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Comparative studies of the response of larch and birch seedlings from two origins to water deficit

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Abstract

Background: Early developmental stages of plants are expected to be a major bottleneck to recruitment. Information on the response of seedling to anticipated water availability is urgently needed in regions where tree seedlings may experience more frequent water deficits. In this paper, we focused on the influence of water deficit on different species (larch vs. birch) and origins (xeric vs. mesic).

Methods: Prince Rupprecht's larch (*Larix gmelinii* var. *principis-rupprechtii* (Mayr) Pilg.) and white birch (*Betula platyphylla* Sukaczew) from northern China were selected to represent the xeric origin. Eastern larch (*Larix laricina* (Du Roi) K. Koch) and paper birch (*Betula papyrifera* Marshall) from eastern Canada were selected to represent the mesic origin. For each species, half of seedlings were assigned to a well-watered treatment (WW, 75% water holding capacity (WHC) and the other half to a low-watered treatment (LW, 35% WHC). After 20 weeks of treatment, we collected data on morphological indexes of shoot height, root collar diameter, total leaf area and total root area; measured photosynthetic rate (A_{sa}), transpiration rate (E), photosynthetic water-use efficiency (WUE) and apparent chlorophyll concentration (Chl); harvested seedlings and measured biomass allocation of leaf, stem and root.

Results: We found that reduced photosynthetic rate, transpiration rate and increased water-use efficiency were physiological responses of the four species to drought, and the responses varied among the species. All larch seedlings appeared no leaf shedding, survived through the drought treatment and showed a longer duration and high tolerance to drought. In low-watered conditions, survival rate, biomass, shoot height, root collar diameter, total leaf area, total root area and chlorophyll concentration of birch seedlings were significantly decreased. The response of seedlings to water deficits also differed according to the seed origins. Xeric origin of Prince Rupprecht's larch was more affected than mesic origin of eastern larch, mainly because Prince Rupprecht's larch originated in a narrower range of mountainous regions, resulting in lower adaptation of this species to water deficits, whereas eastern larch was distributed widely along coastal regions, contributing to higher phenotypic plasticity to variable soil conditions. Similarly, higher plasticity of paper birch may be responsible for its better performance in the low-watered treatment than white birch.

Conclusions: Larch seedlings were less sensitive to water deficit than birch seedlings. Larch seedlings were characteristics of a conservative water-use strategy, whereas birch seedlings responded with a profligate water-use strategy. Plasticity or adaptation of seedlings outweighs the seed origins in determining their drought responses.

Keywords: Climate change, Species origin, Species traits, Water deficit

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Background

The impact of climate change on the structure and function of forest ecosystems has been observed in many places, and this knowledge is continuously expanding to cover biodiversity, distribution, growth, productivity and mortality (Allen et al. 2010; Lei et al. 2016; Kim et al. 2017). Forests have globally and regionally suffered from drought and heat events (Kozyr 2014; Anenkhonov et al. 2015). These include pine and oak species in Mediterranean regions (Ruiz et al., 2013), larch forests and pine forests of the lower forest belt in Southern Transbaikalia, Russia (Kozyr 2014), and coniferous forests in South Korea (Kim et al. 2017). The common causal factors in these examples are elevated temperatures and/or water stress, raising the possibility that the world's forests are increasingly responding to ongoing warming and drying. There is limited opportunity for tree species to adapt to changing climatic conditions due to the combination of rapid climate change and the long life-span of trees (Eilmann et al. 2013). Climate-induced forest mortality seems to be an emerging global phenomenon, and investigation into the physiological mechanisms through which dry and hot climatic conditions drive tree death and forest die-off represent a rapidly growing research area (Allen et al. 2010; Levesque et al. 2013; Anenkhonov et al. 2015). Understanding the impact of future climate change on forest species is important for forest managers to generate adaptation and mitigation strategies.

The sensitivity of trees to climate change differs among species and ecozones (Zhang et al. 2014; Lei et al. 2016). Therefore, knowledge of species- and region-specific responses to climate is needed. Boreal forests have been found to be severely affected by climate change (Chenlemuge et al. 2013; Ashraf et al. 2015). Larches (*Larix* spp.) are widely distributed in cool temperate and boreal regions of the Northern Hemisphere (Hirano et al. 2017) and are some of the most sensitive tree species to climate change (Levesque et al. 2013; Lei et al. 2016; Kim et al. 2017). About half of the carbon that has accumulated in Eurasian forest communities is contained in larch forests (Cai et al. 2013). Precipitation in spring and autumn could affect growth of eastern larch (*L. laricina* (Du Roi) K. Koch) in Manitoba, Canada (Girardin et al. 2005). Future climate changes could facilitate stand growth and accelerate mortality of Changbai larch (*L. olgensis* A. Henry) in north-eastern China (Lei et al. 2016). Increased drought has been found to be detrimental to larch species, such as Dahurian larch (*L. gmelinii* (Rupr.) Kuzen.) in central Siberia (Sidorova et al. 2009) and the lower forest belt in Southern Transbaikalia, Russia (Kozyr 2014), European larch (*L. decidua* Mill.) in Central Europe (Levesque et al. 2013), and Siberian larch (*L. sibirica* Ledeb.) in north-western

Khentey, Mongolia (Dulamsuren et al. 2010; Chenlemuge et al. 2013) and in the South Siberian forest-steppe landscape (Anenkhonov et al. 2015). Differences in the capacity of plants to withstand water deficits may exist among plants native to different climatic conditions (Levesque et al. 2013). Commonly, species adapted to dry environments tend to survive and grow better during drought than mesic-adapted species when grown together in common-garden experiments or in natural ecotones (Engelbrecht et al. 2005).

