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Short-term effects of warming treatment and precipitation manipulation on the ecophysiological responses of *Pinus densiflora* seedlings

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Abstract: The aim of this study was to investigate the short-term effects of warming treatment and precipitation manipulation on the growth, photosynthetic rate, and chlorophyll content of *Pinus densiflora* seedlings based on a climate change scenario in Korea. Two-year-old *P. densiflora* seedlings were planted in a nursery in April 2013. The air temperature of warmed plots (W) was set to increase by 3 °C compared to control plots (C) using an infrared heater in May 2013. The three precipitation manipulations consisted of decreased precipitation using transparent panels (–30%; P^-), increased precipitation using pumps and drip irrigation (+30%; P^+), and a control (0%; P^0). Root collar diameter and seedling height of *P. densiflora* were measured in April and October 2013. Net photosynthetic rate and total chlorophyll contents were measured from June to October 2013. Warming treatment increased the growth of root collar diameter, and the interaction effect of the warming treatment and precipitation manipulation on it was also significant. In contrast, no significant effects were found for the growth of seedling height. These results demonstrate that warming treatment might affect the growth of root collar diameter and seedling height differently. It was also found that WP^- treatment decreased net photosynthetic rates. This pattern might be due to the soil moisture availability, as soil moisture content was lowest in the WP^- treatment and net photosynthetic rate was elevated with increasing soil moisture content. Meanwhile, warming treatment increased the total chlorophyll content. The results suggest that chlorophyll synthesis was promoted by a temperature increase in the leaves. The current study, which simulated a climate change environment in an open field, provides important information for the prediction of the ecophysiological effects of changes in temperature and precipitation on *P. densiflora* seedlings in Korea.

Key words: Climate change, Japanese red pine, precipitation manipulation, seedling growth, warming treatment

1. Introduction

By the end of the 21st century, it is predicted that the average global temperature will have increased by between 1.0 and 3.5 °C (IPCC, 2014) and that precipitation will vary in volume and pattern depending on the region (Fay et al., 2008). Because temperature and precipitation are major factors affecting various processes within an ecosystem (Luo et al., 2008; Luong et al., 2013; Zhou et al., 2013; Kim et al., 2015), the structure and function of ecosystems and plant physiological processes are expected to change with fluctuations in temperature and precipitation caused by climate change (Rustad, 2008; Bettinger et al., 2013; Xu et al., 2014; Khaine and Woo, 2015).

Climate change experiments are one of the most important approaches used to study the response of an ecosystem to climate change. In this type of experiment, one or more environmental factors associated with climate change are experimentally manipulated, and the responses of the ecosystem to that manipulation are measured (Rustad et al., 2001). Climate change factors that are examined in these experiments include the increase in mean temperature, changes in the pattern and volume of precipitation, and the increase in the CO_2 levels of the air (Wu et al., 2011; Xu et al., 2014). Generally, climate change experiments based on a single factor are relatively easy and inexpensive, so this tends to be the preferred methodology worldwide (Luo et al., 2008). In particular, warming experiments based around the predicted increase in temperature, the most prominent consequence of climate change, are the most common focus of attention (Rustad et al., 2001). However, studies need to examine multiple environmental factors because it is difficult to predict the impact of climate change on an ecosystem based on a single factor (Bardgett et al., 2008; Rustad, 2008).

Climate change experiments can be conducted in indoor environments or in open fields. Open-field

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experiments have an advantage in that they can be used to measure responses of an ecosystem that are as close to reality as possible by creating an environment similar to that predicted for climate change (Rustad et al., 2001). However, open-field climate change experiments are far less common than indoor experiments because it is technically difficult to control temperature and precipitation in an open field (Wu et al., 2011). Of the open-field climate change experiments that have been conducted, most have focused on smaller-scale systems such as grasslands, agricultural ecosystems, and seedlings, as opposed to forest ecosystems and trees (Chung et al., 2013).

