Research paper

Urban environment of New York City promotes growth in northern red oak seedlings

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Urbanization is accelerating across the globe, elevating the importance of studying urban ecology. Urban environments exhibit several factors affecting plant growth and function, including high temperatures (particularly at night), CO_2 concentrations and atmospheric nitrogen deposition. We investigated the effects of urban environments on growth in *Quercus rubra* L. seedlings. We grew seedlings from acorns for one season at four sites along an urban–rural transect from Central Park in New York City to the Catskill Mountains in upstate New York (difference in average maximum temperatures of 2.4 °C; difference in minimum temperatures of 4.6 °C). In addition, we grew *Q. rubra* seedlings in growth cabinets (GCs) mimicking the seasonal differential between the city and rural sites (based on a 5-year average). In the field experiment, we found an eight-fold increase in biomass in urban-grown seedlings relative to those grown at rural sites. This difference was primarily related to changes in growth allocation. Urban-grown seedlings and seedlings grown at urban temperatures in the GCs exhibited a lower root : shoot ratio (urban ~0.8, rural/remote ~1.5), reducing below-ground carbon costs associated with construction and maintenance. These urban seedlings instead allocated more growth to leaves than did rural-grown seedlings, resulting in 10-fold greater photosynthetic area but no difference in photosynthetic capacity of foliage per unit area. Seedlings grown at urban temperatures in both the field and GC experiments had higher leaf nitrogen concentrations per unit area than those grown at cooler temperatures (increases of 23% in field, 32% in GC). Lastly, we measured threefold greater ^{13}C enrichment of respired CO_2 (relative to substrate) in urban-grown leaves than at other sites, which may suggest greater allocation of respiratory function to growth over maintenance. It also shows that lack of differences in total R flux in response to environmental conditions may mask dramatic shifts in respiratory functioning. Overall, our findings indicating greater seedling growth and establishment at a critical regeneration phase of forest development may have important implications for the ecology of urban forests as well as the predicted growth of the terrestrial biosphere in temperate regions in response to climate change.

Keywords: allocation, photosynthesis, *Quercus rubra*, respiration, temperature, urbanization.

Introduction

Urbanization is accelerating across the globe, with city dwellers accounting for half the population worldwide (Pickett et al. 2001). The question of how the urban environment affects local plants and wildlife is becoming increasingly important. Urban plants affect local climate, biogeochemical cycling, wildlife habitat and quality of life for city dwellers. Additionally, they sequester anthropogenically produced carbon dioxide and may partially offset a city's carbon footprint. Lastly, urban environments may

be useful as a 'window into the future' in global change research, as they exhibit several environmental factors that are expected in coming decades worldwide (Carreiro and Tripler 2005).

There are several environmental factors associated with the urban environment that likely affect plant growth and function. Cities tend to have greater tropospheric CO_2 concentrations, atmospheric nitrogen deposition and other pollution, and warmer temperatures compared with rural areas. The temperature increase in cities tends to be more pronounced at night: in New York City (NYC), the temperature differential between the city and surrounding rural areas is about twice as great at night than during the day (see the Results section). Extensive areas of concrete, asphalt, metal, stone and brick absorb solar radiation during the day and slowly release the heat overnight and significantly increase surface roughness; this is known as the 'urban heat island effect' (Kuttler 2010).

Fast-growing hybrid clones of *Populus deltoides* have been reported to grow faster in NYC than in surrounding rural areas, an observation attributed to the effects of ozone (Gregg et al. 2003). Higher night-time temperatures in the city may also contribute to this response since they tend to promote plant growth (Camus and Went 1952, Hussey 1965, Patterson 1993, Kanno et al. 2009, Way and Oren 2010). In particular, Kanno et al. (2009) reported that increased night-time temperatures promoted growth in rice plants through increases in both photosynthesis and respiration. Similarly, Turnbull et al. (2002) showed that elevated nocturnal temperatures stimulated night-time respiration in *P. deltoides*, which reduced leaf carbohydrate levels and led to increased photosynthesis the following day.

Physiological responses have also been reported to differ in mature trees growing in NYC compared with rural environments (Searle et al. 2011). These authors showed that leaf respiration rates of mature oaks growing in an urban setting were often higher than those of trees growing in more rural settings. Furthermore, the differences in leaf respiration were associated with changes in respiratory protein abundance (cytochrome and alternative oxidase) and changes in oxygen isotope discrimination reflecting changes in electron partitioning between the two respiratory pathways (Searle et al. 2011). Together these findings suggest that respiratory function and efficiency are altered in the urban environment. The carbon isotope signature of respired CO_2 can provide a tool to further assess such changes in respiratory activity, since a higher production of carbon skeletons from respiratory intermediates can support plant growth and has been shown to enrich the ^{13}C content of the CO_2 released (Rossman et al. 1991, Duranceau et al. 1999, Ghashghaie et al. 2001, Hymus et al. 2005, Barbour et al. 2007, Gessler et al. 2009, Tcherkez et al. 2010).

Here we report on a series of experiments designed to clarify how the elevated night-time temperatures in NYC affect growth in seedlings of northern red oak (*Quercus rubra* L.), a native and regionally dominant tree species. These experiments build on each other. We first replicated the basic experimental protocols used in Gregg et al. (2003) and grew 1-year-old *Q. rubra* seedlings at four sites along an urban–rural transect from Central Park, NYC to the Catskill Mountains in upstate NY. Second, based on the results from this first field experiment, we undertook a growth cabinet (GC) experiment to isolate the effect of temperature from other potentially interacting environmental variables. The GCs simulated natural seasonal temperatures in NYC and at one of our rural sites. *Quercus rubra* seedlings were grown from acorns in this experiment and we measured photosynthesis, respiration and biomass allocation. We hypothesized that high night-time temperatures in the city would stimulate respiration and therefore photosynthesis, promoting growth in urban plants. At the same time we collected respired CO_2 from the field-grown plants to further test our hypothesis that respiratory responses were contributing to the observed growth response. We hypothesized that ^{13}C enrichment would be found at the urban site relative to the others due to a higher production of carbon skeletons for growth from respiratory intermediates. Finally, we returned to the field to assess the biomass allocation response when acorns rather than seedlings are distributed to the four transect locations. Although natural year-to-year variations in the environmental conditions make each experiment unique, the results show many consistencies that allow us to conclude that the reduced diurnal temperature range found in urban environments is likely to contribute to the observed growth response in northern red oak.

