forests

Article

Effects of Drought Stress and Ca Supply on the Biomass Allocation Strategies of Poplar and Mulberry

Tengzi Zhang [1], Guijun Dong [2], Yaguang Tian [3], Yongbin Zhou [1], Yanan Li [1], Songzhu Zhang [1,4,*] and Hui Li [1,4]

[1] College of Forestry, Shenyang Agricultural University, Shenyang 110866, China
[2] Forestry and Grassland Protection Center of Hinggan League, Ulanhot 137400, China
[3] Liaoning Non-Ferrous Geological Exploration and Research Institute Co., Ltd., Shenyang 110013, China
[4] Research Station of Liaohe-River Plain Forest Ecosystem CFERN, College of Forestry, Shenyang Agricultural University, Shenyang 110866, China
* Correspondence: zhangsz@syau.edu.cn

Abstract: In order to investigate the effect of Ca on the biomass allocation strategies of tree species with different growth rates under drought conditions, we treated poplar (*Populus canadensis cv*) cuttings and mulberry (*Morus alba*) seedlings with two soil moisture levels (40 ± 5% and 80 ± 5% maximum water holding capacity) and two soil Ca levels (0 and 200 mg·kg^{-1} Ca^{2+}) in a greenhouse experiment, and then measured the Ca uptake, growth, gas exchange parameters, biomass allocation, and leaf traits. Drought induced a reduction in biomass accumulation of poplar cuttings and mulberry seedlings, and the cuttings and seedlings exhibited different biomass allocation patterns in response to drought stress. Under Ca0 treatment, poplar cuttings allocated more biomass to leaves and less biomass to stems under drought conditions, leading to an increased leaf/stem (L/St) ratio and higher SLA than under moist conditions in order to maintain higher *Pn*, and had enhanced WUE to cope with drought stress. Under the same treatment, mulberry seedlings allocated more biomass to roots and less biomass to stems, leading to an increased root/shoot (R/S) ratio and lower SLA, to improve drought resistance. Ca200 treatment decreased the growth of poplar cuttings and mulberry seedlings, whereas it enhanced the WUE, root growth, and R/S ratio of poplar cuttings and the WUE of mulberry seedlings, and alleviated drought stress in both species.

Keywords: semiarid area; photosynthesis; water use efficiency; biomass allocation; specific leaf area

Citation: Zhang, T.; Dong, G.; Tian, Y.; Zhou, Y.; Li, Y.; Zhang, S.; Li, H. Effects of Drought Stress and Ca Supply on the Biomass Allocation Strategies of Poplar and Mulberry. *Forests* **2023**, *14*, 505. https://doi.org/10.3390/f14030505

Academic Editor: Roberto Tognetti

Received: 14 February 2023
Revised: 1 March 2023
Accepted: 2 March 2023
Published: 3 March 2023

1. Introduction

When trees are exposed to drought stress, new biomass is preferentially allocated to the root system to improve adaptability by increasing the root/shoot (R/S) ratio to decrease water consumption [1–5]. Plants can also activate multiple physiological mechanisms to protect tissues from cellular damage through stomatal closure and protective solutes and proteins [4]. The pattern of biomass allocation from leaf to stem (or root) is irreversible and will affect the tree's assimilation capacity, growth rate, and net primary production, making this an important issue in ecology [6]. Soil nutrients can interactively affect biomass allocation under drought conditions. Nitrogen and phosphorus are the main limiting factors in forest ecosystems, and previous studies have focused on their effects on biomass allocation under drought conditions [7–11], but there are no uniform conclusions on the effects of nutrients on biomass allocation under drought. Under drought conditions, plants tend to take up more nitrogen and phosphorus by distributing biomass to the roots to promote growth under nitrogen treatment [8,10]. However, the nitrogen supply will increase shoot growth, leading to an increase in the plant's transpiration demand [10], thus aggravating the damage and even causing high seedling mortality under drought conditions [12]. It is clear that nutrient application does not always play a positive role in mitigating the negative effects of drought on plant growth by altering biomass allocation patterns.

Calcium (Ca) is another important mineral nutrient that plays a vital physiological role under drought conditions [13,14] by maintaining the stability of cell walls and cell membranes, as well as regulating the process of drought resistance [15–19]. Soil Ca is one of the most variable environmental factors in arid and semiarid regions [20–23], where both low- and high-Ca soil are widely distributed. Plants deficient in Ca are more susceptible to poor growth [18], while excessive Ca in the soil affects the physical, chemical, and biological properties of the soil, and then affects the water use efficiency and photosynthetic characteristics of plants [15]. Ca and water are tightly coupled in plants. Considering that drought conditions and strong heterogeneity of soil Ca are the norm in arid and semi-arid areas of northwestern Liaoning Province, it is important to understand the effect of soil Ca combined with soil moisture on the physiological performance and biomass allocation patterns of plants in the area.

Poplar (*Populus canadensis cv*) and mulberry (*Morus alba*) are cultivated tree species in semi-arid areas of China. Poplars are often selected as plantation trees because of their fast growth, high adaptability [24,25], and resistance to drought [26–28]. Mulberry also shows good adaptability to drought stress [29–31] and environmental friendliness with economic and ecological value [32]. Recently, more studies on the effects of drought on poplar and mulberry have been carried out [33,34]. However, the combined impact of soil moisture and soil Ca concentration on the growth and biomass allocation patterns of native and hybrid fast-growing tree species (mulberry and poplar, respectively) are still poorly understood.

We conducted a greenhouse experiment with two levels of Ca and two levels of soil moisture to investigate the impact of Ca on the biomass allocation patterns of poplar cuttings and mulberry seedlings under drought conditions, focusing on the morphological and physiological performance and the active allocation of biomass among the organs of the two species. We hypothesized the following: (1) Drought alters the physiological performance and allocation patterns of biomass. (2) Poplar cuttings and mulberry seedlings show different allocation strategies in respond to drought stress. (3) Exogenous Ca improves the ability of poplar cuttings and mulberry seedlings to cope with drought stress.

2. Materials and Methods

2.1. Growth Conditions and Experimental Materials

The experimental greenhouse is located at Shenyang Agriculture University, Shenyang, China (41°48′11.75″ N, 123°25′31.18″ E). For the experiment, the ambient temperature was 25 ± 2 °C, relative humidity was 60%, and ambient photon flux density was 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

The soil was selected from a *Pinus sylvestris* forest in Changtu. The soil organic carbon content and water extractable calcium of the soil samples were 20.23 $g \cdot kg^{-1}$ and 50 $mg \cdot kg^{-1}$, respectively. *Zhongliao 1* poplar and *aerxiang* mulberry, which are widely planted in the northwest of Liaoning, were selected as the experimental materials. Poplar cuttings were provided by the Liaoning Poplar Research Institute and mulberry seeds were provided by the forest experiment station of Shenyang Agriculture University.

In early April 2020, the poplar cuttings were put into a pot containing 5.0 kg soil (bottom diameter: 18.3 cm; outer diameter: 22.5 cm; height: 21.5 cm), and each pot was divided into 3 cuttings. Mulberry seeds were sterilized with 1% sodium hypochlorite for 15 min, then washed thoroughly with distilled water before being germinated in moist gauze at 20 °C in the dark. When radicles began to appear, five seeds were sown in pots with 5.0 kg soil. All poplar cuttings and mulberry seedlings were grown in the soil for one month, then superfluous seedlings were removed, leaving only one uniform seedling in each pot. Deionized water was used to maintain the moisture level to prevent the introduction of exogenous Ca from tap water.

2.2. Experimental Design

The experimental layout was completely randomized and consisted of combined water and calcium treatments with six replications for each treatment. There were two independent variables in the experiment: the first one was water control with two levels, the moist condition (maximum water holding capacity of 80 ± 5%) and the drought condition (maximum water holding capacity of 40 ± 5%); and the second one was calcium treatment with two Ca levels, Ca0 (0 $mg \cdot kg^{-1}$ Ca^{2+}) and Ca200 (200 $mg \cdot kg^{-1}$ Ca^{2+}).

When the height of poplar cuttings was about 15 cm and that of mulberry seedlings was about 8 cm (20 May 2020), calcium application and water control treatment were started. Ca treatment was applied to the soil in the form of $CaCl_2$ solution. The solution was divided into several equal parts, and the soil was irrigated to ensure a uniform distribution of $CaCl_2$. Moisture control began after the soil water content fell to the target amount (maximum water holding capacity of 40 ± 5%), and all plants were watered with varying amounts of water at 16:00 to 18:00 every day. All water added during the test was deionized water.

