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Research

Climate warming and tree carbon use efficiency in a whole-tree $^{13}CO_2$ tracer study

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Summary

- Autotrophic respiration is a major driver of the global C cycle and may contribute a positive climate warming feedback through increased atmospheric concentrations of CO_2. The extent of this feedback depends on plants' ability to acclimate respiration to maintain a constant carbon use efficiency (CUE).
- We quantified respiratory partitioning of gross primary production (GPP) and CUE of field-grown trees in a long-term warming experiment (+3°C). We delivered a ^{13}C–CO_2 pulse to whole tree crowns and chased that pulse in the respiration of leaves, whole crowns, roots, and soil. We also measured the isotopic composition of soil microbial biomass and the respiration rates of leaves and whole crowns.
- We documented homeostatic respiratory acclimation of foliar and whole-crown respiration rates; the trees adjusted to experimental warming such that leaf-level respiration rates were not increased. Experimental warming had no detectable impact on respiratory partitioning or mean residence times. Of the ^{13}C label acquired by the trees, aboveground respiration consumed 10%, belowground respiration consumed 40%, and the remaining 50% was retained.
- Experimental warming of +3°C did not alter respiratory partitioning at the scale of entire trees, suggesting that complete acclimation of respiration to warming is likely to dampen a positive climate warming feedback.

Introduction

The respiratory release of CO_2 by plants is a large and globally relevant process that may be affected by climate warming. Autotrophic respiration by land plants (R_a) releases *c.* 60 Gt CO_2 yr^{-1} to the atmosphere, which is around five times the total anthropogenic flux (Le Quéré *et al.*, 2018). Furthermore, plant roots strongly influence microbial respiration in soils via rhizodeposition and carbon (C) allocation to mycorrhizas, which connects heterotrophic and autotrophic processes in soils (Drake *et al.*, 2012; Philippot *et al.*, 2013; Finzi *et al.*, 2015; Kuzyakov & Blagodatskaya, 2015). Typically, R_a increases approximately exponentially in response to short-term (for example hourly) variation in temperature (Atkin & Tjoelker, 2003; Heskel *et al.*, 2016), suggesting a potential positive feedback between climate warming and release of CO_2 from the biosphere of sufficient magnitude to affect future climate (Cox *et al.*, 2000; Frank *et al.*, 2010; Drake *et al.*, 2016).

Autotrophic respiration in trees arises from the combined metabolic activity of leaves, branches, stems, coarse roots, and fine roots. The total R_a flux for an ecosystem depends on the tissue-specific rates of respiration for each organ, as well as the mass of each organ on a land area basis. Absorptive tissues such as leaves and fine roots tend to have higher tissue-specific rates of respiration than supportive tissues, although respiration rates per unit nitrogen (N) converge across organs (Tjoelker *et al.*, 1999, 2005; Reich *et al.*, 2008). At the scale of forest stands, R_a is often 40–50% of gross primary production (GPP; DeLucia *et al.*, 2007; Litton *et al.*, 2007; Zhang *et al.*, 2014), and leaf respiration has been documented to be 28% of R_a in a *Pinus taeda* forest (Hamilton *et al.*, 2002) and *c.* 40% of R_a in boreal forests (Ryan *et al.*, 1997). Therefore while leaf respiration is more commonly studied than other plant tissues, the respiration of fine roots and woody tissues often contributes substantially to total R_a.

There is now widespread evidence that plants adjust their respiratory physiology in response to long-term changes in temperature – a process termed respiratory temperature acclimation. Leaf respiration often acclimates quickly and nearly homeostatically in long-term experimental warming studies, such that leaves exposed to a warmed condition and a control condition have equivalent respiration rates despite the difference in temperature (Bolstad *et al.*, 2003; Drake *et al.*, 2016; Reich *et al.*, 2016). Acclimation results in an altered temperature−response curve for respiration that can involve an adjustment to an exponential parameter (for example the activation energy or Q_{10}) or the basal rate at a reference temperature (Atkin & Tjoelker, 2003). A

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recent review of warming studies found widespread evidence of respiratory temperature acclimation, such that warmed leaves had lower respiration rates than would be expected in the absence of acclimation (Slot & Kitajima, 2015). Relatively few studies have documented the potential for respiratory temperature acclimation in nonleaf tissues such as branches, stems, and roots, but these studies have demonstrated significant acclimation potential across plant organs (Drake *et al.*, 2016, 2017; Jarvi & Burton, 2018). Therefore, respiratory temperature acclimation across all organs may significantly dampen or eliminate a positive feedback with climate warming by preventing R_a from consuming an increasing fraction of GPP as temperatures rise.

