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Afforestation of Mongolian steppe: patterns of biomass partitioning in *Populus sibirica* **and** *Ulmus pumila* **trees in response to management supporting measures**

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ABSTRACT

Climate challenges impose the need for successful afforestation strategies which will increase the amount of carbon sequestered from the atmosphere. In practice, this means evaluation of suitable plant species and management practices for the long-term effects to prove beneficial. In the present study we employed a clasmometric approach to look into biomass partitioning in two tree species, *Populus sibirica* and *Ulmus pumila*, which have been included in the formation of the *Green Belt* project in Asia. Comparing the total biomass comprised of the above ground (AG) and below ground (BG) biomass of trees grown in different irrigation and fertilization regimes, we aim to better understand where the two species invest more biomass as a tool to deal with the environmental challenges. The results suggest that these two tree species prioritize different aspects of development when faced with various challenges. *U. pumilia* tends to be more resistant to drought making it favorable for the semi-arid and arid regions. *P. sibirica* is more sensitive to the lack of water but shows greater potential in terms of biomass production (especially AG biomass) and, therefore, overall higher C-sequestration. The fertilization treatments made no significant impact on tree development on Mongolian steppe soil.

Introduction

To fight global changes, there is the need to establish a planetary positive carbon (C) budget through the input of biomass in forest ecosystems higher than losses due to erosion, mineralization and deforestation (Lal [2018](#page-12-0)). In this scenario, the Paris Climate Agreement recommends a voluntary plan of "4 Per Thousand" (4PT) to sequester C worldwide at the rate of 0.4% annually to 0.4 m (1.3 ft) depth (United Nations Framework Convention on Climate Change (UNFCC) [2015\)](#page-13-0).

The first step of C sequestration is based upon the transfer of atmospheric $CO₂$ into plant biomass through photosynthesis measurable as net primary productivity (NEP) (Lal et al. 2018). Therefore, it is clear that recarbonization of terrestrial biosphere requires the adoption of best management practices (BMPs) (Tang et al. [2018\)](#page-13-1) such as forest landscape restoration (FLR) and afforestation of degraded lands (Stanturf et al. [2014](#page-13-2), [2015,](#page-13-3) [2020\)](#page-13-4).

The Mongolian steppe is included in the list of 3,323 Mha of lands which are suited for afforestation (FAO [2017\)](#page-12-1) as nearly 80% of those are also considered to be exposed to degradation and desertification (Bulgan et al. [2013\)](#page-11-0). This fact

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explains why some authors have highlighted the urgent need for Mongolia to combat desertification along the forest-steppe border zone and to increase its national carbon sequestration capacity (Gradel et al. [2019\)](#page-12-2) as a contribution to mitigating greenhouse gas emission.

In the context of Mongolian activity to fight global changes, of particular importance is the establishment of new forest plantations under the aegis of the *Green Belt* project (Batkhuu et al. [2017](#page-11-1)). Among several others tree species tested for these new plantations, *Populus sibirica* hort. ex. Tausch and *Ulmus pumila* L. remain the most investigated given their good adaptability to such harsh climatic and soil conditions present in these arid and semi-arid Mongolian lands (Khaulenbek and Kang [2017](#page-12-3); Byambadorj et al. [2020,](#page-11-2) [2021](#page-11-3)).

Our research group is interested to understand how biomass is partitioned in *P. sibirica* and *U. pumila* trees used in these new plantations (Cho et al. [2019](#page-11-4); Sungsik et al. [2019;](#page-13-5) Byambadorj et al. 2020). In particular, the effects of watering regimes and fertilization type have been recently observed on the root system through the analysis of the biomass partitioning across different root classes (Nyam-Osor et al. [2021\)](#page-12-4).

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The rationale is that biomass partitioning can be used to evaluate the growth performance (Mooney and Winner [1991](#page-12-5)) of a plant in its environment (Hughes and Bazzaz [1997;](#page-12-6) Yin and van Laar [2005;](#page-13-6) Zhang and Wang [2010](#page-13-7)). Moreover, biomass partitioning is a useful tool to compare growth performance of a) two (or more) species growing under the same environmental conditions (Hay and Porter [2006](#page-12-7)), or b) a single plant species in response to changing environmental conditions. No less important is the role that studies of biomass partitioning assume in understanding plant physiological ecology (Mokany et al. [2006](#page-12-8)) and in measuring biogeochemical cycles (Litton et al. [2007\)](#page-12-9).

The aboveground (AG) and belowground (BG) organs in a plant play different roles despite their shared common objective to ensure optimal growth and reproduction performance; both depend upon the occurrence of a correct functional proportionality in their biomass (Poorter et al. [2015;](#page-13-8) Mensah et al. [2016\)](#page-12-10).

Biomass consists of two main components, namely Carbon (C) and Nitrogen (N) masses, and contains a wide range of elements (Brown et al. [2019\)](#page-11-5). In our studies, we limit our investigations to the analysis of dry mass (DM) as this parameter is the most adequate surrogate to evaluate the amount of C whose assimilation depends on the plant growth (Brown et al. 2019). In this sense, the term "biomass" is considered by us as a parameter that indicates the net dynamic allocation of photosynthesis necessary to foster the metabolic activities of an organ (Shipley and Meziane [2002](#page-13-9)).

