Acclimation of photosynthetic temperature optima of temperate and boreal tree species in response to experimental forest warming

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Abstract

Rising temperatures caused by climate change could negatively alter plant ecosystems if temperatures exceed optimal temperatures for carbon gain. Such changes may threaten temperature-sensitive species, causing local extinctions and range migrations. This study examined the optimal temperature of net photosynthesis (T_{opt}) of two boreal and four temperate deciduous tree species grown in the field in northern Minnesota, United States under two contrasting temperature regimes. We hypothesized that T_{opt} would be higher in temperate than co-occurring boreal species, with temperate species exhibiting greater plasticity in T_{opt}, resulting in better acclimation to elevated temperatures. The chamberless experiment, located at two sites in both open and understory conditions, continuously warmed plants and soils during three growing seasons. Results show a modest, but significant shift in T_{opt} of 1.1 ± 0.21 °C on average for plants subjected to a mean 2.9 ± 0.01 °C warming during midday hours in summer, and shifts with warming were unrelated to species native ranges. The 1.1 °C shift in T_{opt} with 2.9 °C warming might be interpreted as suggesting limited capacity to shift temperature response functions to better match changes in temperature. However, T_{opt} of warmed plants was as well-matched with prior midday temperatures as T_{opt} of plants in the ambient treatment, and T_{opt} in both treatments was at a level where realized photosynthesis was within 90–95% of maximum. These results suggest that seedlings of all species were close to optimizing photosynthetic temperature responses, and equally so in both temperature treatments. Our study suggests that temperate and boreal species have considerable capacity to match their photosynthetic temperature response functions to prevailing growing season temperatures that occur today and to those that will likely occur in the coming decades under climate change.

Keywords: climate change, ecotone, maximum photosynthetic rate, range shifts, thermal plasticity

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Introduction

Continued warming of global land surface temperatures by 1.1–6.4 °C is likely over the next century, with variation at diurnal, regional, and seasonal scales (IPCC, 2013). Range shifts and local extinctions are expected in response to warming, given that range boundaries tend to follow temperature isolines and species may differ in their sensitivity to changing temperature (Sykes & Prentice, 1996; Thomas *et al.*, 2004; Jump *et al.*, 2006; Gunderson *et al.*, 2010). Extreme climate events (i.e., heat waves, severe drought), particularly at warm, lower latitude range limits, may lead to

dieback at the trailing end of a species distribution (Bigler *et al.*, 2006; Zimmermann *et al.*, 2009), while factors such as increased levels of competition at the warmer end of species distributions may also partially determine range limits (Woodward, 1987). The physiological mechanisms that govern geographic range limits are not well understood, which prevents models from accurately characterizing the response of forest systems to a changing climate (Hijmans & Graham, 2006).

There are conflicting results as to whether species' temperature optima vary among species in parallel with differences in their current overall geographic (climate) distribution. A few studies have shown that taxa with lower latitude distributions have higher temperature optima compared to those from cooler, higher latitude locations (Hill *et al.*, 1988; Cunningham & Read,

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2002; Robakowski *et al.*, 2012), but others have found no evidence for temperature optima to be related to climatic distribution (Battaglia *et al.*, 1996; Teskey & Will, 1999; Gunderson *et al.*, 2000, 2010). Future climate warming throughout species' ranges may lead to air and leaf temperatures that exceed current photosynthetic temperature optima, which could lead to reduced photosynthetic capacity and carbon gain, and thus negatively affect not only carbon gain but also potentially rates of growth and survival (Sage *et al.*, 2008).

The ability of species to adjust their photosynthetic temperature optima to changes in temperature (acclimation) could act to limit reductions in gas-exchange rates (Berry & Bjorkman, 1980; Kattge & Knorr, 2007; Gunderson *et al.*, 2010; Ghannoum & Way, 2011). Species growing near their colder, higher latitude range limits may respond positively to warming and such responses may be enhanced by gene transfer (mostly from pollen) from the warmer center of the range (Davis & Shaw, 2001). Conversely, species growing near their lower latitude, warm range limits may have limited potential to respond to warming (Berry & Bjorkman, 1980; Tjoelker *et al.*, 1998; Gunderson *et al.*, 2010; Ghannoum & Way, 2011) and such responses may be retarded by lack of gene flow from populations adapted to warmer temperatures because beyond the range edge individuals do not survive or are out-competed in the unfavorable conditions (Davis & Shaw, 2001). Dillaway & Kruger (2010) compared acclimation of two boreal and two temperate species across a climatic gradient, and found little evidence for temperature acclimation in general, or for differences in acclimation capacities among temperate and boreal species. In contrast, Cunningham & Read (2002) compared acclimation of four temperate and four tropical rainforest species, and observed higher temperature optima and greater acclimation capacities for tropical compared to temperate species. While six of the eight species measured in their study were collected as seedlings from natural populations (the remaining two were grown from seed), the seedlings were grown in a greenhouse for 1 year prior to the experiment, so it is not clear whether these results translate to field conditions.

Acclimation potential (of photosynthesis) has rarely been incorporated into physiological and ecosystem models (Medlyn *et al.*, 2002a,b; Kattge & Knorr, 2007), suggesting that models may over- or underestimate how species respond to temperature change and how ecosystem productivity is affected by climate change (Hanson *et al.*, 2005). It is important to determine the degree of acclimation possible for a wide variety of species, given that the impact of warming may be reduced if species are able to undergo significant shifts in temperature optima that keep their physiology more closely matched to prevailing conditions. Researchers who have investigated temperature acclimation have often studied seedlings in growth chambers kept at uniform day/night temperatures (e.g., Tjoelker *et al.*, 1998; Teskey & Will, 1999) and rarely measured complete temperature response curves (but see Cunningham & Read, 2002). Thus, less is known about the degree and timing of acclimation in natural settings with thermal variability (e.g., Dougherty *et al.*, 1979). To measure species across a wide range of temperatures in natural systems, field studies have made use of latitudinal and altitudinal gradients, as well as seasonal changes in temperature (Slatyer & Ferrar, 1977; Dougherty *et al.*, 1979; Han *et al.*, 2004; Dillaway & Kruger, 2010). While these types of studies often show variation in temperature responses of photosynthesis, there are many other biotic and abiotic factors such as soil moisture, soil nutrient availability, leaf age and ontogeny, and leaf traits like leaf mass per area, that are known to vary seasonally or along latitudinal and altitudinal gradients that may affect the magnitude of acclimation responses (Harris *et al.*, 2006; Homann *et al.*, 2007). While past research using growth chambers and temperature gradients has detected acclimation across wide temperature ranges (i.e., comparisons of plants in growth chambers set 10° apart), it is uncertain whether acclimation will also occur in response to smaller temperature increases (2–5 °C) when plants are subjected to otherwise comparable field conditions (e.g., soils, precipitation, fluctuating baseline temperatures) (but see Gunderson *et al.*, 2010).

The goal of this study was to measure the plasticity of temperature optima, and photosynthetic rates at the optima, to conditions similar to regional predictions of climate change. Mean summer temperatures in central North America are predicted to increase by 3.1–5.1 °C over the next century, with increased frequency and duration of drought conditions (Christensen *et al.*, 2007). We selected tree species that co-occur within the ecotone but have different overall distributions (i.e., boreal vs. temperate) to compare acclimation capacities, given that direct comparisons of species originating from different biomes are rare, particularly comparisons in natural settings with thermal variability.

We measured photosynthetic temperature response curves of two boreal and four temperate broad-leaved tree species that co-occur in North America (Table 1). Thus, we were able to compare acclimation potentials for species from a broad distribution of range limits and habitats. Local ecotypes of each species were exposed to either ambient air temperatures or average midday warming of +2.9 ± 0.01 °C above ambient. Measurements were made at the two sites of a chamberless field warming experiment known as Boreal

Table 1 Study species with southern (lower latitude) range limits and shade tolerance rankings. Shade tolerance rankings were obtained from Niinemets & Valladares (2006)

Species		Southern range limit	Shade tolerance ranking
Acer rubrum L.	Temperate	31.2°N	3.44 ± 0.23
Quercus rubra L.		34.8°N	2.75 ± 0.18
Acer saccharum Marsh.		36.5°N	4.76 ± 0.11
Quercus macrocarpa Michx.		40.7°N	2.71 ± 0.27
Betula papyrifera Marsh.	Boreal	44.2°N	1.54 ± 0.16
Populus tremuloides Michx.		44.3°N	1.21 ± 0.18

Forest Warming at an Ecotone in Danger (B4WarmED), in northern Minnesota, USA within the boreal forest-temperate forest ecotone (Table 2). We tested the following hypotheses: (H1) photosynthetic temperature optima will correlate with species' climate distributions, with temperate species exhibiting higher temperature optima relative to boreal species, (H2a) all species will experience some degree of acclimation in temperature optima and maximum photosynthetic rates in response to 2.9 ± 0.01 °C average warming, but (H2b) boreal species growing near their warmer, lower latitude range limits will have limited acclimation capacities relative to temperate species near their cooler, higher latitude range limits and thus, (H3) the negative impact of warming on photosynthetic rates will be ameliorated less so in boreal species as compared to temperate species.

