Vol. 62 No 03 (243) 2022
DOI: https://doi.org/10.5564/pmas.v62i03.2470
PMAS
Proceedings of the Mongolian Academy of Sciences
ARTICLE

A comparative study of biomass and morpho-physiological traits for different deciduous species in semi-arid afforestation region of Mongolia

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ARTICLE INFO: Received: 14 Oct, 2022; Accepted: 23 Dec, 2022

Abstract: Afforestation practices are useful tools for rehabilitating degraded lands in many parts of the world, as well as in Mongolia for protecting soil, water resources, and the potential of carbon sequestration to mitigate climate change. And so, regular investigation was conducted to determine the response and adaptation of *Populus sibirica*, *Ulmus pumila*, and *Hippophae rhamnoides* in terms of growth characteristics and leaf morpho-physiological traits to suggest and select effective and sustainable afforestation methods to suit Mongolia's conditions. We measured the root collar diameter (RCD), height growth, leaf area (LA), specific leaf area (SLA), leaf biomass (LB), chlorophyll concentration and leaf water potential (ψ) of the selected species. Results showed that *P. sibirica* (135.3±6.81cm) height growth, stem, root and total biomass were higher among the studied species, but leaf and branch biomass was higher in *U. pumila* (93.46±5.10cm). However, leaf morphological parameters and chlorophyll content was higher for *P. sibirica* (330.56±56.81µg/ml) compared with other species. Leaf water potential was found lower for *U. pumila* and higher in *H. rhamnoides*. Therefore, we suggest that *U. pumila* is more adaptable to low mean annual precipitation regions, requiring less water and *H. rhamnoides* provide good financial source for local community as they yield fruits. Our findings are relevant to ensuring the sustainability of afforestation programs in semiarid conditions in Mongolia.

Keywords: *afforestation; biomass; morpho-physiological traits; semi-arid;*

INTRODUCTION

Forests have important ecological functions and provide services to maintain life on land both at the local and global scales [1]. Forests cover only 7.9% of the entire territory of Mongolia, and 84.8% of the forests are boreal and 15.2% - Saxaul forest [2].

According to the national assessment of degradation and desertification, 76.9% of land area of the country have been degraded to some level, from which 23.3% were classified as heavily degraded and desertified [2].

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Several studies emphasized that land degradation and environmental changes caused by overgrazing have become one of the pressing issues in Mongolia [3], which results in increased greenhouse gas (GHG) emissions from agriculture [4]. Studies noted that afforestation is the most effective tool for mitigatiing global warming, especially in dry and semi-arid regions of the world [5].

In the context of Mongolian activity to mitigate climate changes, of particular importance is the establishment of new forest plantations under the Green Belt project and within other similar initiatives. From 2005 to 2017, the "Green Wall" National Program afforested areas in the Gobi-Steppe regions, and since 2008, the “Green Belt” project, which was financed by the Republic of Korea, roughly 3,000 hectares of areas in Umnugobi and Tuv provinces [6-7] were afforested. Since 2007, the “Green Asia Network” NGO planted more than 800,000 trees on 800 ha of afforestation areas in 10 soums of 4 aimags - provinces (Bulgan, Tuv, Dundgovi and Arkhangai) of Mongolia, which were affected by desertification and land degradation [8].

Several tree species were tested for the establishment of plantations and afforestation. The *Populus sibirica* Hort. ex. Tausch., *Ulmus pumila* L., *Tamarix ramosissima* Ledeb., *Elaeagnus moorcroftii* Wall. ex Schtdl, *Haloxylon ammodendron* C.A.Mey., and *Populus euphratica* Oliv., are considered as the most investigated for their adaptation to harsh climate and soil conditions in semi-arid and desert areas in Mongolia [7; 9-13].

Biomass related studies were carried out to understand how biomass is partitioned in *P. sibirica* and *U. pumila* used in afforestation and it was found that *U. pumilia* tends to be more resistant to drought, making it favorable for afforestation in the arid regions. *P. sibirica* is more sensitive to the lack of water or drought, but shows great potential in terms of biomass production, especially in aboveground biomass accumulation [14-15]. According to Bazarsad, the *U. pumila* is considered the most confident tree species that is well adapted to dry-steppe and semi-arid environments of Mongolia incorporated with the intensive development of root systems in the topsoil layer [16].

