

Respiration as a percentage of daily photosynthesis in whole plants is homeostatic at moderate, but not high, growth temperatures

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Summary

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- Here, we investigated the impact of temperature on the carbon economy of two *Plantago* species from contrasting habitats.
- The lowland *Plantago major* and the alpine *Plantago euryphylla* were grown hydroponically at three constant temperatures: 13, 20 and 27 C. Rates of photosynthetic CO₂ uptake (*P*) and respiratory CO₂ release (*R*) in shoots and *R* in roots were measured at the growth temperature using intact plants. At each growth temperature, air temperatures were changed to establish short-term temperature effects on the ratio of *R* to *P* (*R/P*).
- In both species, *R/P* was essentially constant in plants grown at 13 and 20 C. However, *R/P* was substantially greater in 27 C-grown plants, particularly in *P. euryphylla*. The increase in *R/P* at 27 C would have been even greater had biomass allocation to roots not decreased with increasing growth temperature. Short-term increases in air temperature increased *R/P* in both species, with the effects of air temperature being most pronounced in 13 C-grown plants.
- We conclude that temperature-mediated changes in biomass allocation play an important role in determining whole-plant *R/P* values, and, while homeostasis of *R/P* is achieved across moderate growth temperatures, homeostasis is not maintained when plants are exposed to growth temperatures higher than usually experienced in the natural habitat.

Key words: acclimation, biomass allocation, CO₂, *Plantago*, photosynthesis, respiration, temperature.

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Introduction

In leaves of higher plants, the processes of respiratory CO₂ release (*R*) and photosynthetic CO₂ uptake (*P*) are interdependent. On the one hand, *R* relies on *P* substrates, while on the other, *P* is dependent on *R* for the carbon skeletons and for the ATP required for sucrose synthesis plus repair of photosynthetic proteins (Krömer, 1995; Hoefnagel *et al.*, 1998; Padmasree *et al.*, 2002). As a result, the instantaneous *R/P* ratio in individual leaves is often constant, even in plants experiencing contrasting growth temperatures (Gifford,

1995; Ziska & Bunce, 1998; Dewar *et al.*, 1999; Loveys *et al.*, 2003; Atkin *et al.*, 2006). This constancy, if held over extended periods, raises the possibility that global carbon cycle models can assume homeostasis of *R/P* when predicting future CO₂ fluxes between plants and the atmosphere (Gifford, 2003). However, such an assumption requires that *R/P* be homeostatic not only in individual leaves but also in whole plants.

In whole plants, several factors can contribute to differences in *R/P* compared with that observed in individual mature leaves. Firstly, stem and root *R* values contribute to daily respiratory CO₂ release in whole plants; because of this, *R/P*

is greater in whole plants than in individual leaves. Secondly, irradiances experienced by whole shoots are often not saturating or uniform throughout the canopy; as a result, rates of light-saturated P in leaves are often a poor indicator of P in whole shoots (e.g. Evans *et al.*, 2000). Thirdly, in whole plants, mature and immature tissues both contribute to CO_2 exchange. Young tissues respire at higher rates (Collier & Grodzinski, 1996; Radoglou & Teskey, 1997; Millar *et al.*, 1998; Oleksyn *et al.*, 2000; Armstrong *et al.*, 2006) than mature tissues and maximal rates of P are not exhibited until leaves are fully expanded (Evans *et al.*, 2000; Miyazawa & Terashima, 2001). As a result, the presence of young tissues in whole plants can alter R/P ratios.

Because P and R are temperature sensitive, a change in temperature results in an immediate alteration in the rate of R and P , with the extent of that alteration being determined by the temperature coefficient of each process. The temperature sensitivity of P differs from that of R , with the result that R/P is altered following a short-term (i.e. minutes to hours) change in measuring temperature (Dewar *et al.*, 1999; Hansen *et al.*, 2002; Gifford, 2003; Atkin *et al.*, 2006). However, in many species, homeostasis of R/P in individual leaves is re-established when plants experience contrasting temperatures for sustained periods (i.e. as a result of thermal acclimation of specific rates of R and P ; Loveys *et al.*, 2003; Tjoelker *et al.*, 1999a). Given that the degree to which specific rates of R and P acclimate differs among species (Berry & Björkman, 1980; Larigauderie & Körner, 1995; Tjoelker *et al.*, 1998, 1999b; Xiong *et al.*, 1999, 2000; Loveys *et al.*, 2002; Atkin *et al.*, 2006), and among different tissues within individual plants (Atkin *et al.*, 2005; Armstrong *et al.*, 2006), it seems unlikely that all species will exhibit the same degree of homeostasis of whole-plant R/P when grown under contrasting temperatures.

In contrast to the growing number of studies that have investigated the effect of growth temperature on R/P of individual mature leaves, relatively few studies have investigated the impact of growth temperature on R/P in whole plants using actual measurements of whole-plant gas exchange. Gifford (1995) found that daily R/P was constant for potted-grown wheat (*Triticum aestivum*) developed at constant temperatures ranging from 15 to 30 °C. Similarly, soybean (*Glycine max*) grown at a range of growth temperatures between 20 and 35 °C showed no differences in R/P (Ziska & Bunce, 1998). However, in the latter study, growth under an elevated concentration of CO_2 (700 $\mu\text{l l}^{-1}$) did result in reduced R/P , compared with growth at ambient CO_2 (350 $\mu\text{l l}^{-1}$). Similarly, Loveys *et al.* (2002) found that plants grown at 28 °C exhibited higher whole-plant R/P ratios than did the plants grown at 18 and 23 °C. The extent to which whole-plant R/P values remain homeostatic under contrasting growth temperatures therefore appears to be variable.

A factor that could contribute to temperature-mediated changes in R/P is the impact of growth temperature on biomass allocation to roots, stems and leaves. When considered

alone, increased allocation to roots would lead to an increase in whole-plant R/P (as a result, in part, of an increase in overall respiratory carbon release by roots, whose specific rates of R are typically higher than those of stems and leaves). Where increased investment in roots is associated with a decrease in biomass allocation to leaves, reductions in shoot P would further exacerbate increases in R/P . There is evidence that growth at low temperatures can result in increased allocation of biomass to roots in some species (Equiza & Tognetti, 2002), whereas in other species the effect of temperature on root biomass is variable depending on the growth temperatures being compared (DeLucia *et al.*, 1992). To our knowledge, no study has previously established the role temperature-mediated changes in biomass allocation play in determining the whole-plant R/P values.

The objective of our study was to investigate the impact of sustained differences in growth temperature, and short-term changes in air temperature, on the whole-plant carbon economy of two congeneric *Plantago* species from contrasting habitats. The lowland *Plantago major* and the alpine *Plantago euryphylla* differ in maximum relative growth rate (RGR ; Loveys *et al.*, 2002) and in their ability to thermally acclimate specific rates of leaf P and R (Atkin *et al.*, 2006). What is not known, however, is whether differences in the ability to acclimate leaf metabolism are associated with differences in acclimation of P and R and/or homeostasis of R/P at the whole-plant level. By measuring CO_2 exchange in shoots separately from that of roots, we were able to obtain accurate estimates of the temperature dependence of intact shoot and root R , and shoot P . We addressed the following questions. (1) To what extent is homeostasis of whole-plant R/P achieved across a range of growth temperatures in contrasting plant species differing in their ability to thermally acclimate R and P in individual leaves? (2) What role do temperature-mediated changes in biomass allocation and specific rates of R and P play in determining the ratio of R/P in whole plants? (3) To what extent do short-term variations in temperature alter whole-plant R/P values?

