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JIAE AN
HANNA CHANG
SEUNG HYUN HAN
ASIA KHAMZINA
YOWHAN SON

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Differential water-use and growth responses of Pinus densiflora and Larix kaempferi seedlings to microclimate manipulation

Jiae AN1,2, Hanna CHANG3, Seung Hyun HAN4, Asia KHAMZINA1, Yowhan SON1,*  
1Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea  
2Division of Restoration Research, National Institute of Ecology, Yeongyang, Republic of Korea  
3Urban Forest Division, Forest Environment and Conservation Department, National Institute of Forest Science, Seoul, Republic of Korea  
4Forest Technology and Management Research Center, National Institute of Forest Science, Pocheon, Republic of Korea

Abstract: Our study assessed how physiological traits and the growth of seedlings of Pinus densiflora and Larix kaempferi, representative coniferous species of Korea, are affected by warming and precipitation manipulation. A warming and precipitation manipulation experiment was conducted with 1- and 2-year-old seedlings using infrared heaters, transparent panels, and an irrigation system. Plant physiological activities were monitored using a portable photosynthesis measurement system, and seedling growth was investigated by measuring the dry weight of harvested biomass. P. densiflora, an isohydric species, showed an overall decrease in physiological activities under the warming treatment in the first (germination) year. In particular, stomatal conductance decreased to prevent excessive water loss, with minimal decreases in photosynthetic activity, and increased the water-use efficiency in the first year. L. kaempferi, an anisohydric species, maintained stomatal conductance under the warming treatment. Both species maintained physiological activities with their distinct responses to heat in the second year, while the seasonal variation pattern changed in 2-year-old P. densiflora seedlings. The dry weight of L. kaempferi seedlings increased under warming; however, it decreased by warming combined with increased precipitation. These results can enhance the understanding of seedling responses to microclimate manipulation according to the different water-use by species and seedling age. Under the future climate change, these responses should be accounted for in management of seedlings in nurseries or newly afforested areas.

Key words: Climate change, gas exchange, precipitation manipulation, seedling response, warming, water-use strategy

1. Introduction
Global climate change phenomenon has been revealed through various metrics, including mean temperature increases, heat extremes, and heavy precipitation (IPCC, 2018). Considering the current rate of global warming, the global temperature increase is predicted to exceed 1.5 °C between 2030 and 2052 (IPCC, 2018). These changes are expected to affect the carbon cycle, vegetation dynamics, and evapotranspiration in terrestrial ecosystems, especially forest ecosystems (Bonan, 2008).

Considerable changes in atmospheric temperature and water availability caused by climate change will affect tree growth and survival by altering physiological activities and growth rates (Kreuzwieser and Gessler, 2010). Warming and resulting heat and water deficit stresses may significantly impact forest ecosystems by reducing the leaf stomatal conductance and photosynthesis (Reich et al., 2018). Under high temperatures, plant physiological responses are generally reflected in minimization of heat absorption and maximization of its dissipation (Liu et al., 2020b). The opening and closing of the stomata play important roles in warming climates since photosynthetic and transpiration processes are shaped by stomatal functioning (Garcia-Forner et al., 2016). Higher stomatal conductance leads to higher photosynthesis and transpiration rates under adequate water availability but may increase water loss when water is scarce (Liu et al., 2020b). Increase in precipitation could alleviate heat and water stress, and boost photosynthesis by increasing stomatal conductance and reducing leaf temperature (Shrive et al., 1994). However, excessive soil moisture could reduce photosynthesis and stomatal conductance (Fernández, 2006).

The degree of stomatal control of water loss is species-specific. It is strongest in isohydric species, whereas anisohydric species exhibit weak stomatal adjustment capability (Oren et al., 1999, Garcia-Forner et al., 2016). Considering that aforementioned stomatal function of
trees that determine their growth can be either stimulated (Niu et al., 2008, Tang et al., 2016) or hampered (Choat et al., 2012, Chang et al., 2020) by climate change and that the responses to environmental changes vary by tree species, there is a need to elucidate the effects of heat and water stresses related to warming and increased precipitation as well as their interaction of the latter two factors.