Materials and methods

Site and species descriptions

The experiment was located at two sites; the Cloquet Forestry Center, Cloquet MN and at a colder, higher latitude site near Ely, MN, the Hubachek Wilderness Research Center (Table 2).

Both are University of Minnesota field stations and were chosen because of their location within the boreal forest-temperate forest ecotone. At both sites, our warming experiments were located on coarse-textured upland soils in 40–60 year old mixed aspen–birch–fir stands scattered with pine, spruce, and other species.

Treatments were positioned in both closed (≈5–10% of full sunlight) and relatively open (≈40–60% of full sunlight) overstory conditions. Both closed and open plots were exposed to experimental treatments because regeneration in both habitat types is important in determining boreal forest canopy composition, given the spatial and temporal patterns of natural and anthropogenic disturbances (Heinselman, 1973; Grigal & Ohmann, 1975; Frelich & Reich, 1995). In addition, our species vary in light requirements for regeneration so both habitat types were required for a broad test of warming effects on regeneration in southern boreal forests (Table 1).

Our six native deciduous hardwood species included four temperate and two boreal species (Table 1), all of which are present in the ecotonal region. Local ecotypes of all seedlings planted were obtained from the Minnesota Department of Natural Resources; both sites are in the same DNR seed zone. All seeds came from Minnesota sources ≤80 km north or south of the latitude of the Cloquet site. Southern (lower latitude) range limit of each species was defined as the latitude in central North America (west of 86 degrees longitude and east of 100 degrees longitude) above which 95% of individuals are found; these values were calculated using US Forest Service FIA data.

Experimental design

For a detailed explanation of the experimental design, see Rich *et al.* (in review). The overall experimental design was a 2 site × 2 habitat × 2 treatment factorial, with six replicates of each for a total of 48 circular 3 m diameter plots. Treatments included two levels of simultaneous plant and soil warming (ambient and a mean elevation of +3.4 ± 0.01 °C over day and night and the entire growing season) achieved through use of infrared heat lamps and buried soil heating cables (dummy lamps and soil cables in the ambient plot). Although warming in winter can have important impacts on ecosystem processes, the low levels of biological activity at <0 °C, the potential artefactual effects of our warming treatments on snow melt and freeze/thaw cycles, and the high expense of warming year-round in aggregate led us to decide not to warm in winter. Moreover, ambient plot soil data show that soil temperature disassociates with air temperature during winter months, probably due to the insulative snow cover.

Table 2 Site descriptions. Mean minimum and maximum temperatures were measured on site. Reported temperature measurements are the daily minimum of the coldest month and the daily maximum of the warmest month averaged for 2009–2011

Site	Lat/Long	Altitude a.s.l. (m)	Mean annual precipitation (mm)	Mean minimum temperature °C	Mean maximum temperature °C
Cloquet, MN	46°40′N, 92°31W	382	807	−30.6 °C	34.4 °C
Ely, MN	47°56′N, 91°45W	415	722	−34.8 °C	36.5 °C

Chamberless warming was achieved via a feedback control that acted concurrently and independently at the plot scale to maintain a fixed temperature differential from ambient conditions. The rationale for this design was that simultaneous warming above- and belowground provides a more realistic treatment than does either in isolation (as decadal-scale changes in mean growing season soil temperatures are likely to mirror long-term changes in mean air temperatures). Moreover, above- and belowground measurement data show the established warming treatments emulated observed diurnal, seasonal, and annual patterns in temperature and generally matched historical warming during the past half century (in terms of differences among times of day or seasons) (Rich *et al.* in review). The degree of warming diminished slightly as the experiment progressed and increasing amounts of plant biomass transpired greater amounts of water, limiting warming per unit radiation added. Although the amount of energy put toward warming each +3.4 °C plot was similar over the course of each day, we found that the degree of warming was lower during midday hours (09:00–15:00 hours) when ambient air temperatures were warmest (and when the greatest amount of photosynthesis occurs). On average, we achieved warming during midday hours of +2.9 ± 0.01 °C (Table 3) (Rich *et al.* in review). From this point forward, we refer the +2.9 °C warming (rather than the +3.4 °C warming) as it was the mean level of warming achieved during times of day when leaves are most photosynthetically active and thus represents thermal conditions to which temperature optima are likely to be best matched.

Gas-exchange measurements

Thermal responses of photosynthesis were measured using six Li-Cor 6400 portable photosynthesis systems (Li-Cor, Lincoln, NE, USA). A total of 1418 temperature response

curves were measured throughout the growing seasons (June to September) of 2009 through 2011. Not all species were measured each year due to the time-consuming nature of the measurements. Temperature response curves were measured on detached foliage from a subsample of ≈5800 seedlings planted in 2008 at both sites in these two warming treatment levels. Fully expanded, healthy leaves were chosen from individuals planted in open/ambient temperature, open/warmed 2.9 °C, closed/ambient temperature, and closed/warmed 2.9 °C plots. Between 10 and 18 leaf samples were cut each morning and kept hydrated throughout the day using floral water pik tubes in a growth chamber that was used to efficiently achieve a broad range of measurement temperatures (12, 17, 22, 27, 32, and 37 °C) for multiple leaves simultaneously. We chose to measure detached foliage for two reasons: (i) to increase the number of leaves sampled due to rapid heating and cooling of leaf temperatures within the chamber, and (ii) to increase the range of measurement temperatures in each response curve. In a prior study that measured temperature response curves *in situ* using the thermoelectric block and Li-Cor 6400-88 Expanded Temperature Control Kit, leaf temperatures could only be decreased to ≈3–7 °C below the ambient temperature, and substantial amounts of time were spent waiting for target temperatures to be reached (Robakowski *et al.*, 2012). Since summer daytime temperatures generally exceed 23 °C in both Ely and Cloquet, the lower end of each of our response curves would have been impossible to measure. Use of hydrated detached foliage entails several compromises. For example, leaves may have poorer water status than in the field if petioles suffer embolism, or be better hydrated if field plants are in dry soils. Stomatal function will also obviously be decoupled from stems and root signals. Although we thus caution against interpreting the absolute values of net photosynthesis as representative of plants in the field, there is no obvious reason that detachment should change the thermal response, and

Table 3 Ambient air temperatures (± SE) and the degrees above ambient achieved by the 2.9 ± 0.01 °C target warming treatment averaged over each photosynthetic growing season (June 10 through September 27) at both sites and under both canopy conditions. Also shown are air temperatures averaged over the time of day, when plants are most photosynthetically active (09:00–15:00 hours) at the study sites

Year	Site	Overstory condition	Mean ambient 24 h air temperatures (°C)	Mean 24 h warming achieved (°C)	Mean ambient 09:00–15:00 hours temperatures (°C)	Mean 09:00–15:00 hours warming achieved (°C)
2009	Cloquet	Closed	15.6 ± 0.04	4.1 ± 0.01	19.4 ± 0.06	3.8 ± 0.01
		Open	15.7 ± 0.05	3.3 ± 0.01	22.3 ± 0.07	2.6 ± 0.02
	Ely	Closed	15.9 ± 0.04	4.1 ± 0.00	19.0 ± 0.06	3.9 ± 0.01
		Open	16.1 ± 0.05	3.8 ± 0.01	21.7 ± 0.08	3.4 ± 0.02
2010	Cloquet	Closed	16.3 ± 0.04	3.6 ± 0.01	19.2 ± 0.07	3.2 ± 0.01
		Open	16.1 ± 0.05	3.1 ± 0.01	21.6 ± 0.08	2.0 ± 0.02
	Ely	Closed	16.1 ± 0.04	4.0 ± 0.01	18.5 ± 0.08	3.8 ± 0.01
		Open	16.5 ± 0.05	3.3 ± 0.01	21.2 ± 0.09	2.6 ± 0.02
2011	Cloquet	Closed	15.7 ± 0.04	3.0 ± 0.01	18.8 ± 0.07	2.7 ± 0.02
		Open	15.5 ± 0.05	2.6 ± 0.02	20.8 ± 0.08	2.0 ± 0.03
	Ely	Closed	17.0 ± 0.04	3.2 ± 0.01	19.9 ± 0.08	3.0 ± 0.01
		Open	17.1 ± 0.06	2.9 ± 0.01	22.5 ± 0.10	2.5 ± 0.02

more importantly even if the thermal responses were influenced by detachment, it should influence leaves from ambient and warmed treatments similarly.

