ORIGINAL ARTICLE

Responses of photochemical efficiency and shoot growth of alpine dwarf-pine *Pinus pumila* to experimental warming, shading, and defoliation in Japan

Yukihiro Amagai[1,2] · Gaku Kudo[1]

Abstract

Global warming accelerates shrub expansion in high-latitude and high-elevation ecosystems. Over the last several decades, alpine dwarf-pine *Pinus pumila* has expanded its range in northern Japan because of enhanced shoot growth under warm climatic conditions. In alpine regions, local environmental conditions and the length of the growing season, vary depending on the topography, elevation, and snowmelt time. This leads to spatially varying shoot performances that are co-affected by climatic change. We applied a warming, shading, and defoliation treatment to assess how temperature and carbon relations in interaction with habitat type (elevation and snowmelt time) affect shoot growth and photochemical efficiency of needles in this species. Photochemical efficiency (*Fv/Fm*) was maximized during peak growth in the middle of growing season (mid-July–mid-August), and it increased in the shading and warming treatments especially in the early and late season. Shoot growth increased only in the warming treatment, and was not affected by shading and defoliation. These results indicate that shoot growth of alpine dwarf-pine is limited by low temperature, but not by carbon assimilation, i.e., growth is sink- rather than source-limited. Furthermore, the seasonal trend of photochemical efficiency shifted to the late season at higher elevations, and the recovery time of photochemical efficiency took longer in the late-snowmelt habitat, where the growing season was short. Therefore, warmer summers and longer snow-free periods are likely to enhance the growth and areal expansion of alpine dwarf-pine at the expense of the adjacent, species-rich, low-stature alpine plant communities.

Keywords Alpine · *Pinus pumila* · Photochemical efficiency · Shoot growth · Sink demand · Snowmelt time

Introduction

Climate change has already affected the community structure, distribution range, and species diversity of arctic and alpine vegetation (Grabherr et al. 1994; Gottfried et al. 2012; Pauli et al. 2012). In particular, increases in biomass, plant cover, and abundance of shrub species have been recorded in arctic and alpine ecosystems over the last century (Myers-Smith et al. 2011). Positive correlations between shoot growth and summer temperature are reported in these shrubs (Rayback and Henry 2006; Rozema et al. 2009; Forbes et al. 2010; Hallinger et al. 2010) in which warmer temperature and extended growing season enhance the recruitment and growth rates of shrubs (Chapin et al. 2005; Hansen et al. 2010).

Pinus pumila (alpine dwarf-pine) often forms a krummholz belt above the treeline, which is a physiognomically distinct type of alpine vegetation in far-east Asia (Okitsu and Ito 1989). This species occupies the intermediate habitat between exposed, reduced-snow habitats (fellfield) and snow accumulation habitats (snowbed). These compact dwarf-pine thickets become dominant on convex slopes with moderate wind exposure and snow depth (Okitsu and Ito 1984). Previous studies reported that *P. pumila* shoot growth is positively related to the summer temperature (Sano et al. 1977; Takahashi 2003; Wada et al. 2005) and annual shoot growth

✉ Yukihiro Amagai
amagai.yukihiro@nies.go.jp

1 Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Hokkaido 060-0810, Japan

2 Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies (NIES), Tsukuba, Ibaraki 305-8506, Japan

has increased over the years reflecting the recent climatic warming (Wada et al. 2005; Ozeki et al. 2011). In our previous report, we showed that *P. pumila* had expanded its land cover by 14% over the last 32 years in the Taisetsu Mountains of northern Japan (Amagai et al. 2015). *P. pumila* is known for its very high biomass accumulation as compared to non-woody alpine vegetation in Japan (Kajimoto 1989). Therefore, the expansion of *P. pumila* distribution would likely have serious effects on the structure and diversity of alpine plant communities.

For a mechanistic understanding of the *P. pumila* expansion, the shrub's growth and photosynthetic responses to environmental conditions need to be explored. Its vigor may vary among populations from different habitats. For instance, in wind-exposed habitats, Nagano et al. (2009) demonstrated that *P. pumila* produces needles with lower construction cost and shorter lifespan associated with increased photosynthetic nitrogen use efficiency compared to needles from wind-protected habitats. Yet, actual carbon gain in a wind-exposed habitat was restricted by drought stress (Nagano et al. 2013). In addition to the habitat-specific climatic conditions, the length of growing season is determined by local snow conditions in alpine ecosystems (Kudo 1991). Plants are protected from freezing temperatures under snow during the winter. However, a late snowmelt reduces the length of the growing season, resulting in shorter shoots and later bud break (Kammer and Möhl 2002). Snow condition may also affect the stress tolerance of plants. Although alpine plants can commonly tolerate low-temperature extremes and high solar radiation (Manuel et al. 1999; Lütz 2010), an early loss of snow cover at high elevation may increase the risk of photoinhibition (Lehner and Lütz 2003; Öquist and Huner 2003) and/or frost damage (Neuner 2014). Such implications of earlier release from snow have not yet been explored in *P. pumila*.

In addition to direct effects of climatic warming, higher temperatures may also change precipitation and snowmelt regime in alpine ecosystems (Inouye 2008; Nagy and Grabherr 2009). A warmer summer may enhance shoot growth, but early snowmelt and warm temperature may also increase drought stress, resulting in reduced vigor. Furthermore, our previous study demonstrated that summer temperature and sunshine duration were both related to the seasonal shoot extension of *P. pumila* (Amagai et al. 2015). Here, we explore the habitat-specific responses of *P. pumila* shoots to a manipulation of shoot temperature and shoot carbon relations to understand the current expansion of this shrub. Photochemical efficiency, i.e., quantum yield (*Fv/Fm*), has been used as a reliable diagnostic indicator of photosynthetic function in response to light, temperature, and other environmental stresses (e.g., Maxwell and Johnson 2000). Thus, we employed *Fv/Fm* values as an indicator of the state and capacity of the photosynthetic machinery.