Frequent is the use of "allometric" analyses (i.e. allometric equations) to investigate the relationship existing between plant traits and biomass partitioning (Picard et al. [2012;](#page-13-10) Noulekoun et al. [2017](#page-12-11)). More limited is the use of "clasmometric "analysis based on the expression of biomass as a fraction or proportion of the total organismal biomass present at a given time (Poorter et al. 2015; Noulekoun et al. 2017). Clasmometry requires a destructive approach to measure plant biomass (Basuki et al. [2009](#page-11-6)) where AG or BG organs are detached from the rest of the plant body to measure their dry weight (DW) that is then considered as a reliable indicator of C stock (i.e. biomass) (Ketterings et al. [2001\)](#page-12-12). Therefore, it is not surprising that clasmometry is limited to small areas or small tree sample sizes (Dimobe et al. [2018\)](#page-12-13), although it cannot be overlooked that responses of genotypes to a specific environmental condition over time can only be studied by clasmometric analysis (Noulekoun et al. 2017; Byambadorj et al. 2020). Furthermore, clasmometry is of fundamental importance for the modelling pattern of plant growth as a function of the development of all its components (Poorter et al. [2013\)](#page-13-11).

To explain how biomass partitioning in AG and BG organs responds to the availability of resources in the environment, two theories have been advanced: a) optimal partitioning and b) isometric allocation (McCarthy and Enquist [2007\)](#page-12-14). According to the optimal partitioning theory (OPT) (Bloom et al. [1985](#page-11-7)), biomass partitioning between organs responds to the need to maximize growth rate (Chapin et al. [1987](#page-11-8)). The isometric allocation theory proposes that an isometric biomass allocation in AG and BG organs is independent of

differences between plant species or community types (Niklas [2005;](#page-12-15) Cheng and Niklas [2007](#page-11-9)).

The OPT termed functional equilibrium model (FEM) by Bloom et al. (1985), explains well why plants growing in arid (or nutrient-limited) lands present deeper roots (i.e. increase biomass partitioning in BG organs) than those in more humid lands (Chapin et al. 1987; Shipley and Meziane 2002; Deng et al. [2006](#page-12-16); Poorter et al. [2012\)](#page-13-12). Moreover, several studies confirm this theory by showing that differences in biomass partitioning depend also upon plant size (Pino et al. [2002;](#page-13-13) Poorter et al. 2012, Xie et al. [2012\)](#page-13-14), ontogenic trends (Poorter et al. 2012, Xie et al. 2012), species competitive abilities (Ninkovic [2003;](#page-12-17) Dybzinski et al. [2011\)](#page-12-18), species identity and functional traits (McCarthy and Enquist 2007; Poorter et al. 2015), forest structure and competitive interactions (Koslowski et al. [1991](#page-12-19)). On the contrary, the isometric allocation theory cannot explain not only why biomass partitioning changes in the function of the environmental factors (Génard et al. [2008](#page-12-20)), but also why it cannot be considered applicable across all ecosystems (Han and Fang 2003).

In the present work we intend to continue our comparative analysis of growth rate between these two trees species (*P. sibirica* – poplar and *U. pumila* – elm) grown in these new plantations of the *Green Belt* project. In particular, here we present a comparative analysis regarding how biomass is partitioned in AG organs when tress respond to the adoption of management measures (irrigation regimes and fertilization). The data obtained could provide scientific support to establish the most sustainable management practices that avoid the failure of the *Green Belt* project or other similar afforestation initiatives. Moreover, the possibility to measure the amount of C stock present in these plantations could enable the calculation of the potential carbon sink capacity of these lands.

Materials and methods

The study site description

The study site is located in Lun soum, Tuv province, Mongolia (47°52′15.43″N, 105°10′46.4″E) on the right bank of the Tuul river, 135 km west of Ulaanbaatar, at elevation of 1,130m a.s.l [\(Figure 1](#page-3-0)). The site extends for 0.2ha within the forest nursery of the Korea-Mongolia Joint *Green Belt* Plantation project. According to the phytogeographical region of the study area is described as the Middle Khalkha dry steppe, region that has been greatly degraded by intense livestock grazing (Ulziykhutag [1989](#page-13-15)). Vegetation is typical of the genuine dry bunchgrass steppe dominated by xerophytic and mesoxerophytic graminoids (e.g. *Stipa sareptana subsp. krylovii* (Roshev.)*, Cleistogenes squarrosa* (Trin.)*, Agropyron cristatum* (L.) Gaertn*, Artemisia frigida* (Willd.), and, in degraded lands *Artemisia adamsii* (Besser)*, Carex duriuscula* C.A.Mey.*, Leymus chinensis* (Trin.)) (Lavrenko et al. [1991\)](#page-12-21). Soil type is classified as Kastanozems type (Loamic) (IUSS Working Group WRB [2015\)](#page-12-22), characterized by a depth of 1m, weakly developed, and lacking profile differentiation in different horizons. The hardness of the topsoil was 4.5 kg cm−2, while that of the subsoil was 1.7 kg cm−2, as the topsoil is drier than the subsoil.