In addition, a comparison of the morphological and physiological characteristics of *P. sibirica* and *U. pumilia* revealed that they have significantly different response to the water environment, management of plantations and *U. pumila* is considered as a logical choice for use in semi-arid and even in mountainous regions of Mongolia [14; 17-25]. However, several studies have emphasized 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 [26-30]. Several studies on afforestation and plantation forests in the semi-arid regions of Mongolia show that a relatively higher survival of seedlings after transplantation [17] is possible with continuous watering (using a drip irrigation system).

The objectives of the study were (i) to perform a comparative analysis of biomass partitioning at selected plantation area for *U. pumila*, *P. sibirica* and *H. rhamnoides*; and (ii) to estimate morpho-physiological parameters of *U. pumila*, *P. sibirica* and *H. rhamnoides* in the semi-arid steppe region.

MATERIALS AND METHODS

Study site

The study was conducted at the “Green Asia Network” INGO plantation established in Argalant soum, Tuv aimag, Mongolia, 90 km west of Ulaanbaatar (N47°57'10.4", E105°53'11.8"), with an elevation of 1,234 m above sea level (Figure 1).

According to phytogeographical region, the area is described as the Middle Khalkha semi-arid steppe (dry steppe) region, dominated by *Agropyron cristatum, Artemisia adamsii, Artemisia frigida, Bassia prostrata, Elymus sibiricus, Heteropappus hispidus, Oxytropis myriophylla*, and *Poa attenuate* [31]. The soils in the study site are classified as Kastonozems, due to overgrazing in the study area, and different types of land degradation were observed, such as trampling, gully formation,

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and exposure to wind erosion [32-33]. 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. The mean air temperature of growing season was 20.3°C and the mean precipitation was 268 mm, according to Nukhurlul weather station (Figure 2). The highest mean temperature (i.e., 22.5°C) and precipitation (i.e., 146 mm) were detected in July, while the lowest mean temperature (i.e., 11.0°C) and precipitation (i.e., 0.2 mm) during the growing season were in May respectively (Figure 2) [34-35].