We conducted three experiments to test how the physiological activity and shoot growth of *P. pumila* respond to shading, warming, and defoliation treatments. We tested the following hypotheses: (1) shading treatment should decrease shoot growth if light and carbon fixation are limiting factors. (2) Warming treatment should increase shoot growth along with the photochemical efficiency, if low temperature is a limiting factor. (3) Defoliation treatment and resulting reduction of the photosynthetic area should reduce shoot growth, if shoot growth is limited by carbon assimilation. Furthermore, we compared seasonal trends in photochemical efficiency among habitats of different elevation and snowmelt times to assess possible site-specific responses.

Materials and methods

Study sites and climate conditions

This study was conducted in the central part of the Taisetsu Mountains, Hokkaido, northern Japan (43° 32–33′ N, 142° 51–56′ E). Summits in this area are about 1850–2000 m above sea level, and the treeline is located at an elevation of around 1550–1600 m. *Betula ermanii, Picea jezoensis* and *Picea glehnii* are major component species-forming treeline. *P. pumila* is mostly distributed within a range of 1550–1950 m above sea level, in which plant height is commonly 0.5–1.5 m. Climate of the Taisetsu Mountains is characterized by cold, snowy winters, and warm, wet, foggy summers. The mean monthly temperature during the growing season is 8.4 °C in June, 12.1 °C in July, 12.7 °C in August, and 7.8 °C in September. The annual mean temperature is − 1.8 °C (during 2002 and 2015) at an elevation of 1700 m. The mean precipitation is 136 mm in June, 250 mm in July, 368 mm in August, and 244 mm in September. The annual mean temperature in this area has increased at a rate of 0.33 °C per decade (during 1980–2010), and the snowmelt timing has advanced at the rate of 4.1 days per decade (during 1988–2012; G. Kudo unpublished data).

Measurement of potential photosynthetic activity

Chlorophyll fluorescence was measured to assess the photochemical efficiency in the field. Quantum yield (Fv/Fm) is a good indicator of photoinhibition (Kitajima and Butler 1975; Mohammed et al. 1995); it is calculated with Fo, which represents the level of chlorophyll fluorescence in the dark, and Fm, which represents the level of chlorophyll fluorescence in a saturated chemical reaction (Fv = Fm − Fo). Typical leaves have an Fv/Fm value of between 0.80 and 0.83, but damaged leaves exhibit lower values (Demmig and Björkman 1987; Öquist et al. 1992). Measurement was conducted for dark-adapted needles (> 15 min, which stabilizes the *Fv/Fm* value

in our preliminary experiment) using a portable fluorimeter (MINI PAM; Walz, Effeltrich, Germany) from 31 May to 28 September, 2014. Initial fluorescence (Fo) was measured in the Ml-burst mode of the MINI PAM; maximum fluorescence was measured after a saturating light pulse.

Experimental manipulations of temperature and radiation

To quantify the effects of temperature and radiation on physiological activity and shoot growth, experimental manipulations were conducted on an exposed plateau at an elevation of 1670–1690 m (hereafter H site; Table S1, Fig. S1) during 2013 and 2015. At this site, *P. pumila* is distributed as scattered patches, rather than continuous zonation, due to strong wind and little snow cover during the winter, indicating that this site is at the distribution edge adjacent to the fellfield habitat. We selected 20 patches of *P. pumila* and 5 moderately sized shoots in each patch in early June, 2013. All selected shoots were tagged for identification. One was used as an intact control, while the other four were used for manipulations in which lateral branches on the main stem were cutoff at the parts younger than 4 years old. Then, three different manipulations (shading, warming, and defoliation treatments), were conducted along with a manipulation control (no treatment except for the removal of lateral branches; Fig. 1). The lateral branches were removed to simplify the structure of shoots responding to the treatments.

The shading treatment was conducted using black fine-meshed bags with 51% shading ability. In the warming treatment, we used white fabric bags with little shading ability ($< 10\%$) but warming ability. The defoliation treatment involved removing half of the needles on a branch at the parts younger than 4 years old using scissors. The shading and warming treatments, namely, with shoot bagging, were conducted only during the growing season (from 9 June to 10 September in 2013; from 1 June to 28 September in 2014; and from 24 May to 30 September in 2015).

We measured ambient temperature in the shading, warming, and control treatments during the 2013–2015 growing seasons. Measurements were performed at 1-h intervals by automatic small loggers fixed on the manipulated stems (StowAway Tidbit; Onset Co., Bourne, MA, USA). The ambient temperature was measured throughout the year. To evaluate the effects of treatments on shoot growth, we measured annual shoot growth during 2003 and 2015 (10 years before the experiment + 3-year experimental period) using a digital caliper, carefully identifying the internodal length between bud-scar marks on the stem.

The Fv/Fm values of 1-year-old needles were measured in each shoot of each treatment in every patch 10 times during the growing season (from 31 May to 28 September) in 2014. To compare the photochemical efficiency between current-year and 1-year-old needles, Fv/Fm values were measured 6 times from 29 July to 28 September, when they developed. The intact control and the manipulation control showed no significant difference for every variable measured in the preliminary analyses, indicating that branch removal did not affect the performance of terminal branches. Therefore, we used the manipulation control data for the analyses.