[Figure 1.](#page-2-0) Location of study site within the *Green Belt* plantation project in Lun soum, Tuv province, Mongolia.

Climatic characteristics

The area is located in a semi-arid steppe region, with an annual average temperature of 0.6 ± 0.45 °C, and a summer average temperature of 16.29 ± 0.41 °C (May–September). Average annual precipitation during the whole experiment (2000–2019) was 196 mm, according to the Lun soum meteorological station, Mongolia [\(Figure 2\)](#page-4-0). Summer precipitation usually occurs between June and August and accounts for 80–90% of the total annual rainfall. The mean annual potential evapotranspiration is 752.12 ± 30.68 mm (Cao et al. [2018\)](#page-11-10). The mean air temperature of the warmest month (July) is 16 °C, while that of the coldest month (January) is −22 °C. The length of the growing season varies between 110 and 130 days (The National Agency for Meteorology and Environmental Monitoring of Mongolia (NAMEM) [2019](#page-11-11)).

Plant material and management supporting measures

Two-year-old saplings of *U. pumila* (grown from seeds) and *P. sibirica* (obtained from 20 cm cuttings) grown in the greenhouse and acclimated in the open nursery were obtained from the *Green Belt* project nursery and transplanted in May 2011, in 60–70 cm-deep holes with a diameter of 50–60 cm. Immediately after transplanting, all saplings were irrigated using the same amount of water regardless of the treatments for one month during the acclimatization period. Immediately after transplanting, a sufficient level of watering was supplied to individual trees by compensating non- leakage (CNL) button drippers placed 10 cm from the stem of each sapling. After sapling acclimatization, four different irrigation regimes were applied: no irrigation (control, 0L h⁻¹), 2L h⁻¹=0.25 mm m⁻², 4L h⁻¹= 0.5 mm m⁻², 8L h⁻¹=1.0 mm m⁻². The 5-hour duration of watering was done twice a week for the entire vegetative season (from the beginning of May to the end of August). In addition to the different irrigation regimes, two different types of fertilizers, NPK and Compost (120 g and 500 g per tree), were mixed with natural soil to fill the holes before the saplings were transplanted. NPK consisted of solid granules mixture of nitrogen, phosphorus and potassium (12/16/4), whereas Compost consisted of well decomposed sheep manure (pH 7.4; organic matter content in 18.0–25.0%, nitrogen content 5.0–7.0 g/kg, and total Ca, Mg, K, Na contents averaged 9.29, 7.02, 9.18 and 0.05g/kg). Twelve plots per plant species were prepared: one for control+three for irrigation regimes; one for control plus NPK+three3 for NPK with different irrigation regimes: one for control plus Compost+three for Compost with different irrigation regimes. The size of each plot was 20×10 m; trees were planted in rows with a north-south orientation to ensure maximum light availability during the whole day (Johnson and Brandle [2009](#page-12-23)). The distances between rows were 2.5 m and distances

[Figure 2.](#page-3-1) Mean annual temperature and precipitation in the study site from January to December (2000–2019).

between trees were 2.5 m. The number of trees per plot was 32 for control irrigation regime treatments and 16 trees for irrigation regime treatments with addition of fertilizer. At the time of transplanting, the height of the elm seedlings was 51 ± 1.14 cm; with root collar diameter (RCD) of 0.33 ± 0.01 cm $(n=256)$, whereas for the poplar saplings it was 68 ± 2.94 cm; with RCD 0.51 ± 0.02 cm ($n = 256$). After transplanting in the field, the survival rate of the saplings was very high in the case of elm, independently of the treatment used, whereas it was very low for poplar when trees received no water except for the natural amount of rainfall. This was particularly the case for the unwatered trees of poplar which had NPK as a fertilizer treatment and had 100% of mortalily. Nonetheless, data for this treatment was included. The presence of fertilizers in the soil did not significantly change these results (Byambadorj et al. 2020).

Biomass measurement

In August 2019, height and RCD were measured for all surviving trees of elm and poplar of all 12 treatments (*n=*239 and *n*=211) and six trees in each treatment were randomly selected and harvested for destructive sampling for biomass measurement (*n*=135 trees). Following harvesting, fresh mass of leaves, stems and branches was measured on site using portable electronic scales (accuracy 0.01g). Subsamples of all tissues were collected from six trees of each treatment and dried to convert fresh mass to dry mass. Stem disk (5.0cm) subsamples on every 1m segments of stem were taken and root discs with size of 5.0 cm from all harvested trees were taken at ground level, their fresh mass measured and dry mass measured after oven dried at 105°C until constant weight.

Three branches were randomly selected from top, middle and lower parts of crown and their diameter and length was measured; they were then oven dried at 105 °C for 48 hours to convert fresh mass into dry mass. All leaves separated from the branches were subjected to measurement of their fresh and dry mass at 70 °C for 72 hours. Roots were washed carefully to remove all soil particles while ensuring that the fine roots were not damaged or lost. All plant samples were then oven-dried at 70 $^{\circ}$ C for 72h to a constant weight.

Statistical analysis

Statistical analysis was computed by using the SAS software package, version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). One-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT) was used for multiple comparisons among the 2019 data. Permanent plots were considered as independent replicates. At each sampling date and within each plot, trees were measured and data were treated as mean.