Materials and Methods

Plant material and growth conditions

Two *Plantago* species (lowland *Plantago major* L. and alpine *Plantago euryphylla* Briggs, Carolin & Pulley) were used in the experiments. Seeds of *P. major* originated from near-sea-level sites in the Netherlands. *Plantago euryphylla* seeds were collected at a field site in Kosciusko National Park, NSW, Australia at an altitude of 1940 m (G.R. 165687). Seeds of *P. major* were germinated on moist filter paper and then transferred to sand moistened with a half-strength modified 1/8 Hoagland solution in a growth cabinet (25 : 10 °C, 12 : 12 h light:dark; photon flux density (PPFD) 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$). After approx. 14–21 d of establishment, *P. major* seedlings were

transferred to 30-l tanks filled with aerated modified 1/8 Hoagland solution containing 2 mM NO_3^- as the nitrogen source (Poorter & Remkes, 1990; Atkin *et al.*, 2006). Solutions were changed weekly and the pH was adjusted daily to 5.8. Plants were grown under controlled conditions (12 : 12 h day:night; 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$; 70% relative humidity) in three growth cabinets that differed in temperature (constant 13, 20 or 27 C).

Plantago euryphylla seeds were planted on soil and initially grown in a controlled environment chamber with an irradiance of 190 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 23 : 14 C day:night temperature. Once the seedlings were large enough, they were transferred into pots containing Cocopeat and transferred to the 13, 20 or 27 C cabinets described in the previous paragraph. The pots were irrigated with nutrient solution identical to that used for the lowland species, with the exception that the solution contained equal concentrations of both NO_3^- and NH_4^+ . Initial growth of *P. euryphylla* on organic media and provision of NH_4^+ were necessary as *P. euryphylla* produced healthier leaves when grown on organic media than on hydroponic culture and developed nutrient deficiency symptoms when provided with only NO_3^- nitrogen. Once plants had established on the organic media for 35–40 d, they were transferred to hydroponic culture (containing modified Hoagland solution as above, but with 1 mM NO_3^- and 1 mM NH_4^+) for a further 3 wk.

Gas exchange

Whole-plant gas exchange was measured on both species. Whole-shoot photosynthesis (P), shoot respiration (shoot R) in darkness, and root respiration (root R) were measured in four randomly selected plants of each species at each growth temperature (13, 20 and 27 C) when the plants had reached a total fresh mass of approximately 3–6 g (Poorter & Welschen, 1993). Measurements of shoot and root CO_2 exchange were conducted as described in Loveys *et al.* (2002), but with the impact of variable measurement temperatures on CO_2 exchange also being assessed. Intact plants were placed into cuvettes with the roots and shoots in separate compartments (see Den Hertog *et al.* (1993) for additional details of the experimental system); as the shoot compartment was considerably larger than the shoot diameter, leaf display was the same in the compartment as in the growth cabinet. Air within the shoot compartment was well mixed using fans positioned on opposite corners of the compartments, with air passed through the shoot compartment at a flow rate of 12–14 l min^{-1} . The root compartment was filled with 800 ml of aerated nutrient solution (see previous section) plus buffer (10 mM (2-*N*-morpholino)ethanesulfonic acid (MES)), with air passed through the root compartments at a flow rate of 0.9–1.1 l min^{-1} . The irradiance that shoots were exposed to was the same as that in the growth cabinets (provided by HPI-T 400-W high-pressure mercury lamps (Philips Nederland BV, Eindhoven,

the Netherlands) in both cases). Root R in buffered nutrient solution was measured immediately after the P measurement. Shoot R was recorded after the plants had been in darkness for approx. 0.5 h. CO_2 fluxes from the shoot and root compartments were measured using a Li-Cor 6262 infrared gas analyser (Li-Cor, Lincoln, NE, USA) in an open system (air flowing from the root compartments was dried before passing through the gas analyser). In the first set of measurements assessing the impact of shoot temperature on gas exchange, root temperature was set to that experienced by plants in their respective growth cabinet (13, 20 or 27 C). To assess the impact of air temperature on shoot R and P , we exposed shoots sequentially to 20, 27 and then 34 C. At each shoot temperature, P in the light, root R and then shoot R in darkness were measured; root temperature was kept at the growth temperature throughout. Although root respiration at 13 C was measured, measurements of shoot gas exchange at 13 C were technically not possible (because the heat load from the mercury lamps exceeded our capacity to cool the compartment environment). Thus, for the 13 C-grown plants we did not obtain a measurement of shoot R or P at the growth temperature. Estimates of shoot CO_2 exchange at 13 C were made, however, using information on the temperature dependence of leaf R (in darkness and in the light) and leaf P at growth irradiance (Atkin *et al.*, 2006). To establish the short-term temperature dependence of root R over a common measurement temperature range (20–27 C), we conducted an additional set of experiments where roots were first exposed to 20 C and then to 27 C, with root R being measured in each case (with shoots illuminated); in each case, shoots were kept at the growth temperature, with the exception of the 13 C-grown plants, where shoot temperature was set to 20 C.

The impact of growth temperature on the relative growth rate (RGR; $\text{mg g}^{-1} \text{d}^{-1}$) of whole plants was calculated for each species using the following formula:

$$\text{RGR} = \frac{\left(P_{\text{net}} \times \frac{12}{24} \times \text{LMR} \right) - \left(\text{shoot } R_{\text{night}} \times (\text{LMR} + \text{StMR}) \times \frac{12}{24} \right) - \left(\text{root } R \times \text{RtMR} \times \frac{24}{24} \right)}{(\text{CC} \times 1000)} \quad \text{Eqn 1}$$

(P_{net} , the measured specific rate of *net* photosynthesis (i.e. CO_2 uptake by carboxylation minus CO_2 release by photorespiration and shoot R_{day} , the specific shoot R in the light); shoot R_{night} , the specific shoot R in darkness; root R , the specific rate of root R , assumed to be constant over light and dark periods as shown by Scheurwater *et al.* (1998); LMR, StMR and RMR, the leaf, stem and root mass ratios (i.e. proportion of whole-plant biomass allocated to each organ), respectively; CC, the

carbon concentration.) Carbon concentrations at each temperature were predicted using the relationship between CC and growth temperature (T) reported previously ($P. major$, $CC = 48.81 - 0.68T$; $P. eurypphylla$, $CC = 31.01 + 0.03T$; Loveys *et al.*, 2002). For RGR values at 13 C, predicted rates of gas exchange at 13 C were used (see previous paragraph).

Whole-plant R/P_{gross} (i.e. the percentage of daily gross photosynthetic CO_2 uptake released by whole-plant respiration) values were calculated, where daily whole-plant R was taken as root R plus shoot R over a 24-h period, and P_{gross} was the measured rate of P_{net} plus shoot R during the day. When calculating rates of shoot R during the day (shoot R_{day}) for each species, we multiplied the measured rates of shoot R in darkness by the ratio of leaf R in the light to that in darkness (R_{light}/R_{dark}) for each growth temperature/measuring combination reported previously (Atkin *et al.*, 2006). For $P. major$ at 13, 20 and 27 C, mean R_{light}/R_{dark} ratios at each respective growth temperature were 1.25, 0.36 and 0.38, respectively. For $P. eurypphylla$ at 13, 20 and 27 C, mean R_{light}/R_{dark} ratios were 0.84, 0.61 and 0.44, respectively. Data from Atkin *et al.* (2006) were also used to take into account the impacts of short-term changes in measuring temperature on R_{light}/R_{dark} ratios. The equation used to calculate whole-plant R/P_{gross} according to this approach was:

$$R/P_{gross} = \frac{100 \times \left[\begin{array}{l} \left(\text{shoot } R_{day} \times (\text{LMR} + \text{StMR}) \times \frac{12}{24} \right) \\ + \left(\text{shoot } R_{night} \times (\text{LMR} + \text{StMR}) \times \frac{12}{24} \right) \\ + \left(\text{root } R \times \text{RMR} \times \frac{24}{24} \right) \end{array} \right]}{\left(P_{net} + \text{shoot } R_{day} \right) \times \text{LMR} \times \frac{12}{24}} \quad \text{Eqn 2}$$

(12/24, the relative lengths of both the dark and the light periods.) The resultant daily rates were then weighted according to the number of hours over which each gas exchange parameter occurred on each day. For R/P values at 13 C, predicted rates of shoot gas exchange at 13 C were used, these being obtained from measured rates at 20 C and previously measured temperature responses of leaf gas exchange made at growth irradiance (Atkin *et al.*, 2006). To establish the relative contributions of roots and shoots to whole-plant R/P_{gross} , we also calculated root and shoot R/P_{gross} values separately using modified versions of Eqn 2. Similarly, the percentage of whole-plant R taking place in roots and shoots was calculated using modified versions of Eqn 2 (with light impacts on shoot R (Atkin *et al.*, 2006) being taken into account).