Each morning, collected leaves were placed in the growth chamber, which was set to 12 °C. The leaf chamber of each Li-Cor was also placed inside the growth chamber and leaf temperature in the cuvette was set to correspond with the growth chamber temperature. Once both chambers reached the target temperature, leaves were clamped in the leaf chambers for a minimum of 10 min and three measurements were logged at 10 second intervals after readings had stabilized. Light was maintained in the leaf chamber at 1200 μmol m^{-2} s^{-1} using the LED light source for leaves collected from open plots, and 800 μmol m^{-2} s^{-1} for leaves from closed plots. Tests were made to ensure these levels were above those needed for light saturation. Air flow was set at 300–500 μmol s^{-1} and reference CO_2 concentrations were set at 400 μmol mol^{-1}. Photosynthetic rates were measured for each leaf at 12 °C, after which both the growth chamber and leaf temperature were set to 17 °C and the process was repeated for all measurement temperatures in increasing order. Humidity within the leaf chamber was controlled to the best of our ability; we routed incoming air through desiccant at low temperatures to keep relative humidity below 70% and to avoid condensation, and allowed incoming air into the chamber at higher temperatures when humidity levels were lower. However, at 37°, vapor pressure deficit ranged from ~1.5–4.5 kPa and relative humidity often dropped to below 35%, even when attempts were made to increase it. These attempts included adding water to the CO_2 scrub column, lowering the flow rate within the leaf chamber, humidifying the air in the lab, and enclosing a hose attached to the intake valve with damp gauze.

Fitting photosynthetic temperature response curves

The photosynthetic temperature optimum for each measured leaf was estimated using nonlinear regression of the photosynthetic temperature response data:

$$A_{(T)} = A_{opt} - b(T - T_{opt})^2,$$

where $A_{(T)}$ is the measured net CO_2 assimilation rate (μmol m^{-2}s^{-1}) at a leaf temperature T and b is a parameter describing the spread of the parabola (Battaglia *et al.*, 1996). The vertex of each fit is considered the temperature optima of photosynthesis (T_{opt}), and A_{opt} is the rate of photosynthesis at T_{opt}. For a given A_{opt} and T_{opt}, a smaller b describes a relatively broader curve, while a larger b describes a narrower curve. Curves were considered for analysis only when the fits of both the overall curve and the second-order polynomial were at least marginally significant ($P < 0.20$ and $P < 0.15$, respectively) and the R^2-value exceeded 0.75. Thus, of the 1418 curves measured, only 880 curves that met our statistical criteria were used for analyses. We used these relatively nonconservative P-values given that our curves were made up of only six points, but ninety percent of our overall curve fits and 96% of second-order polynomial terms had P-values of <0.10. Curves were then screened to confirm that all points fit within

95% confidence intervals and to ensure that T_{opt} did not occur in the outer 3% of the distribution (15.6 °C < T_{opt} < 33.4 °C). In instances where one measurement point between 17 and 32 °C was negatively affecting the fit of the curve, it was removed and the curve was fit again using five points rather than six. For example, if a leaf had uncharacteristically low conductance and photosynthesis values at 17 °C, its petiole was recut under water and measured again at 22 °C, often recovering to more typical values. Using only curves that met stricter statistical standards did not materially influence the results.

Photosynthetic temperature optima were compared to 1, 3, 5, and 10 days prior temperature histories in ambient and warmed environments to examine how closely T_{opt} matches prevailing temperature conditions. As it is unknown whether acclimation mostly closely matches temperatures experienced on very recent (days) or longer (weeks) time scales, we examined responses across a range of time windows. Results were similar for all, and we use the 5 days prior temperatures both because they had the strongest association with T_{opt} (best match) and because physiologically it seems like a reasonable time frame to use for this purpose (Gunderson *et al.*, 2010; Robakowski *et al.*, 2012).

Contributions of stomatal function to photosynthetic rates

It is important to note that our measures of photosynthetic rates at T_{opt} (A_{opt}) were generally made 3–4 hours after leaves were detached from trees, and although we kept them hydrated in floral water pik tubes, it was possible that stomatal conductance rates, and consequently A_{opt}, were negatively affected by leaf detachment. Thus, we compared rates of A_{opt} reported here with rates of photosynthesis measured under optimal *in situ* conditions within our experimental plots and found that the two were significantly correlated (and close to 1 : 1) (Figure S1). In addition, increased vapor pressure deficit (VPD) at higher temperatures can limit stomatal conductance rates, reducing intercellular CO_2 concentrations (C_i) and photosynthetic rates (A) independent of biochemical effects (Zhang *et al.*, 2001; Hikosaka *et al.*, 2006). Therefore, we examined relationships of leaf diffusive conductance and C_i to leaf temperatures (Figures S2 and S3). The relationship of leaf conductance to temperature roughly matched that of photosynthesis (compare Figure S2 to S4), although conductance appears to peak at cooler temperatures. Moreover, because in most species, C_i was not lower (and often higher) at the highest temperature (37 °C) than at 32 °C, it is unlikely that stomatal closure is responsible for low photosynthetic rates at 37 °C. To more formally address the possible effect of low humidity and high VPD, we followed the procedure of Gunderson *et al.* (2010). Dividing our measures of A by C_i and plotting against temperature revealed nonstomatal decreases in A (Zhang *et al.*, 2001) (Figure S5), and plots with peaks that generally match those of absolute photosynthesis. In other words, the rate of A per unit CO_2 declined at high temperatures, indicating that decreasing C_i caused by stomatal closure is not the singular cause of the decline in A above T_{opt}; and the shapes

of the curves suggest stomatal closure is not the dominant cause – but that there must also be biochemical limitations such as changes in expression and activation of photosynthetic enzymes or leaky membranes (Schrader *et al.*, 2004; Sage & Kubien, 2007). Moreover, analyses that used only air temperatures from 12 °C to 32 °C came to similar conclusions as those using the entire curve. The comparison to *in situ* field measurements and consideration of changes in A/C_i vs. temperature suggest our measurements provide a reasonable estimate of temperature optima of photosynthesis for the studied plants.

Statistical analysis

Mixed effects analyses of variance (ANOVAs) were used to compare photosynthetic temperature optimum (T_{opt}), the photosynthetic rate at T_{opt} (A_{opt}), and the *b* parameter that describes the spread of response curves. Models included the following independent variables: site, species (or southern range limit), overstory condition, warming treatment, and all 2- and 3-way interactions among variables. Plot was added to each model as a random effect with each plot having a unique identifying number. Models were run separately by year since different subsets of species were measured each year (cf. Table 4), but additional models were run across years for a subset of species (*Acer rubrum*, *Betula papyrifera* and *Populus tremuloides*) (Tables S1, S2, and S3). These included year and campaign (early, mid, and late summer), in addition to the independent variables listed above to determine whether treatment effects on these species grew stronger or weaker from year to year or seasonally within years. Results of analyses including southern (lower latitude) range limit as a main effect are shown in the appendix (Tables S4, S5, and S6) but not in the main text as the results are similar to analyses using species as a main effect.

In 2009, two kinds of models were run since three of the seven species were measured at one site only (Table 5). In all cases, we used the fullest model possible, meaning that some species groups were included in more than one analysis (e.g.,

A. rubrum individuals were included in the analysis of species measured at both sites, and the individuals measured in Cloquet were also included in the analysis of species measured at Cloquet only). We added several measures of plot-level temperature and soil volumetric water content data (i.e., averaged over the week prior to the measurement date or by field season) as covariates to this model to see if variation in temperatures within treatments among sites, habitats, or years further explained responses beyond 'treatment' as a nominal variable. None of these covariates was significant, so we interpret treatment effects as largely similar across all of these sources of variation and present models without temperature or soil water covariates included (Tables S1, S2, and S3). All statistical analyses were conducted in JMP statistical analysis software (JMP 10.0, SAS Institute, Cary, NC, USA). See Table 2 for a summary of analyses and where they are shown in the manuscript.

