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Xanthophyll cycle activity in two prominent arctic shrub species

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ABSTRACT

When the capacity for photosynthesis is constrained by unfavorable growing conditions, excess absorbed light is safely lost from leaves via thermal energy dissipation—a photoprotective mechanism ubiquitous among higher plants. The relatively low irradiance conditions yet stressful growing environment of the arctic tundra suggest contrasting hypotheses regarding the necessity for plant investment in photoprotection. To examine these hypotheses, the photoprotective pigments of the xanthophyll cycle were investigated in conjunction with non-photochemical quenching (NPQ) of chlorophyll fluorescence emission in two dominant arctic shrub species, *Salix pulchra* and *Betula nana*. The xanthophyll cycle pool sizes of *S. pulchra* leaves were substantially higher than those reported in most other higher plant species, whereas *B. nana* leaves maintain modestly high xanthophyll cycle pool sizes. In addition, high retention of de-epoxidized xanthophyll cycle pigments in both species and saturation of xanthophyll cycle conversion at low-light intensities were observed and associated with high levels of NPQ. The xanthophyll cycle leaf pigment pools reported are among the first published for arctic plants and support the hypothesis that foliar xanthophyll cycle activity is greater in environments prone to harsher growing conditions.

INTRODUCTION

Plants at high latitudes are exposed to harsh growing conditions characterized by cold temperatures, continuous permafrost, and limited nutrient availability (Nadelhoffer et al., 1990). Combined, these environmental factors limit the photosynthetic capacity for plants to capitalize on the near-continuous sunlight during the short arctic growing season (Chapin et al., 1995; Shaver et al., 1998; Hobbie et al., 2002). Under ideal growing conditions, where a majority of the incoming solar energy is converted into chemical energy by plants, the potential for damage from the absorption of excess light is minimal. However, under stressful conditions like those experienced in the arctic tundra, light may be available in excess of what can be safely used to support photosynthesis; for example, under high irradiance, 50–90% of incident light may go unutilized to drive photochemistry (Björkman and Demmig-Adams, 1994; Demmig-Adams et al., 1995).

It is well understood that the proportion of incoming solar photons not used for photosynthetic electron transport generally increases with exposure to environmental stress (Demmig-Adams and Adams, 2006). Here, plant stress is defined as a decline in plant physiological function caused by environmental conditions that adversely affect plant photosynthetic activity (Chapin, 1991; Niinemets, 2010). When the capacity of a plant to perform photosynthesis is constrained, excess light energy can result in the formation of reactive oxygen species (ROS), which can lead to leaf damage and death (Ledford and Niyogi, 2005). To avoid ROS formation, plants employ a host of photoprotective mechanisms (Demmig-Adams and Adams, 1996; Kanervo et al., 2005; Niyogi et al., 2005; Logan et al., 2014). One of the most ubiquitous mechanisms involves modulation of the efficiency of the conversion of solar energy into chemical energy by constituents of the xanthophyll cycle (Demmig et al., 1987, 1988).

Over the past several decades, a wide body of research has emerged to highlight the role of the xanthophyll cycle in thermal energy dissipation (Demmig-Adams and Adams 2006; Demmig-Adams et al., 2012; Jahns and Holzwarth, 2012). Much of the foundational work was performed by manipulating irradiance and/or nutrient availability of greenhouse or growth chamber environments to examine the role of the xanthophyll cycle in the photoprotection of plants under stressful conditions (e.g., Demmig et al., 1987, 1988; Verhoeven et al., 1997; Logan et al., 1999). These studies found that plants under increasing stress employ a greater level of xanthophyll cycle interconversion, whereby violaxanthin (V) is converted to antheraxanthin (A) and zeaxanthin (Z) via successive, enzyme-catalyzed de-epoxidations (Yamamoto, 1979). Building on the strong empirical evidence supporting a relationship between increased thermal energy dissipation (often measured as non-photochemical quenching of chlorophyll fluorescence emission [NPQ]) and the pigment pool size and conversion state ([A+Z]/[V+A+Z]) of the xanthophyll cycle (Demmig-Adams and Adams, 1994; Demmig-Adams, 1998), some studies are developing an understanding of the molecular mechanisms underpinning thermal energy dissipation (see, e.g., Sylak-Glassman et al., 2014). Field studies have examined xanthophyll cycle conversion throughout the course of a day (e.g., Adams et al., 1999; Barker et al., 2002), the fluctuation of xanthophyll cycle pool size throughout a season (e.g., Adams and Demmig-Adams, 1995; Logan et al., 1998a; Verhoeven et al., 1998; Adams et al., 2002; Porcar-Castell et al., 2008, 2012; Oh et al., 2013), and xanthophyll cycle dynamics under varying light environments (e.g., Logan et al., 1998b; Matsubara et al., 2009). The majority of field-based studies has been conducted in temperate, semi-arid, and Mediterranean climates, with only limited investigations conducted in the end-members of our global climate system, such as tropical rainforest (Königer et al., 1995; Matsubara et al., 2009; Krause et al., 2012); desert (Barker and Adams, 1997; Barker et al., 2002); boreal forest (Ensminger et al., 2004; Yamazaki et al., 2007; Porcar-Castell et al., 2008, 2012); or tundra (this study). Indeed, three recent meta-analyses suggest that total xanthophyll pools and the relative concentration of zeaxanthin are clear indicators of the response of plants to harsh growing conditions or environmental stress (Wujeska et al., 2013; Esteban et al., 2015; Miguez et al., 2015), but these studies lack data reported in more extreme environments, particularly at high latitudes and the tundra biome.