Isotopic pulse–chase studies are a valuable tool to assess C uptake, allocation, and respiratory release as CO_2 (Epron *et al.*, 2012). Specifically, ^{13}C–CO_2 pulse–chase studies have been used to track the movement and respiration of labeled photosynthate in trees growing in natural settings and experimental treatments (Kagawa *et al.*, 2006; Streit *et al.*, 2013; Blessing *et al.*, 2015; Heinrich *et al.*, 2015; Thoms *et al.*, 2017). Several studies have tracked the temporal time courses of respiration of labeled photosynthate in potted saplings (Blessing *et al.*, 2015), small trees (Epron *et al.*, 2016), individual branches (Keel & Schadel, 2010), and whole-tree crowns (Plain *et al.*, 2009). Critically missing from this literature, however, are direct quantifications of the total amount of the assimilated ^{13}C label respired by trees in response to experimental warming. Such measures would enable tests of warming effects on partitioning of GPP and whole-tree respiratory carbon loss. Therefore it is still unclear whether respiratory temperature acclimation allows whole trees to fully adjust to warmer temperatures with a constant carbon use efficiency (i.e. CUE = 1 − R_a/GPP; Drake *et al.*, 2016). This is partly because it is challenging to apply a ^{13}C–CO_2 tracer and then measure its respiration at a whole-tree level. However, our knowledge of C partitioning and CUE would be advanced by experimental warming studies that include whole-tree ^{13}C–CO_2 labeling studies and full documentation of the respiratory fate of the labeled photosynthate.

We used the unique infrastructure of whole-tree chambers (WTCs) to perform a long-term warming experiment with field-grown trees, to deliver a ^{13}C–CO_2 pulse to entire tree crowns, and to chase that pulse in the respiration of leaves, roots, whole crowns, and soil in two temperature treatments. We also followed the pulse into soil microbial biomass. That is, we labeled a discrete 'piece' of GPP, tracked the amount respired aboveground and belowground, and quantified the proportion retained by each tree (CUE). In doing so, our objective was to directly test the hypothesis that autotrophic respiration consumes a larger fraction of GPP in trees exposed to long-term warming of +3°C.

Materials and Methods

Site description

We implemented an experimental warming and ^{13}C–CO_2 pulse–chase experiment at a site in Richmond, New South Wales, Australia (33°36′40″S, 150°44′26.5″E). The site consisted of 12 WTCs, which were large cylindrical structures that enclosed individual trees rooted in soil in a field setting (WTCs: 3.25 m diameter, 9 m height, volume of *c.* 53 m^3; Fig. 1a). Each WTC measured the net exchange of CO_2 and H_2O for an entire tree crown at 15-min resolution while controlling air temperature (T_{air}), relative humidity (RH), and atmospheric CO_2 concentration in the crown air space (Barton *et al.*, 2010; Drake *et al.*, 2016, 2018). A vertical root-exclusion barrier extended

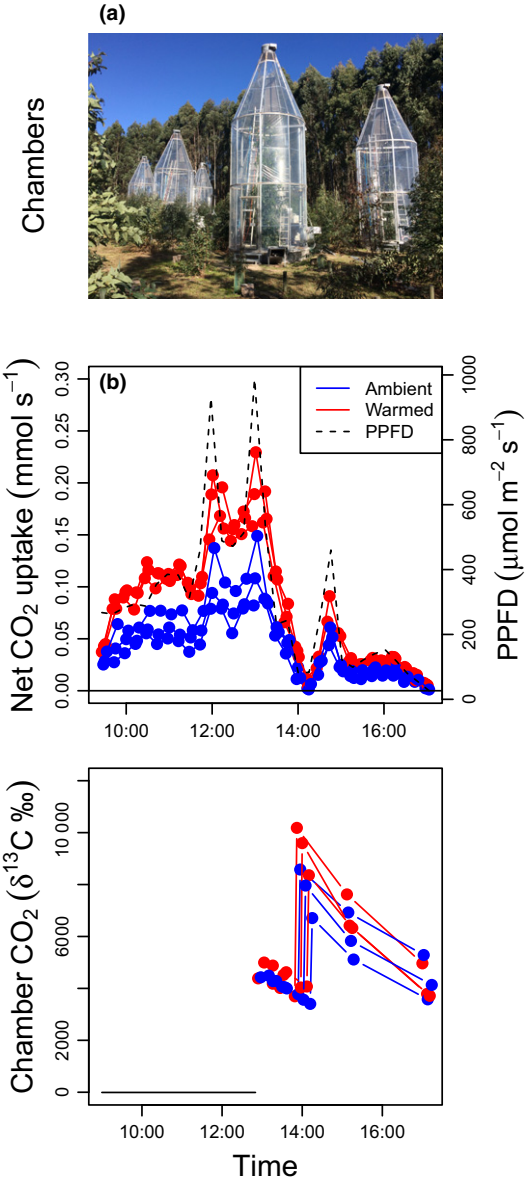

Fig. 1 Delivery of isotopic ^{13}C–CO_2 pulse for an experiment with *Eucalyptus parramattensis*. Whole-tree chambers enclose individual trees rooted in soil in a field setting (a). Net CO_2 uptake by tree crowns followed the external photosynthetic photon flux density (PPFD) and tended to be larger in warmed trees than ambient trees, as warmed trees were larger (b). The isotopic composition of the chamber airspace CO_2 increased from the atmospheric background (solid line at −8‰ and 1.1%) to *c.* 6% following the first injection and increased further to 8–12% following the second injection (c). Data for individual chambers are shown connected by lines.

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belowground for *c.* 1 m into a hard horizontal layer of cemented manganese nodules and clay, which compartmentalized the rooting volume of each tree. A suspended plastic floor sealed around the stem of each tree at 45-cm-height, which subdivided the crown airspace from the subfloor airspace and separated soil gas fluxes from leaf gas fluxes. Soils at the site were low-fertility sandy loams developed on alluvium (Drake *et al.*, 2016). Local climate was warm temperate; mean annual temperature was 17°C and mean annual precipitation was 730 mm (Bureau of Meteorology station 067105, Gimeno *et al.*, 2018).