To assess the impact of variations in night-time shoot temperature alone on R/P_{gross} of whole plants, we included predicted rates of shoot R at 6 and 13 C in Eqn 2 (for plants grown at 13 and 20 C), using second-order polynomial

equations fitted to log-transformed values of shoot R in darkness plotted against measuring temperature:

$$13 \text{ C grown: } \log \text{ shoot } R = -0.3923 + (0.0576 \times T) + (0.00050 \times T^2) \quad \text{Eqn 3}$$

$$20 \text{ C grown: } \log \text{ shoot } R = -0.0172 + (0.0289 \times T) + (0.00009 \times T^2) \quad \text{Eqn 4}$$

(T , measuring temperature.) Measured rates of P_{net} during the day (at 20, 27 and 34 C) and root R at the growth temperature were used in the calculations. We also assessed the impact of daytime shoot temperature (20, 27 and 34 C; with night-time shoot and root temperatures kept constant at 13 C) on R/P_{gross} ; for these calculations, measured rates of shoot and root CO_2 exchange were used, taking into account variations in the ratio of R_{light}/R_{dark} (Atkin *et al.*, 2006).

Biomass allocation and statistical analyses

Dry masses of leaves, stems and roots were determined and used to calculate LMR, StMR and RMR values (see previous section). Tissues were freeze-dried in a Virtus, Unitop 600 SL freeze dryer (Gardiner, New York, NY, USA). Leaf area was determined using a Li-Cor 3100 leaf area meter (Li-Cor), with the ratio of leaf dry mass to leaf area being used to calculate leaf mass per unit area (LMA) values. Statistical analyses were carried out using the SPSS software package version 10 (SPSS Inc., Chicago, IL, USA). Where necessary, proportional data were angular transformed to ensure that data were normally distributed and variances homogeneous before analysis using one- or two-way analysis of variance (ANOVA). In cases where data remained nonparametric (e.g. when comparing $P. major$ root R/P_{gross} values at each growth temperature), the Kruskal–Wallis test was used followed by pair-wise comparison of growth temperatures using the Mann–Whitney U -test. At each growth temperature, comparisons of R/P_{gross} between the two species were made using one-way ANOVA. One-way ANOVAs were also used to compare the values of Q_{10} (i.e. the proportional increase in R for every 10 C rise in measuring temperature) of shoot and root R of the two species.

Results

Biomass allocation at each respective growth temperature

The proportion of total plant dry mass allocated to leaves, stems and roots varied with growth temperature in both species (Fig. 1; Table 1). In $P. major$, 13 C-grown plants allocated a significantly lower proportion of plant mass to leaves (i.e. they exhibited a lower LMR) than plants grown at 27 C (Fig. 1a; Table 1); conversely, growth at 13 C was associated with a significant increase in the RMR (Table 1). Similar effects of

Fig. 1 Effect of growth temperature (13, 20 and 27 °C) on biomass allocation to leaves, stems and roots (a and c) and the percentage of daily whole-plant respiration taking place in roots and shoots (b and d) for *Plantago major* and *Plantago euryphylla*. Leaf, stem and root mass ratios (LMR, StMR and RMR, respectively) are expressed as a proportion of whole-plant biomass; values represent the mean of four individual replicates (\pm standard error (SE)), with SE values for LMR, StMR and RMR being shown at the top of each vertical bar. For each parameter within a species, values associated with different letters to the right of each bar are significantly different (Student–Newman–Keuls post hoc test, $P < 0.05$). In (b) and (d), the rate of respiratory CO₂ release (*R*) in the shoot was calculated taking into account the effects of light on *R* during the daytime ($n = 4$; \pm SE).

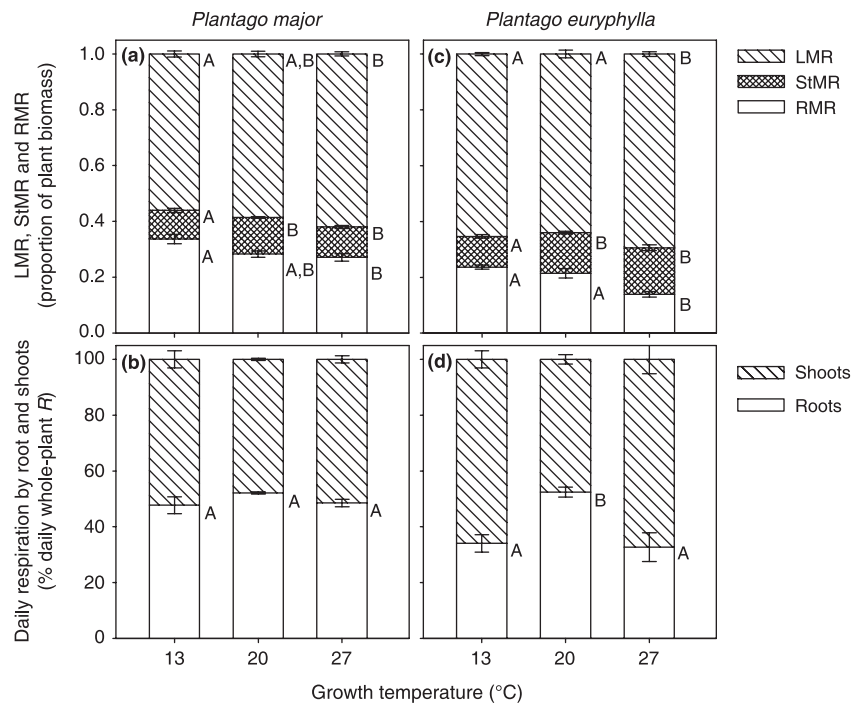


Table 1 Results of tests for differences between parameter values measured at each respective growth temperature for biomass allocation, growth rate, CO₂ exchange data and the Q₁₀ of shoot and root respiration for *Plantago major* and *Plantago euryphylla*

Parameter	<i>P. major</i>	<i>P. euryphylla</i>	Location of data
LMR	**	*	Fig. 1
StMR	*	**	Fig. 1
RMR	*	**	Fig. 1
LMA	***	***	Table 2
Shoot P_{net} (per unit area)	***	***	Table 2
Shoot <i>R</i> (per unit area)	***	**	Table 2
Root <i>R</i> (per unit mass)	***	***	Table 2
RGR	***	***	Table 2
Shoot P_{net} (per unit mass)	***	***	Fig. 2
Shoot <i>R</i> (per unit mass)	***	***	Fig. 2
R/P_{gross}	**	*	Fig. 3/Table 3
Shoot <i>R</i> /whole-plant <i>R</i>	ns	**	Fig. 1
Root <i>R</i> /whole-plant <i>R</i>	ns	**	Fig. 1
Shoot R/P_{gross}	**	**	Results text
Root R/P_{gross}	*	ns	Results text
Q ₁₀ of shoot <i>R</i>	ns	ns	Table 5
Q ₁₀ of root <i>R</i>	ns	ns	Table 5

Relevant figure and table numbers containing data used in each test are shown. For whole-plant and shoot *R* values, the effect of light on shoot respiratory CO₂ release during the daytime was taken into account (where *R* is the rate of respiratory CO₂ release). In most cases, data were analysed using one-way analysis of variance (ANOVA), followed by a Student–Newman–Keuls a posteriori test (see individual figures and tables, and/or Results text). ANOVA *P*-value categories are shown: $P < 0.001$ (***), $P < 0.01$ (**) and $P < 0.05$ (*). For Root R/P_{gross} of *P. major*, data remained nonparametric even after angular transformation, thus necessitating the use of a Kruskal–Wallis test for which the *P*-value is shown. In all cases, four replicates per treatment combination were used in the analysis. A nonsignificant (ns) temperature effect indicates homeostasis for that parameter. LMR, StMR and RMR, leaf, stem and root mass ratios, respectively; LMA, leaf mass per unit area; RGR, relative growth rate; Q₁₀, the proportional increase in *R* for every 10 °C rise in measuring temperature; P_{gross} , the measured specific rate of net photosynthesis plus shoot *R* during the day; P_{net} , the measured specific rate of net photosynthesis.