High-quality seedlings are the basis for reforestation success, as seedling growth determines the establishment of trees (Danby and Hik, 2007), and successfully regenerated forests play an important role in carbon uptake and climate change mitigation (Riikonen and Luoranan, 2018). Seedlings are more sensitive to environmental stressors than mature trees. In particular, warming has a greater impact on the photosynthesis, biomass allocation, and growth of seedlings than those of mature trees because seedlings have limited root systems and carbon reserves (Niinemets, 2010, Khaine and Woo, 2015, Matías et al., 2017). Moreover, seedling responses to environmental change may vary between the germination year and the short period immediately thereafter. In a previous study, no changes were observed in stomatal conductance, transpiration rate, or net photosynthetic rate in the germination year, but these physiological activities were decreased under warming treatment for 2-year-old Larix kaempferi seedlings (An et al., 2016).

In previous studies focusing on temperate coniferous tree species, seedlings showed various responses to the warming and precipitation manipulation as was reflected in leaf emergence, leaf-out rate, growth, and survival (Fisichelli et al., 2014). However, questions remain concerning various response patterns to climate changes by tree species (Lebourgeois et al., 2010). Pinus densiflora and L. kaempferi, key coniferous species used for reforestation in South Korea (Bhusal et al., 2020), were classified as warm and cold temperate conifers with isohydric and anisohydric characteristics, respectively (Matyssek et al., 2014). Their adaptability to environmental changes during the seedling stage is important for producing high-quality planting stock in nurseries and successful establishment after transplanting.

We assessed the physiological characteristics of seedlings, including transpiration rate, net photosynthetic rate, stomatal conductance, and intrinsic water-use efficiency, and the variation of seedling growth by species and plant age with changes in air temperature and precipitation. We hypothesized that (1) physiological activities such as transpiration rate, photosynthetic rate, and stomatal conductance of P. densiflora (isohydric species) would decrease and those of L. kaempferi (anisohydric species) would be less affected by heat and resulting atmospheric or soil moisture stress; the physiological activities of both species would be enhanced by the increased precipitation treatment owing to increased water availability; (2) inhibition of physiological activities due to warming-induced heat and water stress would be alleviated by precipitation increase treatment; and (3) these physiological responses would be reflected in early seedling growth (i.e. the growth of P. densiflora seedlings would be reduced, and the growth of L. kaempferi seedlings would be unaffected).

2. Materials and methods
2.1. Study site
Experimental warming and precipitation manipulation system was installed in the Forest Technology and Management Research Center, Pocheon, Korea in April 2017 (37°45′N, 127°10′E). Our study was conducted from 2018 to 2019. The annual mean air temperature and annual total precipitation for the study area were 11.7 °C and 12 °C and 1500 mm and 1028.1 mm, respectively, in 2018 and 2019 (Korea Meteorological Administration, 2019; Figure 1). The initial soil pH, total carbon concentration, total nitrogen concentration, and cation exchange capacity (CEC) of the soil in the study site were 6.24, 0.24%, 0.16 mg g⁻¹, and 5.57 cmol kg⁻¹, respectively (Jo et al., 2019).

2.2. Experimental design
On April 16, 2018, P. densiflora and L. kaempferi seeds were sown separately in 1 m × 1 m plots spaced 50 cm apart, according to silvicultural guidelines (Korea Forest Service, 2017). The initial seedling rate was as follows: P. densiflora, 15 g m⁻² and L. kaempferi, 20 g m⁻². In April 2019, P. densiflora and L. kaempferi seedlings were transplanted by rearranging the seedlings within the same plot to create an area of approximately 10 cm all-round spacing between the seedlings, in accordance with the silvicultural guidelines (Korea Forest Service, 2017).

Temperature and precipitation were manipulated on the experimental plots to simulate the temperature and precipitation conditions predicted for 2060 based on the Representative Concentration Pathways (RCP) 8.5 climate change scenario (Korea Meteorological Administration, 2005). Treatments included two temperature levels [temperature control (C) and warming (W)], and two precipitation levels [precipitation control (P⁰) and increased precipitation (P⁺)] (4 treatments × 3 plot replicates × 2 species) (Figure 2). There was a problem with blocking precipitation in the decreased precipitation plots by only covering the plot area because the height of the panel above the seedlings was too high, and rainwater might rush to the side. Therefore, the data collected from the decreased precipitation plots was excluded.

