

Article

# Optimal Irrigation Regime for Woody Species Potentially Suitable for Effective and Sustainable Afforestation in the Desert Region of Mongolia

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**Abstract:** Long-term studies on plant response mechanisms to different irrigation regimes will provide a better understanding of the survivability and establishment of plant communities in a desert environment. Thus, across 10 years, we regularly investigated the effects of the rainfall (control), rainfall + 4 L h<sup>-1</sup>, rainfall + 8 L h<sup>-1</sup>, and rainfall + 12 L h<sup>-1</sup> irrigation regimes on the growth and leaf morpho-physiology of *Tamarix ramosissima* Ledeb., *Ulmus pumila* L., *Elaeagnus moorcroftii* Wall. ex Schltdl., and *Hippophae rhamnoides* L. to suggest an optimal irrigation regime for each woody species for effective and sustainable afforestation in Mongolia. We measured the root collar diameter (RCD), annual height growth, survivability, leaf area (LA), specific leaf area (SLA), leaf biomass (LB), total chlorophyll concentration, and predawn ( $\psi_p$ ) and midday ( $\psi_m$ ) leaf water potentials across the treatments and species. Results showed that trees grown at 12 L h<sup>-1</sup> grew taller per year and generally resulted in a higher SLA, but generally resulted in a lower survival rate compared with those in the other treatments in all species. Total chlorophyll content was higher in trees grown under 4 and/or 8 L h<sup>-1</sup>, particularly for *T. ramosissima* and *E. moorcroftii*. Lastly, leaf water potentials were found more negative for trees subjected to 4 L h<sup>-1</sup>, especially in *T. ramosissima* and *U. pumila*, but still resulted in a higher survival rate and LB compared with 12 L h<sup>-1</sup>. *H. rhamnoides* showed higher survivability at 8 and/or 12 L h<sup>-1</sup> than at 4 L h<sup>-1</sup>. Therefore, we suggest 4 L h<sup>-1</sup> to be the optimal irrigation regime for irrigating *T. ramosissima*, *U. pumila* and *E. moorcroftii*, and 8 and/or 12 L h<sup>-1</sup> for *H. rhamnoides*. Our findings are relevant to ensuring the sustainability of afforestation programs in arid and semiarid landscapes in Mongolia.

**Keywords:** arid and semiarid; desertification; *Elaeagnus moorcroftii*; Green belt plantation; morpho-physiology; *Tamarix ramosissima*; *Ulmus pumila*

## 1. Introduction

Drought and desertification are among the major ecological problems in arid and semiarid regions around the world [1,2]. As a response to these problems and compliance to the 2030 Agenda for Sustainable Development, an increasing number of projects have been implemented worldwide to increase the vegetation cover, such as a series of global

afforestation and ecological restoration projects [3,4]. However, water limitation is a major impediment to the establishment of plant communities through afforestation initiatives in arid and semiarid environments with short-lived and erratic rainfall events. Early survival of tree-seedlings is often negatively affected by water shortage in drylands, resulting in reduced total plant biomass production [5–7]. A recent study showed that the duration of drought days equated to the seedling survival in the first year of forest establishment in northern Mongolia [8]. Scientists believe that this situation will be exacerbated by the increasing global temperature and frequency/severity of droughts due to climate change [9,10]. Further, issues on unsustainable consumption of groundwater resources are co-occurring with afforestation and ecological restoration projects in arid regions [2]. For example, increased evapotranspiration, consumption, and extraction of groundwater resources has already been linked to increased vegetation cover, especially in the initial stages of afforestation [11,12]. Afforestation efforts in arid and semiarid areas, thus, face a greater challenge in ensuring the higher survival rate and growth of the newly planted seedlings during the early stage of the forest establishment, while sustainably extracting groundwater resources. Therefore, the adaptation and response mechanisms of plants to different watering regimes and investigation on the optimal irrigation regime for effective and sustainable afforestation in arid and semiarid zones have recently received much attention [13–15].

Some plants have evolved morpho-physiological mechanisms (e.g., thickened leaves, reduced leaf size) that enable them to survive and flourish in areas with adverse ecological conditions (e.g., drought) [16–19]. Physiologically, a photoinhibitory effect has long been attributed to a significant reduction in the total chlorophyll content of two xerophytic shrubs, and this effect further resulted in the limitation on carbon assimilation [20]. The authors further reported that the photosynthetic rate of the species decreased as the leaf water potential ( $\psi$ ) decreased significantly from  $-0.2$  to  $-2.1$  MPa. Several studies have shown that drought-tolerant species remain turgid even at more negative water potentials and the critical physiological processes can still normally function, including photosynthetic gas exchange and leaf hydraulic conductance [21–23]. Further, leaf morpho-physiological traits mentioned above vary with gradients of rainfall or water availability depending on plant taxa and life-history traits [24,25]. Thus, further studies on the responses of leaf morpho-physiological traits to irrigation variability are needed for many woody species thriving in arid and semiarid habitats, such as *Tamarix ramosissima* Ledeb., *Ulmus pumila* L., *Elaeagnus moorcroftii* Wall. ex Schldt., and *Hippophae rhamnoides* L. These species are some of the commonly used woody species in afforestation and reforestation projects in arid and semiarid environments [26–30]. Therefore, it is necessary to study their morpho-physiological responses to varying water/irrigation levels to better understand their suitability to afforestation programs in arid and semiarid regions, such as Mongolia.

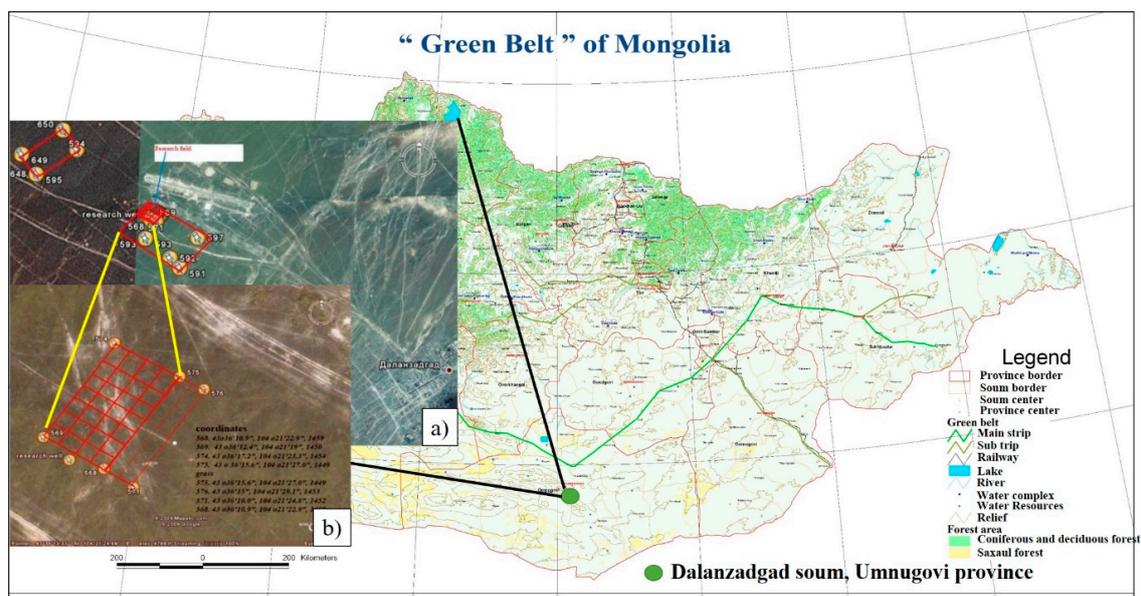
Ecologically, Mongolia is a typical forest-poor arid and semiarid region in Asia, characterized by low precipitation and highly fragile landscapes [31]. While it is an important part of the global biomes, the country suffers from serious desertification that makes success of afforestation or ecological restoration projects a great challenge. The Food and Agriculture Organization (FAO) reported that the annual air temperature in Mongolia has increased by about  $1.6$  °C and the droughts encompassing 50% of the country could happen every 4–5 years, which are attributed to global warming and climate change [32]. These adverse situations explained the higher mortality rate (40%) observed from the annual reforestation of  $4000$  ha year<sup>-1</sup> of degraded lands in the country [32]. The FAO further attributed the low survival rate of seedlings, not only to the harsh environment, but also to the poor quality of seedlings, inadequate site preparation, and poor planting designs. Further, despite the serious problem on water shortage in the country, a very limited number of studies dealing with growth and morpho-physiological responses of trees to irrigation regimes have already been conducted to date. Therefore, studies such as ours are of high significance to Mongolia, or the other arid regions, for effective optimization of an irrigation regime for sustainable afforestation projects.

