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Thermal acclimation of shoot respiration in an Arctic woody plant species subjected to 22 years of warming and altered nutrient supply

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Abstract

Despite concern about the status of carbon (C) in the Arctic tundra, there is currently little information on how plant respiration varies in response to environmental change in this region. We quantified the impact of long-term nitrogen (N) and phosphorus (P) treatments and greenhouse warming on the short-term temperature (T) response and sensitivity of leaf respiration (R), the high-T threshold of R, and associated traits in shoots of the Arctic shrub *Betula nana* in experimental plots at Toolik Lake, Alaska. Respiration only acclimated to greenhouse warming in plots provided with both N and P (resulting in a ~30% reduction in carbon efflux in shoots measured at 10 and 20 °C), suggesting a nutrient dependence of metabolic adjustment. Neither greenhouse nor N+P treatments impacted on the respiratory sensitivity to T (Q_{10}); overall, Q_{10} values decreased with increasing measuring T, from ~3.0 at 5 °C to ~1.5 at 35 °C. New high-resolution measurements of R across a range of measuring Ts (25–70 °C) yielded insights into the T at which maximal rates of R occurred (T_{max}). Although growth temperature did not affect T_{max}, N+P fertilization increased T_{max} values ~5 °C, from 53 to 58 °C. N+P fertilized shoots exhibited greater rates of R than nonfertilized shoots, with this effect diminishing under greenhouse warming. Collectively, our results highlight the nutrient dependence of thermal acclimation of leaf R in *B. nana*, suggesting that the metabolic efficiency allowed via thermal acclimation may be impaired at current levels of soil nutrient availability. This finding has important implications for predicting carbon fluxes in Arctic ecosystems, particularly if soil N and P become more abundant in the future as the tundra warms.

Keywords: Arctic tundra, *Betula nana*, nitrogen, phosphorus, Q_{10}, stems

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Introduction

Rapid warming threatens to alter how carbon (C) is cycled and stored in Arctic tundra ecosystems (Serreze *et al.*, 2000; Shaver *et al.*, 2000; Wookey *et al.*, 2009). The expansion of woody shrub species, one of the most ecologically significant impacts of warming, is increasingly modifying the tundra landscape, influencing both C exchange and climate (Myers-Smith *et al.*, 2011; Bonfils *et al.*, 2012). Evidence from experimental and observational studies shows elevated growth temperature (T) and relaxed soil nutrient limitation promote woody shrub dominance and can greatly alter tundra ecosystem net primary productivity (Shaver *et al.*, 2001; Mack *et al.*, 2004; Walker *et al.*, 2006; Elmendorf *et al.*, 2012). However, to date, little information is available on the underlying physiology controlling C fluxes in woody shrubs, and how this may vary under long-term warming and increased nutrient availability. In particular, we lack crucial data on leaf and stem respiration (R) of woody shrubs and how it varies with T, which can be used to constrain current estimates of the tundra C exchange and aid in the prediction of future rates of C exchange in the rapidly changing Arctic.

As in many low productivity ecosystems, C loss via R can represent a large proportion of C balance in the Arctic, and thus, small variations in R may influence individual species productivity, plant community composition, and overall C storage in Arctic vegetation (Heskel *et al.*, 2013; Hicks-Pries *et al.*, 2013). Both foliar and ecosystem respiratory carbon release can be altered by long-term increases in soil nutrient availability and warming in tundra (Johnson *et al.*, 2000; Arens *et al.*,

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2008; Huemmrich *et al.*, 2010; Heskel *et al.*, 2013). Increases in soil nitrogen (N) availability in this typically N-limited landscape (due to elevated rates of mineralization in warmer soils and to a lesser degree, increased rates of atmospheric deposition) can translate into increased leaf and stem tissue N, influencing rates of plant metabolism and growth and in turn, respiratory C release (Shaver *et al.*, 2001; Weintraub & Schimel, 2005; Heskel *et al.*, 2012). Little is known about leaf and stem respiratory response to N in tundra woody shrub species, nor about the extent to which this *R* response depends on the supply of potentially co-limiting nutrients such as phosphorus (P). While a warmer climate may indirectly increase N availability and likely also *R*, warming itself may be expected to decrease respiratory rates if *R* acclimates to high growth temperatures. Thermal acclimation adjusts rates of respiratory metabolic activity and subsequent C release to compensate for changes in growth temperature and has been identified in Arctic tundra ecotypes along latitudinal and natural environmental gradients (Billings *et al.*, 1971; Chapin & Chapin, 1981; Chapin & Oechel, 1983) and in species grown under experimentally warmed conditions (Atkin & Tjoelker, 2003; Kornfeld *et al.*, 2012). The influence of nutrient availability on thermal acclimation is a complex issue; in roots, acclimation can occur in both N-deficient and N-rich soils (Atkinson *et al.*, 2010), whereas the degree of photosynthetic acclimation may be limited in low-N environments (Martindale & Leegood, 1997). The effect of N supply on thermal acclimation of leaf and/or stem *R* remains unclear. Also, little is known about the impact of altered P availability (either alone or in conjunction with N) on thermal acclimation of *R* in shoots. This may be considerable given the integral role of inorganic P in multiple cellular pathways associated with *R*, as well as the acclimation of photosynthesis, which is closely coupled to acclimation of *R* (Stitt & Hurry, 2002; Atkin *et al.*, 2006; Plaxton & Podesta, 2006). A clear need exists to quantify respiratory fluxes, given the substantial fraction of carbon released by *R* and the unknown, interactive effects of warming and nutrient supply on the C efflux of stems and leaves.

A further unknown is the extent to which warming and nutrient supply influence the instantaneous thermal response and Q_{10} of *R* in tundra plants. Respiratory Q_{10}, the proportional increase in *R* per 10 °C rise in temperature, declines with increasing measurement *T*, with *R*-*T* curves typically measured up to a maximum of ~35 °C predicting a Q_{10} of 1.0 (i.e. where *R* reaches a maximal value, R_{max}) to occur at ~48 °C (Tjoelker *et al.*, 2001). Although this trend appeared to hold across species and biomes, considerable variation in Q_{10} values at any one measuring *T* occurs (Tjoelker *et al.*, 2001; Atkin *et al.*, 2005). Despite knowledge of a dynamic *T*-dependent Q_{10}, many terrestrial C models employ a fixed, constant Q_{10}, which can lead to under-prediction of respiratory rates at low *T* and over-prediction at high *T* (Tjoelker *et al.*, 2001; Wythers *et al.*, 2005; Smith & Dukes, 2013) and the inverse (i.e. over-prediction at low *T* and under-prediction at high *T*) is reported in O'Sullivan *et al.* (2013). Failure to account for the *T* dependence of Q_{10} and environmental influences on respiratory sensitivity to *T* can lead to inaccurate estimations of plant carbon release at the leaf, ecosystem, and global scales (Wythers *et al.*, 2005; King *et al.*, 2006; Atkin *et al.*, 2008; Smith & Dukes, 2013). Further, the short-term *T* response of *R* can vary seasonally and under altered environmental conditions that impact substrate availability (Atkin *et al.*, 2000; Atkin & Tjoelker, 2003; Ow *et al.*, 2010; O'Sullivan *et al.*, 2013). Though previous studies were often confined to few measurement *T*s over a limited range, thus limiting the characterization of the short-term *T* response, recent applications of broad-scale and high-resolution measurement of *R* under continuously increasing temperatures can provide information on both the *T* dependence of Q_{10} and R_{max} (Hüve *et al.*, 2006, 2012; O'Sullivan *et al.*, 2013). Such measurements may thus afford a more detailed analysis of respiratory C exchange and its nuances across species and environmental conditions.