2.3. Sample Collection

The seedlings were harvested on 30 August 2020, and the biomass was separated into leaves, stems, and roots. After harvesting, poplar cuttings and mulberry seedlings were washed with deionized water. After scanning all the leaves, the leaves, stems, and roots of seedlings were put into an oven at 105 °C for 0.5 h and dried to constant weight at 80 °C for determination of dry weight. The plant samples were subsequently milled and passed through a 0.15 mm (100 mesh) sieve.

Soil remaining after the roots were collected was evenly mixed and air-dried. Half of the soil was passed through a 0.85 mm (20 mesh) sieve for determination of soil water extractable Ca.

2.4. Measurements

2.4.1. Biomass

The dry weight of roots, stems, leaves, and total plant biomass was determined with an analytical balance (BSA124S, Sartorius, Göttingen, Germany). The biomass allocation among organs was calculated by dividing the biomass of a specific organ by the total plant biomass [10]. The root/shoot (R/S) ratio was calculated from the biomass of the aboveground and belowground parts.

2.4.2. Leaf Characteristics

Leaves were scanned by a scanner (i800 plus, Microtek, Shanghai, China) and the leaf area was estimated by AutoCAD (Autodesk, San Rafael, CA, USA). The specific leaf area (SLA) was estimated as the ratio of projected leaf area to leaf dry mass [35].

2.4.3. Gas Exchange Parameters

We randomly selected the third functional or fully expanded leaf from the top of plants and labeled them with red thread to measure their photosynthetic parameters. The net photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were measured between 9:00 and 11:00 on 14 July 2020 with a portable photosynthesis system (LI-6400XT, LI-COR Biosciences, Lincoln, NE, USA). A gas exchange system was used to carry out the measurements, with leaf chamber temperature of 25 ± 1 °C, CO_2 concentration of 400 ± 5 $\mu mol \cdot mol^{-1}$, and inner photo flux density of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ chosen to approximate the local environment in the greenhouse. Once the steady-state gas exchange rates were observed in these conditions, net photosynthetic rate (Pn), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) were recorded [36], and WUE was calculate by Pn/Tr [37].

2.4.4. Soil Water Extractable Ca

A 5.00 g subsample (20 mesh) was weighed in a 100 mL conical flask, and 25 mL of double distilled water was added to the soil. The soil suspension was shaken by a rotary shaker for 15 min. All of the extractant solution was spiked with 0.5 mL of 3% $SrCl_2$. The

Ca concentration was measured using a flame atomic absorption spectrometer (ZA3000, Hitachi, Tokyo, Japan) [38].

2.4.5. Leaf, Stem, and Root Ca Concentration

A 100 mg subsample of the sample was digested in 8 mL of a 3:1 acid mixture of HNO_3 and $HClO_4$. All of the digested solution was spiked with 2 mL of 3% $SrCl_2$ before being diluted with double distilled water to 50 mL. The Ca concentration was measured using a flame atomic absorption spectrometer (ZA3000, Hitachi, Tokyo, Japan) [38].

2.5. Statistical Analysis

Data analysis was carried out using the SPSS 22.0 statistical package (SPSS, Inc., Chicago, IL, USA) and OriginPro 2020 (OriginLab, Northampton, MA, USA) was used to draw charts, and the results are expressed as mean ± standard error (SE). A two−way analysis of variance (ANOVA) was performed to determine the significance of the effects of Ca addition and drought and their combination on the physiological parameters and biomass allocation strategies. ANOVA and Duncan's multi-range test ($p < 0.05$) were used to examine the differences among different treatments.

3. Results

3.1. Soil Calcium Concentration

Ca and water treatment had significant effects on water extractable Ca in both species ($p < 0.01$). In both species, water control had a significant effect on water extractable Ca, which was significantly higher under drought conditions compared with moist conditions (Table 1). The water extractable Ca increased with Ca addition and was higher in mulberry seedlings than poplar cuttings, except Ca200 under moist conditions.

Table 1. Effects of water and Ca treatments on soil water extractable Ca of the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of the two−way ANOVA addressing effects of Ca and water treatments are summarized at the bottom. ** indicate that the *F* value is significant at 0.01.

Calcium Treatment	**Ca0**		**Ca200**		F_{Ca}	F_{water}	$F_{Ca \times water}$
Water Treatment	**Moist**	**Drought**	**Moist**	**Drought**			
Mulberry							
Water extractable Ca ($mg \cdot kg^{-1}$)	21.34 ± 0.80 ^{D}	43.28 ± 0.75 ^{C}	175.58 ± 6.71 ^{B}	219.22 ± 16.32 ^{A}	348.951 **	13.764 **	1.507
Poplar							
Water extractable Ca ($mg \cdot kg^{-1}$)	17.4 ± 0.49 ^{C}	36.93 ± 2.89 ^{B}	176.25 ± 1.73 ^{A}	182.71 ± 8.88 ^{A}	1026.796 **	7.469 **	0.176

3.2. Leaf, Stem and Root Calcium Concentration and Content

For mulberry seedlings, we found that Ca, water, and their combination had significant effects on the Ca concentration in leaf and root ($p < 0.01$), while the Ca concentration in stem was affected by Ca and water ($p < 0.01$). The Ca concentrations of the leaf, stem, and root significantly increased with Ca addition (Table 2), and the concentration under drought conditions was significantly higher than that under moist conditions, except the concentration of leaf and root under Ca0 treatment.

For poplar cuttings, Ca treatment and water control had significant interactions on Ca concentration in the leaf and stem ($p < 0.01$), while the concentration in the root was affected by Ca and water treatment ($p < 0.01$). Ca supply increased the Ca concentration of each tissue under the same water regime. Drought treatment had a remarkable effect on tissue Ca concentration; Ca concentrations in the leaf, stem, and root under drought stress were significantly higher than those under moist conditions, except for the concentration in the root under Ca200 treatment. Between the two species, the Ca concentrations in the leaf, stem, and root of mulberry seedlings were higher than that those in poplar cuttings.

Table 2. Effect of water and Ca treatments on Ca concentration in leaf, stem, and root of the two species. Different letters indicate significant differences (p < 0.05) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized at the bottom. ** indicate that the F value is significant at 0.01.

Calcium Treatment	**Ca0**		**Ca200**		F_{Ca}	F_{water}	$F_{\text{Ca} \times \text{water}}$
Water Treatment	**Moist**	**Drought**	**Moist**	**Drought**			
Mulberry							
Leaf (mg·g^{-1})	22.44 ± 1.61 $^{\text{C}}$	24.85 ± 2.43 $^{\text{C}}$	45.80 ± 3.56 $^{\text{B}}$	67.37 ± 5.29 $^{\text{A}}$	86.966 **	12.218 **	7.885 **
Stem (mg·g^{-1})	16.22 ± 1.60 $^{\text{D}}$	22.98 ± 1.02 $^{\text{C}}$	38.51 ± 0.34 $^{\text{B}}$	50.75 ± 3.17 $^{\text{A}}$	182.535 **	26.289 **	2.189
Root (mg·g^{-1})	28.10 ± 1.75 $^{\text{C}}$	26.80 ± 0.48 $^{\text{C}}$	42.74 ± 3.00 $^{\text{B}}$	76.97 ± 4.92 $^{\text{A}}$	114.939 **	29.689 **	34.527 **
Poplar							
Leaf (mg·g^{-1})	7.73 ± 0.34 $^{\text{C}}$	11.25 ± 0.23 $^{\text{B}}$	11.72 ± 0.65 $^{\text{B}}$	22.67 ± 2.92 $^{\text{A}}$	337.870 **	297.421 **	78.177 **
Stem (mg·g^{-1})	4.07 ± 0.19 $^{\text{C}}$	8.20 ± 0.37 $^{\text{B}}$	8.10 ± 0.31 $^{\text{B}}$	15.76 ± 1.21 $^{\text{A}}$	199.705 **	199.705 **	17.987 **
Root (mg·g^{-1})	15.21 ± 0.57 $^{\text{C}}$	21.03 ± 0.44 $^{\text{B}}$	22.00 ± 0.40 $^{\text{AB}}$	25.21 ± 2.17 $^{\text{A}}$	56.103 **	38.049 **	3.168

For the two species, both water and Ca treatments had a significant effect on the Ca content of each tissue (p < 0.01), except for the effect of water treatment on leaf content in poplar, while their interaction affected the leaf, stem, and total Ca content in mulberry seedlings and the stem content in poplar cuttings (Table 3). Drought decreased the Ca content of the two species, and Ca application increased Ca absorption.