In this study, we established 16 experimental plots at the Korea–Mongolia joint “Green Belt” plantation in Dalanzadgad soum, Umnugobi province, Mongolia to (1) investigate the effect of the different irrigation regimes on the growth and leaf morpho-physiological responses of four contrasting tree species planted in the desert region of Mongolia; (2) determine the irrigation regime which resulted in better plant survivability and plant growth; (3) determine the leaf morpho-physiological responses or stress indicators that are highly responsive to irrigation regimes. Based on the adverse ecological condition of an arid environment, we hypothesized that (1) a higher amount of irrigation + rainfall treatments would exhibit higher survival rate, height and root collar diameter (RCD) growth, and a slower decline in water potential compared with the rainfall alone or control treatment in all species; (2) the morpho-physiological responses are similar across treatments regardless of species.

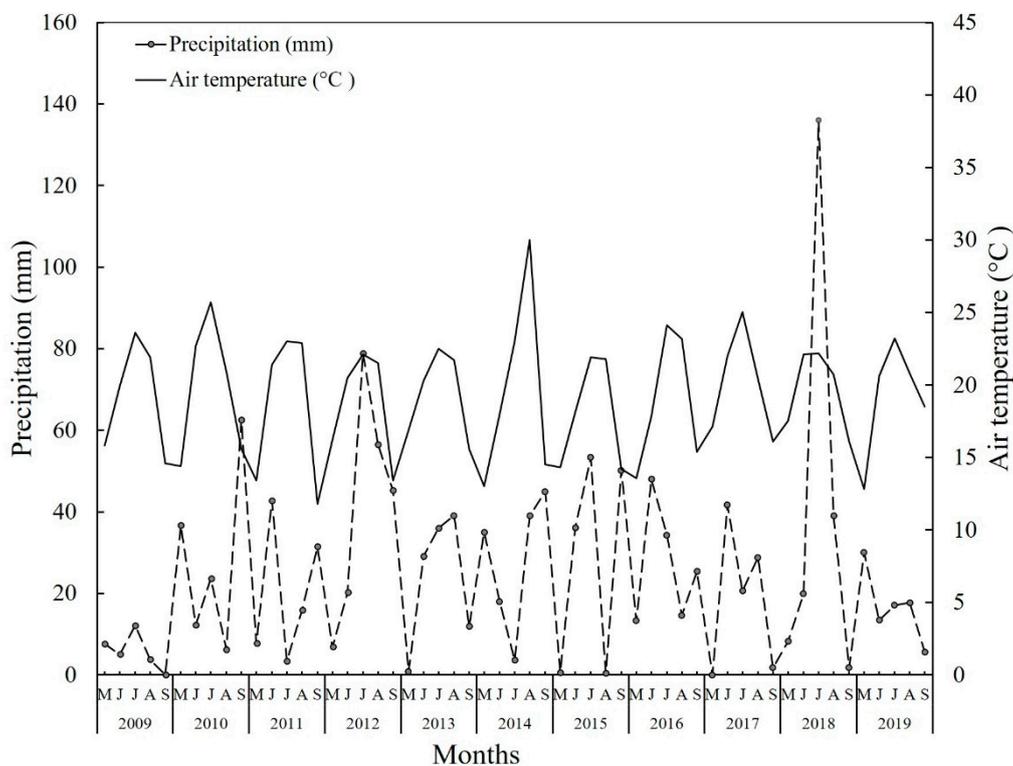
## 2. Materials and Methods

### 2.1. Experimental Site

The study was conducted at the Korea–Mongolia joint “Green Belt” plantation established in Dalanzadgad soum, Umnugobi province, Mongolia. It is about 575 km south of Ulaanbaatar ( $43^{\circ}36'12.74''$  N,  $104^{\circ}21'22.25''$  E), with an elevation of 1468 m above sea level (Figure 1). The area is a desert-steppe characterized by grassland plains or mainly feather grass-onion community with pea shrubs. The dominant species (i.e., 59.7%) includes *Oxytropis aciphylla* Ldb., *Convolvulus ammannii* Desr., *Peganium nigellastrum* Bge, and annual plants, such as *Artemisia pectinata* Pall., *Chenopodium aristatum* L., *Eragrostis minor* Host., *Enneapogon desvauxii* P. Beauv. [33,34]. These species are growing in the area in association with *Allium polyrrhizum* Turcz. ex Rgl, *A. mongolicum* Rgl, *Stipa gobica* Roshev., and *S. glareosa* P. Smirn. The vegetative season usually starts at the onset of the summer rains in May or June and stops at the end of September, with the first frost events [35]. During the growing season months, the mean temperature was  $19.2^{\circ}\text{C}$  and the mean precipitation was 28–200 mm based on our observation using the HOBO climate data logger (Figure 2). The highest mean temperature (i.e.,  $23.1^{\circ}\text{C}$ ) and precipitation (i.e., 130 mm) were detected in July, while the lowest mean temperature (i.e.,  $14.9^{\circ}\text{C}$ ) and precipitation (i.e., 0 mm) during the growing season were in May and September, respectively (Figure 2). Throughout the year, the mean annual temperature and precipitation were  $5.7^{\circ}\text{C}$  and 100–150 mm, respectively (Figure S1).



**Figure 1.** (a) Location and (b) close-up aerial photo of the experimental site within the Green belt plantation in Dalanzadgad soum, Umnugobi province, Mongolia.



**Figure 2.** Mean monthly air temperature (solid line) and precipitation (broken, dotted line) in the experimental site during the growing season (i.e., May–September 2009–2019).

The soil in the study site is classified as Gobi brown soil (Calcisol), which is generally low in organic content, stony, sandy loamy, and high in carbonate content. The occurrence of soil with variable properties in topsoil thickness, presence of stones or gravels, and carbon content is also evident within the experimental site [34].

## 2.2. Experimental Design

We used two-year-old nursery-grown seedlings of four different woody species, namely, *Tamarix ramosissima* Ldb., *Ulmus pumila* L., *Elaeagnus moorcroftii* Wall. ex Schlecht., and *Hippophae rhamnoides* L. The initial root collar diameter (RCD) and the height of the 576 seedlings of the four species were measured (Table 1). Thereafter, they were planted into previously prepared round holes (60–70 cm deep, 50–60 cm diameter), corresponding to different irrigation regimes. All seedlings were first irrigated using the same amount of water regardless of the treatments for 1 month during the acclimatization period. Thereafter, drip irrigation treatments were then applied using the four different irrigation regimes: rainfall (control), rainfall + 84 mm tree<sup>-1</sup> yr<sup>-1</sup> (4 L h<sup>-1</sup>), rainfall + 168 mm tree<sup>-1</sup> yr<sup>-1</sup> (8 L h<sup>-1</sup>), rainfall + 252 mm tree<sup>-1</sup> yr<sup>-1</sup> (12 L h<sup>-1</sup>). The 5-h duration of watering was done twice a week for the entire vegetative season (May to mid-September). Irrigation was supplied to individual trees with two types of drip-emitters (Emitter with CNL Compensating Non-Leakage (CNL) Button Dripper), which was placed 10 cm from the stem of each species. For the for 4 and 8 L h<sup>-1</sup> treatments, we used drip-emitters capable of emitting 4 L (0.5 mm m<sup>-2</sup>) and 8 L (1.0 mm m<sup>-2</sup>) of water, respectively. These two drip-emitters were combined to emit 12 L (1.5 mm m<sup>-2</sup>) of water for the 12 L h<sup>-1</sup> treatment. The irrigation hose system was connected to a water tank with a capacity of 50 tons.