A metaanalysis of the responses of ecosystems to open-field climate change experiments has found that the aboveground biomass, growth, and photosynthesis of plants increased under warming treatments and that plant growth and photosynthesis also increased with elevated precipitation (Wu et al., 2011). Warming treatment and precipitation manipulation showed an interaction effect on soil moisture content (Yun et al., 2014) and standing crop (Bai et al., 2010). A study that simultaneously applied warming treatment and decreased precipitation as treatments also reported that the lack of soil moisture was a primary factor restricting the growth of pine species, with temperature increase a secondary factor (Thiel et al., 2012). Hoeppner and Dukes (2012) investigated warming treatment and decreased precipitation in herbaceous plants and found that plant growth significantly decreased as soil moisture declined. In addition, although the net primary production of plants mostly decreased as precipitation levels fell, the impact varied depending on the precipitation pattern (Cherwin and Knapp, 2012). Pangle et al. (2012) also found that decreased precipitation reduced the physiological function of plants, while increased precipitation accelerated canopy transpiration. In previous experimental climate change studies, the growth responses of trees differed due to various factors such as species, region, and experiment period (Yin et al., 2008). Therefore, understanding differences in the response of trees to climate change experiments according to species and region is very important when predicting the distribution or growth of different species of trees in a climate change-affected environment.

South Korea has a large forest area covering approximately 64% of total land area (100,000 km^2) (Korea Forest Service, 2011). *Pinus densiflora* is a representative native tree species in Korea that accounts for approximately 23% of the entire forest area (Korea Forest Service, 2011) and it is one of the most economically important tree species in Korea for its various wood products and timber (Li et al., 2013). In order to test predictions by observing the responses of *P. densiflora* seedlings subjected to climate change factors, an open-field climate change experiment is thus needed.

The objective of this study was to investigate the effects of warming treatment and precipitation manipulation on the ecophysiological characteristics of *P. densiflora*. For this purpose, the growth, net photosynthetic rate, and chlorophyll content of *P. densiflora* seedlings were measured by designing and operating an open-field warming treatment and precipitation manipulation system. Generally, climate change as temperature rise and precipitation change are expected to affect the growth and ecophysiological response of trees (IPCC, 2014). Therefore, we hypothesized that: 1) warming treatment and precipitation manipulation would alter the growth, net photosynthetic rate, and chlorophyll content of *P. densiflora* seedlings by stimulating the biosynthesis of chlorophyll and increasing (increased precipitation) or reducing (decreased precipitation) water stress, and 2) warming treatment and precipitation manipulation would have an interaction effect on the growth, net photosynthetic rate, and chlorophyll content of *P. densiflora* seedlings because they might have interaction effects on soil conditions such as moisture content (Yun et al., 2014).

2. Materials and methods

2.1. Study area

The experimental nursery site was established in the Korea University Environment and Ecology Arboretum, Seoul, Korea (37°35′36″N, 127°1′31″E). The long-term (1981–2010) mean annual temperature and precipitation were 12.5 °C and 1450.5 mm, respectively (Korea Meteorological Administration, 2011). Prior to establishing the nursery in 2013, the field was plowed up to 50 cm in depth with an excavator. In April 2013, 45 two-year-old *P. densiflora* seedlings were each planted in 1.5 m × 1.5 m plots (n = 18), and soil samples were randomly collected from six points at soil depths of 0 to 10 cm. Soil samples were air-dried and sieved with 2-mm mesh screens (US Standard No. 10). Soil texture was determined using the hydrometer method, and soil texture was determined according to USDA classifications. Soil pH was measured in a 1:5 soil to water suspension using a pH meter (Orion 3 Star, Thermo Fisher Scientific Inc., USA). Total carbon (C) and nitrogen (N) concentrations were determined by dry combustion with an elemental analyzer (Vario Macro Elemental Analyzer, Elementar Analysensysteme GmbH, Germany). Cation exchange capacity (CEC) was extracted with 1 N ammonium acetate at pH 7.0, and the solution was analyzed using an inductively coupled plasma-optical emission spectrometer (Vista-Pro, Varian Inc., USA). The soil type at the site is loamy sand (80% sand, 14% clay, and 6% silt) with a pH of 6.52, 0.22% C, 0.05% N, and a CEC of 3.67 $cmol_c$/kg. Because of the plowing, the soil of the nursery contained greater sand particles compared to the normal forest soil.