Table 3. Effect of water and Ca treatments on Ca content in leaf, stem, and root of the two species. Different letters indicate significant differences (p < 0.05) among treatments. Treatment values shown as mean ± SE (n = 6). Results of the two−way ANOVA addressing effects of Ca and water treatments are summarized at the bottom. *, ** indicate that the F value is significant at $p \leq 0.05$ and 0.01, respectively.

Calcium Treatment	**Ca0**		**Ca200**		F_{Ca}	F_{water}	$F_{Ca \times water}$
Water Treatment	**Moist**	**Drought**	**Moist**	**Drought**			
mulberry							
Leaf (mg·plant^{-1})	118.79 ± 7.82 $^{\text{B}}$	78.58 ± 3.02 $^{\text{C}}$	187.93 ± 10.88 $^{\text{A}}$	111.95 ± 2.76 $^{\text{B}}$	53.516 **	68.739 **	6.513 *
Stem (mg·plant^{-1})	60.95 ± 3.29 $^{\text{B}}$	31.12 ± 2.85 $^{\text{D}}$	125.74 ± 7.65 $^{\text{A}}$	45.29 ± 1.46 $^{\text{C}}$	78.376 **	152.889 **	32.222 **
Root (mg·plant^{-1})	80.29 $^{\text{B}}$ ± 4.99 $^{\text{B}}$	39.08 $^{\text{C}}$ ± 2.58 $^{\text{C}}$	120.31$^{\text{A}}$ ± 6.48 $^{\text{A}}$	74.06 $^{\text{B}}$ ± 5.32 $^{\text{B}}$	55.219 **	75.101 **	0.25
Total (mg·plant^{-1})	267.17 ± 8.29 $^{\text{B}}$	146.76 ± 4.97 $^{\text{C}}$	451.68 ± 19.32 $^{\text{A}}$	230.39 ± 3.69 $^{\text{B}}$	136.994 **	205.251 **	17.41 **
poplar							
Leaf (mg·plant^{-1})	131.84 ± 8.67 $^{\text{B}}$	112.20 ± 6.62 $^{\text{C}}$	192.26 ± 11.67 $^{\text{A}}$	191.91 ± 4.86 $^{\text{A}}$	87.661 **	1.007	2.946
Stem (mg·plant^{-1})	54.82 ± 3.99 $^{\text{B}}$	43.67 ± 2.58 $^{\text{C}}$	95.39 ± 6.68 $^{\text{A}}$	54.32 ± 2.66 $^{\text{B}}$	53.482 **	55.588 **	18.246 **
Root (mg·plant^{-1})	71.74 ± 6.37 $^{\text{B}}$	48.12 ± 1.79 $^{\text{C}}$	102.93 ± 7.46 $^{\text{A}}$	66.58 ± 3.34 $^{\text{B}}$	22.295 **	32.520 **	1.465
Total (mg·plant^{-1})	258.39 ± 9.35 $^{\text{C}}$	203.98 ± 9.47 $^{\text{D}}$	390.22 ± 12.21 $^{\text{A}}$	313.17 ± 13.60 $^{\text{B}}$	112.542 **	33.490 **	0.993

3.3. Photosynthetic Parameters

To demonstrate the effect of Ca and water treatments on the growth of mulberry seedlings and poplar cuttings, we further determined the photosynthetic parameters (Figure 1). We found that the photosynthetic parameters of poplar cuttings and mulberry seedlings showed consistency under water treatment. Water exerted a significant effect on the *P*n, *G*s, and *Tr* values of the two species ($p < 0.01$), and under drought stress these values remarkably decreased compared with moist conditions. However, Ca addition showed a different tendency in the two species under drought conditions. For poplar cuttings, the data show that Ca supply significantly decreased *Pn*, *Gs,* and *Tr* under both water regimes, except *Pn* under moist conditions. For mulberry seedlings, Ca supply had no significant effect on *Pn*, *Gs*, and *Tr* under both water regimes, except *Tr* in drought conditions. Drought significantly increased WUE, except for Ca0 treatment of mulberry seedlings; Ca addition increased the WUE of the two species in both water regimes, except for Ca200 treatment of mulberry seedlings under moist conditions.

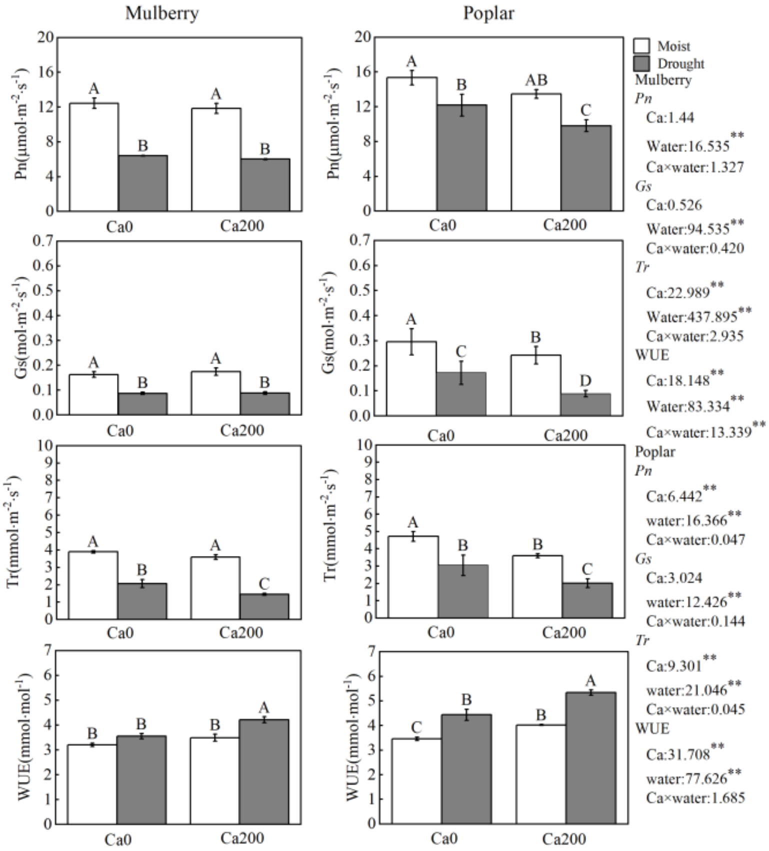

Figure 1. Effects of water and Ca treatments on photosynthetic parameters of the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized on the right side. ** indicate that the *F* value is significant at 0.01.

3.4. Biomass Accumulation

Water and Ca treatments significantly influenced the aboveground biomass compartments (leaf and stem) and the total biomass of the two species, except for the effect of Ca treatment on leaf biomass of mulberry seedlings (Table 4). Ca addition significantly decreased the biomass of leaf, stem, and total biomass under drought conditions in the two species, while Ca addition had no significant influence on leaf, stem, and total biomass under moist conditions. The total biomass and biomass of aboveground compartments of the two species were significantly lower under drought stress. Drought significantly decreased the root biomass of the two species under both Ca regimes. Compared to Ca0, with Ca200, the root biomass of poplar was not altered under moist conditions but increased under drought conditions.

Table 4. Effects of water and Ca treatments on leaf biomass, stem biomass, root biomass, and total biomass of the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized at the bottom. *, ** indicate that the F value is significant at $p \leq 0.05$, and 0.01, respectively.

Calcium Treatment	**Ca0**		**Ca200**		F_{Ca}	F_{water}	$F_{\mathrm{Ca} \times \mathrm{water}}$
Water Treatment	**Moist**	**Drought**	**Moist**	**Drought**			
Mulberry							
Leaf biomass (g/plant)	5.48 ± 0.83 ^{A}	2.03 ± 0.10 ^{B}	4.39 ± 0.48 ^{A}	1.52 ± 0.07 ^{C}	3.279	54.688 **	0.609
Stem biomass (g/plant)	4.1 ± 0.08 ^{A}	1.28 ± 0.07 ^{B}	3.36 ± 0.29 ^{A}	0.91 ± 0.02 ^{C}	12.625 *	278.592 **	1.236
Root biomass (g/plant)	2.8 ± 0.48 ^{A}	1.46 ± 0.1 ^{B}	2.81 ± 0.18 ^{A}	0.95 ± 0.03 ^{C}	3.823	163.652 **	4.044
Total biomass (g/plant)	12.39 ± 1.91 ^{A}	4.77 ± 0.18 ^{B}	10.56 ± 0.64 ^{A}	3.38 ± 0.02 ^{C}	13.309 *	280.881 **	0.237
Poplar							
Leaf biomass (g/plant)	17.03 ± 0.92 ^{A}	10.01 ± 0.61 ^{C}	14.03 ± 1.17 ^{B}	8.48 ± 0.05 ^{D}	5.917 *	1392.677 **	0.188
Stem biomass (g/plant)	14.31 ± 1.05 ^{A}	5.01 ± 0.33 ^{B}	11.83 ± 1.07 ^{A}	3.45 ± 0.48 ^{C}	7.214 *	155.065 **	0.023
Root biomass (g/plant)	4.66 ± 0.46 ^{A}	2.29 ± 0.13 ^{C}	4.67 ± 0.33 ^{A}	2.64 ± 0.13 ^{B}	0.600	89.269 **	0.537
Total biomass (g/plant)	34.97 ± 1.66 ^{A}	16.54 ± 0.98 ^{B}	30.54 ± 2.03 ^{A}	14.57 ± 0.56 ^{C}	6.621 *	174.765 **	0.081

3.5. Biomass Allocation

The biomass allocation of poplar cuttings and mulberry seedlings showed remarkable differences in response to Ca and water treatments (Figure 2). We found that Ca and water treatments, and their combination, significantly affected the SMF and RMF of poplar cuttings and the RMF of mulberry seedlings; the LMF of poplar cuttings and the SMF of mulberry seedlings only responded to water treatment ($p < 0.01$).