The seedlings in the warming plots were warmed to maintain an air temperature 3 °C higher than that in the temperature control plots. Infrared heaters (FTE-1000, Mor Electric Heating Association, USA) were used for the
heating module, whereas dummy heaters were installed in the temperature control plots to simulate the shading effects of the heaters. The infrared heaters were controlled by data loggers (CR-1000, Campbell Inc., USA) and relays (SDM-CD16AC, Campbell Inc., USA) based on the air temperature (AT) monitored by infrared temperature sensors (SI-111, Campbell Inc., USA). Temperature data were collected every 1 min to ensure that the temperature difference between the temperature control and the warming plot was immediately reflected and adjusted. The distance between the infrared heaters and the tops of the seedlings was approximately 70 cm. As the seedlings
The height of the heated infrared was adjusted. Precipitation in P plots was increased through subsurface irrigation with rainwater collected from the coverings in plots next to the P plots, where the rainfall was blocked by 40% of plot areas with V-shaped transparent panels, installed 1.6 m above the ground. Planks were installed to prevent lateral movement of soil water from irrigated plots. Transparent dummy panels (not V-shape) were installed in the P plots. An irrigation pump was operated at a constant rate every day for 10 min beginning at 9:00 AM to 10:00 AM. Soil moisture (SM) was monitored using reflectometers (CS655, Campbell Inc., USA) installed at a depth of 5 cm in the soil of each plot, and the data were recorded every 30 minutes using a data logger.

2.3. Measurements of physiological and growth responses
Leaf gas exchange was measured in June, July, August, and October in 2018 and May, June, August, September, and October in 2019 in the leaves of three randomly selected seedlings. Gas exchange measurements were conducted on a clear morning between 8:30 AM to 11:30 AM using a portable photosynthesis system (LI-6800, LI-COR, USA) and 6800-12A chamber (30 mm × 30 mm) at ambient temperature and ambient relative humidity. During the measurements, photosynthetically active radiation (PAR) and CO₂ concentration were maintained at 1100 μmol m⁻² s⁻¹ and 400 ppm, respectively. The surface areas of the leaves used for gas exchange measurement were analyzed with a scanner (Perfection V700 Photo, EPSON) and WinSeedle software (Regents Inc.). Subsequently, the transpiration rate (E), net photosynthetic rate (A), and stomatal conductance (gₛ) were calculated per unit area of the leaf. The intrinsic water-use efficiency (WUEᵢ) was calculated as the ratio of the net photosynthetic rate to the stomatal conductance.

Three seedlings per plot were carefully excavated in September 2019 using shovels not to damage the roots. The harvested seedlings were brought to the laboratory and separated into leaves, stems, and roots. The roots were washed free of soil and oven-dried at 65 °C. The dry weight of all harvested components was measured.

2.4. Statistical analysis
Repeated Measures ANOVA was used to assess differences in the AT, SM, E, A, gₛ, and WUEᵢ with time included as a random effect for the overall effects of the manipulated warming and precipitation. For each month, the two-way ANOVA was used to analyze the effects of the warming and precipitation treatments on E, A, gₛ, and WUEᵢ. The two-way ANOVA was also used to determine the effects of warming and precipitation manipulation on the dry weight of each component and the total dry weight of the seedlings. All results were considered statistically significant at p < 0.05. Correlation analysis was conducted to determine the relationship between environmental factors (AT and SM) and physiological traits (E, A, gₛ, and WUEᵢ) of P. densiflora and L. kaempferi seedlings. AT, SM, and physiological traits during the entire measurement period were used for correlation analysis by year and species (n = 48 in 2018; n = 60 in 2019). The quadratic relationships between E and gₛ of P. densiflora seedlings to AT were tested with a linear regression (p < 0.05; n = 48 in 2018; n = 60 in 2019). All statistical analyses were performed using SAS 9.4 statistical software (SAS Institute Inc., USA).

3. Results
3.1. Environmental conditions
The mean AT and SM values from the plots of P. densiflora and L. kaempferi in 2018 (April to December) and 2019 (January to October) are presented in Figure 3. In 2019, there was no SM data recorded in April for P. densiflora and April to May for L. kaempferi because of the sensor failure. The AT was significantly increased for both species under the warming treatment in 2018 and 2019. For L. kaempferi, there were interactions between time and precipitation increase treatment (2018) as well as time and warming (2019). The SM significantly increased under the precipitation increase treatment in both species plots in 2018 and only in P. densiflora plots in 2019. There were interactions either between time and warming or between time and precipitation increase treatment in 2018 for both species (Figure 3). For P. densiflora, the mean AT in the W plots was 2.93 °C and 2.78 °C higher than in the C plots in 2018 and 2019, respectively. The mean SM (vol. %) for P. densiflora was 10.84 ± 0.54 and 9.79 ± 0.81 in the P plots and 16.55 ± 0.95 and 12.55 ± 0.99 in the P plots in 2018 and 2019, respectively. For L. kaempferi, the mean AT in the W plots was 3.18 °C and 2.83 °C higher than in the C plots in 2018 and 2019, respectively. The mean SM (vol. %) for L. kaempferi was 10.67 ± 0.39 and 10.61 ± 0.87 in the P plots and 14.03 ± 0.67 and 12.11 ± 1.00 in the P plots in 2018 and 2019, respectively.