Results

After the trees were manually excavated (63 trees of *P. sibirica* and 72 trees of *U. pumila),* as described in Materials and Methods, each tree was disassembled in two main fractions named respectively above ground (AG) and below ground (BG). AG consisted of stem, branches and leaves, whereas BG was formed by all the roots, independently from the relative branching order. Both AG and BG fractions were used to measure biomass and in the case of AG fraction, the biomass was measured separately for each component (i.e. stem, branches, and leaves), whereas in the case of BG, we measured the overall biomass of the root system.

The sum of AG and BG fraction biomass was termed Total biomass or Tree biomass (TB). TB obtained from trees treated with fertilization (NPK or Compost) and/or irrigation regimes (2−4 − 8L h−1) is shown in [Figure 3](#page-5-0). In *Populus*, we found that

[Figure 3.](#page-4-1) Total biomass of *Populus sibirica* and *Ulmus pumila*. The total biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (*p*<0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

[Figure 4.](#page-5-2) Root biomass of *Populus sibirica* and *Ulmus pumila*. The root biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (*p*<0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

only the 4L h⁻¹ and 8L h⁻¹ irrigation regimes showed a significant 5-fold increase in respect to the previous treatment in terms of biomass ([Figure 3A](#page-5-0)). The data variance was significantly influenced by different treatments (*p*<0.0001) The addition of Compost to the soil seemed to reduce the effect of irrigation regimes despite a significant but lower difference with the control continued to remain visible. The reduction effect seemed to be less evident when the highest rate of the irrigation regime (8L h−1) was coupled with fertilization with NPK [\(Figure 3B\)](#page-5-0). In the case of *Ulmus*, a significant increase of biomass was observed when 8L h⁻¹ irrigation regime was applied whereas treatment of soil with NPK or Compost seemed to cancel this biomass increase ([Figure 3B](#page-5-0)). The data variances were not influenced by different treatments ($p=0.0065$).

Root biomass (RB) represented ([Figure 4\)](#page-5-1) the combined biomass of taproot and lateral roots of any branching order (Montagnoli et al. [2018\)](#page-12-24) when the excavated tree was cut into two fractions at the level of the root collar (i.e. the area where the taproot joins the main stem). RB of control trees (i.e. grown without the support of irrigation regime) showed to be formed by the same amount of biomass even when the soil was fertilized with Compost or NPK, independently

from the tree species considered ([Figure 4](#page-5-1)). In *Populus*, a significant (2 fold) biomass increase in the root system was observed when 4L h⁻¹ irrigation regimes was adopted and this increase furtherly increased (4 folds) when the irrigation regime was increased at 8L h⁻¹ ([Figure 4A](#page-5-1)). The data variance was significantly influenced by different treatments (*p* < 0.0001). The effect of 4L h⁻¹ irrigation regime on the increase of root system biomass was cancelled when the soil was treated with both Compost and NPK whereas it remained visible with the highest rate $(8 L h^{-1})$ of the irrigation regime despite it was almost halved when Compost was used as fertilization ([Figure 4A](#page-5-1)). The data referring to RB of *Ulmus* presented in [Figure 4B](#page-5-1) show clearly that only the highest rate of the irrigation regime $(8 L h^{-1})$ induced a significant increase of biomass although this increase was completely cancelled when Compost or NPK were used as fertilizers. The data variance was significantly influenced by different treatments (*p*<0.0001).

The analysis of our data showed that RB values were considerably lower than those obtained by measuring TB. For this reason, we decided to analyze the percentage of RB relative to TB. Regarding this, the data obtained indicated ([Table 1\)](#page-6-0) that in both poplar and elm trees not treated with any management regime presented almost 50% of the total biomass found in the whole trees. It was surprising to find that *Populus* treated with irrigation regimes showed a considerable decrease in the biomass found in the root systems whereas it remained constant in *Ulmus*. Moreover, we found that in *Populus* the fertilization of the soil with Compost or NPK did not cancel the decreasing effect due to the irrigation regimes with the exclusion of fertilization with NPK in pres-ence of the lowest irrigation regime rate (2L h⁻¹) ([Table 1](#page-6-0)).

AG biomass was measured first without any distinction between stem, branches and leaves and termed Total Above Ground Biomass (TAGB). TAGB values are shown in [Figure 5](#page-7-0) and show that in both *Populus* and *Ulmus* TAGB increased considerably when the highest rate of the irrigation regime was considered although the increase seemed to be higher in *Populus*. The addition of fertilizers inhibited this increase in *Ulmus* whereas it was maintained in *Populus* only when the irrigation regime was of 4 and $8L$ h⁻¹. The data variance was significantly influenced by different treatments (*p*<0.0001) for *Populus,* while for *Ulmus* is was not influenced by the different treatments (*p*=0.0510).