2.2. Experimental design

This experimental design established an open-field experimental warming treatment and precipitation manipulation system to simulate the impact of climate change on *P. densiflora* seedlings based on a climate change scenario (RCP 8.5) in Korea. The experiment consisted of six different treatments with three replicates: two levels of warming [(+3 °C (W), control (C)] were crossed with three levels of precipitation [+30% (P^+), –30% (P^-), and control (P^0)] in a factorial design. The temperature was increased in the warming treatment using an infrared heater (FTE-1000, Mor Electric Heating Association, USA). P^- manipulation used a transparent panel to cover 30% of the area of the experimental plot, thus blocking 30% of precipitation from entering the plot. P^+ manipulation collected via a PVC pipe the intercepted precipitation from the P^- treatment in a water tank; using a water-level sensor and pump, it then automatically ran drip irrigation in the increased precipitation plots. P^0 manipulation installed a transparent panel cover that did not intercept precipitation in order to ensure that other environmental factors, outside of precipitation, remained the same as those in the P^+ and P^- treatments. Air temperature was measured by infrared temperature sensors (SI-111, Campbell Scientific, USA), and soil temperature was measured at a depth of 5 cm by temperature sensors (107-L34, Campbell Scientific, USA). Soil moisture content was measured at a depth of 10 cm using reflectometer probes (CS616, Campbell Scientific, USA). All measurements were logged every 30 min, and the data were stored in a data logger (CR3000, Campbell Scientific, USA). The mean air temperature was 2.6 °C higher in warmed plots than control plots and the mean soil temperature was 3.1 °C higher in warmed plots than control plots. The soil moisture content (%) of *P. densiflora* seedlings was highest in CP^+ (10.1 ± 0.2) followed by WP^+ (8.8 ± 0.2), CP^0 (8.1 ± 0.2), WP^0 (7.7 ± 0.2), CP^- (7.5 ± 0.2), and WP^- (6.9 ± 0.2) (Yun et al., 2014). More detailed information (air temperature, soil temperature, and soil moisture content) is provided by Yun et al. (2014).

2.3. Growth

P. densiflora seedlings (n = 45) were planted in each plot (n = 18), and root collar diameter and seedling height were measured for all seedlings. Root collar diameters (2 cm above ground) were measured using a digital caliper (500-101, Mitutoyo, Japan), and seedling heights at the terminal bud were measured using a ruler for all seedlings in April and October 2013. The relative growth rate (mm day^{-1}) of root collar diameter and seedling height were calculated as $(\ln w_2 - \ln w_1) / (t_2 - t_1)$, where w_1 and w_2 are the initial and final measurements, respectively, and t_2 (4 October, 2013) and t_1 (24 April, 2013) represent the time interval.

2.4. Photosynthetic rate and chlorophyll content

Photosynthetic rate and chlorophyll content were measured in six 1-year-old needles from three selected seedlings from June to October, 2013. The net photosynthetic rate was measured using a portable photosynthesis system (CIRAS-2, PP Systems, UK) attached to a conifer leaf cuvette (PLC-6, PP Systems, UK) from 0900 to 1200 hours. The conditions during the measurement were as follows: 1000–1100 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) artificially provided by an LED lamp and 400 ppm of reference CO_2. The net photosynthetic rate was determined at a temperature of 25 °C (Calfapietra et al., 2005; Monclus et al., 2006). In order to measure chlorophyll content, needles were cut into 2-mm pieces and incubated in 5 mL of dimethyl sulfoxide (DMSO). Tubes with DMSO and plant material were incubated at 65 °C for 60 min in the dark. The absorbance of chlorophyll extracts was measured using a spectrophotometer (U-1100, Hitachi, Japan) at 665 nm and 648 nm. Total chlorophyll content (Chl a + b) was then calculated (Barnes et al., 1992).

