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How does the presence and magnitude of air temperature and light variability affect leaf-level dark respiration and photosynthesis respectively of Arctic species?

Background

One of the major objectives in plant ecophysiological research is to determine the connection between physiological processes of plants and their environment[1,2]. In most cases, average constant environmental conditions are used in experiments and the steady state response of plants are taken and assumed to represent and model their performances in these environments. These measurements can then be used to infer mechanistic drivers of plant behavior. Issues arise with such an assumption, mostly because natural environmental conditions are rarely constant, thus introducing the idea of plant responses to variability[3,4]. When variance is present, even if the mean had not changed, it will almost always lead to a different performance value (rate) compared to a constant mean environmental condition. This phenomenon is called Jensen's inequality, the principles of which we can use to predict the effects of environmental variation on non-linear physiological responses. Studying plant responses to environmental variability is a research imperative made even more urgent by the recognition of increased climate variance due to climate change. In this study, we will determine the effects of short-term high-frequency variability on Arctic plant photosynthesis and respiration.

Two physiological processes drive a great deal of plant functioning: photosynthesis and respiration. We will address both of these processes in this study because together they determine the carbon balance of a plant. The main environmental determinant of cellular respiration rate is temperature. The response of respiration to short-term (minutes to days) temperature variability can likely be predicted using Jensen's inequality. Because of the accelerating nature of respiration-temperature curve, Jensen's inequality forecasts a greater respiration rate under variability than the respiration rate at the mean temperature[3–5]. Unfortunately, no experimental studies so far have tested this in plants, making it an urgent research imperative.

For photosynthesis, light is a major driver. The impact of light variability is a topic that has received relatively more attention due to the ecological significance of sun flecks on understory vegetation[6–8]. Change in precipitation patterns including an increase in rainfall variability will also mean more variable cloud cover. High light variation has been shown to produce photosynthetic rates less than what one would expect from the average PFD [6]. One

reason is that the photosynthetic response to light is a decelerating concave down function, meaning light variability should decrease mean photosynthetic rate [3]. The other reason is that plants often are not able to fully utilize transient periods of high light, especially if they have been under lower light conditions[7,8]. Much of the sun fleck research has focused on shade adapted understory plants as sun flecks are the most ecologically relevant to them[9]. However, as climate variability increases and by extension, light variability, sun plants will be exposed to fluctuating light environments as well. Especially in the Arctic, where the plants have experienced constant daylight and often little shading[10], examining the effects of light variability on photosynthetic rate will help inform us of the true extents of global change impacts.

The effects of Jensen's inequality should differ at different points of response curves if the shape of the curve changes e.g., from concave up to linear[11]. With this idea in mind, the average value of the independent variable around which variation occurs should influence the magnitude of difference between the mean with variability and the mean without variability. The effects of variability then depend on the shape of the response curve and the average value of the independent variable. In a respiration to temperature response curve, the initial portion of the curve is concave up but transitions into a linear response at higher temperatures before becoming slightly concave down as respiration approaches a maximum. To put it simply, the Q_{10} of respiration changes according to the temperature[12]. The effects of Jensen's inequality should be the greatest at lower temperatures when the curve is concave up. In addition to respiration, temperature can also affect the shape of the photosynthesis-light response curves by shifting the point of light saturation[13]. Thus, it's important to then examine the effects of variability on respiration at different mean temperatures to fully understand the potential impacts of variability.

In this study, we analyzed the effects of high frequency short term variation of temperature on respiration rate and light on photosynthetic rate in Arctic plants at two different temperatures. We studied three species with increased abundance in the LTER long-term experimental plots (+NP) (*Betula nana*, *Chamaenerion angustifolium*, *Calamagrostis stricta*) belonging to shrub, herbaceous, or grass functional groups respectively. We conducted a preliminary survey of potential differences between the important functional types found in the tundra which can also greatly affect carbon cycling. These species were also specifically chosen due to their intriguing ecological interactions, especially under disturbance. All three species are abundant in high nitrogen and phosphorus treatment plots in a fertilizer experiment, indicating firstly, a superior ability to utilize additional nutrients, and secondly, high adaptability to disturbed environments. The use of fertilizer in these plots also ensures that plant responses are not confounded by nutrient limitation. As warming increases nutrient availability in the Arctic, these interactions may become significant and lead to a shift in vegetation dominance. Incorporating this extra dimension in studying variability effects will make our findings more ecologically relevant as we can examine how these ecologically important species may respond to variability under high nutrient availability.