In poplar cuttings, Ca addition had no influence on LMF under either water regime; LMF was significantly higher under drought conditions than under moist conditions. SMF showed the opposite results: SMF was significantly lower under drought conditions than under moist conditions. Ca addition markedly decreased SMF under drought conditions, while no significant change was observed with Ca addition under moist conditions. Moreover, Ca addition significantly upregulated RMF under both water regimes.

In mulberry seedlings, LMF did not respond to either Ca or water treatment; less than 45% of biomass was allocated to the leaf, and SMF only decreased with Ca0 under drought conditions. Ca supply significantly increased RMF under moist conditions, and drought remarkably increased RMF with both Ca treatments. Compared to poplar cuttings, mulberry seedlings had less biomass allocated to leaf and stem and more biomass to root under drought conditions.

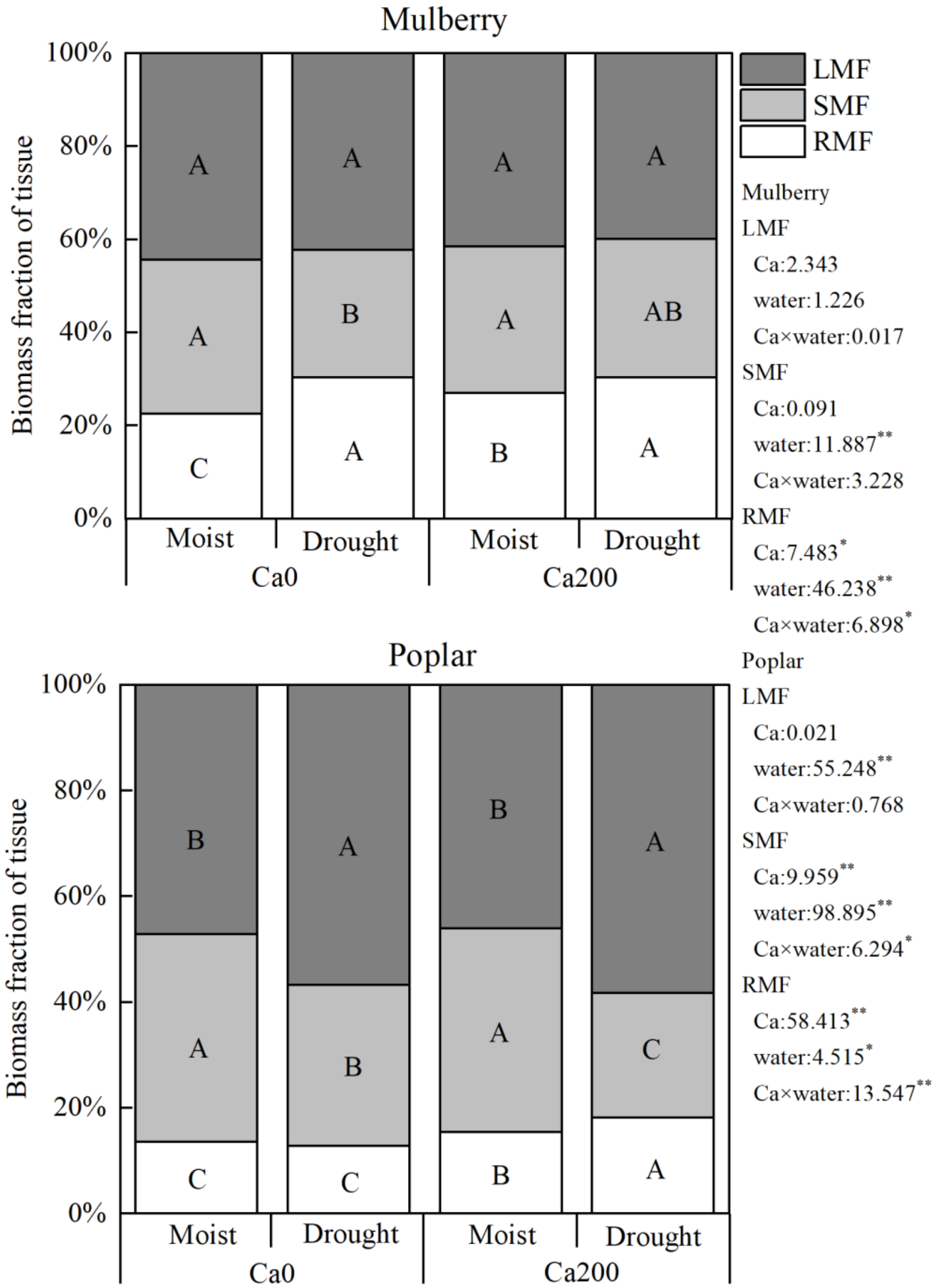

Figure 2. Effects of water and Ca treatments on biomass allocation in organs of the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized on the right side. *, ** indicate that the F value is significant at $p \leq 0.05$ and 0.01, respectively.

Ca, water, and their combination had a significant influence on the root biomass/shoot biomass (R/S) ratio. Ca200 treatment significantly increased the R/S of poplar cuttings under both water regimes, while Ca200 treatment significantly increased the R/S of mulberry seedlings only under moist conditions. Mulberry seedlings had a higher R/S under drought conditions than poplar cuttings (Figure 3). Moreover, the root biomass/stem biomass (R/St) and root biomass/leaf biomass (R/L) ratios of mulberry seedlings and the R/St of poplar cuttings exhibited the same tendency; Ca addition increased the R/St and R/L of the two species, except the R/L of poplar cuttings under moist conditions and the R/St of mulberry seedlings under drought conditions. The stem biomass/leaf biomass (S/L) ratio of the two species showed substantial differences: drought remarkably decreased the S/L of poplar cuttings due to the differences in biomass allocation among poplar cuttings.