2.5. Statistical analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., USA). The parameters related to relative growth rate, photosynthetic rate, and chlorophyll content were subject to analysis of variance (ANOVA) tests, followed by Tukey's multiple range tests when significant ANOVA results were obtained. The effects of warming treatment and precipitation manipulation and their interaction were examined using two-way ANOVA. All results were considered statistically significant at $P < 0.05$ unless otherwise noted.

3. Results

3.1. Growth

The relative growth rates of the root collar diameter and height of *P. densiflora* seedlings varied according to warming treatment and precipitation manipulation. Warming treatment significantly affected the root collar diameter of *P. densiflora* seedlings ($P < 0.05$), and interaction effect was observed ($P < 0.05$) (Table 1). Warming treatment significantly increased the relative growth rate of root collar diameter (mm day^{-1}) (WP^0 0.0012 vs. CP^0 0.0010, WP^- 0.0015 vs. CP^- 0.0010, and WP^+ 0.0014 vs. CP^+ 0.0012) (Table 2), while warming treatment and precipitation manipulation had no significant effect on the relative growth rate of seedling height (mm day^{-1}) (WP^0 0.0020 vs. CP^0 0.0020, WP^- 0.0019 vs. CP^- 0.0019, and WP^+ 0.0020 vs. CP^+ 0.0020) (Table 2).

3.2. Net photosynthetic rate and chlorophyll content

The mean net photosynthetic rate of *P. densiflora* seedlings from June to October 2013 (µmol CO_2 m^{-2} s^{-1}) differed significantly among the six treatments (P <

Table 1. Two-way analysis of variance for the effect of warming treatment and precipitation manipulation on root collar diameter, seedling height, net photosynthetic rate, and total chlorophyll content. Significant effects of warming treatment and precipitation manipulation are shown in bold ($P < 0.05$).

Treatment	df*	Root collar diameter		Seedling height		Net photosynthetic rate		Total chlorophyll content	
		F	P	F	P	F	P	F	P
Warming (W)	1	17.65	**0.0001**	1.45	0.2294	4.15	**0.0429**	8.91	**0.0031**
Precipitation (P)	2	2.41	0.0911	2.13	0.1197	3.61	**0.0287**	0.41	0.6642
W × P	2	4.01	**0.0187**	0.16	0.8542	0.58	0.5630	0.16	0.8531

* df : Degrees of freedom.

Table 2. Relative growth rates of root collar diameters and seedling heights of 2-year-old *Pinus densiflora* in April and October 2013.

Treatment	Root collar diameter (mm)		Relative growth rate (mm day^{-1})	Seedling height (mm)		Relative growth rate (mm day^{-1})
	April	October		April	October	
WP^0	5.78 (0.11)	7.24 (0.13)	0.0012	240.38 (3.79)	350.38 (4.95)	0.0020
CP^0	5.84 (0.12)	7.12 (0.13)	0.0010	236.94 (5.32)	347.94 (6.43)	0.0020
WP^-	5.31 (0.10)	7.07 (0.13)	0.0015	221.80 (3.16)	360.00 (3.68)	0.0019
CP^-	5.86 (0.12)	7.04 (0.12)	0.0010	227.23 (3.84)	340.61 (4.29)	0.0019
WP^+	5.38 (0.11)	7.02 (0.14)	0.0014	235.70 (4.46)	338.83 (6.88)	0.0020
CP^+	5.55 (0.09)	7.03 (0.14)	0.0012	234.43 (4.16)	333.92 (5.63)	0.0019

Note: Numbers in parentheses indicate one standard error of the mean (n = 9). Treatments did not significantly affect the relative growth rate of the root collar diameter and seedling height of 2-year-old *P. densiflora*. All abbreviations are noted in Figure 1.

0.05), decreasing the most in WP^- (11.88 ± 0.45) (Figure 1). Warming treatment and decreased precipitation significantly reduced the mean net photosynthetic rate compared to the other treatments. Warming treatment and precipitation manipulation affected the net photosynthetic rate of *P. densiflora* seedlings, but no interaction effect was observed (Table 1). A correlation analysis found a correlation between the net photosynthetic rate and monthly soil moisture content for warming treatment and precipitation manipulation treatments ($P < 0.05$, $R^2 = 0.57$) (Figure 2). It was found that net photosynthetic rate had a significant and positive correlation with soil moisture, but not with soil temperature. Net photosynthetic rate in each treatment was consistently highest in July and lowest in October, and showed a similar pattern with soil moisture content.