Methods

Sample collection and gas exchange.

We used fresh leaf samples from three high nitrogen + phosphorus treatment plots in the dry heath tundra to test the effects of temperature and light variability. We collected two replicate leaf samples from each plot for a total of six individual leaf samples (from unique plants) for each species and each treatment every morning. Samples were collected during the peak growing season (late-June to mid-July) during the summer of 2023 and 2024. The youngest fully expanded leaf including petiole and a section of stem was cut from the main plant and immediately submerged in water then cut again to preserve the integrity of the vascular system; this allows the leaf to function as usual and is an effective way of conducting field measurements with these species[14]. We then conducted gas exchange measurements on the collected samples. No samples were kept for longer than 12 hours without being measured and measurements were only be taken if the leaf turgor was preserved. To test our hypothesis, we used the Li-6800 portable photosynthesis system (LI-COR Inc.) to conduct respiration and photosynthesis measurements: respiration in response to constant temperature, low variation in temperature (±1°C), and high variation in temperature (±5°C) around baseline temperatures of 15°C and 25°C; photosynthesis in response to constant light, low variation in light (±300 photosynthetic active radiation (PAR)), and high variation in light (±600 PAR) around average light level of 1200 PAR. The variability of temperature and light was controlled using a built-in sin function of the Li-Cor 6800 where the period will be set to 5 minutes. For respiration measurements, leaf samples were kept in the enclosed chamber for 30 mins to acclimate. Relative humidity was controlled at 60%. We matched the infra-red gas analyzers (IRGA) right before starting measurements. Measurements began when respiration values reached steady state and the values were logged every 30 seconds for a period of one hour. For photosynthesis measurements, we acclimated the leaf samples incrementally to increasing light until we reached 1200 PAR. Leaves were put into the chamber under 500 PAR, then when steady state photosynthesis has been reached, light was increased to 800 PAR, then 1000 PAR, then 1200 PAR. When leaf has acclimated and photosynthesis rate was stable, we matched the IRGAs and measurements were taken every 30 seconds for a period of one hour.

Respiration-temperature measurements

To measure respiration-temperature curves, we used collected shoot samples of 5-10 cm in length as measured from the end of the newest leaf. For *C. stricta*, due to having much longer leaves, the shoot sample included at least 2 fully expanded leaves. Sampling shoots allowed the leaves to stay turgid for longer than if only the leaves were removed. The collection process was the same to what was described above. After sampling, the shoots were put into a 15.5 × 11.0 × 6.5 cm glass-topped aluminum chamber that will be covered with a dark cloth to acclimate for 30 mins at ambient temperature. The chamber contains fans that mix the air inside. The temperature of the chamber was controlled with a thermoelectric Peltier module. Leaf temperature was

monitored using a thermocouple in contact with the underside of the leaves inside the chamber which was connected to a Li-6400 external thermocouple adaptor and allowed measurement of leaf temperature by the Li-6400xt portable photosynthesis system (LI-COR Inc.). The air flowing into the aluminum chamber was coming from the air pump of the Li-6400xt which drew from the room air. Through the Li-6400xt, we scrubbed the CO_2 in the incoming air and control the relative humidity. The air flow out from the aluminum chamber was flowing through the "sample" gas line and IRGA of an empty closed Li-Cor chamber. After acclimating for 30 mins, we cooled the chamber temperature to 5°C and measurements began with the temperature increasing in increments of 1°C every minute. Rates of respiration were recorded every 30 seconds until the temperature has reached 35°C.

Photosynthesis-light measurements

Photosynthesis response to light was also measured for all three species at the two temperature values: 15 °C and 25 °C. Leaf samples were collected using the same methods described above. The leaves were allowed to acclimate inside the Li-Cor 6800 chamber to 500 PAR. When steady state gas exchange is reached, we increased PAR in increments of 300 (after steady state has been reached at each new PAR) until we reached 1500 PAR. When measurements are ready to begin, the IRGAs was matched and we initiated an auto-program that progressively set the light levels to 2000, 1800, 1500, 1200, 1000, 800, 600, 300, 150, 50, and 0 PAR. At each new PAR, the auto-program waited for a minimum of 60 seconds and maximum of 180 seconds for acclimation then recorded a measurement. We used these measurement points to construct a photosynthesis-light response curve.