Arctic plants grown under elevated growth *T* exhibit evidence of acclimation through the down-regulation of foliar *R* when measured at a common *T* (Shaver *et al.*, 1998; Kornfeld *et al.*, 2012; Heskel *et al.*, 2013). The *T* response of *R* in tundra vegetation under varied growth conditions is not well characterized, despite the clear implications of climate change for vegetation and carbon storage in this region (Billings *et al.*, 1971). Nevertheless, thermal growth history has the potential to alter the shape of the respiratory *T* response curve and the *T* response of Q_{10} in Arctic plants. For example, one possibility is that plants grown under warmed conditions will exhibit a higher *T* at which R_{max} occurs (i.e. T_{max}). Also, the individual and combined influence of additional soil N and P, under warmed or ambient growth conditions, is not well characterized. High soil N availability is likely to alter respiratory capacity, substrate supply, and demand for respiratory products, which, in concert with other factors, collectively influence Q_{10} (Atkin & Tjoelker, 2003; Atkin *et al.*, 2005). In regions where N is typically limiting to productivity, like the Arctic tundra, the T_{max} of plant *R* may increase due to increased enzyme capacity under fertilization, whereas in less N-limited biomes, this impact may be less pronounced (Chapin, 1983; Chapin & Shaver, 1985).

As warming continues to alter Arctic tundra ecosystems, it is critical to quantify the T response of R in woody shrub species to accurately estimate carbon fluxes. Herein, we address the following objectives: (i) to compare the pattern of T response between stems and leaves; (ii) to examine the extent of variation in R (measured at a common T) and associated traits as a result of altered growth T and nutrient supply; (iii) to determine how nutrient supply and growth T modify the T sensitivity of R across a broad range of Ts; and finally, (iv) to quantify maximum respiration rates and the Ts at which these rates occur and how they may be affected by growth T and nutrient supply. We hypothesize that a long-term warmed growth environment is likely to alter rates of R according to thermal acclimation, though may in turn enable a higher values of T_{max}. We also hypothesize that increased N and P availability will increase rates of R, measured both at a common temperature and at a thermal limit (R_{max}), as possible enzyme limitation will be relaxed in these conditions. We addressed these objectives with high-resolution measurements of R in shoots, leaves and woody stems of *Betula nana*, a deciduous shrub that is common across the Arctic, sampled from global change experimental plots that elevated growth T and soil N and P for 22 years at the Arctic Long Term Ecological Research site at Toolik Lake, Alaska.

Materials and methods

Field site, long-term treatment plots, and plant tissue sampling

The focal species for our study, the deciduous woody shrub *Betula nana nana* L. ('dwarf birch'), is abundant in Northern Alaska and widespread over much of the Arctic region. This study took place in June 2010 at the Arctic Long-Term Ecological Research (LTER) field site near Toolik Lake (68°38′N, 149°36′W, elevation 760 m) on the north slope of Alaska, USA located 254 km north of the Arctic circle. All leaf and stem tissue samples were collected from experimental plots in moist acidic tundra (MAT) established and maintained by the LTER since 1988 [similar to an older experiment described by Chapin *et al.* (1985)]. The MAT site consists of four randomized blocks of 5 × 20 m treatment plots separated by a 1 m buffer and arrayed on a slightly sloped (3–4% slope), poorly drained hillside. The warming treatment plots employ wood-framed greenhouses that are covered with transparent 0.15-mm plastic sheeting and passively increase air T by approximately 5 °C during the growing season, as described in the study by Chapin *et al.* (1995). In the fertilized plots, 10 g m^{-2} of granular NH_4NO_3-N and/or 5 g m^{-2}- of granular P_2O_5-P is applied each year in early June after snow melt, with N-only plots treated only to the N-fertilizer, P-only plots treated only with the P-fertilizer, and combine NP plots treated to the same amounts of N- and P-fertilizers together. In the GH+NP treatment plots, tundra vegetation is enclosed by the same greenhouses as in the GH plots and the same fertilizer treatment is applied as in the NP plots.

Measuring short-term temperature response of dark respiration

To examine the effect of long-term growth conditions on the short-term T response of respiration (R), we sampled mature, sun-exposed shoots of *B. nana* from the LTER manipulated plots. For this study, we define 'shoots' as a 5–10 cm sample from the terminus of a small shoot growing from a longer apical meristem, with approximately 10–20 leaves (25–50 cm^2 leaf area), and care was taken to ensure that all sampled shoots were light-exposed, contained fully expanded leaves and were of a similar size and age. Prior to measurement, shoots were weighed for fresh mass. Shoots were kept in darkness for approximately 30 min prior to measurement and then placed in a 15.5 × 11.0 × 6.5 cm water-jacketed, glass-topped aluminum chamber that was then covered by a dark cloth (Von Caemmerer & Hubick, 1989; Hüve *et al.*, 2012; O'Sullivan *et al.*, 2013) Two fans mixed air within the chamber (Micronel, Fellbrook, CA, USA), and chamber T was maintained and controlled by a connected, programmable, circulating water bath (MRC300, Melcor, Trenton, NJ, USA). Leaf T was continuously monitored via a small gauge wire copper-constantan thermocouple pressed against the lower surface of the leaf, which was attached to a LI-6400 external thermocouple adaptor (LI6400-13, Li-Cor Inc., Lincoln, NE, USA) that enabled the recording of leaf T by a LI-6400*xt* portable gas-exchange system (Li-Cor Inc.). We plumbed the exiting air-stream from the darkened, water-jacketed chamber containing the shoots into the 'sample' gas line and infra-red gas analyzer (IRGA) of the LI-6400*xt* [fitted with an empty, sealed 3 × 2 cm cuvette (LI-6400-02B)]. Net CO_2 exchange (respiration) from the continuously warming, darkened cuvette was calculated via comparison of the 'sample' IRGA values to the 'reference' IRGA values. Flow rates through the water-jacketed chamber (700 mol s^{-1}) and (CO_2) of the incoming air were controlled via the LI-6400*xt* console flow meter and LI-6400-01 CO_2 mixer. Incoming air was passed through the LI-6400*xt* desiccant column to control relative humidity.

After incubating in the darkened chamber for 30 min, dark respiration of shoots (sampled from CT, N, P, NP, GH, and GH+NP treatment plots) was measured first at 10 °C, and then, the shoots were removed from the chamber and kept in darkness as the chamber warmed to 20 °C (10–15 min). The same shoots were then placed back in the darkened chamber to record rates of R at 20 °C. After the shoot R measurement at 20 °C, leaves were excised from the same shoots, and remaining leaf-less stems were then reinserted into the chamber and stem R was measured at 20 °C and then again after cooling, at 10 °C. Rates of R in shoots and stems were recorded after 10 min at either 10 or 20 °C. The difference between R recorded from whole shoots and stems only was attributed to leaves.