Results

Response of T_{opt} to the warming treatment

To determine whether there was evidence that acclimation to the warming treatment differed among years or seasonally within years, we used data for three species; *Acer rubrum*, *Betula papyrifera*, and *Populus tremuloides* (Table 4). These species were measured in all years and all campaigns (early, mid, and late summer). We found that absolute photosynthetic temperature optima (T_{opt}) varied among years, likely due at least in part to yearly variability in ambient temperatures, but the effect of the warming treatment on T_{opt} was similar for all three species in all years and campaigns as evidenced by the lack of treatment interactions (Table S1). As comparisons of the three species among years provide no evidence that plant responses to warming differed across campaigns

Table 4 Summary of species measured by year and site, as well as statistical analyses. Each analysis shown was run three times to test for differences in photosynthetic temperature optima (T_{opt}), photosynthetic rates at T_{opt} (A_{opt}) and the parameter that describes the spread of the response curves (*b*). The solid box represents analyses run among years for the three species measured most often (Tables S1, S2, and S3). The dashed boxes represent analyses run within year for all species measured at both sites (Tables 5–7). The asterisks (*) shown represent a second set of analyses run in 2009 which included only measurements made at the Cloquet site (Table 5). Tables S4, S5, and S6 show results substituting southern (lower latitude) range limit for species in the statistical models

	Species					
Year	*Betula papyrifera*	*Populus tremuloides*	*Acer rubrum*	*Acer saccharum*	*Quercus rubra*	*Quercus macrocarpa*
2009	C*,E	C*,E	C*,E	C*,E	C*,—	C*,—
2010	C,E	C,E	C,E	—,—	C,E	—,—
2011	C,E	C,E	C,E	C,E	C,E	C,E

C, measured at the Cloquet site; E, measured at the Ely site; —, not measured.

Table 5 Mixed effect ANOVA results for measurements made in 2009. Two analyses were run since two of the species were measured at one site only. $P < 0.05$ are indicated in bold; $P < 0.10$ are italicized

Species	Source of variance	T_{opt}		A_{opt}		b	
		F	P-value	F	P-value	F	P-value
(both sites)	Site	**8.12**	**0.008**	**5.79**	**0.022**	1.94	0.177
A. rubrum	Species	**4.24**	**0.006**	**91.81**	**<0.001**	**41.31**	**<0.001**
A. saccharum	Overstory condition	**18.70**	**<0.001**	**38.99**	**<0.001**	**13.54**	**0.001**
B. papyrifera	Warming	**17.28**	**<0.001**	**5.34**	**0.027**	**7.91**	**0.010**
P. tremuloides	Site × Species	1.44	0.231	**2.73**	**0.045**	1.48	0.220
	Site × Overstory Condition	0.48	0.495	0.01	0.938	0.05	0.832
	Site × Warming	1.07	0.310	0.11	0.746	0.10	0.756
	Species × Overstory condition	**5.48**	**0.001**	**5.86**	**<0.001**	**12.02**	**<0.001**
	Species × Warming	0.81	0.490	0.33	0.802	1.10	0.352
	Overstory condition × Warming	1.40	0.245	0.09	0.770	0.94	0.341
	Site × Species × Overstory	1.83	0.143	0.68	0.568	**3.11**	**0.028**
	Site × Species × Warming	1.02	0.387	0.27	0.848	1.57	0.197
	Site × Overstory × Warming	0.23	0.636	**6.57**	**0.016**	**5.02**	**0.036**
	Species × Overstory × Warming	1.37	0.254	1.57	0.199	**4.10**	**0.008**
(Cloquet only)	Species	**2.88**	**0.018**	**32.27**	**<0.001**	**15.10**	**<0.001**
A. rubrum	Overstory condition	**6.69**	**0.016**	**20.93**	**<0.001**	**12.18**	**0.003**
A. saccharum	Warming	**9.90**	**0.006**	1.30	0.273	0.27	0.608
B. papyrifera	Species × Overstory condition	1.45	0.211	**2.34**	**0.047**	**7.32**	**<0.001**
P. tremuloides	Species × Warming	0.75	0.585	0.15	0.978	0.21	0.959
Q. macrocarpa	Overstory condition × Warming	2.37	0.138	1.04	0.326	1.08	0.314
Q. rubra	Species × Overstory × Warming	1.53	0.188	1.31	0.267	*1.92*	*0.097*

Dependent variables: T_{opt} – photosynthetic temperature optimum, A_{opt} – rate of CO_2 assimilation measured at T_{opt}, b – a unitless parameter describing the spread of each temperature response curve.

Table 6 Mixed effect ANOVA results for measurements made in 2010. $P < 0.05$ are indicated in bold; $P < 0.10$ are italicized

Species	Source of variance	T_{opt}		A_{opt}		b	
		F	P-value	F	P-value	F	P-value
A. rubrum	Site	**10.42**	**0.004**	**67.53**	**<0.001**	**20.16**	**<0.001**
A. saccharum	Species	**22.76**	**<0.001**	**62.90**	**<0.001**	**9.85**	**<0.001**
B. papyrifera	Overstory condition	1.90	0.181	**112.06**	**<0.001**	**21.53**	**<0.001**
P. tremuloide	Warming	**11.15**	**0.003**	0.58	0.451	0.36	0.550
Q. macrocarpa	Site × Species	1.41	0.242	0.87	0.458	0.67	0.578
Q. rubra	Site × Overstory condition	2.21	0.150	0.46	0.506	0.04	0.849
	Site × Warming	0.03	0.857	1.26	0.271	0.30	0.588
	Species × Overstory condition	**2.93**	**0.035**	**3.78**	**0.011**	1.71	0.166
	Species × Warming	1.76	0.155	*2.14*	*0.097*	0.33	0.803
	Overstory condition × Warming	0.17	0.680	1.83	0.188	1.53	0.225
	Site × Species × Overstory	0.87	0.456	1.12	0.342	0.76	0.521
	Site × Species × Warming	0.22	0.880	*2.60*	*0.053*	**3.04**	**0.030**
	Site × Overstory × Warming	0.01	0.942	0.56	0.463	0.02	0.899
	Species × Overstory × Warming	0.22	0.885	1.52	0.209	*2.13*	*0.097*

Dependent variables: T_{opt} – photosynthetic temperature optimum, A_{opt} – rate of CO_2 assimilation measured at T_{opt}, b – a unitless parameter describing the spread of each temperature response curve.

within a year or among years of the experiment, in the remaining within-year analyses we compared as many of the six species as were measured in each year, pooling across campaigns (Table 4).

In each year, species differed in T_{opt}, but the rankings of T_{opt} were not related to climate of origin, rejecting H1 (Tables 5–7, Fig. 1). For example, temperate species had both the lowest and highest T_{opt} in 2009 (Fig. 1).

Table 7 Mixed effect ANOVA results for measurements made in 2011. $P < 0.05$ are indicated in bold; $P < 0.10$ are italicized

Species	Source of variance	T_{opt} F	T_{opt} P-value	A_{opt} F	A_{opt} P-value	b F	b P-value
A. rubrum	Site	**8.33**	**0.006**	**26.94**	**<0.001**	**14.51**	**<0.001**
A. saccharum	Species	**6.99**	**<0.001**	**36.95**	**<0.001**	*1.98*	*0.082*
B. papyrifera	Overstory condition	2.75	0.104	**83.10**	**<0.001**	*3.13*	*0.083*
P. tremuloide	Warming	**5.65**	**0.022**	0.58	0.454	0.32	0.573
Q. macrocarpa	Site × Species	**4.08**	**0.001**	*2.07*	*0.069*	1.28	0.272
Q. rubra	Site × Overstory condition	0.85	0.362	0.41	0.525	1.17	0.284
	Site × Warming	0.03	0.868	*3.47*	*0.074*	2.12	0.151
	Species × Overstory condition	*2.18*	*0.056*	**6.01**	**<0.001**	*2.11*	*0.063*
	Species × Warming	0.46	0.804	0.90	0.484	**2.74**	**0.019**
	Overstory condition × Warming	0.31	0.583	0.20	0.655	*3.66*	*0.061*
	Site × Species × Overstory	1.17	0.324	0.74	0.594	1.51	0.184
	Site × Species × Warming	0.65	0.661	0.41	0.840	*2.12*	*0.063*
	Site × Overstory × Warming	0.03	0.857	0.08	0.775	0.40	0.528
	Species × Overstory × Warming	0.21	0.958	0.83	0.531	1.60	0.158

Dependent variables: T_{opt} – photosynthetic temperature optimum, A_{opt} – rate of CO_2 assimilation measured at T_{opt}, b – a unitless parameter describing the spread of each temperature response curve.

There was a consistent acclimation of T_{opt} with T_{opt} shifting upward with warming (P-value of the treatment effect was significant in each case) in each year x 'measured species set' combination (Tables 4–7; Fig. 1). These responses were similar across all species, sites, and overstory conditions as evidenced by the lack of warming treatment interactions in statistical results, consistent with H2a, but rejecting H2b (Tables 5–7). The general trend was for species to adjust their T_{opt} upward in response to the warming treatment by an average of 1.1 ± 0.21 °C (species-level average across all years, Fig. 1). For all species measured in each year, the mean shift for all species was slightly lower in 2011 (0.88 ± 0.12 °C) than in 2009 (1.17 ± 0.31 °C) or 2010 (1.38 ± 0.26 °C). However, the degree of midday warming trees were exposed to in 2011 (2.6 ± 0.01 °C) was also lower than that in 2009 and 2010 (3.1 ± 0.01 °C above ambient in both years).