Statistical Analysis

We compared the means of the respiration and photosynthesis responses using a linear mixed effects model to determine the effects of variability on physiological rates. Using RStudio[15] and the packages lme4[16] and lmerTest[17], we determined the difference between the mean respiration responses and mean photosynthesis rates between the three variability treatments. A separate test was conducted for each temperature for a total of 4 tests. To account for interspecific differences, species was included as a mixed effect while variability treatment was included as a fixed effect. We also integrated the respiration and photosynthetic rate over the duration of each measurement to determine the change in carbon flux as a result of variability.

To measure the potential effects of Jensen's inequality based on the respiration to temperature curves, we fitted a simple exponential model (eq. 1) to the data using hierarchical Bayesian methods.

$$R_d = ae^{bT} \qquad \text{Equation 1}$$

Where R_d is respiration in the day, T is temperature in °C, and a and b are coefficients. The coefficients from the fitting were then used to plot the curves with which we took the points at a

temperature value of 10, 15, 20, 25, and 30°C. Using the two points at 10°C and 20°C, we found a linear equation connecting the two points. Using the linear equation, we were able to calculate the respiration rate at 15°C. The same approach was applied using the two points at 20°C and 30°C to find the respiration rate at 25°C. Then, we compared the actual respiration rate at 15°C to the value predicted from the linear model to find the percentage of increase.

We used a similar approach to predict the effects of Jensen's inequality from the measured photosynthesis-light response curves. We fitted the Ye et al.[18] mechanistic model of photosynthesis light response to our data using hierarchical Bayesian methods. The model is as followed:

$$P_{\mathrm{n}} = \alpha_{\mathrm{p}} \frac{1 - \beta_{\mathrm{p}} I}{1 + \gamma_{\mathrm{p}} I} \mathrm{I} - R_{\mathrm{light}},$$

Equation 2

Where P_n is net CO_2 assimilation rate, I is irradiance, R_{light} is respiration rate in the light, and α_p, β_p, and γ_p are coefficients. Using the fitted model, we found the predicted photosynthetic rates at 600, 1200, and 1800 PAR. We found a linear equation that intercepts the points at 600 and 1800 PAR and found the predicted value for 1200 PAR. This was done for the light response curves at both 15°C and 25°C. The photosynthetic rate at 1200 PAR predicted from the Ye et al. model and from the linear equation was compared to find the predicted effects of Jensen's inequality.

Results

At 15 °C, high variability in temperature had a significant positive effect on average respiration rate as compared to the control treatment ($p < 0.001$; Fig. 1). Respiration rate under high temperature variability was 64% greater than control in *B. nana*, 43% greater in *C. angustifolium*, and 28% greater in *C. stricta* (Table 1). There was no difference between the low variability treatment and control and low variability and high variability. At 25 °C, neither low nor high variability treatments had an effect on respiration rates compared to the control treatment.

For photosynthesis, variability treatment had no effect at 15 °C (Fig. 1). However, high variability treatment decreased photosynthetic rate compared to the control at 25 °C ($p<0.05$). Compared to the control, photosynthetic rate decreased by 5% under high variability in *B. nana*, 12% in *C. angustifolium*, and 39% in *C. stricta* (Table 2). Photosynthetic rate for low variability treatment was not different from the control treatment or the high variability treatment.

For the photosynthesis to light response curves, the three species differed in the shape of the response (Fig. 4). At 15 °C, *C. stricta* reached light saturation around 1000 PAR and experienced a decrease in photosynthesis beyond 1200 PAR. Similarly, *B. nana* also saturated at 1000 PAR and photosynthesis was slightly negatively affected by additional light. *C. angustifolium* did not seem to reach light saturation even beyond 2000 PAR.

