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Research

In situ photosynthetic freezing tolerance for plants exposed to a global warming manipulation in the Rocky Mountains, Colorado, USA

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Summary

• This research tested the hypothesis that experimental infrared warming will reduce photosynthesis for the evergreen shrub *Artemisia tridentata* and the subalpine, herbaceous *Erythronium grandiflorum* exposed to an *in situ* experimental freezing event during the spring snowmelt period.

• Photosynthetic tolerance of freezing was measured for plants growing under infrared (IR) warming at 3050 m in the Rocky Mountains, Colorado, USA. *In situ* freezing was imposed using cold nitrogen gas (from a pressurized container of liquid nitrogen) passed through a heat exchanger placed on top of stems and leaves.

• Plant water potential, photosynthetic CO_2 assimilation, and stomatal conductance to water vapor were higher for both species on IR-warmed compared with control plots. For *A. tridentata*, IR warming caused enhanced tolerance of *in situ* freezing temperatures. There was no difference in freezing tolerance for *E. grandiflorum* on control vs IR plots.

• These results suggest that some species will not be negatively affected by freezing, whereas others may exhibit enhanced tolerance of subzero air temperatures, under a future warmer climate in which snowmelt occurs earlier in the year.

Key words: *Artemisia tridentata* (sagebrush), chlorophyll *a* fluorescence, CO_2 assimilation, *Erythronium grandiflorum*, freezing, glacier lily, stomatal conductance, water potential.

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Introduction

It is likely that increased levels of CO_2 and other greenhouse gases will result in a 1.4°C to 5.8°C increase in global air temperatures by the middle of this century compared to pre-industrial times (Schneider, 1989; Watson *et al.*, 1990; Kacholia & Reck, 1997; Field *et al.*, 1999). Such warming will alter air and soil temperatures, resulting in dramatic effects on ecosystems (Peters & Lovejoy, 1992). These effects are expected to include altered establishment, survival, and reproduction of plants. At higher levels of organization, atmospheric warming will enhance extinction or geographic migration of certain species, change community composition, and affect rates of ecosystem processes (Peters & Lovejoy, 1992; Bachelet *et al.*, 2003).

Freezing temperatures are an important filter on plant recruitment, survival, productivity, and geographic distribution (Grace, 1987; Sakai & Larcher, 1987; Woodward & Williams, 1987; Nobel, 1988; Woodward, 1992; Larcher, 1995; Pockman & Sperry, 1997; Inouye, 2000). Episodic subzero air temperature events represent transient, nonsteady-state conditions, and can have dramatic impacts on plants at cellular to ecosystem scales. Episodic, low-temperature freezing events are more likely than average minimum air temperatures to cause plant mortality and to impact geographic distributions (Nobel, 1988; Loik & Nobel, 1993; Larcher, 1995;

Pockman & Sperry, 1997; Smith *et al.*, 1997). Many models indicate that freezing temperatures are critically important in predicting plant responses to global change (Koch & Mooney, 1996; Körner & Bazzaz, 1996; Walker & Steffen, 1996; Luo & Mooney, 1999). Minimum nighttime air temperatures are warming faster than daytime maximal temperatures (at least in the Northern Hemisphere; Alward *et al.*, 1999), yet despite this nocturnal warming, the 1.5°C–4.8°C predicted increase in average air temperatures (Schneider, 1989; Houghton & Bolin, 1992; Watson *et al.*, 1996; Field *et al.*, 1999) is not likely to eliminate the occurrence of episodic freezing events for many regions of the United States. For example, the summers of 1998 and 2001 were some of the hottest since the start of weather record-keeping, yet episodic freezing episodes in each subsequent winter caused a combined estimated $1.2 billion in damage to agricultural crops in California alone (California Department of Agriculture, 1999).

In situ experiments on the effects of warming temperatures on plant and ecosystem processes have employed electric soil heating cables, passive shelters, and as in the experiment reported here, overhead infrared heaters (Shaver *et al.*, 2000). This research examined the effects of IR warming that simulates conditions associated with a warmer atmosphere on the ability of photosynthesis to tolerate freezing temperatures. The research was conducted at the ecotone between Great Basin Desert sagebrush scrub and subalpine meadow communities of the Rocky Mountains, Colorado, USA, where overhead IR heaters have continually simulated atmospheric forcing of the future (Harte *et al.*, 1995). The IR-treatment has been shown to advance the timing of snowmelt by 1–2 wk, increase soil temperatures, and decrease soil water content (Harte & Shaw, 1995; Harte *et al.*, 1995; Loik & Harte, 1996, 1997; Loik *et al.*, 2000b; Shaw *et al.*, 2000). These effects on microclimate lead to lowered plant water potential, altered high-temperature tolerance, reduced photosynthetic CO_2 uptake, and a down-regulation of Photosystem II in summer for subalpine, herbaceous geophyte species (Loik & Harte, 1996, 1997; Loik *et al.*, 2000b; Shaw *et al.*, 2000). Despite these impacts of IR warming on summertime physiology, little is known about the potential effects of warming on boundary layer microclimate and photosynthetic physiology in winter. Snow provides insulation for overwintering plant parts from episodic freezing temperatures; earlier snowmelt coupled with changes in soil surface temperature and water content may alter the timing of development, such as emergence from underground overwintering structures or new leaf production. The occurrence of low-temperature episodes could be independent of snowmelt timing (i.e. the frequency and intensity of low-temperature episodes are based on high-pressure systems driven by the jet streams, and are thus somewhat stochastic in time.) Changes in snowmelt timing, altered PPFD (Photosynthentic Photon Flux Density; 400–700 nm) near the soil surface, increased soil temperature, or altered soil water content caused by IR warming may cause plants to come out of dormancy earlier in the season. Because physiologically active plants are more vulnerable than dormant plants to a transient low-temperature event (Larcher, 1995), earlier acclimation to warmer temperatures and onset of photosynthetic activity under the IR warming treatment may make plants more susceptible to damage that reduces overall functional leaf surface area and productivity.