To initiate the R-T response measurements (made on shoots from a subset of the previously sampled plots: CT, NP, GH

and GH+NP), a sampled shoot of *B. nana* was placed into the chamber in darkness at ambient *T* (approximately 20 °C), and subsequently cooled to 5 °C prior to the start of each measurement run. After cooling, chamber air *T* was heated at a rate of 1°C min^{-1}, though evaporative water loss resulted in a slower rate of increase in shoot/leaf *T*. Rates of net CO_2 exchange were recorded every 30 s as the shoot was warmed to 35 °C. Earlier experiments using CT plot shoots revealed that the resultant *R*-*T* curves were fully reversible over the 5–35 °C range (i.e. no hysteresis effect of warming; data not shown).

A separate set of measurements was made to quantify maximum *R* rates (R_{max}) and the high *T* at which maximal rates of shoot (both leaves and stems) *R* occurred (T_{max}) in a subset of samples from CT, NP, GH, and GH+NP treatment plots. These measurements required the substitution of the MRC300 water bath used in the previously described *T* response measurements with a thermostatically controlled circulator and micropressor (model F32-HL, JULABO Labortechnik GmbH, Seelbach, Germany), where air *T* within the chamber was monitored using an integrated Pt100 Lemosa-type stainless steel external sensor (JULABO, Labortechnik GMBH). Similar to the earlier measurements, *T* inside the cuvette increased from 25 °C to 70 °C at a rate of ~1 °C min^{-1}, driving an increase in rates of dark *R* until a peak rate (R_{max}) was reached at a specific *T* (T_{max}), after which *R* rates decreased. Leaf *T* and net CO_2 exchange were measured and recorded according to the same methods for the previous low-*T* (i.e. 5–35 °C) measurements, and flow rate, (CO_2), and relative humidity were controlled in the same manner via the LI-6400*xt*. At the end of each curve experiment, measured leaves and stems were oven-dried for a minimum of 2 d at 70 °C and then weighed so that rates could be expressed on a dry mass basis (nmol CO_2 g^{-1} s^{-1}). Prior to drying, leaf area was measured (LI-3100C leaf area meter, Li-Cor Inc.).

Quantifying temperature response curves

High-resolution *T* response curves measured between 5 and 35 °C were fitted with curve fitting software (Excel Solver function, Microsoft Excel 2007, Microsoft Corporation, Richmond, WA, USA) maximizing the r^2 value of the relationship between measured and modeled data. Modeled *R* was calculated using the a third-order polynomial fit, from log values of *R* versus *T*, according to the following equations (as used in Atkin *et al*., 2005 and O'Sullivan *et al*., 2013):

$$\log_e(R) = a + bT + cT^2 \quad (1)$$

and

$$R = e^{a+bT+cT^2} \quad (2)$$

In these equations, *T* is leaf *T* (°C), and *a*, *b*, and *c* are the polynomial coefficients that describe the *T*-response of the natural log of *R*. The differential equation of Eq. 2 can be then applied to model the Q_{10} at any given value of *T*, as follows:

$$Q_{10} = e^{10*(b+2cT)} \quad (3)$$

The *T* at which maximum rates of *R* is reached, T_{max}, was predicted from the polynomial fit of the 5–35 °C *R*-*T* curve and then compared with directly measured T_{max} from the subsequent high-*T* curves that surveyed shoot *R* across a larger range of temperatures.

Leaf structure and chemistry

Leaf fresh (FM) and dry mass (DM) and area were used to calculate leaf structural traits: leaf dry mass per area (L_A, g DM m^{-2}), leaf fresh mass per area (F_A, g FM m^{-2}), leaf, stem, and shoot dry matter content (*DMC*, g DM g FM^{-1}) and the ratio of leaf dry mass to shoot dry mass (L_M/St_M). F_A is a good indicator of leaf thickness (Dijkstra & Lambers, 1989). Subsequently, leaves and stems were ground in a ball mill and analyzed for tissue N and P using a Technicon Auto-analyzer II (Bran & Luebbe Pty. Ltd, Norderstedt, Germany) and Kjeldahl acid digests (Allen, 1974). Ground tissue material was also used to analyze soluble sugars, starch, and the resulting total nonstructural carbohydrate content, as described in Loveys *et al.* (2003).

Statistical analyses

Respiratory measurements and leaf traits were analyzed using a one-way analysis of variance (ANOVA), assigning treatment as factors. Differences between treatments were determined with a post hoc Tukey's test. To evaluate the influence of leaf and shoot structure on *R* rates at 10 and 20 °C, we compared the r^2 and *P* values of step-wise linear regression models of single trait variables on fluxes. Statistical analyses were performed in R (v2.7.0, The R Foundation for Statistical Computing). For all analyses, *P*-values <0.05 were considered significant.

Results

Long-term treatment impacts on leaf structure and chemistry

Sampled leaf dry mass per unit area (L_A) did not vary significantly among the growth treatments (Table 1); however, leaf fresh mass per area values (F_A) exhibited directional treatment effects, with less thick leaves under long-term warming, and thicker leaves when P was added. Values of leaf, stem, and shoot DMC were lowest when grown under long-term N and P fertilization, and highest under long-term warming only, though differences were only significant for stems and shoots (Table 1). The ratio of leaf to shoot dry mass, L_M/St_M, did not vary significantly, but values were greatest under warming (Table 1). Nitrogen and phosphorus concentrations were greatest in shoots, stems, and leaves under long-term fertilization, as would be expected (Fig. 1a and b, Table 2), though this effect was modified in the presence of long-term warming, where

Table 1 Impact of long-term environmental treatments at Toolik Lake, Alaska, on average (±SE, n = 3-4) values of leaf dry mass per unit area (L_A), leaf fresh mass per unit leaf area (F_A), leaf dry matter content (*DMC*), stem *DMC*, shoot *DMC* and the ratio of leaf dry mass to stem dry mass (L_M/St_M) in sampled individual shoots. $P > 0.05$ were considered not significant (i.e. homeostasis of values across treatments for that parameter), and alphabetical notation denotes significance

	Treatment						*P*-value
Parameter	CT	+P	+N	+NP	GH	GH+NP	
L_A (g_{DM} m^{-2})	76.5 ± 5.7^{a}	80.1 ± 1.7^{a}	71.5 ± 3.0^{a}	79.5 ± 3.3^{a}	72.7 ± 1.3^{a}	71.7 ± .9^{a}	ns
F_A (g_{FM} m^{-2})	202.1 ± 7.3^{ab}	217.0 ± 5.6^{ab}	194.1 ± 4.7^{ab}	226.1 ± 7.7^{b}	182.2 ± 4.7^{a}	186.0 ± 17.9^{ab}	*
Leaf DMC (g_{DM} g_{FM}^{-1})	0.38 ± 0.02^{a}	0.37 ± 0.01^{a}	0.37 ± 0.01^{a}	0.35 ± 0.01^{a}	0.40 ± 0.02^{a}	0.39 ± 0.03^{a}	ns
Stem DMC (g_{DM} g_{FM}^{-1})	0.49 ± 0.01^{ab}	0.43 ± 0.01^{db}	0.50 ± 0.02^{ab}	0.40 ± 0.01^{dc}	0.52 ± 0.02^{ab}	0.43 ± 0.02^{bcd}	***
Shoot DMC (g_{DM} g_{FM}^{-1})	0.43 ± 0.01^{ab}	0.40 ± 0.01^{ab}	0.42 ± 0.02^{ab}	0.38 ± 0.01^{a}	0.45 ± 0.02^{b}	0.41 ± 0.02^{ab}	*
L_M/St_M ratio	1.16 ± 0.14^{a}	1.24 ± 0.09^{a}	1.27 ± 0.10^{a}	1.28 ± 0.15^{a}	1.47 ± 0.17^{a}	1.48 ± 0.19^{a}	ns

CT, control treatment; GH, greenhouse; +N, high nitrogen treatment, +P, high phosphorus treatment, +NP, high N plus P treatment; GH+NP, greenhouse-grown plants provided with high N and P. One-way analysis of variance *P*-values are shown for comparisons among treatments.
***$P < 0.001$; *$P < 0.05$.