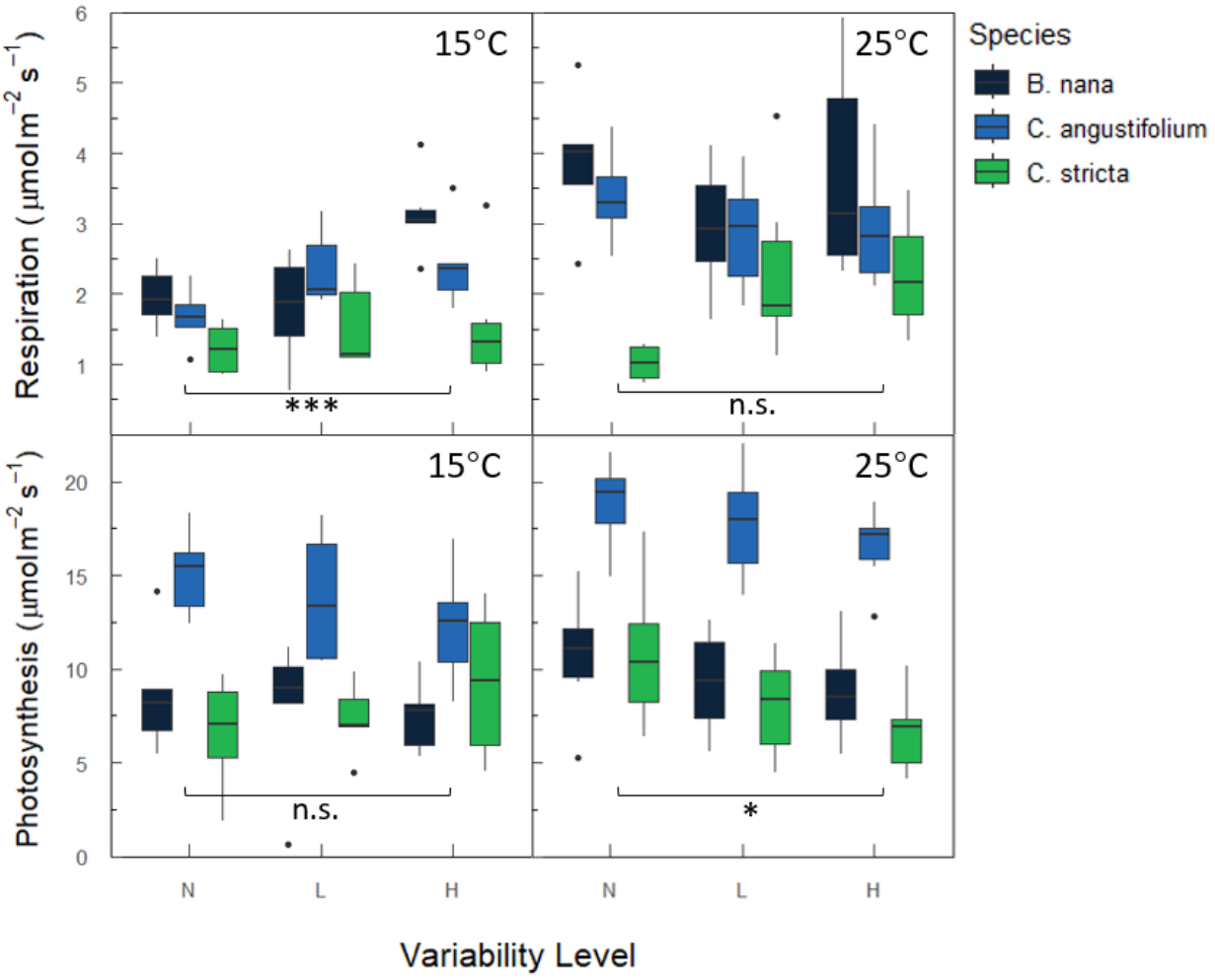

Figure 1: The response of respiration and photosynthesis to variability in temperature and light respectively. Boxplots represent the average value of the three species examined.

Table 1: The respiration response of *Betula nana*, *Chamanaerion angustifolium*, and *Calamagrostis stricta* to temperature variability at two mean temperatures.

Species	Temperature (°C)	Variability Treatment	Respiration
B. nana	15	None	1.92 ± 0.02
		Low	1.81 ± 0.02
		High	3.14 ± 0.04
	25	None	3.88 ± 0.04
		Low	2.95 ± 0.03
		High	3.70 ± 0.05
C. angustifolium	15	None	1.68 ± 0.01
		Low	2.35 ± 0.02
		High	2.41 ± 0.02
	25	None	3.39 ± 0.02

	25	Low	2.87 ± 0.02
		High	2.95 ± 0.03
C. stricta	15	None	1.22 ± 0.02
		Low	1.54 ± 0.03
		High	1.56 ± 0.03
	25	None	1.02 ± 0.01
		Low	2.34 ± 0.04
		High	2.15 ± 0.04

Table 2: The photosynthesis response of *Betula nana*, *Chamaenerion angustifolium*, and *Calamagrostis stricta* to temperature variability at two mean temperatures.