**Table 1.** The initial age and growth of the tree species used in the experiment.

Species	Age (Year)	Height (cm)	RCD (mm)	Root Length (cm)
<i>Tamarix ramosissima</i>	2	83.6	7.7	34.5
<i>Ulmus pumila</i>	2	51.4	4.4	37.2
<i>Elaeagnus moorcroftii</i>	2	52.1	5.3	33.5
<i>Hippophae rhamnoides</i>	2	39.4	3.8	21.9

Sixteen plots (25 × 12.5 m) were established following a 4-m distance between trees and a 2-m distance between rows. There were a total of 36 seedlings planted in each plot [29,34]. Further, replacement of dead seedlings in control plots was done in spring 2010 due to high mortality rate [36]. We made sure that the seedlings used for replacement were healthy and had similar height and RCD growth compared with the original seedlings.

### 2.3. Growth Measurements

The annual RCD and height were regularly measured every end of each vegetative season (i.e., September) for 10 years (i.e., 2009–2019). The RCD was measured at 5 cm above the base of the stem using a digital Vernier caliper, while the height was determined by measuring the length of the highest shoot from the base of the stem of each seedling of each species. At the end of the experimental period, relative growth rate (i.e.,  $RGR = \frac{\text{Growth}_2 - \text{Growth}_1}{\text{Time}_2 - \text{Time}_1}$ ) across treatments was computed for each species based on the height and RCD values relative to those at initial planting.

### 2.4. Measurements of Morpho-Physiological Responses

In this study, 720 healthy and fully expanded leaves (i.e., 20 leaves × 3 replicates × 3 treatments × 4 species) were used in the determination of leaf area (LA), specific leaf area (SLA), and leaf biomass (LB). Leaf samples were sealed in plastic bags and stored in a cold container to avoid water loss during transport until further processing in the laboratory. The leaves were photo-scanned using an HP LaserJet scanner (M1132 MFP, Idaho, USA) with a 600 dpi resolution. The LA of the samples was then analyzed using the ImageJ processing software, following the procedure in Schneider et al. [37]. In the case of scale-like leaves *T. ramosissima*, we detached the leaf samples from the stem very carefully and followed the same measurement and analysis procedure with that of the other species.

We measured the LB and SLA by weighing the leaves using the high precision electronic scale (d = 0.001 g, Discovery Semi-Micro and Analytical Balance, Ohaus Corp., Switzerland) after oven drying in a convection oven (OF-11E, Jeio Tech., Republic of Korea) at 65 °C for 3 days. The SLA was expressed as the ratio between LA and leaf dry mass (LDM) ( $\text{cm}^2 \text{g}^{-1}$ ) following the procedure and equation (i.e.,  $SLA = LA (\text{cm}^2)/LDM (\text{mg})$ ) in Li et al. [38].

A total of 36 trees (i.e., 3 trees × 3 replicates × 4 species) were randomly selected, from which 1–5 healthy, fully expanded and sun-exposed leaves were collected for chlorophyll content analysis. Leaf samples were first stored in an ice-cooled box until further analysis in the laboratory. Following the procedure in Kousar et al. [39], a known amount of leaf tissue (0.10 g) was ground and then suspended in 10 mL of 80% acetone, mixed well and kept at 4 °C overnight in the dark. The solution was centrifuged (5000 rpm, 2 min) and the supernatant was obtained. Thereafter, the absorbance was read at 663 and 646 nm in Spectrophotometer (Genesys 10S UV-Vis Thermo Fisher Science Inc, Madison, WI, USA). The total chlorophyll and chlorophyll *a* and *b* concentrations were calculated based on the equation used in Lichtenthaler and Wellburn and Wellburn [40,41], that is:

$$\begin{aligned}
 \text{Chlorophyll } a (C_a, \mu\text{g/mL}) &= 12.21 A_{663} - 2.81 A_{646} \\
 \text{Chlorophyll } b (C_b, \mu\text{g/mL}) &= 20.13 A_{646} - 5.03 A_{663} \\
 \text{Total chlorophyll } (C_{a+b}, \mu\text{g/mL}) &= C_a + C_b \\
 \text{Chlorophyll } a/b \text{ ratio} &= C_a/C_b
 \end{aligned}
 \tag{1}$$

Predawn ( $\psi_p$ ) and midday ( $\psi_m$ ) leaf water potentials (MPa) were determined using a pressure chamber (Model 1505D EXP, PMS Instrument Company, Albany, OR, USA) in fully expanded sun-exposed apical leaves (c.a., 1.5 m above the ground), following the procedures in Scholander et al. [42]. Here the  $\psi_p$  and  $\psi_m$  were assumed to represent the daily maximum of turgor condition and the daily minimum of turgor condition, respectively. Randomly, we selected three healthy trees, which were replicated three times, from each treatment and species for  $\psi$  measurement.

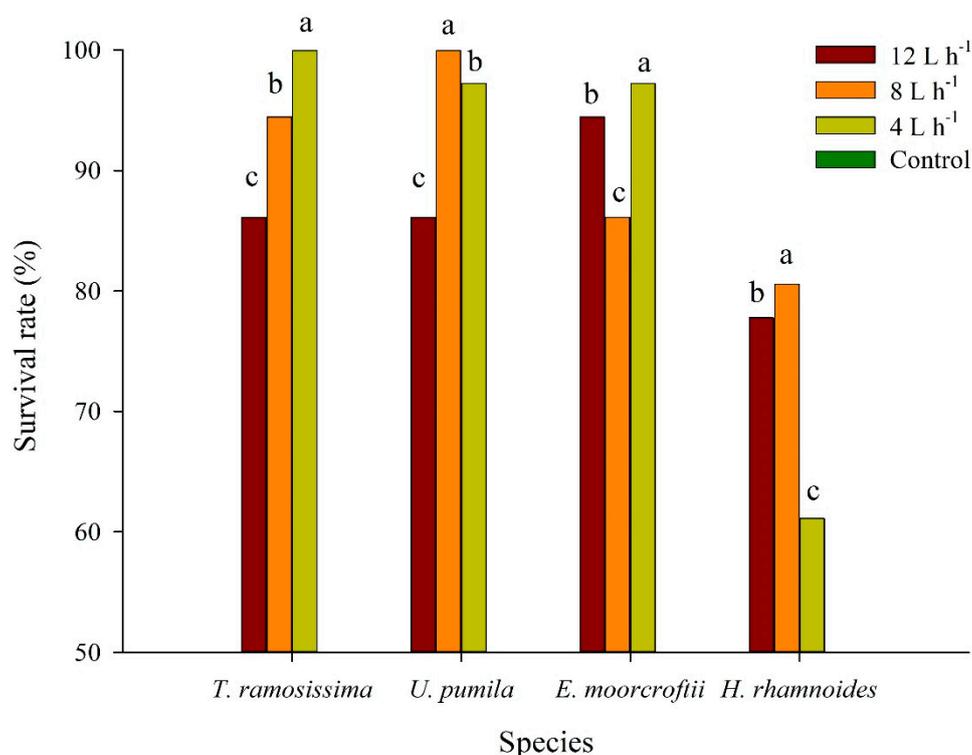
### 2.5. Statistical Analysis

All the statistical analyses were performed using the Statistical Analysis Software (SAS) package [43]. Repeated measures analysis of variance (RM ANOVA) was carried out to evaluate the significant difference in the treatment effects on height and RCD growth across time for each species. One-way ANOVA was run for each species to determine the effects of different irrigation treatments on the other parameters measured. Duncan's multiple range test (DMRT) was used to evaluate multiple comparisons among the treatments. The graphs were produced using the SigmaPlot software [44].