Materials and methods

First transect study

We utilized four sites along a roughly 150 km urbanization gradient from NYC to the Catskill Mountains. The 'urban' site was located on the east of Central Park in NYC (40.780°N, 73.970°W), the 'suburban' site at Lamont-Doherty Earth Observatory in Palisades, NY (41.005°N, 73.950°W), the 'rural' site at Black Rock Forest near Cornwall, NY (41.430°N, 74.020°W) and the 'remote' site near the Ashokan Reservoir in the Catskill Mountains (41.925°N, 74.248°W). There was no replication of planting sites at each location. Each of the sites was exposed to full, direct sunlight, and was fenced to exclude large herbivores. Temperature data at the urban and remote sites were logged every 30 min using properly shielded HOBO pendant temperature/light data loggers at a height of 1 m (Model 8K-UA-002-08 Onset, Bourne, MA, USA). Long-term temperature records for Central Park are also available from a National Oceanic and Atmospheric Administration (NOAA)-operated weather station at Belvedere Castle in Central Park,

NYC (www.ncdc.noaa.gov). Temperature at the rural site was measured hourly with a properly shielded thermistor (Model 107L thermistor and model 41303-5A 6-plate Gill Radiation Shield, Campbell Scientific Inc., Logan, UT, USA) in a standard weather station maintained by the Black Rock Forest Consortium. Temperature data at the suburban site were obtained from a personal weather station in the nearby town of Haworth, NJ, USA (www.wunderground.com/). Average daily minimum and maximum temperatures from the 2002 to 2006 growing season are reported in Figure 1. Although soil temperatures were not measured, they were likely similar to air temperatures as plants were grown in pots. Ambient CO_2 concentrations in NYC have been previously reported to be ~390–400 ppm during the summer, excluding the morning rush hour, compared with 370–390 ppm measured at our suburban site (Hsueh 2009).

This study utilized acorns collected in the spring of 2001 and planted in garden boxes at Black Rock Forest in 2002. In 2004, they were transplanted into 26.5 l pots filled with soil
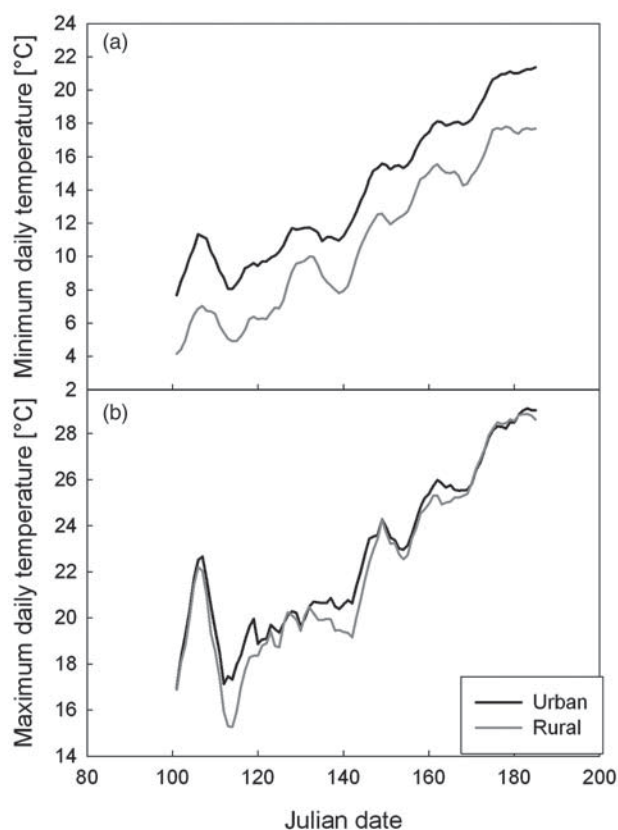

Figure 1. Five-year (May–July, 2002–06) mean air temperatures from the urban (Central Park, New York, NY, USA) and rural (Black Rock Forest, Cornwall, NY, USA) field sites of the urban to rural transect. (a) Minimum and (b) maximum daily field temperature. Data from NOAA (Belvedere Castle Station CLINYC (http://www.erh.noaa.gov/okx/climate_cms.html)) and The Black Rock Forest Consortium (Open Lowland Station (http://blackrock.ccnmtl.columbia.edu/portal/browse/portal/station/objects/1/)).

from the rural site and 20 pots were distributed to each site along the urban–rural gradient. At the time of planting, an allometric relationship was established between stem diameter at the root crown and seedling mass by harvesting 25 seedlings spanning the entire range of planted diameters (total dry mass (g) = base diameter$^{3.42}$ (mm), $r^2 = 0.95$). Ten seedlings from each site were harvested in fall, 2006 and the leaf nitrogen concentration was determined. Preliminary gas-exchange measurements were made on these plants and have been presented elsewhere (Searle et al. 2007).

Growth cabinet study

Based on the observed responses from the first field study, two nearly identical GC experiments were conducted to further isolate the response to temperature. In the first, acorns of *Q. rubra* collected from the rural site in the fall of 2007 were planted in 'Cone-tainers' of vermiculite in an indoor greenhouse at Barnard College (New York, NY, USA) in January 2007. Once the first pair of leaves had emerged in most plants, the seedlings were transplanted to 6 l pots of sand in GCs (Conviron model E-15, Winnipeg, Manitoba, Canada) at the Lamont-Doherty Earth Observatory. In the second experiment, germinating acorns collected at the rural site in April 2007 were planted in 6 l pots of sand in GCs. In both experiments, four GCs were used in a split-plot design, with two cabinets set to simulate urban temperature regimes, and two to simulate rural temperatures. Ten plants were present in each chamber, for a total of 20 plants per treatment. The temperature regimes duplicated the measured hourly springtime field temperatures at the urban and rural sites averaged over the five previous years from 2002 to 2006 (Figure 1; rural site data obtained from the Black Rock Forest Consortium, and urban site data from the National Oceanic and Atmospheric Administration (NOAA (www.ncdc.noaa.gov)). Hourly measurements were averaged over a 1-week interval (see Table S1 available as Supplementary Data at *Tree Physiology* Online), and the temperature regime in the GCs was updated each week to preserve the natural diurnal and seasonal temperature patterns. To keep each GC experiment consistent, the temperature regime was set to 9 April at the start and progressed seasonally from there. Relative humidity in the GCs was maintained at 50% at all temperatures. The first GC experiment ran for 52 days; the second ran for 89 days. Seedlings were watered every other day and were fertilized weekly. Light levels in the GCs were $350 \pm 25\ \mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation, measured at the top of the canopy. Seedlings grown at urban temperatures will henceforth be referred to as 'warm-grown,' and seedlings grown at rural temperatures will be referred to as 'cool-grown.'

Ten seedlings from each GC (20 seedlings per treatment) were harvested in late July 2007 when the seedlings were 3 months of age. Stems were cut at the base. All leaves were

removed and were analyzed on a leaf area meter (LI-3000, Li-Cor, Lincoln, NE, USA). Roots were removed from the pots and carefully washed to remove sand grains. Fine roots as well as coarse roots were included. Tissues were then bagged separately and dried in an oven at 65 °C for 3 days for leaves, and for 1 week for stems and roots. All dry tissues were then weighed. Root, stem and leaf mass were summed for total plant biomass.