Table 2 Rates of shoot net photosynthesis per unit area (P_a), shoot respiration per unit area (R_a), root respiration per unit root mass (root R), leaf mass per unit area (LMA) and calculated relative growth rates (RGRs) at each respective growth temperature (i.e. 13, 20 or 27 °C) of two contrasting *Plantago* species

Parameter	<i>Plantago major</i>			<i>Plantago euryphylla</i>		
	13 C	20 C	27 C	13 C	20 C	27 C
Shoot P_a ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	11.2 \pm 0.3 ^A	13.7 \pm 0.5 ^B	9.8 \pm 0.2 ^C	8.8 \pm 0.3 ^A	12.1 \pm 0.7 ^B	8.0 \pm 0.3 ^A
Shoot R_a ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	0.8 \pm 0.3 ^A	1.2 \pm 0.0 ^C	1.1 \pm 0.0 ^B	1.0 \pm 0.1 ^A	1.5 \pm 0.1 ^B	1.8 \pm 0.1 ^C
Root R ($\text{nmol CO}_2 \text{ g}^{-1} \text{ DM s}^{-1}$)	23.0 \pm 1.5 ^A	39.2 \pm 1.7 ^B	41.7 \pm 1.4 ^B	16.9 \pm 0.8 ^A	41.2 \pm 4.1 ^B	26.4 \pm 2.8 ^C
RGR ($\text{mg g}^{-1} \text{ d}^{-1}$)	79 \pm 2 ^A	173 \pm 11 ^B	168 \pm 12 ^B	69 \pm 4 ^A	117 \pm 5 ^B	68 \pm 7 ^A
LMA (g DM m^{-2})	62.9 \pm 0.6 ^A	44.6 \pm 1.6 ^B	38.1 \pm 0.8 ^C	83.6 \pm 2.7 ^A	66.8 \pm 0.8 ^C	75.5 \pm 2.4 ^B

Values are the mean of four individual replicates (\pm standard error). Within each species, gas exchange and LMA values or RGRs associated with different letters are significantly different (Student–Newman–Keuls post hoc test, $P < 0.05$). See Table 1 for P -values (as analysed by one-way analysis of variance).

growth temperature on LMR and RMR were observed in *P. euryphylla*, with plants grown at 13 and 20 °C exhibiting significantly lower LMR and higher RMR values than their 27 °C-grown counterparts (Fig. 1c; Table 1). Moreover, 13 °C-grown *P. euryphylla* plants allocated less biomass to petioles (included in the StMR) compared with their warm-grown counterparts (Fig. 1c; Table 1). Thus, in both species, RMR increased with decreasing growth temperature at the expense of other plant organs.

Biomass allocation within leaves was also temperature dependent in both species, as shown by the significantly different LMA values among the three growth temperatures (Table 1). In *P. major*, LMA increased with decreasing growth temperature, with the increase in LMA being most marked when comparing plants grown at 13 and 20 °C. For *P. euryphylla*, LMA values were also significantly greater in 13 °C-grown plants (compared with their counterparts grown at 20 and

27 °C); however, LMA values were also significantly greater at 27 °C than at 20 °C for this species (Table 2). Moreover, the relative difference in LMA values among the growth temperatures was considerably greater for *P. major* than for *P. euryphylla*. Therefore, while temperature influenced LMA values in both species, the magnitude and direction of the temperature effect differed between the species.

Whole-plant CO₂ exchange at each respective growth temperature

In both species, rates of shoot net CO₂ exchange in the light (i.e. P_{net} expressed on a dry mass basis) at each respective growth temperature were significantly greater in plants grown at 20 °C than in plants grown at 13 and 27 °C (Table 1, and symbols connected with thick solid lines in Fig. 2a,c). Similarly, rates of P_{net} expressed per unit leaf area at each

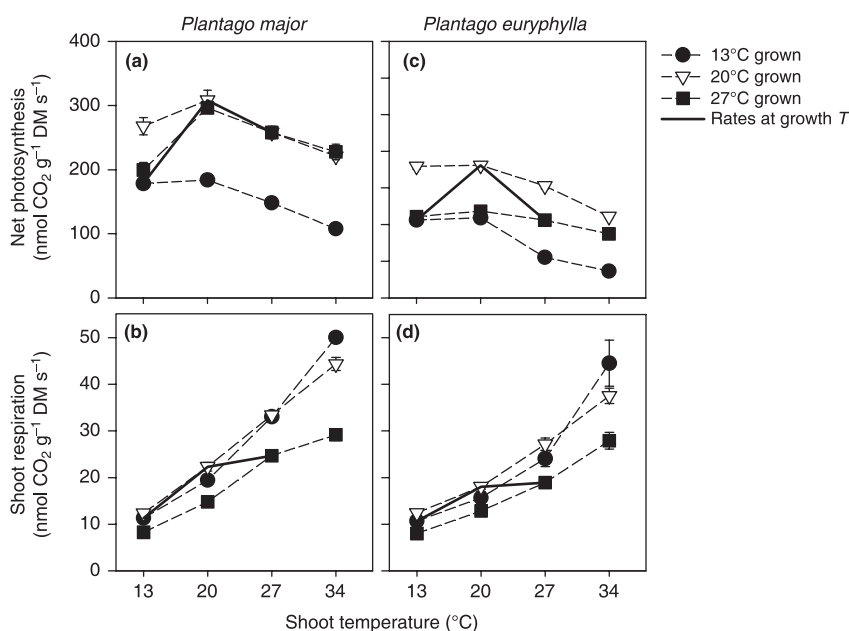
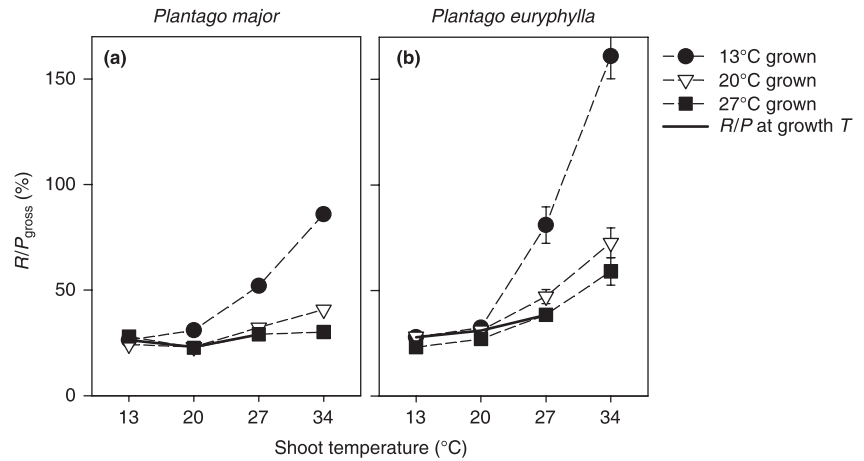


Fig. 2 Net photosynthesis (a, c) and respiration in darkness (b, d) by intact, whole shoots of (a, b) *Plantago major* and (c, d) *Plantago euryphylla* grown at three constant temperatures (13, 20 and 27 °C). For both photosynthesis and shoot respiration, rates are expressed on a dry mass (DM) basis. Values are the mean of four individual replicates (\pm standard error). The thick lines in each figure connect values at each respective growth temperature. For shoot gas exchange at 13 °C, values were predicted from determinations made at a measurement temperature of 20 °C and scaled to 13 °C using temperature response functions for photosynthesis and respiration determined on individual leaves (Atkin *et al.*, 2006).