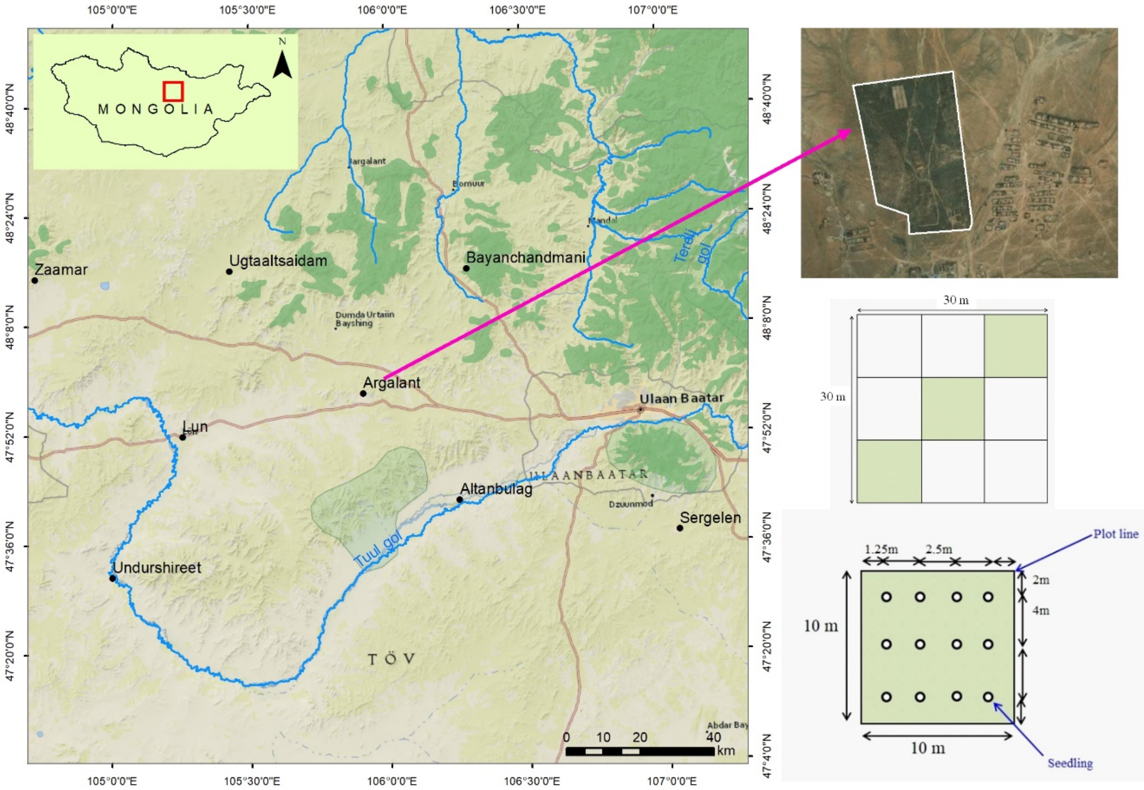

Figure 1. The location of study site, Argalant soum, Tuv aimag in central Mongolia. Planting scheme of the afforestation site (30✕30m). Green rectangular area (10✕10m) indicates the plot with 12 seedlings

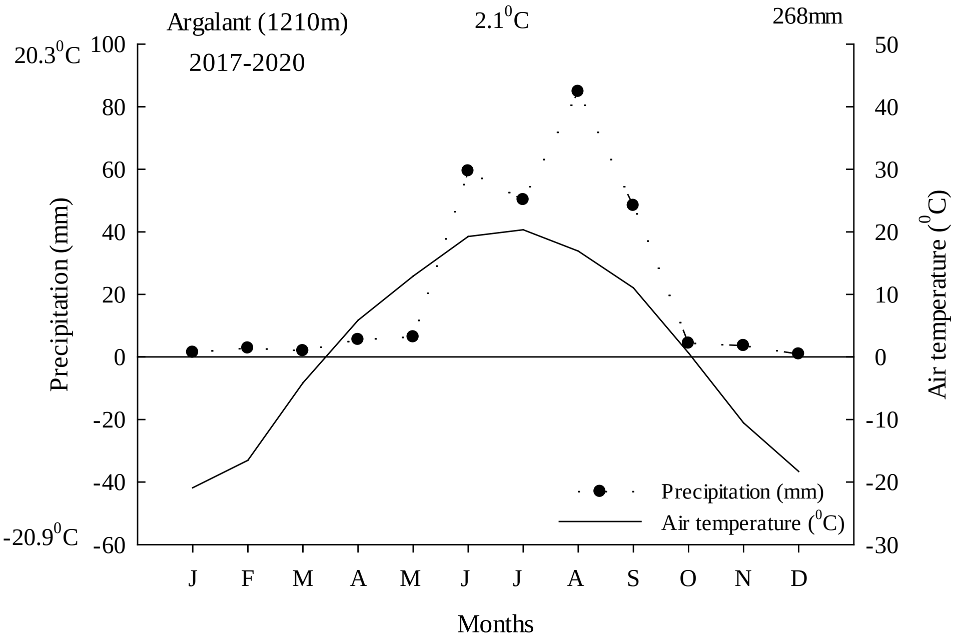

Figure 2. Climagram of study site (2017-2020), Nukhurlul weather station

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Planting design

The plantation was established in 2017 using two-year-old seedlings of three different woody species, namely, *Populus sibirica* Hort. Ex Tausch., *Ulmus pumila* L., and *Hippophae rhamnoides* L. Seedlings were planted in holes (60-70 cm deep, 50-60 cm in diameter) with 2.5-m distance in-between the trees and 4-m distance in-between the rows. All seedlings were hose equipped with an irrigation system and continuously watered three times a month during the growing seasons. The irrigation hose system was connected to a water tank with a capacity of 25 tons. Three plots (30×30 m), which has nine subplots (10×10 m), were established within the plantation using completely randomized sampling consisting of 12 trees (Figure 1).

Growth and biomass measurement

The RCD and height were measured during vegetative season (June-August) in 2020. 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 tree of each species [36].

In August, 2020, six trees in each species were randomly selected and harvested for destructive sampling for biomass measurement (18 trees). Following harvesting, fresh mass of leaves, branch, stem and roots were weighed with an accuracy of 0.001 g, using a portable balance (Discovery Semi-Micro and Analytical Balance-DVG215CD, Ohaus Corp., Switzerland). All samples were subjected to dry biomass measurement after drying at 70°C for 72 hours (Forced Convection Oven-OF-21E, Lab Comp., Korea). The sum of all parts of biomass samples was termed Total biomass [14].