Photosynthetic capacity (A_{max}) measurements and dark respiration measurements were made on 28 March 2007 and 27 March 2007, respectively. All gas-exchange measurements in the GC experiment were performed on seedlings *in situ* in the GCs, manipulating GC temperature to produce temperature response curves of respiration, as described below.

Respiratory CO_2 isotope study and ^{13}C analysis

To avoid the possibility of root binding the original transect seedlings were replaced with a second cohort of red oak, which was planted in 2007. These plants came from germinating acorns (with a 0–2-inch radicle) collected at the rural site in April 2007, and were transported to each of the four transect sites. They were then planted in 26.5 l pots containing soil from the rural site. Seedlings were watered weekly throughout the growing season.

Three plants per site were used for this experiment and all ^{13}C gas samples used in this analysis were collected between the hours of 21:00 and 24:00, when plants were in natural darkness. Incubation and gas sampling for isotopic analysis followed the approach of Hemming et al. (2005) and Hymus et al. (2005). Three healthy, mature leaves from each plant were placed in a darkened respiration chamber with a volume of 470 cm³. Three respiration chambers were connected in series with each other, and in sequence with a dessicant tube, a CO_2 infrared gas analyzer (LI-6200, Li-Cor, Inc.) and a gas collection vial. Carbon dioxide-free air was flushed through each of the chambers at a rate of ~1 l min⁻¹ for 3 min, after which the chambers were sealed. After half an hour of respiration, the chamber air was eluted by flushing CO_2-free air through the first chamber, desiccant tube, CO_2 infrared gas analyzer and collection vial. When the peak CO_2 concentration (300–800 ppm) was detected on the infrared gas analyzer, the collection vial was presumed to be full of sample gas (based on preliminary tests) and was immediately sealed. This process was repeated at time intervals for the other chambers.

After collecting respired CO_2, the same leaves were used for bulk organic and soluble sugar analyses. The midribs were removed. Half the material was placed in an oven at 60 °C for 3 days; this material was later ground with a ball mill and analyzed for bulk organic carbon isotope composition. The other half of the material was wrapped in aluminum foil, snap frozen in liquid nitrogen and placed in a freezer at −80 °C.

Approximately 1 g of this material was used for soluble sugar extraction according to the protocol of Duranceau et al. (1999) and Xu et al. (2004). For each 0.1 g of leaf material, 1 ml of deionized water was added and the mixture was ground in a mortar and pestle chilled with liquid nitrogen. The resulting extract was kept at 0 °C for 20 min and then centrifuged at 12,000 g for 10 min. The supernatant was boiled for 3 min and centrifuged again. The water-soluble fraction was then mixed with Dowex-50 (H⁺) and Dowex-1 (Cl⁻) resins in sequence to remove amino acids and organic acids, respectively. The eluate has previously been shown to have a carbon isotopic composition representative of leaf-soluble sugars (Brugnoli et al. 1988). The eluate was then dried in an oven at 60 °C for a day.

Isotopic composition of respired gas and soluble extracts was determined at the Weizmann Institute as described earlier (Hemming et al. 2005, Hymus et al. 2005). Briefly, organic matter ^{13}C composition was determined on oven-dried and mechanically ground samples. Two replicates from each sample of ~0.3–0.4 mg were weighed into 3×5 mm tin foil capsules (Elemental Microanalysis Ltd, Exeter, UK) and combusted in an elemental analyzer (Carlo Erba 1108; Carlo Erba, Milan, Italy, precision ± 0.5‰) connected online to a continuous-flow isotope ratio mass spectrometer (Optima; Micromass Ltd, Manchester, UK). For analysis of respired CO_2, an aliquot of 1.5 ml was removed from each flask into a sampling loop and the CO_2 cryogenically trapped using helium as a carrier gas. It was then passed through a Carbosieve G packed column at 70 °C to remove N_2O and analyzed on a Europa 20–20 continuous-flow isotope ratio mass spectrometer (Crewe, UK). Batches of 15 flasks were measured at one time from a manifold system, with five flasks of a standard gas being measured for every 10 samples. The CO_2 concentration of each flask was determined by removing an additional 40 ml from the flask into a mechanical bellows and then passing the sub-sample through an infrared gas analyzer (LI-6262, Li-Cor Inc.). Two sub-samples were taken in series from each flask, with the value from the second sample being used to reduce memory effects between samples. Precision was ± 0.1 ppm.

Stable isotope ratios are expressed in the conventional delta (δ) notation in ‰ relative to an international standard:

$$\delta = \left(\frac{R_{sam}}{R_{std}} - 1 \right) \times 1000 \qquad (1)$$

where δ is $\delta^{13}C$, and R_{sam} and R_{std} are the isotope ratios ($^{13}C/^{12}C$) of the sample and standard, respectively, and the standard was Vienna Pee Dee Belemnite. Calibration of each batch run was done by measuring four samples of the acetanilide (Elemental Microanalysis Ltd.) international standard at the start of each run and two samples of a cellulose laboratory working standard for every 12 sample capsules, making a correction for a blank cup. Precision was ±0.1‰. Discrimination

between respired CO_2 (δ_R) and soluble sugars ($\delta_{substrate}$) during processes examined in this study was expressed as

$$\Delta^{13}C = \frac{(\delta_{substrate} - \delta_R)}{(1 + \delta_R / 1000)} \qquad (2)$$

Second transect study

Acorns collected at the rural site in the fall of 2007 were planted at each site along the transect in pots containing soil from the rural site in May 2008. Seedlings were watered weekly over the growing season and fertilized with slow-release fertilizer (Osmocote, Marysville, OH, USA). From May to August, 2008, seedlings were sprayed weekly with insect repellant. Biomass, respiration, photosynthesis and leaf nitrogen measurements are presented for each of the five plants from this cohort. Five seedlings at each site were harvested in mid-August 2008 (at ~5 months of age). The number of leaves per seedling was counted in 10 seedlings at each site (except for the rural site, where 7 were measured) on 16–17 July.

Temperature regimes at the four field sites were measured as described above (Figure 2). The year 2008 was an anomalous
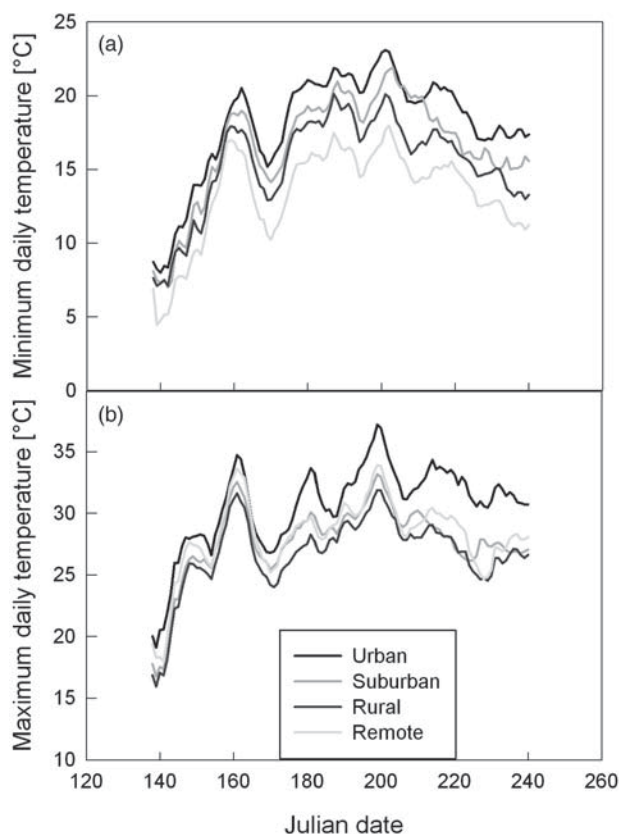