The mean total chlorophyll content (mg g^{-1}) of *P. densiflora* seedlings measured from June to October 2013 did not significantly differ among the six treatments ($P = 0.0771$), though it showed a pattern of increase under the warming treatment (Table 3). The total chlorophyll content showed a similar pattern with changes in air temperature and soil temperature by warming treatment and precipitation manipulation. Thus, a correlation was found between total chlorophyll content and net photosynthetic rate ($P < 0.05$, $R^2 = 0.79$), air temperature ($P < 0.05$, $R^2 = 0.50$), and soil temperature ($P < 0.05$, $R^2 = 0.44$) (Figure 3). Warming treatment significantly affected the total chlorophyll content, but no interaction effect was observed (Table 1). Precipitation manipulation did not significantly affect the total chlorophyll content while warming treatment significantly increased it.

4. Discussion

4.1. Growth responses

We hypothesized that warming treatment and precipitation manipulation would shift the growth of *P. densiflora* seedlings and would have an interaction effect on the growth. Our findings partly agree with the hypotheses because the effects of warming treatment and the interaction appeared significant only for growth

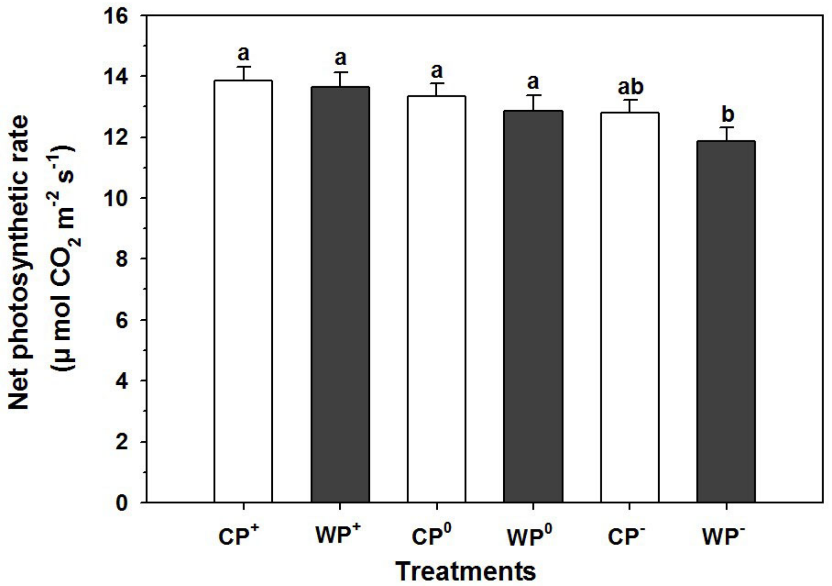

Figure 1. Mean net photosynthetic rate for warming treatment and precipitation manipulation. WP^0 - warmed and precipitation control plots, CP^0 - temperature control and precipitation control plots, WP^- - warmed and decreased precipitation plots, CP^- - temperature control and decreased precipitation plots, WP^+ - warmed and increased precipitation plots, and CP^+ - temperature control and increased precipitation plots. Vertical bars indicate the standard errors, and different letters above the bars indicate significant differences among the treatments ($P < 0.05$).