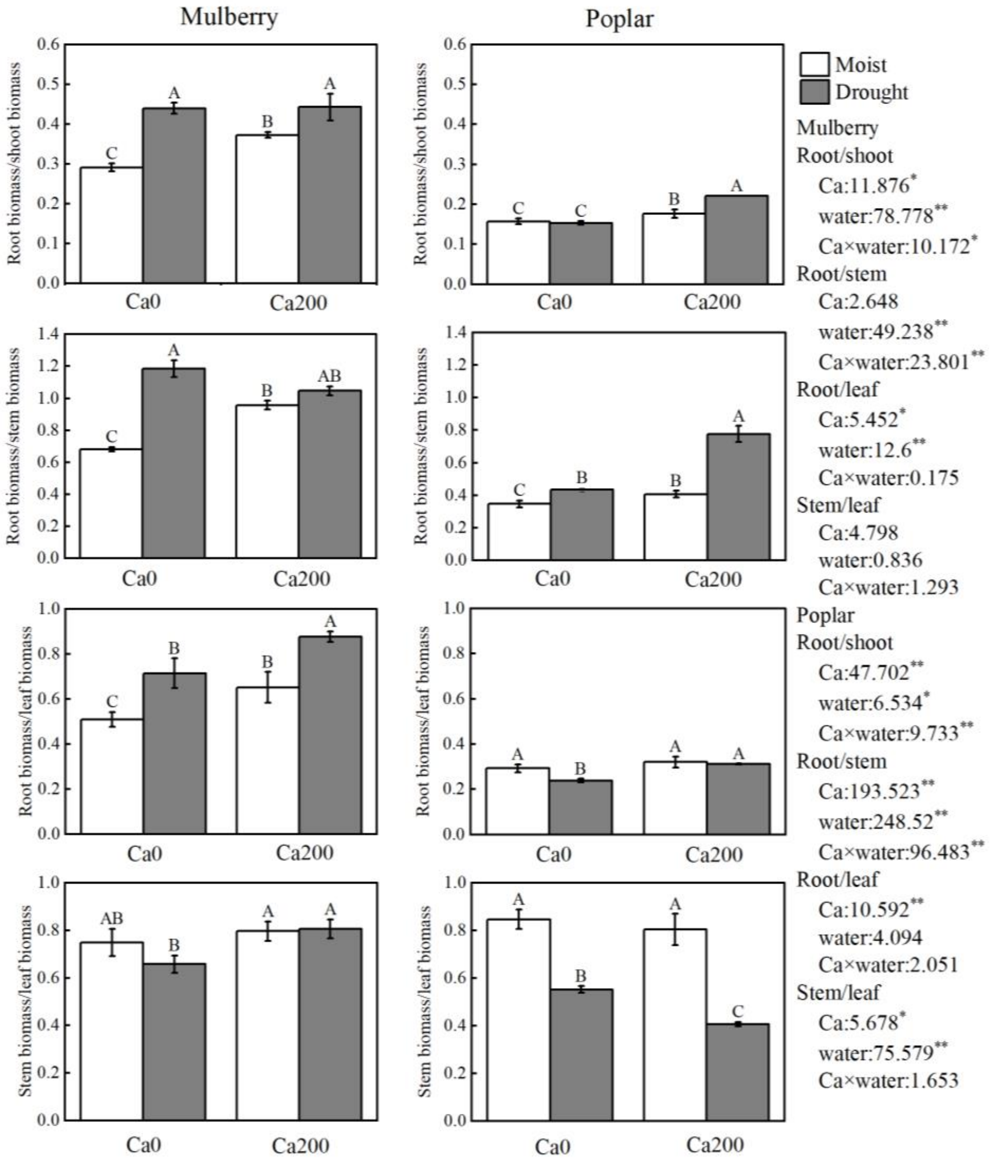

Figure 3. Effects of water and Ca treatments on biomass allocation of organs in the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized on the right side. *, ** indicate that the *F* value is significant at $p \leq 0.05$ and 0.01, respectively.

3.6. Leaf Characteristics

The leaf area was significantly influenced by Ca and water ($p < 0.01$) and their interaction ($p < 0.05$) in the two species (Figure 4). Ca supply had no significant effect on the leaf area of poplar cuttings but remarkably decreased the leaf area of mulberry seedlings under moist conditions. Under drought conditions, the leaf area of the two species remarkably decreased with Ca addition. We found that drought decreased the SLA of mulberry seedlings under Ca0 treatment, while it increased the SLA of poplar cuttings. SLA was significantly higher for mulberry seedlings compared with poplar cuttings.

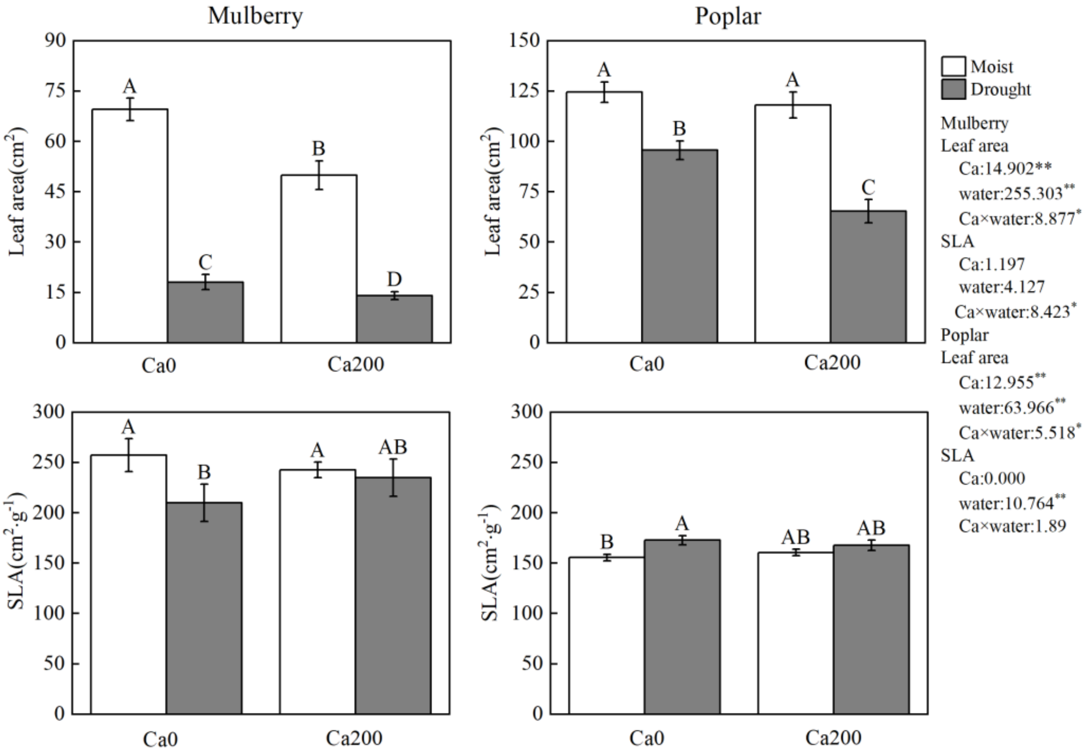

Figure 4. Effects of water and Ca treatments on leaf characteristics of the two species. Different letters indicate significant differences ($p < 0.05$) among treatments. Treatment values shown as mean ± SE (n = 6). Results of two−way ANOVA addressing effects of Ca and water treatments are summarized on the right side. *, ** indicate that the F value is significant at $p \leq 0.05$ and 0.01, respectively.

4. Discussion

4.1. Responses of Plant Ca Uptake to Drought and Ca Treatments

Water-extracted Ca is a form that can be directly absorbed and utilized by plants [39]. Soil water-extracted Ca is transported by mass flow of the soil solution to the root surface and absorbed through the apoplastic space in roots, by the only acropetal transportation in the xylem vessels and immobility in the phloem; therefore, plant transpiration and soil Ca availability are the main determining factors of Ca uptake [15,40,41]. According to the Ca uptake characteristics, plants can absorb more Ca^{2+} from soil under normal moisture conditions than drought conditions. In our study, the water availability and transpiration rate were reduced under drought conditions, and the Ca uptake (Ca content of the plant) was reduced in the two species. Moreover, Ca addition increased the tissue calcium content of poplar cuttings and mulberry seedlings when transpiration was inhibited under drought conditions. Exudates from root and rhizosphere microorganisms, such as organic acids, enzymes, and other molecules, can improve the soil Ca availability under drought conditions by inducing Ca^{2+} desorption [42] and accelerating soil weathering [43,44].

In our study, under moist conditions, the Ca content of poplar cuttings and mulberry seedlings was comparable, while the slower growth rate of mulberry than poplar resulted in a higher tissue Ca concentration in mulberry seedlings. Previous studies found variable responses to Ca among tree species [45,46]. Our results show that the Ca content was higher in mulberry seedlings than poplar cuttings after Ca addition, which may be due to the different Ca tolerance between the two species. Under drought conditions, there was a lower reduction in biomass and transpiration rate in poplar cuttings than mulberry seedlings, and according to the Ca uptake characteristics [15,40,41], poplar cuttings absorb more Ca into their tissues than mulberry seedlings.

4.2. Growth and Leaf Gas Exchange Responses to Drought and Ca Treatments

Growth and biomass are the most basic indices for assessing the growth of plants. Water deficiency seriously affects the growth and dry matter content of plants [47–49]. Our results show that the growth and biomass of poplar cuttings and mulberry seedlings remarkably decreased in drought conditions without Ca application. The results of a few studies are consistent with our results. For example, Shang [50] showed, in a potted plant experiment, that drought stress stimulated leaf biomass, root biomass, and total biomass. In our study, the slow growth of the two species with Ca addition was associated with physiological processes that are regulated by the combination of excess calcium ions and drought, indicating that Ca200 treatment is detrimental to the biomass accumulation of poplar and mulberry during the seedling growth stage under drought conditions. However, our results show that Ca promoted root growth, and increased root biomass always increases water uptake [2,3,5], allowing roots to take up deeper groundwater to absorb more Ca^{2+} and reduce the effects of stress under drought conditions [1].