Measurements of leaf morpho-physiological traits

All morpho-physiological traits were immediately measured in July, 2020. 540 healthy and fully expanded leaves (i.e., 20 leaves × 3 replicates × 3 trees × 3 species) were used in determining the leaf area (LA), specific leaf area (SLA), and leaf biomass (LB). Collected samples were sealed in plastic bags and stored in a cold container to avoid water loss during transportation 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 were then analysed using the ImageJ software, following the procedure in [17-18]. In terms of LB and SLA, LB was measured after drying at 72°C for 48 hours (Forced Convection Oven-OF-21E, Lab Comp., Korea). Oven-dried leaf samples were weighed with an accuracy of 0.001 g using a high-precision electronic scale (Discovery Semi-Micro and Analytical Balance-DVG215CD, Ohaus Corp., Switzerland). The SLA was calculated using the procedure and equation (i.e., SLA = LA (cm^2)/LB (mg)) in [37, 39].

A total of 27 trees (i.e., 3 trees × 3 replicates × 3 species) were randomly selected, from which 5 healthy, fully expanded and sun-exposed leaves were collected for chlorophyll content analysis. The hand-held leaf-clip chlorophyll meter was used (MC-100, Apogee Instruments, Inc., Logan, UT, USA). The respective measurement values are chlorophyll content index (CCI) [38].

To determine the plant water status, three healthy trees with three replicates from each species were randomly selected for measuring leaf water potential (ψ, MPa). Here pre-dawn (ψ_p) and mid-day (ψ_m) were assumed to represent the daily maximum of turgor condition and the daily minimum of turgor condition, respectively. Pre-dawn and mid-day leaf water potentials 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) [17-18].

Statistical analysis

One-way ANOVA was employed to determine the interacting effects of species on the growth and morpho-physiological traits, and multiple comparisons among the species, Duncan's multiple range test (DMRT) was used. Statistical analysis was computed by using the SAS software package, version 9.4 [40].

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DOI: https://doi.org/10.5564/pmas.v62i03.2470

RESULTS AND DISCUSSION

Growth characteristics

Statistically significant differences were observed in the height and RCD growth in all studied species (Table 1).

Table 1. ANOVA of growth characteristics of studied species

Source	DF	H, cm		RCD, mm	
		F value	Pr>F	F value	Pr>F
Species	2	48.69	<.0001	47.00	<.0001
Month	2	13.80	<.0001	17.13	<.0001

P. sibirica (135.3±6.81cm) showed higher value in height growth, while lower value was observed for *U. pumila* (93.46±5.10cm) and *H. rhamnoides* showed intermediate growth performance among the studied species. The RCD was higher in *P. sibirica* (29.68±0.89mm) and the lowest value was in *U. pumila* (93.46±1.41mm) (Figure 3).