## 3. Results

### 3.1. Survival Rate across Irrigation Treatments

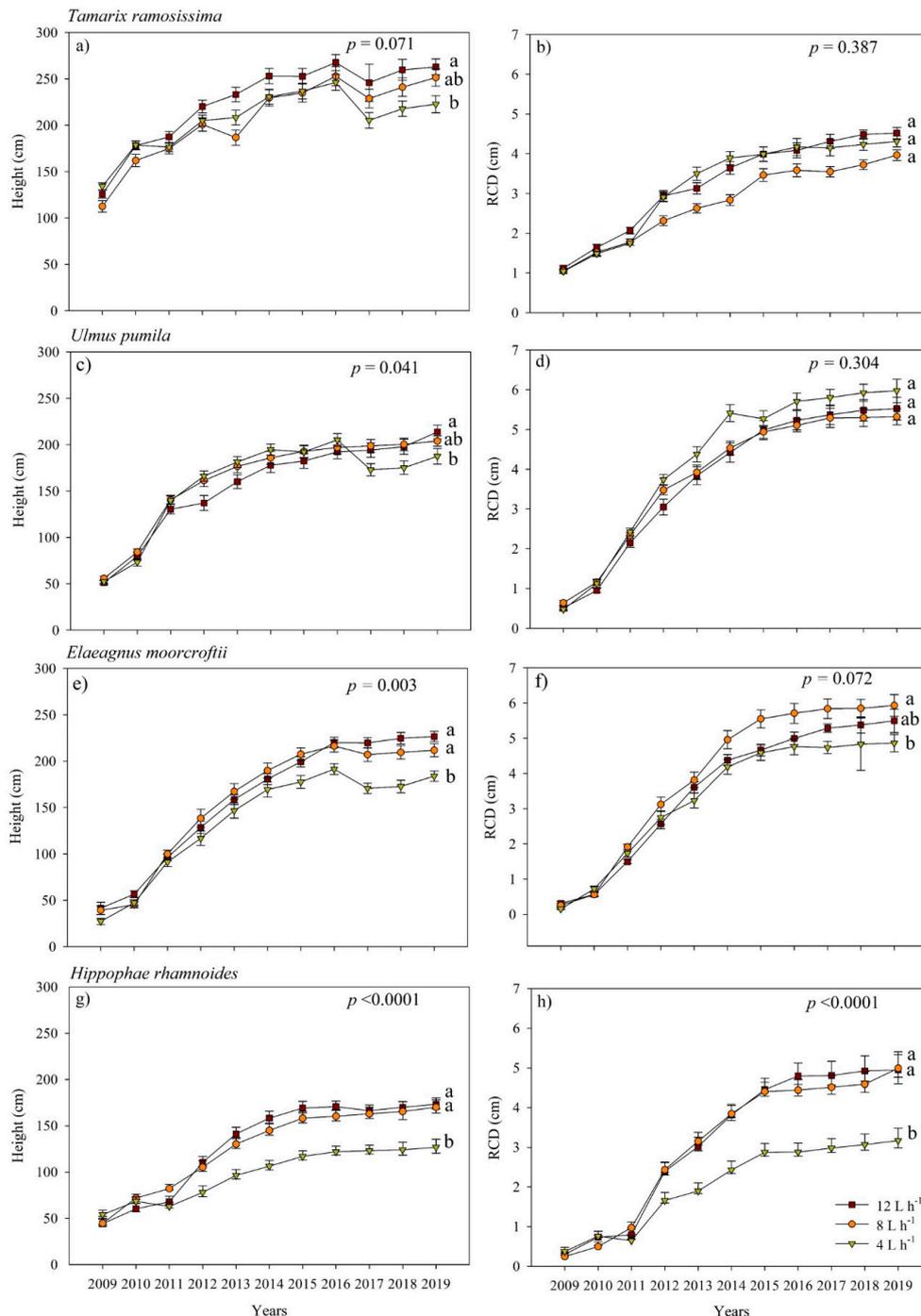
As expected, trees at irrigation + rainfall treatments showed a significantly higher survival rate compared with control in the last growing season, but the rate significantly varied by treatment in all species (Figure 3). All trees at rainfall alone treatment (control) showed 100% mortality. Generally, trees grown under  $12 \text{ L h}^{-1}$  had the lowest survival rate, while the highest was observed from those under 4 and/or  $8 \text{ L h}^{-1}$  for *T. ramosissima* ( $4 \text{ L h}^{-1} > 8 \text{ L h}^{-1} > 12 \text{ L h}^{-1}$ ), *U. pumila* ( $8 \text{ L h}^{-1} > 4 \text{ L h}^{-1} > 12 \text{ L h}^{-1}$ ), and *E. moorcroftii* ( $4 \text{ L h}^{-1} > 12 \text{ L h}^{-1} > 8 \text{ L h}^{-1}$ ). Survival rates of *T. ramosissima*, *U. pumila*, and *E. moorcroftii* ranged from 92% to 94% on average across the treatments. *H. rhamnoides* seedlings had only an average of <80% survival rate after the treatments.



**Figure 3.** Survival rate of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii* and *Hippophae rhamnoides* across different irrigation treatments (i.e., control, 12, 8, and  $4 \text{ L h}^{-1}$ ) measured in 2019. The control had 100% mortality rate in all species. Different lowercase letters within each species indicate significant differences among the treatments at  $\alpha = 0.05$ .

### 3.2. Root Collar Diameter (RCD) and Height Growth

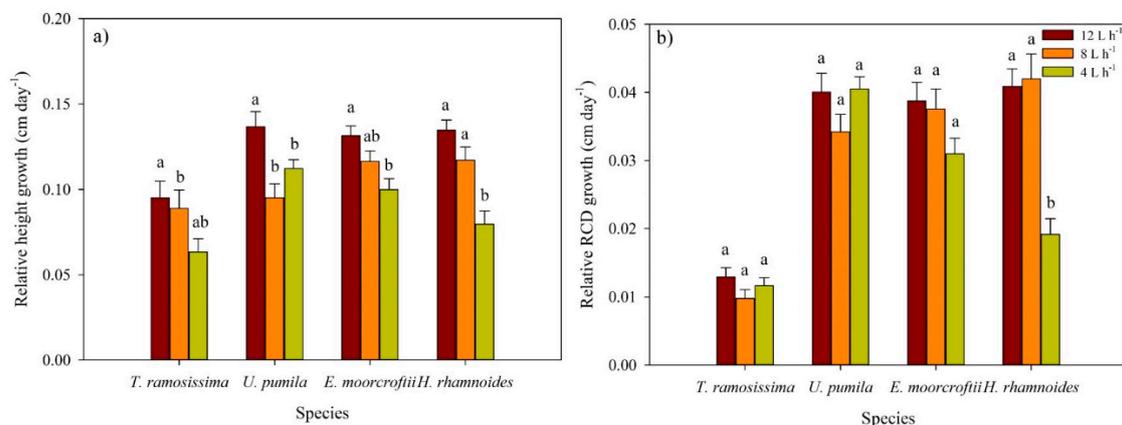
The effect of the irrigation treatments on the annual RCD growth of *T. ramosissima* and *U. pumila* was generally similar across growing seasons (Table S1, Figure 4). In the case of *E. moorcroftii* and *H. rhamnoides*, significant treatment effects on RCD were first detected in the second to third growing seasons (Table S1, Figure 4). The pattern of effects of the treatments on annual height growth were generally consistent across years (2009–2019), such that 4 and/or 8 L h<sup>-1</sup> treatments had lower annual height growth compared with that of 12 L h<sup>-1</sup> in all species, except for *U. pumila*.