Species	Temperature (°C)	Variability Treatment	Photosynthesis
B. nana	15	None	8.70 ± 0.1
		Low	8.04 ± 0.08
		High	7.48 ± 0.05
	25	None	9.36 ± 0.14
		Low	9.28 ± 0.09
		High	8.84 ± 0.08
C. angustifolium	15	None	15.16 ± 0.09
		Low	13.75 ± 0.07
		High	12.33 ± 0.06
	25	None	18.84 ± 0.07
		Low	17.81 ± 0.08
		High	16.66 ± 0.07
C. stricta	15	None	6.62 ± 0.79
		Low	7.36 ± 0.06
		High	9.27 ± 0.12
	25	None	10.86 ± 0.11
		Low	8.04 ± 0.08
		High	6.67 ± 0.09

We also examined the predicted Jensen's inequality effect under high temperature variability around means of 15°C and 25°C. We found that based on the measured respiration to temperature response, there was no difference in the predicted effect of Jensen's inequality between 15°C and 25°C. Jensen's inequality predicted a 7% increase for both 15°C and 25°C in respiration rate based on the curvature of the response. For photosynthesis response to light, Jensen's inequality predicted a greater negative effect of light variability under 25°C than 15°C.

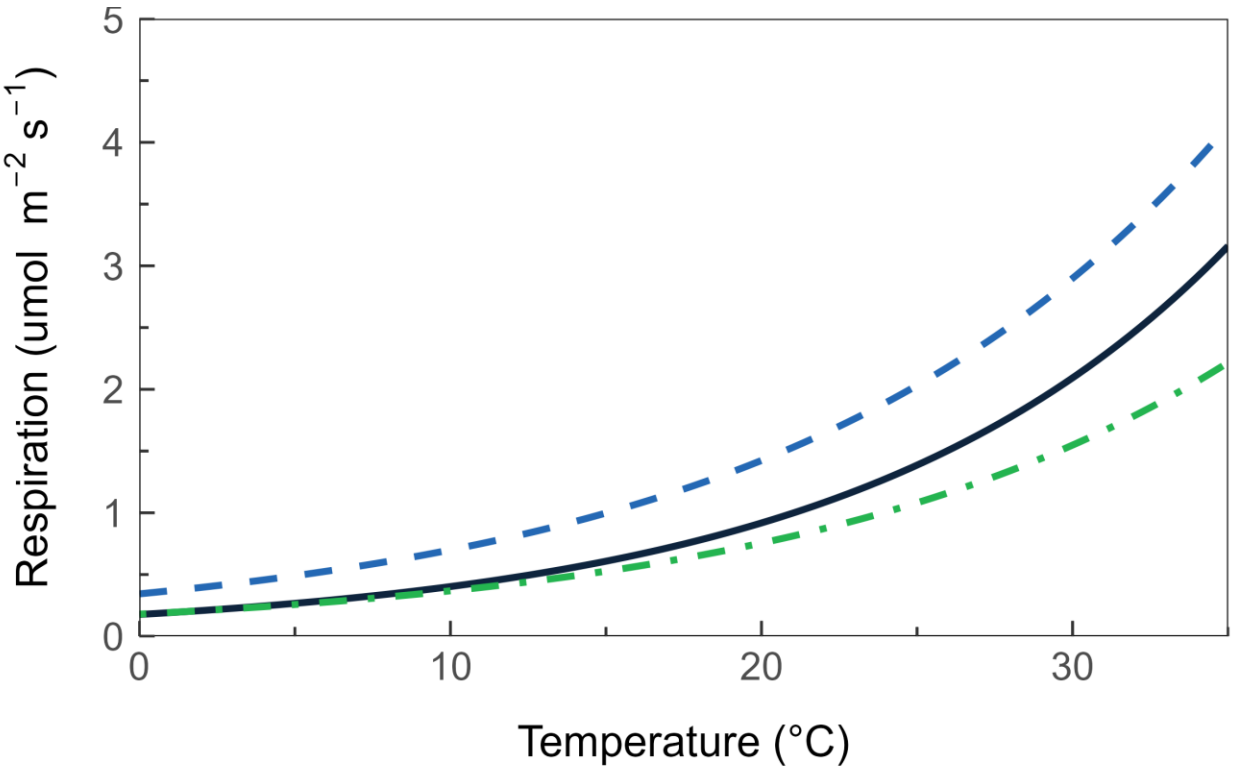

Figure 2. The temperature response of dark respiration rates for *Betula nana* (dark solid line), *Chamaenerion angustifolium* (blue broken line), and *Calamagrostic stricta* (green dotted line).