Survival of seedlings is often considered the most important factor affecting recruitment, which would significantly affect future forest stand development processes and dynamics (Cai et al. 2013; Xiang et al. 2016), and significant climatic effects on tree recruitment have also been detected. Temperature conditions associated with water availability were found to be an important determining factor affecting tree recruitment in semi-natural *Larix-Picea-Abies* forests in north-eastern China (Xiang et al. 2016). The Siberian larch forests in Central Asia have experienced a lack of regeneration attributed to decreasing summer precipitation in the course of climate change (Dulamsuren et al. 2010; 2013). Drought-induced tree mortality appeared to be more likely for either young or old trees. Seedlings are the most critical development stage because they are more vulnerable to environmental constraints than mature trees due to their poorly developed root systems (Allen et al. 2010; Walck et al. 2011; Galiano et al. 2013). Therefore, information about seedling response to anticipated decrease in water availability is urgently needed, especially in regions likely to experience more frequent water deficits for the coming decades (Walck and Dixon 2009; Baeten et al. 2010; Walck et al. 2011).

Larches are the fourth most abundant tree genus in China, accounting for 6.5% of the forest area and 6.8% of the forest volume in this country (Lei et al. 2016). Prince Rupprecht's larch (*L. gmelinii* var. *principis-rupprechtii* (Mayr) Pilg.) is one of dominant species of cold temperate coniferous forests in northern China, mainly distributed at mountainous regions with elevations of 1400–2800 m (Di et al. 2014). The species plays significant ecological roles in water conservation, ecotourism and biodiversity, and it is also relatively fast growing with good-quality timber (Li et al. 1997; Zhang and Meng 2004). Eastern larch is native to North America. It has a continuous distribution in Canada from the Yukon in the west to Newfoundland in the east and south to the northern and north-eastern USA (Berg and Chapin 1994). It is a commercial species, particularly for short-rotation pulpwood plantations. Prince Rupprecht's larch and eastern larch are both of ecological and commercial importance but responses of seedlings of these species to water deficits are still unknown. Populations of Prince

Rupprecht's larch have experienced serious drought since the 1990s (Qian and Zhu 2001). Eastern larch is mainly confined to hydric sites in Canada, so is tolerant to spring flooding in wetlands (Islam and Macdonald 2004).

Investigating multiple species and comparing how each would respond to the same soil moisture regime are beneficial to understanding how a single species responds to differences in soil moisture (Nishimura and Laroque 2011; Jansons et al. 2016). Larch species are commonly found to be mixed with birch species in natural forest (Cai et al. 2013; Di et al. 2014; Kozyr 2014; Anenkhonov et al. 2015; Xiang et al. 2016; Jansons et al. 2016; Schaedel et al. 2017; Hiranoa et al. 2017). Larch and birch species have been reported to respond differently to climate change. For example, in Southern Transbaikalia, Russia, the habitats of the subalpine belt had also become drier, particularly in the upper parts of the slopes where populations of larch (*L. dahurica* L.) was consequently less stable and was in a declining state; while at the foot of the slopes, dwarf birch (*Betula nana* L.) showed an increase in height due to increased soil moisture (Kozyr 2014). In a study of plasticity comparisons of climate-growth relationships of seven tree species, European larch was sensitive to the temperature in the dormant period and silver birch (*B. pendula* Roth) appeared to be the most robust against fluctuations in weather (Jansons et al. 2016). In northeastern China, increases in the severity and frequency of fires due to climate change may prompt shifts from a larch-dominated forest to an increasingly birch-dominated landscape (Cai et al. 2013). Water crises are expected to occur as a consequence of warmer temperatures and lower levels of precipitation in northern China (Zhang et al. 2014), so may affect species pair of Prince Rupprecht's larch and white birch (*B. platyphylla* Sukaczew) in mountainous forests of northern China. In eastern subarctic Québec, Canada, warmer temperatures since the 1990s have triggered shrub expansion of tundra dwarf birch (*B. glandulosa* Michx.) and might have hindered seedling establishment of eastern larch (Dufour-Tremblay et al. 2012). Also, climate change could affect the species pair of eastern larch and paper birch (*B. papyrifera* Marshall) in boreal forests of eastern Canada.

In this paper, we selected Prince Rupprecht's larch and white birch of Chinese seed origin, eastern larch and paper birch of Canadian seed origin to compare their drought responses. Considering the different original habitats of the two species pairs, we hypothesised that: (i) the growth of birch seedlings is more likely to be affected by water deficiency than that of larch seedlings; and (ii) seedlings of larch and birch originating from a xeric region will grow better than those from a mesic region in response to water deficit.

Methods

Study species and seed origin

Two pairs of larch and birch species were selected, respectively, from eastern Canada and northern China to represent mesic and xeric site origins, the geographical range and sites, Fig. 1.

Mesic origins: Seeds of eastern larch and paper birch were collected from Fredericton in New Brunswick (45.60–46.11° N, 63.90–65.20° W), eastern Canada, in 2012, and mature seeds were obtained from the National Tree Seed Centre of Natural Resources Canada in September 2013. The climate of the site is characterised by mesic conditions with a mean annual temperature of 5.8 °C and a mean annual precipitation total of 1144 mm (mean data from 1971 to 2000) being distributed rather uniformly over the year (Fig. 2a).

Xeric origins: Mature seeds of Prince Rupprecht's larch and white birch were collected from Guandi Mountain (Shanxi Province, northern China, 37.79°–37.93° N, 111.37°–111.54° E) in October, 2012. The climate of the site is dry with a mean annual temperature of 9.9 °C and a mean annual precipitation of 427 mm (mean data from 1971 to 2000) with a high probability of water deficit during the early and late growing season (Fig. 2b).