N and P values were similar to those in leaves from the control plots. The ratio of N to P was lowest under P addition, and only sensitive to treatment in leaves and shoots, not stems (Fig. 1c; Table 2). Values of mean N/P in stems and leaves were only greater than 10 under the N-only fertilization, and not in either of the combined nutrient treatments (NP or GH+NP). Leaf, stem, and shoot soluble sugar concentrations were consistent across treatments (Fig. 1d) and did not vary significantly (Table 2). Starch concentration showed greater variation across warming and nutrient treatments, with lower values under N, P, and the combined fertilizer treatment in leaves (Fig. 1e). Conversely, in stems, warming significantly lowered starch concentrations compared to control and fertilized samples, and a similar, though not significant, trend was observed in shoots. Treatment effects on total nonstructural carbohydrates mirrored those on soluble sugars, given their higher relative contribution to its calculation (Fig. 1f; Table 2).

Environmental and structural controls on shoot respiration

Respiration measured at 20 and 10 °C was greatest in shoots, stems, and leaves grown under long term N and P addition, though this effect was only significant across tissue types in rates measured at 20 °C; for measurements made at 10 °C, only differences in shoots were significant (Fig. 2; Table 2). The interaction of warming negated the positive effect of fertilization on R at either measurement T – rates from shoots and leaves sampled from the GH+NP plots showed a marked decrease compared to those from NP plots (Fig. 2). Warming alone had no effect on R, nor did P alone – and the slight increases in R under N addition were further enhanced under the combined fertilizer treatment, suggesting N-limitation. While similar general trends were observed across treatment conditions, rates of R were greatest in leaves, likely attributable to the greater available concentrations of leaf N. One-way ANOVA showed no significant differences among treatments in Q_{10} values (Table 2), and thus, overall Q_{10} values (across the 10–20 °C range) were 2.33 ± 0.07 (shoot), 1.80 ± 0.07 (stem) and 3.13 ± 0.28 (leaf).

An additional motivation of this study was to determine which leaf, stem, and shoot structural and chemical traits best correlate with R rates in shoots of *B. nana*. Linear regression fits of shoot R at 10 and 20 °C (y-axis) and shoot traits (x-axis) were ranked based on r^2 and P values (Table 3). Parameters indicated that in a one-component model, shoot DMC accounted for 56% and 47% of variation in shoot R_{20} and shoot R_{10}, respectively (shoot R_{20} = 56.1–87.50*Shoot DMC; shoot R_{10} = 24.60–40.63*Shoot DMC), with R rates at 20 °C decreasing with increasing shoot DMC (Fig. 3). Adding other parameters to the model did not significantly increase the explanatory power, suggesting that variations in shoot R_{20} and shoot R_{10} can be largely explained by shoot DMC, with variations in shoot DMC being underpinned by variations in the DMC of both stems and leaves. Leaf fresh mass per unit area (F_A) when correlated with shoot DMC also yielded a

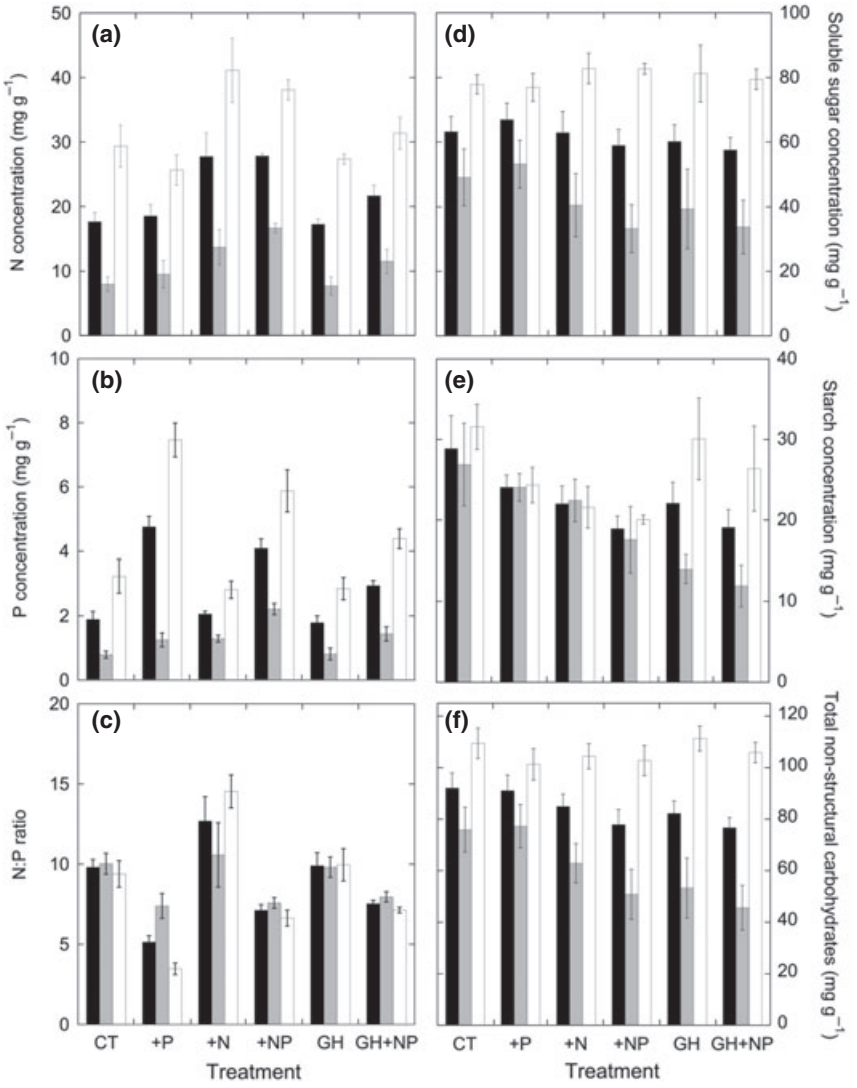

Fig. 1 Impact of long-term environmental treatments at Toolik Lake, Alaska, on the concentration (mg g^{-1}) of (a) total nitrogen, (b) phosphorus, (c) N/P ratios, (d) concentration (mg g^{-1}) of soluble sugars (i.e. sucrose, fructose, and glucose), (e) starch, and (f) total nonstructural carbohydrates (i.e. soluble sugars and starch) of *B. nana* stems, leaves and shoots (the latter being calculated from relative concentrations in stems and leaves) (n = 4, ±SE). CT, control treatment; GH, greenhouse; +N, high nitrogen treatment, +P, high phosphorus treatment, +NP, high N plus P treatment; GH+NP, greenhouse grown plants provided with high N and P. See Table 2 for statistical analysis results, alphabetical notation of statistical results were not included for clarity.

strong negative relationship (r^2 = 0.489, P < 0.001; Fig. 3). When individual components of DMC were regressed to shoot DMC, strong positive relationships were observed (Fig. 3); stem DMC co-varied with shoot DMC (r^2 = 0.825, P < 0.0001) to a stronger degree than leaf DMC (r^2 = 0.551, P < 0.0001), likely due to its greater contribution to DMC in terms of mass.

