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Coupling of respiration, nitrogen, and sugars underlies convergent temperature acclimation in *Pinus banksiana* across wide-ranging sites and populations

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Abstract

Patterns and mechanisms of short-term temperature acclimation and long-term climatic adaptation of respiration among intraspecific populations are poorly understood, but both are potentially important in constraining respiratory carbon flux to climate warming across large geographic scales, as well as influencing the metabolic fitness of populations. Herein we report on leaf dark respiration of 33-year-old trees of jack pine (*Pinus banksiana* Lamb.) grown in three contrasting North American common gardens (0.9, 4.6, and 7.9 °C, mean annual temperature) comprised of identical populations of wide-ranging geographic origins. We tested whether respiration rates in this evergreen conifer acclimate to prevailing ambient air temperatures and differ among populations. At each of the common gardens, observed population differences in respiration rates measured at a standard temperature (20 °C) were comparatively small and largely unrelated to climate of seed-source origin. In contrast, respiration in all populations exhibited seasonal acclimation at all sites. Specific respiration rates at 20 °C inversely tracked seasonal variation in ambient air temperature, increasing with cooler temperatures in fall and declining with warmer temperatures in spring and summer. Such responses were similar among populations and sites, thus providing a general predictive equation regarding temperature acclimation of respiration for the species. Temperature acclimation was associated with variation in nitrogen (N) and soluble carbohydrate concentrations, supporting a joint enzyme and substrate-based model of respiratory acclimation. Regression analyses revealed convergent relationships between respiration and the combination of needle N and soluble carbohydrate concentrations and between N-based respiration (R_N, μmol mol N^{-1} s^{-1}) and soluble carbohydrate concentrations, providing evidence for general predictive relationships across geographically diverse populations, seasons, and sites. Overall, these findings demonstrate that seasonal acclimation of respiration modulates rates of foliar respiratory carbon flux in a widely distributed evergreen species, and does so in a predictable way. Genetic differences in specific respiration rate appear less important than temperature acclimation in downregulating respiratory carbon fluxes with climate warming across wide-ranging sites.

Keywords: acclimation, adaptation, biogeography, carbohydrates, climate change, common garden, dark respiration, intraspecific variation, jack pine (*Pinus banksiana*), nitrogen

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Introduction

Respiration is a critical determinant of plant, ecosystem, and global carbon exchange (Ryan, 1991; Cox *et al.*, 2000; Valentini *et al.*, 2000), as well as a trait that influences metabolic fitness (Walters & Reich, 2000) and is integral to a broader multidimensional trait syndrome (Wright *et al.*, 2004). The predicted doubling of atmospheric concentrations of carbon dioxide (CO_2) and greenhouse warming of 1.1–6.4 °C within the century will exceed both the magnitude and rate of climate and atmospheric

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change over the last 1000 years (Solomon *et al*., 2007). Climate warming may alter the carbon balance of plants and terrestrial ecosystems through direct effects of increasing temperature on respiration. However, temperature acclimation may constrain respiratory carbon exchange rates in plants (Atkin & Tjoelker, 2003; Gifford, 2003), soils (Luo *et al*., 2001), and alter ecosystem carbon pools (Wythers *et al*., 2005; King *et al*., 2006) in response to climate warming. At present, it is not possible to predict the capacity of plant species to acclimate to temperature change, nor do we fully understand the relative importance of genetic constraints on respiratory and related traits of species distributed across broad climate gradients (Mooney & Billings, 1961; Billings *et al*., 1971; Davis & Shaw, 2001). Understanding temperature acclimation in respiratory traits and its potential biogeographic variation may aid in predicting respiration across temporal and spatial scales and the response of plant species to future climate change.

In the very short-term (seconds to minutes), specific rates of dark respiration in plants increase as an approximate exponential function of ambient temperature (Atkin & Tjoelker, 2003). However, respiration rates acclimate (a type of phenotypic adjustment) to the temperature of the growth environment (Strain & Chase, 1966; Rook, 1969; Larigauderie & Körner, 1995; Tjoelker *et al*., 1999a, b; Loveys *et al*., 2003). There is increasing evidence of a rapid short-term acclimation of respiration, occurring within 1 or 2 days, associated with changes in ambient air temperature (Teskey & Will, 1999; Atkin *et al*., 2000, 2005a; Atkin & Tjoelker, 2003; Bolstad *et al*., 2003; Lee *et al*., 2005). Temperature acclimation results in a reversible shift in the shape or elevation of the temperature respiration response curve (i.e. a downward adjustment of respiration rates at warmer temperatures and upward adjustment in rates with cooler temperatures when compared at a standard measurement temperature). Consequently, respiration rates do not differ nearly as much as they otherwise would across thermal environments, although complete homeostasis across temperatures is uncommon (Atkin & Tjoelker, 2003).

Temperature acclimation may, in effect, constrain both temporal (e.g. days to seasons) and spatial variation (e.g. along climate gradients) in respiration rates in a manner that differs from predictions based on measured short-term response functions (Tjoelker *et al*., 1999a, b; Atkin & Tjoelker, 2003). This may help to explain the similar respiration : photosynthesis ratios of species and communities in widely differing climate zones (Reich *et al*., 1998; Amthor, 2000). Additionally, acclimation of respiration to thermal environment may be important in modulating carbon exchange rates in plants (Atkin *et al*., 2000; Atkin & Tjoelker, 2003; Bolstad *et al*., 2003; Atkin *et al*., 2005a; Lee *et al*., 2005) and soils (Luo *et al*., 2001), and thus influence net ecosystem exchange. Respiratory temperature acclimation has the potential to influence ecosystem carbon fluxes and carbon pools in ways that differ from simple Q_{10}-based (or Arrhenius functions) extrapolations (Gifford, 2003; Wythers *et al*., 2005; Davidson *et al*., 2006), potentially increasing carbon storage in terrestrial ecosystems globally (King *et al*., 2006). The extent to which temperature acclimation alters dark respiration rates in field-grown plants across sites and species remains an open question.

Temperature is a key selection factor contributing to population differentiation along altitudinal and latitudinal clines. Consequently, for tree species with large geographic distributions, respiratory traits may be genetically differentiated among diverse populations and arrayed along climatic clines. Indeed, specific respiration rates are often higher in populations native to high altitudes and latitudes compared with counterparts from lower altitudes and latitudes when grown in common garden (Mooney & Billings, 1961; Ledig & Korbobo, 1983), reflecting underlying genotypic differentiation. Leaf dark respiration (in common gardens) increased along latitudinal clines in *Pinus sylvestris* (Reich *et al*., 1996) and altitudinal clines in *Picea abies* (Oleksyn *et al*., 1998). In the two latter studies, rates of net CO_2 exchange were correlated with leaf nitrogen (N) concentration that covaried along latitudinal and altitudinal clines in plants grown in uniform environments in common gardens. In contrast, common-garden studies of geographically contrasting seed sources of *Pinus taeda* (Teskey & Will, 1999), *Quercus alba, Q. rubra, Acer rubrum* (Bolstad *et al*., 2003; Lee *et al*., 2005), and *Acer saccharum* (Gunderson *et al*., 2000; but see Ledig & Korbobo, 1983) show little evidence of genetic differentiation in leaf dark respiration rates. Despite the apparent lack of broad genetic differences, three of the same species, *Q. alba, Q. rubra,* and *A. rubrum* covaried in respiration and leaf N *in situ* along altitudinal gradients in North Carolina, USA (Mitchell *et al*., 1999). Collectively, these studies indicate that both environment and genotype shape observed phenotypic variation in respiratory traits in wide-ranging tree species.