Figure 2. 2008 May–August air temperatures from the four sites of the urban to rural transect (Urban = Central Park, New York, NY USA, Suburban = Lamont-Doherty Earth Observatory, Palisades, NY, USA, Rural = Black Rock Forest, Cornwall, NY, USA, Remote = Ashokan Reservoir, Shokan, NY, USA). (a) Minimum and (b) maximum daily field air temperature.

year with daytime temperature differences that were larger than the measured 2002–06 trends that were used to set the environmental conditions used in the GC experiment. Ozone concentrations were measured at weather stations near the urban and remote sites in 2008 and were found to be similar and relatively low (yearly averages of 30 ppb at each site) (New York State Department of Environmental Conservation 2008).

Photosynthetic capacity (A_{max}) measurements were taken on 11 July 2008 and leaf dark respiration (R_d) measurements were taken on 12–15 August 2008. Photosynthesis measurements were performed on seedlings in situ in the field; however, seedlings and pots were transported to the GCs at the Lamont-Doherty Earth Observatory for respiration measurements in order to manipulate the temperature of the seedlings with the GCs. All gas-exchange measurements were performed as described below.

Gas-exchange measurements

Photosynthesis and dark respiration measurements were made using an LI-6400 (Li-Cor). Measurements of A were made at 30 °C. Measurements of R_d were made at 5, 10, 15, 20, 25 and 30 °C in the GC experiment, and at 10, 14, 18, 22 and 26 °C in the field study. Relative humidity was controlled at 50–75%. Fully expanded mature leaves were chosen randomly and the same leaf was used for all gas-exchange measurements on each replicate plant. Leaves were present in the cuvette for at least 3 min before measurements to allow equilibration in the chamber. Between 5 and 20 measurements were taken per plant at each temperature, and these values were averaged. Light intensity was set to 1500 μmol m^{-2} s^{-1} for measurements of A. Plants were allowed to stabilize for 30 min after each temperature change in the GCs before respiration measurements.

The temperature response of respiration was modeled as previously described in Atkin et al. (2005):

$$R = R_{10}Q_{10}^{(T_1 - T_0 / 10)} \qquad (3)$$

where R is the respiration rate, R_{10} is the respiration rate at a reference temperature (here 10 °C), T_0 is the reference temperature (10 °C), T_1 is the measurement temperature of R and Q_{10} is the increase in the respiration rate with a doubling of temperature (i.e., the temperature sensitivity of respiration). Non-linear curve fitting was performed using the Marquardt–Levenberg algorithm (Sigma Plot, software version 8.0, SPSS Inc., Chicago, IL, USA).

Leaf nitrogen

Ten leaf samples from each site in both transect studies and in each of the treatments in the first GC experiment were analyzed for leaf N. Samples were ground with a ball mill and were analyzed for %N at the Stable Isotope Laboratory at Washington

State University, or at the Lamont-Doherty Earth Observatory. Percent leaf nitrogen is reported on a mass basis.

Statistical analysis

Statistical analyses were performed using R 2.4.0 (R Development Core Team). All data were tested for normality and ln transformed where needed (R : S ratio). An analysis of variance (ANOVA) was used to test differences between sites in the field study, and a Tukey post hoc test was used to determine the specific differences between sites. Tree size was added as a co-variate in the analysis of the root : shoot ratio between sites and treatments and was not found to influence results. A two-way ANOVA was used to test differences in the masses of plant components between treatments and chambers in the GC experiments, while a t-test was used to test differences in leaf nitrogen, A, and respiratory parameters between treatments in the GC experiments due to small sample size ($n = 5$). All comparisons were considered significant if $P < 0.05$. All error terms reflect the standard error of the mean.

Results

First transect study

Average daily minimum and maximum temperatures at the urban and rural field sites from early May to early July, 2002–06 are shown in Figure 1. The diurnal temperature range was significantly lower at the urban than at the rural site (6.7 vs. 10.4 °C, $P < 0.05$). The primary difference was at night with the minimum city temperatures nearly 4.5 °C warmer than the rural and remote temperatures. The daytime maximum temperature difference was much smaller, often 1 °C or less with a maximum of 2.0 °C. These 5-year average trends were replicated in the growth chamber experiments, which began in 2007, the year immediately following this record.

After 1 year of growth, seedlings from the urban site accumulated aboveground biomass at nearly twice the rate of the seedlings from the other three sites (Figure 3). There were no significant differences among the non-urban sites. Leaf nitrogen (N) was significantly different between sites along the transect, with urban-grown leaves having significantly greater N than leaves grown at other sites (Table 1).

Growth cabinet experiment

Total dry biomass, stem mass and leaf mass were significantly greater in warm- than in cool-grown seedlings in the second GC experiment (Table 2). Root mass was not significantly different between treatments. The root : shoot ratio and total leaf area were also significantly higher in cool-grown plants relative to warm-grown plants (Figure 4a and b, respectively). The percent leaf nitrogen was significantly greater in seedlings grown at warm temperatures relative to those grown at cool
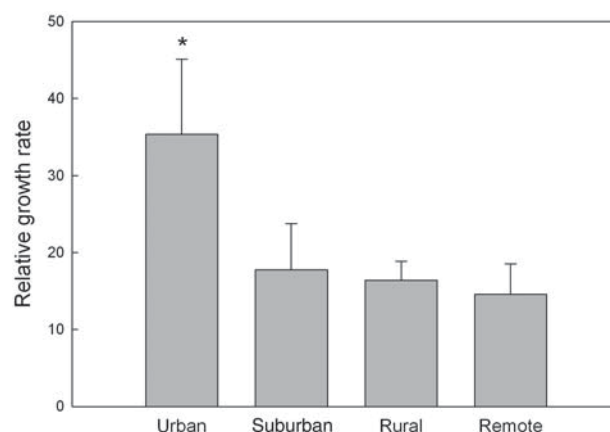

Figure 3. The relative growth rate of above-ground biomass in *Q. rubra* seedling from the 2006 urban to rural transect field experiment. Values shown are mean ± SEM.