Temperature response of shoot R (measured from 5 °C to 35 °C and then modeled from Eqs 1 and 2) increased in amplitude in *B. nana* shoots grown under long term NP addition (Fig. 4a). Similar to the effect observed from 'snapshot' R values at 10 and 20 °C, elevated growth T limited the increasing impact of fertilization, with rates of R under warming lower than those of shoots grown in control plots. Shoots grown under warming alone did not exhibit the same degree of effect on the R-T curve. The T dependence of Q_{10} between 5 and 35 °C (Fig. 4b), though dynamic within the measurement T range, did not vary significantly in shoots grown under different long-term environmental treatments.

Predicted T_{max} from the modeled polynomial R-T curves (Fig. 4a) was highest in shoots grown under warming (GH = 56.0 ± 4.1 °C, GH+NP = 51.3 ± 1.3

Table 2 Results of analysis of variance tests for differences among the six long-term environmental treatments for tissue (shoots, stems, and leaves) chemical composition parameters, respiration rates (measured at two temperatures) and corresponding Q_{10} values of respiration. Significance values are shown. $P > 0.05$ were considered not significant (i.e. homeostasis of values across treatments for that parameter). See Figs 1 and 2 for values

	Tissue type		
Parameters	Shoot	Stem	Leaf
Nitrogen	**	*	**
Phosphorus	***	***	***
N/P ratio	***	0.116	***
Soluble sugars	0.814	0.575	0.927
Starch	0.108	*	0.183
TNC	0.243	0.113	0.902
R at 10 °C	**	0.196	0.114
R at 20 °C	**	*	*
Respiratory Q_{10}	0.377	0.535	0.616

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

°C), lowest in shoots grown under NP treatment only (48.8 ± 2.2 °C), with control shoots falling between these values (52.8 ± 2.8 °C). One-way ANOVA found no differences between these predicted T_{max} values, and thus, the overall mean predicted T_{max} for *B. nana* shoots was 52.2 ± 1.5 °C. However, actual measured values of T_{max} acquired from the subsequent high-T response curves (Fig. 5), differed from the predicted values. Unlike estimates from the 5–35 °C T response, shoots grown in NP plots had higher actual T_{max} ($P < 0.05$; NP = 58.4 ± 0.5 °C; GH+NP = 57.8 ± 0.8 °C) than shoots from unfertilized plots (CT = 49.3 ± 1.8 °C; GH = 53.3 ± 1.7 °C). There was no evidence showing a long-term elevated temperature effect on actual T_{max} values, unlike that predicted from the earlier 5–35 °C modeled R-T curves. Maximum R rates (Fig. 5) at T_{max} were significantly lower in shoots grown under long-term warming (GH = 40.7 ± 5.2 nmol g^{-1} s^{-1}; GH+NP = 54.1 ± 2.3 nmol g^{-1} s^{-1}) compared with those grown in CT (57.2 ± 5.4 nmol g^{-1} s^{-1}) and fertilization only plots (73.2 ± 3.7 nmol g^{-1} s^{-1}; Fig. 6). Further, fertilization substantially increased R_{max} (Fig. 6), which corresponds with findings from the lower T response curve (Fig. 5a).

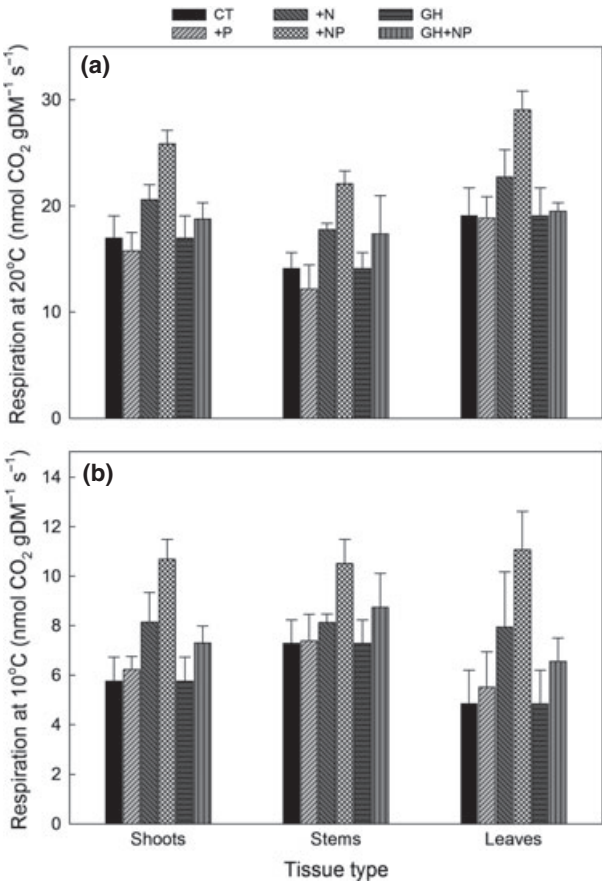

Fig. 2 Rates of dark respiration (R, nmol CO_2 gDM^{-1} s^{-1}) in whole shoots, stems, and leaves of *B. nana* (n = 4, ±SE) grown under long-term environmental treatments. Rates of R were measured at (a) 20 °C and (b) 10 °C. Measurements were initially made at 20 °C and then 10 °C in whole shoots, followed by measurements at both temperatures for stems alone (following removal of leaves from the shoot). Rates of leaf R were calculated from using data on specific rates of shoot and stem R, and relative amounts of stem and leaf dry mass in each shoot (see L_M/St_M ratios in Table 1). CT, control treatment; GH, greenhouse; +N, high nitrogen treatment, +P, high phosphorus treatment, +NP, high N plus P treatment; GH+NP, greenhouse-grown plants provided with high N and P. See Table 2 for statistical analysis results.

Discussion

Our study examined the variation in structural and chemical traits and T response of R in shoots of *B. nana* grown under long-term soil fertilization and warming treatments in Arctic Alaska. The study took place in the broader context of characterizing plant C efflux and metabolic controls in an increasingly dominant tundra species under current and predicted future consequences of climate change. Our findings suggest a significant thermal acclimation of R in *B. nana* shoots that is mediated by soil nutrient status, and environmental effects on respiratory T thresholds.