**Figure 4.** Annual growth pattern of plant height (cm) and root collar diameter (RCD, cm) of (a,b) *Tamarix ramosissima*, (c,d) *Ulmus pumila*, (e,f) *Elaeagnus moorcroftii*, and (g,h) *Hippophae rhamnoides* across irrigation treatments (12, 8, and 4 L h<sup>-1</sup>). Different lowercase letters within each species indicate significant differences among the treatments at  $\alpha = 0.05$ . Vertical bars represent standard errors.

At the end of the experiment, there were significant species, treatment, and species  $\times$  treatment effects on the RCD growth (Figure 4, Tables S2 and S3). The RCD growth was generally similar across the treatments for *T. ramosissima*, *U. pumila*, and *E. moorcroftii*, while that for *H. rhamnoides* was significantly higher at both 8 and 12 L h<sup>-1</sup> compared with 4 L h<sup>-1</sup>. The height growth also varied significantly across treatments, except for *T. ramosissima* in 2019 (Figure 4, Tables S2 and S3). Generally, 8 and 12 L h<sup>-1</sup> had a similar higher effect on height growth compared with 4 L h<sup>-1</sup>, especially in *E. moorcroftii*, and *H. rhamnoides*.

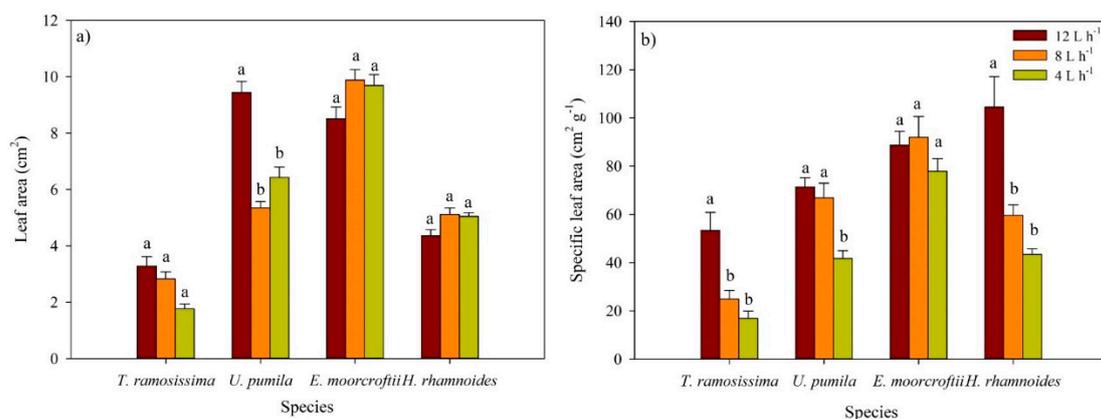
The relative RCD growth increments were similar across the irrigation treatments in *T. ramosissima*, *U. pumila*, and *E. moorcroftii*, but it varied significantly for *H. rhamnoides*, i.e., 8 and 12 L h<sup>-1</sup> had a similar higher effect than that of 4 L h<sup>-1</sup> (Figure 5). In contrast, the relative height varied considerably across the treatments in all species, such that trees planted at 12 L h<sup>-1</sup> showed the highest values (i.e., 0.10–0.13 cm day<sup>-1</sup>).



**Figure 5.** (a) Relative height growth rate (cm day<sup>-1</sup>) and (b) relative root collar diameter growth rate (cm day<sup>-1</sup>) of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across different irrigation treatments (12, 8, 4 L h<sup>-1</sup>) measured from the period of 2009–2019 growing seasons. Different lowercase letters within each species indicate significant differences among the treatments at  $\alpha = 0.05$ . Vertical bars represent standard errors.

### 3.3. Leaf Area and Biomass and Specific Leaf Area across Irrigation Treatments

The leaf area (LA) was not statistically different across the treatments, except in *U. pumila* (Figure 6a). In this species, the effect of 12 L h<sup>-1</sup> was significantly greater than that of 8 or 4 L h<sup>-1</sup>. The pattern of the effect of the irrigation treatments on specific leaf area (SLA) was generally similar in all species, i.e., 12 L h<sup>-1</sup> resulted in the highest SLA (range: 53.34–104.48 cm<sup>2</sup> g<sup>-1</sup>) among the three treatments (range: 16.84–99.99 cm<sup>2</sup> g<sup>-1</sup>) (Figure 6b).



**Figure 6.** (a) Leaf area (LA) and (b) specific leaf area (SLA) of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across different irrigation treatments (12, 8, 4 L h<sup>-1</sup>) measured in July 2016. Different lowercase letters within each species indicate significant differences among the treatments at  $\alpha = 0.05$ . Vertical bars represent standard errors.

### 3.4. Changes in Leaf Water Potential across Irrigation Treatments

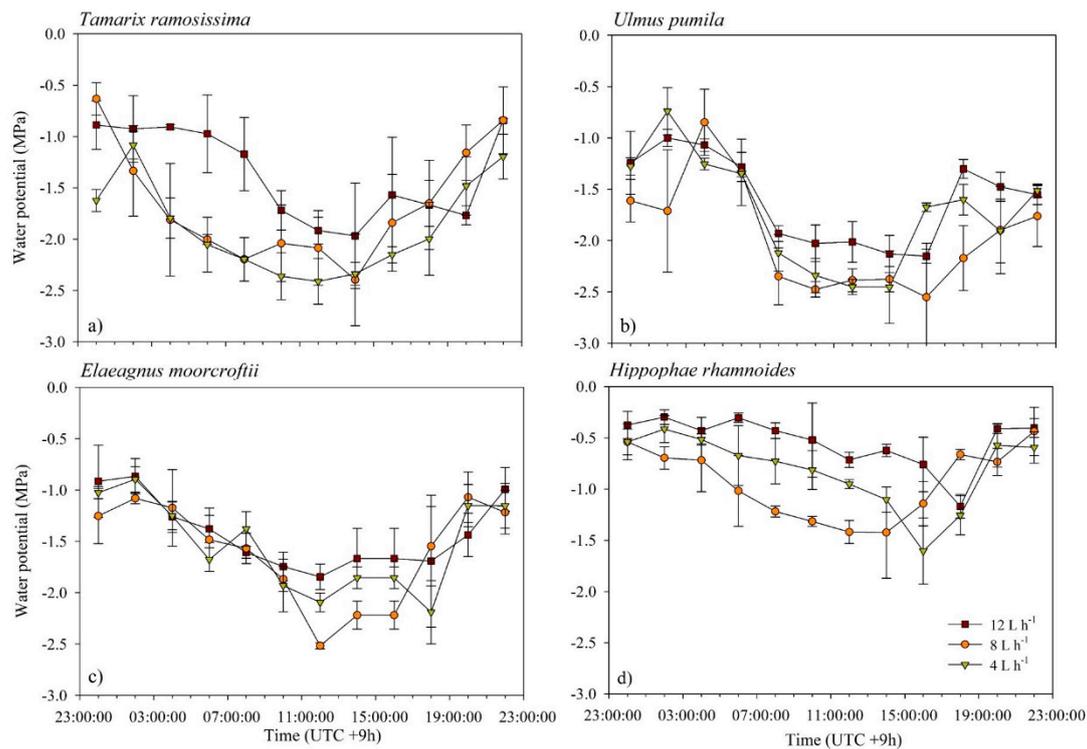
Generally, the irrigation treatments significantly affected the predawn ( $\Psi_p$ ) and mid-day ( $\Psi_m$ ) leaf water potential of the species, but the significance of the effects differed by month (Table 2). The leaf  $\Psi_p$  of 4 L h<sup>-1</sup> of *T. ramosissima* and *U. pumila* was found to be the lowest (i.e., more negative) in June (−0.673 MPa) and September (−1.064 MPa), respectively. In contrast, the highest leaf  $\Psi_p$  was observed at 12 L h<sup>-1</sup> among the treatments in the said species and months, that is, −0.380 MPa for *T. ramosissima* and −0.618 MPa for *U. pumila*. In terms of leaf  $\Psi_m$ , 4 L h<sup>-1</sup> generally decreased significantly in August for *T. ramosissima* and July and September for *U. pumila*. Overall, both  $\Psi_p$  and  $\Psi_m$  tended to decrease progressively from June to August in all species. The hottest month in the study area is July, and our study revealed that the effects of irrigation treatments on  $\Psi_p$  during this month were similar across the treatments in almost all species (Figure 7; Table 2).