Fig. 1 Experimental manipulations of *Pinus pumila*. **a** Control shoot in which lateral branches on the main stems were cutoff within parts younger than 4 years old (white line). **b** Shading treatment was conducted using black fine-meshed bags after cutting the lateral branches. **c** Warming treatment was conducted using white fabric bags after cutting the lateral branches. **d** Defoliation treatment involved removing half of the needles on the stem of parts younger than 4 years old after cutting the lateral branches

(a) Control

(b) Shading

(c) Warming

(d) Partial defoliation
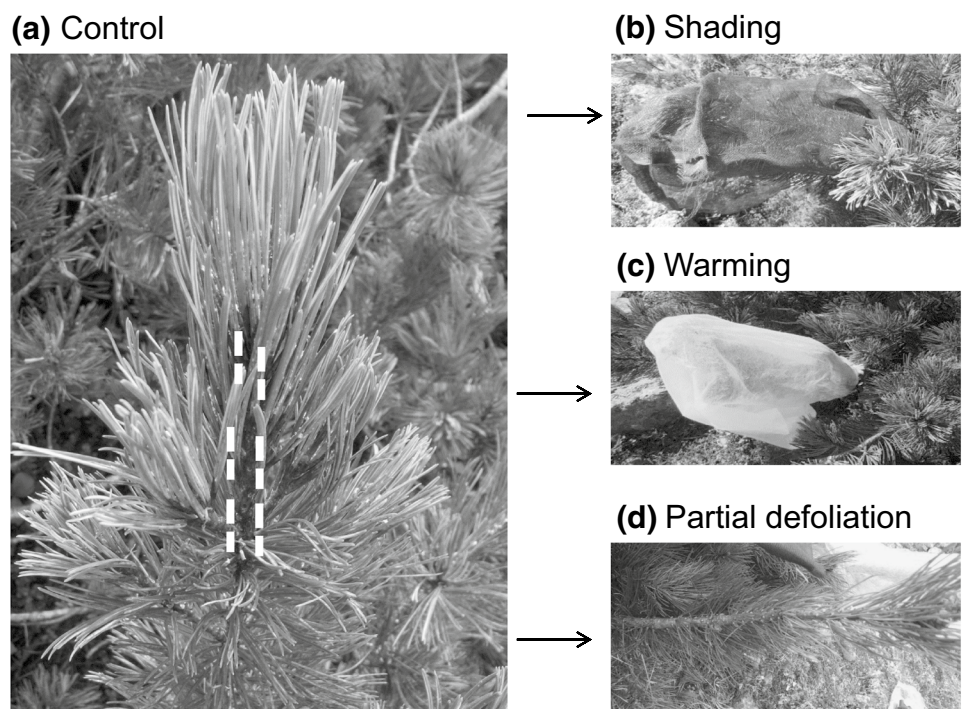
Effects of elevation and snowmelt time on the seasonality of photochemical efficiency

We measured Fv/Fm values at Goshikigahara (hereafter G site) and the southern slope of Mt. Kaun (hereafter K site; Table S1, Fig. S1). We set five plots in each location across the gradients of snowmelt time (18th–27th weeks of the year) and elevation (1550–1950 m), and arbitrarily selected six or seven shoots and tagged them in each plot. We measured Fv/Fm values of 1-year-old needles 10 times during the growing season (from 1 June to 29 September) in 2014. The actual number of measurements per plot varied depending on the snowmelt timing and weather condition; 5 times in G site and 8–10 times in K site. The Fv/Fm values of intact control shoots at H site were included in the analysis, resulting in totally 11 plots across 3 sites.

Data analysis

Seasonal trends of Fv/Fm values of 1-year-old needles were compared among the treatments using the generalized linear mixed-effect model (GLMM), postulating a gamma error distribution with log-link functions. In the GLMM, assigned patch (hereafter ID patch) was included as a random intercept. We used treatment and seasonal progress (number of weeks since the first measurement) as explanatory variables, in which a quadratic term of week was also included. The effects of needle age (current-year vs. 1-year-old) and treatment on Fv/Fm values were compared by the GLMM, postulating a gamma error distribution with log-link functions, in which age, seasonal progress (week and week^2), and treatment were included as explanatory variables, and ID patch as a random intercept.

Next, the effects of treatments on shoot growth were analyzed using the GLMM, postulating a Gaussian error distribution with identity-link functions, in which ID patch was included as a random intercept. Before the analysis, annual shoot growth data from 2003 to 2015 were standardized in each shoot. To eliminate yearly variation and endogenous effects of individual stems, we included the experiment year (2013, 2014, and 2015) and mean value of annual shoot growth during 2003 and 2012 in the explanatory variables, in addition to the treatment effect. The GLMMs were performed using the functions glmer (gamma error distribution) and lmer (Gaussian error distribution) included in the R package lme4 (Bates et al. 2015) using the R statistical software version 3.2.4. Furthermore, between-year variation of annual growth of control shoots was compared with summer temperature (mean values of 1 June to 31 August temperature) of the previous year by Pearson's correlation test. This is because previous studies reported that the shoot growth of *P. pumila* is positively correlated with summer

temperature of previous year (Takahashi 2003; Wada et al. 2005).

Finally, the effects of elevation and snowmelt time on Fv/Fm values during the growing season were analyzed for the data from 11 plots across 3 locations (H, G, and K sites) in 2014 using the generalized linear model (GLM) postulating a gamma error distribution with log-link functions. In the GLM, elevation, snowmelt time, and seasonal progress (week and week^2) were treated as explanatory variables. The interaction terms between week and elevation and between week and snowmelt time were included to test the seasonal variations in physiological activities with reference to the elevation and snowmelt time. The best-fit model was obtained by selecting explanatory variables based on Akaike's information criterion (AIC).

Results

Effectiveness of the warming treatment

According to the weather station data at H site, mean air temperatures during the summer (from 1 June to 31 August) were 12.9 °C, 11.8 °C, and 11.0 °C in 2013, 2014, and 2015, respectively. The length of growing season, i.e., the period from first day to final day on which daily mean temperature exceeded 5 °C, ranged from 146 days to 184 days. The number of freezing days (daily mean temperature < 0 °C during the growing season) was 0, 7, and 6 days in 2013, 2014, and 2015, respectively.