Response of A_{opt} and the b parameter to the warming treatment

The photosynthetic rate measured at T_{opt} (A_{opt}) of all species was unaffected by the warming treatment in most cases (Tables 5–7; Fig. 2). This result rejects H3, both because we did not observe a negative impact of warming on A_{opt} (due to temperatures exceeding T_{opt}) and because boreal and temperate species were similarly affected. In 2009, A_{opt} of the two boreal and two temperate species measured at the Cloquet site showed a positive response to the warming treatment (Table 5; Fig. 2). This result also rejects H3, again because all species responded similarly and because A_{opt} was positively affected by

warming. Leaf nitrogen and specific leaf area were unaffected by the temperature treatments (data not shown).

Despite the general lack of warming treatment effects on A_{opt}, there were significant species differences in A_{opt} in all years that corresponded to species' successional status. Averaged across years and overstory conditions, shade tolerant and midtolerant species such as *Acer saccharum* and *A. rubrum* exhibited the lowest rates of A_{opt} (3.4–5.7 μmol m^{-2} s^{-1}), while light-demanding species such as *Betula papyrifera* and *Populus tremuloides* had rates that were two to three times higher on average (9.4–11.1 μmol m^{-2} s^{-1}). These rates of A_{opt} are significantly correlated (and slightly lower, but close to the 1 : 1 line) with light-saturated photosynthetic rates measured *in situ* (on attached leaves) in the experimental plots as part of our larger experiment (Figure S1).

The b parameter also differed significantly among species (Tables 5–7; Fig. 3), with the two *Acer* species having the smallest b in all years. This indicates a lower temperature sensitivity and a broader optimal range. *A. saccharum* and *A. rubrum* had b values that averaged 0.017 ± 0.001, whereas the two *Quercus* species, *B. papyrifera*, and *P. tremuloides* all averaged 0.028 ± 0.001 across treatments and years. The b parameter was affected by the warming treatment in a few cases (i.e., warming increased b for *B. papyrifera* in 2009 and for *P. tremuloides* in 2011), but these results appeared to be minor and had negligible effects on photosynthetic rates.
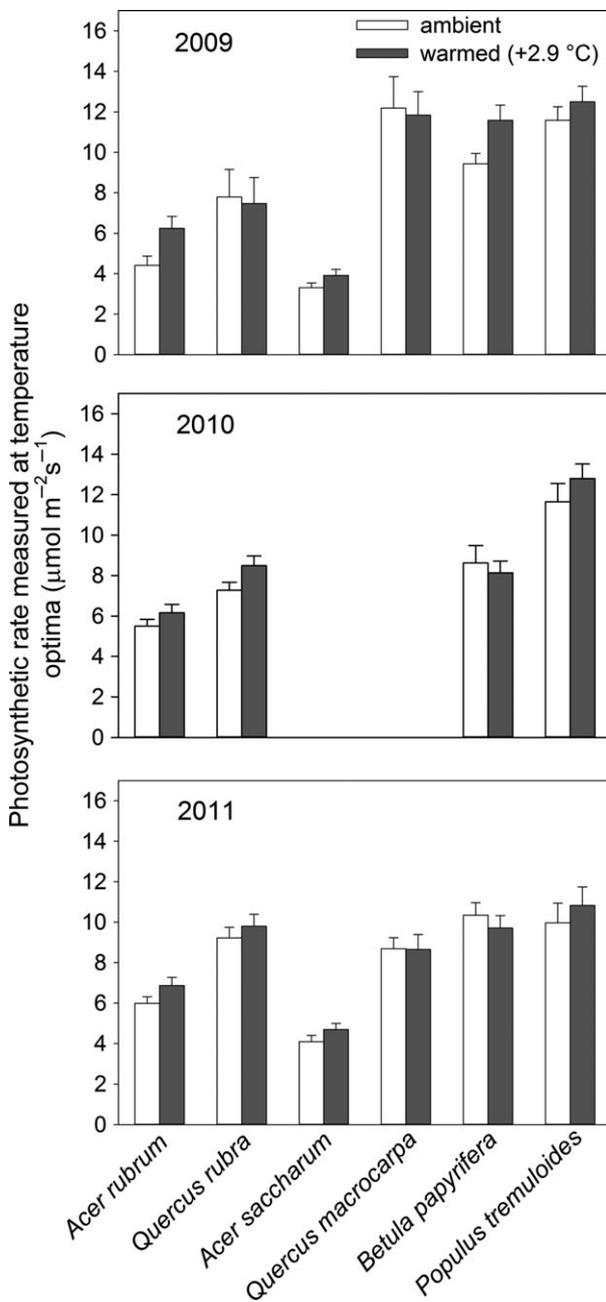
Acclimation response to temperature history

In general, the T_{opt} of ambient and warmed plants was close to but not perfectly matched to recent
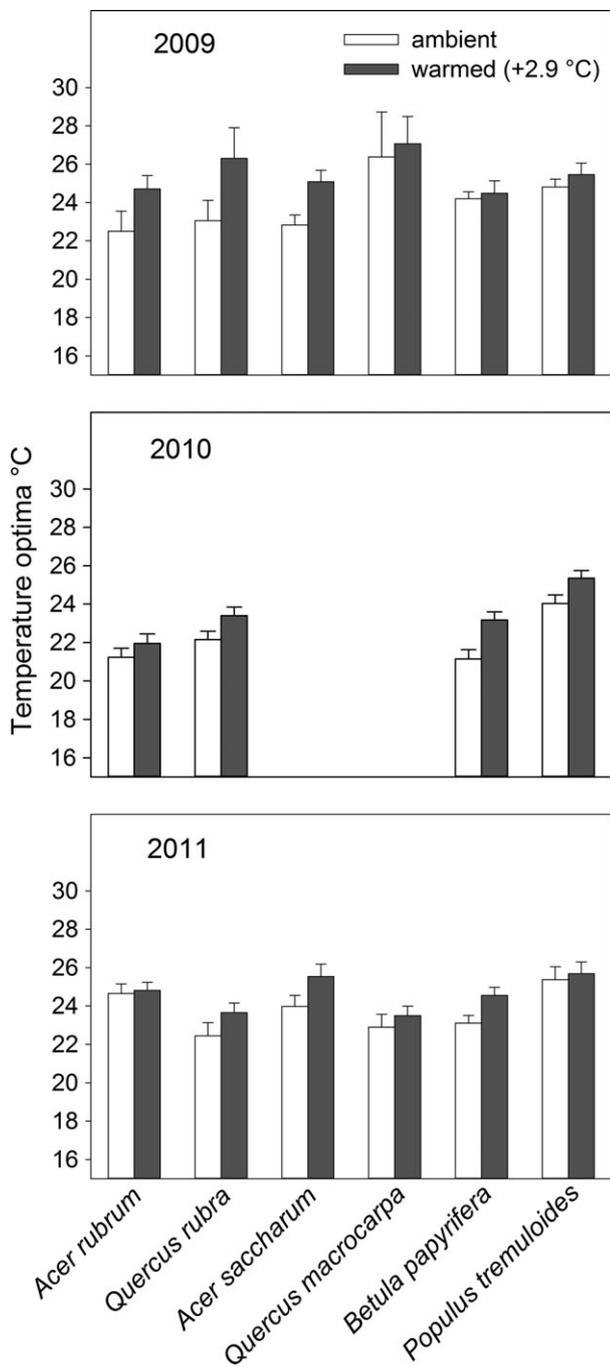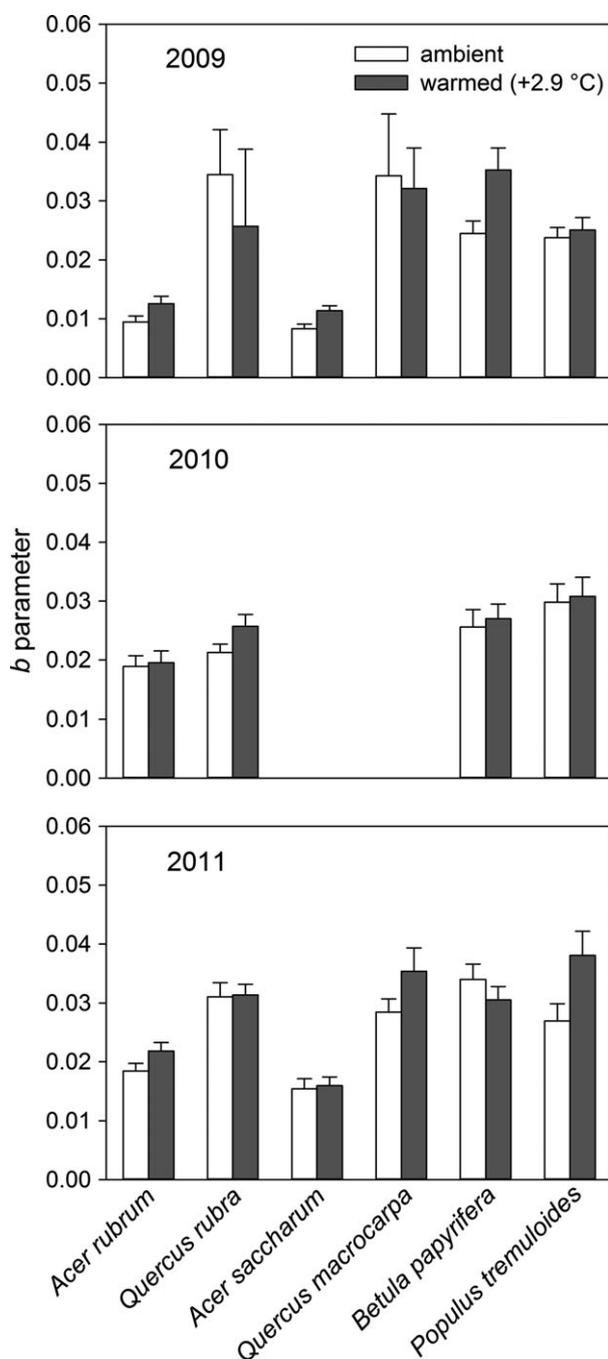
Fig. 1 Mean temperature optima ($T_{opt} \pm$ SE) for juvenile trees of four temperate and two boreal species grown at two temperatures, ambient and 2.9 ± 0.01 °C above ambient. Species are shown in order of their southern (lower latitude) range limits, with the most southern species on the left. *A. saccharum* and *Q. macrocarpa* were not measured in 2010. T_{opt} estimates were averaged for individuals growing in open and closed plots. T_{opt} was estimated from temperature response curves.