Cross-tissue accordance in environmental effects. Our study presents the first measurements of dark R in both stems and leaves in *B. nana* under long-term environmental treatments. Previous studies have focused on

Table 3 Bivariate relationships among leaf traits for *B. nana* shoots sampled from six long-term environmental treatments. Relationships between shoot respiration measured at two temperatures (20 and 10 °C) on the *y*-axis, and a range of structural and chemical composition traits on the *x*-axis are shown. Coefficients of determination (r^2) and significance values (P) of each bivariate relationship are shown. $P > 0.05$ was considered not significant (i.e. there was no significant relationship between the *x*- and *y*-axis parameter). *x*-axis leaf traits: M_A: leaf dry mass per unit area; F_A: leaf fresh mass per unit area; leaf DMC: leaf dry mass per unit leaf fresh mass; stem DMC: stem dry mass per unit stem fresh mass; shoot DMC: shoot dry mass per unit shoot fresh mass; shoot (sugar): concentration of total soluble sugars per unit shoot dry mass; shoot (starch): concentration of starch per unit shoot dry mass; shoot (N): concentration of total nitrogen per unit shoot dry mass; shoot (P): concentration of total phosphorus per unit shoot dry mass.

y-axis	*x*-axis	r^2	*P*
Shoot R_{20}	M_A (g_{DM} m^{-2})	0.001	0.860
(nmol g_{DM}^{-1} s^{-1})	F_A (g_{DM} m^{-2})	0.247	*
	Leaf DMC (g_{DM} g_{FM}^{-1})	0.340	**
	Stem DMC (g_{DM} g_{FM}^{-1})	0.515	***
	Shoot DMC (g_{DM} g_{FM}^{-1})	0.556	***
	Shoot (sugar) (mg g_{DM}^{-1})	0.006	0.713
	Shoot (starch) (mg g_{DM}^{-1})	0.049	0.297
	Shoot (N) (mg g_{DM}^{-1})	0.153	0.058
	Shoot (P) (mg g_{DM}^{-1})	0.015	0.564
Shoot R_{10}	M_A (g_{DM} m^{-2})	0.001	0.880
(nmol g_{DM}^{-1} s^{-1})	F_A (g_{DM} m^{-2})	0.143	0.068
	Leaf DMC (g_{DM} g_{FM}^{-1})	0.198	*
	Stem DMC (g_{DM} g_{FM}^{-1})	0.535	***
	Shoot DMC (g_{DM} g_{FM}^{-1})	0.470	***
	Shoot (sugar) (mg g_{DM}^{-1})	0.011	0.618
	Shoot (starch) (mg g_{DM}^{-1})	0.002	0.838
	Shoot (N) (mg g_{DM}^{-1})	0.093	0.147
	Shoot (P) (mg g_{DM}^{-1})	0.034	0.391

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

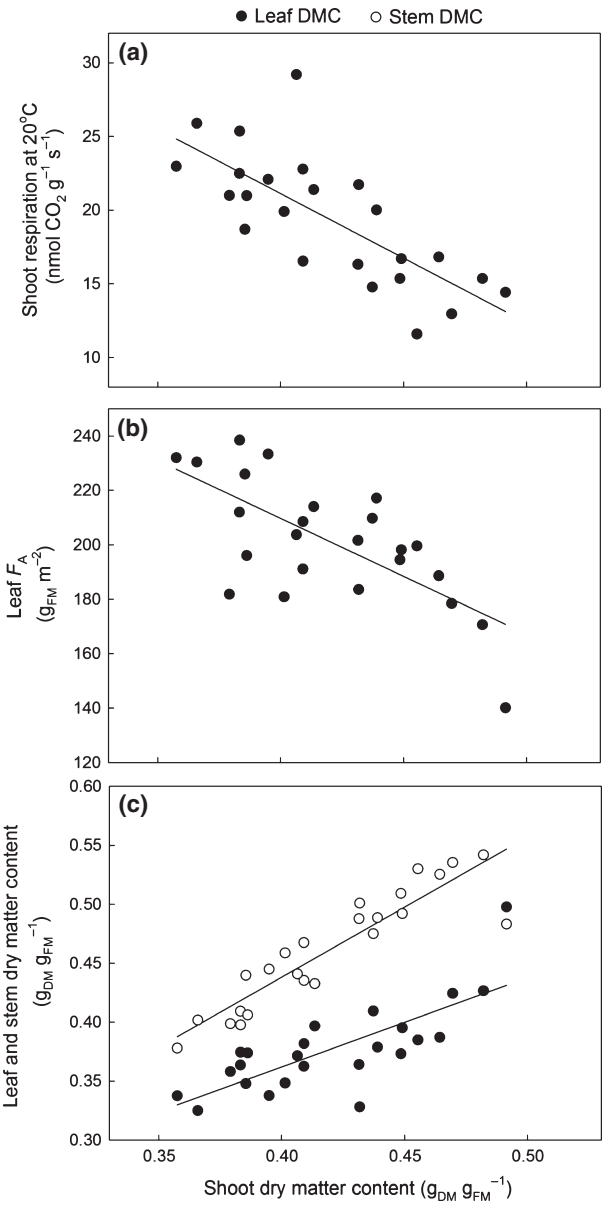

Fig. 3 Shoot dark respiration at 20 °C [*R*, panel (a)], leaf fresh mass per unit leaf area [F_A, panel (b)], and leaf and stem dry matter content [DMC, panel (c)] plotted against corresponding shoot DMC values. Individual replicate values are shown. Coefficients of determination (r^2) and *P* values: Shoot R_{20} – Shoot DMC, $r^2 = 0.56$ ($P < 0.001$); F_A – Shoot DMC, $r^2 = 0.48$ ($P < 0.001$); (c) Leaf DMC – Shoot DMC, $r^2 = 0.58$ ($P < 0.001$); Stem DMC – Shoot DMC, $r^2 = 0.83$ ($P < 0.001$).

either foliar fluxes (Kornfeld *et al.*, 2012; Heskel *et al.*, 2013; Van De Weg *et al.*, 2013) or ecosystem fluxes where *B. nana* is dominant within the chamber footprint (Mack *et al.*, 2004; Cahoon *et al.*, 2012). The structural and chemical compositional responses of both tissues varied in a similar manner to experimental treatments. In our study, L_A was not altered by increased N and P availability, unlike the thinner leaves of *B. nana* after 15 years of the same fertilization reported by Shaver *et al.* (2001), and this incongruence may have implications for how net primary productivity is calculated from leaf traits. Stem DMC reflected the influence of warming and fertilization more than leaf DMC (Table 1), though mass-based N and P concentrations were influenced by treatments in degree and direction in both tissues (Fig. 1; Table 2). Despite the addition of N and P fertilizers and warming treatment, only leaves grown in the N-only addition exceed conditions of N-limitation according to N : P > 10 (Güsewell, 2004). Leaves, more metabolically active than stems, contained higher concentrations of non-structural

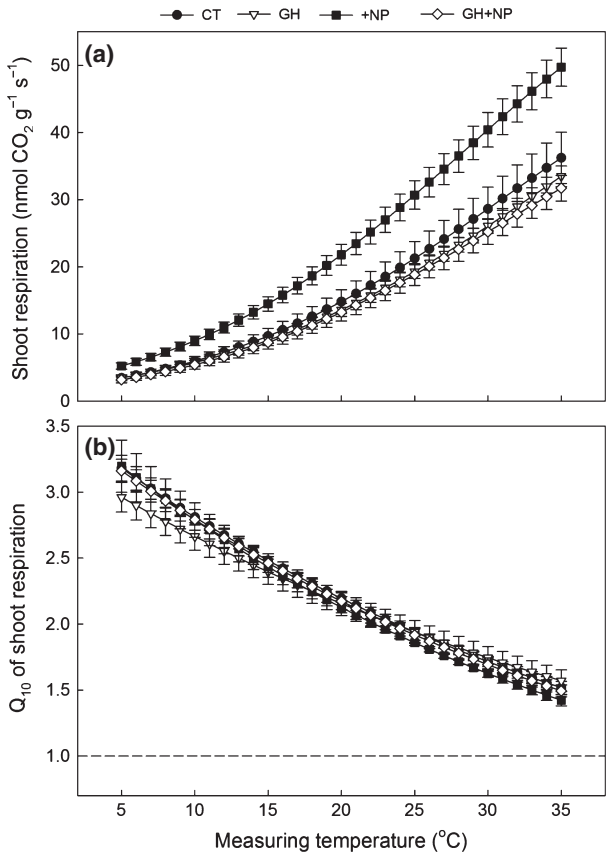

Fig. 4 Temperature dependence of (a) dark respiration (R, nmol CO_2 g_{DM}^{-1} s^{-1}) and (b) Q_{10} of *B. nana* shoots. Measurements were made between the 5–35 °C range. Symbols represent the modeled specific rates of R and Q_{10} values at 1.0 °C intervals (n = 3–4, ±SE). CT, control treatment; GH, greenhouse; +NP, high N and P treatment; GH+NP, greenhouse grown plants provided with high N and P. In (B), the dashed line shows Q_{10} values of 1.0 for reference. Leaves were heated at 1.0 °C min^{-1}.