If xanthophyll cycle pool size and conversion state are indeed indicators of environmental stress, we can hypothesize that increasing xanthophyll cycle pool sizes would be observed across an increasingly stressful environmental gradient where substantial sunlight exists. For this reason, we focused on the arctic tundra, a biome characterized by long winters, large temperature variability, low annual precipitation, continuous permafrost, perched water tables, and short and cool growing seasons, all of which leads to limited opportunities for plant nutrient uptake (Nadelhoffer et al., 1990; Hobbie et al., 2002). Potentially compounding these stresses is the near-complete 24 hours per day of sunlight during the arctic growing season, leading one to hypothesize that the availability of light energy exceeds the capacity for photosynthetic carbon assimilation, increasing a need for photoprotection. However, of that sunlight, the fraction of diffuse to total sunlight is also high, with over 80% of days during a typical summer in northern Alaska exceeding a diffuse/total irradiation fraction of 0.8 (68.63°N, −149.60°W; Williams et al., 2014; this study). This value for the diffuse fraction of sunlight in the Arctic is on the high end of the global range and combined with the low solar angles at high latitudes produces a relatively low average summer midday photosynthetic photon flux density (PPFD) of 1032 μmol m^{-2} s^{-1} above vegetation canopies under sunlit conditions, and 351 μmol m^{-2} s^{-1} during diffuse conditions (Williams et al., 2014). Williams et al. (2014) found that net ecosystem productivity in northern Alaska (same field site as this study) saturates at around 600 μmol m^{-2} s^{-1}, and that at this photon flux density canopy-level photosynthesis is enhanced under diffuse radiation conditions by 17% relative to sunlit conditions. Williams et al. (2014) explained that this could be due to a greater proportion of irradiance reaching the bottom part of the canopy under diffuse conditions. Further, the mean leaf-level diurnal

photon flux for an arctic shrub is relatively low (modeled at ~15 mol m^{-2} d^{-1} during peak growing season, reported in Magney et al., 2016a) and would suggest that bulk xanthophyll cycle pools (per Chl) would fall among the lower 25th percentile globally according to a recent meta-analysis (Esteban et al., 2015). From this, one could hypothesize that arctic plants may not need to invest heavily in photoprotective mechanisms because of their lack of extended exposure to high light.

Overall, opposing hypotheses suggest (1) high xanthophyll cycle pool size resulting from long days in a stressful environment, or (2) low xanthophyll cycle pool size due to the high fraction of diffuse sunlight and relatively low maximal irradiance. These potentially contradictory hypotheses provide motivation to investigate the role of the xanthophyll cycle in higher plants of the Arctic, and to compare xanthophyll cycle characteristics of arctic species to species growing in other ecosystems that represent a wide range of growing conditions. Further, because changes in visible (400–700 nm) spectral reflectance on the leaf surface are mainly driven by plant pigments, the remote sensing community has taken a keen interest in observing and interpreting changes in plant pigments over space and time (Ollinger, 2010; Ustin and Gamon, 2010). In particular, there has been growing interest in relating spectral indices to plant function due to, for example, the strong link between xanthophyll cycle pigments and the rates of photosynthesis and CO_2 uptake, as well as light–use efficiency (LUE) (Gamon et al., 1992; Peñuelas et al., 1995; Garbulsky et al., 2011; Hilker et al., 2012). However, before these relationships can be used to extrapolate remotely sensed plant function, it is important that the theory is well established and validated using leaf-level pigment data (Garbulsky et al., 2011). A step forward would involve a better understanding of how the relationship between xanthophyll cycle components and spectra vary (Gamon and Berry, 2012) in response to environmental conditions, in particular in the arctic tundra (Boelman et al., 2016). For these reasons, as well as the increasing interest in understanding the response of plants to climate change in the Arctic, our objectives were twofold:

• Quantify xanthophyll cycle pool sizes, dark–acclimated zeaxanthin retention, and xanthophyll cycle conversion of the abundant arctic shrub species *Salix pulchra* and *Betula nana* during peak growing season.

• Compare xanthophyll cycle constituents and NPQ across light environments within *S. pulchra* and *B. nana* canopies, and against 'unstressed' greenhouse-grown crops.

METHODS

Arctic Study Site and Leaf Sampling

Field measurements took place during peak growing season (6–16 July in 2014 and 2015) in northern Alaska, near the arctic Long Term Ecological Research (LTER) site at Toolik Lake (68.63°N, −149.60°W). We sampled leaves from *S. pulchra* and *B. nana*, which are two of the primary species associated with climate-driven deciduous shrub expansion in the Arctic (Myers–Smith et al., 2011). We sampled fully expanded leaves selected using a stratified random design to ensure the inclusion of sun, shade, and a mix of sun/shade leaves. Sun/shade conditions were quantified using a LI–COR model LAI-2000 (Lincoln, Nebraska, U.S.A.), and data were collected during diffuse sky conditions when irradiance was (1) not rapidly changing, and (2) relatively uniform across the sky hemisphere. This allowed us to apply common algorithms to derive canopy fraction vs. sky fraction (Bréda, 2003). To encompass the top, middle, and lower third of leaf cohorts in terms of leaf area index (LAI), LAI values < 0.25 m^2 m^{-2} were considered sun leaves, 1.0 > LAI > 0.25 m^2 m^{-2} were considered mixed, and LAI values > 1.0 m^2 m^{-2} were considered shade leaves. In 2014, we sampled 8 large *S. pulchra* shrubs with a mean height of ~1 m from moist acidic tundra complexes near Toolik Lake. In 2015, we selected 10 *S. pulchra* and 10 *B. nana* shrubs ranging from 0.2 m to 1.2 m tall in the same region. Xanthophyll activity from 4 of the 2014 *S. pulchra*, which run through the dark–light transition experiment, are reported in a spectral analysis by Boelman et al., 2016. Moist acidic tundra has an average soil pH < 5.5 and is dominated by deciduous shrubs (*S. pulchra*, *B. nana*) and graminoids (primarily *Eriophorum vaginatum* and *Carex bigelowii*). Mean annual temperature for this region is −8.73 °C, and mean annual precipitation is about 300 mm, whereas the mean growing-season temperature is 10 °C. Hourly environmental conditions (air temperature, soil temperature, precipitation, and photosynthetically active radiation) during the 2014 and 2015 growing seasons, and the timing of sampling, are all shown in Figure 1. We show hourly fluctuations in Figure 1 to provide information on the range of conditions experienced. During the 10-day period in which the leaves were sampled, air temperature ranged from 5–15 °C, and PPFD ranged from 6 to 1022 µmol m^{-2} s^{-1} in 2014; and 2–18 °C and 25 to 1245 µmol m^{-2} s^{-1} PPFD in 2015 (Fig. 1).