The overall goal of this research was to assess the effect of simulated global warming on the ability of photosynthesis to tolerate a transient freezing episode during the late-winter snowmelt period. We tested the following hypotheses: water potential and photosynthesis are higher for plants on IR-heated plots compared to nonwarmed control plots, reflecting an earlier onset of physiological activity caused by IR treatment during the late winter when air temperatures are low but soil moisture is high because of snow melt; freezing temperatures in heated plots lead to a greater reduction in photosynthesis compared to plants on adjacent nonheated control plots, reflecting a greater vulnerability of photosynthetically active plants to episodic freezing; and the effects of IR warming on photosynthesis is greater for the lower-elevation species *Artemisia tridentata* in comparison to the subalpine geophyte *Erythronium grandiflorum*. These two species were chosen for comparison because *E. grandiflorum* is an ephemeral species of high elevations in the Rocky Mountains and Sierra Nevada that emerges, flowers, fruits, then senesces over a 2 wk period during snowmelt, and may be especially vulnerable to changes in the timing of snowmelt and episodic freezing. On the other hand, *A. tridentata* is a geographically widespread species and exhibits considerable ability to tolerate cold, drought, and high-temperatures (Smith *et al.*, 1997). Photosynthesis – measured as CO_2 uptake, stomatal conductance to water vapor, and quantum efficiency of Photosystem II – was compared for plants of both species on control and IR heated plots. Measurements were conducted before, during, and after an *in situ* experimentally imposed episodic freezing event.

Materials and Methods

Study site

This study was conducted at the Rocky Mountain Biological Laboratory near Crested Butte, Colorado, USA (lat. 38°53′ N, long. 107°02′ W, elev. 2920 m), in a meadow that contains elements of the intermountain Great Basin Desert shrub and Rocky Mountain subalpine floras. Climatic conditions for the period 1961–1990 (Fig. 1) indicate that minimum air temperatures average approx. −20°C from December through February, yet extreme minimum temperatures can reach −40°C. During May (the time of year of this research), nighttime minimum air temperatures average −7°C and extreme minimum temperatures can reach −15°C (Fig. 1); minimum air temperatures to −30°C occur about once every 10 yr. Average maximum temperatures are below

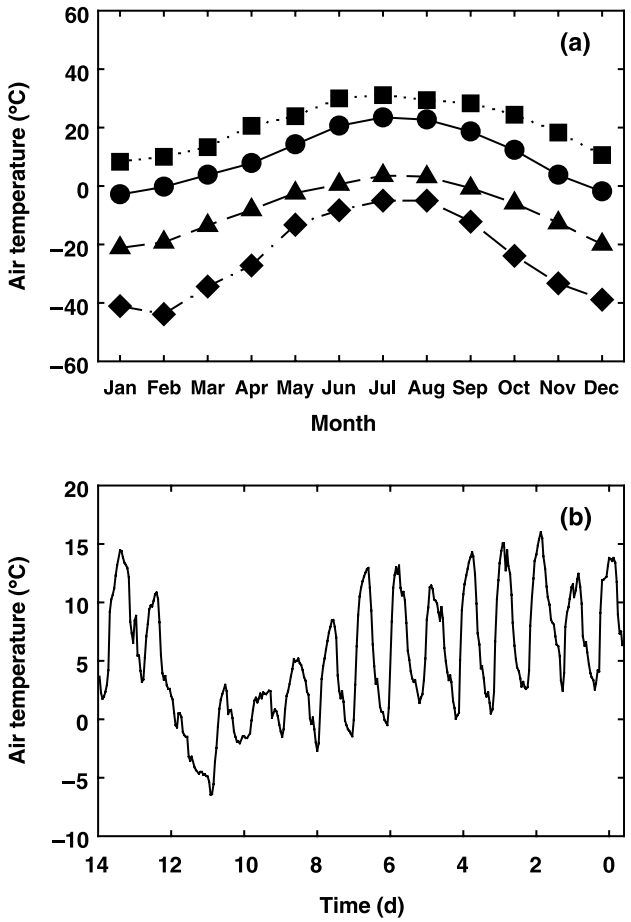

Fig. 1 Monthly temperature for Crested Butte, Colorado (a) averaged for 1961–90 (National Climatic Data Center http://www.ncdc.noaa.gov/oa/ncdc.html). Data are monthly average extreme maximum air temperatures (squares), average maximum air temperatures (circles), average minimum air temperatures (triangles), and extreme minimum air temperatures (diamonds). (b) Daily course of air temperatures for the 2-wk period before experiments. Data were measured approximately 100 m from the study site.

freezing in winter and are approx. 20°C in summer. For the 2-wk period before experiments, the minimum air temperature was −7°C (Fig. 1). Snow is generally present from December through March, but melting can occur as late as June depending on the amount of snowfall in the previous winter, and snowfall has been recorded for every day of the year. Snow represents approx. 80% of the annual precipitation input, with summer monsoon rainfall making up the balance. The Hadley Climate Model (HadCM2) predicts that this region of Colorado will warm by approx. 2°C in spring and 3°C in summer by 2100. Spring and autumn precipitation are predicted to increase by approx. 10% and winter snowfall is predicted to increase by 20%–70% (EPA, 1997).