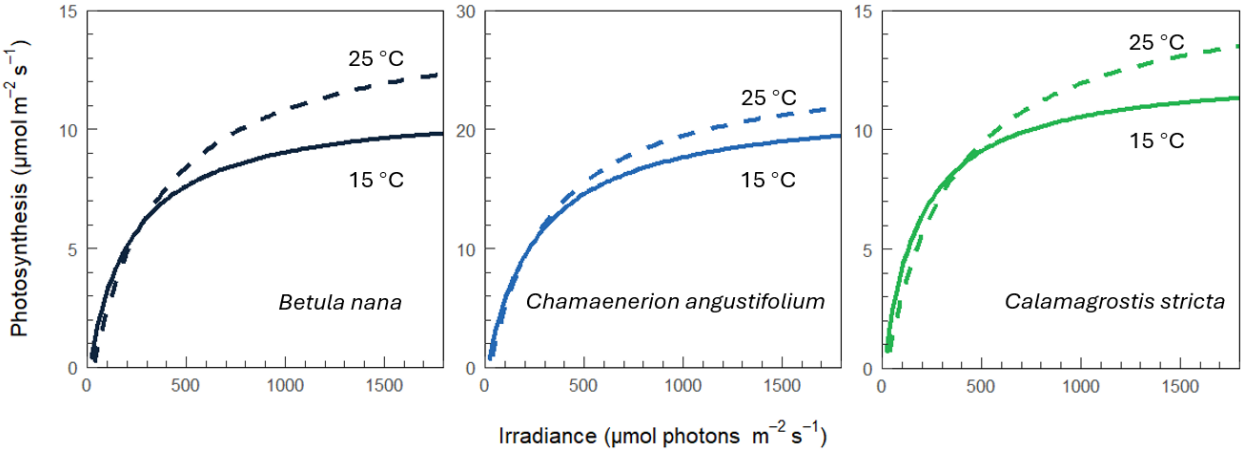

Figure 3. The response of net CO_2 assimilation rate to irradiance at 15°C (solid lines) and 25°C (broken lines) for *Betula nana*, *Chamaenerion angustifolium*, and *Calamagrostic stricta*.

References

1. Billings, W. D. The Environmental Complex in Relation to Plant Growth and Distribution. *The Quarterly Review of Biology* **27**, 251–265 (1952).
2. Billings, W. D. Physiological Ecology. *Annual Review of Plant Physiology* **8**, 375–392 (1957).

3. Ruel, J. J. & Ayres, M. P. Jensen's inequality predicts effects of environmental variation. *Trends in Ecology & Evolution* **14**, 361–366 (1999).
4. Denny, M. The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen's inequality. *J Exp Biol* **220**, 139–146 (2017).
5. Dowd, W. W., King, F. A. & Denny, M. W. Thermal variation, thermal extremes and the physiological performance of individuals. *J Exp Biol* **218**, 1956–1967 (2015).
6. Smith, W. K. & Berry, Z. C. Sunflecks? *Tree Physiology* **33**, 233–237 (2013).
7. Way, D. A. & Pearcy, R. W. Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiology* **32**, 1066–1081 (2012).
8. Pearcy, R. W. Sunflecks and Photosynthesis in Plant Canopies. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 421–453 (1990).
9. Watling, J. R., Ball, M. C. & Woodrow, I. E. The utilization of lightflecks for growth in four Australian rain-forest species. *Funct. Ecol.* **11**, 231–239 (1997).
10. Min, E. *et al.* Using structure to model function: incorporating canopy structure improves estimates of ecosystem carbon flux in arctic dry heath tundra. *Environ. Res. Lett.* **18**, 065004 (2023).
11. Vasseur, D. A. *et al.* Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20132612 (2014).
12. Atkin, O. K. & Tjoelker, M. G. Thermal acclimation and the dynamic response of plant respiration to temperature. *Trends in Plant Science* **8**, 343–351 (2003).
13. Coles, J. F. & Jones, R. C. Effect of temperature on photosynthesis-light response and growth of four phytoplankton species isolated from a tidal freshwater river. *Journal of Phycology* **36**, 7–16 (2000).
14. Heskel, M. *et al.* Differential physiological responses to environmental change promote woody shrub expansion. *Ecol. Evol.* **3**, 1149–1162 (2013).
15. Posit team. RStudio: Integrated Development Environment for R. Posit Software, PBC (2023).
16. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**, 1–48 (2015).
17. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82**, 1–26 (2017).
18. Ye, Z.-P., Suggett, D. J., Robakowski, P. & Kang, H.-J. A mechanistic model for the photosynthesis–light response based on the photosynthetic electron transport of photosystem II in C3 and C4 species. *New Phytologist* **199**, 110–120 (2013).