In our study, mulberry seedlings and poplar cuttings displayed a lower carbon assimilation (*Pn*) response to drought stress, their carbon assimilation was significantly reduced, and they showed a sharp decrement of *Pn*, in line with previous studies [10,49]. The reduction in leaf area, *Tr*, and *Gs,* led to a reduction in *Pn* of poplar cuttings and mulberry seedlings. In terms of the two species, the degree of *Pn* inhibition was less for poplar cuttings than mulberry seedlings under drought conditions. As a fast-growing species, poplar maintains a higher *Pn* for growth, so poplar cuttings had a smaller proportion of decreased biomass than mulberry seedlings under drought conditions. Ca is a signaling substance that regulates stomatal closure; high Ca can induce stomatal closure and thus reduce photosynthesis [49]. In our study, Ca treatment inhibited *Pn* in poplar cuttings by suppressing *Gs*, and poplar cuttings were more sensitive to Ca treatment under drought conditions.

4.3. Biomass Allocation Patterns in Response to Drought

Plants usually change their morphology and biomass allocation under water deficiency, which may be a primary mechanism to cope with drought stress [12]. SLA reflects the adaptation of plants to their environment [51], and plants growing in an arid habitat always have smaller SLA [52]. Previous studies mainly suggested that drought causes decreased leaf biomass allocation and leaf area, together with lower SLA, to reduce the surface area for transpiration and thus prevent water loss [10,53]. The mulberry in this study is a native tree species in the semi-arid climate zone of China, with higher biomass allocated to root and lower leaf area and SLA, suggesting that it is more advantageous to reduce transpiration and increase drought resistance.

High SLA or leaf biomass allocation implies rapid assimilation for plant growth and production [54], but it may increase the vulnerability of plants to adverse environmental conditions [55]. In our study, poplar cuttings allocated more biomass to their leaves and had increased SLA under drought stress. Similar aggressive countermeasures against drought were also found in tomato (*Solanum lycopersicum*), in which the mean specific leaf area tended to be higher in plants under mild drought treatment than control plants [56]. Beikircher et al. [57] reported that some apple cultivars maintained stomatal conductance and leaf gas exchange longer to support higher productivity, increasing the risk of hydraulic failure under drought stress. It seems that these aggressive countermeasures against drought are based on the selection of high-yield cultivars under an optimized water supply [57]. The poplar in this study is a hybrid, and its higher L/S and SLA possibility contribute to its rapid growth. In summary, our results show that poplar cuttings and mulberry seedlings have different strategies for coping with drought.

4.4. Ca Increases Drought Resistance via Biomass Allocation and Plant WUE

In our study, poplar cuttings exposed to drought had significantly increased LMF and RMF and decreased SMF under Ca200 treatment. It seems that exogenous Ca promotes relatively aggressive biomass allocation patterns in poplar while increasing the drought resistance by increasing the RMF and R/S. Ca^{2+} can enhance drought stress tolerance in plants by maintaining membrane stability, osmotic homeostasis, and cell signaling [58–61]; it seems that the increased RMF by Ca signaling is a recognized response of plants to drought. The Ca addition had no significant effect on the biomass partitioning of mulberry seedlings under drought conditions, and combined with the above results, this suggests that the effect of Ca on biomass partitioning of mulberry seedlings was not as great as under drought conditions.

Plant WUE reflects the relationship between water consumption and dry matter production and is an important index of drought resistance [62,63]. Ca can regulate stomatal closure, which can directly affect the transpiration rate and CO_2 uptake, thereby increasing WUE [39,64–67]. In this study, the WUE of poplar cuttings increased under drought stress without Ca application, suggesting a certain compensatory modification of plant water use; as observed in previous studies, poplar had an adaptive mechanism to cope with drought stress. Interestingly, Ca increased the WUE of mulberry seedlings and poplar cuttings under drought conditions, in agreement with Li [39] and Yin [68].

5. Conclusions

In this study, Ca addition and drought treatment significantly influenced the Ca absorption, growth, leaf gas exchange, biomass accumulation, and leaf traits of mulberry seedlings and poplar cuttings. We demonstrated that poplar cuttings and mulberry seedlings had different biomass allocation strategies in response to drought, and Ca addition enhanced the drought resistance of both. Drought induced reduced growth of poplar cuttings and mulberry seedlings. Poplar cuttings allocated more biomass to leaves and had increased SLA in order to maintain higher *Pn*, and they had enhanced WUE to cope with drought stress. Mulberry seedlings allocated more biomass to root and had reduced SLA under drought conditions in order to improve drought resistance. With Ca application, the growth of poplar cuttings and mulberry seedlings decreased under drought, while Ca application further improved the drought resistance of poplar cuttings by increasing R/S and WUE values, and it improved the drought resistance of mulberry seedlings only via enhanced WUE. These findings give us a deeper understanding of the adaptation and response mechanisms of poplar cuttings and mulberry seedlings to drought and Ca availability in arid and semi-arid areas of Northwest Liaoning, China.

Author Contributions: Conceptualization, S.Z.; data curation, T.Z., G.D. and Y.L.; funding acquisition, S.Z. and Y.Z.; investigation, T.Z., Y.T. and H.L.; methodology, G.D. and Y.Z.; resources, T.Z. and Y.T.; software, T.Z.; supervision, Y.Z. and H.L.; validation, S.Z. and Y.Z.; visualization, H.L.; writing—original draft, T.Z., G.D. and Y.L.; writing—review and editing, T.Z., Y.T. and S.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Liaoning Province Scientific Research Funding Project, grant number LSNZD202002, Science and Technology Program of Liaoning Province, grant number 2020020287-JH1/103-05-02, and the National Natural Science Foundation of China, grant number 31800364.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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

