesis rate, vein density (*VD*), and leaf hydraulic conductance (K_{leaf}) were higher in long-shoot leaves than in short-shoot leaves. Similar to the long shoots of other species, which extended their canopy by taking advantage of elevated radiation conditions, long-shoot leaves might achieve rapid growth through higher gas exchange rates and an adequate leaf water supply [4]. The positive relationships among *VD*, K_{leaf}, and net photosynthesis rate further support the findings of Leigh et al. [4].

When improving the water conductivity or maintaining a high photosynthesis rate, air bubbles may invade the vascular system and subsequently increase the risk of cavitation [4,34]. As an important hydraulic trait, the water potential of 50% loss in leaf hydraulic conductance (leaf *P50*) was closely associated with plant drought tolerance [35,36]. The long-shoot varieties showed higher leaf *P50* than their short-shoot counterpart, indicating a lower resistance to embolism (lower hydraulic safety). However, long-shoot leaves might reduce the risk of embolism through coordination between economical traits and other hydraulic traits. Specifically, long-shoot leaves possessed higher *VD* and leaf thickness and lower specific leaf area (*SLA*) than short-shoot leaves under the same external conditions, and these three traits were tightly related. The higher *VD* implied that when leaves were at risk of embolism, long-shoot leaves could transport water through non-embolized veins, achieving a more flexible water transport strategy [37]. Moreover, it has been suggested that selection stress in drought conditions favors plants with low *SLA* due to their higher leaf dry matter content [38,39], and a previous study reported that *G. biloba* could enhance the water retention ability by keeping higher leaf thickness when facing drought and/or heat stress [40]. Hence, the coordination between leaf functional traits might help long-shoot leaves acclimate to future drought conditions.

In addition to the above discussion, the leaf thickness, tracheid number, stomatal density (*SD*), and *VD* were higher in long-shoot leaves than in short-shoot leaves, and these traits in these two leaf types decreased from basal to apical regions (Figures 3 and 4), indicating that there was a strong variation in leaf structural traits. The trade-off between the tracheid size and number (more abundant and narrower tracheids) of long-shoot leaves might also represent an adaptation for increased safety during periods of high evaporative demand in summer. Moreover, we also found that long-shoot leaves could achieve the trade-off between hydraulic efficiency (higher K_{leaf}) and hydraulic safety.

4.2. Variation in Leaf Functional Traits between Males and Females

Inconsistent with our initial hypothesis, our analysis demonstrated that the difference in leaf *P50* between males and females was not significant (Figure 6). The low variation exhibited by leaf *P50* between sexes was in agreement with the results reported for gymnosperms and reinforced the conservative nature of this trait [23,41]. Another reasonable explanation was that the differences in sexes might be obscured by the differences in leaves within single-sex species. Thus, leaf *P50* might not be the main factor affecting the difference in drought-resistance capacity between males and females under natural conditions. Interestingly, the Ψ_{pd} and *Tr* of female short-shoot leaves were lower than those of the other leaves. Such phenomena might be attributed to the finding that the water transport efficiency of female short-shoot leaves was lower than those of other leaves. Compared with male long- and short-shoot leaves, the female short-shoot leaves also showed lower leaf thickness, *VD*, WUE_{i}, and K_{leaf}.