Fig. 3 Effect of short-term changes in shoot temperature on whole-plant R/P_{gross} (where R is the rate of respiratory CO_2 release and P_{gross} is the measured specific rate of net photosynthesis plus shoot R during the day), as dependent on growth temperature (13, 20 and 27 C) for (a) *Plantago major* and (b) *Plantago euryphylla*. The thick lines in each figure connect values at each respective growth temperature. In all cases, roots were kept at their respective growth temperature (see Table 2 for specific rates of root R at each respective growth temperature). Values are the mean of four individual replicates (\pm standard error). Whole-plant R/P_{gross} values were calculated using Eqn 2 as outlined in the Materials and Methods.



respective growth temperature were significantly greater in plants grown at 20 C than in plants grown at 13 and 27 C (Tables 1, 2). Shoot R (measured after 30 min in darkness) at each respective growth temperature was also temperature dependent (Table 1), being significantly lower in 13 C-grown plants (compared with plants grown at 20 and 27 C), irrespective of whether R was expressed on a dry mass (see symbols connected with thick, solid lines in Fig. 2b,d) or area (Table 2) basis. Similarly, root R measured at each respective growth temperature differed significantly among the growth temperatures (Table 1), being lowest in 13 C-grown plants (Table 2); in *P. major*, plants grown at 20 and 27 C exhibited similar rates of root R , whereas in *P. euryphylla* root R was significantly lower in 27 C-grown plants (compared with plants grown at 20 C). While such results do not exclude a degree of thermal acclimation of P_{net} , shoot R and root R , they show that complete homeostasis of specific rates of CO_2 exchange at the whole-plant level was not achieved across the 13–27 C growth temperature range.

Whole-plant RGRs for both species grown at 13, 20 and 27 C were calculated using Eqn 1. As expected, maximal RGR values were higher for *P. major* than for *P. euryphylla*, particularly at 20 at 27 C (Table 2). In contrast, the two species exhibited relatively similar RGR values when grown at 13 C. There was no significant difference between RGR values exhibited by *P. major* at 20 and 27 C (Table 2); in contrast, growth at 27 C resulted in significantly lower RGR values of *P. euryphylla* (compared with 20 C-grown plants). The temperature optimum of RGR was thus lower for the alpine than for the lowland species.

Figure 3 (symbols connected with thick solid lines) and Table 3 show the extent to which whole-plant R/P_{gross} remained homeostatic at each respective growth temperature. For *P. major*, whole-plant R/P_{gross} was significantly lower in the 20 C-grown plants than in their counterparts grown at 27 and 13 C (Fig. 3a; Table 3). Although it was not surprising that R/P_{gross} was significantly higher at 27 than at 20 C, what was unexpected was the rise in whole-plant R/P_{gross} at 13 C. The rise

Table 3 Effect of growth temperature on the percentage of CO_2 fixed by gross photosynthesis which is subsequently respired by whole plants over a 24-h period (i.e. whole-plant R/P_{gross} , where R is the rate of respiratory CO_2 release and P_{gross} is the measured specific rate of net photosynthesis plus shoot R during the day) at each respective growth temperature (i.e. 13, 20 or 27 C) for the two contrasting *Plantago* species

Species	Growth temperature		
	13 C	20 C	27 C
<i>Plantago major</i>	26.3 \pm 0.7 ^B	23.1 \pm 0.3 ^A	29.1 \pm 1.7 ^B
<i>Plantago euryphylla</i>	27.8 \pm 1.6 ^A	31.0 \pm 1.3 ^A	38.5 \pm 2.9 ^B

Daily whole-plant R was taken as root R over a 24-h period plus shoot R over a full 24-h period (taking into account temperature-dependent changes in the ratio of $R_{\text{light}}/R_{\text{dark}}$ as outlined in the Materials and Methods) and photosynthesis during the daytime was calculated on a gross basis (i.e. the measured specific rate of net photosynthesis plus shoot R during the day). Values are the mean of four individual replicates (\pm standard error). P -values (as analysed by one-way analysis of variance) are shown in Table 1. Within each row of data, values associated with different letters are significantly different (Student–Newman–Keuls post hoc test, $P < 0.05$). To ensure data were normally distributed and the variances homogeneous, we performed an angular transformation of proportional data before analysis (untransformed data are shown).

in whole-plant R/P_{gross} at 13 C reflected the fact that leaf $R_{\text{light}} > R_{\text{dark}}$ at the low growth temperature (Atkin *et al.*, 2006), thus increasing daily shoot R . In *P. euryphylla*, whole-plant R/P_{gross} was significantly higher in 27 C-grown plants compared with their counterparts grown at 13 and 20 C (Fig. 3b; Table 3). Comparison of the whole-plant R/P_{gross} values of the two species at each growth temperature showed that, while R/P_{gross} was significantly higher in *P. euryphylla* than in *P. major* when plants were grown at 20 and 27 C ($P < 0.01$ and $P < 0.05$, respectively, when interspecific data at each growth temperature were analysed with a one-way ANOVA), there were no significant differences in whole-plant R/P_{gross}

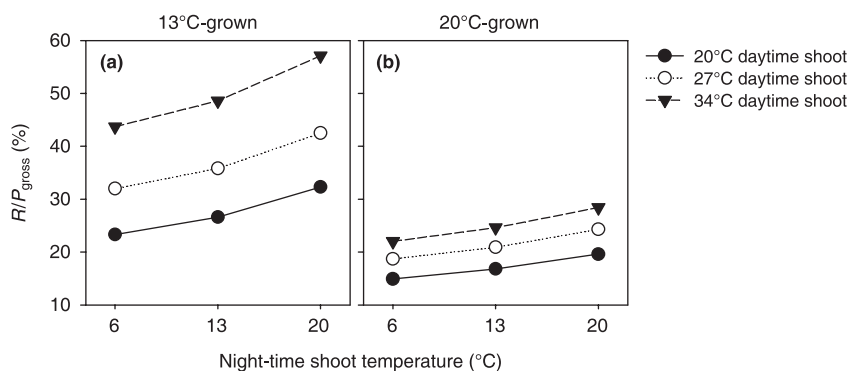


Fig. 4 Modelled temperature-dependent changes in the percentage of daily gross photosynthetic CO_2 uptake released by whole-plant respiration (i.e. R/P_{gross} , where R is the rate of respiratory CO_2 release and P_{gross} is the measured specific rate of net photosynthesis plus shoot R during the day) for *Plantago major*. (a) and (b) show (for 13 C- and 20 C-grown plants, respectively) R/P_{gross} values where night-time shoot temperature alone was varied (with daytime shoot temperatures at 20, 27 or 34 C) and roots experiencing their respective growth temperatures during the day and night. Whole-plant R/P_{gross} values were calculated using Eqn 2 as outlined in the Materials and Methods.

when 13 C-grown plants were compared ($P=0.436$; Fig. 3; Table 3).

To assess whether the contributions of roots and shoots to whole-plant R differed between the species and/or among growth temperatures, we calculated the contributions of roots and shoots to whole-plant R (Fig. 1b,d). In both species, specific rates of respiratory CO_2 release were on average about 1.8 times higher in roots (Table 2) compared with shoots (Fig. 2), when compared at each respective growth temperature. However, as the allocation of biomass to roots (i.e. RMR) was less than the allocation of biomass to shoots (i.e. LMR plus StMR; Fig. 1), roots did not represent the dominant component of whole-plant R (Fig. 1b,d). Rather, shoot R represented 47–52% of whole-plant R in *P. major* (Fig. 1b) and 47–67% in *P. euryphylla* (Fig. 1d).