Specific and total rates of leaf and root dark respiration often correlate with tissue N concentration and content across diverse taxa and environments (Ryan, 1995; Reich *et al*., 1998, 2006; Burton *et al*., 2002; Tjoelker *et al*., 2005; Wright *et al*., 2006). In addition, the role of carbohydrates in respiratory metabolism has received increased attention as a potential mechanism underpinning temperature acclimation of dark respiration in plants (Dewar *et al*., 1999; Atkin & Tjoelker, 2003), particularly with regard to constraining the ratio of respiration to photosynthesis in leaves and plants grown in various environments (Amthor, 2000; Gifford, 2003; Whitehead *et al*., 2004). Temperature acclimation of foliar dark respiration was related to differences in both leaf N and carbohydrate contents in seedlings of five boreal tree species grown under contrasting temperatures (Tjoelker *et al*., 1999b) and to changes in both leaf N and carbohydrate contents of trees of three deciduous species that occurred following 3-day shifts in ambient temperature (Lee *et al*., 2005). Respiration is often greater in leaves and roots containing higher concentrations of soluble carbohydrates (Azcón-Bieto *et al*., 1983; Covey-Crump *et al*., 2002; Xu & Griffin, 2006; but see Atkin *et al*., 2000; Griffin *et al*., 2002). Consequently, changes in both N and nonstructural carbohydrate contents may be associated with observed temperature acclimation responses of respiration.

Herein, we address the following questions: first, to what extent do foliar dark respiration rates acclimate to prevailing ambient temperatures in a widely distributed evergreen species at contrasting sites? Second, do populations of diverse geographic origins differ in respiration rates and, if so, are respiratory traits arrayed along climatic clines? Finally, to what extent do respiration rates covary with foliar N and carbohydrate concentrations across seasons and sites? The overall aim was to examine environmental and genetic sources of variation in leaf dark respiration in field-grown trees. To this end, we used a series of common-garden studies along a climate gradient that enabled us to separate genetic and environmental sources of variation that are often confounded by concomitant changes in both species and environment in field surveys and data compilations. We measured rates of needle dark respiration in 33-year-old trees of 20 geographically diverse jack pine (*Pinus banksiana* Lamb.) populations (44–57°N), spanning much of the native climatic range of the species (−1.1 to 7.2 °C, mean annual temperature) and grown together in three contrasting common gardens in southern Michigan and northern Minnesota, USA and in northwestern Ontario, Canada.

Materials and methods

Common-garden sites

We used common-garden plantations of jack pine (*P. banksiana* Lamb.) that were part of a provenance study coordinated by Mark J. Holst (Rudolph & Yeatman, 1982; Mátyás & Yeatman, 1992) and established with 99 seed sources collected from native stands throughout the northern temperate and boreal range of the species and planted at sites in the United States and Canada. We selected three sites along a latitudinal climate gradient in Allegan, MI, USA (42.56°N, 86.00°W, 228 m), Cloquet, MN, USA (46.70°N, 92.51°W, 385 m), and Red Lake, ON, Canada (50.87°N, 93.76°W, 395 m). The mean annual temperature (1971–2000) at these sites declines from 7.9 to 4.6 and 0.9 °C with increasing latitude. From south to north, the mean January temperatures are −5.8, −12.7, and −19.6 °C, and mean July temperatures are 20.9, 19.6, and 18.1 °C. The number of days with minimum temperatures ≤ 0 °C increases from south to north from 156 to 192 and 203 days across sites. From south to north, mean annual precipitation is 1027, 807, and 640 mm. The soil textures were sands or loamy sands and of typical fertility for the species.

The sites were planted in spring of 1966 with 2-year-old bare-root seedlings of identical seed lots grown in local nurseries. Seedlings were planted at 2.4 m spacing in row plots of four trees of each seed source in randomized complete blocks in Allegan, MI (92 seed sources in each of 10 blocks) and Cloquet, MN (90 seed sources in each of five blocks). The Red Lake, ON site was planted at 1.8 m spacing in row plots of five trees of each seed source in eight blocks that each contained 53 provenances.

Across-site measurements of dark respiration

At each site, we identified 20 common provenances from throughout the geographic range of the species (Table 1). For each provenance, we randomly selected six trees from among at least two replicate blocks, avoiding overtopped or visibly damaged or diseased individuals. We used a pole pruner to cut a single branch comprised of several years of shoot growth from the upper, sunlit portion of the crown of each tree. We transported the cut branches to a nearby air-conditioned room and placed them in the dark at room temperature.

We cut current-year twigs from each branch and removed and weighed 4 g (fresh mass) of needle fascicles and placed them in paper bags in a darkened controlled-environment chamber at 20 °C. This sampling strategy ensured a comparable amount of needle tissue among samples, adequate CO_2 differentials, and removed any contribution of twig or bud respiration. Following a temperature equilibration period of about 20 min, rates of net CO_2 efflux were measured using an infrared gas analyzer and cuvette (LCA-3 and PLC-C, Analytical Development Co. Ltd, Hoddesdon, UK), operating in an open configuration. We removed water vapor from the analyzer air stream using desiccants in order to standardize measurement conditions and eliminate concerns about the accuracy of measuring and correcting for water vapor effects. At measurement, we

Table 1 Location and climate of origin of geographically diverse seed sources of *Pinus banksiana* selected for study at three common-garden plantations in the United States and Canada

				Temperature (°C)		
Provenance	State or province	Latitude (°N)	Longitude (°W)	January	July	Mean annual
Fort McMurray	Alberta	56.63	111.88	−21.1	16.7	−1.1
Sandy Lake	Ontario	53.05	93.25	−22.2	17.8	−1.1
Kississing Lake*	Manitoba	55.12	101.15	−21.7	18.9	−0.6
Nipekamew River*	Saskatchewan	54.20	104.92	−20.0	17.8	0.0
Red Lake†	Ontario	51.02	94.12	−20.0	18.9	0.6
Macdowall*	Saskatchewan	53.12	106.07	−18.3	18.3	1.1
Lac la Biche*	Alberta	55.23	111.92	−17.2	17.2	1.7
Hadashville	Manitoba	49.50	95.75	−18.3	18.3	1.7
Kenora	Ontario	49.78	94.50	−16.7	19.4	2.2
Port Alfred	Quebec	48.25	70.88	−16.1	17.8	2.2
Capitachouane River	Quebec	47.75	76.70	−14.4	17.2	2.2
Fort Frances†	Ontario	48.77	93.50	−15.6	18.3	2.8
Chateau d'Eau	Quebec	46.85	71.42	−12.2	19.4	3.9
St Louis de France	Quebec	46.42	72.58	−12.2	20.0	4.4
Petawawa Plains*	Ontario	45.78	77.38	−13.3	19.4	4.4
Spencer Lake	Maine	45.48	70.23	−10.6	18.3	4.4
Gladstone*	Michigan	46.00	86.50	−7.8	19.4	5.6
Miller Lake	Ontario	45.13	81.45	−5.6	20.0	6.7
Fife Lake*†	Michigan	44.55	85.37	−6.1	20.0	6.7
Mosinee*	Wisconsin	44.83	89.67	−8.3	22.2	7.2

Provenances are ordered by increasing mean annual temperature at seed origin, based on historic climate data obtained before seed collection.

*Northern and southern populations selected for repeated measures at the intermediate common garden, Cloquet, MN, USA.

†Designated nearest local provenance for each of three common-garden plantations (see 'Materials and methods').

placed the needle sample completely inside the sealed cuvette to minimize leaks around the gaskets and placed the cuvette inside the darkened chamber at 20 °C. The reference CO_2 concentration averaged 380 μmol mol^{-1} and was stabilized by sampling air through a void volume. On average, steady-state measures were recorded after 6 min. The needles were frozen in a commercial freezer and later oven-dried (65 °C). On each sampling date, we collected branches between 08:00 and 12:00 hours and completed measures of respiration within a 3–4-h period the same day using two identical calibrated gas exchange systems operating concurrently.

We visited each of the three common-garden sites in 2-day measurement campaigns in September of 1997 and the following May and August. For each sampling period, we calculated 3-day (includes 1–2 days prior) mean, minimum, and maximum air temperatures (Table 2). The same needle age-class cohort was measured in the September and May measurement campaigns. A new current-year cohort was sampled in August. All 20 provenances were sampled at each site in May and September, except for September at Cloquet in which 16 of the 20 provenances were sampled. In August, we sampled the same subset of 10 (eight at Cloquet) populations at each site.