Table 1. Percent leaf nitrogen of *Q. rubra* seedlings from three sequential experiments: 2006 urban to rural field transect, 2007 GC study and 2008 urban to rural field transect. Bold letters indicate statistical differences among sites within an experiment; *P* values are given for differences among treatments or sites for each study. Values shown are mean ± SEM.

Experiment	Site/treatment	% N	Significance
2006 field transect	Urban	2.22 ± 0.07 a	$P < 0.001$
	Suburban	1.92 ± 0.06 b	
	Rural	1.80 ± 0.02 b	
	Remote	1.81 ± 0.06 b	
2007 GC	Warm grown	1.37 ± 0.08 a	$P = 0.025$
	Cool grown	1.04 ± 0.08 b	
2008 field transect	Urban	2.13 ± 0.05 a	n.s.
	Suburban	2.16 ± 0.06 a	
	Rural	2.17 ± 0.05 a	
	Remote	2.22 ± 0.10 a	

temperatures (Table 1). Leaf-scale photosynthetic capacity was not different between treatments (Figure 5a) nor were R_{10} or Q_{10} values, although on average warm-grown seedlings tended to respire at a greater rate than did cool-grown seedlings (Figure 5b; Table 3).

Respiratory CO$_2$ isotope study

$\delta^{13}C$ values of respired CO_2 were highest (least negative) in leaves from the urban site and lowest in leaves from the rural site, although these differences were not significant (Figure 6a). Differences in $\delta^{13}C$ of bulk organic material and the soluble sugar fraction among sites were also not significant (data not shown). However, there was a threefold difference in ^{13}C discrimination by leaf respiration (the difference in $\Delta^{13}C$ between respired CO_2 and the soluble sugar fraction was assumed to represent the main substrate of respiration;

Table 2. Total, root, stem and leaf dry masses of *Q. rubra* seedlings. Values are mean ± SEM. Within columns, values with different letters are significantly different at $P < 0.05$ (note separate tests for the GC and field experiment); $n = 2$ replicate growth chambers or $n = 5$ individual seedlings per site from the field study.

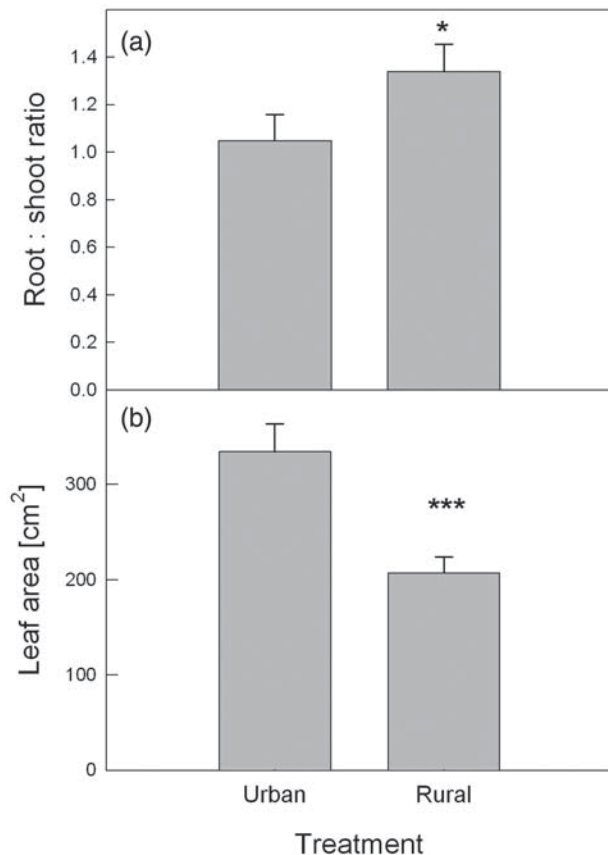| Experiment	Site/treatment	Total mass (g)	Root mass (g)	Stem mass (g)	Leaf mass (g)
2007 GC	Warm grown	4.95 ± 0.30 a	2.36 ± 0.09 a	0.47 ± 0.06 a	2.12 ± 0.43 a
	Cool grown	4.09 ± 0.27 b	2.28 ± 0.17 a	0.35 ± 0.09 b	1.47 ± 0.22 b
2008 field transect	Urban	125.4 ± 23.6 a	56.5 ± 10.1 a	42.9 ± 10.5 a	26.1 ± 3.3 a
	Suburban	90.3 ± 6.9 a	44.0 ± 7.4 a	23.8 ± 3.4 a	22.5 ± 2.4 a
	Rural	17.0 ± 5.6 b	10.6 ± 3.0 b	3.8 ± 1.2 b	2.8 ± 1.4 b
	Remote	15.4 ± 3.5 b	8.5 ± 2.1 b	3.2 ± 0.8 b	3.6 ± 1.0 b

Figure 4. *Quercus rubra* seedling biomass allocation in the GC experiment. Experimental conditions mimic the 5-year average temperature trends (Fig. 1) for the urban or rural sites: (a) root : shoot ratio and (b) total leaf area. Values shown are mean (of two replicate GCs) ± SEM. Asterisks indicate statistical significance: *$P < 0.05$, ***$P < 0.001$.
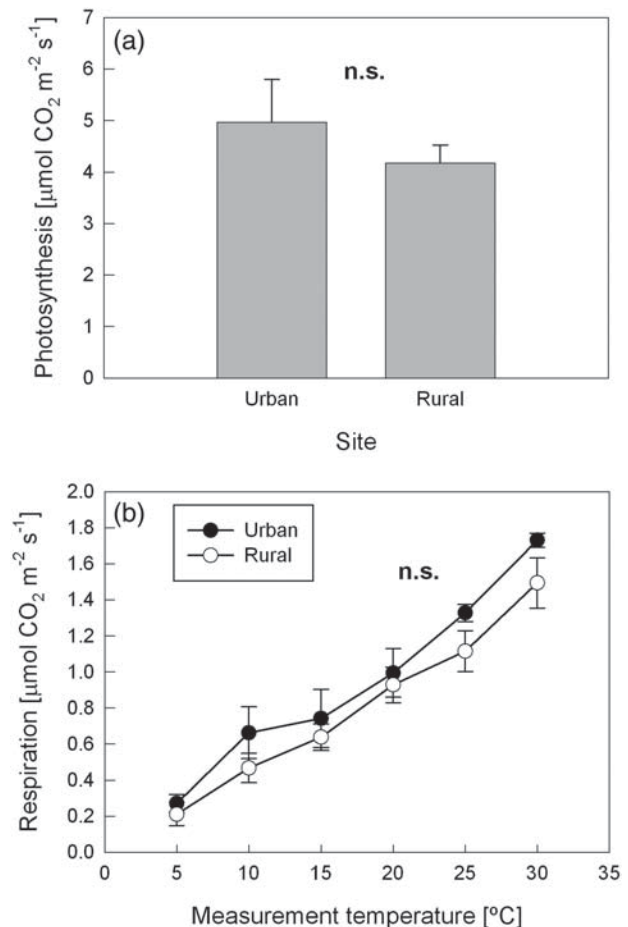

Figure 5. (a) Maximum rate of photosynthesis in *Q. rubra* seedling leaves measured at 30 °C and (b) temperature response curves of respiration in the 2007 GC experiment. Experimental conditions mimic the 5-year average temperature trends (Fig. 1) for the urban or rural sites. Values shown are mean (of two replicate GCs) ± SEM. There were no significant differences between treatments.