Did the greater RMR in 13 C-grown plants (compared with plants grown at 20 and/or 27 C; Fig. 1) increase the contribution of root R to daily whole-plant R ? In *P. major*, growth temperature had no significant effect on the contribution of root R (or shoot R) to whole-plant R (Fig. 1b; Table 1); thus at 13 C, the greater RMR (Fig. 1a) was compensated by lower specific rates of root R in *P. major* (Table 2). In *P. euryphylla*, growth temperature significantly altered the contribution of root R (and shoot R) to daily whole-plant R (Fig. 1d; Table 1); however, the ratio of root R to whole-plant R was significantly greater at 20 C compared with plants grown at 13 and 27 C (Fig. 4d). Therefore, the higher RMR in 13 C-grown *P. euryphylla* (Fig. 1b) did not result in a concomitantly higher contribution of root R to whole-plant R (Fig. 1d), because of the large decline in specific rates of root R exhibited by 13 C-grown *P. euryphylla* (relative to its 20 C-grown counterparts; Table 2). Similarly, the percentage of whole-plant R taking place in roots was lower in 27 C-grown plants compared with 20 C-grown *P. euryphylla*, because of a combination of a lower specific rate of root R and lower RMR.

Variations in whole-plant R/P_{gross} (Fig. 3; Tables 1, 3) reflect the sum of R/P_{gross} by roots over a 24-h period (i.e. root R/P_{gross}) plus that by shoots over a full day (i.e. shoot R/P_{gross}); root R/P_{gross} and shoot R/P_{gross} are, in turn, dependent on

specific rates of CO_2 exchange and the allocation of biomass to roots vs shoots (see Eqn 2). Shoot R/P_{gross} was sensitive to growth temperature in both species (Table 1); in *P. major*, shoot R/P_{gross} was significantly lower in 20 C-grown plants (shoot $R/P_{\text{gross}} = 10.0\%$) than in plants grown at 13 C (15.4%) and 27 C (14.9%), whereas in *P. euryphylla* shoot R/P_{gross} was significantly higher at 27 C (29.3%) than at 20 C (18.3%) and 13 C (18.4%). In contrast to the shoot R/P_{gross} values, no significant differences were found in root R/P_{gross} of *P. euryphylla* among the three growth temperatures (Table 1), with root R/P_{gross} values being in the 9.1–12.6% range. In *P. major*, root R/P_{gross} was significantly lower in 13 C plants (11.3%) compared with plants grown at 20 C (12.9%) and 27 C (14.2%).

Impact of short-term changes in temperature on CO_2 exchange of whole plants

The impact of short-term changes in temperature on shoot net CO_2 exchange (i.e. P_{net} measured under growth irradiance, and shoot R measured after 30 min in darkness) in both species is shown in Fig. 2. A two-way ANOVA revealed that P_{net} was sensitive to the short-term changes in shoot temperature in both species, with the response of P_{net} to shoot temperature (over the 13–34 C range) being dependent on growth temperature (Table 4). Although exposure to high measuring temperatures invariably decreased P_{net} in both species (irrespective of the growth temperature), the high-temperature-induced decline in P_{net} occurred at lower measuring temperatures in plants grown at 13 C than in plants grown at 20 and 27 C (i.e. growth temperature affected the temperature optima of P_{net}) (Fig. 2a,c). Shoot R in darkness was also sensitive to short-term changes in measuring temperature in both species (Fig. 2b,d; Table 4). Rates of shoot R were significantly lower in the 27 C-grown plants at any given measuring temperature (compared with both the 20 C- and 13 C-grown plants) in both species (Fig. 2; Table 4); by contrast, the plants grown at 20 and 13 C exhibited relatively similar rates of shoot R at any given measuring temperature. (Note: in these measurements, root R was always measured at

Table 4 Results of a two-way analysis of variance with factors growth temperature (GT) and measuring temperature of shoots (shoot MT), with the interaction term shown as GT × shoot MT (significant interactions indicate that the effect of measuring temperature differed among the growth temperature treatments)

Parameter	<i>Plantago major</i>			<i>Plantago euryphylla</i>		
	GT	Shoot MT	GT × shoot MT	GT	MT	GT × shoot MT
P_{net}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Shoot R	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	ns
Root R	< 0.001	ns	ns	< 0.001	ns	ns
Whole-plant R/P_{gross}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Shoot gas exchange was measured at five temperatures (20, 27 and 34 °C) using plants grown at three growth temperatures (13, 20, and 27 °C) ($n = 4$ in all cases), with root temperatures being kept constant in all cases (at the respective growth temperature of each treatment). P -values are presented, with significant values (< 0.05) being shown. To ensure that data were normally distributed and the variances homogeneous, we performed an angular transformation of proportional data before analysis (untransformed data are shown). Data used in the analysis are shown in Figs 2 and 3.

R , the rate of respiratory CO_2 release; P_{gross} , the measured specific rate of net photosynthesis plus shoot R during the day; P_{net} , the measured specific rate of net photosynthesis.

Table 5 Q_{10} values of respiration (Q_{10} being the proportional increase in the rate of respiratory CO_2 release (R) for every 10 °C rise in measuring temperature) for intact roots and intact shoots of two contrasting *Plantago* species

Organ	<i>Plantago major</i>			<i>Plantago euryphylla</i>		
	13 °C	20 °C	27 °C	13 °C	20 °C	27 °C
Shoot	2.15 ± 0.10	1.79 ± 0.16	2.09 ± 0.17	1.85 ± 0.07	1.69 ± 0.03	1.75 ± 0.05
Root	2.38 ± 0.73	2.10 ± 0.16	2.19 ± 0.10	1.48 ± 0.03	1.63 ± 0.14	1.47 ± 0.15

Q_{10} values were calculated using the slope of linear first-order regressions fitted through $\log R$ plotted against measuring temperature and the equation $Q_{10} = 10^{(\text{slope} \times 10)}$ for roots and shoots. The temperature range used to calculate Q_{10} values was 20–27 °C for both roots and shoots. Values are the mean of three or four replicates (\pm standard error). As growth temperature did not have a significant effect on the Q_{10} of both roots and shoots in either species (Table 1), we could assess, within each species, whether the Q_{10} values differed between roots and shoots using grouped data from the three growth temperatures (see text).

the respective growth temperature of each treatment (i.e. 13, 20 or 27 °C); in neither species did short-term changes in shoot temperature significantly affect rates of root R expressed on a dry mass basis (irrespective of the growth temperature; Table 4); we therefore calculated the average rates of root R (shown in Table 2).

Table 5 shows Q_{10} values for both shoot R in darkness (calculated using data shown in Fig. 2) and root R (also calculated using data obtained from short-term changes in root temperature; see Table 2 for reference values at each respective growth temperature). To enable a comparison of Q_{10} values of roots and shoots over a common measurement temperature range (given that estimates of the Q_{10} of R are highly dependent on the measurement temperature range used in calculations; Tjoelker *et al.*, 2001), we calculated average Q_{10} values between 20 and 27 °C. Despite some variability, growth temperature had no significant effect on the average Q_{10} of shoot or root R in either species (Table 1). As a result, we could assess within each species whether the Q_{10} values differed between roots and shoots using grouped data from the three growth temperatures.