**Table 2.** Seasonal variation of the predawn ( $\psi_p$ ) and midday ( $\psi_m$ ) leaf water potentials in *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across different irrigation treatments measured in 2016. Different lowercase letters indicate significant differences among the treatments at  $\alpha = 0.05$ .

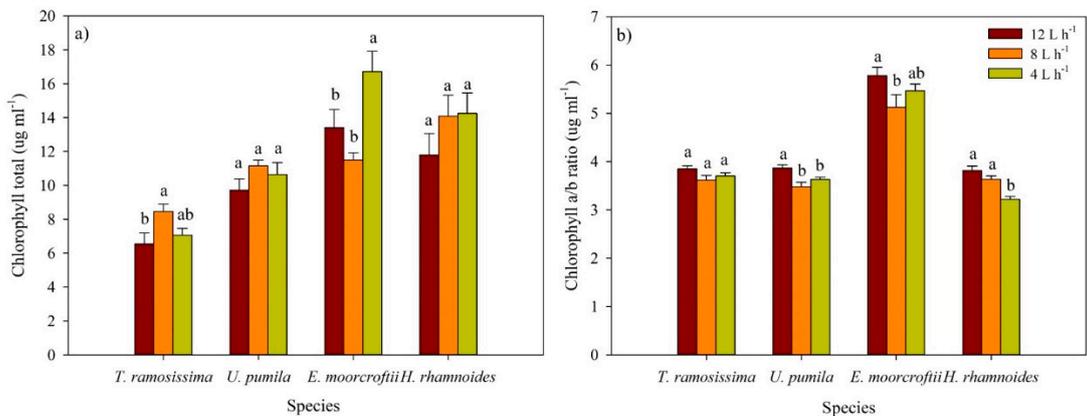
Species	Treatment	June	July	August	September	
<i>Tamarix ramosissima</i>	$\psi_p$	12 L h <sup>-1</sup>	−0.38 <sup>a</sup>	−0.91 <sup>a</sup>	−0.77 <sup>a</sup>	−0.98 <sup>a</sup>
		8 L h <sup>-1</sup>	−0.45 <sup>ab</sup>	−1.81 <sup>a</sup>	−0.84 <sup>a</sup>	−0.94 <sup>a</sup>
		4 L h <sup>-1</sup>	−0.67 <sup>b</sup>	−1.79 <sup>a</sup>	−1.12 <sup>a</sup>	−0.67 <sup>a</sup>
	$\psi_m$	12 L h <sup>-1</sup>	−1.05 <sup>a</sup>	−1.91 <sup>a</sup>	−1.28 <sup>a</sup>	−2.38 <sup>a</sup>
		8 L h <sup>-1</sup>	−1.23 <sup>a</sup>	−2.08 <sup>a</sup>	−1.45 <sup>a</sup>	−1.84 <sup>a</sup>
		4 L h <sup>-1</sup>	−1.76 <sup>a</sup>	−2.33 <sup>a</sup>	−2.59 <sup>b</sup>	−1.84 <sup>a</sup>
<i>Ulmus pumila</i>	$\psi_p$	12 L h <sup>-1</sup>	−0.31 <sup>a</sup>	−1.07 <sup>a</sup>	−0.39 <sup>a</sup>	−0.61 <sup>a</sup>
		8 L h <sup>-1</sup>	−0.39 <sup>a</sup>	−0.84 <sup>a</sup>	−1.01 <sup>a</sup>	−0.96 <sup>ab</sup>
		4 L h <sup>-1</sup>	−0.51 <sup>a</sup>	−1.25 <sup>a</sup>	−0.56 <sup>a</sup>	−1.06 <sup>b</sup>
	$\psi_m$	12 L h <sup>-1</sup>	−1.62 <sup>ab</sup>	−2.13 <sup>a</sup>	−2.52 <sup>a</sup>	−1.88 <sup>a</sup>
		8 L h <sup>-1</sup>	−1.89 <sup>b</sup>	−2.37 <sup>ab</sup>	−1.97 <sup>a</sup>	−1.86 <sup>a</sup>
		4 L h <sup>-1</sup>	−1.29 <sup>a</sup>	−2.45 <sup>b</sup>	−2.13 <sup>a</sup>	−2.64 <sup>b</sup>
<i>Elaeagnus moorcroftii</i>	$\psi_p$	12 L h <sup>-1</sup>	−0.27 <sup>a</sup>	−1.26 <sup>a</sup>	−0.27 <sup>a</sup>	−0.31 <sup>a</sup>
		8 L h <sup>-1</sup>	−0.37 <sup>a</sup>	−1.17 <sup>a</sup>	−0.37 <sup>a</sup>	−0.79 <sup>a</sup>
		4 L h <sup>-1</sup>	−0.40 <sup>a</sup>	−1.25 <sup>a</sup>	−0.40 <sup>a</sup>	−0.71 <sup>a</sup>
	$\psi_m$	12 L h <sup>-1</sup>	−1.20 <sup>a</sup>	−1.84 <sup>a</sup>	−0.99 <sup>a</sup>	−1.91 <sup>a</sup>
		8 L h <sup>-1</sup>	−1.35 <sup>a</sup>	−2.51 <sup>a</sup>	−1.11 <sup>a</sup>	−2.23 <sup>a</sup>
		4 L h <sup>-1</sup>	−1.09 <sup>a</sup>	−2.09 <sup>a</sup>	−1.09 <sup>a</sup>	−2.46 <sup>a</sup>
<i>Hippophae rhamnoides</i>	$\psi_p$	12 L h <sup>-1</sup>	−0.24 <sup>a</sup>	−0.42 <sup>a</sup>	−0.49 <sup>a</sup>	−0.22 <sup>a</sup>
		8 L h <sup>-1</sup>	−0.36 <sup>a</sup>	−0.71 <sup>a</sup>	−0.71 <sup>a</sup>	−0.35 <sup>a</sup>
		4 L h <sup>-1</sup>	−0.51 <sup>a</sup>	−0.51 <sup>a</sup>	−0.46 <sup>a</sup>	−0.54 <sup>a</sup>
	$\psi_m$	12 L h <sup>-1</sup>	−0.97 <sup>a</sup>	−0.71 <sup>a</sup>	−1.18 <sup>a</sup>	−1.68 <sup>a</sup>
		8 L h <sup>-1</sup>	−0.80 <sup>a</sup>	−1.41 <sup>b</sup>	−1.36 <sup>a</sup>	−2.31 <sup>a</sup>
		4 L h <sup>-1</sup>	−1.64 <sup>a</sup>	−0.95 <sup>a</sup>	−1.57 <sup>a</sup>	−2.37 <sup>a</sup>