Greenhouse experiment

We conducted a controlled experiment to evaluate the responses of the species origins and species traits to water deficit in a greenhouse at Lakehead University, Thunder Bay, Ontario. In late October 2013, seeds were soaked for 48 h and sown in 50 cm × 25 cm × 5 cm bedding trays, filled with peat moss and vermiculite (3:1 by volume). Most seedlings emerged about 15–20 days later. In December, seedlings were transplanted in 12 cm × 12 cm × 15 cm containers, uniformly filled with an equal weight of above growing medium. On 1 January 2014, healthy 6-week-old seedlings of similar sizes were randomly selected for the experiment. We used a completely randomised design with four different species and two moisture regimes ending up with eight experimental treatments. We had 30 replicates (experimental units) for each species and water regime combination. Half of the seedlings of each species were assigned to a well-watered treatment (WW) and the other half to a low-watered treatment (LW). All the seedlings were watered with tap water using a 150mL beaker to allow the water to permeate into peat moss slowly and thus minimising gravimetric water loss. Well-watered seedlings were watered every 3 days to container capacity, the actual water content (AWC, %) of the substrate was restored to 75% of the water holding capacity (WHC, %) (Fini et al. 2013), whereas low-watered seedlings were watered every 6 days to keep AWC near 35% WHC until the end of the drying cycle (Wu et al. 2010;

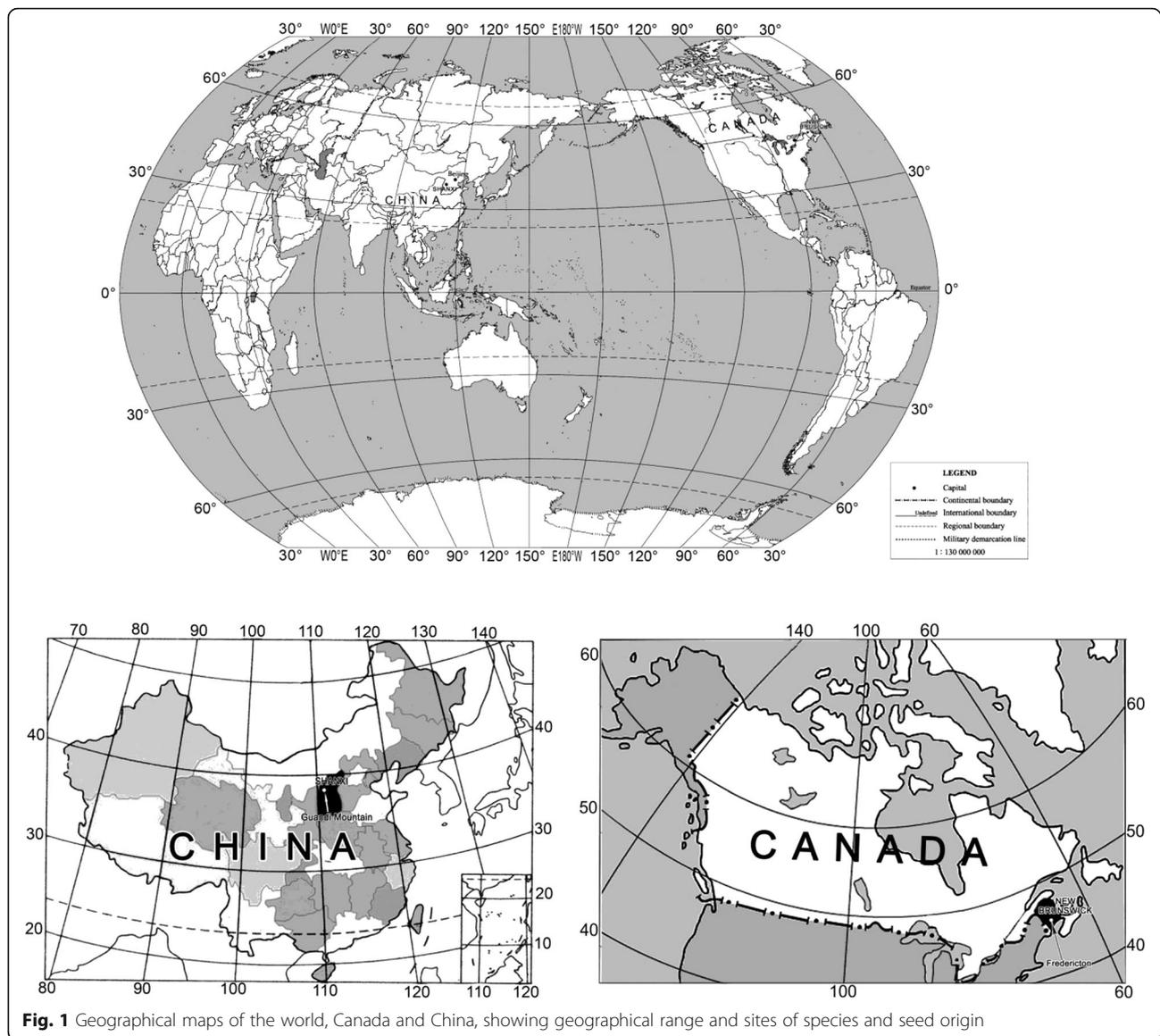


Fig. 1 Geographical maps of the world, Canada and China, showing geographical range and sites of species and seed origin

Ambebe and Dang 2010). During the experiment, relative humidity was approximately 50% and temperature fluctuated from 20 °C (night) to 25 °C (day). Supplemental light was provided to maintain photosynthetic active radiation (PAR) above the seedlings at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Fifty millilitres of mixed nutrient solution (N:P:K, 15:15:10) at a concentration of 5 g L^{-1} was added to the water once every 2 weeks. The containers were randomly moved periodically to reduce edge effects.