In addition, we measured the biomass in the three AG fractions: stem, branches, and leaves. The data relative to the leaf biomass (LB) is shown in [Figure 6](#page-7-1) and each value represents the mean of LB obtained from six trees. Regarding *Populus* LB it is evident that only the use of at least 4L h⁻¹ irrigation regimes can induce a difference of LB in respect to control. A fertilization management measure by itself did not induce any variation in LB independently from the fact that Compost or NPK was used whereas an increase was observed when fertilization with Compost was associated to the highest irrigation regime (8L h⁻¹). When NPK was used as fertilizer, the increase resulted to be already evident with 4L h⁻¹ irrigation regime [\(Figure 6A\)](#page-7-1). The data variance was significantly influenced by different treatments (*p*<0.0001). No increments were observed in LB of *Ulmus* even in the presence of supporting measures as shown in [Figure 6B](#page-7-1). The data variance was not influenced by different treatments (*p*=0.3229).

The considerable differences (with a ratio 1 to 10 between the leaves of *Ulmus* and *Populus, respectively*) existing

Table 1. Ratio between root biomass and total biomass, leaves biomass and woody biomass according to the two studied plant species (*U. pumila* and *P. sibirica*).

Plant species	Irrigation regimes	%, RB/TB	%, LB/WB
Ulmus pumila	$0 L h^{-1}$	52.1 ± 3.7^a	39.4 ± 6.1 ^a
	$2L h^{-1}$	41.7 ± 13.7 ^{ab}	38.7 ± 11.2 ^a
	4 $L h^{-1}$	50.4 ± 5.7 ^{ab}	34.0 ± 5.3 ^a
	$8L h^{-1}$	48.0 ± 7.1^{ab}	$17.6 \pm 4.1^{\circ}$
	NPK-0L h^{-1}	40.9 ± 2.5^{ab}	45.3 ± 22.1 ^a
	$NPK-2L h^{-1}$	41.7 ± 3.9 ^{ab}	31.1 ± 4.1^{ab}
	NPK-4L h^{-1}	43.3 ± 5.9 ^{ab}	37.3 ± 3.2 ^a
	NPK-8L h^{-1}	42.6 ± 14.5^{ab}	30.2 ± 5.5^{ab}
	COMP-0L h^{-1}	$30.0 \pm 7.6^{\rm b}$	37.7 ± 24.1 ^a
	COMP-2L h^{-1}	41.3 ± 9.8 ^{ab}	31.8 ± 10.1^{ab}
	COMP-4L h^{-1}	42.1 ± 8.9^{ab}	31.0 ± 8.1^{ab}
	COMP-8L h^{-1}	39.4 ± 8.1^{ab}	40.8 ± 13.1^a
Populus sibirica	$0 L h^{-1}$	50.6 ± 10.8 ^{ab}	13.6 ± 7.2^b
	$2L h^{-1}$	28.6 ± 6.8 ^c	21.5 ± 9.1^{ab}
	$4L h^{-1}$	39.3 ± 3.4 ^{bc}	26.0 ± 6.2 ^a
	$8L h^{-1}$	34.5 ± 2.3 ^c	26.1 ± 3.3^a
	$NPK-OL h^{-1}$	n.d.	n.d.
	$NPK-2L h^{-1}$	$54.5 \pm 19.5^{\circ}$	23.1 ± 9.2^{ab}
	NPK-4L h^{-1}	29.3 ± 4.8 ^c	26.1 ± 12.1^a
	NPK-8L h^{-1}	29.3 ± 3.3 ^c	17.6 ± 2.5^{ab}
	COMP-0L h^{-1}	47.2 ± 9.7 ^{ab}	$15.3 \pm 5.1^{\circ}$
	COMP-2L h^{-1}	33.4 ± 13.8 ^c	26.0 ± 4.1 ^a
	COMP-4L h^{-1}	33.2 ± 8.1 ^c	19.0 ± 8.1^{ab}
	COMP-8L h^{-1}	33.5 ± 4.4^c	22.5 ± 3.1^{ab}

The irrigation regimes tested were 0 (control), 2, 4, and 8L h⁻¹. Soil was fertilized with Compost or NPK. Each value and SD was obtained as a mean of six trees $(n=6)$.

Letters indicate significant differences (*p*<0.05) among factors for each species. RB, root biomass; TB, total biomass; LB, leaves biomass; WB, woody biomass; n.d, not determined.

[Figure 5.](#page-6-1) Total aboveground biomass of *Populus sibirica* and *Ulmus pumila*. The total aboveground biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (p <0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

[Figure 6.](#page-6-2) Leaf biomass of *Populus sibirica* and *Ulmus pumila* in response to irrigation regimes and fertilization. The leaf biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (*p*<0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

between the leaf area of the two tree species examined here, suggested that biomass investment necessary to ensure the mechanical support required by the leaf weight could be different and could respond differently to the management supporting measures. For this reason, we decided to collect separately the branches from the stem to analyze comparatively the effect of irrigation regimes and fertilization with NPK and Compost fertilizers. The results of this investigation are presented in [Figure 7](#page-8-0), where it is shown that in *Populus* the biomass invested in branches did not change in the presence of either type of fertilizer unless fertilization is not accompanied by at least a 4L h^{-1} or 8L h^{-1} irrigation regime [\(Figure 7A\)](#page-8-0). The data variance was significantly influenced by different treatments ($p = 0.0008$). In the case of *Ulmus* ([Figure 7B\)](#page-8-0), we did not observed any differences relative to controls, irrespective of the type of management supporting measures used. The data variance was not influenced by different treatments ($p = 0.2754$).