### 3.5. Variation in Chlorophyll Content

As shown in Figure 8a, a significant variation in the total chlorophyll content was detected across the treatments in some species, particularly in *T. ramosissima* (i.e., 8 L h<sup>-1</sup> > 4 L h<sup>-1</sup> > 12 L h<sup>-1</sup>) and *E. moorcroftii* (i.e., 4 L h<sup>-1</sup> > 8 L h<sup>-1</sup> = 12 L h<sup>-1</sup>). Respectively, these two species were also the ones with the lowest (range: 6.53–8.45  $\mu\text{g mL}^{-1}$ ) and highest (range: 11.50–16.71  $\mu\text{g mL}^{-1}$ ) total chlorophyll content among the four species in all the treatments. The chlorophyll a/b ratio also varied considerably across the treatments, except in *T. ramosissima* (Figure 8b). Generally, trees under 12 L h<sup>-1</sup> had a higher chlorophyll a/b ratio compared with the other treatments.



**Figure 7.** Diurnal variation in leaf water potential ( $\Psi$ ) of (a) *Tamarix ramosissima*, (b) *Ulmus pumila*, (c) *Elaeagnus moorcroftii*, and (d) *Hippophae rhamnoides* across different irrigation treatments (12, 8, and 4 L h<sup>-1</sup>) measured in July 2016. Vertical bars represent standard errors.



**Figure 8.** (a) Total chlorophyll content and (b) chlorophyll a/b ratio of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across different irrigation treatments (12, 8, 4 L h<sup>-1</sup>) measured in July 2016. Different lowercase letters within each species indicate significant differences among the treatments at  $\alpha = 0.05$ . Vertical bars represent standard errors.

#### 4. Discussion

##### 4.1. Effects of Different Levels of Irrigation on Plant Survivability and Growth of Four Contrasting Species

As expected, trees grown in rainfall alone treatment (control) would have the highest mortality rate compared with those in rainfall + irrigation treatments, which support the report of Zhang et al. [2] that millions of newly planted trees in arid and semiarid areas in Mongolia could hardly survive due to adverse ecological conditions, particularly water shortage or short-lived rainfall events. Dying of newly germinated seedlings has long been observed in revegetation activities in arid and semiarid regions [45]. Here we found that survival rate was generally higher in trees grown in 4 and/or 8 L h<sup>-1</sup> compared with that

in  $12 \text{ L h}^{-1}$ , except for *H. rhamnoides* which tended to survive better in 8 and/or  $12 \text{ L h}^{-1}$  than in  $4 \text{ L h}^{-1}$  treatment. This suggests that these four species can respond to varying water availability, consistent with the results of the previous studies [46–48].

In terms of growth performance, we showed that when trees underwent different irrigation regimes, all showed increments in height and RCD, although the observed RCD growth was generally similar across the treatments and years. This result is expected because, in general, height is more responsive to fluctuations in resource availability, increases more rapidly than RCD when a tree is young, and tends to slow down with age based on the hydraulic limitation hypothesis [49,50]. This implies that the growth pattern of the three species conforms to the general pattern of trees growing in normal conditions. Therefore, results further imply that a normal growth pattern for the three species can already be achieved by supplying only  $4 \text{ L h}^{-1}$  for the three species.

The relative height varied significantly across the treatments in most of the studied species, i.e., trees planted at  $12 \text{ L h}^{-1}$  tended to grow taller than those at the other treatments. Although they grew taller, trees under  $12 \text{ L h}^{-1}$  had relatively lower survival rate compared with the other irrigation regimes, and this is evident throughout the growing seasons. These results can be explained by life-history theory, which states that plants grown or adapted to the low-resource environment tend to exhibit low phenotypic plasticity, thereby growing slowly even if the ideal resource supply is available because they divert resources to other functions, particularly storage and defense [51]. Consequently, trees grown under  $4 \text{ L h}^{-1}$  regime may have conserved resources under limited water supply, which may have resulted in higher survival rate at the expense of vegetative growth, whereas trees grown under  $12 \text{ L h}^{-1}$  may have invested more on the vegetative growth rather than on storage and mechanical functions under high-water availability condition. Ryan and Yoder [50] had long concluded that plants in resource-poor environments (e.g., a lower amount of water) have greater hydraulic resistance for a given tree height than plants in resource-rich environments. Moreover, trees of *T. ramosissima*, *U. pumila*, and *E. moorcroftii* grown under  $4 \text{ L h}^{-1}$  have shown increases in height in as early as first growing season, but generally declined starting from 2016, which could indicate that they have traded their present growth investment to increased longevity and future reproductive capacity as they grow with age. This gradual decline in height growth seems to be favorable for tree survival of these three species even with low amount of water supply because slow shoot growth requires less amount of water, thereby decreasing transpiration rate. The result could also exemplify the dormancy in growth, which is a characteristic typical of plants growing in arid regions, such as the xerophytes or drought evaders [52,53].

#### 4.2. Responses of Morpho-Physiological Traits of Four Contrasting Species to Different Irrigation Regimes

In this study, the LA of the broad-leaved *U. pumila* varied significantly across the irrigation treatments, i.e., 8 or  $4 \text{ L h}^{-1}$  had significantly lower LA compared with  $12 \text{ L h}^{-1}$ . This result is consistent with the study of Liu and Dickmann [54] who found that a lower amount of soil water resulted in a decreased number of expanded leaves. The authors cited that the drought-induced decrease in LA was merely due to reduced rates of cell division and cell enlargement [19]. Trees under 8 or  $4 \text{ L h}^{-1}$ , however, have shown higher survival rate compared with those in  $12 \text{ L h}^{-1}$ . From a physiological viewpoint, such a result can be due to minimization of water loss by transpiration and risk of xylem embolism in leaves. The present study, therefore, suggests that the LA growth of *U. pumila* may be highly dependent on water availability. Here we also found that the LA of the shrub species (*T. ramosissima*, *E. moorcroftii*, and *H. rhamnoides*) did not vary significantly across the irrigation treatments, implying that these species may have a wide adaptability to water variability or fluctuations. Further, these shrubs may have a similar magnitude of phenotypic plasticity to varying irrigation regimes, resulting in similar LA across the treatments. Here all the species we used are commonly found tree species in arid and semiarid environments and have already adapted to harsh conditions [26, 30]. Consequently, we could logically speculate that these species may have responded

plastically to variable irrigation levels, by either reducing or increasing their total leaf area depending on the irrigation level and environmental conditions. Thus, our result agrees with the general strategies of plants in arid and semiarid habitats to cope with severe environmental pressures or oligotrophic conditions [55–57].