In week 20, the number of dead seedlings was counted, and the shoot height and root collar diameter of the remaining live seedlings were measured to 1 and 0.1 mm precision, respectively. Photosynthesis and transpiration were measured on three mature leaves for each species of the three randomly selected seedlings using an open gas-exchange system (LI-6400, Li-Cor,

Inc., Lincoln, NE) at 25 °C, a vapour pressure deficit of 1.5–1.9 KPa and an irradiation of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$. We used saturating light for all gas-exchange measurements to ensure they would not be biased by daily light fluctuations and that photosynthetic capacity would not be limited by suboptimal light. Leaf area was calculated from leaf dimensions. We recorded instantaneous light saturated photosynthetic rate (A_{sat}) and transpiration rate (E) at ambient CO_2 concentration at 370 $\mu\text{mol mol}^{-1}$ controlled by a CO_2 injector. We also calculated water-use efficiency (WUE), defined as the ratio of photosynthetic rate to transpiration rate (A/E). We measured apparent chlorophyll concentration (Chl) on the five youngest fully expanded leaves for each seedling with a portable chlorophyll meter (SPAD 502, Minolta Inc., Ramsey, NJ).

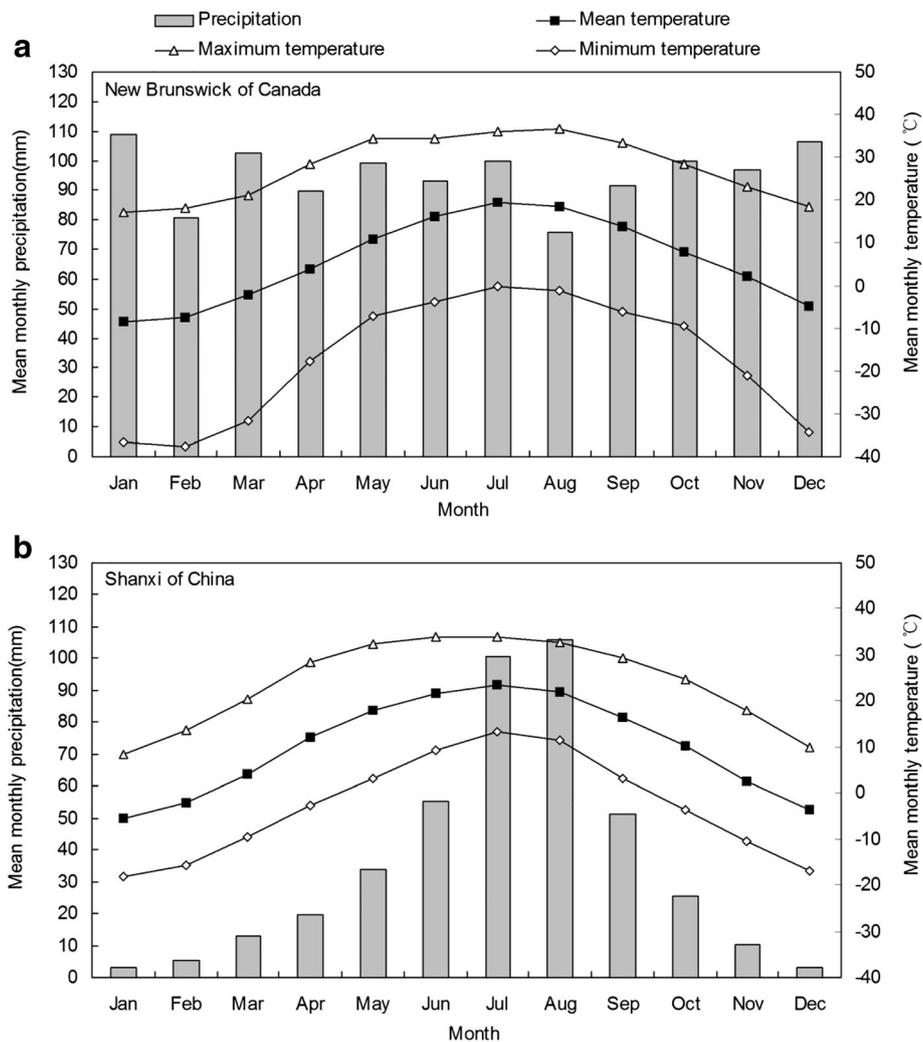


Fig. 2 Mean monthly precipitation and temperature (mean, maximum and minimum) of seed origins from 1971 to 2000. Eastern larch and paper birch are from NB (New Brunswick), eastern Canada (**a**); Prince Rupprecht’s larch and white birch are from Shanxi, northern China (**b**)

Seedlings were then harvested and divided into leaf, stem and root. The roots were washed with tap water and meticulously cleaned. Total leaf area and total root area were determined by software of WinFolia for birch, WinSeedle for larch and WinRhizo for roots of the four species, respectively (Regent Instruments, Quebec City, Canada). All plant samples were dried in an oven at 70 °C for 48 h to obtain leaf, stem and root biomass to 0.01 g precision. For biomass allocation, leaf biomass ratio, stem biomass ratio and root biomass ratio were calculated as ratio of each part biomass divided by the total biomass, and root-to-shoot ratio was also calculated as below-ground biomass divided by above-ground biomass.

We calculated relative survival, relative biomass, relative biomass allocation, relative morphological performance and relative photosynthesis capacity of each species

by the means of the difference in absolute values between the two water treatments divided by the value in the well-watered treatment.

Statistical analysis

A two-way ANOVA was used to test the significant differences of seedling indexes between the two water treatments and four species. If the interaction was tested to be significant ($P < 0.05$), one-way ANOVA was used to test the effects of water treatments on seedling indexes, and multiple comparison was used otherwise. The least significant difference method (LSD) was used to test the significant differences of all data at level of 0.05. All statistical analyses were carried out using the SPSS version 22.0 software package (SPSS, Chicago, IL, USA).

Results

Seedling survival rate and biomass

Seedlings of the broad-leaved birch species were more severely affected by the drought treatment than the larch species, which are conifers. All Prince Rupprecht's larch and eastern larch seedlings survived over the period of 20 weeks in both water treatments (Table 1). Survival of white birch and paper birch was less than 100% even in the well-watered treatment (95.8 and 81.8%, respectively), and survival was still lower for both species following low-water treatment (54.5 and 59.1%, respectively). Thus, the relative survival of white birch and paper birch was 43.1 and 27.8%, respectively (Table 1).