3.2. Physiological responses of seedlings
The warming treatment significantly affected E, A, gₛ, and WUEᵢ in P. densiflora seedlings in 2018; there was no warming or increased precipitation effects observed in 2019 (Figure 4). In 2018, E, A, and gₛ were lower overall by 13.1, 8.9, and 16.2% under the warming treatment, respectively, while WUEᵢ was 12.3% higher under warming than under the temperature control. The time factor was significant throughout the observation period, indicating considerable temporal variations. The interaction effect of time × warming or time × precipitation increase treatment was significant for E in 2018. There was an interaction between time and warming for WUEᵢ in 2018.

Over the course of the observation period, gₛ of P. densiflora was lower under the warming treatment in June.
and July of 2018 by 14.4% and 31.2%, respectively (Figure 4). Warming significantly decreased E in July 2018 by 23.2%, and WUE increased by 34.8% and 12.3% in July 2018 and August 2019 (Figure 4). In the second year, the physiological activities such as E, A, and gs were generally higher compared to the first year in August. There was no observed effect from precipitation manipulation on any of the physiological traits of *P. densiflora* seedlings in either year. There was no effect of the warming-only or the precipitation increase treatment on the physiological traits of *L. kaempferi* (Figure 5). The time factor was significant throughout the observation period, indicating considerable temporal variations. The interaction effect of time and the warming treatment was significant for E in 2018. In August 2018, A decreased by 22.4% under the warming treatment, and there was an interaction on the warming and precipitation increase treatment. In May 2018, E and gs were increased by 38.1% and 39.3%, respectively, by the warming treatment, and there were interactions of warming and increased precipitation.

In *P. densiflora*, the correlation between AT and SM was negative in 2018 and positive in 2019. A and gs were negatively correlated with AT in 2018, while E and gs were positively correlated with AT in 2019. Additionally, E and gs were positively correlated with SM in 2019 (Table). In *L. kaempferi*, there was a negative correlation between AT and SM in 2018. gs was negatively correlated with AT in 2018 and 2019. E and A were positively and negatively correlated with AT, respectively, in 2019 (Table). All of the above-stated correlations were significant (p < 0.05) with a correlation coefficient ranging from 0.27 to 0.72 and from −0.28 to −0.54 (Table). The linear regression test showed that E decreased with increasing AT above a certain temperature, around 23 °C, and gs also decreased as the temperature increased for 1-year-old *P. densiflora*. However, E and gs of 2-year-old *P. densiflora* increased with increasing AT (Figure 6).

### 3.3. Growth of seedlings

For 2-year-old *P. densiflora* seedlings, there were no statistically significant effects of warming, increased precipitation, or their interaction on individual organs.
For L. kaempferi, all of the components and the total dry weights were significantly decreased by the precipitation increase treatment, and root dry weight was increased by the warming treatment. Leaf, stem, root, and the total dry weight of the seedlings in P+ plots were 46.7%, 48.2%, 43.1%, and 46.3% lower than in P0 plots. Root dry weight was higher by 63.6% in W plots than in C plots. There was an interaction of warming and increased precipitation for leaf, stem, and the total dry weight (Figure 7).

4. Discussion

4.1. Differences in physiological responses by species

Newly germinated P. densiflora seedlings have shown decreased physiological activities under warming treatment. In contrast, in the case of 2-year-old seedlings, Repeated Measures ANOVA revealed no response to the warming. The decrease of gS in 1-year-old P. densiflora seedlings might be explained by specific water-use mechanisms of this isohydric species (Matyssek et al., 2014) in response to heat stress resulting from the warming treatment (Collatz et al., 1991, Tardieu and Simonneau, 1998, Denham et al., 2021). As leaf temperature increases, transpiration might be accelerated to cool off the leaf surface as warming increases the vapor pressure deficit (VPD) between the leaf and air (Will et al., 2013). In the meantime, isohydric species are able close the stomata to lower the stomatal conductivity, preventing excessive water loss (Farquhar and Sharkey, 1982).