Fig. 2 Mean maximum photosynthetic rates ($A_{opt} \pm$ SE) for juveniles trees of four temperate and two boreal species grown at two temperature, ambient and 2.9 ± 0.01 °C above ambient. Species are shown in order of their southern (lower latitude) range limits, with the most southern species on the left. *A. saccharum* and *Q. macrocarpa* were not measured in 2010. A_{opt} estimates were averaged for individuals growing in open and closed plots. A_{opt} was measured at the photosynthetic temperature optima of temperature response curves.

temperatures. Under closed canopy conditions, T_{opt} in both temperature treatments best matched maximum recent temperatures, while T_{opt} of ambient and warmed

plants in the open best matched mean midday temperatures (Figs 4 and 5). Similar trends were observed when these comparisons were made by year, species, or time

Fig. 3 Mean value of unitless b parameter (which denotes the shape of the temperature response curve, $\pm$ SE) for juveniles trees of four temperate and two boreal species grown at two temperature, ambient and 2.9 ± 0.01 °C above ambient. Species are shown in order of their southern (lower latitude) range limits, with the most southern species on the left. *A. saccharum* and *Q macrocarpa* were not measured in 2010. The b parameters were averaged for individuals growing in open and closed plots.

of year (Tables S7–S9). Thus, despite showing only a 1.1 ± 0.21 °C difference in mean T_{opt} between ambient and warmed plants (for a 2.9 ± 0.01 °C temperature

difference), the T_{opt} of plants in the warmed treatment matched recent temperatures they experienced as closely as the T_{opt} of plants in the ambient treatment matched their recent temperatures. In other words, using the degree of matching to experienced temperatures as a measure, the ambient and warmed treatment plants were equally well acclimated in terms of their photosynthetic optimas. Moreover, in both temperature treatments, T_{opt} was at a level where realized photosynthesis was within 90–95% of optimal (Table 8).

Discussion

Documenting photosynthetic temperature responses and acclimation to changes in temperature provides critical information for projecting the impacts of climate change on ecological systems. In particular, acclimation could act to limit potential reductions in gas-exchange rates (Berry & Bjorkman, 1980; Kattge & Knorr, 2007; Gunderson *et al.*, 2010) associated with higher growth temperatures. Such information can enhance ecosystem carbon balance models by representing climate change responses more accurately. All six species consistently showed acclimation responses to warming. These acclimation responses were robust across years, canopy conditions, and species differences in tolerance (to shade and drought) and in geographic distribution. Moreover, we found that acclimation in plants grown under warmer temperatures served to match T_{opt} to prevailing temperatures as well as did ambient plants. T_{opt} under both ambient and warmed conditions was not perfectly matched to the range of experienced temperatures, but was close (average $\approx$90–95% the photosynthesis that would occur with a perfect T_{opt} match to recent midday temperatures).

Acclimation potential of T_{opt} to warming

Acclimation of photosynthesis has been shown in leaves formed at one growth temperature and later exposed to different temperatures (i.e., leaves formed in cool spring temperatures and later exposed to warmer midsummer temperatures) (Loveys *et al.*, 2003; Gunderson *et al.*, 2010), though some research suggest that full acclimation requires that leaves are formed at the new growth temperature (Veres & Williams, 1984; Atkin *et al.*, 2006). Our results documented an acclimation response (a 1.1 ± 0.21 °C shift in T_{opt} on average) to a temperature increase that roughly approximated regional predictions of climate change.

There are contrasting ways one can view these results. Compared with the 2.9 ± 0.01 °C mean difference in daytime temperatures, it appears that plants did not fully acclimate to the warmer temperature

Table 8 Mean ± SE percent of photosynthetic rate at T_{opt} at prevailing 5 days temperatures under ambient and 2.9 ± 0.01 °C warming by canopy condition

	Percent of photosynthetic rate at T_{opt} at prevailing 5 days temperatures			
	Open		Closed	
	Ambient	Warmed	Ambient	Warmed
Year				
2009	97 ± 0.8	96 ± 0.8	89 ± 1.2	94 ± 0.8
2010	95 ± 1.2	95 ± 0.6	92 ± 1.2	95 ± 0.8
2011	93 ± 1.1	92 ± 1.3	89 ± 1.3	92 ± 1.1
years combined	94 ± 0.6	94 ± 0.6	90 ± 0.7	93 ± 0.6
Species				
Acer rubrum	92 ± 1.8	94 ± 1.3	93 ± 1.1	94 ± 0.9
Acer saccharum	96 ± 1.3	93 ± 1.8	89 ± 2.9	93 ± 1.7
Betula papyrifer	97 ± 0.6	94 ± 1.1	90 ± 1.4	94 ± 1.0
Populus tremuloides	93 ± 1.3	95 ± 1.3	86 ± 2.2	90 ± 2.2
Quercus macrocarpa	91 ± 3.1	93 ± 4.2	85 ± 3.8	91 ± 2.8
Quercus rubrum	95 ± 1.8	94 ± 1.8	93 ± 1.3	95 ± 1.1
Campaign				
Early summer	93 ± 1.3	91 ± 1.5	92 ± 1.2	93 ± 1.0
Midsummer	98 ± 0.5	95 ± 0.7	93 ± 0.9	96 ± 0.5
Late summer	94 ± 1.1	95 ± 0.8	86 ± 1.5	90 ± 1.4
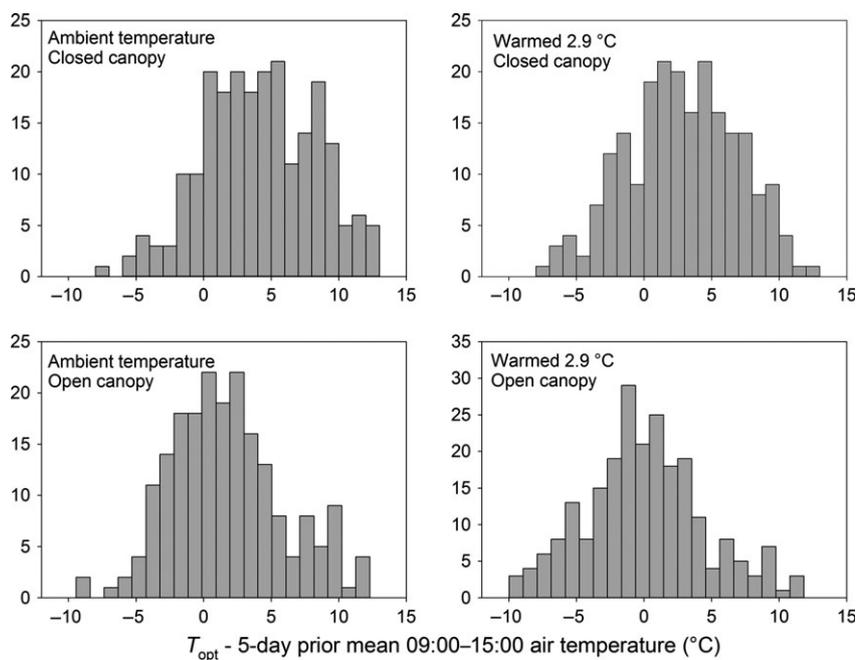
Fig. 4 The distribution of photosynthetic temperature optima (T_{opt}) minus the 5 days prior mean air temperature during the measurement period (09:00–15:00 hours) under open and closed canopy and ambient and warmed 2.9 ± 0.01 °C conditions.