Seedling biomass was significantly different between species, water treatments and their interactions (Table 2). Leaf, stem, root and total biomass of low-watered seedlings were significantly decreased in Prince Rupprecht's larch, white birch and paper birch compared with the well-watered treatment ($P < 0.01$, Fig. 3). The biomass of leaf, shoot and root of Prince Rupprecht's larch decreased by 48.6, 34.6 and 39.4% from well-watered to low-watered treatments. The biomass of eastern larch was slightly decreased (7.4, 7.4 and 11.1%) from well-watered to low-watered, and the effect of each part is insignificant. The biomass of leaf, shoot and root of white birch in low-watered condition were more than half (53.7, 55.3 and 57.7%) of those in the well-watered treatment. The corresponding biomass decreases for paper birch were less than those for white birch; the decreases of leaf, shoot and root were 38.7, 40.1 and 40.6%, respectively (Fig. 3).

The four indexes of biomass allocation calculated were all significantly different between species ($P < 0.01$, Table 2). Biomass allocation of seedlings differed between the two water treatments, with root biomass ratio and root-to-shoot ratio being significantly different ($P < 0.05$, Table 2). In the low-watered treatment, root biomass allocation of Prince Rupprecht's larch seedlings increased from 24.2 to 27.5%, and eastern larch increased from 21.1 to 23.6%. Root biomass ratio of Prince Rupprecht's larch and eastern larch seedlings was increased by 13.7 and 12.1%, respectively. These increases corresponded to decreased allocation to the leaves. Leaf biomass allocation of Prince Rupprecht's larch reduced from 50.4 to 48.0% and eastern

larch reduced from 51.0 to 49.6%. While the two birch species responded differently, both increased leaf biomass allocation, from 54.0 to 59.0% for white birch and from 58.2 to 60.1% for paper birch, but with a little change of root biomass allocation. Root-to-shoot ratio of Prince Rupprecht's larch and eastern larch was increased by 21.9 and 14.8%, respectively; that of two birch species changed slightly. These results might indicate that both of larch species allocated more biomass in root than broad-leaved species in low-watered conditions.

Seedling morphological characteristics

Morphological characteristics of seedlings were significantly different among species, water treatment and their interactions ($P < 0.001$, Table 2). Birch species were more severely affected by low-water conditions than larch species. No obvious needle loss was observed for the two larch species by low-water treatment but low-watered seedlings of white birch began to drop leaves in week 5, and paper birch shed leaves in week 9. Total leaf area, shoot height, root collar diameter and total root area of Prince Rupprecht's larch and eastern larch were all slightly reduced by the low-watered treatment ($P > 0.05$, Fig. 4). All the parameters for both birch species were all significantly decreased. In low-watered condition, total leaf area was reduced from 1319.9 to 619.5 cm² for white birch and reduced from 994.3 to 729.9 cm² for paper birch, and reduced by 53.1 and 26.6%, respectively. The shoot height of white birch was decreased by 23.5% and was very close to that of paper birch (24.9%). The root collar diameter was reduced from 6.05 to 4.19 cm for white birch and reduced from 5.51 to 4.31 cm for paper birch, and decreased by 30.7 and 21.8%, respectively. The total root area was reduced from 241.2 to 151.6 cm² for white birch and from 221.5 to 131.5 cm² for paper birch, and reduced by 37.1 and 40.6%, respectively.

Seedling photosynthesis capacity

Chlorophyll content of seedlings was significantly different among species and water treatment ($P < 0.001$, Table 2), while no significant interactions between water treatment and species ($P > 0.05$, Table 2). Photosynthetic rate, transpiration rate and water-use efficiency of seedlings

Table 1 Sensitivity of Prince Rupprecht's larch (PL), eastern larch (EL), white birch (WB) and paper birch (PB) to water deficit

Species	WW survival (%)	LW survival (%)	Relative survival (%)	Relative biomass (%)	Relative biomass allocation (%)	Relative morphological performance (%)	Relative photosynthesis capacity (%)
PL	100	100	0	42.2	14.6	18.0	30.8
EL	100	100	0	7.4	8.5	5.1	20.0
WB	95.8	54.5	43.1	56.0	6.3	36.1	47.6
PB	81.8	59.1	27.8	40.2	2.1	28.5	39.9

Relative values are calculated as the mean of the difference in absolute values between the two water treatments divided by the value in the well-watered treatment

Table 2 *F* values of the effects of species, water treatment and interaction on seedling biomass, biomass allocation, morphological performance and photosynthesis capacity using a two-way ANOVA