Infrared warming manipulation

Ten 3 × 10 m plots were demarked in 1990 with their long axis on the east-facing slope of a ridge to minimize water runoff from higher elevations during snowmelt. The ridge is relatively xeric at the top and grades into a moist willow swale at the bottom, with an elevational difference of approx. 1 m. Three heaters (1.6 m in length and 12 cm wide; Kalglo, Inc., Lehigh, PA, USA) were suspended over alternating plots in 1991 using a system of towers and cables, and set to enhance the downward infrared radiation by 22 Wm^{-2} in 1993. This flux was chosen because it is comparable to the level predicted to occur for a doubling of CO_2, and includes the flux associated with feedback effects, such as an increased amount of atmospheric water vapor (Harte *et al.*, 1995). The heaters give off no radiation in the visible range (400–700 nm), and in the far red (700–800 nm) the flux equals approximately 10^{-6} of solar input. Custom reflectors above the heating element ensure a uniform heating of the plots, as determined using an infrared radiometer. Soil moisture and temperature were measured with thermocouples and gypsum resistance blocks at 5, 12 and 25 cm depth (Harte *et al.*, 1995). Access to the plants and soil of each plot is by way of a series of wooden walkways suspended over each plot in order to minimize soil compaction. For further details of the experimental manipulation, see Harte *et al.* (1995).

Plant material

We compared the impacts of IR warming on photosynthetic freezing tolerance for *Artemisia tridentata* (Asteraceae) Nutt. and *Erythronium grandiflorum* (Liliaceae) Pürsh as model evergreen and early season ephemeral herbaceous geophyte species, respectively. One plant of each species was randomly chosen from each plot to be used for experimental measurements, resulting in a sample size of $n = 5$ plants on heated plots and $n = 5$ plants on control plots for both species. This sample size was chosen in part because of the amount of liquid nitrogen that could be transported to the site. For *A. tridentata*, plants averaged 35 ± 4 cm in height and 41 ± 6 cm in diameter; plants on heated plots were producing silver-gray-green new leaves in contrast to plants on control plots. Plants of *E. grandiflorum* produce two leaves before producing flower buds. All plants used were at the two-leaf stage and all plants on control plots had unopened flower buds, whereas four of the five plants on heated plots had open flowers.

Measurement strategy

Comparisons of photosynthetic responses to freezing for plants under IR heaters and on control plots were made before, during, and after an imposed *in situ* freezing event.

The first set of experiments were conducted before the imposed freezing event to test the hypothesis that the heaters cause enhanced levels of photosynthesis during the late winter when air temperatures are low but soil moisture is high (as a

result of snow melt.) Photosynthetic assimilation, stomatal conductance to water vapor, and quantum yield of Photosystem II (PSII) were measured for $n = 5$ plants of both species on control and heated plots. Measurements were conducted every 2 h from 0700 to 1800 h. Soil and plant water potential were measured the same day at 0730 h.

The second set of experiments were conducted during an imposed *in situ* freezing event to test the hypothesis that photosynthesis for plants under the IR heaters was less freezing tolerant (i.e. photosynthetic processes had de-acclimated because of the warming) compared to plants on control, nonheated plots. Individual plants were exposed to freezing air temperatures as described below. Simultaneous, repeated measurements of leaf temperatures and chlorophyll *a* fluorescence from Photosystem II were used to assess the real-time responses of leaves to the imposed freezing treatment.

The third set of experiments were conducted 2 d later to test the hypothesis that the impacts of freezing temperatures would cause a greater decrease in photosynthesis for plants on heated plots (i.e. indicating a greater degree of freezing sensitivity because of acclimation under the heaters) compared to plants on control, nonheated plots. Instantaneous measurements of photosynthetic assimilation, stomatal conductance to water vapor, and quantum yield of Photosystem II (PSII) were measured for plants of both species on control and heated plots to assess the postfreezing treatment effects.

Water potential

Plant water potential measurements were made for 10-cm-long stem segments of *Artemisia tridentata* that were removed with a sharp knife, re-cut with a razor blade, and then rapidly inserted into a pressure chamber. Shoot water potential was measured between 0800 and 1000 h with a PMS Scientific (Corvallis, OR, USA) Scholander-type pressure chamber. For *Erythronium grandiflorum*, for which leaves could not be inserted into the pressure chamber, water potential was measured using a psychrometric technique (Loik & Harte, 1997). Samples (one per plant) were removed from the middle of leaf blades using a number 9 cork borer (1.3 cm dia) and immediately sealed into the sample chambers of a Decagon SC-10 thermocouple psychrometer (Decagon Devices, Pullman, WA, USA). The water content of the leaf samples and the water vapor pressure of the headspace above the samples were allowed to equilibrate for 3 h. The voltage across the thermocouple was then measured with a Decagon NT-3 nanovoltmeter, and water potential was calculated based on the van't Hoff relation and NaCl calibration samples within the SC-10 (Loik & Nobel, 1991). These two methods for measuring water potential have been compared in the past for *Artemisia tridentata* stems ranging between −0.15MPa and −4.10 MPa (Loik & Harte, 1997), and show close agreement ($r^2 = 0.96$, $P < 0.01$, $n = 10$).

Photosynthetic gas exchange

Photosynthetic gas exchange was compared for *Artemisia tridentata* and *Erythronium grandiflorum* in terms of: diurnal patterns of CO_2 assimilation and stomatal conductance to water vapor over a typical day during the snowmelt period at this site; and responses of assimilation following an experimentally imposed freezing event (described below).

All measurements were conducted for five, randomly selected plants of each species in both the control and heated plots. For diurnal measurements, leaves were marked so that the same leaves were used throughout the day. For *A. tridentata*, three leaves were used and carefully arranged to avoid overlapping; for *E. grandiflorum* one leaf per plant was used.

CO_2 assimilation, stomatal conductance to water vapor, and transpiration were measured using a LI-6400 open-mode portable photosynthesis system (Li-Cor, Inc., Lincoln, Nebraska, USA). Vapor pressure deficit within the chamber was maintained at premeasurement ambient levels by passing a portion of the incoming airstream through 10–20 mesh $CaSO_4$. The CO_2 concentration within the leaf measurement chamber was maintained at a constant level (360 μmol mol^{-1}) by scrubbing the incoming airstream with soda lime, and the subsequent addition of a precise amount of CO_2 via injection from an external cartridge. Leaf temperatures were recorded with a copper-constantan thermocouple appressed to the abaxial surface of the leaf within the cuvette. Leaf area for *A. tridentata* measurements was determined by tracing the leaf onto paper and subsequent comparison of the image to a known area and weight of paper; for *E. grandiflorum*, the 2 cm × 3 cm area of the cuvette was filled by the leaf.