A partially different pattern of biomass allocation with respect to LB and branch biomass (BB) patterns was found

[Figure 7.](#page-7-2) Branch biomass of *Populus sibirica* and *Ulmus pumila*. The branch biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (*p*<0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

[Figure 8.](#page-8-2) Stem biomass of *Populus sibirica* and *Ulmus pumila* in response to irrigation regimes and fertilization. The stem biomass was measured in trees treated with different irrigation regimes (0, 2, 4, and 8 L h−1) and fertilizers (Compost and NPK). Each value represents the mean of six samples (*n*=6) ± 1 SE. Letters indicate significant differences (*p*<0.05) among irrigation regimes and fertilization type. The solid horizontal line at the center of each box is the median value and the dotted line is the mean.

when the pattern of stem biomass (SB) was examined. Regarding this, we show in [Figure 8](#page-8-1) that the biomass investment by *Populus* in their stems is evident when trees were treated with the highest irrigation regime. Completely insensitive seems to be the biomass increase when Compost or NPK fertilizers were used ([Figure 8A\)](#page-8-1). The data variance was significantly influenced by different treatments ($p = 0.0019$). In the case of stem biomass of *Ulmus*, the only significant increase was observed with no fertilization and the highest irrigation regime (8 L h⁻¹) ([Figure 8B](#page-8-1)). The data variance was significantly influenced by different treatments (*p*< 0.0001).

The rationale of checking for a correlation between the variation of LB and the biomass allocated in both branches and stem was that the AG organs (i.e. stem and branches) represent the mechanical support of leaves. Therefore, any variation of biomass taking place in the leaves could require a related variation in the biomass of their mechanically supporting structures. This is especially relevant when considering the great difference existing between the leaf area (LA) of *Populus* and *Ulmus*. For this reason, we decided to sum the biomass present in the branches and stem in a single parameter termed woody biomass (WB) and then

we investigated the ratio between LB and WB fractions ([Table 1](#page-6-0)).

As shown in [Table 1](#page-6-0), in control trees the ratio LB/WB was greater in *Ulmus* than in *Populus*. In both trees, these differences remained unaltered even when the management supporting measures (irrigation regime and fertilization) were adopted. The two tree species responded differently to the different management supporting measures ([Table 1](#page-6-0)). In fact, in *Populus*, we observed an increase in ratio when the two management supporting measures were adopted. In *Ulmus*, a decrease of the ratio was observed only when the highest rate $(8 L h^{-1})$ of the irrigation regime was adopted. This decrease was abolished when the highest irrigation regime was applied together with NPK fertilization. The addition of Compost seemed to leave the ratio unaltered, regardless of the presence or absence of any additional irrigation regime.

Discussion

The temperature increase affecting the world is particularly evident in Central Asia that is now considered one of the climate change hotspots at a global level (Mannig et al. [2013,](#page-12-25) Unger-Shayesteh et al. [2013](#page-13-16)). Mongolia represents the center of this hot spot (Gradel et al. 2019), and the models predict a future of climate worsening with warmer and drier conditions (Karthe et al. [2014](#page-12-26); Oyuntuya et al. [2015\)](#page-13-17). Therefore it is not surprising that the degraded condition affecting the majority of arid and semiarid lands in Mongolia calls for urgent interventions of reforestation and afforestation (Byambadorj et al. 2020; Stanturf et al. 2020). In these scenarios, recarbonization of terrestrial ecosystems (soil and vegetation) through Forest Landscape Restoration (FLR) (Stanturf et al. 2014, 2015, 2020) is the most efficient strategy to mitigate climate change effects (Lal et al. 2018). The *Green Belt* project launched jointly from the Government of Mongolia and South Korea represents an example of FLR where several experimental new plantations have been established in both steppe and desert lands (Batkhuu et al. [2012](#page-11-12); Byambadorj et al. 2021).

In one of these *Green Belt* plantations situated in Lun soum, our research group is investigating the growth performance of two tree species: *Populus sibirica* and *Ulmus pumila*. Regarding this, several physiological and morphological traits of these two trees have already been investigated (Batkhuu et al. 2012) and the present work aims to continue these studies by taking into consideration the patterns of biomass partitioning in AG and BG organs of these two tree species. Indeed, in terms of root biomass partitioning, *P. sibirica* and *U. pumila* clearly employ different strategies as higher sensitivity to watering and fertilization regimes, respectively (Byambadorj et al. 2020; Nyam-Osor et al. 2021). The rationale of the work is that this new investigation could help to evaluate better several physiological and ecological (Mensah et al. 2016) aspects of the growth and development of the trees. The hope from a practical point of view is that extensive knowledge of the behavior of these trees could help to design the best management strategy to ensure not only tree survival, but also the long-term success of these afforestation initiatives.