We selected a locally endemic woodland tree species (*Eucalyptus parramattensis*) for this experiment and acquired seed from Harvest Seeds and Native Plants (Terry Hills, NSW, Australia). Seed was germinated in a local shade house when six potted seedlings were placed into each WTC when the experimental warming treatment was initiated on 28 October 2015. One seedling was planted into the soil within each WTC on 23 December 2015; at that time average seedling height was 60 cm.

Warming experiment

We began an experimental warming treatment on 28 October 2015. Six of the 12 chambers were assigned to the 'ambient' treatment, in which T_{air} and RH tracked the natural variation observed at the site. The other six chambers were assigned to the 'warmed' treatment, which tracked the ambient T_{air} and RH with an additional +3°C of warming. The average warming achieved was +2.9°C (± SD of 0.6 across 265 d) for T_{air} in the crown compartment, +2.6°C (± 0.6) for T_{air} in the subfloor airspace, +2.9°C (± 0.8) for soil temperature (T_{soil}) at 5-cm depth, +3.0°C (± 0.5) for T_{soil} at 10-cm depth, +2.4°C (± 0.2) for T_{soil} at 20-cm depth, +1.7°C (± 0.3) for T_{soil} at 30-cm depth, and +1.6°C (± 0.2) for T_{soil} at 50-cm depth. All chambers tracked ambient CO_2 concentration and were irrigated equally every 2 wk at half of the mean monthly rainfall measured over the past 30 yr. This particular warming experiment has been described previously in detail (Drake *et al.*, 2018) and is similar to previous warming experiments at this site with different tree species (Aspinwall *et al.*, 2016; Drake *et al.*, 2016; Crous *et al.*, 2017).

Isotopic labeling with ^{13}C–CO_2

While the warming experiment included 12 trees ($n = 6$ in ambient and warmed), practical issues constrained the isotopic labeling to six trees ($n = 3$ in ambient and warmed). Within each treatment, we excluded the largest and smallest tree and randomly selected three of the four remaining possible trees. Each of these six chambers was isotopically labeled with the injection of *c.* 2 l of 98 atom % ^{13}C–CO_2 during the afternoon of 5 August 2016. This day had a mean midday T_{air} of 15.9°C, a day length of 10.5 h, and a maximum photosynthetic photon flux density (PPFD) of *c.* 1000 μmol m^{-2} s^{-1} (Supporting Information Fig. S1). The normal operation of the WTCs was interrupted during the 4-h labeling period by disabling the fresh air input fan, disabling the normal injection of CO_2 from tanks, and sealing the fresh air input tube. The internal circulating fans and air handling units continued to operate, maintaining mixing of the crown airspace and T_{air} regulation (tracking ambient and +3°C). Two chambers that did not receive the isotopic label were used as references for background isotopic values throughout the study.

The ^{13}C–CO_2 was injected twice into the crown airspace of each of the six chambers; each injection was *c.* 1 l. The isotopic composition of the chamber air was sampled seven times per chamber during the labeling event. Chamber air was collected in Tedlar bags via a hand-operated vacuum pump; bags were filled and evacuated twice with the chamber air, and the third sample was analyzed on a cavity ring-down spectroscopy (CRDS) analyzer (G2201i; Picarro, Santa Clara, CA, USA). The first injection of 98 atom % ^{13}C–CO_2 occurred at *c.* 13:00 h on 5 August 2016 and resulted in C isotopic compositions of *c.* 5.3 atom % ($\delta^{13}C = +4000‰$). The second injection occurred at *c.* 14:00 and increased this further, to *c.* 10 atom % ($\delta^{13}C = +9000‰$). The $\delta^{13}C$–CO_2 value of the chamber airspace tended to decline between injections, perhaps because of dilution by respired CO_2 from the tree crowns or atmospheric CO_2 leaking into the chambers. The chambers were forcibly and rapidly vented with outside air using the WTC air handling units at 17:00 h, yielding a 4-h ^{13}C–CO_2 labeling time. Note that isotopic labeling was performed in the crown airspace only; the suspended plastic floor that sealed around the stem of each tree likely prevented significant ^{13}C–CO_2 labeling of soil pore spaces.

We calculated the total quantity of ^{13}C–CO_2 label taken up via photosynthesis for each tree using three methods. The methods were: (1) quantification of CO_2 uptake with the WTC system; (2) quantification of CO_2 uptake based on independent measurements of the CO_2 concentration inside the chamber airspaces; and (3) measurement of the $\delta^{13}C$ values of leaves collected immediately after labeling. Method (1) took advantage of the measurements of the net canopy CO_2 exchange provided by the WTC system at 15-min intervals (Barton *et al.*, 2010). The measured rate of CO_2 uptake was linearly interpolated to every minute and multiplied by the measured chamber air ^{13}C–CO_2 value (on an atom percent basis), which was also linearly interpolated. This resulted in a direct estimate of the ^{13}C–CO_2 taken up by each tree. For method (2), we installed CO_2 probes (Vaisala GMP 343, Helsinki, Finland) in the air handling unit of each WTC and calculated estimates of CO_2 uptake every minute from the change in CO_2 concentration over time. These data were also multiplied by the measured chamber air ^{13}C–CO_2 to estimate the ^{13}C–CO_2 taken up by each tree. Methods (1) and (2) were similar, but utilized different CO_2 analyzers to account for potential differences in the isotopic sensitivity of these instruments (Epron *et al.*, 2012). Both of these methods utilized the following equation:

$$\text{Uptake} = -1 \times \text{CO}_2\text{uptake} \times \frac{\text{AP} - \text{AP}_{\text{natural}}}{100} \times 1000, \quad \text{Eqn 1}$$

where Uptake reflects the rate of ^{13}C uptake in mmol min^{-1} and $AP_{natural}$ is the atom percent isotopic composition of background air (−10‰ or 1.10685%). All isotopic values were expressed on an atom percent basis (Fry, 2007):