Across-season measurements of respiration at Cloquet

We used the plantation site at Cloquet, MN to make a more detailed assessment of potential temporal changes in needle dark respiration, using eight of the 20 provenances. We determined the short-term temperature-response functions (5–30 °C) on five of the six sampling periods at this site. In September respiration was determined at 20 °C only. Temperature responses, including variation in basal respiration rates and Q_{10}, are reported in detail elsewhere (M. G. Tjoelker, unpublished results). Herein, we report respiration rates measured at 20 °C to enable comparisons across seasons and sites.

For each population, we marked four trees randomly selected for repeated sampling of branches (as described above) six times between September and the following August. The sampling dates encompassed mean daily air temperatures ranging from −6.6 °C in November to 20.4 °C in August. In addition to the September, May, and August sample dates (Table 2),

Table 2 Three-day mean, minimum, and maximum air temperatures (°C) of the sampling dates for needle respiration of *Pinus banksiana* seed sources at each of three common-garden plantations in the United States and Canada

			September 1997		May 1998		August 1998	
Site	Latitude (°N)	Longitude (°W)	Date	Mean (minimum, maximum)	Date	Mean (minimum, maximum)	Date	Mean (minimum, maximum)
Allegan, MI, USA	42.56	86.00	24–26	13.2 (6.0, 18.7)	5–7	16.7 (8.7, 24.1)	25–27	21.7 (15.7, 28.5)
Cloquet, MN, USA	46.70	92.51	10–12	12.8 (4.3, 21.3)	11–13	13.4 (5.2, 21.7)	3–5	20.4 (13.9, 26.8)
Red Lake, ON, Canada	50.87	93.76	16–18	11.6 (6.6, 16.9)	19–21	12.6 (3.1, 20.8)	9–11	22.3 (12.6, 27.4)

the sample dates and corresponding mean, minimum, and maximum air temperatures (3-day mean) were: October 15–16 (4.0, −2.6, 10.6 °C), and November 12–13 (−6.6, −10.2, −3.0 °C), February 12–13 (−4.1, −7.8, −0.4 °C). Rates of net CO_2 efflux at 20 °C were measured in a controlled-environment chamber using infrared gas analyzers as described above, but on detached, intact twigs sealed inside the cuvette to enable repeated measures at the different temperatures. Paired measures of respiration rates of intact twigs and detached needles revealed that the respiratory CO_2 efflux from the woody twig constituted, on average only 5.4% (± 2.3% SEM, $n = 32$) of the total CO_2 efflux of combined twig and needles. Consequently, respiration rates were expressed on a needle dry mass basis (R_{mass}, nmol g^{-1} s^{-1}) and needle N basis (R_{N}, μmol mol N^{-1} s^{-1}).

To examine the degree to which *in situ* respiration rates were altered by temperature acclimation, we estimated *in situ* respiration rates at the mean daily temperature of each of the sample dates using observed respiration rates at 20 °C and Q_{10} (5–30 °C, M. G. Tjoelker, unpublished results) and the following equation:

$$R = R_{20}Q_{10}^{(T-20/10)},$$

where R_{20} is the measured specific respiration rate, R_{mass}, at the reference temperature of 20 °C and T is the mean daily temperature (Atkin *et al*., 2005b). We compared *in situ* respiration rates of acclimating foliage using observed values of R_{mass} at 20 °C with estimates of nonacclimating foliage that were simulated using a fixed value of R_{mass} at 20 °C (September value).

Needle N and carbohydrates

Dried and powdered samples of the needles on which we measured respiration were used for determination of N concentration by coupled CN and mass spectroscopy (University of California – Davis Stable Isotope facility, Davis, CA, USA). We determined N concentrations in samples ($n = 6$ trees) of all 20 provenances at each of three sites sampled in May. In addition, we measured needle N and carbohydrates of the eight provenances ($n = 4$ trees) repeatedly sampled throughout the 12-month period at Cloquet. Soluble sugars and N at Cloquet will be summarized in regression relationships with temperature-response parameters elsewhere (M. G. Tjoelker, unpublished results). Herein we report the entire N and carbohydrate dataset to examine seasonal patterns and enable comparisons across sites not reported elsewhere.

Total nonstructural carbohydrates were determined using the methods of Haissig & Dickson (1979) and Hansen & Møller (1975). Soluble sugars were extracted from oven-dried tissue powder in a mixture of methanol, chloroform, and water (12 : 5 : 3 by volume). Sugar concentrations in the extracts were determined colorimetrically with anthrone at 625 nm. Starch in the residue was gelled by adding ethanol and sodium acetate-NaF buffer and boiling. Starch and converted to glucose with amyloglucosidase, incubating at 50 °C for 24 h. Glucose was measured with glucose oxidase by mixing the sample with peroxidase-glucose oxidase-*o*-dianisidine dihydrochloride. Absorbance was measured at 450 nm after a 30-min incubation at 25 °C. Absorbances were determined with a spectrophotometer (Secomam S750, Alés, France). Concentrations of soluble sugars and starch are expressed as a percentage of needle dry mass. Soluble sugar concentrations were calculated from linear regressions based on glucose standard solutions. We measured carbohydrates on the same samples for which we determined N concentrations with the exception that a subset of three trees from each of the 20 populations sampled in May at each site were randomly selected for carbohydrate analysis. We calculated needle dark respiration at 20 °C on a total soluble carbohydrate basis (R_{sugar}, nmol g^{-1} s^{-1}).

Data analysis

For data obtained from the across-season measurements at the Cloquet site, we used a repeated measures analysis of variance to examine the effects of provenance (7 df), sampling date (5 df), and provenance × sampling date (35 df) on measured respiration rates and needle chemistry. The *F* statistic for the between-population effect used the nested effect of tree within provenance as the expected mean square for the denominator. In addition, we tested preplanned contrasts of northern vs. southern populations within the analysis of variance. The *F* statistic for month and the provenance × sampling date effects used the residual error term.

For the across-site study, we used analysis of variance to examine the effects of provenance (19 df) on respiration rates for each site and measurement campaign separately and in separate models that examined the provenance × site interaction effects. In addition, hypothesized relationships between climate variables of seed-source origin (e.g. mean annual temperature, latitude) and response variables of population means were examined using correlation and Type I regression analyses. In all of our data analyses, we used statistical analysis software (JMP 5.01 for Macintosh, SAS Institute, Cary, NC, USA).

We used transfer functions to quantify the effects of movement of the geographically diverse seed-sources to the contrasting common-garden sites (e.g. Rehfeldt *et al*., 1999). We determined the proportional differences in respiration (R_{mass} at 20 °C) among the 20 populations compared with the nearest local provenance at each of the three common gardens (R_{mass} provenance R_{mass} local^{-1}) –1. The local provenance is assigned a value of 0. Transfer distances were calculated as the mean annual temperature at each common garden minus the mean annual temperature at seed-source origin. We compared relative differences in R_{mass} among populations within and across sites and in a regression relationship with climatic distances (the independent variable) between seed-source origins and the three common gardens. In this analysis the null transfer distance represents the response of the populations in climatic conditions comparable to their seed-source origin.

Results

Comparison of respiration rates among diverse P. banksiana *populations across three common gardens*

Mean specific rates of needle dark respiration (R_{mass}) at 20 °C of populations originating from throughout the species native geographic range were not consistently related to latitude or climate of seed-source origin at any of three common-garden sites (Fig. 1a–c). There was a provenance × site interaction effect in the September measurement period ($P = 0.03$). In September, the populations differed in R_{mass} at Allegan ($P = 0.005$) and Cloquet ($P = 0.009$), but not Red Lake ($P = 0.25$); however, at no site was R_{mass} related to any geographic or climatic variable of seed-source origin. In May, the populations differed only at the coldest, northernmost Red Lake site ($P < 0.0001$) where mean R_{mass} was positively correlated with mean annual temperature of seed-source origin (Fig. 1b). In August, R_{mass} was positively correlated with mean annual temperature of seed-source origin at the southernmost, Allegan site (Fig. 1c).