The comparative study of biomass partitioning presented here is necessary as it is known that C allocation to plant organs as a metabolic function must be continuously adjusted to maximize both growth rate and coordinated development of the whole plant (Zhang et al. [2012;](#page-13-18) Thornley and Parsons [2014\)](#page-13-19). Therefore, it is interesting to understand if the two tree species are using the same pattern of biomass partitioning in all AG and BG organs. Our choice to use the clasmometric (i.e. measuring biomass fractions) (Poorter et al. 2015; Noulekoun et al. 2017) analysis, is due to the possibility this approach offers to compare C allocation with a level of detail that other approaches, such as allometric analysis, do not allow (Brown et al. 2019).

We present here not only the pattern of biomass partitioning between AG and BG portions, but in the case of AG, we present also a distinction between biomass patterns in stem (SB), branches (BB), and leaves (LB). In particular, the subdivision of AG biomass in three fractions (SB, BB, LB) offers the possibility to make a physiological distinction between biomass invested in source-organs (leaves) or sink-organs (stem, branches, and roots). Moreover, clasmometric analysis is possible to measure also the percentage of biomass allocated in every single fraction in respect to the total biomass present in the whole tree (TB).

TB data in control (i.e. in absence of irrigation regime and fertilization treatments) trees of *Populus* and *Ulmus* indicate the presence of a slightly lower amount of biomass in respect to values found in trees treated with the lower rate $(2L h^{-1})$ of irrigation regime. This finding confirms that both tree species are adapted to survive in this climate even when no additional water is provided to them (Byambadorj et al. 2020). Nevertheless, the increase of TB observed when trees were treated with higher irrigation regimes (8L h⁻¹) suggests that additional irrigation in both tree species enhanced productivity through an increased photosynthetic activity sustained by the higher availability of water in the soil (i.e. a higher rate of gas exchange taking place in the leaves) (Cernusak [2020\)](#page-11-13). The finding that this biomass increase is cancelled when the trees are treated with the fertilizers suggests that probably fertilization alters the chemical, biological, and physical nature of the soil. This hypothesis is supported by several works showing that establishment of a new plantation induces changes in the soil, such as alteration of microbial (Lauber et al. [2013\)](#page-12-27) and fungal communities (Helgason et al. [2009\)](#page-12-28), and increase of soil organic carbon (SOC), total nitrogen (TN) and total phosphorous (TP) (Yang et al. [2018\)](#page-13-20). Moreover, we cannot exclude that fertilization affects rhizosphere characteristics (Menyailo et al. [2002\)](#page-12-29), the root exudate cycle (Cullings et al. [2003;](#page-12-30) Scheibe et al. [2015\)](#page-13-21), and root turnover rate (Prescott and Grayston [2013\)](#page-13-22). Therefore, our data suggest that given the type of soil present in Lun soum plantations, soil fertilization should be avoided so as not to abolish the stimulatory effect of *Populus* and *Ulmus* tree growth obtainable with an increase of water availability. The lack of biomass increase as a consequence of fertilization results, also from the analysis of biomass pattern in all the fractions examined, indicate that the negative effect is widespread in all compartments of the plant body. In *Ulmus* the outcome from the treatment of NPK with 2L h⁻¹ watering

regime has provided TB increase comparable with the next higher watering regime (i.e. 4 L h⁻¹). The same pattern for *Ulmus* had been noted in terms of root biomass (Nyam-Osor et al. 2021) and general higher survival rate of species in reduced water availability (Byambadorj et al. 2020). This observation supports a feasible option for *Ulmus* management within the *Green Belt* plantations via increased fertilization but reduced water input as a strategy for water conservation.

The data referring to the analysis of the percentage of RB to TB, reveal that in *Populus* RB decreases when we increase water availability in the soil. This decrease could result from the action of two "concomitant" factors. The first factor could be represented by the fact that AG organs require the allocation of more biomass to step up their photosynthetic productivity in the presence of more water in the soil. In consequence of that, the increment of biomass in BG organs is diminished. The second factor could be represented by the waiver of roots to demand more biomass investments given the decreased need to expand their water uptake effort thanks to the higher availability of water in the soil (Di Iorio et al. [2011](#page-12-31); Montagnoli et al. [2012a](#page-12-32), [2012b,](#page-12-33) [2014,](#page-12-34) [2019\)](#page-12-35). A support to this scenario could derive from previous findings that show how *Populus* from the same plantations are characterized by a much higher photosynthetic activity related to the occurrence of a larger leaf area index (LAI) as compared with leaves of *Ulmus* (Cho et al. 2019). The lack of variation in the percentage of RB to TB observed in *Ulmus* (in presence of more water) could be explained with the fact that this tree is characterized by a higher value of water use efficiency (WUE) in respect to *Populus* (Cho et al. 2019). The higher WUE in *Ulmus* probably cancels the need of this tree to invest more biomass to produce more leaves in order to step up the photosynthetic productivity (Cho et al. 2019). In this sense, data presented here suggest that the small leaves of *Ulmus* make this tree better adapted to dry climates whereas *Populus* is more adapted to cool and moist climates with its large leaves (Givnish [1984](#page-12-36); Ackerly et al. [2002](#page-11-14)).