Experiment Protocol: Sampling Procedure

Small branches with mature, fully expanded leaves (~4–6 leaves) were clipped from a wide range of canopy positions on 8 *S. pulchra* canopies in 2014, 10 *S. pulchra*
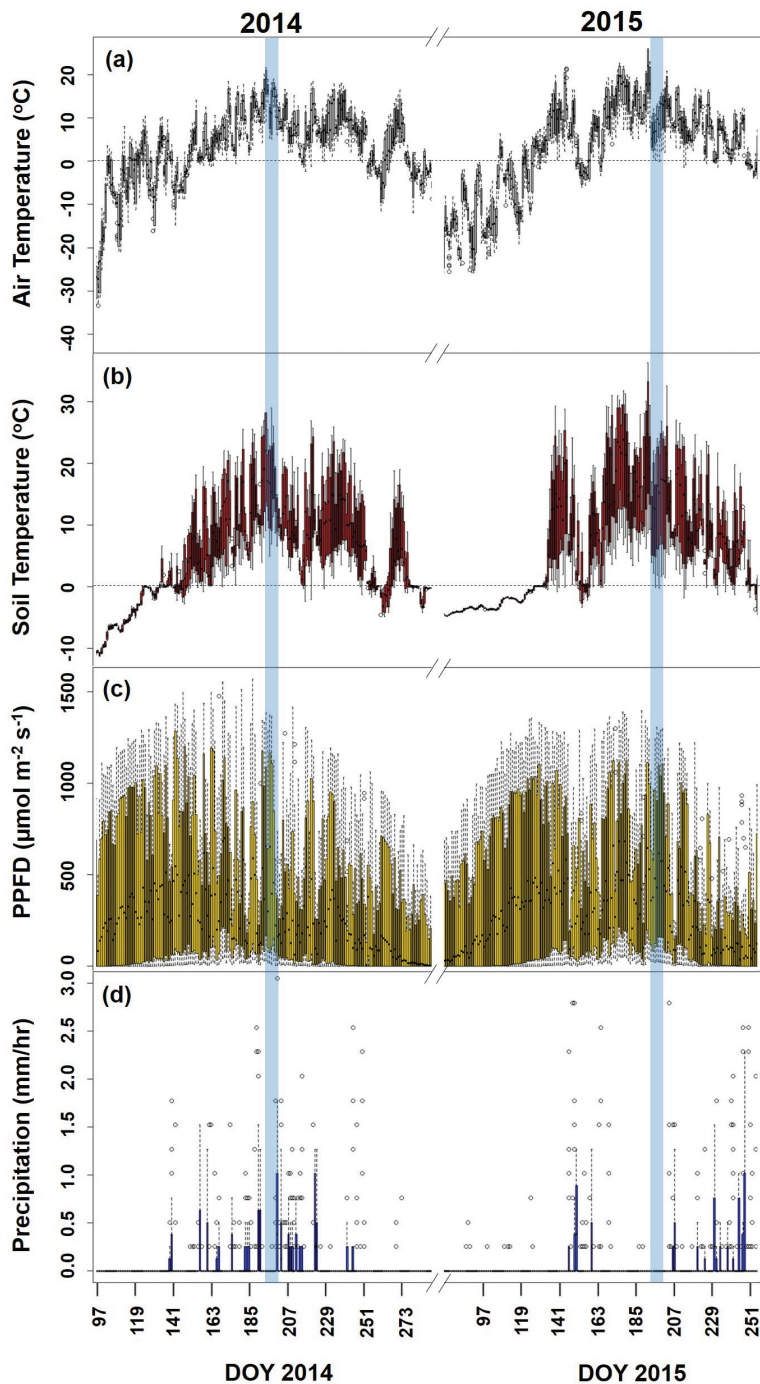
FIGURE 1. Hourly (a) air temperature at 5 m, (b) soil temperature at −5 cm, (c) photosynthetic photon flux density (PPFD), and (d) precipitation. Data were collected during 10 d periods as indicated by the blue rectangles. Meteorological data from the Toolik Lake Long-Term Ecological Research Station Environmental Data Center Team (2016) were collected at a metrological tower located within 1 km of sampling locations. In all subplots, the bars represent one standard deviation, and the tails represent 2 standard deviations from the daily mean.

canopies in 2015, and 10 *B. nana* canopies in 2015 to determine xanthophyll cycle pool sizes of leaves exposed to different light environments. Leaf and stem material was collected at ~midday, and the cut ends of the stems were placed immediately in a test tube with water and brought to the laboratory to be subjected to either (1) a dark-light transition experiment to determine levels of xanthophyll cycle conversion and NPQ, or (2), in 2014 only, measurements of gas exchange and xanthophyll cycle pool size. Following each set of experiments,

a 0.25 cm² disc of leaf tissue was removed using a cork borer and frozen at −80°C for pigment analysis.