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$$AP = \left(\frac{\delta^{13}C}{1000}\right) / \left(\delta^{13}C + 1000 + \frac{1000}{ARC}\right) \times 100, \quad \text{Eqn 2}$$

where $\delta^{13}C$ is the measured $\delta^{13}C$ isotopic value (‰) and ARC is the absolute $^{13}C/^{12}C$ ratio of Vienna Pee Dee Belemnite (VPDB: 0.0111803). These estimates of Uptake for each minute were then summed for each chamber for the 4-h labeling event.

Method (3) was independent of methods (1) and (2). Immediately after labeling, six leaves were sampled on each tree, freeze dried, ground, and measured for $\delta^{13}C$ by elemental analysis and continuous flow isotope ratio mass spectrometry (University of Wyoming, Delta V isotope ratio mass spectrometer; ThermoFisher Scientific, Waltham, MA, USA). The leaf isotopic composition (as atom %) was multiplied by an estimate of total leaf mass, which was derived from direct measurements of entire tree leaf counts, average leaf size, and specific leaf area, exactly as previously (Drake *et al.*, 2016). We acknowledge that method (3) is potentially biased towards high values, as leaves were sampled from the outer portions of the canopy where photosynthetic rates were expected to be most rapid.

$^{13}C–CO_2$ of crown and soil respiration

We quantified the $\delta^{13}C$ value of crown respiration (R_{crown}) using the crown compartment of the WTCs as large cuvettes. We measured the rate and isotopic composition of R_{crown} 11 times throughout the month following labeling. Weather conditions during this chase period were moderate but showed fluctuations across days (Fig. S2). These measurements were done at night to avoid confounding photosynthetic CO_2 gain from respiratory CO_2 loss. The fresh air fan and CO_2 injectors were turned off and the fresh air input was sealed, creating a large closed chamber that was continuously mixed by the WTC air handling units, maintaining the ambient and +3°C treatments (Drake *et al.*, 2016). The CO_2 concentration within the chambers was measured every minute by the WTC system and concentrations increased over time as CO_2 was respired by the leaves, branches, and stem material inside the chamber. Samples of the chamber air were collected three times over a period of *c.* 4 h in Tedlar bags via a hand-operated vacuum pump. Bags were filled and evacuated twice with the chamber air; the third sample was kept and analyzed on the CRDS analyzer.

The rate and isotopic composition of soil respiration (R_{soil}) were measured 16 times during the chase period using the entire subfloor compartment as a large cuvette (cylindrical air volume 0.45-m tall by 8.3 m^2 area). The fresh air input and export ports were closed, creating a closed chamber. The rate of CO_2 accumulation in the headspace of this subfloor compartment was measured every minute using Vaisala probes (model GMP 343) and the rate of R_{soil} was calculated utilizing an asymptotic exponential model (function *SSasymp* in R). Samples of the headspace air were collected into Tedlar bags three times over a period of 60 min using the same procedure used for the crown air measurements and analyzed via CRDS. Note that the subfloor space included the base of the stem, so the R_{soil} measurements presented here may contain some stem CO_2 efflux as well.

Keeling plots were utilized to estimate the isotopic composition of crown and soil respired CO_2 (Pendall *et al.*, 2001). The intercept of the relationship between sample $\delta^{13}C$ value and the inverse of sample CO_2 concentration was used to estimate the isotopic composition of respired CO_2. The median r^2 value for crown and soil respiration Keeling plots was 0.91 and 0.99, respectively. Many of the Keeling plots with low r^2 values for R_{crown} actually fit the data well but had a flat line, and therefore a low r^2 value, when the $\delta^{13}C$ value of R_{crown} was similar to that of the atmosphere. The total amount of labeled $^{13}C–CO_2$ respired by the crown and soil was calculated as the cumulative sum of respired $^{13}C–CO_2$ over the month following labeling.

Respiratory fractions and carbon use efficiency

We utilized the isotopic label and the R_{crown} and R_{soil} measurements to calculate respiratory partitioning and CUE. CUE is the ratio of net primary production to GPP (CUE = NPP/GPP = 1 − R_a/GPP; DeLucia *et al.*, 2007), which is approximately equivalent to:

$$CUE = 1 - \frac{R_{crown}}{GPP} - \frac{R_{soil}}{GPP}. \quad \text{Eqn 3}$$

For Eqn 3 to be valid, we considered all belowground respiration of the ^{13}C label to be 'autotrophic', be it root or microbial respiration (i.e. microbial respiration of recent root exudates is defined as 'autotrophic'; Hanson *et al.*, 2000). The method employed here used the $^{13}C–CO_2$ to isotopically label a discrete piece of GPP and tracked the cumulative amount of isotopic label that was respired by the crown and the soil. The amount of isotopically labeled GPP that was not respired was assumed to be retained by the tree. The partitioning terms of Eqn 3 (R_{crown}/GPP and R_{soil}/GPP) were calculated by dividing the total integrated sum of ^{13}C label respiration of crowns and soil by the total quantity of ^{13}C label uptake for each tree, using units of mg ^{13}C excess. CUE was then calculated directly (Eqn 3). We calculated CUE for each of the three label uptake methods as an estimate of uncertainty. We present each of these calculated results in the Supporting Information, and we present the average of the three methods in the Results below.