To further quantify the apparent genotype × environment effect, we examined the proportional differences in respiration (R_{mass} at 20 °C) of the 20 populations relative to the nearest local provenance at each of the three common gardens. Proportional differences in respiration rate were negatively correlated with climatic transfer distance for all months pooled (Fig. 1d) and separately (not shown) as follows: September ($r = -0.38$, $P = 0.004$, $n = 56$), May ($r = -0.69$, $P < 0.0001$, $n = 60$), and August ($r = -0.52$, $P = 0.003$, $n = 28$). A reduced model forcing the intercept through the origin (the null transfer distance) for all data pooled provided a slope estimate of -0.0160 ± 0.00194 (SEM). This analysis revealed that proportional differences in R_{mass} of a given provenance relative to the mean of the local provenance increase (and are positive) upon transfer to colder climates and decrease (and are negative) with transfer to warmer climates than that of their seed origin.

Acclimation of respiration to prevailing ambient temperatures

At the intermediate site in Cloquet, MN, rates of needle dark respiration (R_{mass}) were compared at a standard temperature (20 °C) to assess the degree to which respiration acclimated throughout a 12-month period. R_{mass} differed among the six sampling dates ($P < 0.0001$), rising from September values to peak values in October, November, and February and declining in May and August of the following year (Fig. 2a). Mean R_{mass} differed between contrasting northern and southern population groups (Table 1) for the November sampling date (population × sampling date effect, $P = 0.0086$) and not the other dates. Overall, R_{mass} at 20 °C exhibited a declining linear relationship with increasing mean daily temperatures of the 3-day sampling periods (Fig. 2b).

Given evidence of seasonal acclimation of R_{mass} to prevailing ambient temperatures at the intermediate Cloquet site, we examined the measured respiration rates for the same eight populations at each of the three sites and all sampling dates. Mean R_{mass} at 20 °C averaged across the eight populations declined linearly with increasing 3-day mean ambient temperatures

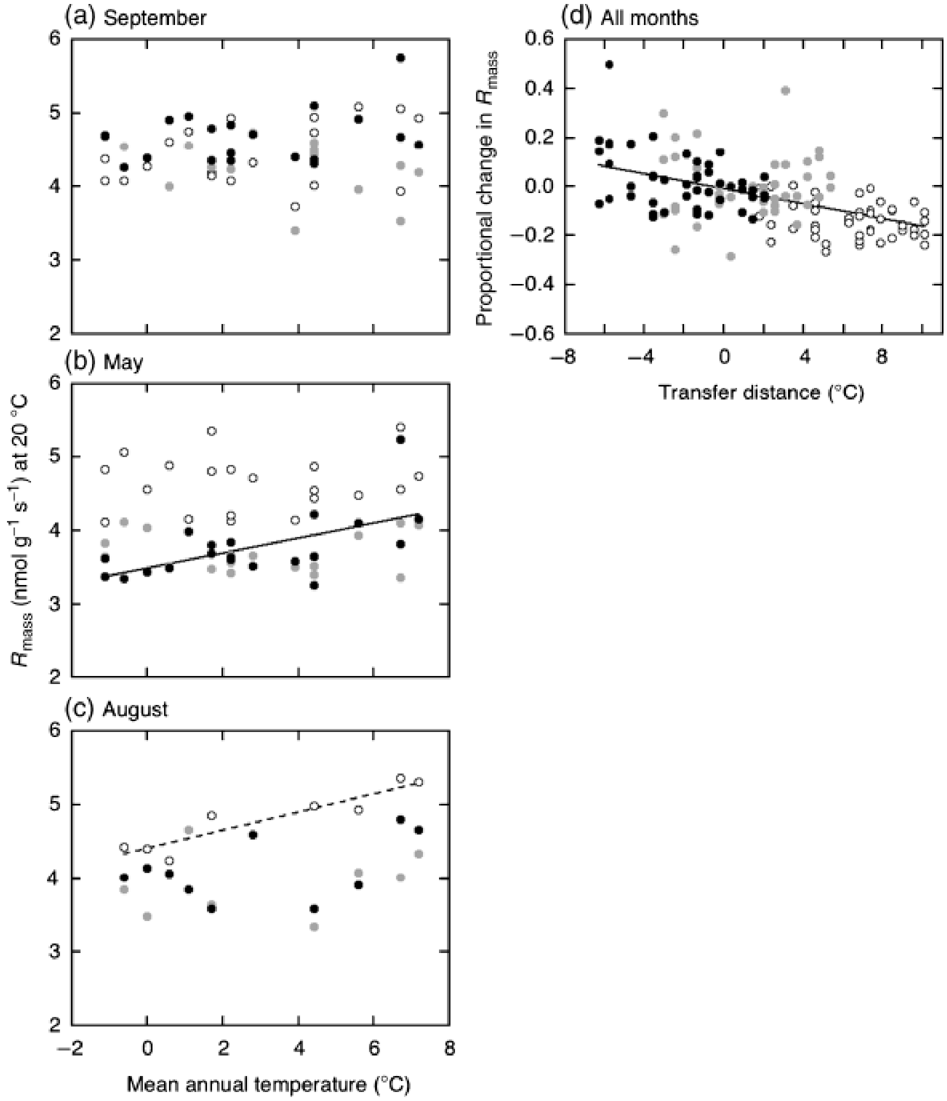

Fig. 1 Mean needle dark respiration at 20 °C (R_{mass}, nmol g^{-1} s^{-1}) in relationship to mean annual temperature (MAT) of seed-source origin among 20 geographically diverse populations of *Pinus banksiana* grown at each of three contrasting common gardens in Allegan, MI (○, 42.56°N, 7.9 °C), Cloquet, MN (●, 46.70°N, 4.6 °C), and Red Lake, ON (●, 50.87°N, 0.9 °C). Mean values ($n = 6$ trees) are shown for each population measured in (a) September, (b) May (Red Lake: $R_{mass} = 3.49 + 0.101$ MAT, $r^2 = 0.36$, $n = 20$, $P = 0.005$), and (c) August (Allegan: $R_{mass} = 4.42 + 0.123$ MAT, $r^2 = 0.85$, $n = 10$, $P = 0.0002$). (d) The relative responses of R_{mass} (at 20 °C) of populations to transfer to contrasting climates were calculated as proportional differences in respiration relative to the local provenance in each of three common gardens for all months pooled (see 'Materials and methods'). The Type I linear regression relationship shown is: $Y = -0.0112–0.0151$ transfer distance ($r^2 = 0.26$, $P < 0.0001$, $n = 144$).

across sites and dates (Fig. 3a). Subdividing the data by individual measurement dates for each 2-day measurement campaign revealed a comparable linear relationship (Fig. 3b), suggesting that the relationship was robust for both 1- and 3-day average values of ambient air temperature. Overall, R_{mass} at 20 °C was negatively correlated with mean daily air temperatures ranging from −10 to 20 °C.