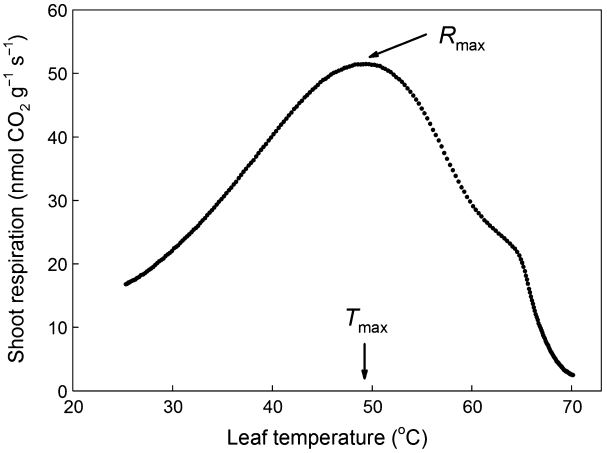

Fig. 5 Example *R-T* curve over the larger range of measurement *T*s showing impacts of continuous heating on rates of whole shoot respiration of a single replicate *B. nana* plant grown under control conditions (i.e. no fertilizer or greenhouse treatments). Data points show measured rates over the 25–70 °C measurement range, with data collected at 15 s intervals; leaves were heated at 1 °C min^{-1}. The maximal rate of leaf R (R_{max}) at the corresponding T_{max} are noted.

carbohydrates in the form of soluble sugars and starch, as well as N and P (Fig. 1).

Shoots, stems, and leaves all exhibited highest rates of R (at 10 and 20 °C) under NP treatment expressed on a mass basis. In turn, when soil nutrients are abundant, a substantial increase in input of CO_2 by this species to ecosystem carbon efflux can be anticipated, given its adaptive developmental plasticity and resulting community dominance (Bret-Harte *et al*., 2001; Heskel *et al*., 2013). The lack of significant response in R in shoots grown under N or P separately (when measured at a common temperature), further underscores the co-limitation of N by P in controlling R in *B. nana* (Van De Weg *et al*., 2013). The elevated rates in shoots grown under combined N and P additions may be attributed to structural changes in tissues – as stem, shoot, and leaf DMC were lowest and F_A greatest, suggesting the 'thickest' leaves contained proportionally greater metabolically active cell material (Fig. 3, Table 1). This response implies a shift in leaf cell growth and organization leading to higher R per unit leaf mass, perhaps via increased proportional investment in mesophyll cells at the expense of structural cell types (e.g., schlerenchyma).

Recent work has reported *B. nana* optimizes R under increased N and P through up-regulating the energy efficient cytochrome pathway, spurring metabolism needed for whole organism growth (Kornfeld *et al*., 2012). Rates of R in stems were greater under the NP treatment, suggesting increases in 'metabolically expensive' processes such as non-structural carbohydrate conversion, and solute assimilation and transport (Ryan, 1991; Amthor, 2000; Cannell & Thornley, 2000; Scheurwater *et al*., 2000; Bouma, 2005; Reich *et al*., 2008). Respiration also supports the highly elevated growth rates in stems of *B. nana*, which result in greater annual growth ring width and longer individual ramets in plants subjected to long-term fertilization (Bret-Harte *et al*., 2002). In contrast to the stimulating response of fertilization, warming did not induce significant shoot structural, chemical, or respiratory responses, and values were similar to those of control-grown plants. While warming promotes microbial activity in tundra soils and increases in mycorrhizal networks (which may in turn be negatively impacted by N addition) that facilitate the expansion of *B. nana* (Weintraub & Schimel, 2005; Deslippe & Simard, 2011; Deslippe *et al*., 2011), our observations did not indicate these effects impact R or physical traits in shoots.

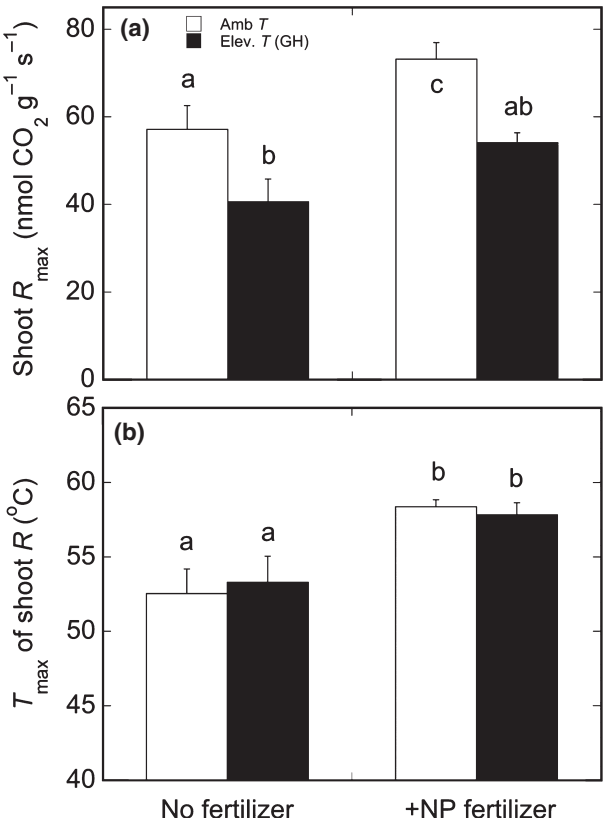

Fig. 6 Impact of long-term environmental treatments on (a) maximal rates of shoot dark respiration (R_{max}, nmol CO_2 gDM^{-1} s^{-1}) measured at a high T (T_{max}), and (b) the T_{max} value of each treatment. R_{max} was determined during continuous heating assays of R-T curves over the 25–70 °C range. Open bars show R_{max} and T_{max} for plants grown under ambient T conditions, whereas solid bars show R_{max} for plants grown under the elevated T conditions experienced in greenhouses (GH) (n = 3–4, ±SE). Values are shown for plants grown in the absence and presence of additional nitrogen and phosphorus fertilizer. Significantly, different values are shown indicated by different letters ($P < 0.05$).