Eq. (2)) between the remote and urban sites (from ~3 to ~9‰; Figure 6b).

Second transect study

Daily minimum and maximum temperatures at all field sites from 15 May to 31 August 2008 are shown in Figure 2. Comparison of Figures 1 and 2 reveals that 2008 had an unusual temperature pattern as compared with our initial and longer-term 2002–06 records. The 2008 average differential in maximum temperatures between sites (i.e., the difference in temperatures between the urban and remote sites) was 2.4 °C, while the average differential in minimum temperatures was 4.6 °C. The 2008 Central Park maximum temperatures were warm relative to 2002–06 records, particularly during the second half of the summer. Another atypical feature is that the Black Rock Forest minimum temperatures were often lower than the remote site

Table 3. R_{10} (respiration rate at 10 °C) and Q_{10} (temperature sensitivity of respiration) of *Q. rubra* seedlings from two sequential experiments: 2007 growth cabinet study and 2008 urban to rural field transect. Values shown represent mean ± SEM. There were no significant differences among treatments of the GC experiment or the sites in the field transect study.

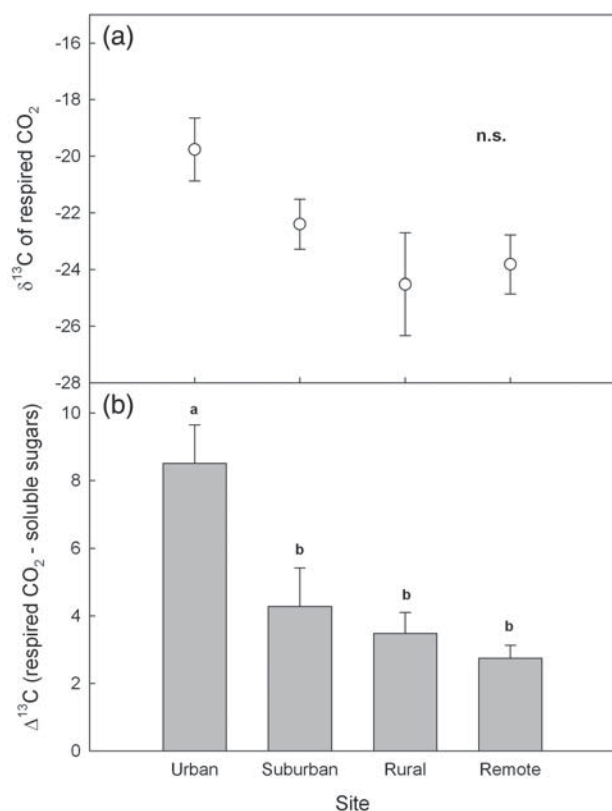| Experiment	Site/treatment	R_{10}	Q_{10}
2007 GC	Warm-grown	0.60 ± 0.13	2.0 ± 0.1
	Cool-grown	0.45 ± 0.06	1.9 ± 0.1
2008 field transect	Urban	0.47 ± 0.06	2.3 ± 0.1
	Suburban	0.55 ± 0.05	1.9 ± 0.1
	Rural	0.64 ± 0.56	2.0 ± 0.0
	Remote	0.65 ± 0.09	2.1 ± 0.1

Figure 6. (a) $\delta^{13}C$ of respired CO_2 in *Q. rubra* seedling leaves at each site along the urban–rural transect. (b) The discrimination in ^{13}C by respiration (i.e., the difference in $\Delta^{13}C$ between respired CO_2 and soluble sugars, assumed to be the substrate of respiration). Values shown are mean ± SEM. Letters indicate statistical differences.
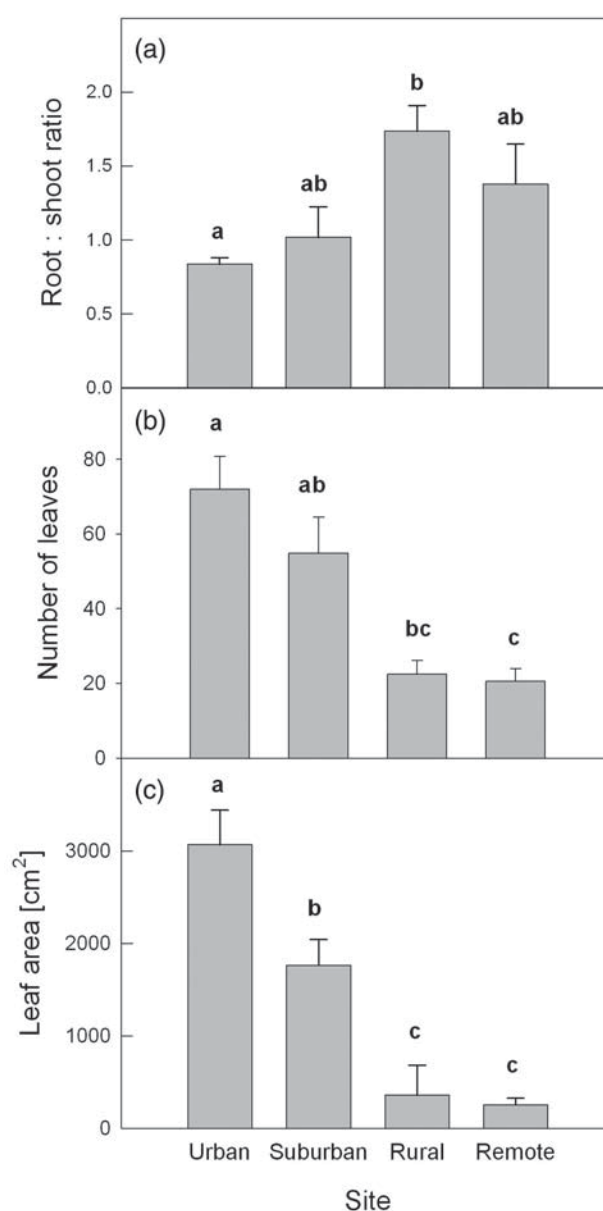

Figure 7. *Q. rubra* seedling biomass allocation measured during the 2008 urban to rural transect field experiment (a) root : shoot ratio, (b) number of leaves per plant and (c) total leaf area in field experiment. Values shown are mean ± SEM. Letters indicate statistical differences.

minimum temperatures in 2008 (a 3.9 °C differential). This abnormally warm growing season distinguishes the second transect study from both the first transect study and GC study.

In this experiment total dry biomass was greatest in seedlings grown at the urban site, and decreased significantly with increasing distance from the city (Table 2). Similarly, leaf, stem and root mass were greatest in urban-grown seedlings and decreased significantly towards the remote site (Table 2).