To account for potential statistical leveraging of the R_{mass}–mean daily temperature relationships (Fig. 3a and b) owing to site differences in R_{mass}, we calculated the proportional differences in mean R_{mass} and absolute differences in daily ambient air temperatures across the consecutive measurement dates at each site. Across all sites, proportional differences in R_{mass} at 20 °C were negatively related to differences in mean air temperatures for both 3-day means across months (Fig. 3c) and 1-day means within sample periods (Fig. 3d). Using reduced models that forced the intercept (which did not differ from 0, $P \geq 0.25$) through the origin, slope esti-

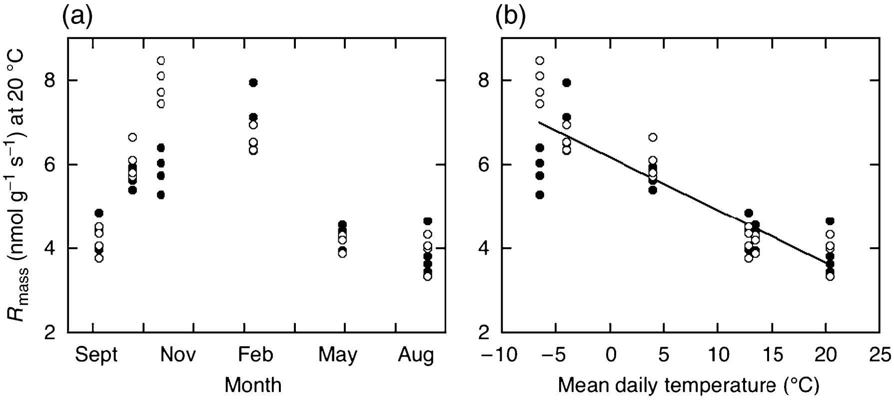

Fig. 2 Seasonal changes in specific rates of respiration at 20 °C (R_{mass}, nmol g^{-1} s^{-1}) in needles of eight contrasting populations of *Pinus banksiana* grown in a common garden in Minnesota, USA. Means for each of four northern (●) and southern (○) populations are shown (see Table 1) for (a) each month and in relation to (b) the mean daily temperature of the 3-day sample period (*T*). R_{mass} at 20 °C = 6.18–0.125*T*, $r^2 = 0.80$, $n = 48$ ($P < 0.0001$).

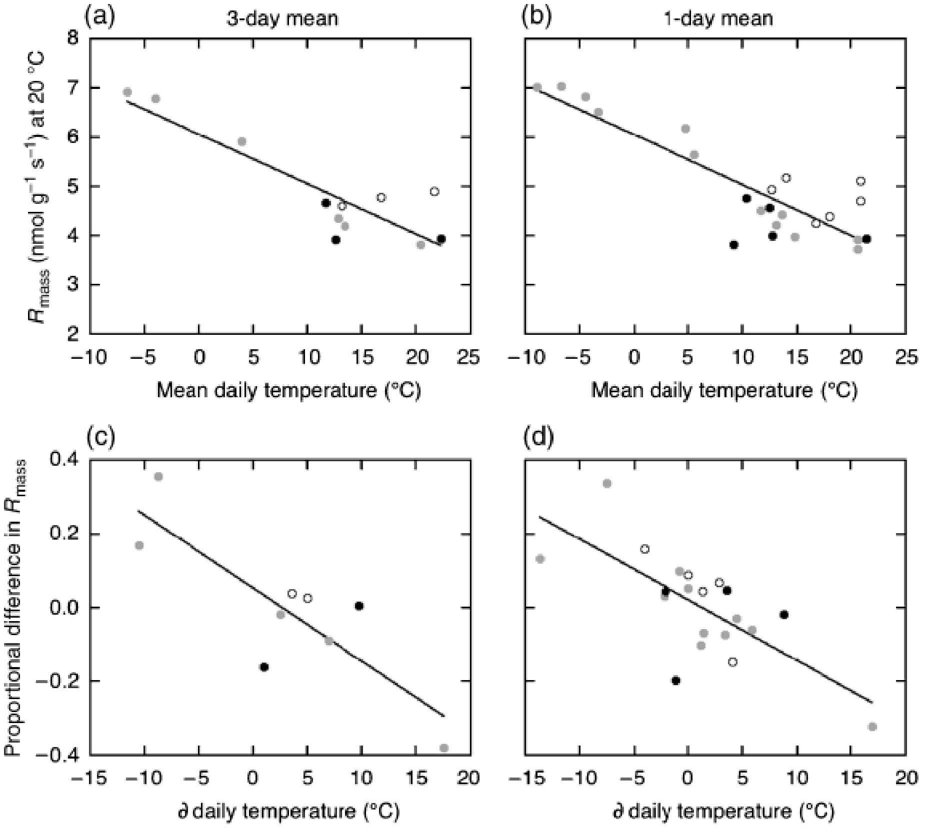

Fig. 3 Acclimation of needle dark respiration of *Pinus banksiana* to prevailing ambient air temperatures in three common gardens in Allegan, MI (○), Cloquet, MN (●), and Red Lake, ON (●). (a) Mean respiration rates (R_{mass} at 20 °C) of eight populations of *P. banksiana* grown and measured at each location in relation to mean daily air temperature (*T*) of the 3-day sampling period ($R_{mass} = 6.07–0.101T$, $r^2 = 0.78$, $n = 12$, $P < 0.0001$). (b) R_{mass} in relation to 1-day mean temperature of each separate sampling date ($R_{mass} = 6.058–0.102T$, $r^2 = 0.76$, $n = 23$, $P < 0.0001$). (c) Proportional differences in R_{mass} at 20 °C of eight common populations are shown in relation to differences in 3-day mean ambient temperature for trees grown and measured at each location: $Y = 0.0527–0.0197\ \partial T$, $r^2 = 0.70$, $n = 9$, $P = 0.0048$. (d) Proportional differences in R_{mass} at 20 °C in relation to differences in 1-day mean ambient temperature: $Y = 0.0226–0.0165\ \partial T$, $r^2 = 0.52$, $n = 20$, $P = 0.0003$.

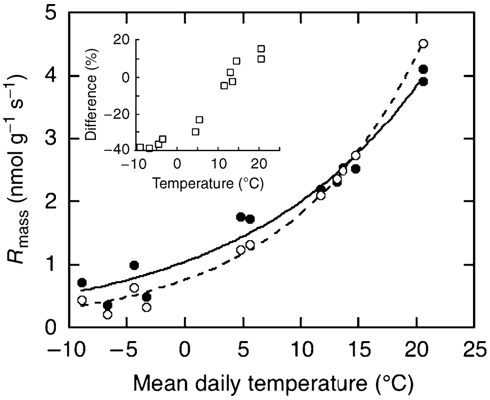

Fig. 4 Estimated *in situ* specific rates of respiration (R_{mass}, nmol g^{-1} s^{-1}) at the mean daily temperature of the sample dates for *Pinus banksiana* grown in a common garden in Minnesota, USA. Rates were predicted using a fixed reference value of R_{mass} (20 °C) for all dates, using the measured September mean value across populations to represent a nonacclimating scenario (○): $R_{\mathrm{mass}} = 4.32\ (2.38)^{(T-20/10)}$, $r^2 = 0.99$, $n = 12$. For the observed, acclimating scenario (●), rates were predicted with observed reference values for each date separately: $R_{\mathrm{mass}} = 3.83\ (1.91)^{(T-20/10)}$, $r^2 = 0.97$, $n = 12$. The inset shows the proportional difference in predicted *in situ* R_{mass} between the two estimates [(nonacclimating−observed/observed) 100].

mates indicated that R_{mass} at 20 °C changed by 1.8% (±0.5 SEM) for 3-day and 1.6% (±0.4 SEM) for 1-day intervals for each 1.0 °C change in mean daily air temperature.

Using pooled 1-day means of R_{mass} at 20 °C (Fig. 3b) and measured Q_{10} (5–30 °C, M. G. Tjoelker *et al.*, unpublished results), we estimated *in situ* rates of R_{mass} at the mean ambient air temperature for each sample date at the Cloquet site (Fig. 4). To examine the degree to which *in situ* respiration rates were altered by temperature acclimation of the reference respiration (R_{mass} at 20 °C), we compared *in situ* R_{mass} with rates estimated using a fixed reference respiration set at the September value. On colder dates in autumn and winter (<10 °C), estimates of *in situ* R_{mass} were up to 40% lower in nonacclimating foliage compared with observed values. On warmer dates in August, *in situ* respiration rates were overpredicted by about 15% in the nonacclimating scenario compared with observed values. Acclimating foliage had a lower temperature sensitivity of *in situ* R_{mass} than nonacclimating foliage with a long-term Q_{10} equivalent of 1.91 vs. 2.38 over the temperature range −10 to 20 °C.