$^{13}C–CO_2$ of leaf and root respiration

The isotopic composition of leaf and root respiration (R_{leaf} and R_{root}) were measured to quantify the mean residence time of labeled C in these components. In the absence of respiratory temperature acclimation, we expect respiratory reactions to be sped up by experimental warming such that these mean residence times would be reduced. We quantified the $\delta^{13}C$ value of R_{leaf} 23 times during the month following labeling, including daytime and nighttime measurements. Two leaves per tree were sampled and placed in Tedlar bags. The bags were flushed three times

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with CO_2-free air containing N_2 and O_2 at atmospheric concentrations and then filled with 240 ml of CO_2-free air. The samples were incubated for *c.* 30 min inside insulated containers placed within the WTCs, such that the incubation temperature matched the *in situ* temperature of each treatment. Samples were then analyzed by CRDS to provide an estimate of the R_{leaf} $\delta^{13}C$ value. The mean CO_2 concentration of these incubated samples was 647 ± SD of 362 ppm.

Fine root samples (roots < 2 mm in diameter) were obtained by crawling under the plastic subfloor and excavating fine root systems from the surface soils (0–15 cm depth) using a hand trowel. Fine roots were washed with water, blotted dry, and incubated in Tedlar bags flushed with CO_2-free air containing N_2 and O_2 at atmospheric concentrations, similar to the R_{leaf} measurements. Fine roots were incubated at lab temperatures of *c.* 25°C for *c.* 1 h before the CO_2 concentrations were high enough to measure accurately; the mean CO_2 concentration of these incubated samples was 499 ± SD of 199 ppm. Roots were incubated at slightly higher temperatures than leaves, which facilitated CO_2 accumulation in the root incubations. While roots and leaves were incubated slightly differently, we do not expect this temperature difference to meaningfully affect the isotopic composition of respiration during this pulse–chase.

The isotopic composition of microbial biomass C

Soil samples were collected in association with root sampling and processed for microbial ^{13}C–C using chloroform fumigation extraction. After removing roots, soils were stored at 4°C for up to 2 d when fumigated (72 h) and unfumigated subsamples were extracted in 0.05 M K_2SO_4. The resulting extracts were analyzed for soluble C (TOC-LCSH/CPH Shimadzu, Wood Dale, IL, USA) and a subsample was oven dried (60°C) and then analyzed on an Elementar Vario EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). For further details of extraction and calculations of microbial ^{13}C, see Carrillo *et al.* (2018).

Leaf and crown respiration rates throughout the warming experiment

We measured R_{leaf} and R_{crown} rates throughout the warming experiment to assess respiratory acclimation to growth temperature. Note that these measurements were nonisotopic and were performed over 8 months, including the 1-month ^{13}C–CO_2 pulse−chase period. These methods closely followed a previous experiment from this facility (Aspinwall *et al.*, 2016; Drake *et al.*, 2016).

We measured R_{leaf} 13 times across the experiment (at 3–4 wk intervals) by sampling three fully expanded mature leaves from the upper third of the crown of each tree ($n = 6$) after dusk. The aggregate sample of three leaves was placed in a large gas exchange chamber (LI-6400-22L; Li-Cor Inc., Lincoln, NE, USA). The block temperature was maintained at 15°C, the [CO_2] of the reference cell was set at 400 ppm, and the flow rate was set to 350 ml min^{-1}. Leaves were incubated in the chamber for > 10 min to achieve stable gas concentrations, after which data were logged every 10 s for 2 min and subsequently averaged. All measurements were performed at least 1 h after dusk to standardize time of measurement and avoid postillumination respiratory artifacts. The aggregate leaf area of each sample of three leaves was measured with a benchtop leaf area meter (LI-3100C; Li-Cor). Leaves were dried to a constant mass at 70°C to measure leaf area per unit dry mass (specific leaf area; SLA). Physiological acclimation of R_{leaf} to seasonal and treatment differences in temperature were assessed with the set temperature method (Atkin *et al.*, 2005), as all respiration measurements were performed at 15°C.

The rate of CO_2 efflux from entire tree crowns (R_{crown}; including leaves, branches, and stems) was measured using the WTC system, which solves a mass-balance equation to calculate the net CO_2 exchange rate of each tree crown at 15-min resolution (Barton *et al.*, 2010; Drake *et al.*, 2016). Flux measurements began on 28 February 2016 when plastic floors were sealed around the stem of each tree, and finished on 23 November 2016 when the trees were harvested. We excluded flux measurements before 1 May 2016, as the estimated rates were unreliable. We calculated the median net CO_2 release for each tree for the remaining 205 nights of the experiment; we use the median as it is relatively robust against outliers. Net CO_2 release from tree crowns increased over time as the trees got larger. To account for plant size, we calculated the standing leaf area of each tree on each night (following previously published methodology exactly; Drake *et al.*, 2016) and expressed R_{crown} as nighttime net CO_2 release per unit leaf area. This produced 2460 data points of *in situ* R_{crown}, which we investigated for thermal acclimation by comparing the *in situ* temperature dependence across the ambient and warmed treatments.