Chlorophyll *a* fluorescence

Measurements of chlorophyll fluorescence were obtained with a pulse-modulated fluorimeter (Model FMS1, Hansatech, Kings Lynn, UK). A custom script was written to automatically record F_O, F_M, F'_M, and F_S (van Kooten & Snel, 1990). Leaves were dark adapted for at least 15 min using leaf clips designed for use with the FMS1. Following dark adaptation, the efficiency of excitation transfer was measured as $F_V/F_M = (F_M - F_O)/F_M$, using a saturating beam of 14 256 μmol m^{-2} s^{-1} for 0.7 s. During the *in situ* freezing treatments, F_V/F_M was measured at five minute intervals. Based on preliminary experiments in the absence of freezing, this frequency did not result in a reduction of F_V/F_M. The quantum efficiency of Photosystem II (ΦPSII) 2 d after *in situ* freezing was calculated as $(F'_M - F_S)/F'_M$.

ICE treatment

The *In situ* Cold Experiment (ICE) treatment in the field was conducted using a liquid nitrogen source, a heat exchanger, and an instrument cluster. For *A. tridentata*, stems containing

at least three terminal leaves per target plant were enclosed in a cylindrical styrofoam chamber (25 cm dia × 30 cm deep). Leaves were covered with a Hansatech dark-adapting leaf clip for at least 15 min before the onset of the freezing treatment. For *E. grandiflorum*, the entire plant was enclosed in the chamber, owing to the small stature of this species. The fluorescence probe was attached to the leaf clip, and a 0.5 mm dia copper constantan thermocouple (Type T, 16 gauge) was held to the underside of the leaf by the leaf clip. Leaf and air temperatures were monitored with a digital microprocessor thermometer (Omega Model HH21, Omega Engineering, Stamford, CT). Liquid nitrogen was stored to pressurize overnight in a 70 L Dewar flask to 85 kPa. Air temperature adjacent to leaves and stems was lowered by adjusting a valve on the Dewar to vent cold nitrogen vapor through a coiled copper tubing heat exchanger (10 coils, 25 cm dia × 20 cm tall) inside the styrofoam chamber. The ICE episodes were conducted at a rate comparable to air temperature changes measured at the site (4°C h^{-1}); air and leaf temperatures were continually decreased, as would occur during a natural episodic freezing event. Temperature inside the styrofoam chamber was started at ambient air temperature (*c.* 10°C during experiments) and adjusted to −30°C over 10 h (extreme minimum air temperatures in May reach −30°C for this site about once every 10 yr; Western Regional Climate Center http://www.wrcc.dri.edu). The freezing program was the same for all plants. Real-time measurements of chlorophyll *a* fluorescence were obtained *in situ* with a Hansatech FMS1 pulse-modulated fluorometer (van Kooten & Snel, 1990). The freezing treatment was performed with the leaves in darkness, with periodic exposure to weak excitation light and saturating beams for calculation of F_V/F_M. The fluorometer recorded F_V/F_M during the freezing event as an indicator of low-temperature stress within PSII (Loik & Harte, 1996; Huxman *et al.*, 1998; Hamerlynck *et al.*, 2000; Loik *et al.*, 2000a).

Recovery following ICE

The ability of leaves to recover from the ICE treatment was assessed via subsequent measurements of the functional recovery of Photosystem II assessed as F_O, F_M, F'_M, and F_S in order to calculate PSII efficiency under a down-regulated energy dissipation state (as F_V/F_M for dark-adapted leaves) and to calculate PSII efficiency (as ΦPSII) under ambient light levels, which has been shown to be sensitive to global change treatments during winter (Hymus *et al.*, 1999; Roden *et al.*, 1999). Following *in situ* freezing treatment, leaves were left to recover overnight, then were exposed to ambient light averaging 1000 μmol m^{-2} s^{-1} over the next 2 d.

Recovery was also assessed as the ability of leaves that had been freeze-treated during ICE to conduct photosynthetic CO_2 assimilation. Gas exchange measurements were conducted with the Li-Cor LI-6400 programmable gas exchange system as described above. CO_2 uptake measurements were conducted under ambient PPFD; VPD and temperature inside the chamber were set to track ambient conditions based on energy balance. Photosynthetic recovery following freezing were characterized in terms of net assimilation (A), and stomatal conductance to water vapor (g_s).

Statistical analyses

Repeated measures ANOVA was used to measure the diurnal patterns of CO_2 assimilation and stomatal conductance before the freezing treatment for plants on control and IR-heated plots. Water potential for plants on control and heated plots was compared using an unpaired *t*-test. Linear regression was used to assess the relationship between assimilation and stomatal conductance for plants on control vs heated plots. An unpaired *t*-test was used to test for statistical differences in freezing tolerance (LT_{50}, the half-maximal reduction in PSII chlorophyll fluorescence). The potential for recovery of photosynthesis was assessed as assimilation, stomatal conductance, and ΦPSII using separate two-way ANOVA for each measurement for plants exposed to freezing compared to nonfrozen plants, and to plants frozen or not on control vs heated plots. Throughout, means and standard errors are reported and $P < 0.05$ is considered significant. Statistical tests were conducted using StatView vs 5.0 (SAS Institute, Inc., Cary, NC).

Results

For the 2 wk period before the onset of physiological measurements, soil temperature in the upper zone (the primary region where *Artemisia tridentata* occurs) of the heated plots was higher than in the control plots, however, the difference was not significant ($t = 1.898$, $P = 0.0698$). In the lower zone where *Erythronium grandiflorum* mainly occurs, soil temperature was 7.3 ± 4.1°C on heated plots and 3.9 ± 4.7°C on control plots ($t = 1.963$, $P = 0.0613$). Soil moisture was significantly higher for the upper zone of the plots ($t = 2.523$, $P = 0.0187$). For the lower zone, soil moisture averaged 11%–13%, and there was no significant difference caused by the IR treatment.