In 2014, for the analysis of xanthophyll cycle pool size and photosynthetic capacity, a total of 94 leaf samples including 33 sun-exposed leaves, 39 mixed sun/shade leaves, and 22 shade leaves were analyzed. Of these samples, 40 leaves (including a similarly representative fraction of sun, mixed, and shade leaves) were taken back to the laboratory for the dark-light transition experiment in which the samples underwent dark acclimation and

subsequent exposure to the equivalent of arctic full sunlight to induce xanthophyll cycle de-epoxidation and NPQ. Gas exchange was measured from the remaining 54 leaves prior to cold storage for pigment analysis (but only for total pigment pools, not xanthophyll cycle conversion). In 2015, all analyzed samples went through the dark–light transition experiment, resulting in a total of 31 *S. pulchra* leaf samples, including 13 sun-exposed leaves, 12 mixed sun/shade leaves, and 6 shade leaves. In addition, 55 *B. nana* leaves were analyzed, including 26 sun-exposed leaves, 17 mixed sun/shade leaves, and 12 shade leaves. Of these, there were two pigment samples per leaf representing the dark- and light-exposed state of each leaf.

Experiment Protocol: Dark–Light Transition—Arctic Species

The experimental protocol differed slightly in 2014 and 2015. In both years, leaves were dark acclimated for >2 h to allow relaxation of thermal energy dissipation. In 2014, while still in the dark, Chl fluorescence emission was measured (described below) and leaf discs were collected from five leaves on each leaf whorl and placed in the −80 °C freezer. With the stem still immersed in water, the leaf cluster was placed under a bank of four lights (high–intensity discharge metal halide lamps) situated in a semicircle around the leaf clump. After >6 min exposure, Chl fluorescence emission was remeasured and leaf discs were collected and the corresponding irradiance for each disc was quantified as PPFD measured by a quantum sensor at the leaf surface. Owing to nonuniform angles of individual leaves relative to the light source, leaf-level irradiance ranged from 200 μmol m^{-2} s^{-1} to 1200 μmol m^{-2} s^{-1}, which coincides with the range typically experienced in this arctic growth environment. Light intensities were subsequently binned into low (<300 μmol m^{-2} s^{-1}), medium (800 > PPFD > 300 μmol m^{-2} s^{-1}), and high light (PPFD > 800 μmol m^{-2} s^{-1}). In 2015, a bank of high–intensity LED lights was placed 30 cm above a single leaf on a lab bench. The 2015 study therefore only allowed for the quantification of incident PPFD at two levels (darkness, and a PPFD of ~1200 μmol m^{-2} s^{-1}). Temperature at the leaf level in 2014 and 2015 was monitored to ensure that xanthophyll cycle conversion was not the result of increased leaf temperature (Havaux and Tardy, 1996). After exposure to light for at least 3 min, we assumed that a significant amount of xanthophyll conversion had occurred, although we cannot ensure that steady state was achieved (Demmig-Adams et al., 2012); therefore, our measurements of xanthophyll cycle conversion state may be conservative. At this time, a PPFD measurement was recorded and a leaf pigment sample was collected near to the region of leaf where a sample was collected in darkness previously, and immediately frozen between two blocks of dry ice. These leaf discs remained in a cooler of dry ice for less than 0.5 h before being transferred to a −80 °C freezer. Chlorophyll fluorescence emission was measured from leaves after dark acclimation and after exposure to each light intensity.

Experiment Protocol: Dark–Light Transition—Greenhouse Crops

Data from greenhouse-grown crops ('unstressed' environment) serve as a comparison with the 2014 dark–light transition experiment, as both experiments were performed under four binned light intensities. A similar protocol was employed for the greenhouse-grown species, following Magney et al. (2014). The species used for comparison were wheat, *Triticum aestivum* L. ($n = 19$), and sunflower, *Helianthus annuus* L. ($n = 18$). Both of these annual crops were grown in a greenhouse with replete water and nutrients. Half of the *T. aestivum* and *H. annuus* plants were placed under a shade cloth three weeks prior to measurements. The light intensity in the greenhouse peaked at ~1500 μmol m^{-2} s^{-1} above the canopy of "sun" exposed plants, while midday PPFD under the shade cloth peaked at ~500 μmol m^{-2} s^{-1}. These intensities are similar to (though slightly higher than) the observed light conditions for *S. pulchra* sun and shade leaves during peak growing season. Daily photon irradiance was ~20 mol m^{-2} day^{-1} for shade leaves and ~50 mol m^{-2} day^{-1} for sun leaves in the greenhouse.

Pigment Analysis

Leaf discs were stored at −80 °C until extraction in acetone according to Adams and Demmig-Adams (1992). Pigment separation and quantification were achieved by high–performance liquid chromatography (HPLC), as described in Gilmore and Yamamoto (1991), using an Agilent 1100 series HPLC with an Agilent 1100 diode array detector (Agilent Technologies, Palo Alto, California, U.S.A.) set to record at 445 nm and a YMC Carotenoid™ C30 reverse phase column (5 μm particle size, 250 mm × 4.6 mm I.D.) (YMC America, Inc., Allentown, Pennsylvania, U.S.A.). Prior to injecting each sample, the column was equilibrated with acetonitrile:methanol:water (86.4:10:3.6 [v/v], solvent A) for 10 min at a flow rate of 2.0 mL min^{-1}. After each sample was injected, solvent A was pumped for another 1.5 min (2 mL min^{-1}) followed by a linear gradient over the next 5

min to a 4:1 mixture of methanol and hexanes (solvent B). Solvent B was then pumped (2 mL min^{-1}) for the next 8.5 min until beta carotene eluted from the column. This method resulted in the separation of the major leaf carotenoids (V, A, Z, neoxanthin, lutein, and beta carotene) and leaf chlorophylls (a, b) in 15 min. The organic solvents used were HPLC grade supplied by either Pharmco-AAPER (Brookfield, Connecticut, U.S.A.) or Fisher Scientific (Pittsburgh, Pennsylvania, U.S.A.). The water used was 0.2 μm filtered Type I DI. Bulk xanthophyll cycle pool size (V+A+Z) was expressed on a per chlorophyll (a + b, Chl_{a+b}) basis (mmol V+A+Z mol Chl_{a+b}^{-1}) to allow cross-comparison to other studies, since xanthophyll cycle pool size per unit area is influenced by leaf thickness and possibly by extraction efficiency. The level of de-epoxidation of the xanthophyll cycle was expressed as the conversion state, i.e., as a fraction of the total xanthophyll cycle pool (Z+A)/(V+A+Z), because of the proposed involvement of A, along with Z, in energy dissipation (Gilmore and Yamamoto, 1993).