The root : shoot ratio was significantly greater in plants grown at the urban site relative to the rural site, although it was not statistically different in plants grown at the suburban and remote sites relative to the other sites (Figure 7a). Number of leaves per plant and total leaf area were greatest at the urban site and decreased significantly towards the remote site (Figure 7b and c). Leaf nitrogen did not vary among the transect locations averaging 2.17 ± 0.03% across all sites.

Leaf-scale photosynthetic capacity was not different between sites in the third cohort (Figure 8a; $P = 0.96$). Temperature response curves of respiration are shown in Figure 8b; Table 3 shows values of R_{10} (respiration at a
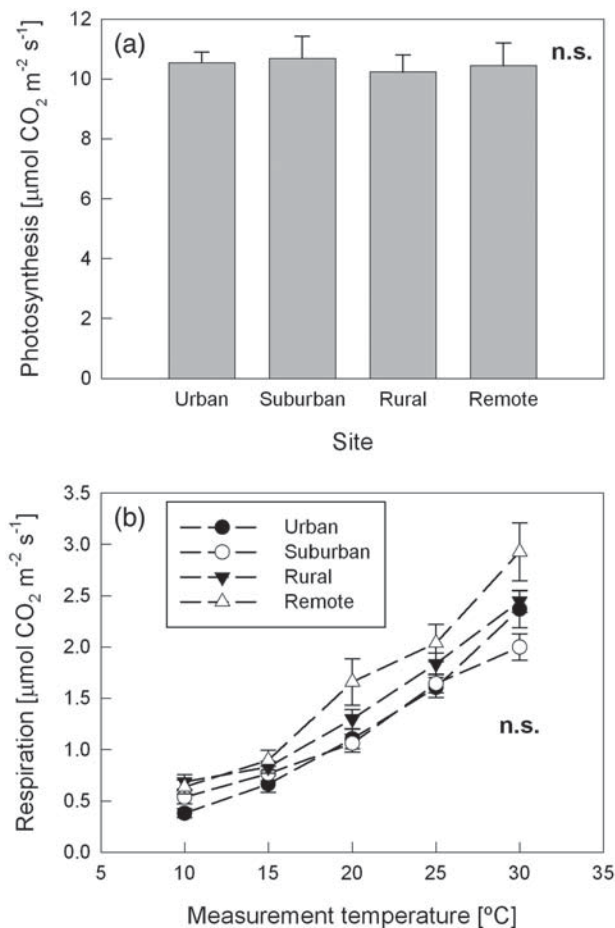
Figure 8. (a) Maximum rate of photosynthesis at 30 °C and (b) temperature response curves of respiration in field experiment. Values shown are mean ± SEM. There were no significant differences between treatments.

set-point temperature of 10 °C) and Q_{10} (the temperature sensitivity of respiration). There were no significant differences in either R_{10} or Q_{10} among sites.

Discussion

Along an urban–rural transect originating in NYC, we found that city-grown *Q. rubra* seedlings had a greater relative growth rate in shoot biomass than seedlings grown in more rural areas. This result confirms the observations of Gregg et al. (2003) who working with *P. deltoides* clones reported a doubling of shoot biomass along a similar urban to rural transect. Since our experiment began with 2-year-old seedlings, we express our results from this first transect study as relative growth rates, and find a similar doubling of the rate of biomass accumulation in the urban compared with rural seedlings (note that Gregg et al. 2003 similarly began with cuttings). *Populus deltoides* is a recent arrival to the forests of New York (Schuster et al. 2008) and remains a very modest influence on the ecology of these systems; thus, extending the previous findings to

Q. rubra, the single most dominant species in the local forests, is significant.

The mechanism behind the growth increase observed in this first transect study cannot be determined. Many environmental variables are changing along this transect. For example, in addition to temperature, changes in atmospheric trace gases like CO_2 or O_3, rates of nitrogen or particulate deposition, and small changes in rainfall, vapor pressure deficit or light may all influence growth both singly and interactively.

Leaf nitrogen concentrations were highest in the urban seedlings, perhaps suggesting that nitrogen deposition may be playing a key role. Also 2005 O_3 levels were lowest near Central Park and increased with distance from the city, perhaps suggesting that ozone could be affecting seedling growth. However, O_3 levels at the rural site were still quite moderate and these levels have typically not elicited a response in growth (Samuelson and Edwards 1993, Kelly et al. 1995, Wullschleger et al. 1996, Samuelson et al. 1996) or photosynthesis in oak seedlings (Samuelson and Edwards 1993, Hanson et al. 1994, Kelly et al. 1995, Wullschleger et al. 1996). Furthermore, shoot relative growth rate was not correlated to O_3, nor were photosynthetic rates in preliminary gas-exchange measurements (Searle et al. 2007). All this led us to undertake growth chamber experiments to increase the level of environmental control as we further explored the potential effects of temperature. Specifically we tested the hypothesis that increased night-time temperatures will result in increased rates of respiration, which will have the effect of increasing both photosynthesis and growth (cf. Turnbull et al. 2002).

Our GC experiment replicated the temperature differential between the urban and rural sites as recorded in the 4 years prior to the experiment (2002–06) while controlling the CO_2, O_3, nitrogen, RH, light and length of the growing season. In this experiment warm-grown seedlings (i.e., grown at urban temperatures) also had greater biomass than cool-grown seedlings (grown at rural temperatures; Table 2), consistent both with the previous work of Gregg et al. (2003) and with our first transect study. These results support our hypothesis and clearly indicate that the warmer temperatures associated with the urban environment, especially high night-time temperatures, can lead to enhanced growth when all other variables are held constant. Although the response of seedlings in the GC experiment provides support for a number of findings of the field experiment, the differences in growth between sites in the field study are much greater than the differences between treatments in the GC experiment. This is likely due to much lower light levels experienced by plants in the GCs.

Similar to our first transect study, leaves from the GC study had significantly more nitrogen. While in the transect study this was likely partly due to the fact that NYC has twice the inorganic nitrogen deposition as surrounding rural areas (Lovett

et al. 2000), this was not the case for the GC study where fertilization remained constant, and thus there is also evidence that the thermal growing regime of these *Q. rubra* seedlings affected either their nitrogen allocation or uptake. This result is similar to the finding of Kanno et al. (2009) that elevated night-time temperatures lead to increased allocation of nitrogen to leaves in rice. The same effect may have occurred in our GC experiment, where warm-grown leaves had a higher specific leaf area than cold-grown leaves.

The physiological measurements did not support our hypothesis that the mechanism enabling increased growth with elevated night-time temperatures would be increased respiration and photosynthesis sensu Turnbull et al. (2002). Instead, despite appropriate trends in area-based leaf photosynthesis and respiratory parameters, the rates were not significantly different between treatments, leaving us to reevaluate the cause of the differences in growth. Physiological processes like photosynthesis and respiration are known to acclimate relatively quickly to ambient environmental conditions (Berry and Björkman 1980, Atkin et al. 2000) and thus perhaps the short-term responses observed during brief manipulative ecosystem warming (Turnbull et al. 2002) are not indicative of the longer-term responses to persistent warming.