The daily mean ambient temperatures of control shoots (mean of 2013–2015) were 9.8, 13.1, 12.6, and 8.1 °C in June, July, August, and September; the daily minimum temperatures were 5.0, 8.3, 8.7, and 4.3 °C; and the daily maximum temperatures were 15.9, 19.1, 17.5, and 13.3 °C, respectively. The warming effects of the shading treatment and the warming treatment relative to the control were 0.10–0.13 °C for daily mean and 0.45–0.58 °C for daily maximum in the shading treatment, while they were 0.38–0.86 °C and 1.7–3.1 °C in the warming treatment, respectively (see Table S2). Thus, our warming treatment effectively created warm conditions, but the warming effect of the shading treatment was negligible.

Responses of photochemical efficiency to the treatments

The Fv/Fm values of 1-year-old needles varied seasonally ($P < 0.001$); it retained high values during the middle of summer (from mid-July to mid-August), but exhibited lower values in the early and late season (Fig. 2; Table 1). The Fv/Fm values increased in the shading ($P < 0.001$) and warming treatments ($P < 0.001$), especially in the early and late

Fig. 2 Photochemical efficiency (Fv/Fm) of 1-year-old needles of the control, shading, warming, and partial defoliation treatments during the 2014 growing season. Box-and-whisker plot presents the 75th, 50th, and 25th percentiles with whiskers from the 90th to 10th percentiles. $n = 20$ patches
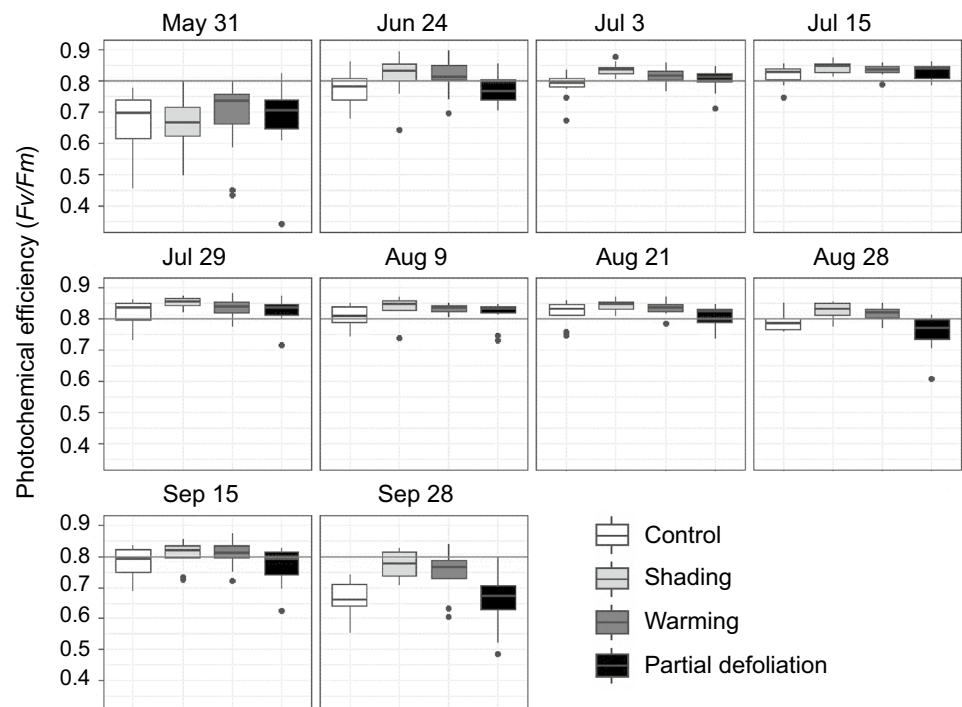

Table 1 GLMM results for the effects of treatments (control, shading, warming, and partial defoliation) on photochemical efficiencies (Fv/Fm) of 1-year-old needles during the growing season in 2014

Variables	Coefficient	SE	t	P
Intercept (control)	−2.65	0.027	−99.82	< 0.001
Shading	0.049	0.009	5.22	< 0.001
Warming	0.041	0.009	4.42	< 0.001
Partial defoliation	−0.001	0.009	−0.14	0.89
Season (week)	0.16	0.001	165.28	< 0.001
Season^2 (week^2)	−0.003	< 0.001	−167.38	< 0.001

growing season, while there was no change in the defoliation treatment ($P = 0.89$; Table 1).

The Fv/Fm values of current-year needles were significantly smaller than those of 1-year-old needles ($P < 0.001$;

Table 2). Similar to the 1-year-old needles, the Fv/Fm values of current-year needles increased in the shading ($P < 0.001$) and warming treatments ($P < 0.001$), but there was no significant difference between the control and defoliation treatments ($P = 0.41$; Table 2; Fig. 3). Although Fv/Fm values decreased in the late growing season ($P < 0.001$) for both current-year and 1-year-old needles, significant interactions between needle age and season ($P < 0.001$) indicated that the seasonal decline in photosynthetic capacity was more apparent in current-year needles.

Responses of shoot growth to the treatments

Annual growth of the control shoots oscillated with a period of 5–6 years during the last 13 years; it was large in 2006–2007 and 2011–2012 but small in 2003–2004, 2008–2009, and 2014–2015 (Fig. 4). Annual shoot

Table 2 GLMM results for the effects of needle age (current-year and 1-year-old) and treatments (control, shading, warming, and partial defoliation) on photochemical efficiencies (Fv/Fm) during the latter half of the growing season (from late July to late September) in 2014

Variables	Coefficient	SE	t	P
Intercept (1-year-old needle, control)	−2.98	0.059	−50.6	< 0.001
Current-year needle	−3.80	0.076	−50.1	< 0.001
Shading	0.062	0.010	6.4	< 0.001
Warming	0.077	0.010	8.0	< 0.001
Partial defoliation	−0.008	0.010	−0.8	0.41
Season (week)	0.18	0.002	92.8	< 0.001
Season^2 (week^2)	−0.003	< 0.001	−152.1	< 0.001
Current needle × season (week)	0.24	0.002	108.0	< 0.001
Current needle × season^2 (week^2)	−0.004	< 0.001	−438.1	< 0.001