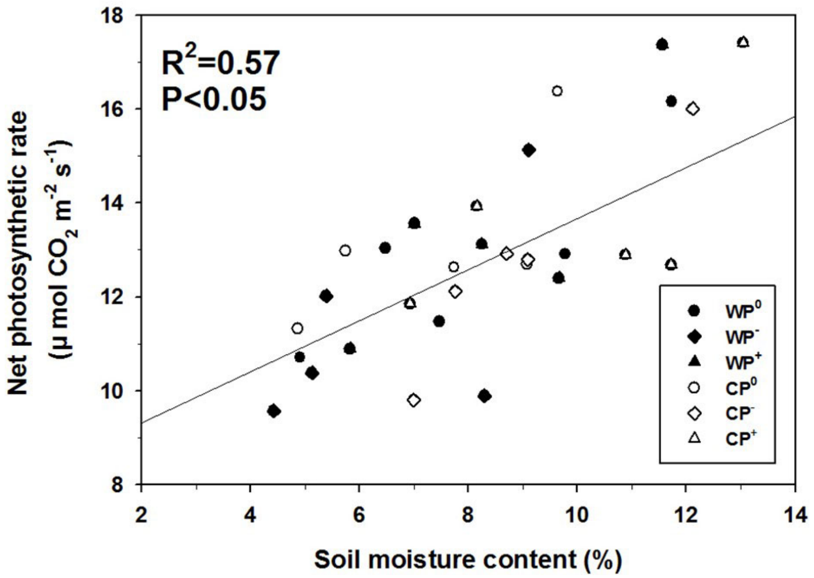

Figure 2. Relationship between monthly mean net photosynthetic rate and monthly mean soil moisture content of 2-year-old *Pinus densiflora* seedlings subjected to warming treatment and precipitation manipulation. All abbreviations are noted in Figure 1.

of root collar diameter. No significant changes in the seedling height might result from the fact that the height growth of *P. densiflora* shows a fixed growth pattern and is affected by environmental conditions (i.e. air temperature and precipitation) in the previous year (Kozlowski, 1964; Kramer, 2012). The growth of root collar diameter generally

Table 3. Total chlorophyll content (mg g[-1]) of 2-year-old *Pinus densiflora* seedlings from June to October 2013.

Treatment	Total chlorophyll content (mg g^{-1})					
	June	July	August	September	October	Mean
WP^0	1.37 (0.07)	1.68 (0.07)	1.37 (0.06)	1.44 (0.07)	1.13 (0.04)	1.40 (0.04)
CP^0	1.36 (0.08)	1.52 (0.07)	1.26 (0.05)	1.27 (0.06)	1.0 (0.06)	1.28 (0.04)
WP^-	1.37 (0.07)	1.72 (0.09)	1.40 (0.08)	1.40 (0.08)	1.17 (0.08)	1.41 (0.04)
CP^-	1.34 (0.09)	1.59 (0.08)	1.39 (0.05)	1.33 (0.05)	1.02 (0.06)	1.33 (0.04)
WP^+	1.48 (0.07)	1.72 (0.04)	1.36 (0.04)	1.37 (0.05)	1.02 (0.03)	1.39 (0.05)
CP^+	1.46 (0.05)	1.59 (0.06)	1.29 (0.05)	1.22 (0.04)	1.00 (0.05)	1.30 (0.05)

Note: Numbers in parentheses indicate one standard error of the mean (n = 9). Treatments did not significantly affect total chlorophyll content of 2-year-old *P. densiflora* seedlings. All abbreviations are noted in Figure 1.