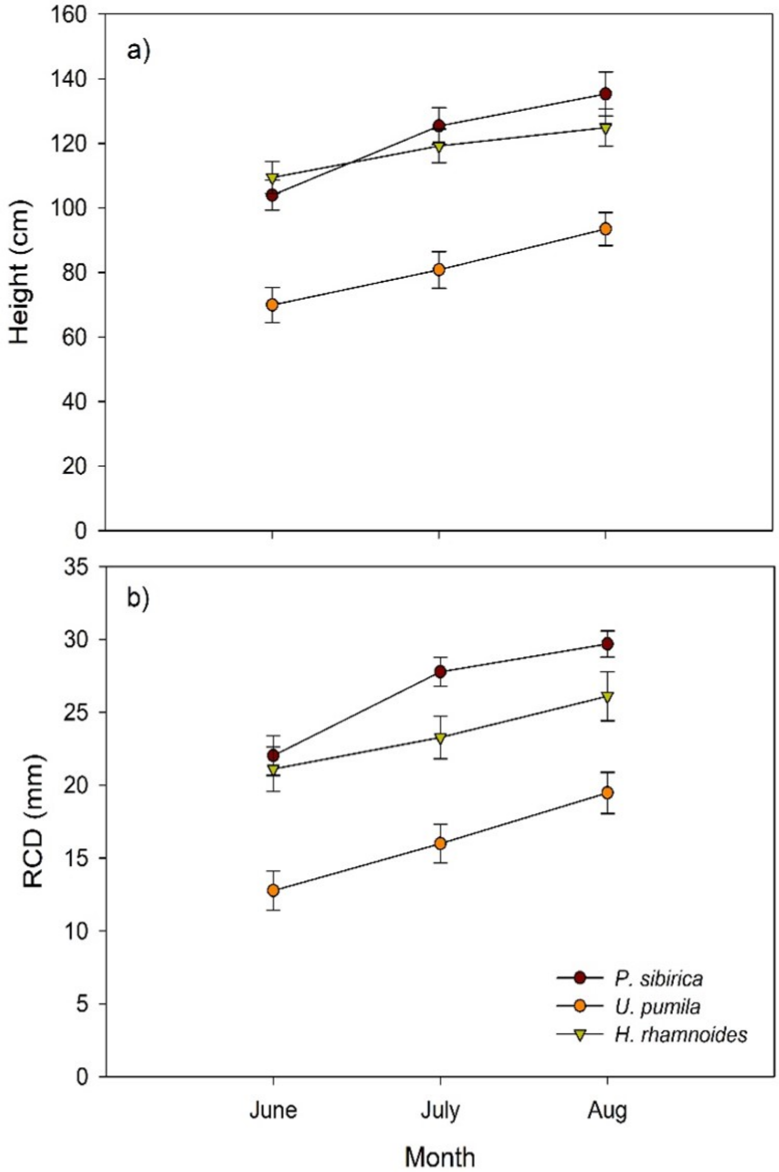

Figure 3. Growth characteristics of studied species (a) height and (b) root collar diameter. Line with marker represent standard errors

Biomass partitioning

Significant difference was found only in stem biomass fragments (p=0.0002) (Table 2).

Table 2. The ANOVA of biomass partitioning of the studied species

Source	DF	Leaf biomass		Branch biomass		Stem biomass		Root biomass		Total biomass	
		F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Species	2	0.27	0.7699	0.80	0.4731	18.96	0.0002	1.25	0.3212	1.65	0.2320

Biomass partitioning studied species are shown in Table 3, the highest value for leaf biomass (190.04±43.12gr), and branch biomass (230.48±51.60gr) were measured in *P. sibirica*, higher biomass accumulation in terms of stem (210.40±24.49gr), root (514.00±46.10gr), and total biomass (1016.03±74.13gr) was measured in *U. pumila*, while, lowest total biomass was measured in *H. rhamnoides* (778.26±75.86gr).

Table 3. The biomass partitioning of studied species (n=18)

Species	Leaf biomass (gr)	Branch biomass (gr)	Stem biomass (gr)	Root biomass (gr)	Total biomass (gr)
P. sibirica	190.04±43.12^a	230.48±51.60^a	59.80±14.42^b	365.00±139.80^a	845.32±126.48^a
U. pumila	134.92±63.22^a	156.70±38.40^a	210.40±24.49^a	514.00±46.10^a	1016.03±74.13^a
H. rhamnoides	165.09±51.75^a	172.76±39.13^a	104.40±11.78^b	336.00±14.69^a	778.26±75.86^a

* *Different lowercase letters indicate significant differences across the species at α=0.05*

Tree biomass allocation is shown in Figure 5. The results show that root biomass consists 43.2-50.6% of total biomass.

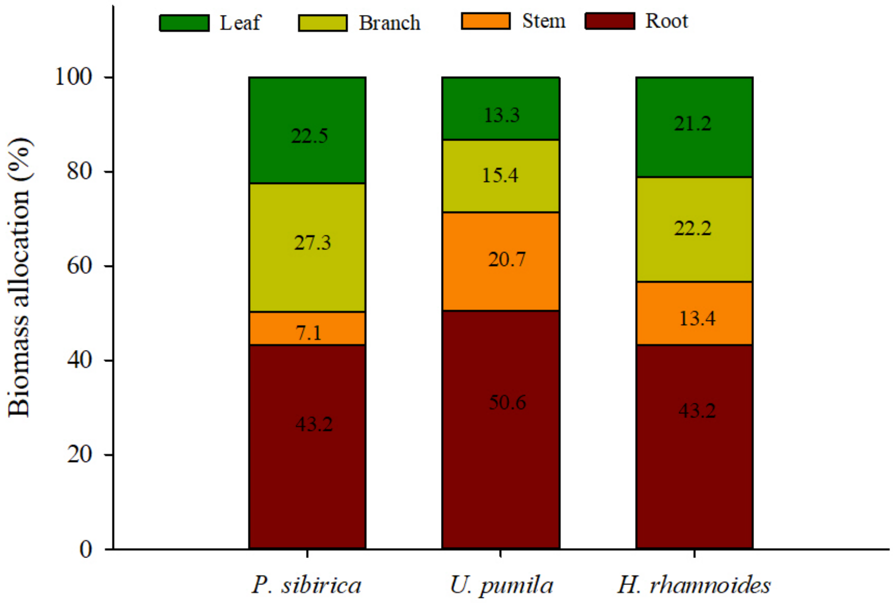

Figure 4. Tree biomass allocations (%)