treatment. This interpretation assumes that T_{opt} under ambient conditions was very well matched to recent temperature experienced by the plants. This was not the case. Although the T_{opt} of ambient plants was relatively well matched to recently experienced temperatures, they were no better matched than the T_{opt} of
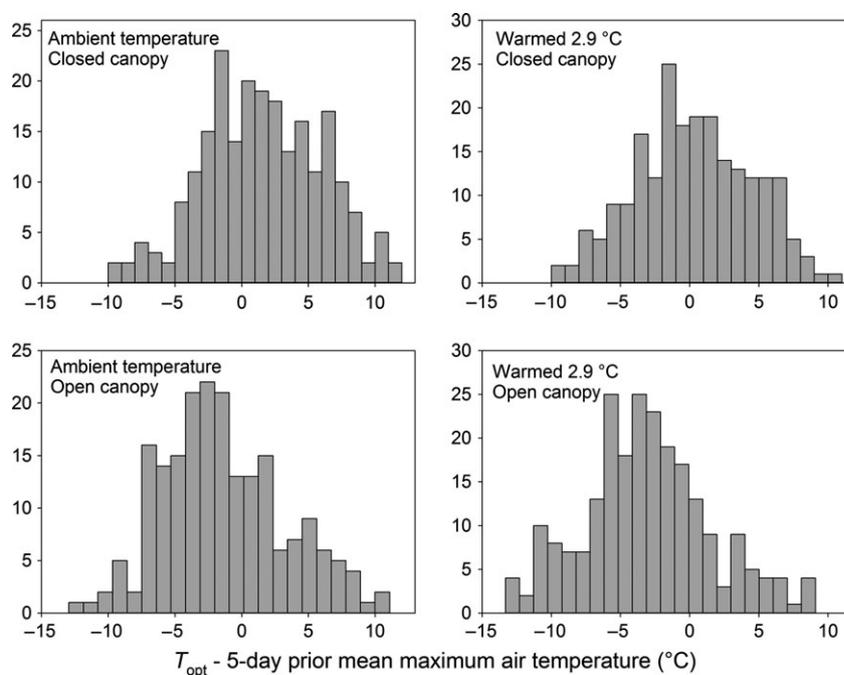
Fig. 5 The distribution of photosynthetic temperature optima (T_{opt}) minus the 5 days prior maximum air temperature under open and closed canopy and ambient and warmed 2.9 ± 0.01 °C conditions.

warmed plants. For example, the mean T_{opt} of warmed plants under open canopy conditions was 24.0 °C in 2009, 23.1 °C in 2010, and 24.3 °C in 2011, while 5 days prior mean temperatures were 24.1 °C, 23.8 °C, and 24.0 °C, respectively. In contrast, mean T_{opt} for the ambient treatment under open canopy conditions was 23.4 °C in 2009, 22.1 °C in 2010, and 23.5 °C in 2011, while 5 day prior mean temperatures were 21.6 °C, 21.3 °C, and 21.0 °C, respectively. Moreover, in both warming treatments, T_{opt} was close enough to realized temperatures that realized photosynthesis was within 90–95% of optimal (Table 8) because the shape of the photosynthesis-temperature response curve is not sharply peaked.

To determine whether leaves operate near their maximum capacity throughout the growing season (rather than just on average), we also compared T_{opt} to prior mean temperatures in early, mid-, and late summer and found that seasonal temperature differences (which themselves were modest and only varied by approximately ±4 °C) did not have a significant effect on T_{opt} overall (Table S9). This suggests that seedlings of all species in both warming treatments, in both canopy conditions, and across the growing season were close to optimizing photosynthetic temperature responses; and warmed-treatment plants were as effective at doing this as plants growing under ambient conditions. Viewed from this perspective, regardless of only a 1.1 °C difference in T_{opt} among warming treatments, acclimation to

warming was highly effective, as warmed plants came as close to optimizing photosynthesis (in terms of temperature) as the unheated ambient plants. We argue that this perspective is the more reasonable one, as there was no evidence (and no *a priori* theory) that ambient plants under current conditions would have T_{opt} that guaranteed photosynthetic rates to be near 100% of those possible.

Species and interannual differences in T_{opt} and acclimation potential

We found that acclimation capacities (measured as the difference in T_{opt} between ambient and warmed treatments) were statistically similar across all years for three species and among all species in each year, contrary to our hypothesis (H1) that boreal species would have limited acclimation capacities relative to temperate species. We made this prediction based on the idea that species growing near their warmer, lower latitude range limits where boundaries are determined in part by thermal limitations (Berry & Bjorkman, 1980; Tjoelker *et al.*, 1998; Gunderson *et al.*, 2000; Davis & Shaw, 2001) or increased levels of competition (Woodward, 1987) may be constrained in their potential to acclimate to warming, whereas species growing near their colder, higher latitude range limits may respond more strongly to warming. Instead, our results suggest that local ecotypes may be similarly adapted to local temperatures in

terms of their photosynthetic temperature sensitivity, and therefore each of the six species measured in this study, whether boreal or temperate, may have similar acclimation capacities. Whether this kind of local adaptation is common is not well understood, since the body of literature is limited and shows conflicting results. A few studies have found that T_{opt} is related to climate of origin in both intra- (Robakowski *et al.*, 2012) and interspecific comparisons (Hill *et al.*, 1988; Cunningham & Read, 2002), but others determined that there is no evidence for intra- (Teskey & Will, 1999; Gunderson *et al.*, 2000) or interspecific (Gunderson *et al.*, 2010) adaptation of T_{opt} to climatic distribution. The studies that found significant relationships either measured mature trees that had spent their entire lifespan in differing temperatures (Robakowski *et al.*, 2012) or seedlings that were collected directly from broad latitudinal ranges (Hill *et al.*, 1988; Cunningham & Read, 2002). Studies finding no relation between T_{opt} and climate of origin used seedlings purchased from commercial nurseries or propagated plants from seeds or root segments (Teskey & Will, 1999; Gunderson *et al.*, 2000, 2010), but it is unclear whether the young plants experienced differing temperature regimes prior to the start of the experiment.

The similar T_{opt} and T_{opt} acclimation capacities observed in boreal and temperate species could potentially be explained in part by leaf habit. It has been proposed that the degree of plasticity in temperature sensitivity corresponds with the range of temperatures to which foliage is typically exposed (Berry & Bjorkman, 1980; Read, 1990; Bunce, 2000). Each of the six species measured in the current study was winter deciduous and therefore exposed to similar temperature regimes given that leaf out of all species occurs within a narrow window of time in the spring. Consequently, leaves of all winter deciduous species may have similar T_{opt}s and may be limited in their ability to acclimate, leading to similar responses. A comparison with evergreen species, whose tissues are exposed to a much wider range of temperatures, would be necessary to test whether this holds true.

Because our study, along with most studies of photosynthetic acclimation potential (Hill *et al.*, 1988; Battaglia *et al.*, 1996; Teskey & Will, 1999; Gunderson *et al.*, 2000; Cunningham & Read, 2002; Dillaway & Kruger, 2010), measured juvenile trees, we are unsure whether the responses observed will translate to mature trees. Only Gunderson *et al.* (2010) made direct comparisons of acclimation potential for seedling-sapling size trees and mature trees. They found that tree size and age had no effect on acclimation responses of *Quercus rubra* (northern red oak) or *Liquidambar styraciflua* (sweetgum), suggesting that our results are similarly translatable.

We were unsure at the outset of this project whether relative differences in T_{opt} between ambient and warmed plots would remain stable or change with time, as most studies looking at photosynthetic temperature acclimation were not long-term experiments and instead examined short-term responses following abrupt changes in air temperature (Cunningham & Read, 2002; Ow *et al.*, 2008; Gremer *et al.*, 2012). We found that acclimation capacity was consistent across years and was slightly lower in 2011, likely because the degree of warming that trees were exposed to in that year was less than it had been in 2009 and 2010. The consistency in relative differences in T_{opt} across years simplifies incorporating acclimation in ecosystem to global models to improve carbon balance algorithms.