References

1. Poorter, H.; Niklas, K.J.; Reich, P.B.; Oleksyn, J.; Poot, P.; Mommer, L. Biomass allocation to leaves, stems and roots: Meta-analyses of interspecific variation and environmental control. *New Phytol.* **2012**, *193*, 30–50. [CrossRef]
2. Bahn, M.; Lattanzi, F.A.; Hasibeder, R.; Wild, B.; Koranda, M.; Danese, V.; Brüggemann, N.; Schmitt, M.; Siegwolf, R.; Richter, A. Responses of belowground carbon allocation dynamics to extended shading in mountain grassland. *New Phytol.* **2013**, *198*, 116–126. [CrossRef]
3. Zang, U.; Goisser, M.; Grams, T.E.E.; Haberle, K.-H.; Matyssek, R.; Matzner, E.; Matzner, E.; Borken, W. Fate of recently fixed carbon in European beech (*Fagus sylvatica*) saplings during drought and subsequent recovery. *Tree Physiol.* **2014**, *34*, 29–38. [CrossRef]
4. Brunner, I.; Herzog, C.; Dawes, M.A.; Arend, M.; Sperisen, C. How tree roots respond to drought. *Front. Plant Sci.* **2015**, *6*, 547. [CrossRef]
5. Liu, Y.; Li, P.; Wang, T.; Liu, Q.; Wang, W. Root respiration and belowground carbon allocation respond to drought stress in a perennial grass (*Bothriochloa ischaemum*). *Catena* **2020**, *188*, 104449. [CrossRef]
6. Chen, R.; Ran, J.; Hu, W.; Dong, L.; Ji, M.; Jia, X.; Lu, J.; Gong, H.; Aqeel, M.; Yao, S.; et al. Effects of biotic and abiotic factors on forest biomass fractions. *Natl. Sci. Rev.* **2021**, *8*, nwab025. [CrossRef]
7. Hamidou, F.; Heynikoye, M.; Halilou, O.; Upadhyaya, H.D.; Vadez, V. Drought (WS) and low phosphorus (LP) stress in groundnut: Water extraction pattern and tolerance related traits for breeding program. In Proceedings of the InterDrought, Hyderabad, India, 21–25 February 2017.
8. Shi, H.; Ma, W.; Song, J.; Lu, M.; Rahman, S.U.; Bui, T.T.X.; Vu, D.D.; Zheng, H.; Wang, J.; Zhang, Y. Physiological and transcriptional responses of *Catalpa bungei* to drought stress under sufficient- and deficient-nitrogen conditions. *Tree Physiol.* **2017**, *37*, 1457–1468. [CrossRef]
9. Bauw, P.D.; Vandamme, E.; Lupembe, A.; Mwakasege, L.; Senthilkumar, K.; Dramé, K.N.; Merckx, R. Anatomical root responses of rice to combined phosphorus and water stress-relations to tolerance and breeding opportunities. *Funct. Plant Biol.* **2019**, *46*, 1009–1022. [CrossRef]
10. Zhang, Z.; Tariq, A.; Zeng, F.; Graciano, C.; Zhang, B. Nitrogen application mitigates drought-induced metabolic changes in *Alhagi sparsifolia* seedlings by regulating nutrient and biomass allocation patterns. *Plant Physiol. Biochem.* **2020**, *155*, 828–841. [CrossRef]
11. Meena, S.K.; Pandey, R.; Sharma, S.; Gayacharan, K.T.; Singh, M.P.; Dikshit, H.K. Physiological basis of combined stress tolerance to low phosphorus and drought in a diverse set of mungbean germplasm. *Agronomy* **2021**, *11*, 99. [CrossRef]
12. Wu, F.; Bao, W.; Li, F.; Wu, N. Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of sophora davidii seedlings. *Environ. Exp. Bot.* **2008**, *63*, 248–255. [CrossRef]
13. Broadley, M.R.; Bowen, H.C.; Cotterill, H.L.; Hammond, J.P.; Meacham, M.C.; Mead, A.; White, P.J. Variation in the shoot calcium content of angiosperms. *J. Exp. Bot.* **2003**, *54*, 1431–1446. [CrossRef]
14. Gruber, B.D.; Giehl, R.F.H.; Friedel, S.; Wirén, N.V. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol.* **2013**, *163*, 161–179. [CrossRef]
15. Hirschi, K.D. The calcium conundrum. Both versatile nutrient and specific signal. *Plant Physiol.* **2004**, *136*, 2438–2442. [CrossRef]
16. Wang, C.Q. Exogenous calcium alters activities of antioxidant enzymes in *Trifolium repens* L. leaves under PEG-induced water deficit. *J. Plant Nutr.* **2010**, *33*, 1874–1885. [CrossRef]
17. He, L.; Lu, X.; Tian, J.; Yang, Y.; Li, B.; Li, J.; Guo, S. Proteomic analysis of the effects of exogenous calcium on hypoxic-responsive proteins in cucumber roots. *Proteome Sci.* **2012**, *10*, 42. [CrossRef]
18. Dolatabadian, A.; Sanavy, S.A.M.M.; Gholamhoseini, M.; Joghan, A.K.; Majdi, M.; Kashkooli, A.B. The role of calcium in improving photosynthesis and related physiological and biochemical attributes of spring wheat subjected to simulated acid rain. *Physiol. Mol. Biol. Plants* **2013**, *19*, 189–198. [CrossRef]
19. Liang, C.; Zhang, B. Effect of exogenous calcium on growth, nutrients uptake and plasma membrane H^+-ATPase and Ca^{2+}-ATPase activities in soybean (*Glycine max*) seedlings under simulated acid rain stress. *Ecotoxicol. Environ. Saf.* **2018**, *165*, 261–269. [CrossRef]
20. Lawrence, G.B.; David, M.B.; Shortle, W.C. A new mechanism for calcium loss in forest-floor soils. *Nature* **1995**, *378*, 162–165. [CrossRef]
21. Likens, G.E.; Driscoll, C.T.; Buso, D.C.; Siccama, T.G.; Johnson, C.E.; Lovett, G.M.; Fahey, T.J.; Reiners, W.A.; Ryan, D.F.; Martin, C.W.; et al. The biogeo-chemistry of calcium at Hubbard Brook. *Biogeochemistry* **1998**, *41*, 89–173. [CrossRef]
22. Page, B.D.; Bullen, T.D.; Mitchell, M.J. Influences of calcium availability and tree species on Ca isotope fractionation in soil and vegetation. *Biogeochemistry* **2008**, *88*, 1–13. [CrossRef]
23. Zhou, Y.B.; Zou, X.M. From matching site with trees towards matching calcium with trees. *J. Nanjing For. Univ. Nat. Sci. Ed.* **2017**, *41*, 1–8. [CrossRef]
24. Ji, Y.; Zhou, G.; Li, Z.; Wang, S.; Zhou, H.; Song, X. Triggers of widespread dieback and mortality of poplar (*Populus* spp.) plantations across northern china. *J. Arid Environ.* **2019**, *174*, 104076. [CrossRef]
25. Song, X.; Gao, X.; Wu, P.; Zhao, X.; Zhang, W.; Zou, Y.; Siddique, K.H.M. Drought responses of profile plant-available water and fine-root distributions in apple (*Malus pumila* Mill.) orchards in a loessial, semi-arid, hilly area of China. *Sci. Total Environ.* **2020**, *723*, 137739. [CrossRef] [PubMed]

26. Jiang, D.; Wang, Y.; Dong, X.; Yan, S. Inducible defense responses in *Populus alba berolinensis* to Pb stress. *S. Afr. J. Bot.* **2018**, *119*, 295–300. [CrossRef]
27. Xu, S.; Li, B.; Li, P.; He, X.; Chen, W.; Yang, K.; Lia, Y.; Wang, Y. Soil high Cd exacerbates the adverse impact of elevated O_3 on *Populus alba 'Berolinensis'* L. *Ecotoxicol. Environ. Saf.* **2019**, *174*, 35–42. [CrossRef] [PubMed]
28. Wang, H.L.; Yang, Q.; Tan, S.; Wang, T.; Zhang, Y.; Yang, Y.; Yin, W.; Xia, X.; Guo, H.; Li, Z. Regulation of cytokinin biosynthesis using PtRD26pro-IPT module improves drought tolerance through PtARR10-PtYUC4/5-mediated reactive oxygen species removal in *Populus*. *J. Integr. Plant Biol.* **2022**, *64*, 771–786. [CrossRef]
29. Liu, X.; Zhang, H.; Wang, J.; Wu, X.; Ma, S.; Xu, Z.; Zhou, T.; Xu, N.; Tang, X.; An, B. Increased CO_2 concentrations increasing water use efficiency and improvement PSII function of mulberry seedling leaves under drought stress. *J. Plant Interact.* **2019**, *14*, 213–223. [CrossRef]
30. Zhang, H.; Wang, Y.; Li, X.; He, G.; Che, Y.; Teng, Z.; Shao, J.; Xu, N.; Sun, G. Chlorophyll synthesis and the photoprotective mechanism in leaves of mulberry (*Morus alba* L.) seedlings under NaCl and $NaHCO_3$ stress revealed by TMT-based proteomics analyses. *Ecotoxicol. Environ. Saf.* **2020**, *190*, 110164. [CrossRef]
31. Zhang, H.; Li, X.; Guan, Y.; Li, M.; Wang, Y.; An, M.; Zhang, Y.; Liu, G.; Xu, N.; Sun, G. Physiological and proteomic responses of reactive oxygen species metabolism and antioxidant machinery in mulberry (*Morus alba* L.) seedling leaves to NaCl and $NaHCO_3$ stress. *Ecotoxicol. Environ. Saf.* **2020**, *193*, 110259. [CrossRef]
32. Li, R.; Liu, L.; Dominic, K.; Wang, T.; Fan, T.; Hu, F.; Wang, Y.; Zhang, L.; Lia, L.; Zhao, W. Mulberry (*Morus alba*) MmSK gene enhances tolerance to drought stress in transgenic mulberry. *Plant Physiol. Biochem.* **2018**, *132*, 603–611. [CrossRef] [PubMed]
33. Sun, S.; He, C.; Qiu, L.; Li, C.; Zhang, J.; Meng, P. Stable isotope analysis reveals prolonged drought stress in poplar plantation mortality of the Three-North Shelter Forest in Northern China. *Agric. For. Meteorol.* **2018**, *252*, 39–48. [CrossRef]
34. Cao, X.; Shen, Q.; Liu, L.; Cheng, J. Relationships of growth, stable carbon isotope composition and anatomical properties of leaf and xylem in seven mulberry cultivars: A hint towards drought tolerance. *Plant Biol.* **2020**, *22*, 287–297. [CrossRef] [PubMed]
35. Wang, Z.; Guo, D.; Wang, X.; Gu, J.; Mei, L. Fine root architecture, morphology, and biomass of different branch orders of two Chinese temperate tree species. *Plant Soil* **2006**, *288*, 155–171. [CrossRef]
36. Fang, X.; Li, Y.; Nie, J.; Wang, C.; Huang, K.; Zhang, Y.; Zhanga, Y.; Shea, H.; Liud, X.; Ruana, R.; et al. Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (*Fagopyrum esculentum* M.). *Field Crops Res.* **2018**, *219*, 160–168. [CrossRef]
37. Ou, L.J.; Wei, G.; Zhang, Z.Q.; Dai, X.Z.; Zou, X.X. Effects of low temperature and low irradiance on the physiological characteristics and related gene expression of different pepper species. *Photosynthetica* **2015**, *53*, 85–94. [CrossRef]
38. Lu, R.K. *Analytical Methods for Soil and Agro-Chemistry*; China Agricultural Science and Technology Publishing House: Beijing, China, 2000; pp. 197–317.
39. Li, Y.; Zhang, T.; Zhou, Y.; Zou, X.; Yin, Y.; Li, H.; Liu, L.; Zhang, S. Ectomycorrhizal symbioses increase soil calcium availability and water use efficiency of *Quercus acutissima* seedlings under drought stress. *Eur. J. For. Res.* **2021**, *140*, 1039–1048. [CrossRef]
40. White, P.J. The pathways of calcium movement to the xylem. *J. Exp. Bot.* **2001**, *52*, 891–899. [CrossRef]
41. White, P.J.; Broadley, M.R. Biofortifying crops with essential mineral elements. *Trends Plant Sci.* **2005**, *10*, 586–593. [CrossRef]
42. Liese, R.; Lübbe, T.; Albers, N.W.; Meier, I.C. The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. *Tree Physiol.* **2017**, *38*, 83–95. [CrossRef]
43. Blum, J.D.; Klaue, A.; Nezat, C.A.; Driscoll, C.T.; Johnson, C.E.; Siccama, T.G.; Eagar, C.; Fahey, T.J.; Likens, G.E. Mycorrhizal weathering of apatite as an important calcium source in base-poor forest ecosystems. *Nature* **2002**, *417*, 729–731. [CrossRef] [PubMed]
44. Quirk, J. Effects of Evolutionary Advances in Plant-Mycorrhiza Associations on Biological Weathering. Ph.D. Thesis, University of Sheffield, Sheffield, UK, 2012.
45. Bigelow, S.W.; Canham, C.D. Nutrient limitation of juvenile trees in a northern hardwood forest: Calcium and nitrate are preeminent. *For. Ecol. Manag.* **2007**, *243*, 310–319. [CrossRef]
46. Liu, T.W.; Wu, F.H.; Wang, W.H.; Chen, J.; Li, Z.J.; Dong, X.J.; Patton, J.; Pei, Z.M.; Zheng, H.L. Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain. *Tree Physiol.* **2011**, *31*, 402–413. [CrossRef] [PubMed]
47. Anjum, S.A.; Ashraf, U.; Tanveer, M.; Khan, I.; Hussain, S.; Shahzad, B.; Zohaib, A.; Abbas, F.; Saleem, M.F.; Ali, I. Drought induced changes in growth, osmolyte accumulation and antioxidant metabolism of three maize hybrids. *Front. Plant Sci.* **2017**, *8*, 69. [CrossRef]
48. Barutcular, C.; Dizlek, H.; EL-Sabagh, A.; Sahin, T.; Elsabagh, M.; Islam, S. Nutritional quality of maize in response to drought stress during grain-filling stages in Mediterranean climate condition. *J. Exp. Biol. Agric. Sci.* **2016**, *4*, 644–652. [CrossRef]
49. Li, H.; Huo, Y.; Weng, X.; Zhou, Y.; Sun, Y.; Zhang, G.; Zhang, S.; Liu, L.; Pei, J. Regulation of the growth of mongolian pine (*Pinus sylvestris* var. *mongolica*) by calcium-water coupling in a semiarid region. *Ecol. Indic.* **2022**, *137*, 108736. [CrossRef]
50. Shang, Y. Effects of elevated ozone and water deficit on poplar saplings: Changes in carbon and nitrogen stocks and their allocation to different organs. *For. Ecol. Manag.* **2019**, *441*, 89–98. [CrossRef]
51. Wilson, P.J.; Thompson, K.; Hodgson, J.G. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytol.* **2010**, *143*, 155–162. [CrossRef]