Leaf temperatures at 0700 h at the beginning of the experimental period averaged 10.2 ± 2.0°C for *A. tridentata* and 8.6 ± 4.6°C for *E. grandiflorum* and leaf temperatures did not significantly differ for either species on control compared to heated plots. Plant water potential (Ψ) averaged −3.14 ± 0.56 MPa for *A. tridentata* on control plots, and −2.26 ± 0.32 MPa on heated plots ($t = 3.038$, $P = 0.016$). For *E. grandiflorum*, Ψ averaged −1.19 ± 0.9 MPa on control plots and −1.47 ± 0.08 MPa on heated plots ($t = 5.239$, $P = 0.001$).

For plants measured before the *in situ* freezing treatment, photosynthetic CO_2 assimilation (A) was higher for *A. tridentata*

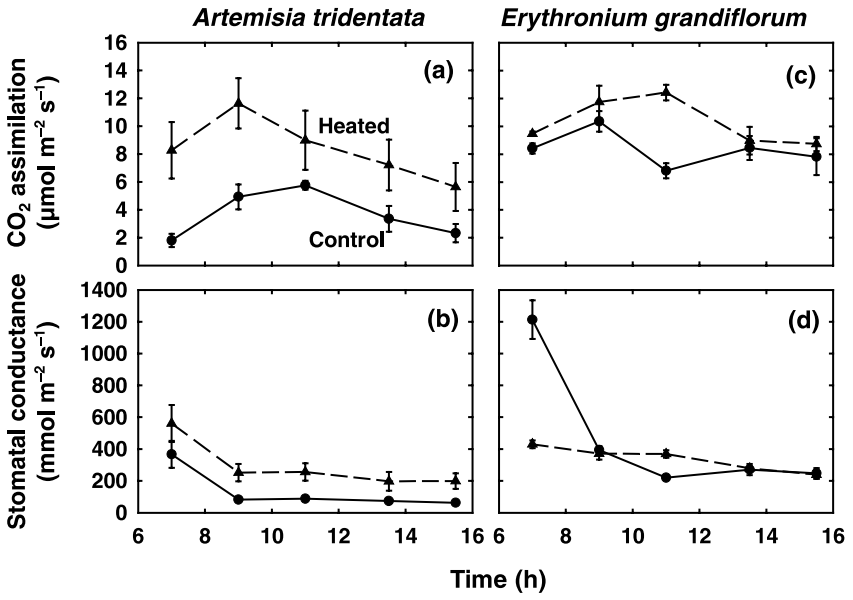

Fig. 2 Photosynthetic CO_2 assimilation (a, c), and stomatal conductance to water vapor (b, d), for *Artemisia tridentata* (a, b) and *Erythronium grandiflorum* (c, d) on control (circle) and heated (triangle) plots. Data are means⁺-standard errors for $n = 5$ plants on each of control and heated plots.

on heated plots compared to plants on control, nonheated plots (Fig. 2a; repeated measures ANOVA for control vs heated plots: $F = 104.2$, $P < 0.001$). Maximal rates of A occurred at 09.00 h for plants on heated plots compared to 11.00 h for plants on control plots (Repeated Measures ANOVA for time: $F = 3.24$, $P = 0.0155$). Stomatal conductance to water vapor (g_s) was maximal early in the morning for plants on both heated and control plots (Fig. 2b), and was higher for plants on heated compared to control plots (Repeated Measures ANOVA: $F = 877$, $P < 0.001$). For *E. grandiflorum*, pronounced differences in assimilation occurred at 11.00 h and stomatal conductance was only different at the beginning of the measurements. In the overall repeated measures statistical model, A was significantly higher for plants on heated compared to control plots (Fig. 2c; $F = 553$, $P < 0.001$), and there was a slight offset in the timing of maximal CO_2 uptake. Plants on heated plots had a higher g_s compared to plants on control plots (Fig. 2d; $F = 341$, $P < 0.001$), primarily because of greater stomatal opening on heated plots early in the morning.

Before the imposed freezing treatment, the relationship between A and g_s was higher for *A. tridentata* on control compared to heated plots (Fig. 3A). However, the slope of A vs g_s was lower and the range of values was less for plants on control vs heated plots, indicating an overall reduced level of photosynthetic gas exchange for control compared to heated plants. For *E. grandiflorum*, the slope of A vs g_s was slightly higher on heated plots, and assimilation rates were higher on heated compared to control plots, but the range of stomatal conductance values were roughly the same (Fig. 3b).

Leaf temperatures for both species tracked air temperatures within the ICE chamber (Fig. 4a); both species exhibited a freezing exotherm between temperatures of 0 and −10°C, and *A. tridentata* had a secondary exotherm around −25 to