Foliar Gas Exchange

Gas exchange measurements were collected on the 54 *S. pulchra* leaves in 2014 that were not used in the dark–light transition experiment described above. In the lab, three prepped and calibrated gas exchange analyzers (LI-6400XT Portable Photosynthesis System, LI-COR Inc., Lincoln, Nebraska, U.S.A.) were used to obtain CO_2 fluxes of photosynthesis and respiration for leaves on each of the branch tips. Leaf selection was determined based on the size (closest to the cuvette size of 6 cm^2) and flatness of the leaf. The gas–exchange method from Heskel et al. (2013) was used to obtain light–response curves for each sample. To determine photosynthetic capacity, CO_2 assimilation was measured under ambient (400 ppm) CO_2 concentration at 26 PPFDs that fully encompass the range of irradiance in this growth environment (Heskel et al., 2012), with greater sampling density between 0 and 100 μmol m^{-2} s^{-1}. The cuvette block temperature was set to 20 °C. Using corrections provided in the LI-COR 6400 manual, we accounted for potential diffusion though the gasket and in and out of the cuvette. All measurements were collected at a relative humidity of approximately 40–60%. Following light–response curve measurements, leaf area was measured using a leaf area meter (LI-3100, LI-COR Inc., Lincoln, Nebraska, U.S.A.). Maximum light-saturated net photosynthetic rate (A_{max}) was estimated by fitting the data to a rectangular hyperbolic function (Excel Solver, Microsoft, Redmond, Washington, U.S.A.) (Heskel et al., 2013).

Analyses of Chlorophyll Fluorescence Emission

The level of thermal energy dissipation was quantified as NPQ of chlorophyll fluorescence using an FMS2 fluorometer (Hansatech Instruments, Kings Lynn, Norfolk, U.K.). Measurement of the maximal fluorescence emission (F_m) during exposure to a 0.8-s saturating pulse of light (>3000 μmol m^{-2} s^{-1}) generated by the instrument was collected at the end of dark acclimation periods. During illumination, maximal fluorescence (F_m') was measured during the saturation pulse after steady-state fluorescence had achieved steady state (~2–5 min). NPQ was calculated as $[(F_m/F_m') - 1]$ according to Bilger and Björkman (1990).

Statistical Analysis

Data were compared using analysis of variance (ANOVA) to identify statistically significant differences ($p < 0.05$) between xanthophyll cycle pool sizes, A_{max}, and NPQ (Figs. 2, 3, and 4) among light environments and species. As this study primarily focuses on the conversion states of the xanthophyll cycle of arctic species, an ANOVA was only conducted between annual crop species and 2014 *S. pulchra* (which had similar dark–light sampling procedures; i.e., Fig. 3).

RESULTS

Xanthophyll Cycle Pool Size and Photosynthetic Capacity

Xanthophyll cycle pool size (V+A+Z)/(Chl_{a+b}) increased with increasing sun exposure in *S. pulchra* (Fig. 2, parts a and b) and *B. nana* (Fig. 2, part c), although statistically significant differences were only observed between sun and shade *S. pulchra* leaves in 2014 and 2015. Means and standard deviations of 2014 *S. pulchra* (V+A+Z)/(Chl_{a+b}) pools were 86 ± 14.5, 108 ± 17.8, and 168 ± 24.3 mmol mol^{-1} for shade, mixed, and sun leaves, respectively. Means and standard deviations of the 2015 *S. pulchra* xanthophyll cycle pools (per Chl) were 78 ± 13.1, 101 ± 20.3, and 120 ± 26.9 mmol mol^{-1} (Fig. 2, part b), and for *B. nana* were 51 ± 13.2, 71 ± 16.5, and 85 ± 32.8 mmol mol^{-1} (Fig. 2, part c) for shade, mixed, and sun leaves, respectively. There was modest, not statistically significant, difference in the photosynthetic capacity (A_{max}) of these same *S. pulchra* leaves in 2014; photosynthetic capacities were 12.4 ± 1.88, 15.1 ± 3.09, and 15.2 ± 2.89 μmol m^{-2} s^{-1} for shade, mixed, and sun *S. pulchra* leaves, respectively.

Xanthophyll Cycle Pigment Conversion and Retention

Exposure to varying light intensities during the dark-light transition experiment in 2014 and the greenhouse experiment are shown for comparison in Figure 3. At dark to low, and low to medium light, a significant increase in (Z+A) in *T. aestivum* and *H. annuus* shade leaves was observed (Fig. 3, part a). Only a significant increase at the lowest light level was observed in shade leaves of *S. pulchra* (Fig. 3, part a). A similar pattern was observed with a greater conversion state in sun-grown plants, where a general stepwise increase in conversion to (Z+A) occurred with increasing PPFD, with statistically significant increases at low PPFD for *T. aestivum*; low, medium, and high PPFD for *H. annuus*; and low PPFD for *S. pulchra* (Fig. 3, part b). There was a statistically significant difference between sun and shade plants at all light levels in each species except for *T. aestivum* (Fig. 3, parts a and b).

There was a statistically significant difference at all light intensities between the *S. pulchra* and the annual crop species (*T. aestivum* and *H. annuus*), with high intensities of (Z+A) in the dark-acclimated *S. pulchra* (Fig. 3, parts a and b). Saturation of (Z+A) concentration at low-light intensities occurred at low-light levels for *S. pulchra*, with no apparent saturation in either annual crop species (*T. aestivum* and *H. annuus*) (Fig. 3, parts a and b). NPQ increased with light intensity across all species, but did not reach saturation at low-light intensities in *S. pulchra* (Fig. 3, parts c and d). Similarly, the absolute magnitude of NPQ in *S. pulchra* was generally higher than the greenhouse-grown crops, but only significantly when compared with sun-exposed leaves of *T. aestivum* and *H. annuus*.