We found that males had higher *VD*, K_{leaf}, and leaf thickness than females. The correlation analysis emphasized the fact that the coordination between these anatomical and hydraulic traits might play an important role in water transport (Figure 7). The higher *VD* might be a major factor in increasing K_{leaf} by improving the water exchange area between the xylem and the surrounding mesophyll cells and reducing the distance of water passing through the mesophyll space [42,43]. Furthermore, we observed that males sustained higher gas exchange rates in summer by maintaining higher *VD*, K_{leaf}, and instantaneous water use efficiency (WUE_{i}). A previous study suggested that the change in K_{leaf} is usually linked with stomatal closure and, accordingly, affects the photosynthesis of plants [44], because CO_2 and water exchange between leaves and air share a common route through stomatal pores. When the ability of CO_2 assimilation is reduced, this, in turn, limits the development of leaf veins, indicating that there is a trade-off between the investment and return of leaf structure [34]. Hence, it was not surprising that males tended to show greater net photosynthesis rate, K_{leaf}, and *VD* than females. These results indicate that males might achieve an ecological advantage in summer due to their higher gas exchange rates and hydraulic efficiency. Many previous studies have demonstrated sex-specific differences in resistance to environmental stress, nutrient uptake, and utilization strategies, and consequently, male individuals perform better than female individuals [20,45,46]. We suspected that the sex-specific differences in the hydraulic traits and resource utilization of ginkgoes might result in a situation where females are more prone than males to future urban drought conditions, thus ultimately leading to a population decline. Our study revealed that the net photosynthesis rate differed between males and females. Similar results were also reported by Correia and Barradas [47], who found that the net photosynthesis rate and stomatal conductance (g_s) of *Pistacia lentiscus* L. (Anacardiaceae) under natural environments in summer were higher in males than that in females. However, the pattern of leaf photosynthesis in dioecious trees might be complex. Jin et al. [48] reported that the leaf net photosynthesis of female Ginkgoes was higher than that of males in April, whereas Shi et al. [49] found that the leaf net photosynthesis of male ginkgoes was higher in August. Such phenomena suggest that sex-related differences in photosynthetic patterns are probably related to the measurement time due to the different reproductive costs of dioecious trees. Therefore, the dynamic changes in leaf gas exchange between male and female ginkgoes, and the hydraulic and economic traits of other dioecious trees need further research.

5. Conclusions

Our results showed that long-shoot leaves were thicker, with lower *SLA* and higher average tracheid number, Ψ_{md}, net photosynthesis rate, and *Tr* than short-shoot leaves in male and female *Ginkgo biloba*. In addition, due to its unique fan-shaped leaves, there was also considerable heterogeneity in the anatomical traits of different positions of the leaves. The two leaf types of males could achieve the trade-off between hydraulic efficiency and safety in that lower K_{leaf} was accompanied by more negative leaf *P50*. Although no significant difference in leaf *P50* was observed, males may perform better in summer via higher leaf hydraulic efficiency and gas exchange rates than females. We found discrepancies in hydraulic and carbon economic traits between the two leaf types in males and females *G. biloba*. It should be noted that this study had a small sample size and was conducted on only one site. Future studies are necessary to explore the hydraulic and economic traits of dioecious plants over a large-scale environmental gradient.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14030535/s1. Figure S1: The climate conditions of study area in 2020 (a) and 2021 (b), respectively; Figure S2: Principal component analyses on long shoot and short shoot leaves physiological and anatomical traits of males and females *Ginkgo biloba*. *SD*: stomatal density; *VD*: vein density; g_s: stomatal conductance; *A*: net photosynthesis rate; WUE_i: instantaneous water use efficiency; *SLA*: specific leaf area; *LDI*: leaf dissection index; K_{leaf}: leaf hydraulic conductance; Ψ_{md}: midday leaf water potential; Ψ_{pd}: predawn leaf water potential; *P50*: water potential of 50% loss in leaf hydraulic conductance; *TN*: average tracheid number; *TD*: average tracheid diameter; Table S1: An overview table of all measurements including the name, the unit, total number of measured leaves from each tree, and the number of final replicates; Table S2: Results from independent-samples t-test for the difference between males and females *Ginkgo biloba* on leaf anatomical traits; Table S3: Information about the statistical output from two-way nested ANOVA.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Datasets are available from the corresponding author upon reasonable request.

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Abbreviations

SD	stomatal density
LT	leaf thickness
VD	vein density
WUE_i	instantaneous water use efficiency
g_s	stomatal conductance
A	net photosynthesis rate
Tr	transpiration rate
SLA	specific leaf area
LDI	leaf dissection index
K_{leaf}	leaf hydraulic conductance
Ψ_{md}	midday leaf water potential
Ψ_{pd}	predawn leaf water potential
$P50$	water potential of 50% loss in leaf hydraulic conductance
TN	average tracheid number
TD	average tracheid diameter

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