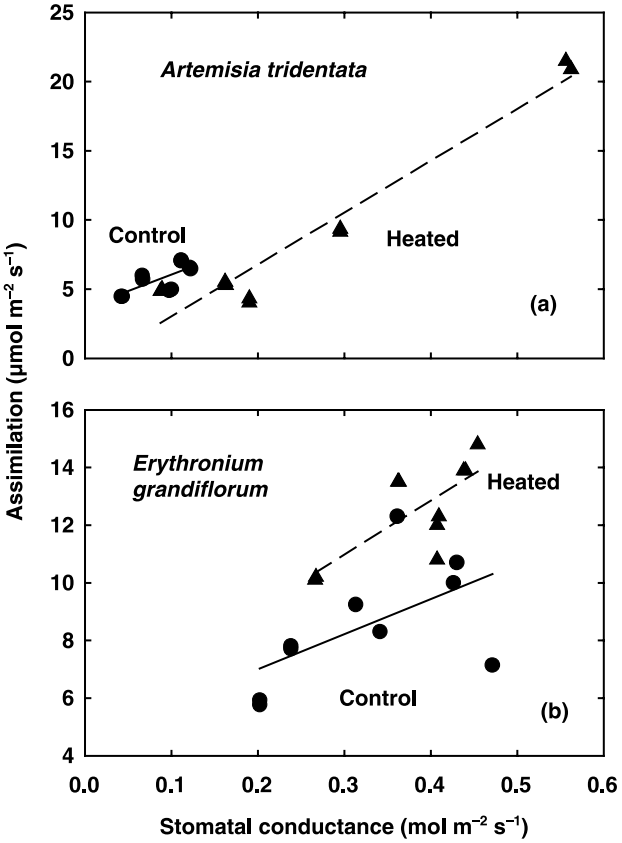

Fig. 3 Photosynthetic CO_2 assimilation as a function of stomatal conductance to water vapor for plants on control (circles) and heated (triangles) plots for *Artemisia tridentata* (a) and *Erythronium grandiflorum* (b). For *A. tridentata*, the relationship is $A = 24(g_s) + 3.65$, $r^2 = 0.53$ ($F = 9.125$, $P = 0.0165$) on control plots and $A = 38(g_s) - 0.71$, $r^2 = 0.94$ ($F = 118$, $P < 0.001$). For *E. grandiflorum*, the relationship is $A = 12(g_s) + 4.57$, $r^2 = 0.34$ ($F = 4.09$, $P = 0.77$) on control plots, and $A = 19(g_s) + 5.4$, $r^2 = 0.57$ ($F = 10.4$, $P = 0.012$)

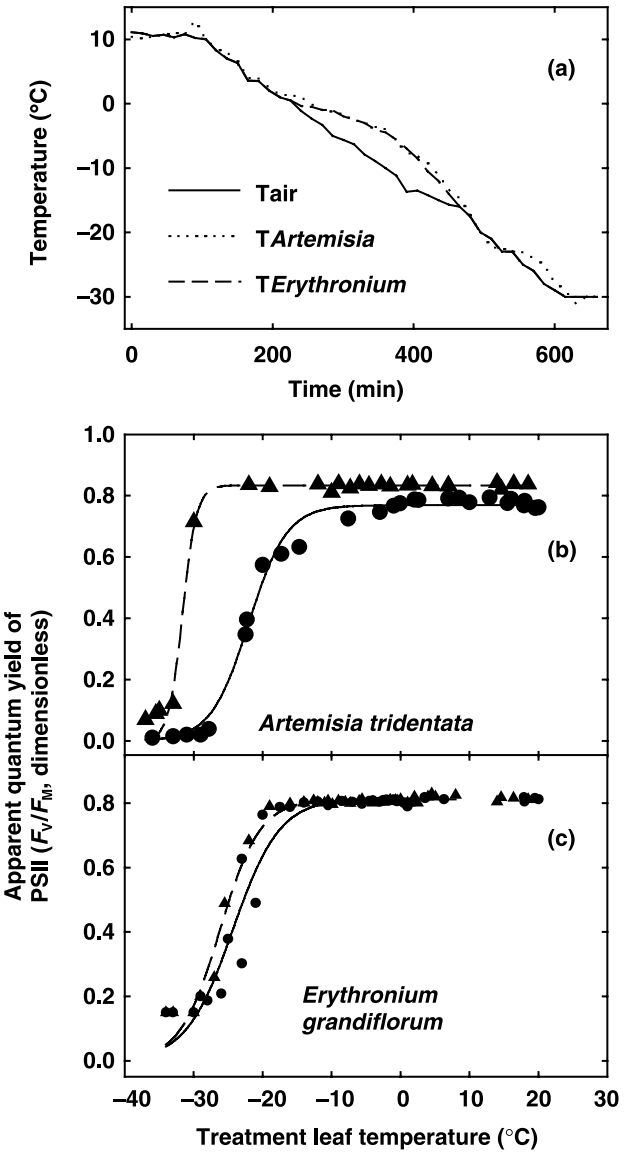

Fig. 4 Air and leaf temperatures (a), and PSII chlorophyll fluorescence responses (b, c), of *Artemisia tridentata* (b) and *Erythronium grandiflorum* (c) during the *in situ* freezing treatment. For responses of PSII chlorophyll fluorescence (F_V/F_M) to *in situ* air temperature treatments, data are from control (circles) and heated (triangles) plots, and are composites of three to five leaves per plant, and $n = 5$ plants for each species and treatment combination.

−30°C. Freezing tolerance – measured as the response of chlorophyll *a* fluorescence from Photosystem II to *in situ* freezing temperatures – differed for *A. tridentata* on control compared to heated plots (Fig. 4b). Specifically, F_V/F_M was maximal (approx. 0.8) from ambient air temperatures to approx. −10°C for plants on control plots, and to − 20°C for plants on heated plots. The rate of low-temperature induced decrease in F_V/F_M was more pronounced for plants on control compared to heated plots. The temperature leading to a decrease in F_V/F_M to half-maximal levels (LT_{50}) was −24°C for plants on control plots, and −33°C for heated plots (unpaired *t*-test: $t = 4.466$, $P = 0.011$). For *E. grandiflorum*, the response of F_V/F_M to *in situ* freezing was similar for plants on heated and control plots (Fig. 4c). Maximal fluorescence was sustained to approx. −10°C to −12°C, and decreased at comparable rates for plants on both control and heated plots. Based on half-maximal decreases in F_V/F_M, LT_{50} was −25°C for plants on control plots, and −27°C on heated plots, but did not significantly differ for the two treatments ($t = 0.265$, $P = 0.804$).