Nutrient-dependent thermal acclimation. One of the main objectives of our study was to determine the interactive effects of warming and fertilization on R, and how they may relate to thermal acclimation. Despite the constant rate of addition of N and P in the GH+NP treatment, leaves, stems, and shoots of *B. nana* exhibited lower concentrations of both nutrients when compared to shoots sampled from the NP-only plots, suggesting a top-down physiological control of nutrient assimilation driven by lowered metabolic rates. Thermal acclimation, the warming-induced adjustment in respiratory rate, was only observed when N and P fertilizers were added (in the GH+NP plots); there was no evidence for acclimation in shoots grown under only long-term warming without the combined fertilization treatment (GH only plots; Figs 2 and 4). This nutrient-dependent acclimation effect implies a strong control on metabolic physiology in *B. nana* in the tundra; the efficiency allowed via thermal acclimation may be impaired at current levels of soil nutrient availability. However, with continued permafrost thaw and corresponding increased microbial activity releasing greater amounts of soil nutrients, we may observe an acclimation effect under a future climate. Though limited or non-existent acclimation in *B. nana* under long-term warming implies a greater respiratory release of CO_2 to the atmosphere and greater energy production via R, which may be used to support growth. It should be noted that this study only evaluated dark R; respiration in the light measured in leaves of *B. nana* under the same warming treatment was diminished due to a strong inhibitory effect by light, in turn reducing carbon loss (Heskel *et al.*, 2013). Currently, it is unknown if light inhibition of R varies under short-term T response.

The variation in short-term T response of shoots of *B. nana* under warming, fertilization and the combined treatment underscores the significant promotion of R under NP addition and the amplification of the thermal acclimation effect when nutrients are not limiting (Fig. 4a). The increasing disparity between rates of R when comparing the response curves of NP and GH+NP shoots measured under warming temperatures indicates Type II acclimation and emphasizes the likely higher enzyme capacity of shoots grown under NP (Atkin & Tjoelker, 2003). Despite the controlling influence of nutrient addition on R-T responses, the thermal sensitivity of respiration, Q_{10}, did not vary significantly between long-term environmental treatments, though subtle differences in Q_{10}-T relationship of GH-grown shoots demonstrate a flatter response, which is reflected in shorter-term warming, such as that over the annual cycle (Crous *et al.*, 2011; O'Sullivan *et al.*, 2013). Considering the increasingly shrubby and warming tundra in some Arctic regions, and its highly variable T regime, these values of a T-dependent Q_{10} can be incorporated for more accurate description of the tundra carbon balance (Tjoelker *et al.*, 2001; Wythers *et al.*, 2005).

Environmental influences on the thermal limits of R. In addition to comparing respiratory responses within a range of Ts experienced by *B. nana* during the growing season, we present the first examination of maximal rates of R (R_{max}) and the T at which that rate was reached (T_{max}) in tundra species and the first published report of how these maximal rates vary under long-term environmental treatments. Though Arctic tundra is rarely subjected to Ts beyond 35 °C, the respiratory performance at these threshold Ts can reveal

information about underlying controls on physiological capacity in extreme environments. R_{max} and T_{max} in *B. nana* were influenced by the availability of N and P; values of both variables were significantly greater under long-term fertilization (Fig. 6). Warming, on the other hand, lowered shoot R_{max} under control and fertilized conditions, suggesting an acclimation effect not only apparent at lower *T*s (as at 10 and 20 °C), but at threshold *T*s. However, while limiting R_{max}, warmer growth conditions did not affect the *T* at which R_{max} was reached, considering both control and NP treatments (Fig. 6). While we hypothesized that growth under warming may shift *T* thresholds of *R* [as has been reported for photosynthetic metabolism (Berry & Björkman, 1980)], there was no observable impact of enhanced growth *T*; a similar conclusion was reached in a recent comparison of winter and summer acclimated evergreen trees in Australia (O'Sullivan *et al.*, 2013). By contrast, T_{max} values were higher in leaves of plants grown under NP fertilization (without warming) – one possible explanation for this is that the higher T_{max} values were associated with NP-grown plants exhibiting thicker and less dense (i.e. greater water content) leaves, which in turn may have slowed detrimental desiccation and membrane dissolution at high *T*s. Higher leaf sugar concentrations and resulting higher leaf osmotic potential, can also protect against heat stress, allowing for a more gradual increase in *R* and photosynthesis at high temperatures (Hüve *et al.*, 2006, 2012;). However, this effect may only be detectable at saturating levels under laboratory conditions, and not necessarily within the range of sugar concentrations in field-grown plants.

Another goal of our study was to compare predictions of R_{max} and T_{max} made from modeled curves from a lower *T* range (5–35 °C) with actual measured values at the thermal limits. By extrapolating the Q_{10}-*T* relationship in Tjoelker *et al.* (2001) (which was typically based on measurements of leaf *R* up to a maximum of ~35 °C), the predicted globally-averaged T_{max} of leaf *R* is 48 °C, irrespective of the thermal environment to which the plants are adapted and/or acclimated. However, our study suggests that actual values of T_{max} can differ markedly from values predicted from *T*-response curves made over a restricted range of *T*s below T_{max}. The shape of the *R*-*T* curve is sufficiently variable at high *T*s that curve fits at lower *T* ranges do not correctly predict T_{max}. In our study, we found that using *R*-*T* curves over the 5–35 °C range resulted in an under-prediction of T_{max} for shoots grown in NP plots by approximately 6–10 °C, and an over-prediction of T_{max} for shoots grown in CT and GH plots by approximately 2.5–3.5 °C. These discrepancies suggest different controlling mechanisms on the *R*-*T* relationship under extreme *T*s that may not be important within a normal *T* range, such as increased membrane permeability (Vacca *et al.*, 2004; Hüve *et al.*, 2006). Though the dearth of studies evaluating these predictive relationships limit our interpretation, our results highlight the need for caution when predicting T_{max} values from *T*-response curves made over a range of low leaf *T*s. Importantly, the results also highlight the high *T* tolerance of respiratory metabolism in leaves of a dominant Arctic woody shrub, *B. nana*.

Implications for a warming Arctic. As warming and warming-driven environmental change continue to transform the ecology of the tundra landscape, quantitative characterization of the C emitted from *R* in the leaves and shoots of the increasingly dominant woody shrub *B. nana* will contribute to more accurate descriptions of terrestrial C cycling where this species occurs. In our study, we report for the first time the nutrient-mediated thermal acclimation of *R* and the significant impact of warming and soil nutrient availability on maximal measured respiratory rate and *T* at which that rate was reached in shoots of *B. nana* grown under 22-year environmental treatments. The lack of thermal acclimation effect in shoots grown under the warming-only treatment implies an expected increase in aboveground plant respiratory C release with increased growth and spread of *B. nana*. Although, if continued warming enhances N and P turnover and permafrost thaw to the point where they become more abundant, thermal acclimation of shoot *R* may slow the release of aboveground C, and potentially balance the corresponding loss of belowground C under high soil nutrient addition (Mack *et al.*, 2004). However, though *T*s of maximal shoot *R* are far from predicted ranges experienced in the Arctic tundra-growing season, the steeper *T*-response curve under NP treatment suggests the potential amplification of shrub-respired C with only small increases in ambient temperature. Further, as the response of C uptake via photosynthesis may be decoupled from respiratory responses under environmental change, ecosystem carbon storage is likely to lessen (Baddeley *et al.*, 1994; Arens *et al.*, 2008; Heskel *et al.*, 2012, 2013). In conclusion, given the environmentally sensitive respiratory responses of the ecologically important woody shrub, *B. nana*, we recommend that estimations of C cycling in Arctic plants integrate the influence of soil N and P and the inclusion of a *T*-dependent Q_{10}.

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