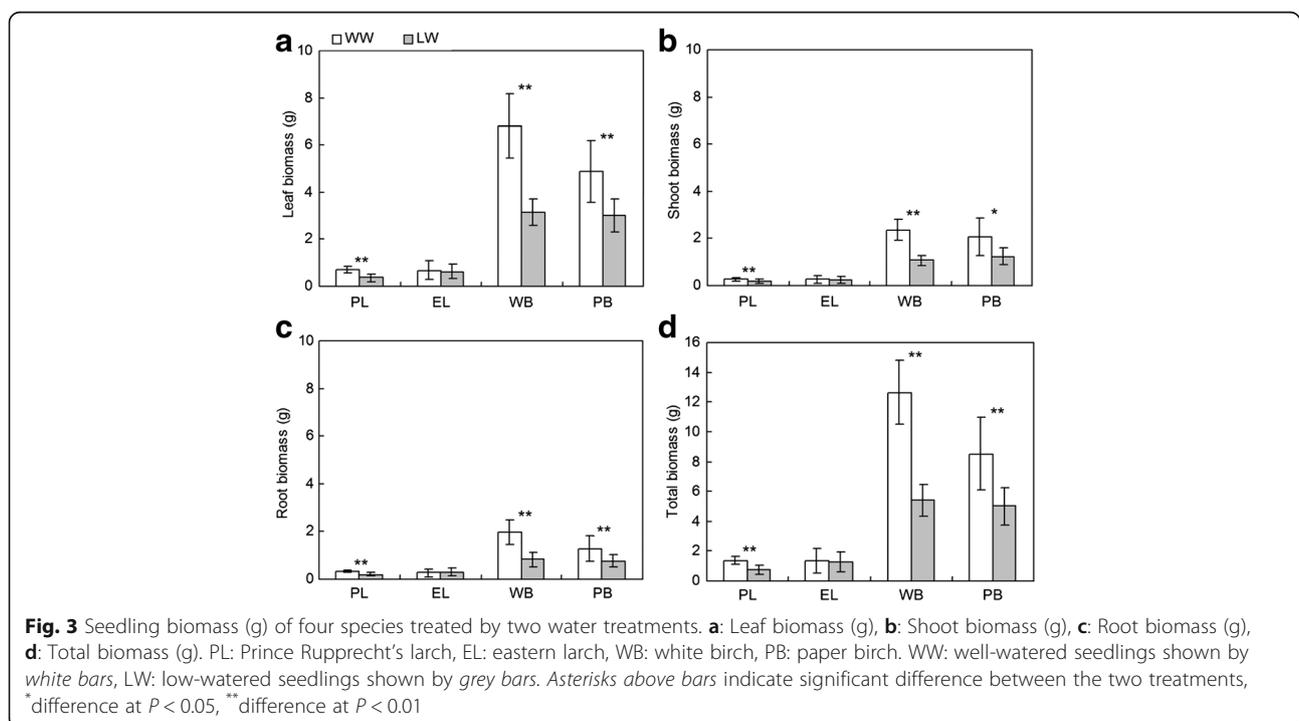
Source	Biomass				Biomass allocation			
	<i>LB</i>	<i>SB</i>	<i>RB</i>	<i>TB</i>	<i>LBR</i>	<i>SBR</i>	<i>RBR</i>	<i>RSR</i>
Species	363.70***	198.30***	121.10***	324.40***	30.12***	12.43***	68.89***	58.80***
Treatment	146.50***	86.60***	69.19***	158.30***	0.80 ns	3.52 ns	4.78*	5.27*
Spe. × tre.	51.71***	29.07***	27.45***	59.39***	4.11**	1.99 ns	2.23 ns	2.54 ns
Source	Morphological indexes				Photosynthesis indexes			
	<i>TLA</i>	<i>SH</i>	<i>RCD</i>	<i>TRA</i>	<i>Chl</i>	<i>A_{sat}</i>	<i>E</i>	<i>WUE</i>
Species	99.71***	299.10***	21.23***	88.28***	47.77***	9.70***	11.63***	6.93***
Treatment	38.22***	33.95***	36.86***	47.40***	24.83***	13.32**	21.81***	24.12***
Spe. × tre.	14.07***	12.54***	7.44***	13.21***	2.10 ns	8.88***	4.74**	0.24***

Biomass includes *LB* leaf biomass, *SB* stem biomass, *RB* root biomass and *TB* total biomass. Biomass allocation includes *LBR* leaf biomass ratio, *SBR* stem biomass ratio, *RBR* root biomass ratio and *RSR* root-to-shoot ratio of biomass; Morphological indexes include *TLA* total leaf area, *SH* shoot height, *RCD* root collar diameter and *TRA* total root area; Photosynthesis indexes include *Chl* chlorophyll content, *A_{sat}* photosynthetic rate, *E* transpiration rate and *WUE* water-use efficiency. *Ns* indicates no significance; *, ** and *** indicate significant difference at level of 0.05, 0.01 and 0.001, respectively

were significantly affected by species, water treatment and their interactions ($P < 0.01$, Table 2).

The low-water treatment had an inhibitory effect on chlorophyll content, photosynthetic rate and transpiration rate, while improving water-use efficiency (Fig. 5). In the low-water treatment, chlorophyll content of birch seedlings was significantly decreased by 15.2% ($P < 0.01$), while changes in the chlorophyll content of larch seedlings was not significant (Fig. 5a, $P > 0.05$). The photosynthetic rates of Prince Rupprecht’s larch, eastern larch, white birch and paper birch were significantly reduced by 12.4, 12.9, 51.9 and 10.0%, respectively (Fig. 5b, $P < 0.05$). Transpiration rate

and water-use efficiency of the four species differed greatly between the two water treatments (Fig. 5c, d, $P < 0.01$, Table 2). In the low-water treatment, transpiration rate of Prince Rupprecht’s larch and eastern larch was decreased by 42.8 and 30.0%, respectively; white birch and paper birch decreased by 66.7 and 43.7%, respectively. Water-use efficiencies of these four species were all significantly increased in low-watered condition (Fig. 5d, $P < 0.05$, Table 2). Water-use efficiencies of Prince Rupprecht’s larch and eastern larch was increased by 58.2 and 33.1%, respectively; white birch and paper birch increased by 60.6 and 90.9%, respectively. Overall, photosynthetic capacities of the two birch species