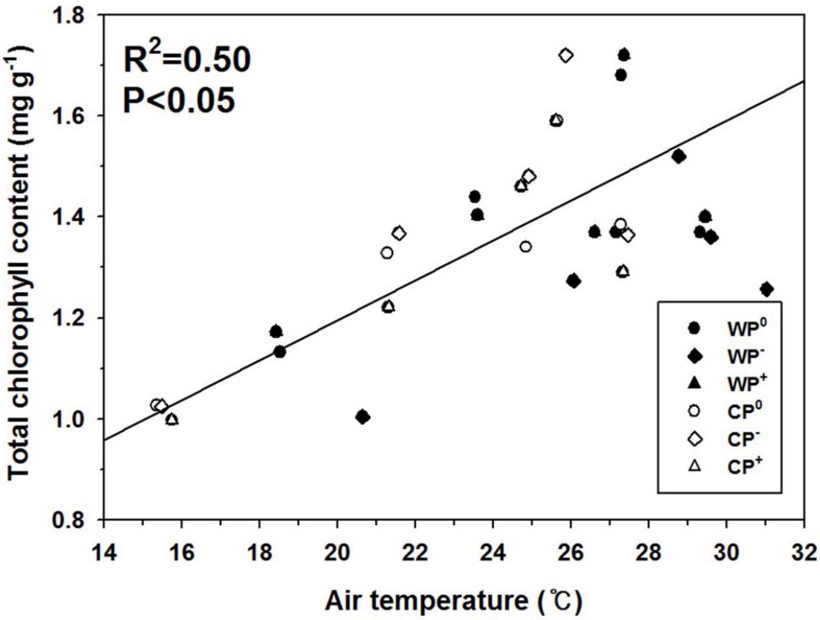

Figure 3. Relationship between monthly mean total chlorophyll content and monthly mean air temperature under warming treatment and precipitation manipulation. All abbreviations are noted in Figure 1.

originates from the activity of the cambium, which starts with the growth in seedling height in spring and continues to grow even after the growth in seedling height stops. In contrast, the growth of seedling height is related to the fact that the stem formed in the previous year elongates at the beginning of spring and stops growing in the early spring (Kozlowski, 1964; Kramer, 2012). It is speculated that the difference in the growing periods of root collar diameter and seedling height might result in the inconsistent effect of warming treatment on the growth of root collar diameter and seedling height. Therefore, a difference in the growth of seedling height would be expected in the year following this study due to the effects of continued warming treatment and precipitation manipulation. This speculation could be supported by the fact that warming treatment resulted in an increasing pattern of seedling height by 7.97%–8.98% at the same study site in 2014, though the increase was not statically significant (P >

0.05) (Park et al., 2016). These findings imply that growth response of seedling height might not be immediately affected due to climate change, at least in the short-term. In addition, a previous study that performed warming treatment and precipitation manipulation on *Pinus nigra* suggested that the growth in the height of seedlings in the first year was not affected by warming treatment and decreased precipitation, but growth in the second year was reduced with decreased precipitation (Thiel et al., 2012). This finding is similar to that observed in our case. However, other studies have observed different results, including increased height and basal diameter in 3-year-old *Picea asperata* and 2-year-old *Pinus tabulaeformis* in the first experimental year under warming treatment using an infrared heater (Zhao and Liu, 2009). These contradictory results may be due to a variety of reasons, including seedling age, environmental differences, and genetic differences (Barber et al., 2000; Llorens et al., 2004). The growth responses of plants to warming treatment and precipitation manipulation have been reported to vary depending on the experimental site and tree species. Hoeppner and Dukes (2012) suggested that the growth of herbal species decreased under warming and reduced precipitation. Barber et al. (2000) also reported that the root collar diameter of seedlings decreased under warming treatment because of temperature and moisture stress.

4.2. Net photosynthetic rate and chlorophyll content

We hypothesized that warming treatment and precipitation manipulation would influence the photosynthetic rate of *P. densiflora* seedlings (hypothesis 1) and would have an interaction effect on the photosynthetic rate (hypothesis 2). Our findings demonstrate that the pattern in photosynthetic rate was consistent with hypothesis 1, but did not agree with hypothesis 2. Particularly, the obtained results suggest that the combination of warming treatment and decreased precipitation might result in a decreased net photosynthetic rate. The observed decrease in the photosynthetic rate due to warming treatment with decreased precipitation might be accompanied with altered moisture availability, because either warming treatment or decreased precipitation could reduce soil moisture content (Yun et al., 2014) and might consequently cause moisture stress (Callaay et al., 1994; Roden and Ball, 1996; Gunderson et al., 2000; He and Dong, 2003). It has been proven that temperature and moisture stress reduced photosynthesis due to a negative effect on the synthesis of chlorophyll and photopigments (Zhang et al., 2002; Zhao and Liu, 2009; Idrees et al., 2011). It has also been reported that moisture stress decreases photosynthesis because it blocks the stomatal pore between the guard cells of a leaf (Yordanov et al., 2000) and suppresses metabolism due to decreased RuBisCo activity (Parry et al., 2002; Tezara et al., 2002). It has also been demonstrated that warming treatment and decreased precipitation instigate a decrease in the net photosynthetic rate of *Leymus chinensis* due to changes in nitrogen metabolism and increased lipid peroxidation (Xu and Zhou, 2006).