52. Reich, P.B.; Ellsworth, D.S.; Walters, M.B.; Vose, J.M.; Gresham, C.; Volin, J.C.; Bowman, W.D. Generality of leaf trait relationships: A test across six biomes. *Ecology* **1999**, *80*, 1955–1969. [CrossRef]
53. Liu, F.; Stützel, H. Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Sci. Hortic.* **2004**, *102*, 15–27. [CrossRef]
54. Vitra, A.; Deléglise, C.; Meisser, M.; Risch, A.C.; Signarbieux, C.; Lamacque, L.; Delzon, S.; Buttler, A.; Mariotte, P. Responses of plant leaf economic and hydraulic traits mediate the effects of early-and late-season drought on grassland productivity. *AoB Plants* **2019**, *11*, plz023. [CrossRef]
55. Agathokleous, E.; Paoletti, E.; Saitanis, C.J.; Manning, W.J.; Sugai, T.; Koike, T. Impacts of ethylenediurea (EDU) soil drench and foliar spray in *Salix sachalinensis* protection against O_3-induced injury. *Sci. Total Environ.* **2016**, *573*, 1053–1062. [CrossRef]
56. Nilsen, E.T.; Freeman, J.; Grene, R.; Tokuhisa, J. A rootstock provides water conservation for a grafted commercial tomato (*Solanum lycopersicum* L.) line in response to mild-drought conditions: A focus on vegetative growth and photosynthetic parameters. *PLoS ONE* **2014**, *9*, e115380. [CrossRef]
57. Beikircher, B.; Cesare, D.C.; Mayr, S. Hydraulics of high-yield orchard trees: A case study of three *Malus domestica* cultivars. *Tree Physiol.* **2013**, *33*, 1296–1307. [CrossRef] [PubMed]
58. Mohamed, A.M.; Hashim, A.L.; Mohamed, S. Ameliorative Effects of Calcium Sprays on Yield and Grain Nutritional Composition of Maize (*Zea mays* L.) Cultivars under Drought Stress. *Agriculture* **2021**, *11*, 285. [CrossRef]
59. Shao, H.B.; Song, W.Y.; Chu, L.Y. Advances of calcium signals involved in plant anti-drought. *Comptes Rendus Biol.* **2008**, *331*, 587–596. [CrossRef] [PubMed]
60. He, L.; Yu, L.; Li, B.; Du, N.; Guo, S. The effect of exogenous calcium on cucumber fruit quality, photosynthesis, chlorophyll fluorescence, and fast chlorophyll fluorescence during the fruiting period under hypoxic stress. *BMC Plant Biol.* **2018**, *18*, 180. [CrossRef] [PubMed]
61. Ranty, B.; Aldon, D.; Cotelle, V.; Galaud, J.P.; Thuleau, P.; Mazars, C. Calcium sensors as key hubs in plant responses to biotic and abiotic stresses. *Front. Plant Sci.* **2016**, *7*, 327. [CrossRef]
62. Beer, C. Impact of soil freezing and thawing dynamics on soil organic carbon stocks in permafrost regions. *IOP Conf. Ser. Earth Environ. Sci.* **2009**, *6*, 052013. [CrossRef]
63. Ahmadi, S.H.; Solgi, S.; Sepaskhah, A.R. Quinoa: A super or pseudo-super crop? Evidences from evapotranspiration, root growth, crop coefficients, and water productivity in a hot and semi-arid area under three planting densities. *Agric. Water Manag.* **2019**, *225*, 105784. [CrossRef]
64. Jones, H.G.; Tardieu, F. Modelling water relations of horticultural crops: A review. *Sci. Hortic.* **1998**, *74*, 21–46. [CrossRef]
65. Pei, Z.M.; Murata, Y.; Benning, G.; Thomine, S.; Klüsener, B.; Allen, G.J.; Grill, E.; Schroeder, J.I. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature* **2000**, *406*, 731–734. [CrossRef] [PubMed]
66. Mahajan, S.; Tuteja, N. Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.* **2005**, *444*, 139–158. [CrossRef] [PubMed]
67. Wang, Y.; Kang, Y.; Ma, C.; Miao, R.; Wu, C.; Long, Y.; Ge, T.; Wu, Z.; Hou, X.; Zhang, J.; et al. Cngc2 is a Ca^{2+} influx channel that prevents accumulation of apoplastic Ca^{2+} in the leaf. *Plant Physiol.* **2017**, *173*, 1342–1354. [CrossRef] [PubMed]
68. Yin, Y.; Zhou, Y.B.; Li, H.; Zhang, S.Z.; Fang, Y.; Zhang, Y.J.; Zou, X. Linking tree water use efficiency with calcium and precipitation. *Tree Physiol.* **2022**, *12*, 2419–2431. [CrossRef] [PubMed]