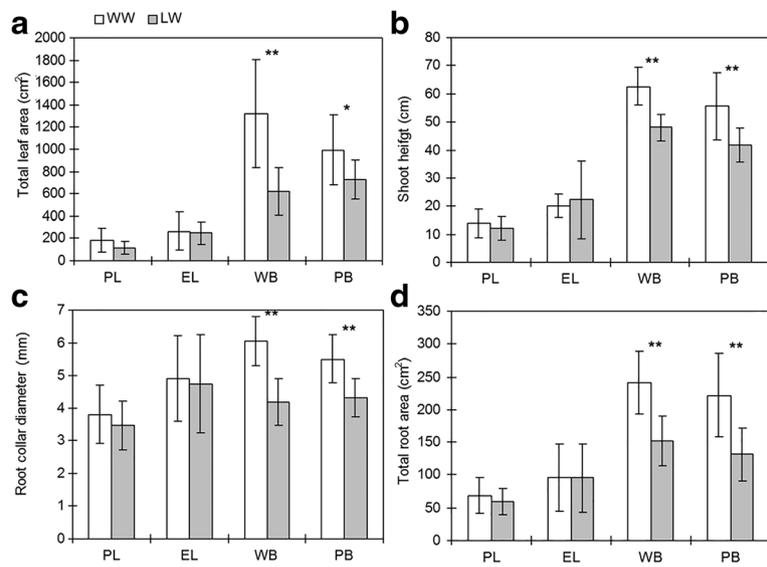


Fig. 4 Morphological indexes of four species treated by two water treatments. **a:** Total leaf area (cm²), **b:** Shoot height (cm), **c:** Root collar diameter (mm), **d:** Total root area (cm²). PL: Prince Rupprecht's larch, EL: eastern larch, WB: white birch, PB: paper birch, WW: well-watered seedlings shown by open bars, LW: low-watered seedlings shown by shaded bars. Asterisks above bars indicate significant difference between the two treatments, * difference at $P < 0.05$, ** difference at $P < 0.01$

were more affected by water deficit than for the two larch species tested.

Discussion

Seedling general response

In our study, restricted levels of water resulted in higher root biomass allocation and high biomass investment to

the root system for the two larch species tested. In contrast, significant decreases in biomass, leaf area, shoot height, root collar diameter and root area occurred in the two birch species tested to minimise water losses. The morphological changes of the four species to drought were consistent with the general responses of other species. Leaf shedding of birch seedlings in low-water environments has

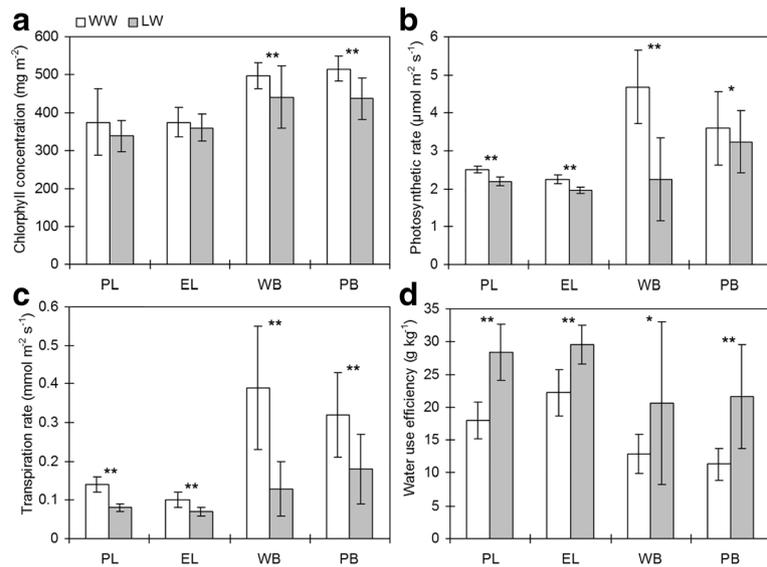


Fig. 5 Photosynthesis capacity of four species treated by two water treatments. **a:** Chlorophyll concentration (mg m⁻²), **b:** Photosynthetic rate (µmol m⁻² s⁻¹), **c:** Transpiration rate (mmol m⁻² s⁻¹), **d:** Water use efficiency (g kg⁻¹). PL: Prince Rupprecht's larch, EL: eastern larch, WB: white birch, PB: paper birch, WW: well-watered seedlings shown by open bars, LW: low-watered seedlings shown by shaded bars. Asterisks above bars indicate significant difference between the two treatments, * difference at $P < 0.05$, ** difference at $P < 0.01$

been shown to occur to limit evaporative losses when seedlings can no longer maintain a positive carbon balance (Levesque et al. 2013; Ruiz et al. 2013; Mantovani et al. 2014). During drier periods, reduction in available soil water results in closure of stomata and decrease of stomatal conductance C_i to limit evaporative losses. Drought also reduces carbon dioxide uptake, thereby reducing both photosynthetic rate and transpiration rate. This, in turn, induced shedding of older foliage and decreased biomass. Water-use efficiency significantly increased in the low-water treatment as the plants attempted to fix the most carbon per unit of water transpired (Mantovani et al. 2014; Waghorn et al. 2015). Reduced photosynthetic rate, transpiration rate and increased water-use efficiency were physiological responses observed in these four species to reduce the risk of soil drought. These responses to water deficits, in turn, constrain the growth of seedlings (Waghorn et al. 2015).

Species-specific response

The ability of tree species to respond to drought is heritable and shows a wide interspecific variation. Larch is one of the most sensitive tree species to climate change (Levesque et al. 2013; Lei et al. 2016; Kim et al. 2017). The distribution, growth, reproduction and regeneration of Dahurian larch, Siberian larch, and European larch have all suffered from increased drought and are all known to be rather drought sensitive (Sidorova et al. 2009; Dulamsuren et al. 2010; Chenlemuge et al. 2013). Paper birch and river birch (*B. nigra* L.) have been reported to have inherently low tolerance to drought (Ambebe and Dang 2010). Tree recruitment responses to climate change were also found to be species-specific traits and environmental constraints. Larch recruitment might be aided by lower winter temperatures, while low winter-spring temperature to be a chronic stressor causing hardwoods (white birch, etc.) sapling shoot damage, increasing sapling mortality. Larch recruitment could occur under shaded and xeric micro-topography, while birch populations expanded at the foot of the slopes (Kozyr 2014; Xiang et al. 2016). It indicated that the birch recruitment is more likely to be affected by climate change than larch.