Here, a higher amount of irrigation resulted in higher SLA, particularly trees grown under 12 L h<sup>-1</sup>. Urban and Restrepo-Diaz [58] reported a similar result, that is, leaves of well-watered trees expand at a much faster rate and tend to have higher SLA compared with those leaves produced during a period of limited water supply. SLA is a good proxy of plant growth because it can determine how much new leaf area to be allocated for each unit of biomass produced. Thus, high-SLA leaves will usually enhance plant growth; however, studies have shown that high SLA may trade-off plant longevity and tolerance to abiotic stress [59–61]. Consequently, this could be another explanation to the observed lowest survival rate of trees grown under 12 L h<sup>-1</sup>, particularly for *T. ramosissima*, *U. pumila*, and *E. moorcroftii*. This result can be further supported by the result on LB, i.e., 8 and/or 4 L h<sup>-1</sup> generally resulted in higher LB compared with 12 L h<sup>-1</sup> in all species (Figure S2), which is expected because of the higher survival rate of trees in the former treatments than those in the latter treatment. The reduction in plant leaf biomass of trees grown under 12 L h<sup>-1</sup> may have been due to lower leaf mass growth or higher SLA, which means a lower allocation of biomass to thicker leaves or mechanical leaf tissues. Leaf mass, a leaf morphological trait, has already been regarded as a good indicator of carbon gain, leaf chemical composition, photosynthesis rate, and resource acquisition rate [62,63]. Moreover, several studies have shown a strong positive correlation of leaf mechanical strength and stiffness with leaf mass growth across different species and communities [64–66]. This assumption can be supported by our result on total chlorophyll content, that is, 4 and/or 8 L h<sup>-1</sup> had significantly higher chlorophyll content than 12 L h<sup>-1</sup>, particularly in *T. ramosissima* and *E. moorcroftii*. Studies have shown that thicker leaves generally have higher chlorophyll and N contents and photosynthetic enzymes per unit leaf area, thereby improving the net photosynthetic rate [67,68]. Therefore, leaves of trees grown at 4 and/or 8 L h<sup>-1</sup> may be thicker than those in 12 L h<sup>-1</sup>, containing higher chlorophyll concentration or probably photosynthetic enzymes. It has long been reported that thicker leaves are associated with water deficits [69].

Lastly, the observed generally higher survival rate and LB and lower SLA (or higher leaf mass) at 4 L h<sup>-1</sup> compared with 12 and/or 8 L h<sup>-1</sup> for almost all species are reflective of the ubiquitous characteristic of plants typical of drylands. In our study, the  $\Psi_p$  and  $\Psi_m$  leaf water potentials were found the lowest (i.e., more negative) for trees subjected to 4 L h<sup>-1</sup> treatment, especially in *T. ramosissima* and *U. pumila* during the summer season, and this may have enabled the species to increase their photosynthesis rate amid decreasing  $\Psi$ . Model calculations have illustrated that decreasing leaf water potential, can reduce the transpiration rate by lowering the vapor pressure of water and can increase the solubility of CO<sub>2</sub>, thereby leading to increased leaf CO<sub>2</sub> assimilation rate [70]. The author further noted that the plant water use efficiency (WUE) was enhanced up to 10% at a leaf water potential of -2 MPa.

#### 4.3. Optimal Irrigation Regime for Each Woody Species Potentially Suitable for Effective and Sustainable Afforestation in Mongolia

In our study, it was revealed that 4 L h<sup>-1</sup>, which is an irrigation regime with the lowest amount of water among the treatments, was found to be the most appropriate irrigation regime for watering the seedlings of *T. ramosissima*, *U. pumila*, and *E. moorcroftii*, with 92%–94% survival rate. In the case of *H. rhamnoides*, we found that the irrigation regime suitable for the species are/is 8 and/or 12 L h<sup>-1</sup>, with <80% survival rate, which partly agrees with the study of Tian et al. [71]. Here we showed that 12 L h<sup>-1</sup> generally did not result in a higher survival rate and better plant growth for most of the studied species. Our result is, therefore, relevant to ensuring the sustainability of afforestation programs in arid and semiarid landscapes in Mongolia, by carefully determining the appropriate amount of water to be supplied to the newly planted seedlings. Amid climate change, water is

becoming increasingly scarce in Mongolia, and this scarcity is also becoming a critical impediment to afforestation efforts in the country, in which millions of newly planted seedlings struggle to survive [2,12]. In the initial stages of afforestation, water to be used for irrigation is normally sourced from groundwater, which could make the availability of water in the afforestation sites even more scarce. Several studies have also shown that successful afforestation projects could increase the consumption of groundwater resources due to increased vegetation cover and extraction of water from the ground [2,11,12]. Consequently, we could also say that consumption of groundwater resources in afforestation sites can be managed sustainably in the long term when trees have fully grown by knowing the optimal irrigation needs of each studied species, thereby ensuring the sustainability of afforestation programs in arid and semiarid areas in Mongolia.

In terms of the tree species potentially suitable to the arid condition, we found that the three species, *T. ramosissima*, *U. pumila*, and *E. moorcroftii* under the 4 L h<sup>-1</sup> irrigation regime have shown some leaf morpho-physiological responses that are indicative of plant characteristics growing in arid and semiarid habitats. A decrease in LA and SLA and the ability to recover from water deficit has already been reported for *T. ramosissima* as a rapid response to altered water availability [72]. The authors further implied that the observed trait in *T. ramosissima* may also facilitate the proliferation of the species in zones characterized by frequent groundwater fluctuations. A recent transcriptomic study has also detected distinct differential gene expression in flaccid leaves of *T. ramosissima* in response to water deficit [73]. Studies have also investigated the response of *U. pumila* to the different water table and drought stress treatments and reported some morpho-physiological traits that may provide advantages to the species in arid and semiarid zones (e.g., [74,75]). Specifically, the study of Xiongwen [76] showed that among the water table treatments (i.e., underwater, 3, 9, 15, and 22 cm distance from the soil surface to the water surface), *U. pumila* sapling grown under 9-cm and 15-cm treatments had higher net photosynthesis rate and increment in stem height than 3-cm, and 22-cm treatments, which partially agrees with our results. Lastly, the suitability of *E. moorcroftii* to 4 L h<sup>-1</sup> irrigation regime agrees with the study of Sun et al. [77] who reported a positive response of *E. angustifolia*, a synonym of *E. moorcroftii*, to heavy drought stress treatment.

## 5. Conclusions

In the present study, different irrigation regimes positively and negatively influenced the survivability, growth, and leaf morpho-physiology of the studied species, particularly *T. ramosissima*, *U. pumila*, and *E. moorcroftii*. Among the four treatments, 4 L h<sup>-1</sup> irrigation regime showed to be the most effective irrigation regime because it generally resulted in the positive effect on most of the parameters measured in *T. ramosissima*, *U. pumila*, and *E. moorcroftii*, whereas the 8 and/or 12 L h<sup>-1</sup> showed to be the most suitable for *H. rhamnoides*. Here we presented that higher amount of irrigation level generally did not result in better plant survival and growth performance, and most of the morpho-physiological traits were generally unresponsive to irrigation treatments, particularly in *T. ramosissima*, *U. pumila*, and *E. moorcroftii*. The morpho-physiological traits that were found responsive to varying irrigation regimes were SLA, total chlorophyll content, and leaf water potential ( $\Psi$ ), to which the higher survival rate of trees grown at 4 L h<sup>-1</sup> could be attributed. Under 4 L h<sup>-1</sup> irrigation regime, *T. ramosissima*, *U. pumila*, and *E. moorcroftii* have shown growth and morpho-physiological traits, which are considered typical characteristics of plants growing in xeric environments. Therefore, a constant application of the 4 L h<sup>-1</sup> irrigation regime could be employed to enhance and ensure the high survivability and growth performance of trees in the on-going and future afforestation efforts in Mongolia using the three potentially feasible tree species that were identified. Our findings are conducive to effective and sustainable afforestation in Mongolia, while also sustainably managing groundwater resources.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-445X/10/2/212/s1>, Figure S1: Mean annual temperature in the experimental site obtained from the period of January to December (2000–2019); Figure S2: Leaf biomass (LB) of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across different irrigation treatments; Table S1: Annual root collar diameter growth (RCD) and height of *Tamarix ramosissima*, *Ulmus pumila*, *Elaeagnus moorcroftii*, and *Hippophae rhamnoides* across treatments measured from 2009 to 2019; Table S2: *p* values estimated by two-way analysis of variance (ANOVA) for RCD and height growth across treatments and species; Table S3: Repeated measures analysis of variance for transformed heights and RCD from all measurement dates.

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