Fig. 3 Photochemical efficiency (Fv/Fm) of current needles of the control, shading, warming, and partial defoliation treatments during the growing season in 2014. Refer to the caption of Fig. 2 for details. $n = 20$ patches
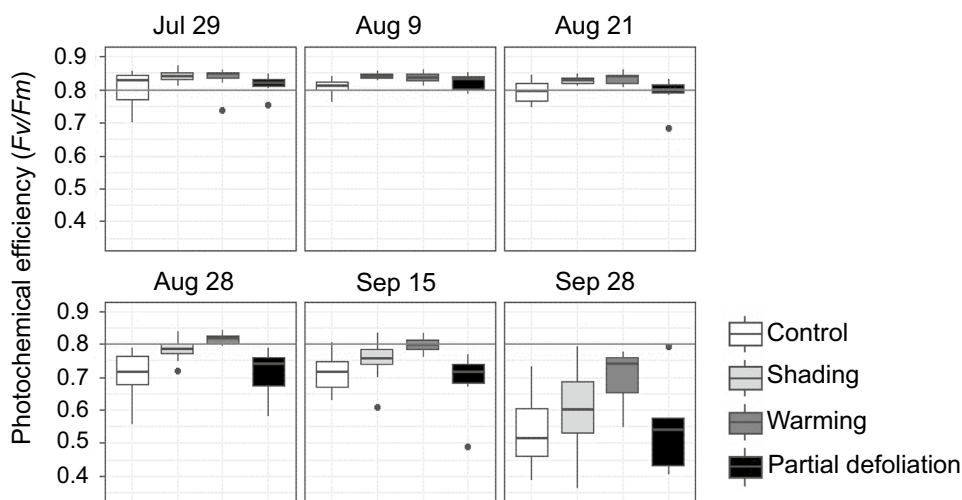

Fig. 4 Annual shoot growth patterns before the experiment (2003–2012) and during the experimental period (2013–2015) for shoots with manipulations (control, shading, warming, and partial defoliation treatments). These data from 2003 to 2015 were standardized in each shoot (vertical bars standard error). Summer temperature (mean values of June 1 to August 31, at 1.5 m height) recorded at H site is shown in thick gray line
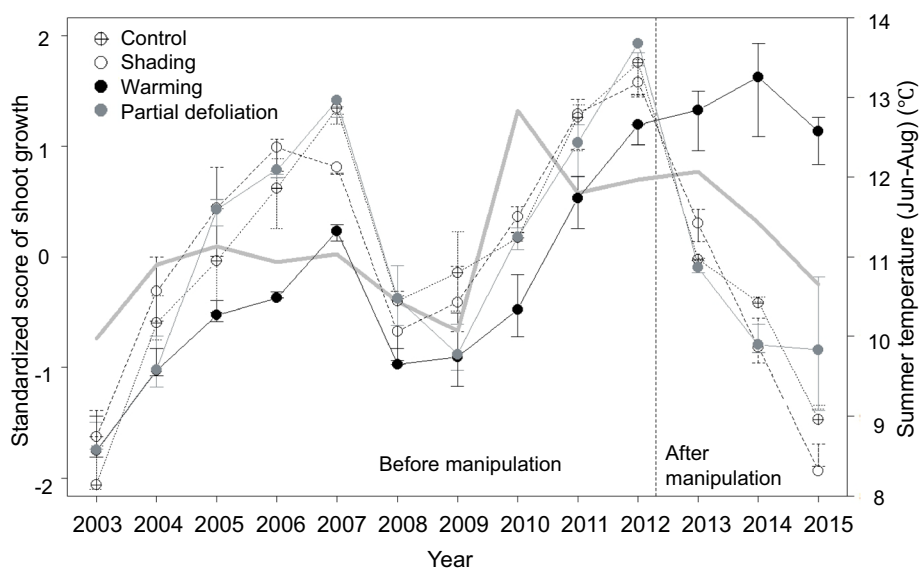
growth significantly increased by the warming treatment ($P < 0.001$), but there was no significant difference between the shading and defoliation treatments ($P > 0.1$; see Table S3 for statistical details). Annual shoot growth during the experimental period was negatively related to the mean shoot growth during the last decade (2003–2012; $P < 0.001$, Table S3), indicating that shoots that grew slower in the current test were growing fast in earlier years. Shoots under the warming treatment retained high growth activity during the experimental period (2013–2015), while shoots under other treatments, including the control, showed a continuous decreasing pattern from 2013 to 2015 (Fig. 4). The trend of annual growth of intact and manipulation control shoots was positively correlated with the summer temperature (1 June to 31 August) of previous year ($r = 0.580$, $P = 0.038$; and $r = 0.478$, $P = 0.099$, respectively).

Variations in photochemical efficiency among habitats

The Fv/Fm values across 11 plots from three sites (H, G, K) tended to show lower values in the early season, increased in the middle season, and decreased again in September (Table 3; Fig. S2). The GLM result indicated the decreases in Fv/Fm values at higher elevations ($P < 0.001$) and later snowmelt places ($P < 0.001$), while site difference was excluded by AIC (Table 4). Significant interactions with seasonal progress were detected for elevation and snowmelt time (both $P < 0.001$), indicating that seasonal trend in photochemical efficiency varied along the elevation gradients and snowmelt times. The Fv/Fm values recovered slowly after overwintering at higher elevations, while the values decreased in the late season

Table 3 Summary of photochemical efficiencies (Fv/Fm) along the elevation gradients (**a**) and snowmelt times (**b**) during the growing season in 2014. Mean ± SD (sample size)