Photosynthetic CO_2 assimilation for *A. tridentata* was higher for plants exposed to freezing on heated compared to control plots (Fig. 5a; Two-way ANOVA: $F = 46.61$, $P < 0.001$). For comparison, *A* for plants that had not been exposed to freezing was higher than for plants exposed to *in situ* freezing. Based on the two-way ANOVA that compares all combinations of heated vs control and frozen vs nonfrozen plants, there was no significant difference in g_s for any combination of IR heating and *in situ* freezing treatments likely caused by the small sample size and high variance (Fig. 5b). The quantum yield of PSII (ΦPSII) was significantly lower for *A. tridentata* exposed to *in situ* freezing on control plants, in comparison to plants on heated plots, and in comparison to plants not exposed to freezing (Fig. 5c; $F = 16.87$, $P < 0.001$).

Photosynthetic CO_2 assimilation was significantly lower for *E. grandiflorum* exposed to *in situ* freezing for plants on both heated and control plots in comparison to nonfrozen plants (Fig. 5d; two-way ANOVA for all combinations of heated vs control and frozen vs nonfrozen plants: $F = 2046$, $P < 0.001$). The patterns of stomatal conductance were different across freezing and IR treatments, but not parallel to the effects of either treatment combination on *A* (Fig. 5e; $F = 2.59$, $P = 0.12$). By contrast, ΦPSII was significantly lower for plants on both control and heated plots exposed to *in situ* freezing in comparison to nonfrozen plants (Fig. 5f; $F = 7.48$, $P < 0.001$).

Discussion

Our results indicate that the infrared warming treatment led to a significant increase in photosynthetic tolerance of an experimentally imposed *in situ* freezing event for the Great Basin Desert evergreen shrub *Artemisia tridentata*. This was in contrast to our hypothesis that photosynthetic tolerance of freezing for *A. tridentata* would be reduced by infrared warming because of advanced acclimation for plants on heated plots during the snowmelt period. *A. tridentata* becomes physiologically capable of photosynthesis soon after snowmelt to take advantage of available soil moisture despite low air temperatures (Caldwell, 1985; Evans & Black, 1993). Although few studies have examined the ability of *A. tridentata* to tolerate freezing temperatures during the earliest parts of the growing season, DePuit and Caldwell (1973) showed that net CO_2 uptake occurs at 0°C, and carbon fixation during winter was demonstrated by Caldwell *et al.*

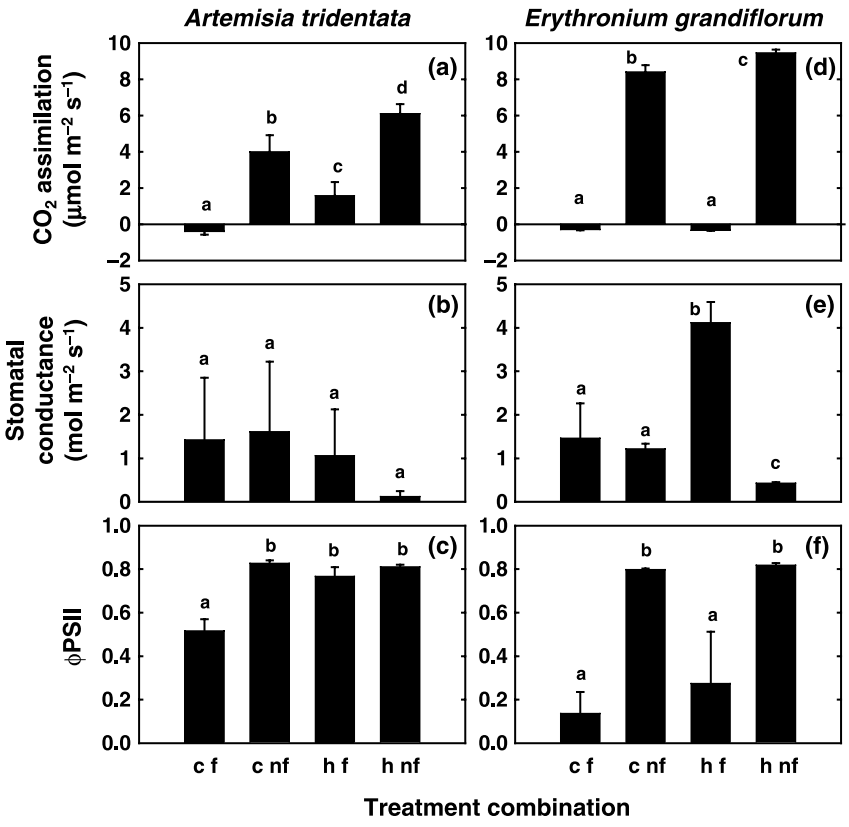

Fig. 5 Recovery of leaves from experimentally induced *in situ* episodic freezing. (a,d) Photosynthetic CO_2 assimilation (b,e) stomatal conductance to water vapor (c,f) and quantum yield of chlorophyll *a* fluorescence from PSII, for leaves of *A. tridentata* and *E. grandiflorum* from control (c) and heated (h) plots. Data are means⁺-standard error for $n = 5$ leaves for leaves not frozen (nf), or exposed to *in situ* freezing (f). Superscript letters indicate significant differences at the $P = 0.05$ level based on Tukey's posthoc comparisons for a two-way ANOVA.

(1975). For seedlings of *A. tridentata* from the eastern slope of the Sierra Nevada of California, low-temperature tolerance measured by vital stain uptake and reductions in F_V/F_M is −15.5°C (Loik & Redar, 2003). Our results were obtained during May when average minimum air temperatures from 1961 to 1990 were −2°C, extreme minimum air temperatures average −13°C, and rarely can reach −30°C. The lowest air temperature experienced during the 2-wk period before experiments was −6°C 11 d before measurements, therefore we reject the *ad hoc* hypothesis that results occurred because of a recent low-temperature episode.