Calculations and statistical analyses

Mean residence times of ^{13}C were calculated with a three parameter, two pool exponential model (Wedin & Pastor, 1993; Pendall *et al.*, 2011) of the form:

$$R_t = C_l \times k \times e^{-kt} + r, \quad \text{Eqn 4}$$

where R_t is the respiration rate of the ^{13}C label at time t, C_l is the labile C pool, k is the intrinsic decay constant of the labile pool, and the asymptote value r reflects a constant background respiration rate of a resistant C pool. The mean residence time was calculated as the inverse of the exponential decay coefficient k. This model was fit to isotopic composition data expressed on an atom percent basis, normalized by the total quantity of ^{13}C–CO_2 taken up by each chamber. We found that it was important to normalize for ^{13}C–CO_2 uptake given the differences in the quantity of label acquired by trees of different size.

The temperature-response of R_{crown} was assessed by fitting a simple two-parameter Q_{10} model to the data for each treatment and assessing treatment differences by the 95% confidence intervals of parameters.

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$$R_{crown} = R_{15} \times Q_{10}^{\frac{T_{air}-T_{ref}}{10}}. \quad \text{Eqn 5}$$

In this equation, R_{15} reflects the basal rate of respiration at a reference condition of 15°C (this reference temperature was chosen because it is an intermediate value in our dataset), Q_{10} reflects the multiplicative change in R_{crown} that is achieved for a 10°C change in temperature, T_{air} reflects the measured air temperature, and T_{ref} is the reference temperature (15°C). The Q_{10} model (Eqn 5) was fit with the *nls* function and the confidence intervals were assessed with the PROPAGATE package of R. Physiological acclimation to the warming treatment could manifest as a change in either the Q_{10} or R_{15} parameters (Atkin & Tjoelker, 2003; Atkin *et al.*, 2005), but most previous work has documented change in the basal rate parameter rather than the Q_{10} directly (Drake *et al.*, 2016; Heskel *et al.*, 2016; Reich *et al.*, 2016).

Statistical analyses were done as simple one-way analyses of variance (ANOVAs) with a single categorical variable with two levels (ambient vs warmed treatment) using the *lm* function in R.

Results

Isotopic labeling with ^{13}C–CO_2

Tree crowns acquired ^{13}C–CO_2 at high rates immediately upon label delivery (Fig. 1), but net CO_2 uptake declined when clouds arrived at the site in the early afternoon (14:00 h, Fig. 1b). The second injection of ^{13}C–CO_2 further enriched the isotopic composition of chamber CO_2, such that late afternoon photosynthesis also contributed to isotopic label uptake (Fig. 1c). Note that the warmed trees had *c.* 50% more leaf biomass than the ambient trees on the labeling day, so their crowns had higher net CO_2 uptake rates (Fig. 1b). Method (1) of calculating label uptake based on the WTC flux calculations suggested that the ambient trees took up 252 ± 13 (SE) while the warmed trees took up 525 ± 34 mg ^{13}C–CO_2. Method (2) of calculating label uptake based on the Vaisala CO_2 data suggested that the ambient trees took up 328 ± 17 while the warmed trees took up 439 ± 22 mg ^{13}C–CO_2. Method (3) of calculating label uptake based on the isotopic composition of leaves immediately after labeling suggested that the ambient trees took up 757 ± 76 (SE) while the warmed trees took up 1184 ± 135 mg ^{13}C–CO_2. Therefore, these methods agree that the warmed trees took up more ^{13}C–CO_2 but do not agree on the absolute magnitude.

^{13}C–CO_2 of crown and soil respiration

The CO_2 produced by respiration of the tree crowns and soils (R_{crown} and R_{soil}) reflected rapid transport and metabolism of the isotopic label (Fig. 2). The absolute rate of R_{crown} per unit ground area of the WTC was significantly higher in the warmed treatment relative to the ambient treatment (Fig. 2a), which we primarily attribute to the warmed trees having more leaf mass than the ambient trees, although the 3°C difference in temperature