It is possible that altered respiratory metabolism significantly affected the seedling growth, despite the lack of measured differences in the net carbon flux. The $\delta^{13}C$ of respired CO_2 was enriched relative to the presumed substrate (the soluble fraction) at all sites along the urban–rural transect. This result agrees with several previous studies reporting enrichment of ^{13}C by leaf respiration (Duranceau et al. 1999, Ghashghaie et al. 2001, Xu et al. 2004, Barbour et al. 2007, Gessler et al. 2009, Tcherkez et al. 2010), but the degree of enrichment in leaves at the urban site (8.5‰) was relatively large compared with values reported in the studies cited here (3–6‰) and elsewhere. The cause of this enrichment is thought to be related to the carbon isotope distribution that exists among the carbon atoms in glucose, with the C-3 and C-4 atoms being enriched relative to the other carbon atoms (Rossman et al. 1991, Tcherkez et al. 2010). As the C-3 and C-4 atoms are the first to be released in the decarboxylation of pyruvate, the simplest explanation for enrichment in ^{13}C of respired CO_2 relative to the respiratory substrate is incomplete oxidation of glucose in the tricarboxylic acid cycle (Rossman et al. 1991, Gessler et al. 2009). These carbon skeletons would presumably be allocated to the creation of new compounds and tissues, i.e., growth. This provides a suggestive link between respiratory function in leaves of urban-grown plants in the present study and their high growth rate. Although total respiration rates appear to be relatively insensitive to this gradient, our findings (along with those in a related study investigating oxygen isotope discrimination during respiration in oak trees along this transect; Searle et al. 2011)

highlight the importance of assessing different respiratory flux components in developing a full understanding of plant responses to the environment.

Further examination of the GC experiment results suggests that biomass allocation may provide some insight into the potential mechanism of the observed growth response. The root : shoot ratio was significantly lower in warm-grown seedlings relative to cool-grown plants, and negatively correlated ($P < 0.05$) with total biomass, consistent with many previous studies that report warm temperatures decrease the root : shoot ratio in various taxa (Teskey and Will 1999, Atkin et al. 2007, Way and Sage 2008, Way and Oren 2010). The low root : shoot ratio in plants grown at warmer urban temperatures has significant implications for their carbon balance as roots are an important carbon sink. Supporting these findings, seedlings grown at warm temperatures also had significantly greater leaf area than cool-grown seedlings. These results agree with previous studies linking higher night-time temperatures with increased growth, leaf area and leaf number (Patterson 1993, Kanno et al. 2009). We suggest that increased growth is not the result of increased leaf-level carbon gain, but simply a result of greater photosynthetic area.

It is also possible that other environmental factors associated with the urban environment of NYC, aside from elevated temperatures, affected the growth of the urban seedlings. Ozone was likely not a factor, as discussed above. It is possible that herbivores, specifically leaf-feeding insects, negatively affected rural-grown plants, as these elements are much less prominent in the city. Lastly, while urban centers often have elevated CO_2 concentrations that could conceivably alter plant physiology and growth, the CO_2 concentrations in NYC have recently been shown to be only ~390–400 ppm during the summer (Hsueh 2009) and so are unlikely to have significantly enhanced growth in our seedlings.

Based on the combination of results from the first transect study and the GC study we chose to conduct a second transect study to assess the role of biomass allocation in the field when the plant material is propagated from seed (acorns). This experiment was conducted during the 2008 growing season, which turned out to be unusually warm, with larger differences in maximum daytime temperatures, particularly during the second half of the growing season. Similar to our first transect study, we found markedly higher growth rates in the urban compared with the rural environments. It is possible that the slightly shorter growing season at the rural and remote sites may account for some of the differences in growth, although budding dates and observed leaf expansion in 2008 were only a few days different among sites.

Biomass allocation did vary along the urban to rural transect in a manner consistent with the GC experiment and we suggest that it may be responsible for the observed difference in biomass accumulation. The root : shoot ratio increased from

the urban towards the rural end of the transect, with a negative relationship between root : shoot ratio and total mass. As with the GC experiment, decreased allocation to the roots allowed greater allocation to the shoot, and in particular, to leaves, as urban-grown seedlings had both greater leaf area and number of leaves than seedlings grown at more rural sites.

As with the GC experiment, we found no support for our hypothesized mechanism of temperature-stimulated respiration leading to increased photosynthetic carbon gain. The gas-exchange rates were unchanged along the transect suggesting that acclimation had occurred. These were also higher in the field than in the GC experiment, again consistent with the relatively low light in the GC environment.

All sites in the 2008 field study were fertilized and thus all exhibited high leaf N. Interestingly, this contrasts with the results of the GC experiment, which both had much lower leaf N levels and showed a clear effect of temperature, perhaps suggesting that thermal effects on leaf N are more likely to occur under nitrogen limitation. Low light levels in the GC experiment may have limited the energy available for nitrate reduction.

The high 2008 temperatures and somewhat unusual diurnal and seasonal patterns during the 2008 transect study mean that both the daytime and night-time temperatures were warmer at the urban site than at the rural sites, potentially confounding our efforts to test the effect of night-time temperatures. In one of the early 'phytotron' experiments, Camus and Went (1952) grew *Nicotiana tabacum* under 27 different temperature cycles and at various light conditions. From this extraordinary experiment, they concluded that night-time temperature is 'the most critical factor influencing developmental processes such as growth rate, leaf characteristics, flowering and final weight.' Thus, we suggest that our results, like those of these early phytotron experiments, demonstrate the importance of night-time temperatures in controlling the growth of tree seedlings in urban environments.

Taken together, the results of these experiments lead us to several important conclusions. First, the key observation of Gregg et al. (2003), that phytoindicators found in urban locations grow much faster than in rural areas, can be extended to include the regionally important species *Q. rubra*. Second, because differences in growth can occur when other environmental variables such as CO_2, O_3, light, RH, water and soil N are held constant and only the temperature varies, diurnal temperature range should be added to the list of urban environmental variables such as O_3 (Gregg et al. 2003) capable of affecting plant growth. Third, the proximate mechanism resulting in altered growth is not leaf gas exchange as we hypothesized, which instead appears to have acclimated, but rather is more likely a function of total leaf area as influenced by biomass allocation. Fourth, while bulk respiratory carbon flux may

not have changed, the isotopic signal of respired CO_2 and previous work on respiratory pathways (Searle et al. 2011) suggest that important alterations of respiratory efficiency and function may be occurring. Finally, we caution that there is little reason to believe that any of these environmental variables are necessarily acting completely independently and suggest that much more research is needed to clarify the multitude of potential effects and interactions influencing plant growth in urban environments.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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