Season[a]	Term 1	Term 2	Term 3	Term 4	Term 5
(a) Elevation (m)					
Low (< 1700)	0.67 ± 0.08 (12)	0.76 ± 0.06 (38)	0.82 ± 0.03 (62)	0.77 ± 0.03 (52)	0.67 ± 0.07 (26)
Middle (1700–1800)	—	0.78 ± 0.04 (21)	0.82 ± 0.02 (21)	0.80 ± 0.02 (42)	0.71 ± 0.05 (21)
High (> 1800)	0.61 ± 0.06 (17)	0.75 ± 0.06 (54)	0.83 ± 0.02 (120)	0.79 ± 0.03 (60)	0.74 ± 0.04 (30)
(b) Snowmelt time (week of the year)					
Early (18–19)	0.65 ± 0.07 (18)	0.78 ± 0.03 (36)	0.83 ± 0.02 (72)	0.78 ± 0.03 (36)	0.70 ± 0.08 (18)
Middle (21– 23)	0.59 ± 0.06 (11)	0.78 ± 0.04 (45)	0.83 ± 0.02 (69)	0.80 ± 0.02 (66)	0.71 ± 0.05 (33)
Late (25–27)	—	0.70 ± 0.05 (32)	0.81 ± 0.02 (62)	0.78 ± 0.03 (52)	0.72 ± 0.06 (27)

[a]Within-season sampling periods; 1: late May–early June, Term 2: late June–early July, Term 3: mid-July–mid-August, Term 4: late August–mid-September, Term 5: late September

Table 4 GLM results for the effects of elevation and snowmelt time on the photochemical efficiencies (Fv/Fm) during the growing season in 2014

Variables	Estimate	SE	t	P
Intercept	5.560	0.265	20.97	< 0.001
Elevation (10^3 m)	−0.449	0.134	−3.36	< 0.001
Snowmelt time	−0.057	0.006	−9.68	< 0.001
Season (week)	0.135	0.010	13.54	< 0.001
Seasonality^2 (week^2)	−0.0031	0.0001	−30.03	< 0.001
Elevation × week	0.017	0.004	4.24	< 0.001
Snowmelt time × week	0.0016	0.0002	8.88	< 0.001

rapidly at lower elevations (Table 3a). Seasonal patterns of the Fv/Fm values varied among plots with different snowmelt time, with a peak in mid-summer (Table 3b).

Discussion

Seasonal pattern of photochemical efficiency

Our study demonstrated clear seasonal dynamics of physiological activity of *P. pumila* in terms of photochemical efficiency. The Fv/Fm values of needles were commonly high in the middle of growing season, while they were low early and late in the season. Because evergreen conifers often retain needles for several years, needles have to survive severe winters through the induction of physiological hardiness in autumn and recover the photosynthetic function after overwintering (Öquist and Huner 2003). Therefore, the increase in Fv/Fm values early in the season reflects a physiological recovery process following growth initiation. In contrast, the decrease in Fv/Fm values in September reflects the decline in growth and the beginning of cold-hardening (Öquist and Huner 2003; Yamazaki et al. 2003, 2011).

Although the responses of current-year needles to the treatments were similar to those of 1-year-old needles, the Fv/Fm values of the current-year needles were lower than those of 1-year-old needles. The significant interaction between needle age and the seasonal trend in Fv/Fm (Table 2) indicates an age-specific seasonality of photosynthetic activity: current-year needles showed a peak later in the season, corresponding to the ongoing maturation process (Kajimoto 1990) as was reported for *Pinus sylvestris* (Troeng and Linder 1982). Nevertheless, photochemical efficiency decreased earlier in the current-year needles than one-year-old needles in autumn, presumably reflecting an earlier onset of hardening, perhaps because current-year needles are more sensitive to freezing temperature.

Responses of shoot growth to the treatments

Our experiment revealed that growth of *P. pumila* is limited by low temperature during the summer because annual shoot growth was increased by the warming treatment, while shoot growth was not influenced by the defoliation treatment. This means that shoot growth (sink activity) is not limited by carbon assimilation (source activity) but by meristematic processes (Körner 2015). Low temperature is restricting sink activity of trees at their higher elevation limits (Hoch and Körner 2009; Dawes et al. 2015). In the warming treatment, mean ambient temperatures during the growing season (2013–2015) were 10.5 °C–12.8 °C, approximately 0.4 °C–0.9 °C higher than the control (Table S2). Therefore, shoot growth of *P. pumila* strongly depended on ambient temperature during the growing season under natural conditions (Fig. 4), and photochemical efficiency was higher in the warming treatment (Figs. 2, 3).

The growth initiation and duration of woody production in conifers inhabiting higher elevations strongly depend on temperature, and thermal limits of growth activity were around 6–8 °C (Rossi et al. 2007). At H site, the first record

of a daily mean temperature above 0 °C was observed on April 24, the last record of freezing temperature in spring was on May 19, and daily mean temperature had exceeded 6 °C after May 28 during the summer in 2014. Because our experiment was conducted in the beginning of June, our treatments might affect the environmental conditions within the main growing season without affecting the timing of growth initiation.

As reported in previous studies (Takahashi 2003; Wada et al. 2005), current shoot growth was also positively related to the temperature of previous summer (Fig. 4). Kajimoto (1993) reported that current shoots develop rapidly from June to July and final shoot size correlates positively with the initial bud size produced in the previous summer. It can be assumed that shoot length in a given season is determined by the growth conditions in the previous late season through the number of nodes, i.e., the number of preformed needle primordia produced per bud.

Little impact of the shading treatment on shoot growth was an unexpected response because our previous study demonstrated that sunshine duration (not radiation intensity) was related to the shoot growth (Amagai et al. 2015). Although the *Fv/Fm* values increased under shaded conditions, there was no significant difference in shoot growth between control and shaded shoots, indicating that shoot growth is sink- and not source-limited.