Drought response comparisons between larch and birch species are crucial considering strong differences in growth patterns and utilisation of resources among co-occurring species (Simard et al. 2004; Mitchell et al. 2013). In a greenhouse experiment of short (4-day-long) and progressive (3-week-long) soil drought, intact 1-year-old white birch was less tolerant than Dahurian larch (Mao et al. 2004). Conversely, silver birch appeared to be more robust against weather fluctuations than European larch (Jansons et al. 2016). During our 20-week experiment, neither obvious needle loss nor mortality was observed for larch seedlings in the low-water treatment while leaf

defoliation and higher mortality of birch seedlings did occur. It suggested that larch species could survive longer with low-water availability and that birch seedlings were more sensitive to water stress. Our first hypothesis was supported by the results.

Birches have been found to have mechanisms for detecting and responding to changes in ambient CO_2 concentration, then imparting a greater capacity to optimise water-use efficiency (Brodrribb et al. 2009). Our result was in accordance with the conclusions of Mao et al. (2004), Pittermann et al. (2006) and Ambebe and Dang (2010). With the larch species, no leaf shedding and mortality occurred, but photosynthetic rate and transpiration rate significantly decreased and water-use efficiency greatly increased. It might imply that growth performance and morphological adaptations of larch were less sensitive than the physiological performance, which could also be found in another study (Gao et al. 2015). Different water-use strategies may be employed in larch and birch species. The larch species are characteristic of conservative water-use strategy, which is associated with slow intrinsic growth rates and high capacity for drought tolerance, which resulted in a longer duration prior to mortality (Zhang et al. 2005; Mitchell et al. 2013). While the birch species are fast-growing trees with profligate water use, rapid loss of hydraulic conductivity for fast growth rates means that they succumb to drought rapidly (Mao et al. 2004; Ambebe and Dang 2010). The different water-use strategies of larch and birch species, in turn, determined their differences in environmental recruitment constraints.

Site-specific response

Tree drought-responses are commonly regarded as site-specific limited to how far they can be adjusted (Levesque et al. 2013). Tree responses to water deficit that are out of their natural environments depend on their native habitats (Otieno et al. 2005; Ruiz et al. 2013; Jansons et al. 2016). It has been observed that in a common water stress environment, seedlings originating from drier areas tend to display a better performance than those from wetter areas (Ruiz et al. 2013; Levesque et al. 2013). In our study, seed origin of eastern larch and paper birch was of mesic conditions with a mean annual precipitation sum of 1144 mm, being distributed rather uniformly over the year (Fig. 2a); seed origin of Prince Rupprecht's larch and white birch was characteristic of xeric conditions with a mean annual precipitation sum of 427 mm (Fig. 2b). In Table 1, the relative biomass, relative biomass allocation, relative morphological performance and relative photosynthetic capacity of Prince Rupprecht's larch were all greater than eastern larch; the relative survival, relative biomass, relative biomass allocation, relative

morphological performance and relative photosynthetic capacity of white birch were greater than paper birch. It indicated that Prince Rupprecht's larch is more affected by water deficit than eastern larch, and white birch is more affected than paper birch. The result conflicts with our second hypothesis, as well as the conclusions of Otieno et al. (2005) and Ruiz et al. (2013).

Tree drought-response is partly plastic and phenotypic plasticity results from environmental factors should be another explanation of our findings. Eastern larch is from NB, where it is characterised by mixture of continental climate and maritime climate. Species from these areas are likely to have high tolerance to changing weather conditions (Islam and Macdonald 2004; Lukkarinen et al. 2009; Dufour-Tremblay et al. 2012). Effective spring drought avoidance by eastern larch partially explains why it dominates eastern Siberian forests with their cold, dry continental winter climate (Berg and Chapin 1994). Larger natural range of eastern larch contributes to its higher adaptation to the temporally and spatially variable soil conditions, including water availability. Prince Rupprecht's larch is native to the mountainous regions of 1400–2800 m elevation, distributed in a scattered manner, with a smaller area in Shanxi and Hebei provinces of northern China. It has been well known that temperature and water supply (especially precipitation) were critical drivers for seedling germination (Walck et al. 2011; Xiang et al. 2016). So, we deduced that frequently occurring spring drought in northern China is the limiting factor to the tree recruitment, which in turn resulted in the narrower distribution of Prince Rupprecht's larch, and lower adaptation to water deficits compared to eastern larch. Similarly, paper birch has a wide range. It is found in interior and south-central Alaska and in all provinces and territories of Canada, as well as the northern continental USA, south to Pennsylvania and Washington. White birch is mainly distributed in East Asia, native to China, Korea and Japan. Wider distribution of paper birch also resulted in higher adaptation to water deficits compared to white birch.

These findings demonstrate that the species-specific response and environment constrains to water deficit in the current climate regime may finally lead to growth adjustments to drier conditions, e.g., by enhanced root growth to access water resources in deeper soil layers (Feichtinger et al. 2014). Thus, the response of trees to abrupt changes in water availability is decreasing with time due to growth adjustments. Current knowledge on the seedlings' responses to water deficit is based on manipulation experiments, which have several limitations when extrapolating to field conditions. Therefore, long-term field experiments and observations are needed to reveal the mechanisms behind growth adjustments to drought.

Conclusions

Seedlings showed varied responses to water deficit according to the species origins and the species traits. Birch seedlings are more sensitive to water deficit than larch. In low-watered condition, no mortality is occurred in larch seedlings, whereas survival rate of birch seedlings is reduced by 35.4%. Biomass, shoot height, root collar diameter, total leaf area, total root area, and chlorophyll concentration of two birch species were all significantly decreased. Contrary to common opinion, species pairs of xeric origins are more affected by water deficit than those of mesic origins, probably because the narrower range in mountainous regions of Prince Rupprecht's larch results in lower adaptation to water deficits, while larger distribution along coastal regions of eastern larch contributes to its higher phenotypic plasticity of variable soil conditions. Better performance of paper birch might also be a result of higher adaptation due to its larger range than white birch. That is, plasticity or adaptation of seedlings outweighs seed origins in determining their drought responses.

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Authors' contributions

RG was the primary author and conducted the majority of the greenhouse work. XS undertook the data analysis and assisted with the writing of the paper. JRW reviewed and edited the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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