How biomass is partitioned among the components of AG biomass (stem, branches and leaves) does not emerge from the analysis of TAGB pattern, but when LB pattern is examined it is evident that *Ulmus*, independently from the irrigation regime rate and fertilizer considered, do not show biomass variations. This finding agrees with indications emerging from the TB pattern analysis and by the lack of variation observed in the percentage in the RB-to-TB ratio. The increase of LB found in *Populus* with a higher rate of the irrigation regime (4 and 8 L h⁻¹), could be due to factors such as a) the presence of an increase in the number of leaves, and/or b) an increase of leaf area index (LAI). Both hypotheses agree with the reduction of percentage observed in LB when compared to TB but our data are not sufficient to establish the occurrence of equivalence or prevalence of one factor over the other. In this regard, it will be interesting to investigate in future the leaf traits of *Populus* to identify the trait responsible for LB variation reported in this work.

Data regarding the analysis of BB patterns in the two tree species are very similar to data discussed above for LB patterns and this fact suggests the possibility that a direct correspondence occurs between these two AG biomass fractions. Regarding this, it cannot be overlooked that an increase of leaf size requires an increase of biomass in petioles and branches as a consequence of an increased need for mechanical support. In this sense our data regarding the ratio between LB and WB in *Populus* also suggest that the increased water availability induces an increase in biomass allocation to the leaf fraction with a consequent increase of the LB:WB ratio. The effect is absent or much less evident in *Ulmus*. This hypothesis is supported by literature data showing that leaf size is directly correlated to biomass investments in branches (i.e. larger leaves associate with thicker branches) (Westoby et al. [2002;](#page-13-23) Pickup et al. [2005\)](#page-13-24). However, as mentioned above, data presented here cannot confirm at present that in *Populus* an increase in LB depends upon an increase in the leaf size rather than upon the number of leaves. If a future investigation of LA in our experiment will show the presence of a variation of the leaf size in response to irrigation regimes, then it will be clear that our data agree with the findings of other authors that report that increase in leaf size modifies biomass allocation in stems, petioles, and mid-ribs (Niinemets et al. [2006\)](#page-12-37). Moreover, it has been shown that leaf size is directly correlated to biomass investments with larger leaves being associated with thicker branches (Westoby et al. 2002; Pickup et al. 2005).

The pattern of SB from the two tree species presents differences with all the other biomass patterns discussed above. The most interesting difference regards the fact that in *Populus*, unlike the other biomass fractions, the increase of SB is not affected by fertilization. In *Ulmus*, again SB shows that in analogy with all the biomass fractions the increased allocation of biomass in the stem is cancelled in presence of fertilization treatments. The reason for the occurrence of a biomass partitioning difference in AG fractions between the two tree species examined here remains unexplored. However, it is known that given the same amount of transporting elements (xylem vessels and phloem tubes) in the stem, plant species with larger leaves (such as *Populus*) have a greater fraction of total shoot biomass invested in leaves than plant species with smaller leaves such as *Ulmus* (Pickup et al. 2005).

Conclusions

In this work, we used a clasmometric approach to investigate biomass partitioning in *P. sibirica* and *U. pumila* trees used for new plantations in the Mongolian steppe. Data collected confirm 1) that both tree species present a good growth and development performance (Byambadorj et al. 2020), and 2) that irrigation regimes, applied at their medium or high rates $(4 L h⁻¹ – 8 L h⁻¹)$, increase biomass allocation in all biomass fractions with prevalent investment in leaves in *Populus*.

The differences found between the patterns of biomass partitioning are still insufficient to outline the net prevalent reliability of one tree species as compared with the other. The greater productivity of *Populus* is compensated by the higher drought resistance of *Ulmus* whose small leaves have more optimized heat and gas exchange with the environment

(Givnish 1984; Ackerly et al. 2002). Therefore, the indication emerges that in presence of a worsening of drought conditions, *Ulmus* species should be preferred to *Populus* species to establish new plantations. At the same time, decision-makers should consider that by replacing *Populus* with *Ulmus*, there will be a considerable loss in terms of biomass production and therefore a decrease in C sequestration potential of these new plantations.

Data presented here call for investigation regarding the effect of age upon biomass partitioning in these two tree species. It has been reported that as trees age the plant allocates proportionately less biomass to leaves and more to stems (Givnish [1995](#page-12-38)), and a variation of SLA (Specific Leaf Area) takes place during growth for higher photosynthetic activity necessary to enable the growth of above and below ground organs (Tomlinson et al. [2014\)](#page-13-25). For these reasons, the comparative analysis of biomass partitioning should also examine the time factor that in the present study has been ignored. Indeed, including the time factor would allow to calculate the effect of treatments not only in terms of biomass partitioning but also in a more precise quantification of biomass increment, in different plant compartments over time (i.e. C sequestration patterns).

Finally, these studies are important for enabling to achieve a complete picture of the C sequestration potential per tree and per year achievable in these new plantations. These data could enable allometric equations to model the potential C sequestration capacity obtainable from afforestation initiatives to be established in degraded lands in Mongolia.

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Author contributions

Conceptualization, B.N., D.C., and AM.; methodology, D.C., A.M., B.N., S.B.; software and data analysis, S.B.; data collection, S.B., Ts.A.; writing original draft preparation, S.B., G.SS., A.D. D.C.; writing - review and editing, B.N., B.B.P., A.D.; supervision, B.N., D.C.; funding acquisition, B.N. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

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

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