Leaf morpho-physiological traits

Significant differences in leaf biomass, specific leaf area and leaf area were observed in all species (Figure 5-6). Higher leaf biomass accumulation was observed in *P. sibirica* (0.32±0.01gr) and twice lower values were measured in *U. pumila* and *H. rhamnoides*. Highest SLA value was measured in *P. sibirica* (31.08±0.94cm^2/gr) and values for *U. pumila* (14.03±2.07 cm^2/gr) and for *H. rhamnoides* (5.66±1.18 cm^2/gr) were lower as compared to *P. sibirica* (Figure 5b). Similar to SLA, higher leaf area was measured in *P. sibirica* (9.83±0.24 cm^2), followed by *U. pumila* (0.98±0.13 cm^2) and *H. rhamnoides* (0.48±0.1 cm^2) (Figure 5c).

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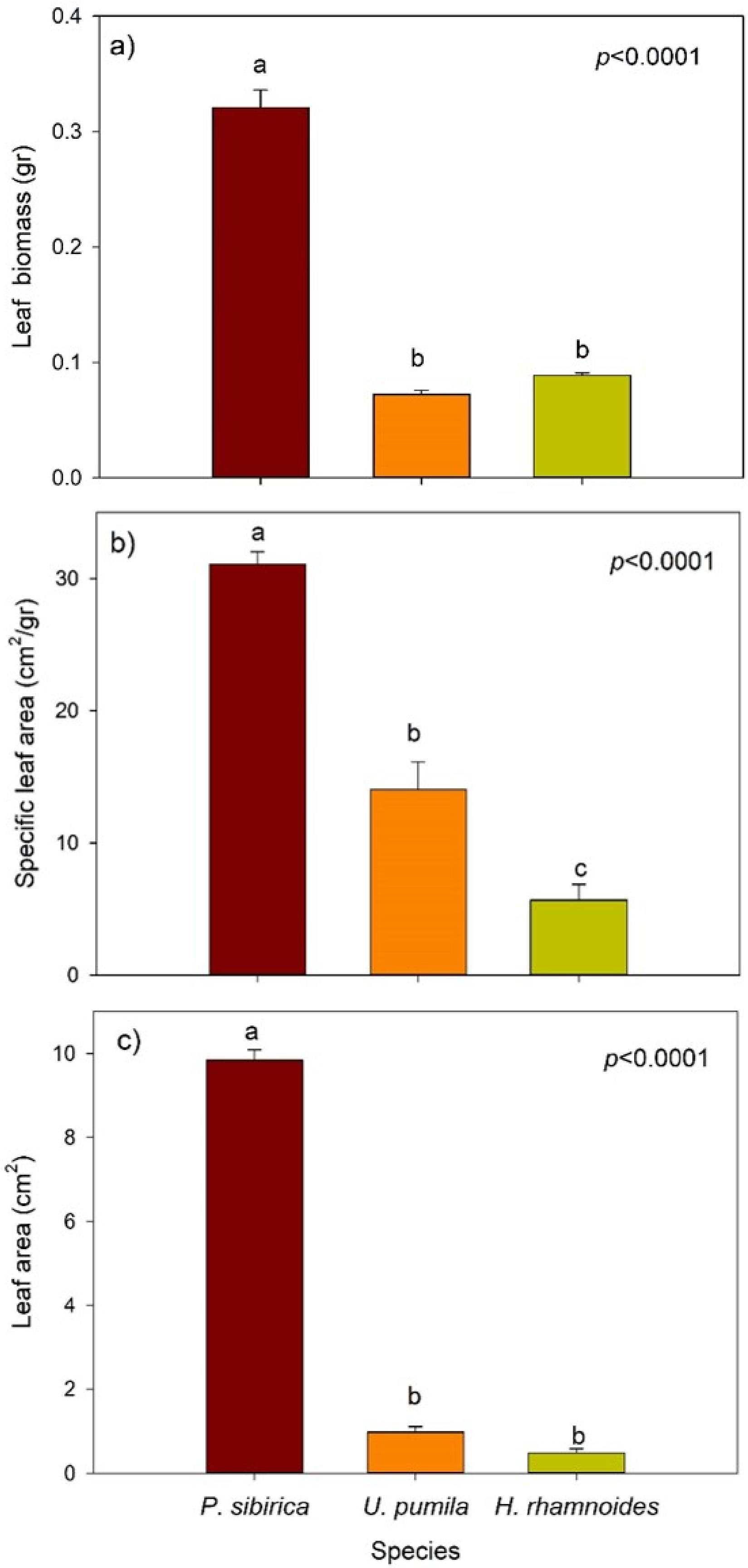