In previous studies, the net photosynthetic rate of seedlings was positively correlated with soil moisture content (Llorens, 2003; Zhang et al., 2005) and accumulated rainfall (Llorens, 2003). In addition, Schwarz et al. (1997) reported that the total chlorophyll content of *Picea rubens* was highly correlated with both air temperature and soil temperature. The net photosynthetic rate under warming treatment and precipitation manipulation was similar in pattern to the changes in soil moisture content by warming treatment and precipitation manipulation. Mean soil moisture content (%) was significantly higher in CP^+ (10.1 ± 0.2) than in other treatments and lower in WP^- (6.9 ± 0.2) than in other treatments (Yun et al., 2014).

We found that the pattern in total chlorophyll content was partly in agreement with hypothesis 1 but did not correspond to hypothesis 2 since only warming effect was significant for the chlorophyll content. These results indicate that increased total chlorophyll content is closely related to the elevated temperature, which suggests that our choice of temperature increase might stimulate pigment biosynthesis (Wang et al., 2012). This is likely because warming accelerated chlorophyll synthesis (Ormrod et al., 1999). Moreover, the chlorophyll content increased as the concentration of pigment needed for photosynthesis rose due to the increased temperature (Yin et al., 2008). In a previous study of *Q. variabilis*, the total chlorophyll content increased due to the early development of leaves as the leafing in the warming treatment plots preceded the control plots (Jo et al., 2011; Lee et al., 2013). It is suggested that the total chlorophyll content increased in this study for the same reason. In addition, a study of *Larix kaempferi* and *Betula costata* suggested that the total chlorophyll content decreased due to heat stress in leaves under warming treatment (Han et al., 2012). Mihailović et al. (1997) examined the effect of drought on *Triticum aestivum* and showed that the chlorophyll content decreased with increasing chlorophyllase due to drought. These significant changes in chlorophyll content might impact growth responses of seedlings, as observed in the growth of root collar diameter in the current study.

4.3. Implications for changes in *P. densiflora* forests

Though long-term monitoring is essential to assess the effect of climate change, our findings offer implications on climate change because 1-year-old and 2-year-old seedlings are the most widely used seedlings for establishing plantations in Korea. Therefore, the observed ecophysiological responses imply that the altered air temperature and precipitation might impact the *P. densiflora* plantations in terms of seedling growth. Moreover, the responses during the seedling stage are closely related to the subsequent growth and production of mature trees (Mardani et al., 2014), and seedlings are more sensitive to the shifts in air temperature and precipitation than mature trees (Chung et al., 2013); thus, it is speculated that the observed shifts in the initial seedling growth might accompany further changes in *P. densiflora* forests. However, it remains uncertain why the pattern of net photosynthetic rate was inconsistent with those of other responses; accordingly, further studies should address elucidation of the mechanisms behind the inconsistent patterns observed in the current study.

4.4. Conclusion

This study investigated initial responses in the growth, net photosynthetic rate, and total chlorophyll content of *P. densiflora* seedlings under warming treatment and precipitation manipulation. We found significant changes in the growth of root collar diameter, photosynthetic rate, and total chlorophyll content following the treatments despite the short study period. Although the effect of treatment on the growth of seedling height was not apparent, the significant changes in other characteristics after the treatments might accompany further shifts in ecophysiological responses of seedlings. The response of the net photosynthetic rate and total chlorophyll content of *P. densiflora* seedlings to warming treatment and precipitation manipulation was closely associated with soil moisture content and air/soil temperature, respectively. Therefore, a difference in the growth of the seedling height would be expected in the year following this study due to the effects of continued warming treatment and precipitation manipulation. Nevertheless, the results of this study, which simulated a climate change environment in an open field, provide important information for the prediction of the ecophysiological effects of changes in temperature and precipitation on *P. densiflora* seedlings in Korea.

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