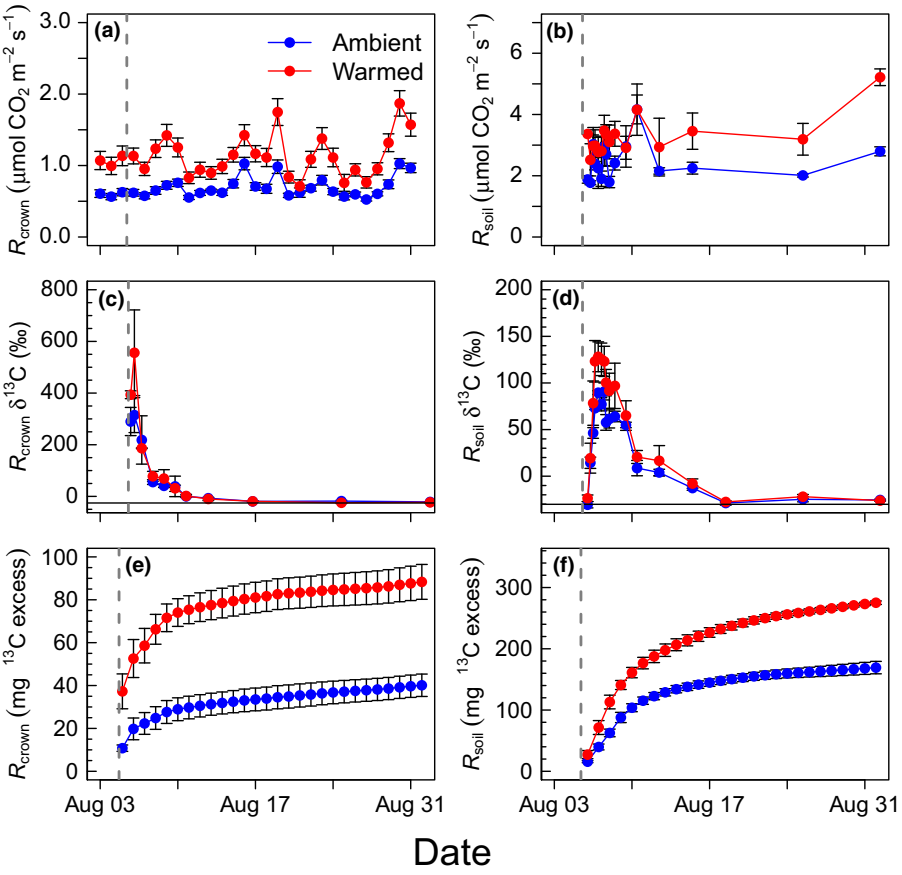

Fig. 2 Crown and soil respiration (R_{crown} and R_{soil}) for a ^{13}C–CO_2 pulse–chase experiment with *Eucalyptus parramattensis*. The rates of R_{crown} and R_{soil} expressed per unit ground area were higher in warmed trees ($P < 0.05$), as trees were larger and temperatures were +3°C (a, b). The isotopic composition of R_{crown} was highly enriched during the evening following ^{13}C–CO_2 labeling and rapidly decayed towards background values observed in unlabeled chambers, shown as the solid horizontal line (c). The vertical dashed lines denote the ^{13}C–CO_2 labeling and error bars reflect ± 1 SE. The isotopic composition of R_{soil} was not strongly affected by ^{13}C–CO_2 labeling until 12 h after labeling, after which it was highly enriched for *c.* 2 wk before decaying towards background values observed in unlabeled chambers, shown as the solid horizontal line (d). The cumulative excess amount of ^{13}C–CO_2 label that was respired by the crown and the soil increased to approach an asymptote after *c.* 1 month (e, f). Note the disparity in *y*-axis scales.

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may also have played a role. The absolute rate of R_{soil} was also significantly higher in the warmed treatment (Fig. 2b), which may also reflect treatment-level differences in biomass and temperature.

The isotopic composition of R_{crown} was higher in labeled chambers than unlabeled chambers just 6 h after labeling, with high $\delta^{13}C$ values of *c.* +300‰ in the ambient and +400‰ in the warmed treatment (Fig. 2c). The R_{crown} $\delta^{13}C$ value increased slightly 4 h later on the same night, but then declined rapidly on subsequent nights and returned to background levels 11 d after labeling (Fig. 2c). The isotopic composition of R_{soil} did not differ between labeled and unlabeled chambers during the first measuring point, 12 h after labeling (Fig. 2d). The R_{soil} $\delta^{13}C$ value increased over time to a maximum of *c.* +80‰ in the ambient treatment and +125‰ in the warmed treatment *c.* 48 h after labeling. R_{soil} $\delta^{13}C$ values remained high for a slightly longer period than R_{crown}; R_{soil} $\delta^{13}C$ values declined towards background levels after 13 d, but did not fully return to background levels within the study period (Fig. 2d).

We combined the measurements of respiration rates and isotopic composition to calculate the total amount of the ^{13}C–CO_2 label that was respired by the tree crowns and soil (Fig. 2e,f). We linearly interpolated the respiration rates (Fig. 2a,b) and the isotopic composition data (Fig. 2c,d) to calculate daily estimates of the ^{13}C–CO_2 label respired by R_{crown} and R_{soil}. Cumulative sums of R_{crown} and R_{soil} ^{13}C–CO_2 approached an asymptote after *c.* 1 month, indicating that the majority of the respiration of the label was captured, although low levels of label respiration may have continued longer for R_{soil} (Fig. 2e,f). In total, the ambient treatment respired 40 mg ^{13}C in R_{crown} and 169 mg ^{13}C in R_{soil}, while the warmed treatment respired 88 mg ^{13}C in R_{crown} and 275 mg ^{13}C in R_{soil} (Fig. 2e,f). The mean residence time of the ^{13}C–CO_2 label in R_{crown} and R_{soil} was not affected by experimental warming (Table 1).

Table 1 The mean residence time in hours (SE) of the ^{13}C–CO_2 label in the respiration of individual leaves (R_{leaf}), whole-tree crowns (R_{crown}), fine roots (R_{root}), and the entire soil system (R_{soil}), as well as microbial biomass (MB) for *Eucalyptus parramattensis* trees grown under ambient or warmed (+3°C) conditions ($n = 3$).