Figure 5. a) Leaf biomass (LB), b) specific leaf area (SLA) and c) leaf area (LA) of studied species measured in July 2020. Different lowercase letters indicate significant differences across the species at α=0.05. Vertical bars represent standard errors

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The chlorophyll content index showed highest value for *P. sibirica* (330.56±56.81µg/ml), while it showed the lowest value for *H. rhamnoides* (72.17±4.77 µg/ml) (Figure 6).

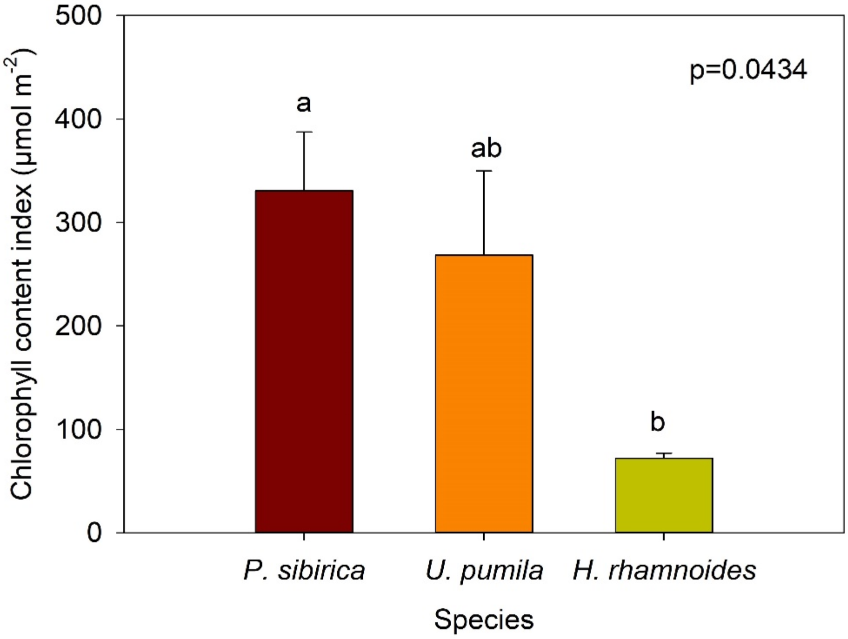

Figure 6. Chlorophyll content index of studied species measured in July 2020. Different lowercase letters indicate significant differences across the species at α=0.05. Vertical bars represent standard errors

The leaf water potential was not affected before dawn and was more pronounced or dehydrated at noon. According to the daily water potential of *U. pumila* leaves, the minimum value of leaf water potential (-1.75±0.18MPa) between 22:00-04:00 hours was not affected by water shortage (Figure 7).