In 2015, when the dark-light transition experiment consisted of only two light regimes, a significant increase in the concentrations of (Z+A)/(V+A+Z) was observed in both species across all three binned can-opy positions when dark-acclimated leaf samples were exposed to light (Fig. 4, parts a and b). A statistically significant difference in the concentrations of (Z+A)/(V+A+Z) was observed between the dark-acclimated sun and shade leaves from both *S. pulchra* and *B. nana*; no statistically significant differences were observed among the light-acclimated leaf samples within each species. Both *B. nana* and *S. pulchra* exhibited similar dark-acclimated antheraxanthin and zeaxanthin retention (i.e., no statistically significant difference), whereas (Z+A)/(V+A+Z) in *S. pulchra* (Fig. 4, part a) was higher than for *B. nana* for shade, mixed, and sun leaves (Fig. 4, part b).

In the 2015 dark-light transition experiment, we found no significant differences in NPQ regardless of light environment, though a slight decrease was observed from shade to sun leaf exposure in *S. pulchra* (Fig. 4, part c). The level of NPQ was significantly higher in *S. pulchra* than in *B. nana* (Fig. 4, parts c and d), coincident with greater xanthophyll cycle conversion states (Fig. 4, parts a and b), and pool sizes (Fig. 2, parts b and c) in *S. pulchra* than in *B. nana*.

DISCUSSION

Our findings, taken together, suggest substantial deployment of photoprotective thermal energy dissipation in two dominant arctic shrub species and thus support the hypothesis that the extreme environmental conditions and lengthy growing-season photoperiods of the Arctic lead to environmental stress—to which plants acclimate with high xanthophyll cycle pool sizes and conversion to Z+A. Xanthophyll cycle pool sizes of sun leaves of *S. pulchra* reported here fall on the far high end (near the 95th percentile) of the spectrum when compared to a recent meta-analysis on global drivers of pigment contents by Esteban et al. (2015, and their Fig. 3, part e); *B. nana* xanthophyll cycle pool
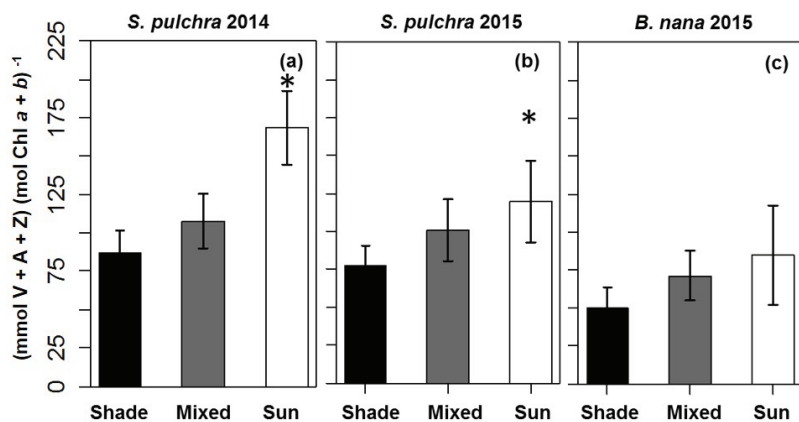

FIGURE 2. Xanthophyll cycle pool size ([mmol V + A + Z] [mol Chl a + b]$^{-1}$) in shade (leaf area index [LAI] > 1.0), mixed (1.0 > LAI > 0.25), and sun (LAI < 0.25) locations on *Salix pulchra* in (a) 2014 and (b) 2015, and *Betula nana* in (c) 2015. Data are means ± 1 standard deviation. Asterisk indicates significant difference between light environments.
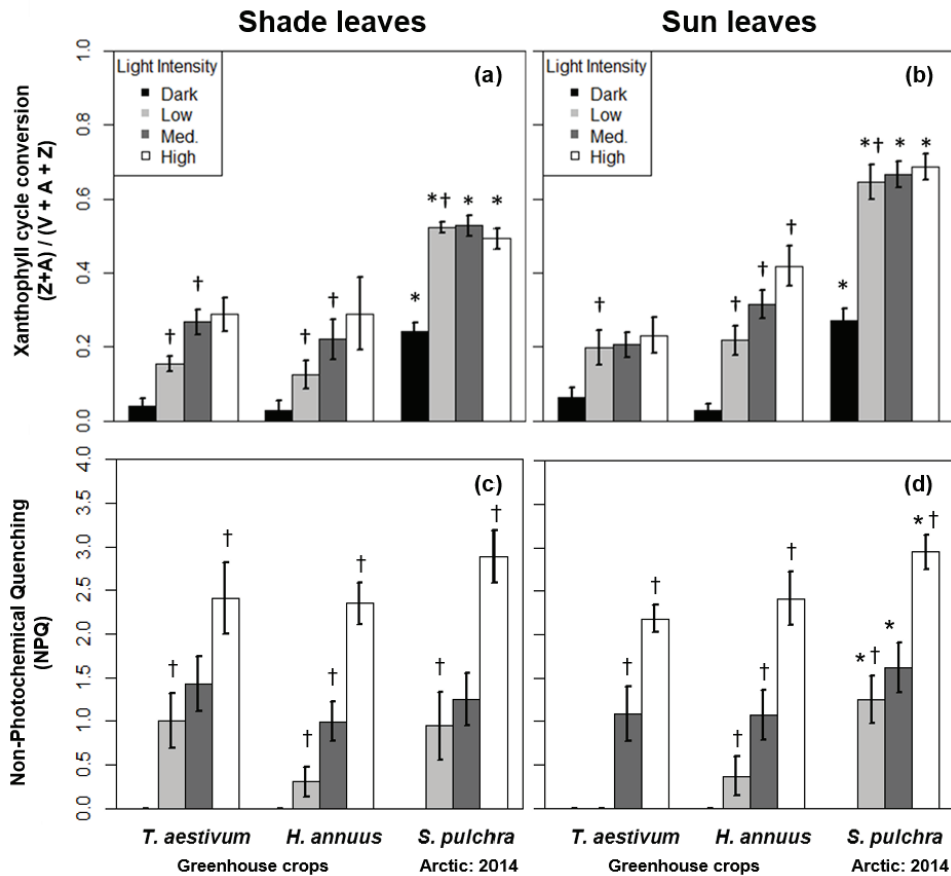
Shade leaves   **Sun leaves**