Site and seasonal patterns in needle N and total soluble carbohydrates

Mean needle N and soluble sugar concentrations differed among the three sites ($P<0.0001$) and among the 20 populations with the exception of needle N at Cloquet ($P = 0.25$). At each of the three common-garden sites sampled in May, these traits were unrelated to latitude or climate of seed origin. At the Cloquet site, needle N and soluble sugar concentrations differed among dates for the eight populations sampled throughout the 12-month study ($P<0.0001$, Fig. 5). Soluble sugar concentrations increased in autumn, peaking in November and subsequently declining in February and May (sample date effect, $P<0.0001$), exhibiting a negative relationship with increasing mean daily temperature. Overall, N concentrations did not differ among populations ($P = 0.56$) at this site; while soluble sugar concentrations were higher in southern than northern populations (group contrast, $P = 0.0003$). Starch concentrations were generally present in trace amounts (<5 mg g^{-1}) except in May and August when values were comparatively higher (data not shown).

Relationships between respiration, N, and carbohydrates

Across sites sampled in May for which complete N and carbohydrate data were available, mean R_{mass} increased linearly with needle N concentration among intraspecific populations of *P. banksiana*, suggesting that site differences may, in part, be related to needle N concentration (Fig. 6a, Table 3). However, the R_{mass}–N relationship across seasons for the eight populations measured in Cloquet was weakly statistically significant ($P = 0.01$, $r^2 = 0.14$, Fig. 6b), and combining both the across-site (May only) and across-season (Cloquet only) data, no R_{mass}–N relationship was evident (Fig. 6c). In addition, we found linear relationships between R_{mass} and soluble carbohydrate concentrations across seasons and in the combined data, but not across sites (Table 3). In contrast, for all data subsets and data pooled, there were linear relationships between N-based respiration rates, R_{N} (μmol mol N^{-1} s^{-1}) and soluble carbohydrate concentrations (Fig. 6d–f), as well as between R_{sugar} (nmol g^{-1} soluble sugar s^{-1}) regressed against N (Fig. 6g–i). Overall, R_{mass} covaried with foliar N and soluble sugar concentrations (Fig. 7). A multiple regression model including both N and soluble sugars explained 63% of the variance in R_{mass} at 20 °C (Table 3).

Discussion

Do dark respiration rates acclimate similarly to prevailing ambient temperatures at contrasting sites?

We tested whether or not needle respiration in field-grown trees of the evergreen conifer, *P. banksiana*, showed evidence of seasonal acclimation. Comparisons of respiration rates at a standard temperature (here

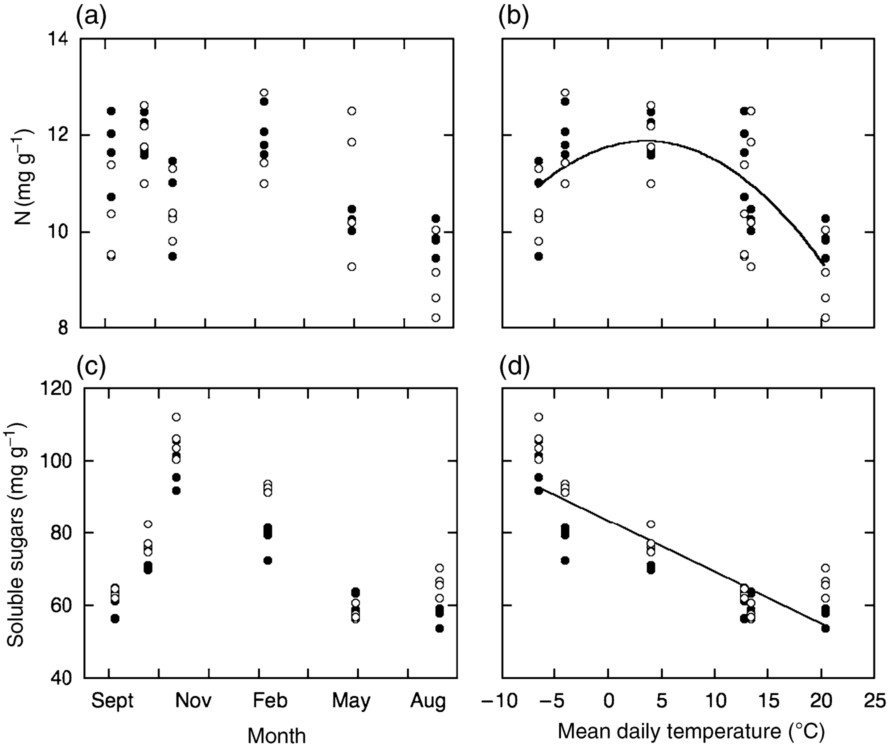

Fig. 5 Seasonal changes in (a, b) nitrogen (N) and (c, d) soluble sugar concentrations among contrasting populations of *Pinus banksiana* grown in a common garden in Minnesota, USA. Means of northern (●) and southern (○) populations are shown in relation to month and mean daily temperature (T) of the 3-day sampling period (see Table 2). The regression relationships: (b) $\mathrm{N} = 12.2–0.0559T–0.00915 (T–6.65)^2$, $r^2 = 0.47$, $n = 48$, ($P < 0.0001$); (d) soluble sugars $= 84.3–1.48T$, $r^2 = 0.78$, $n = 48$, ($P < 0.0001$).

20 °C) provide a basis for determining the degree of acclimation in contrasting thermal environments. We found evidence of respiratory acclimation to prevailing ambient air temperatures across a wide range of temperatures (−10 to 20 °C mean daily temperature) in trees grown at sites of contrasting temperate and boreal climates, ranging from 0.9 to 7.9 °C mean annual temperature. In addition to controlled-environment studies with seedlings of various plant species (Covey-Crump *et al.*, 2002; Loveys *et al.*, 2003), including *P. banksiana* (Tjoelker *et al.*, 1999b), there is increasing evidence that temperature acclimation of respiration occurs in field-grown trees, including evergreen *P. abies* (Stockfors & Linder, 1998), *Eucalyptus* species (Atkin *et al.*, 2000; Bruhn *et al.*, 2007), and deciduous *Acer* and *Quercus* species (Gunderson *et al.*, 2000; Lee *et al.*, 2005; Xu & Griffin, 2006).

We found that R_{mass} values at 20 °C were reduced about 18% for each 10 °C increase in ambient daily mean temperature, ranging from −10 to 20 °C across sites and seasons. Thus, acclimation through its compensatory effect alters *in situ* foliar respiration rates throughout the course of the year and in effect dampens the long-term temperature sensitivity of *in situ* foliar respiration rates. Indeed, *in situ* rates on individual dates at Cloquet differed by as much as 40% between acclimated and nonacclimated estimates over the same temperature range. Consequently, model extrapolations based on a fixed temperature-response function will lead to integrated respiratory losses that differ from *in situ* rates (Atkin *et al.*, 2000; Atkin & Tjoelker, 2003; Wythers *et al.*, 2005), likely underestimating wintertime respiration and overestimating summertime needle respiration in this evergreen species. In comparison, specific respiration rates declined by about 46% with a 10 °C increase in prevailing ambient temperatures (15–25 °C) in a study of three deciduous broadleaved species (Lee *et al.*, 2005); based on these and other studies, it appears that the magnitude of acclimation differs among species (Larigauderie & Körner, 1995; Atkin & Tjoelker, 2003; Atkin *et al.*, 2005a, b).