Seasonal variations in physiological activity among habitats

Fv/Fm values were negatively related to the elevation and snowmelt time of individual habitats. At higher elevations, recovery of photochemical efficiency in the early growing season might progress slowly due to a low carbon demand under cool conditions (Hoch and Körner 2009). In contrast, the decrease in Fv/Fm values in the late growing season occurred earlier at lower elevations. The retention of photochemical efficiency until late season at high elevations may be a compensatory response to thermally limited growing season, when the development of buds progresses slowly and continues until later in the growing season (Kajimoto 1993), i.e., exerting a longer sink demand by maturing tissues. Similarly, a seasonal pattern of photochemical efficiency is expected along the snowmelt time, in which later growth initiation in late-snowmelt habitats results in a significant interaction between snowmelt time and the progress of bud/shoot development (Table 4). Furthermore, lower photochemical efficiency at the late-snowmelt habitat might because response to longer recovery time from winter dormancy (Lehner and Lütz 2003), resulting in less shoot growth at the late-snowmelt habitat (Rossi et al. 2011).

The distribution of *P. pumila* has expanded toward both sides of fellfield and snowbed habitats during the last few decades in the Taisetsu Mountains (Amagai et al. 2015). The expansion toward the fellfield (as seen with H site) might be due to the increasing temperature during the growing season, while the expansion toward the snowbed (as seen with G site) might reflect both, the earlier snowmelt and the warmer summer temperature. The results of our experiment suggest that *P. pumila* will increase its shoot growth and expand its range under continued climatic warming. However, warm spring and earlier release from snow cover may increase the risk of frost damage and needle browning (Maruta et al. 1996; Ishida et al. 2001; Nakamoto et al. 2012), which may impede the growth of *P. pumila* especially around the fellfield habitat. It is important to monitor *P. pumila*'s range dynamics under climate change because it can out-compete species-rich alpine plant communities, given its taller stature and massive biomass accumulation.

Compliance with ethical standards

Ethical Statement The authors declare that observance Ethical Standards.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The *Pinus pumila* is not an endangered and endemic species.

Informed consent The investigation at national park was conducted by obtaining due permission.

References

Amagai Y, Kaneko M, Kudo G (2015) Habitat-specific responses of shoot growth and distribution of alpine dwarf-pine (*Pinus pumila*) to climate variation. Ecol Res 30:969–977. https://doi.org/10.1007/s11284-015-1299-6

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw 67:1–48. https://doi.org/10.18637/jss.v067.i01

Chapin FS, Sturm M, Serreze MC et al (2005) Role of land-surface changes in Arctic summer warming. Science 310:657–660. https://doi.org/10.1126/science.1117368

Dawes MA, Philipson CD, Fonti P, Bebi P, Hättenschwiler S, Hagedorn F, Rixen C (2015) Soil warming and CO₂ enrichment induce

biomass shifts in alpine tree line vegetation. Glob Chang Biol 21:2005–2021. https://doi.org/10.1111/gcb.12819

Demmig B, Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O₂ evolution in leaves of higher plants. Planta 171:171–184. https://doi.org/10.1007/BF00391092

Forbes BC, Fauria MM, Zetterberg P (2010) Russian Arctic warming and 'greening'are closely tracked by tundra shrub willows. Glob Chang Biol 16:1542–1554. https://doi.org/10.1111/j.1365-2486.2009.02047.x

Gottfried M, Pauli H, Futschik A et al (2012) Continent-wide response of mountain vegetation to climate change. Nat Clim Change 2:111–115. https://doi.org/10.1038/nclimate1329

Grabherr G, Gottfried M, Pauli H (1994) Climate effects on mountain plants. Nature 369:448–448. https://doi.org/10.1038/369448a0

Hallinger M, Manthey MM, Wilmking M (2010) Establishing a missing link: warm summers and winter snow cover promote shrub expansion into alpine tundra in Scandinavia. New Phytol 186:890–899. https://doi.org/10.1111/j.1469-8137.2010.03223.x

Hansen J, Ruedy R, Sato M, Lo K (2010) Global surface temperature change. Rev Geophys 48:1–29. https://doi.org/10.1029/2010RG000345

Hoch G, Körner C (2009) Growth and carbon relations of tree line forming conifers at constant vs. variable low temperatures. J Ecol 97:57–66. https://doi.org/10.1111/j.1365-2745.2008.01447.x

Inouye DW (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. Ecology 89:353–362. https://doi.org/10.1890/06-2128.1

Ishida A, Nakano T, Sekikawa S, Maruta E, Masuzawa T (2001) Diurnal changes in needle gas exchange in alpine Pinus pumila during snow-melting and summer seasons. Ecol Res 16:107–116. https://doi.org/10.1046/j.1440-1703.2001.00376.x

Kajimoto T (1989) Aboveground biomass and litterfall of Pinus pumila scrubs growing on the Kiso mountain range in central Japan. Ecol Res 4:55–69. https://doi.org/10.1007/BF02346943

Kajimoto T (1990) Photosynthesis and respiration of Pinus pumila needles in relation to needle age and season. Ecol Res 5:333–340. https://doi.org/10.1007/BF02347008

Kajimoto T (1993) Shoot dynamics of Pinus pumila in relation to altitudinal and wind exposure gradients on the Kiso mountain range, central Japan. Tree Physiol 13:41–53. https://doi.org/10.1093/treephys/13.1.41

Kammer PM, Möhl A (2002) Factors controlling species richness in alpine plant communities: an assessment of the importance of stress and disturbance. Arct Antarct Alp Res 34:398–407. https://doi.org/10.2307/1552197

Kitajima M, Butler WL (1975) Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. Biochim Biophys Acta 376:105–115. https://doi.org/10.1016/0005-2728(75)90209-1

Körner C (2015) Paradigm shift in plant growth control. Curr Opin Plant Biol 25:107–114. https://doi.org/10.1016/j.pbi.2015.05.003

Kudo G (1991) Effect of snow-free period on the phenology of alpine plants inhabiting snow patches. Arct Alp Res 23:436–443. https://doi.org/10.2307/1551685

Lehner G, Lütz C (2003) Photosynthetic functions of cembran pines and dwarf pines during winter at timberline as regulated by different temperatures, snowcover and light. J Plant Physiol 160:153–166. https://doi.org/10.1078/0176-1617-00798

Lütz C (2010) Cell physiology of plants growing in cold environments. Protoplasma 244:53–73. https://doi.org/10.1007/s00709-010-0161-5