FIGURE 3. (a, b) Xanthophyll conversion states of greenhouse-grown *T. aestivum* and *H. annuus* plants compared to *S. pulchra* samples from 2014 under increasing light intensities in the laboratory. Plants were dark acclimated for >2 h and then placed under increasing light intensities ranging for low (PPFD <300 µmol m^{-2} s^{-1}), medium (800> PPFD > 300 µmol m^{-2} s^{-1}), and high light (PPFD > 800 µmol m^{-2} s^{-1}). (c, d) Non-photochemical quenching (NPQ) data are provided for shade and sun leaves, respectively, for each species. Asterisk indicates a significant difference using ANOVA between *S. pulchra* and other species, and † indicates significant differences within species from prior light regime.

sizes are near the 70th percentile. Although only moderately high xanthophyll cycle pools were observed in *B. nana*, high xanthophyll de-epoxidation and (Z+A) retention were observed in both *B. nana* and *S. pulchra*. When compared with unstressed, greenhouse-grown crops, both arctic shrub species exhibited greater dark-acclimated retention of Z+A, greater xanthophyll cycle conversion states at high light, saturation of xanthophyll cycle conversion state at lower light intensities, and increased NPQ.

Whereas this study reports the first xanthophyll cycle data on higher plants in the Arctic that we are aware of, previous studies on Antarctic mosses at 66°S (Lovelock and Robinson, 2002; Robinson et al., 2005), Antarctic angiosperms in maritime Antarctica (Bascu-ñan-Godoy et al., 2012) and subarctic pine forests at 66°N (Louis et al., 2005; Slot et al., 2005) also reported high xanthophyll cycle pool sizes and zeaxanthin retention, although in most cases not as high as those observed in *S. pulchra* in this study. Although it is difficult to compare xanthophyll interconversion rates among studies, our (Z+A) dark-retention data (Figs. 3 and 4) from arctic species are in line with similarly collected data from species growing in harsh environments as reported in the meta-analysis by Miguez et al. (2015,

and their Fig. 2, part b). Miguez et al. highlighted that studies where the mean annual winter temperature falls below zero degrees generally have (Z+A) retention rates greater than 0.5, as was observed in this study, which is notable considering data in this study were collected during the least stressful time of the growing season (Fig. 1). In a study similar to ours, xanthophyll cycle pools reported in *Abies koreana* collected from a similar growing environment (high alpine) during peak growing season are nearly half of those in Alaskan tundra *S. pulchra*, and up to 25% lower than those reported for *B. nana* (Oh et al., 2013). However, one should note that the experimental design and nature of the above studies are dissimilar enough from ours that direct comparison is difficult.

Xanthophyll cycle pool sizes similar and/or greater than what we report here for *S. pulchra* have been reported for evergreen species from more temperate climates; however, only when examined during the winter, when cold temperatures inhibit primary metabolism (Verhoeven et al., 1999, 2009; Adams et al., 2002, 2004; Verhoeven, 2014). For example, in many overwintering evergreens, large xanthophyll cycle pool sizes and high levels of nocturnal Z+A retention are accompanied by a substantial downregulation in photosynthetic capac-
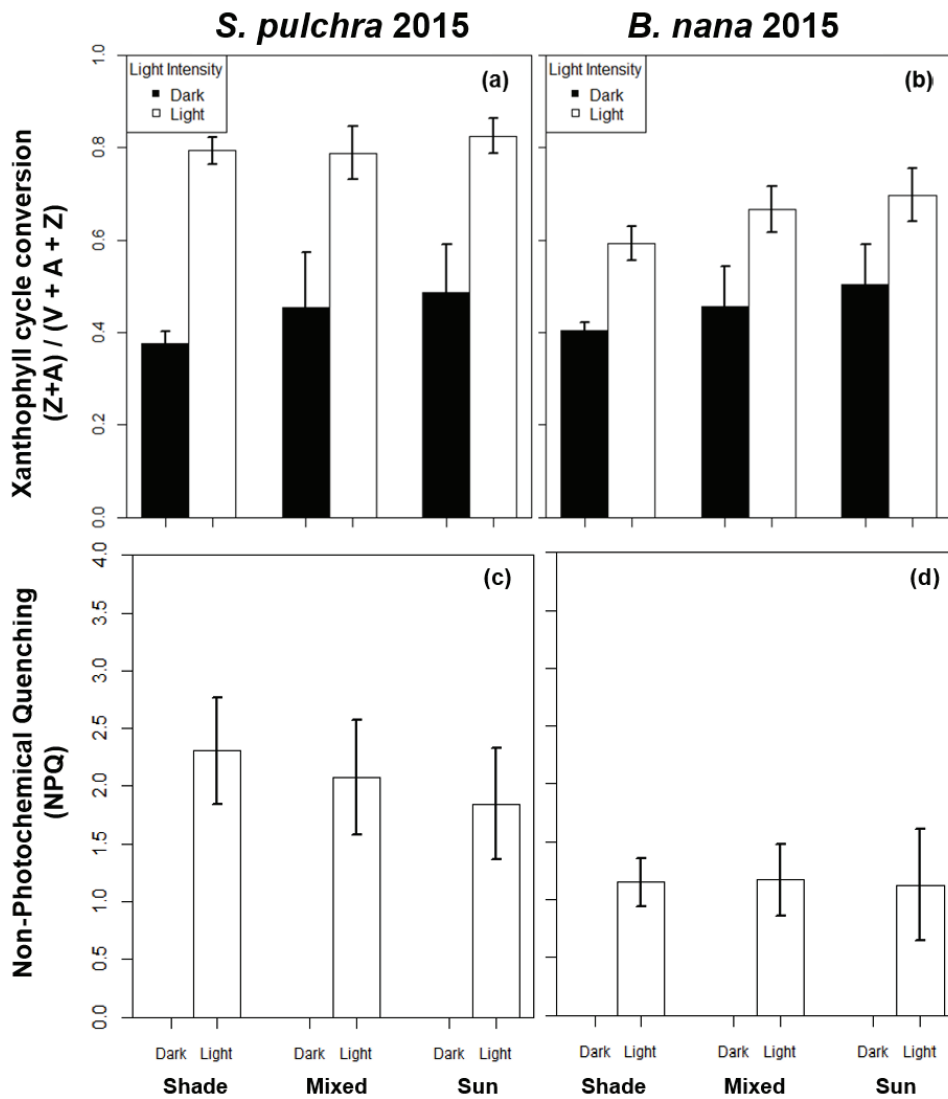
S. pulchra 2015   B. nana 2015