Parameter b and A_{opt}

The b parameter characterizes sensitivity to short-term fluctuations in temperature, with a lower value indicating a broader response curve and less temperature sensitivity. The lowest values of b of all the species in our study were in the two *Acer* species (Fig. 3), indicating that they may have inherently lower sensitivity to short-term temperature changes. Values of b were also significantly lower in mid- and late summer as compared to early summer, suggesting that sensitivity to changing temperatures is more acute early in the growing season. Despite these differences in the shape of the temperature response curves, the *Acer* species were no more effective at optimizing photosynthetic rates (in terms of temperature) compared to the other species measured, nor were plants less effective at optimization in early summer (Table 8) or differentially responsive to warming in terms of T_{opt} or A_{opt} (Fig. 2). However, in contrast to the similar response of T_{opt} or A_{opt} to warming for the six species studied here, response of warming to *in situ* light-saturated net photosynthetic rates averaged across the entire growing season (including periods of non-optimal conditions) did differ for species with different climate origins, with rates increasing in temperate oaks and maples and decreasing in boreal species (Reich *et al.*, 2015). All species in all years and times of year seemed to be able to acclimate and maintain T_{opt} close enough to realized temperatures that realized photosynthesis was within 90–95% of optimal. Nevertheless, rarely were plants in either temperature treatment completely acclimated to prevailing temperatures (Figs 4 and 5), indicating that the temperature responses of photosynthesis are broad enough that it is not necessary for these species to invest resources in keeping T_{opt} fully acclimated to current temperatures.

Mechanisms of photosynthetic acclimation

This study was designed to determine the extent that photosynthetic acclimation occurs in response to temperature changes similar to regional predictions of climate change, not to determine the underlying mechanisms of acclimation. Nonetheless, we can use published literature to make inferences about possible mechanisms. Models used to investigate the effect of climate warming on plant function often incorporate the Farquahar *et al.* (1980) model of C_3 photosynthesis (Medlyn *et al.*, 2002a,b; Kattge & Knorr, 2007). The Farquhar model has the potential to accurately characterize photosynthetic response to increased temperatures based on the acclimation response of the potential rate of electron transport (J_{max}) and the maximum rate of Rubisco activity (V_{cmax}). Both parameters have been shown to acclimate to plant growth temperature and thus act to limit potential reductions in photosynthetic rates (Bernacchi *et al.*, 2003; Onoda *et al.*, 2005; Kositsup *et al.*, 2009), but further research would be required to determine if this is the underlying cause of acclimation in our study.

Predicting future consequences of climate warming will require information on acclimation responses of photosynthesis, but we realize this is only one piece of the puzzle. Respiration rates of plants, soils, and microbes as well as nutrient cycling will also be modified by increasing temperatures, and will be affected indirectly by the decline in soil moisture that will likely accompany higher temperatures. Phenological responses such as earlier budbreak in the spring may increase growing season length, while potentially causing plant-herbivore asynchrony if herbivores respond differently to changes in climate as seen in B4WarmED (Schwartzberg *et al.*, 2014). Assuming that these, or other, responses are species-specific, relative rates of growth and competitiveness may also change.

Most empirical succession models assume that future vegetation will follow shifts in climate (Davis & Shaw, 2001) based on projected shifts in 'suitable' habitats (Iverson & Prasad, 1998), defined by the climate envelope of each species. These models sometimes predict considerable changes in species distributions, including poleward migrations and species eliminations from the warmest parts of a range (Pastor & Post, 1988; Schenk, 1996; Iverson & Prasad, 1998). However, locations into which a species might migrate may not contain the same suite of correlated conditions. These variations would most likely affect tree species during early life stages, since they are more sensitive to environmental conditions when small. The degree to which forest composition and productivity are affected by warming, as well as the relative range of 'suitable' habitats (Iverson & Prasad, 1998) species will be able to survive in, will partly depend on the ability of species to acclimate (Ghannoum & Way, 2011). In this study, the roughly equivalent differences between T_{opt} and realized temperatures under both ambient and warmed conditions led to realized photosynthetic rates that were 90–95% of optimal in both warming treatments, demonstrating that plants subjected to experimental warming were as well-matched to their environment as ambient plants. Thus, despite the modest shift in T_{opt} of 1.1 ± 0.21 °C in response to the warming treatment, acclimation was relatively complete, suggesting that direct negative impacts of modest climate warming on photosynthesis will be ameliorated as plants come near to optimizing photosynthesis with respect to temperatures experienced. However, indirect effects mediated through alterations of soil moisture will likely occur independent of but simultaneous with these thermal response shifts. Thus, omitting temperature acclimation from physiological and ecosystem models could result in predictions of carbon balance, ecosystem productivity, and species regeneration that are less realistic than can be achieved given the state of collective knowledge.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationship between mean ($\pm$ SE) photosynthetic rates measured at temperature optima (A_{opt}) and light-saturated photosynthetic rates (A_{max}) measured *in situ*. A_{opt} was measured on detached foliage (3–4 h following removal from the tree, on average) and A_{max} was measured on attached foliage.

Figure S2. Mean ($\pm$ SE) rates of leaf diffusive conductance at six leaf temperatures (12, 17, 22, 27, 32, 37 °C) for juvenile trees of four temperate and two boreal species. Open symbols, dashed lines represent species averages from the open canopy treatments and closed symbols, solid lines represent species averages from the closed canopy treatments.

Figure S3. Mean ($\pm$ SE) internal leaf CO_2 concentrations at six leaf temperatures (12, 17, 22, 27, 32, 37 °C) for juvenile trees of four temperate and two boreal species. Open symbols, dashed lines represent species averages from the open canopy treatments and closed symbols, solid lines represent species averages from the closed canopy treatments.

Figure S4. Mean ($\pm$ SE) rates of net photosynthesis at six leaf temperatures (12, 17, 22, 27, 32, 37 °C) for juvenile trees of four temperate and two boreal species. Open symbols, dashed lines represent species averages from the open canopy treatments and closed symbols, solid lines represent species averages from the closed canopy treatments.

Figure S5. Mean ($\pm$ SE) rates of photosynthesis divided by internal leaf CO_2 concentrations at six leaf temperatures (12, 17, 22, 27, 32, 37 °C) for juvenile trees of four temperate and two boreal species. Open symbols, dashed lines represent species averages from the open canopy treatments and closed symbols, solid lines represent species averages from the closed canopy treatments.

Table S1. ANOVA results for photosynthetic temperature optima (T_{opt}) of *Acer rubrum*, *Betula papyrifera*, and *Populus tremuloides* measured in 2009, 2010, and 2011.

Table S2. ANOVA results for photosynthetic rates at T_{opt} (A_{opt}) of *Acer rubrum*, *Betula papyrifera*, and *Populus tremuloides* measured in 2009, 2010, and 2011.

Table S3. ANOVA results for the *b* parameter (which denotes the shape of the temperature response curve) of *Acer rubrum*, *Betula papyrifera*, and *Populus tremuloides* measured in 2009, 2010, and 2011.

Table S4. ANOVA results for photosynthetic temperature optima (T_{opt}) of all species by year in which the site, species' southern (lower latitude) range limits, overstory condition, warming treatment, and all 2- and 3-way interactions were the sources of variance.

Table S5. ANOVA results for photosynthetic rates at T_{opt} (A_{opt}) of all species by year in which the site, species' southern (lower latitude) range limits, overstory condition, warming treatment, and all 2- and 3-way interactions were the sources of variance.

Table S6. ANOVA results for the *b* parameter (which denotes the shape of the temperature response curve) of all species by year in which the site, species' southern (lower latitude) range limits, overstory condition, warming treatment, and all 2- and 3-way interactions were the sources of variance.

Table S7. Mean ($\pm$ SE) photosynthetic temperature optima under ambient and +2.9 $\pm$ 0.01 °C warming by year and canopy condition. Temperature data shown are averaged across the 5 days prior to the measurement day. Mean air temperatures represent the time of day when plants are most photosynthetically active (09:00–15:00 hours).

Table S8. Mean ($\pm$ SE) photosynthetic temperature optima under ambient and +2.9 $\pm$ 0.01 °C warming by species and canopy condition. Temperature data shown are averaged across the 5 days prior to the measurement day. Mean air temperatures represent the time of day when plants are most photosynthetically active (09:00–15:00 hours).

Table S9. Mean ($\pm$ SE) photosynthetic temperature optima under ambient and +2.9 $\pm$ 0.01 °C warming by time of year and canopy condition. Temperature data shown are averaged across the 5 days prior to the measurement day. Mean air temperatures represent the time of day when plants are most photosynthetically active (09:00–15:00 hours).