In *P. banksiana*, temperature acclimation was observed in a controlled-environment study in which needle respiration rates at the reference temperature declined by 24% with a 12 °C increase in growth temperature from 12 to 24 °C (Tjoelker *et al.*, 1999b); in the present study, respiration rates declined by a similar 21% for a 12 °C increase in ambient temperature over the same temperature range. This comparison suggests that long-term acclimation of respiration to fixed differences in

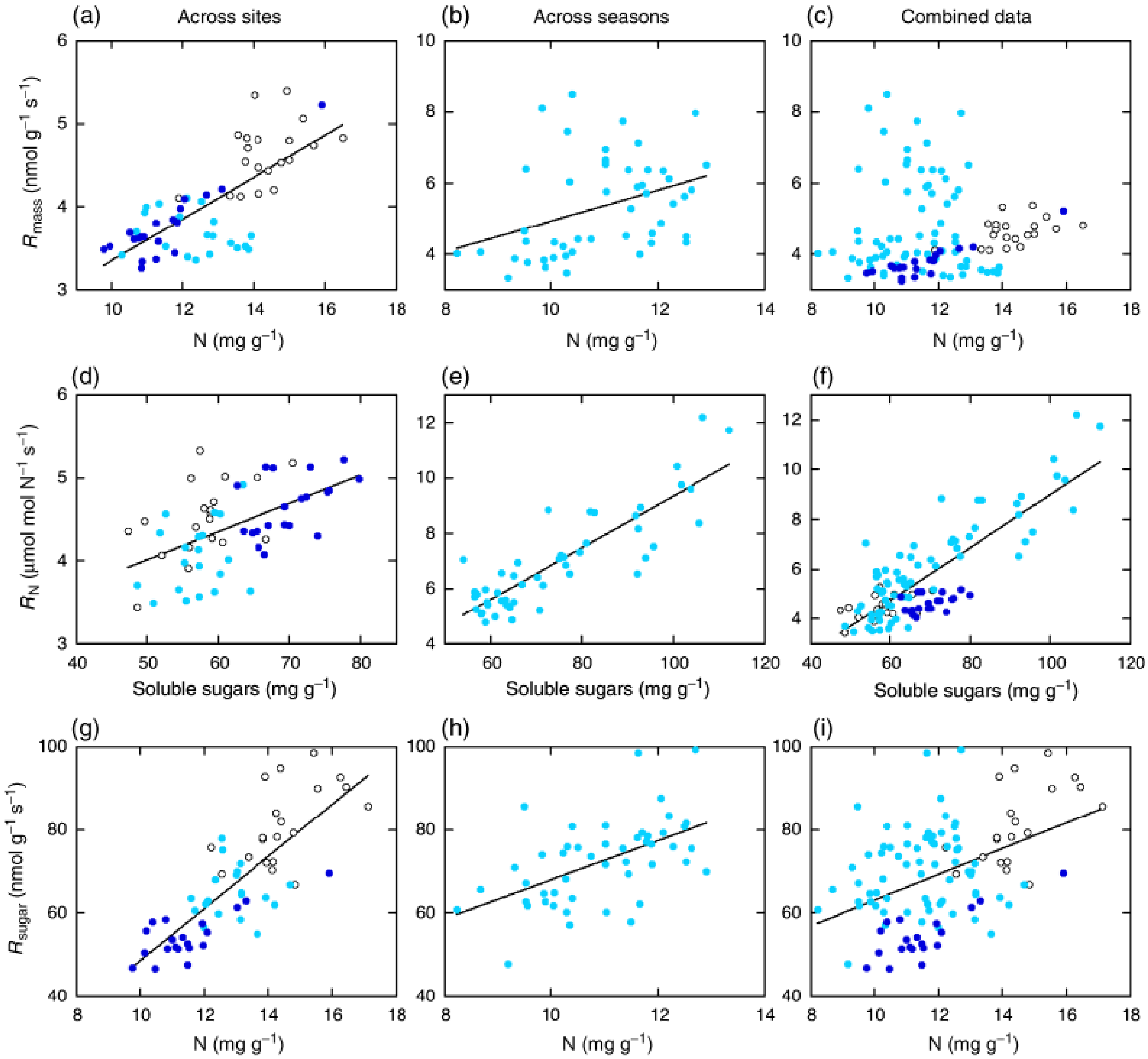

Fig. 6 Mean needle dark respiration at 20 °C expressed on a (a–c) mass basis (R_{mass}, nmol g^{-1} s^{-1}), (d–f) nitrogen basis (R_N, µmol mol N^{-1} s^{-1}), and (g–i) soluble carbohydrate basis (R_{sugar}, nmol g^{-1} s^{-1}) in relationship to nitrogen (N) and soluble sugar concentrations among 20 geographically diverse populations of *Pinus banksiana* grown at each of three contrasting common gardens in Allegan, MI (○), Cloquet, MN (●), and Red Lake, ON (●). Mean values are shown for each of 20 populations in May (across sites), eight populations at the intermediate Cloquet site measured on six dates (across seasons), and for both datasets combined (combined data). Equations for the Type I regression fits are shown in Table 3.

growth temperature and seasonal acclimation to prevailing ambient temperatures were of similar magnitude in this species. However, this was not necessarily expected given that temperature effects on leaf development (morphological plasticity) are thought to have larger effects on gas exchange physiology than shorter-term temperature effects mediated by physiological plasticity (Atkin & Tjoelker, 2003; Loveys *et al.*, 2003; Armstrong *et al.*, 2006).

Do populations of diverse geographic origin differ in respiratory traits?

We found evidence that temperature acclimation was more important than inherent biogeographic variation in respiratory traits in *P. banksiana* in influencing respiration rates, despite the large native geographic range of the studied populations. The absence of consistent trait-climate origin correlations in individual

Table 3 Regression relationships of needle dark respiration at 20 °C, expressed on the basis of dry mass (R_{mass}, nmol g^{-1} s^{-1}), nitrogen (R_{N}, μmol mol N^{-1} s^{-1}), and soluble sugar concentrations (R_{sugar}, nmol g^{-1} soluble sugar s^{-1}) of geographically diverse seed sources of *Pinus banksiana* at three common garden plantations in the United States and Canada

Regression relationship (independent –dependent variable)	Intercept	Slope	r^2	*n*	*P*
Across sites					
R_{mass}–N	0.844	0.251	0.54	60	<0.0001
R_{mass}–soluble sugar					ns
R_{N}–soluble sugar	2.34	0.0336	0.29	60	<0.0001
R_{sugar}–N	−13.8	6.24	0.64	60	<0.0001
Across seasons					
R_{mass}–N	0.574	0.437	0.14	48	0.0097
R_{mass}–soluble sugar	0.098	0.070	0.70	48	<0.0001
R_{N}–soluble sugar	0.0576	0.0928	0.74	48	<0.0001
R_{sugar}–N	21.3	4.68	0.29	48	<0.0001
Combined data					
R_{mass}–N					ns
R_{mass}–soluble sugar	0.278	0.0645	0.55	108	<0.0001
R_{N}–soluble sugar	−1.55	0.106	0.68	108	<0.0001
R_{sugar}–N	32.0	3.11	0.21	108	<0.0001
Multiple regression					
R_{mass}–N, soluble sugar	−2.79	0.203 N + 0.074 soluble sugar	0.63	108	<0.0001

common gardens suggests that R_{mass} of the 20 populations was not strongly genetically differentiated along geographic or climatic clines, as would be predicted if climatic selection had shaped variation in these traits. Population differences in R_{mass} (relative to each common garden) converged to a linear relationship when arrayed along climatic transfer distances. This suggests that the comparatively small genotype × environment effects on R_{mass} were largely explained by climatic distance from seed-source origins.

The transfer of an individual population to a warmer climate than that of its seed-source origin resulted in decreased respiration rates at the reference temperature relative to the local provenance, whereas movement to a cooler climate increased respiration rates relative to the local provenance. To the extent that seed-source transfer is a surrogate for climate change (Rehfeldt *et al*., 1999), this suggests that climate warming will lead to decreases in respiration rates (at a standard temperature) in extant *P. banksiana* throughout its current geographic range. For example, for a 4 °C increase in mean annual temperature, specific respiration rates at the reference temperature of 20 °C will be reduced by 6.4% compared with local provenances (using the reduced model on pooled data). A reduction in the reference respiration rate of this magnitude would result in declines *in situ* respiration rates of respiration comparable in magnitude to that observed with temperature acclimation across seasons. The seed-source transfer effect is independent, and thus added to that of the temperature acclimation response. The transfer relationships although consistent across sample dates, were leveraged by site, and thus, potential confounding factors across sites such as soils, photoperiod (75 min difference in daylength at summer solstice), and perhaps other factors preclude strong inferences attributed to climate warming alone. Nonetheless, differences in foliar N among populations and sites along with the observed correlation between R_{mass} and N suggest that the effect of transfer on R_{mass} was linked, at least in part, to foliar N.