FIGURE 4. Xanthophyll cycle conversion states for the 2015 dark-light transition experiment for sun-exposed, mixed sun/shade, and shade leaves of (a) *S. pulchra* and (b) *B. nana*. Dark bars represent dark-acclimated leaf samples, and light bars represent light-acclimated leaf samples under PPFD of ~1200 μmol m^{-2} s^{-1}. (c, d) NPQ data are provided for shade and sun leaves, respectively, for each species.

ity during winter, a season when photosynthetic activity would otherwise be greatly inhibited by low temperatures (Adams et al., 2002, 2004; Verhoeven 2014). Thus, so-called "chronic photoinhibition," which is commonly observed in overwintering evergreens, may represent the manifestations of acclimation to winter conditions and not the result of light-mediated damage to the photosynthetic apparatus, per se (Adams et al., 2004). Sustained retention of Z+A during the peak of the growing season, as we report here, is less commonly observed. However, it has been reported in spinach grown at high irradiance and low nitrogen availability (Verhoeven et al., 1997). Close et al. (2003) reported that, in *Eucalyptus nitens* grown in shaded and fully sunlit conditions, low soil nitrogen availability intensified decreases in F_v/F_m, which can be an indicator of sustained thermal energy dissipation. Thus, low nutrient availability typical of arctic soils (Nadelhoffer et al., 1990; Hobbie et al., 2002) might contribute to high growing-season Z+A reten-

tion in arctic shrubs. To enable a better understanding of the seasonality of both xanthophyll cycle activity and its relationship to photosynthesis, future studies should consider the seasonal dynamics of these processes in the Arctic.

The difference in xanthophyll cycle pool size between *S. pulchra* and *B. nana* may be explained by species differences in photosynthetic activity. CO_2 assimilation rates of *B. nana* leaves reported in Heskel et al. (2012, 2013) are modestly higher, ~15–25 μmol m^{-2} s^{-1}, than those we report here for *S. pulchra* in 2014, albeit under different sampling conditions. Similarly, Prager et al. (2017) also reported that at the canopy scale, when species composition becomes more dominated by *B. nana* under increased nutrient fertilization, gross primary production increases significantly. These findings support earlier work by Bret-Harte et al. (2001), who suggested that *B. nana* may dominate altered environments more than *S. pulchra* because of increased developmental

plasticity. In addition, unpublished data from Formica, Griffin, and Boelman (2013) indicate that *B. nana* leaves maintain higher rates of maximum electron transport and greater percent nitrogen when compared to those of *S. pulchra* at this same field location. Combined, this could suggest greater photochemical LUE in *B. nana* as compared with *S. pulchra*, thereby leading to a comparatively lesser need for photoprotection in *B. nana*. This is consistent with the observed higher levels of NPQ in *S. pulchra* when compared with *B. nana* under similar conditions.

There is little debate that fluctuations in xanthophyll cycle pool size and conversion state are robust indicators of photoprotection, canopy LUE, and exposure to stress (Demmig-Adams and Adams, 2006). A global compilation of baseline xanthophyll cycle activity over a wide range of biomes could provide researchers with new techniques to monitor environmental change (similar to Esteban et al., 2015). Given our current data set, we cannot identify the exact stressor(s) or biochemical mechanism(s) contributing to enhanced levels of photoprotection in the Arctic, but the xanthophyll cycle pool sizes reported here could serve as a baseline for improving interpretation of plant responses to environmental conditions. This work could have implications for understanding the ecological significance of photoprotective mechanisms as a response to light and growing conditions, and in particular, how two prominent arctic shrub species may respond to increased warming in the northern high latitudes (as has been reported in detail in Myers-Smith et al., 2011).

In summary, this data set serves as an end-member on the global spectrum of reported xanthophyll cycle pool size and activity (Esteban et al., 2015), and data from this study in conjunction with spectral information might improve the extrapolation of xanthophyll cycle constituents and infer within-canopy partitioning (Boelman et al., 2016). Within this framework, remote sensing techniques that quantify xanthophyll cycle pool sizes using a 'dark-state' photochemical reflectance index, PRI_o, (Gamon and Berry, 2012; Magney et al., 2016b) could provide a rapid indicator of how arctic plants are, or are not, responding to changing environmental conditions (Magney et al., 2016a, Boelman et al., 2016). As PRI becomes an increasingly popular tool, it is important to continue to improve our understanding of the complex patterns and controls on xanthophyll cycle pool size and interconversion before we can be confident that remotely sensed data can explain variability in photosynthetic LUE across large spatial and temporal scales (i.e., Hilker et al., 2012; Hall et al., 2012).

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