No significant differences were found between Q_{10} values of roots and shoots of *P. major* (overall mean (\pm s.e.) Q_{10} values of roots and shoots for *P. major* were 2.24 ± 0.25 and 2.01 ± 0.06 , respectively); however, significant differences were found between the Q_{10} values of roots and shoots of *P. euryphylla* (overall mean Q_{10} values of roots and shoots for *P. euryphylla* were 1.54 ± 0.04 and 1.81 ± 0.05 , respectively; $P < 0.001$). Moreover, the Q_{10} values of shoot R and root R were significantly higher in *P. major* than in *P. euryphylla* (Q_{10} of shoot R , $P < 0.05$; Q_{10} of root R , $P < 0.05$).

To explore the extent to which whole-plant R/P_{gross} values varied with short-term changes in shoot temperature, we calculated whole-plant R/P_{gross} using the shoot CO_2 exchange data (Fig. 2) and rates of root R (Table 2) using Eqn 2. Short-term increases in shoot temperature increased whole-plant R/P_{gross} in all cases (Fig. 3; Table 4); however, the impact of increasing measuring temperature was most pronounced in 13 °C-grown plants, particularly in *P. euryphylla* (because of the detrimental impact that exposure to high shoot temperatures had on shoot photosynthesis; Fig. 2c). The data in Fig. 3 are

for plants where roots were kept at their growth temperature; if both root and shoot temperatures had been altered, the result would have been even greater temperature-dependent changes in whole-plant R/P_{gross} .

To assess what impact short-term changes in night-time temperature would have on whole-plant R/P_{gross} , we calculated R/P_{gross} for 13 C- and 20 C-grown *P. major* plants experiencing a wide range of shoot temperatures at night (6–20 C) and three different shoot temperatures during the day (20, 27 and 34 C); calculations were made for roots experiencing their respective growth temperatures. Increases in night-time shoot temperature increased the percentage of daily P_{gross} released by whole-plant R , with whole-plant R/P_{gross} being highest in plants also experiencing high daytime temperatures (Fig. 4). Importantly, whole-plant R/P_{gross} values were markedly higher in plants grown at 13 C than in those grown at 20 C, illustrating that the impacts of variable night-time and daytime temperatures on whole-plant R/P_{gross} are highly dependent on the thermal history of a plant.

Discussion

Sustained differences in mean daily temperature: impacts on whole-plant R/P

The fact that respiratory metabolism and photosynthetic metabolism are interdependent (Krömer, 1995; Hoefnagel *et al.*, 1998; Padmasree *et al.*, 2002) has led the authors of previous studies (Gifford, 1995; Ziska & Bunce, 1998) to hypothesize that whole-plant R/P should be constant across a wide range of growth temperatures. Although there was movement towards homeostasis of whole-plant R/P_{gross} in both species when grown at 13 and 20 C, statistically significant differences were nevertheless found at these temperatures in *P. major* (Table 3). These differences reflected, in part, the interactive effects of temperature and irradiance on daytime shoot R (Atkin *et al.*, 2006) with the result that whole-plant R/P_{gross} was significantly higher at 13 C than at 20 C (Table 3). However, the *absolute* differences in whole-plant R/P_{gross} exhibited by 13 C- and 20 C-grown *P. major* plants remained relatively minor, demonstrating that R/P_{gross} was essentially homeostatic in the 13–20 C growth temperature range in both species. Although some ontogenetic and seasonal variation in R/P_{gross} is likely (Gifford, 2003), it remains possible that carbon cycle models might be able to assume homeostasis of whole-plant R/P_{gross} when considering carbon fluxes in cool–moderate temperature environments. Further work is needed to test whether this assumption holds over long time periods in plants experiencing cool–moderate temperatures. The role changes in biomass allocation and/or specific rates of R and P play in maintaining homeostasis of R/P_{gross} over extended time periods also needs to be established.

In contrast to the near homeostasis of whole-plant R/P between 13 and 20 C, whole-plant R/P_{gross} values were markedly

greater (both in statistical and in absolute terms) at 27 C (particularly in *P. eurypyphylla*); thus, homeostasis of whole-plant R/P_{gross} is not always achieved. Increases in whole-plant R/P_{gross} at high growth temperatures have been reported previously (Tjoelker *et al.*, 1999a; Loveys *et al.*, 2002). Accounting for temperature-induced increases in whole-plant R/P_{gross} is necessary if carbon cycle models are to accurately predict future CO_2 fluxes between plants and the atmosphere under conditions of unusually high mean daily temperatures, the likelihood of which is expected to increase in the future (Hansen *et al.*, 2006).

Given that allocation to roots decreases with increasing growth temperature (Fig. 1), the increase in whole-plant R/P_{gross} in 27 C-grown plants was not simply a result of increased respiratory CO_2 release brought about by increased RMRs. Rather, temperature-mediated increases in whole-plant R/P_{gross} (Table 3) suggest that the respiratory costs for ion uptake, cellular maintenance and/or biosynthesis increase with increasing growth temperature, and/or that the efficiency of respiratory ATP synthesis (i.e. the ratio of ATP produced per unit respiratory CO_2 released) decreases with increasing growth temperature, with the result that higher rates of respiratory CO_2 are required for a given rate of photosynthetic CO_2 uptake. While there is little evidence of the specific costs of biosynthesis being temperature dependent (Cannell & Thornley, 2000), maintenance and ion uptake costs are likely to be increased with increasing temperature (Rachmilevitch *et al.*, 2006). The efficiency of ATP synthesis is dependent on the extent to which electron transport in the mitochondrial inner membrane occurs via phosphorylating (i.e. complexes I, III and IV) and nonphosphorylating (i.e. uncoupling protein (UCP), complex II, external NAD(P)H dehydrogenases, rotenone-insensitive internal NADH dehydrogenase and the alternative oxidase (AOX)) pathways. Available evidence suggests that relative engagement of the AOX does not increase at high temperatures (Vanlerberghe & McIntosh, 1992; González-Meler *et al.*, 1999; Ribas-Carbó *et al.*, 2000; Atkin *et al.*, 2002; Kurimoto *et al.*, 2004; Fiorani *et al.*, 2005). Moreover, there is no evidence that the external NADH dehydrogenase activity (Svensson *et al.*, 2002) or UCP protein concentration increases with increasing temperature (Laloi *et al.*, 1997; Kurimoto *et al.*, 2004; Sluse *et al.*, 2006). Thus, the increase in whole-plant R/P in high-temperature-grown plants is not likely to reflect decreases in the efficiency of ATP synthesis. Rather, increases in the specific costs of cellular maintenance and/or ion uptake are probably responsible. Further research is needed to establish whether this hypothesis is correct.

The extent to which growth at 27 C increased whole-plant R/P_{gross} was greater in the slow-growing *P. eurypyphylla* than in the fast-growing *P. major* (Table 3), demonstrating that the ability to maintain homeostasis of whole-plant R/P_{gross} at high growth temperatures is not identical in all plant species. Tjoelker *et al.* (1999a) reported interspecific variation in the degree of thermal homeostasis of whole-plant R/P . Moreover,

our past work has shown that individual leaves of *P. eurypphylla* exhibit lower thermal acclimation of R and P , and reduced homeostasis of R/IP_{gross} , than leaves of the faster growing *P. major* (Atkin *et al.*, 2006). What is less clear, however, is whether there are systematic differences in the degree of R/IP homeostasis (both in individual leaves and in whole plants) among contrasting species. Our results obtained using one alpine and one lowland species suggest that exposure to unusually high growth temperatures may have a greater adverse effect on the balance between R and P in selected species adapted to colder climates than in species from warmer habitats. Given the importance of annual values of R/IP as a driver in climate models (Gifford, 2003), it is important that future research establish the extent to which a larger number of contrasting plant species (from both cold and warm habitats) differ systematically in their ability to maintain homeostatic R/IP_{gross} values when experiencing high growth temperatures.