When P. densiflora seedlings were affected by high temperatures and the gS was decreased, A was not reduced by a comparable portion as the observed increase in WUEi (expressed as the ratio of A to gS) (Yi et al., 2019). Nonetheless, the assimilated CO2 reserves might quickly be used up, and the seedlings begin to starve if the gS and A are not recovered (Ruehr et al., 2016). In the second year, as seedlings grew and adapted to the changes in environmental conditions, photosynthetic activities were similar under temperature control and the warming treatment; WUEi similarly was not affected by warming.

Unlike the P. densiflora seedlings, the L. kaempferi seedlings in 2018 showed no physiological response to warming treatment (as reflected by the E, A, and gS values), which might be explained by relatively weak stomatal control by L. kaempferi as an anisohydric species. In response to heat stress caused by warming, anisohydric species maintain gS by opening stomata to an extreme near-death state and continuously maintaining photosynthesis.
These results suggest that *L. kaempferi* seedlings maintain physiological activity under warming conditions.

In the second year of the experiment, when the canopy increased as the *P. densiflora* seedlings grew, the seasonal characteristics of summer (high temperature and heavy precipitation) seem to have impacted the correlation between AT and SM, superimposing the effects of the warming treatment on SM. In the *L. kaempferi* plots, there was no correlation between AT and SM in the following year.

Figure 5. Transpiration rate (E, a), net photosynthetic rate (A, b), stomatal conductance (gs, c), and intrinsic water-use efficiency (WUEi, d) of *Larix kaempferi* seedlings in 2018 and 2019. There was a combination treatment of warming (temperature control (C) and warming (W)) and precipitation (precipitation control (P0) and increased precipitation (P+)). Error bars are the standard errors of the means. T and W with the upper asterisk (*) indicate significant differences in time and warming by the repeated measures ANOVA, respectively. The asterisk (*) and multiplication (×) above the graph lines indicate significant differences by the warming and the interaction between the warming and the precipitation manipulation of each month, respectively (*p < 0.05; **p < 0.01; ***p < 0.001).

Table. Correlation analysis results on physiological traits of *Pinus densiflora* and *Larix kaempferi* seedlings with air temperature and soil moisture during the entire measurement period (n = 48 in 2018; n = 60 in 2019). Bold values indicate the statistical significance of the correlation between the variables.

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Variable</th>
<th>AT</th>
<th>SM</th>
<th>E</th>
<th>A</th>
<th>g_s</th>
<th>WUEi</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus densiflora</em></td>
<td>2018</td>
<td>AT</td>
<td>-</td>
<td>-</td>
<td>-0.35</td>
<td>0.0145</td>
<td>0.18</td>
<td>0.2084</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SM</td>
<td>-0.35</td>
<td>0.0145</td>
<td>-</td>
<td>-</td>
<td>-0.07</td>
<td>0.6138</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>AT</td>
<td>0.41</td>
<td>0.0012</td>
<td>0.72</td>
<td>&lt;0.0001</td>
<td>-0.06</td>
<td>0.6391</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SM</td>
<td>0.41</td>
<td>0.0012</td>
<td>0.72</td>
<td>&lt;0.0001</td>
<td>-0.06</td>
<td>0.6391</td>
</tr>
<tr>
<td><em>Larix kaempferi</em></td>
<td>2018</td>
<td>AT</td>
<td>-0.34</td>
<td>0.0166</td>
<td>0.22</td>
<td>0.1244</td>
<td>-0.24</td>
<td>0.0934</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SM</td>
<td>-0.34</td>
<td>0.0166</td>
<td>0.22</td>
<td>0.1244</td>
<td>-0.24</td>
<td>0.0934</td>
</tr>
<tr>
<td></td>
<td>2019</td>
<td>AT</td>
<td>0.46</td>
<td>0.0002</td>
<td>-0.28</td>
<td>0.0323</td>
<td>-0.32</td>
<td>0.0128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SM</td>
<td>0.46</td>
<td>0.0002</td>
<td>-0.28</td>
<td>0.0323</td>
<td>-0.32</td>
<td>0.0128</td>
</tr>
</tbody>
</table>

AT: air temperature, SM: soil moisture, E: transpiration rate, A: net photosynthetic rate, g_s: stomatal conductance, WUEi: intrinsic water-use efficiency

(Matyssek et al., 2014). These results suggest that *L. kaempferi* seedlings maintain physiological activity under warming conditions.

In the second year of the experiment, when the canopy increased as the *P. densiflora* seedlings grew, the seasonal characteristics of summer (high temperature and heavy precipitation) seem to have impacted the correlation between AT and SM, superimposing the effects of the warming treatment on SM. In the *L. kaempferi* plots, there was no correlation between AT and SM in the following year.
year also. For *P. densiflora*, possibly because of the peak values of E and gₛ in August and the high temperature combined with heavy precipitation in summer, the positive relationships between environmental variables with E or gₛ were also observed despite a lack of warming and precipitation manipulation effect in 2019.