Very weak evidence of a positive correlation of R_{mass} and mean annual temperature of seed-source origin observed here (in two of nine cases, Fig. 1), differs from strong patterns found in studies of geographically diverse seed sources of *P. sylvestris* (Reich *et al*., 1996) or altitudinal seed sources of *P. abies* (Oleksyn *et al*., 1998). Why these three evergreen conifer species should differ in this respect is not clear. Correlations of R_{mass} with climate, altitude, or latitude of seed origin may be influenced by concomitant differences among populations in foliar N and soluble carbohydrates, perhaps reflecting phenology, shifting source-sink relationships, and time of year (Oleksyn *et al*., 2000). However, the response in jack pine observed here is consistent with a common-garden study of bole respiration in *P. banksiana* that found no clinal differences (Lavigne, 1996). Moreover, common-garden studies of geographically con-

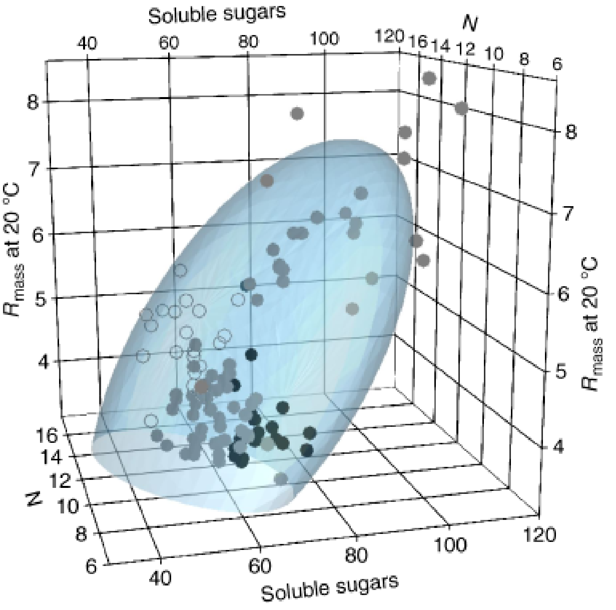

Fig. 7 Mean needle dark respiration at 20 °C expressed on a mass basis (R_{mass}, nmol g^{-1} s^{-1}) in relationship to nitrogen (N, mg g^{-1}) and soluble sugar concentrations (soluble sugars, mg g^{-1}) among 20 geographically diverse populations of *Pinus banksiana* grown at each of three contrasting common gardens in Allegan, MI (○), Cloquet, MN (●), and Red Lake, ON (●). A 90% normal contour ellipsoid is shown.

trasting seed sources of deciduous angiosperms *A. saccharum* (Gunderson *et al.*, 2000) or *Q. alba*, *Q. rubra*, and *A. rubrum* (Bolstad *et al.*, 2003; Lee *et al.*, 2005) also showed little evidence that respiration or its response to temperature depended upon genetic adaptation to climate of origin.

Do respiration rates covary with foliar N and carbohydrates across seasons and sites?

The positive correlations between R_{mass} at 20 °C and needle N concentrations, R_{N} at 20 °C and total soluble sugar concentrations (as well as R_{sugar} at 20 °C vs. N concentrations) across the geographically diverse provenances, sample dates, and sites suggests that both enzyme levels and substrate availability affect base respiration rates, with carbohydrate concentrations especially important during colder autumn and winter months when they increased markedly in concert with decreasing ambient air temperatures. To be sure, respiratory metabolism is not a simple function of substrate availability, but also involves feedbacks from adenylate demand (Atkin & Tjoelker, 2003; Atkin *et al.*, 2005a). The linear relationships between R_{mass}, N, and soluble carbohydrates are similar to those reported earlier for *P. banksiana* seedlings (among other boreal tree species) grown in contrasting cold-temperature environments (Tjoelker *et al.*, 1999b). These patterns suggest that the rates of respiration per unit N are linked to substrate concentrations, while in turn, the rates of respiration per unit substrate are well linked to N concentration, a useful index of enzyme concentrations and activities. The relationship of R_{mass} with both N and soluble sugars was robust, and thus suggestive of a more general predictive relationship than R_{mass}–N relationships alone (Ryan, 1991, 1995; Reich *et al.*, 1998; Vose & Ryan, 2002; Wright *et al.*, 2006) as this dataset encompasses both variation in thermal environment and wide-ranging populations across contrasting sites.

Reduced temperatures may result in increased carbohydrate concentrations and substrate availability for respiration, as well as altered adenylate demand (Atkin *et al.*, 2005a). Cold temperatures often have a greater relative effect on carbohydrate translocation and carbon use than on carbon assimilation, resulting in an altered balance between substrate supply and use and leading to increased carbohydrate concentrations (Farrar & Williams, 1991). In addition, cold hardening in evergreen conifers is associated with active maintenance of elevated concentrations of soluble carbohydrates, even when photosynthesis has ceased at subfreezing temperatures (Ögren *et al.*, 1997, 1999a, b). Our findings are consistent with a cold acclimation response, resulting in increased concentrations of soluble carbohydrates (Oleksyn *et al.*, 2000) and concomitant increases in respiration rates in controlled environments (Tjoelker *et al.*, 1999b; Atkin *et al.*, 2000; Covey-Crump *et al.*, 2002) and in the field (Lee *et al.*, 2005).

Needle N concentrations differed seasonally as well as among diverse populations of *P. banksiana*. N was generally higher in trees measured in the cooler than warmer months (but not in mid-winter), although the association was not as pronounced as for soluble carbohydrates. This finding is consistent with the prediction that short-term thermal acclimation is not primarily related to adjustments in N concentration (Atkin *et al.*, 2005a). However, controlled-environment and field studies show increased N concentrations in foliage with decreasing growth temperatures or prevailing ambient temperatures over longer time periods (weeks to months and in some instances days), suggesting that adjustments in foliar N concentrations may also represent a physiological acclimation response to lower air temperature (Tjoelker *et al.*, 1999b; Weih & Karlsson, 2001; Lee *et al.*, 2005) with the largest changes occurring in tissues that *develop* in contrasting thermal environments (Loveys *et al.*, 2003). In our study, both needle carbohydrates and N changed across seasons throughout the year. Overall, respiration rates at a standard temperature were correlated with changes in both carbohydrate and N concentrations, suggesting that seasonal variation in base respiration rates may be predicted from these and related leaf traits (Xu & Griffin, 2006; Xu *et al*., 2007). These findings support the idea that a joint substrate- and enzyme-based model of respiratory acclimation may be useful in modeling temporal changes in respiration and provide a linkage to photosynthesis and carbon balance (Dewar *et al*., 1999; Turnbull *et al*., 2002; Whitehead *et al*., 2004). Thus, foliar N and carbohydrate concentrations may be useful in modeling seasonal respiration rates among taxa in contrasting environmental conditions.

Summary

We used a series of common-garden plantations of geographically diverse populations of *P. banksiana* arrayed along a climate gradient to determine the extent of thermal acclimation of respiration and intraspecific differences in respiratory traits. The temporal pattern and correlation of respiration rates at 20 °C with mean daily temperature were indicative of a consistent temperature acclimation response associated with changes in ambient temperatures across seasons and sites for this evergreen conifer. Respiration rates of the populations were not strongly or consistently arrayed along climatic clines. Instead, across populations and sites, respiration rates at the reference temperature decreased with increasing transfer distances of populations to warmer climates than that of their seed-source origin. Respiration rates covaried with foliar N and soluble carbohydrate concentrations, providing evidence for a general predictive relationship across geographically diverse populations, seasons, and sites.

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