The infrared warming treatment did not reduce the ability of the subalpine herbaceous geophyte *Erythronium grandiflorum* to tolerate *in situ* freezing, in contrast to our hypothesis that it would exhibit altered tolerance of freezing temperatures because of its early emergence and growth following snowmelt, when it should be tolerant of episodic low temperature events. Rates of photosynthetic CO_2 assimilation and stomatal conductance to water vapor for *E. grandiflorum* measured here are similar to those measured by Hamerlynck & Smith (1994) and Germino & Smith (2001). Our post-freezing results for *A* and ΦPSII (Fig. 5d–f) are indicative of chronic photoinhibition of photosynthesis (Osmond, 1994), likely because of the combination of exposure to freezing followed by PPFD levels around 1000 μmol m^{-2} s^{-1}. This is in contrast to the results of Germino & Smith (2000), who found diurnal photoinhibition in response to natural freezing episodes in the Medicine Bow Mountains of Wyoming, USA. The photosynthetic apparatus of *E. grandiflorum* begins to develop before leaves emerge from the snowbed, and gas exchange occurs soon after leaves emerge from the snow (Hamerlynck & Smith, 1994). This species occurs in microsites that result in leaf temperatures below 0°C for 38% of summer nights, resulting in little effect on CO_2 assimilation during subsequent daytime periods (Germino & Smith, 2001). Because photosynthetic development and growing season length are determined in part by date of snowmelt, earlier melt dates induced by warming could result in greater carbon gain for *E. grandiflorum*. However, this could be counteracted by negative effects on carbon gain because of low-temperature induced photoinhibition (Hamerlynck & Smith, 1994; Krause, 1994; Germino & Smith, 2000).

Both species exhibited higher rates of photosynthetic CO_2 assimilation and stomatal conductance to water vapor on heated compared to control plots, indicative of advanced physiological activity as a result of the infrared warming treatment. The IR warming treatment leads to an earlier onset of snowmelt, warmer soil temperatures and lower soil moisture content during summer, and enhanced above-ground biomass accumulation for shrubs in comparison to forbs (Harte *et al.*, 1995; Harte & Shaw, 1995). In summer, the warming treatment affects photosynthetic physiology for some plants

through effects on soil moisture and water potential, rather than through direct effects on leaf temperatures. During summer, warming causes no differences in CO_2 assimilation or stomatal conductance to water vapor, but does result in reduced ΦPSII for *A. tridentata* and the herbaceous geophyte *Erigeron speciosus* (Loik *et al.*, 2000b). Moreover, tolerance of high-temperature stress was decreased for *A. tridentata* on heated compared to control plots, possibly through effects on leaf pubescence and energy budgets (Loik & Harte, 1996).

We hypothesize that enhanced tolerance of episodic freezing is a result of enhanced physiological activity, in particular, higher plant water potentials and photosynthetic gas exchange caused by the IR treatment effects on soil water content and soil temperature. Such effects may occur via an increased level of photosynthates available for allocation to cryoprotection or other acclimation mechanisms (Iba, 2002). In this regard, soluble carbohydrates within roots and stems of *A. tridentata* increase in spring (Coyne & Cook, 1970). It is also possible that enhanced tolerance of freezing is based on effects of warming on photosynthetic physiology in the previous summer, but photosynthesis declines during the drought of summer (Shaw *et al.*, 2000), and much of the effect of warming on *A. tridentata* production appears to be caused by the earlier onset of snowmelt and shrub growth (Perfors *et al.*, 2003). It is also important to note that not all species at this site are likely to undergo the same effects of warming on tolerance of episodic freezing. For *Delphinium nelsonii*, snow depth provides protection from freezing air temperatures and affects flower production (Inouye & McGuire, 1991). For *Helianthella quinquenervis* which emerges from underground storage organs in June and is vulnerable to freezing events, one of the effects of the IR treatment has been to provide greater survival of episodic freezing (De Valpine & Harte, 2001). Similar effects of snow depth, exposure to freezing temperatures, and subsequent production of inflorescences have been shown for *Delphinium barbeyi* (Inouye *et al.*, 2002).

Other factors that are affected by the infrared warming treatment, such as soil nitrogen availability, could have impacted plants over the 8 yr span of the experiment, and thereby influenced our results. Reduced snow accumulation leads to lower soil temperatures and a greater depth of freezing (Hardy *et al.*, 2001). Because most of the perennial herbaceous species overwinter as bulbs, tubers, or corms, greater frequency of soil freezing or lower soil temperatures under bare soil could potentially alter survival and subsequent growth rates. The abundance and flowering of *Delphinium nuttalianum* at the same study site as our experiments are correlated with snow pack depth, which affects both soil temperature and soil water content in the subsequent growing season (Saavedra *et al.*, 2003).

The infrared warming treatment enhanced photosynthetic tolerance of freezing for *Artemisia tridentata*, but had no effect on *Erythronium grandiflorum*. Protection from freezing damage by warming may thus lead to enhanced survival for some species, neutral effects on others, and possibly negative impacts on still other species. On the other hand, the effects of freezing on plant processes may take on a lesser importance as global air temperatures continue to warm to higher levels. Species-specific responses to IR warming have been shown for above-ground biomass accumulation, photosynthetic physiology, and reproductive phenology (Harte & Shaw, 1995; Loik & Harte, 1996, 1997; Price & Waser, 1998; Loik *et al.*, 2000b). Changes in physiological performance during the early snowmelt period may be complicated by changes in competitive and facilitative interactions with other species (Callaway *et al.*, 2002). Also, our measurements were conducted with adult individuals of *A. tridentata*, and for individuals of *E. grandiflorum* originating from underground bulbs of unknown age. Seedling establishment occurs in episodic years at this site, and few recruits survive beyond the first summer drought or subsequent winter cold. Because seedlings are often more sensitive to abiotic stresses compared to established adults (Smith & Nowak, 1990; Larcher, 1995; Loik *et al.*, 2000a; Loik & Redar, 2003), the impacts of warming on freezing tolerance for seedlings may have an important impact on recruitment success. Effects of warming on snow melt timing, exposure to episodic freezing air temperatures, as well as intensity, frequency and depth of soil freezing, would also likely affect soil microorganisms and soil nutrient turnover. As a result, a number of community and ecosystem based processes could be altered by effects of IR warming during the late winter or early spring snowmelt period.

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