In Gifford (1995), estimates of daily gross P at different temperatures were obtained using measurements of net CO_2 uptake by entire plants (which includes respiratory CO_2 release by roots and shoots), with adjustments being made for assumed rates of whole-plant respiratory CO_2 release taking place during the daytime; respiratory CO_2 release was assumed to be identical in the light and in darkness. Given that rates of leaf R are rarely the same in darkness as in the light (Brooks & Farquhar, 1985; Villar *et al.*, 1994; Hurry *et al.*, 1996; Atkin *et al.*, 1997, 1998, 2000, 2006; Tcherkez *et al.*, 2005), reliance on night-time R alone to obtain estimates of gross P and shoot R during the daytime will almost certainly lead to errors in estimates of gross P , as well as whole-plant R/IP_{gross} ratios. By measuring shoot and root CO_2 exchange independently and by correcting shoot CO_2 exchange in the daytime for light-mediated changes in leaf R (Atkin *et al.*, 2006), we sought to obtain estimates of whole-plant R/IP_{gross} that took into account the light-dependent changes in shoot R . While not without its weaknesses, such an approach does highlight the importance of light-mediated changes in shoot R in determining R/IP_{gross} values in whole plants, particularly at high temperatures (where accounting for light inhibition of leaf R results in a reduction in whole-plant R/IP_{gross}).

Importance of temperature-mediated changes in biomass allocation

Intuitively, the lack of significant differences in root R/IP_{gross} in *P. major* plants grown at 20 and 27 °C (Table 1) may seem surprising given that specific rates of root R were near constant over the 20–27 °C range of growth temperatures in this species (Table 2), whereas rates of photosynthesis were lower at 27 °C than at 20 °C (Fig. 2a). Given such fluxes, we expected root R/IP_{gross} to increase significantly between 20 and 27 °C in *P. major*. Why didn't root R/IP_{gross} increase significantly? The answer lies in the fact that 27 °C-grown plants allocated a slightly greater proportion of plant mass to leaves (i.e. they

exhibited a higher LMR) than their 20 °C-grown counterparts (Fig. 1a); this resulted in a greater rate of whole-plant photosynthetic carbon gain in 27 °C-grown plants. In *P. eurypphylla*, the constancy of root R/IP_{gross} across all temperatures (Table 1) reflected the decrease in rates of root R (Table 2) combined with the significant decline in allocation of biomass to roots in plants grown at 27 °C (i.e. lower RMR; Fig. 1c). These results highlight the importance of temperature-mediated changes in biomass allocation (Fig. 1), as well as changes in specific rates of P and shoot R (Fig. 2) and root R (Table 2) in determining the contribution of shoots and roots to whole-plant R/IP_{gross} values.

Inter-specific differences in whole-plant R/IP : an artefact of high growth temperatures?

Past studies have shown that, when plants are grown at a single moderate temperature (e.g. 20 °C), plant species characteristic of 'unfavourable' habitats typically exhibit lower RGR and greater whole-plant R/IP values than their counterparts characteristic of more 'favourable' habitats (Poorter *et al.*, 1990; Atkin *et al.*, 1996; Tjoelker *et al.*, 1999a; Amthor, 2000; Loveys *et al.*, 2002). Underpinning the higher whole-plant R/IP_{gross} values in the slow-growing species are higher ATP costs associated with ion uptake and exchange in roots compared with their fast-growing counterparts (Van Der Werf *et al.*, 1992; Scheurwater *et al.*, 1999; Scheurwater *et al.*, 2000). What remains unclear from past work, however, is whether growth at lower average temperatures closer to that often experienced in nature (e.g. < 20 °C for many temperate species and even lower for alpine species) alters the difference in whole-plant R/IP values exhibited by fast- and slow-growing species. Our results show that the alpine *P. eurypphylla* exhibited significantly higher whole-plant R/IP_{gross} values than the lowland *P. major* when grown at 20 and 27 °C. However, no significant differences in whole-plant R/IP_{gross} were found between the species when 13 °C-grown values were compared (Fig. 2; Table 4). Such results highlight the extent to which our current understanding of respiratory (and photosynthetic) metabolism in contrasting species is biased by studies conducted at average growth temperatures well above that normally experienced in nature. The results also highlight the need for further work comparing respiratory characteristics of a larger number of plant species (from contrasting habitats) at low growth temperatures.

Impacts of short-term changes in temperature on CO_2 exchange in whole plants

Our results show that short-term increases in air temperature increased R/IP_{gross} in both species, with the effects of air temperature being most pronounced in cold-grown plants (Fig. 3), and that short-term exposure to warm nights increased R/IP_{gross} , particularly when combined with high daytime

temperatures (Fig. 4). Given that warm nights and high daytime temperatures are becoming more common (Easterling *et al.*, 1997), one might predict that average daily R/P_{gross} values will increase in the coming decades. Crucial to the extent to which steady-state R/P_{gross} values do change will be the magnitude of diurnal fluctuations in air and soil temperature and the average growth temperature at which roots and shoots develop (which determines the allocation of biomass above and below ground, as well as the extent of metabolic acclimation of shoot P and R , and root R).

Another factor that is likely to be crucial in determining average daily R/P_{gross} values is the impact of growth temperature and/or genotype on the short-term temperature dependence (i.e. Q_{10}) of shoot and root R . In a recent literature review, Atkin *et al.* (2005) concluded that growth temperature had no consistent effect on reported Q_{10} values of individual leaves or detached roots/root pieces; however, no data were available on the Q_{10} of intact, attached whole shoots and roots. With this in mind, we assessed whether the Q_{10} of R of intact, attached whole shoots and roots varies systematically with long-term differences in growth temperature. We found that, when compared over a common measurement temperature range, growth temperature had no significant effect on the Q_{10} of R in either whole shoots or roots (Table 2). Although average Q_{10} values differed between the species, being greater in the fast-growing, lowland *P. major* than in the slow-growing, alpine *P. euryphylla* (Table 2), past work by us using 16 contrasting species (Loveys *et al.*, 2003) showed that there was no *systematic* relationship between the Q_{10} of R (in detached whole roots or individual mature leaves) and RGR. Moreover, in a recent comparison of Arabidopsis leaves at different stages of development, Armstrong *et al.* (2006) reported no differences in the Q_{10} of R in immature and mature leaves, suggesting that measurements made on individual leaves should, in all likelihood, yield similar Q_{10} values to those exhibited by intact whole shoots. Thus, it may be possible to assume constant Q_{10} values (over defined measurement temperature intervals) across species, irrespective of the thermal history of individual plants, with variations in Q_{10} resulting from changes in measurement temperature being predicted by algorithms such as that reported by Tjoelker *et al.* (2001).

Concluding statements

There is growing acceptance that scaling relationships linking rates of whole-plant R to rates of whole-plant P need to be better understood if the impacts of climate change on carbon exchange between plants and the atmosphere are to be accurately predicted (Atkin & Tjoelker, 2003; Gifford, 2003; Wythers *et al.*, 2005; King *et al.*, 2006). Our results and those of previous studies (Gifford, 1995; Ziska & Bunce, 1998) suggest that homeostasis of whole-plant R/P_{gross} is approached across the low–moderate growth temperature range, irrespective of the inherent maximum growth rate and/or environmental

origin of the species. Importantly, however, our results show that whole-plant R/P_{gross} values increase substantially when plants are grown at daily average temperatures greater than those normally experienced in their natural environment, with the increase in whole-plant R/P_{gross} being most pronounced in the slow-growing alpine *P. euryphylla*. Underpinning the increase in whole-plant R/P_{gross} at high growth temperatures are likely to be increases in the specific costs of cellular maintenance and/or ion uptake. By measuring shoot and root gas exchange separately, our study has also highlighted the importance of temperature-mediated changes in biomass allocation in controlling whole-plant R/P_{gross} values and in determining the proportion of whole-plant R taking place in roots and shoots. This finding has implications for carbon cycle models predicting the effects of rising temperatures on future CO_2 fluxes between plants and the atmosphere – in such models, it is essential that temperature-mediated changes in metabolism and biomass allocation be taken into account.

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