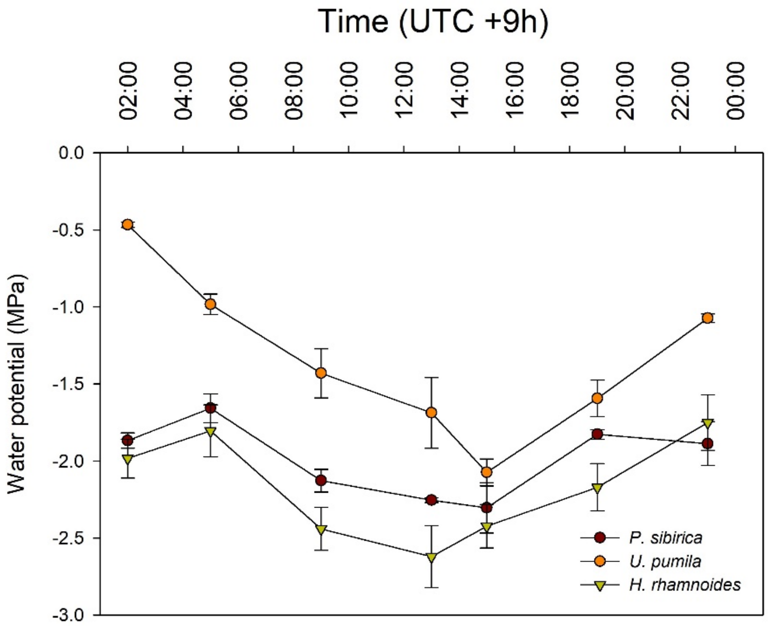

Figure 7. Diurnal variation in leaf water potential (Ψ) of studied species measured in July 2020. Line with marker represent standard errors

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However, after sunrise at 06:00 AM, the value of water potential gradually increases, and between 13:00 and 15:00 hours reached the maximum and was subjected to water shortage. In the case of *P. sibirica*, the results were similar to those in *U. pumila*. *H. rhamnoides* suffers from minimal water shortages even with regular drip irrigation (Table 4).

Table 4. Seasonal variation of the pre-dawn (Ψ_p) and mid-day (Ψ_m) leaf water potentials in studied species measured in 2020

Species	Time	Jun	Jul	Aug
P. sibirica	Predawn	-4.8	-1.07	-2.67
	Midday	-3.33	-2.01	-2.01
U. pumila	Predawn	-1.01	-1.78	-2.55
	Midday	-1.96	-1.89	-2.25
H. rhamnoides	Predawn	-5.6	-2.19	-3.02
	Midday	-2.0	-2.95	-3.31

Three woody species, namely, *P. sibirica*, *U. pumila*, and *H. rhamnoides*, planted in semi-arid regions, were subjected to measurement of their growth characteristics, biomass partitioning and leaf morpho-physiological traits.

According to the results of this study, each tree species had a different biomass accumulation in terms of leaves, branch, stem, root and total biomass fragments, and it was found that the *P. sibirica* had the highest total biomass, meanwhile, leaf and branch biomass accumulation was higher in *U. pumila*. The obtained results were in line with the research results, which are conducted in the semi-arid steppe region [14-15].

Further morpho-physiological traits studies, which are affecting plant growth, are carried out, for example, drought alters the photosynthetic machinery of plants, chlorophyll synthesis, biomass allocation, and other major physiological activities of plants [17-18; 38].

An important method for assessing the photosynthetic capacity under water stress is the chlorophyll content, which is used to determine drought tolerance [41]. In terms of leaf morphometry and chlorophyll content, the *P. sibirica* had positive characteristics.

The LB, LA and SLA in *U. pumila* have the lowest values and this confirms the results of several studies on drought effects on leaf traits [15-17].

Drought is a likely contributing factor as chlorophyll concentrations tended to be higher in irrigated versus non-irrigated subplots, although only once a statistically significant difference was observed [42].

The *H. rhamnoides* cultivated in that environment was more exposed to water stress and can inhibit photosynthesis, create an imbalance between light absorption and utilization, and reduce chlorophyll levels [17]. Our results suggest that chlorophyll level of *H. rhamnoides* were lower than other species and similar studies were found [17].

According to the diurnal measurements of leaf water potential, all studied species were subjected to water stress between 13:00 and 16:00 hours and recovered after 20:00 p.m., similar observation was confirmed in plantations established in semi-arid and desert-steppe regions of Mongolia [15, 18; 21-22].

Overall, *U. pumila* had a better adaptive response in accordance with our observations and similar research results we obtained in trees of *U. pumila* growing in the sand dune area of Inner Mongolia [43-45].

CONCLUSIONS

In order to establish successful afforestation in semi-arid areas, the importance of selection of species cannot be overlooked, and specific consideration should be given to native species, which had more adaptive responses to the site.

Based on our study, *U. pumila* has higher adaptive characteristics in arid regions as they require less water, while, *P. sibirica* showed better biomass accumulation, which can be employed as a species for carbon sequestration. *H. rhamnoides* has been considered as an

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economic species for the local communities if regular irrigation is conducted, which can increase the yield of fruits. Our findings are relevant to ensuring the sustainability of afforestation programs in semi-arid landscapes in Mongolia.

Data availability statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Acknowledgments: This work was financially supported by the Fellowship Grant by the "Green Asia network" INGO.

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

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