The peak values of E and gₛ of 2-year-old *P. densiflora* in summer are in contrast to the results of the 1-year-old *P. densiflora*, i.e. the seasonal variation pattern of physiological activities differed by seedling age. E and gₛ decreased with increasing AT (above 23 °C for E) in the germination year, but both increased with increasing AT
4.2. Combined effect of warming and increased precipitation on seedling growth

The growth response to the environment changes lags behind the physiological responses (Chapin and Shaver, 1996). In previous studies using the same microclimate manipulation system, changes in 1- to 4-year-old *P. densiflora* seedling growth (root collar diameter and seedling height) due to the warming or precipitation manipulation treatment were not significant, which is in accordance with our results (Lee et al., 2013, Chang et al., 2019). Meanwhile, Sherry et al. (2008) have reported the suppression of aboveground biomass production of tallgrass prairie by the warming and doubled precipitation treatment nine months after the conclusion of the treatment. Thus, monitoring of posttreatment effects might bring new insights.

In the case of *L. kaempferi*, the weight of leaves was greatly increased by the warming treatment. In our study, the findings indicate that an extended growing period under warming treatment may result in increased leaf growth through advanced spring phenology and delayed leaf fall (Saxe et al., 2001). Consequently, despite no difference in the photosynthetic rate per unit leaf area, the total photosynthesis of the seedling as a whole seems to have increased since the total possible photosynthetic leaf was increased under the warming treatment. Also, the increase in total photosynthesis would have caused an increase in overall growth.

Bao et al. (2022) has reported that the increase in precipitation offset the negative effects of warming on plant growth. However, in our study the stimulating effect of warming on seedling growth was weakened by increased precipitation. The leaf, stem, root, and the total dry weight of *L. kaempferi* decreased when warming treatment and increased precipitation were implemented simultaneously. Kwon et al. (2020) reported that when the precipitation increased along with increase in temperature, the seedling growth was adversely affected by the combined stress of heat and excessive humidity for *P. densiflora*. Observation of coniferous species of genus *Abies* under the similar microclimate manipulation system have shown that increased precipitation did not mitigate the heat stress caused by the warming treatment (Jo et al. 2022).

However, even the moisture stress was understood as an important key to interpreting the interaction effect between the warming and the increased precipitation treatment on the growth of *L. kaempferi* seedlings, the influence of atmospheric water demand or soil moisture stress was not analyzed separately. Both cases have been reported when the soil moisture deficit is dominant and when the effect of enhanced VPD is dominant on the moisture stress (Yuan et al., 2019; Liu et al., 2020a). Moreover, Restaino et al. (2016) suggested a method of analyzing climatic water deficit and VPD for measuring the relationship between plant evaporative demand and available soil moisture and atmospheric deficits, respectively. Therefore, further studies are needed on the mechanism of how a certain level of atmospheric or soil moisture affects the growth of coniferous plants under high-temperature conditions, respectively.

5. Conclusions

In this study, *P. densiflora* and *L. kaempferi* seedlings showed different responses by species and seedling age to heat stress caused by warming. During the germination year under the warming treatment, seedlings of *P. densiflora* reduced and those of *L. kaempferi* maintained physiological activities determined by stomatal function. In the second year, neither species was affected by the warming nor the precipitation increase treatment, while *P. densiflora* showed a different seasonal variation pattern in physiological activities compared to the first year. Since the above-ground biomass production of *L. kaempferi* was decreased by the combined treatment of warming and increased precipitation, factor measurements should be expanded to investigate the interaction between warming and precipitation manipulation treatment more closely. Further analysis of plant physiological activities and growth responses in relation to atmospheric VPD and the root-zone soil moisture conditions imposed by microclimate manipulation would deepen the understanding of mechanisms of stomatal function and changes in growth under warming, increased precipitation and combination of these treatments. Moreover, the current study was conducted with two species for 2 years, while the seedling responses may vary not only by water-use strategies of each species but also by seedling age or environmental conditions of each year. Therefore, longer-term and iterative experiments would be helpful to figure out the responses of *P. densiflora* and *L. kaempferi* seedlings to climate change. Also, the revealed physiological and growth responses of *P. densiflora* and *L. kaempferi* to climate change need to be considered for management of nursery seedlings and in afforestation decisions.

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