Manuel N, Cornic G, Aubert S, Choler P, Bligny R, Heber U (1999) Protection against photoinhibition in the alpine plant Geum montanum. Oecologia 119:149–158. https://doi.org/10.1007/s004420050771

Maruta E, Nakano T, Ishida A, Iida H, Masuzawa T (1996) Water relations of Pinus pumila in the snow melting season at the alpine region of Mt. Tateyama Proc NIPR Symp Polar Biol 9:335–342

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668. https://doi.org/10.1093/jexbot/51.345.659

Mohammed GH, Binder WD, Gillies SL (1995) Chlorophyllfluorescence: a review of its practical forestry applications and instrumentation. Scand J For Res 10:383–410

Myers-Smith IH, Forbes BC, Wilmking M et al (2011) Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environ Res Lett 6:045509. https://doi.org/10.1088/1748-9326/6/4/045509

Nagano S, Nakano T, Hikosaka K, Maruta E (2009) Needle traits of an evergreen, coniferous shrub growing at wind-exposed and protected sites in a mountain region: does Pinus pumila produce needles with greater mass per area under wind–stress conditions? Plant Biol 11:94–100. https://doi.org/10.1111/j.1438-8677.2009.00253.x

Nagano S, Nakano T, Hikosaka K, Maruta E (2013) Pinus pumila photosynthesis is suppressed by water stress in a wind-exposed mountain site. Arct Antarct Alp Res 45:229–237. https://doi.org/10.1657/1938-4246-45.2.229

Nagy L, Grabherr G (2009) The biology of alpine habitats. Oxford University Press, New York

Nakamoto A, Ikeda T, Maruta E (2012) Needle browning and death in Pinus pumila in the alpine region of central Japan were not related to mechanical damage of cuticle and cuticle thickness. Can J For Res 42:167–178. https://doi.org/10.1139/x11-153

Neuner G (2014) Frost resistance in alpine woody plants. Frontiers Plant Sci 5. https://doi.org/10.3389/fpls.2014.00654 (Article 654)

Okitsu S, Ito K (1984) Vegetation dynamics of the Siberian dwarf pine (Pinus pumila Regel) in the Taisetsu mountain range. Hokkaido Japan Vegetatio 58:105–113. https://doi.org/10.1007/BF00044934

Okitsu S, Ito K (1989) Conditions for the development of the Pinus pumila zone of Hokkaido, northern Japan. Vegetatio 84:127–132. https://doi.org/10.1007/BF00036513

Öquist G, Huner NP (2003) Photosynthesis of overwintering evergreen plants. Annu Rev Plant Biol 54:329–355. https://doi.org/10.1146/annurev.arplant.54.072402.115741

Öquist G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186:450–460. https://doi.org/10.1007/BF00195327

Ozeki M, Hamada T, Iijima Y (2011) Shoot elongation of alpine dwarf pine (Pinus pumila) in Senjojiki cirque, central Japan. Bull Nagano Environ Conserv Res Inst 7:39–42

Pauli H, Gottfried M, Dullinger S et al (2012) Recent plant diversity changes on Europe's mountain summits. Science 336:353–355. https://doi.org/10.1126/science.1219033

Rayback SA, Henry GHR (2006) Reconstruction of summer temperature for a Canadian high arctic site from retrospective analysis of the dwarf shrub, Cassiope tetragona. Arct Antarct Alp Res 38:228–238. (https://doi.org/10.1657/1523-0430(2006)38[228:ROSTFA]2.0.CO;2)

Rossi S, Deslauriers A, Anfodillo T, Carraro V (2007) Evidence of threshold temperatures for xylogenesis in conifers at high altitudes. Oecologia 152:1–12. https://doi.org/10.1007/s00442-006-0625-7

Rossi S, Morin H, Deslauriers A (2011) Multi-scale influence of snowmelt on xylogenesis of black spruce. Arct Antarct Alp Res 43:457–464. https://doi.org/10.1657/1938-4246-43.3.457

Rozema J, Weijers S, Broekman R et al (2009) Annual growth of Cassiope tetragona as a proxy for Arctic climate: developing correlative and experimental transfer functions to reconstruct past

summer temperature on a millennial time scale. Glob Chang Biol 15:1703–1715. https://doi.org/10.1111/j.1365-2486.2009.01858.x

Sano Y, Matano T, Ujihara A (1977) Growth of *Pinus pumila* and climate fluctuation in Japan. Nature 266:159–161. https://doi.org/10.1038/266159a0

Takahashi K (2003) Effects of climatic conditions on shoot elongation n of alpine dwarf pine (*Pinus pumila*) at its upper and lower altitudinal limits in central Japan. Arct Antarct Alp Res 35:1–7. **(https://doi.org/10.1657/1523-0430(2003)035[0001:EOCCOS]2.0.CO;2)**

Troeng E, Linder S (1982) Gas exchange in a 20-year-old stand of Scots pine. Physiol plant 54:7–14. https://doi.org/10.1111/j.1399-3054.1982.tb00569.x

Wada N, Watanuki K, Narita K, Suzuki S, Kudo G, Kume A (2005) Climate change and shoot elongation of alpine dwarf pine (*Pinus pumila* Regel): Comparisons between six Japanese mountains. Phyton 45:253–260

Yamazaki JY, Ohashi A, Hashimoto Y, Negishi E, Kumagai S, Kubo T, Oikawa T, Maruta E, Kamimura Y (2003) Effects of high light and low temperature during harsh winter on needle photodamage of *Abies mariesii* growing at the forest limit on Mt. Norikura in Central Japan. Plant Sci 165:257–264. https://doi.org/10.1016/S0168-9452(03)00169-9

Yamazaki JY, Maruta E, Nakano T (2011) Acclimation to the various environments in *Rhododendron brachycarpum* growing at the subalpine zone on Mt. Fuji Mount Fuji Res 5:25–32