Flux	Ambient	Warmed	*P*-value
R_{leaf}	11 (1)	13 (1)	0.15
R_{crown}	43 (6)	39 (3)	0.55
R_{root}	64 (10)	51 (8)	0.38
R_{soil}	102 (6)	98 (16)	0.48
MB	142 (122)	42 (42)	0.49

Respiratory fractions and carbon use efficiency

We calculated the respiratory fractions and CUE using the estimates of label uptake, R_{crown} and R_{soil} sums, and Eqn 3. The warmed treatment trees took up more ^{13}C–CO_2 label than the ambient treatment trees, so the total quantities of ^{13}C–CO_2 label respired and retained by the trees were higher in the warmed treatment (Fig. 3a). The three methods of calculating ^{13}C–CO_2 uptake resulted in widely divergent estimates of CUE, ranging from a mean across treatments of 0.23 when using the WTC flux estimates of ^{13}C–CO_2 uptake, to 0.70 when using the leaf biomass estimates of ^{13}C–CO_2 uptake (Fig. S3). The three methods also resulted in slightly different estimates of the warming treatment effect (Fig. S3), although the treatment effect was not statistically significant in any case ($P > 0.05$). As no single method is 'best', we present the average estimates across the three methods.

On average, the proportions of these C fluxes relative to ^{13}C–CO_2 label uptake were equivalent across the treatments (Fig. 3b–d). Here, *c.* 40% of the ^{13}C–CO_2 label acquired by each tree was released back to the atmosphere as R_{soil} (Fig. 3b), with no difference between treatments ($P > 0.8$); *c.* 10% of the ^{13}C–CO_2 label acquired by each tree was released back to the atmosphere as R_{crown} (Fig. 3c), with a trend towards a higher proportion in the warmed treatment that was not statistically significant ($P = 0.11$). By contrast, *c.* 50% of the ^{13}C–CO_2 label acquired by each tree was retained through the 1-month chase period (Fig. 3d), with no difference between treatments ($P > 0.4$).

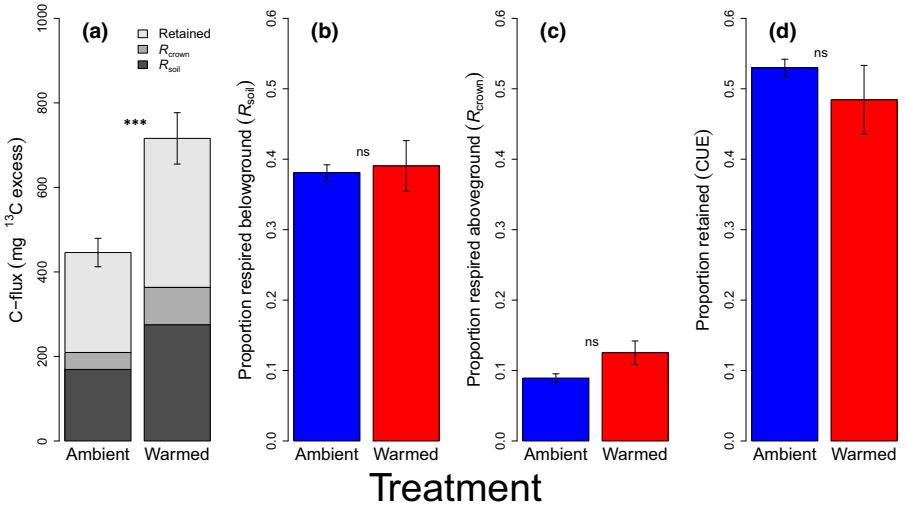

Fig. 3 Carbon partitioning of a ^{13}C–CO_2 pulse–chase within a warming experiment with *Eucalyptus parramattensis*. The excess amount of ^{13}C–CO_2 label that was respired belowground (R_{soil}), respired aboveground by the crown (R_{crown}), and the amount of label retained by trees was different between treatments, because the warmed trees were larger and acquired more ^{13}C–CO_2 (a). However, the proportion of the ^{13}C–CO_2 label that was respired by R_{soil} (b), R_{crown} (c), or retained by the trees (d) was not different between the ambient and warmed treatments ($P > 0.05$). Error bars reflect ± 1 SE. *** Denotes statistical significance at $P < 0.01$, and 'ns' refers to differences that are not statistically significant. CUE, carbon use efficiency.

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Therefore, these trees allocated photosynthate in a similar manner, despite the 3°C difference in growth temperature between the ambient and warmed treatments. However, we recognize the uncertainty in these results given the disparity in the three estimates of $^{13}C–CO_2$ uptake.

$^{13}C–CO_2$ of leaf and root respiration

Leaf respiration (R_{leaf}) had high $\delta^{13}C$ values of *c.* +1000‰ just 4 h after the initiation of labeling (Fig. 4a). The isotopic composition of R_{leaf} declined strongly afterwards, with a short mean residence time of just 10.8 ± 1 (SE) and 12.6 ± 1 h in the ambient and warmed treatments, respectively (Table 1; $P = 0.15$). One month after labeling, the $\delta^{13}C$ value for R_{leaf} was still modestly higher (−24‰) than background levels in unlabeled chambers (−28‰). The mean residence time of $^{13}C–CO_2$ from R_{leaf} was *c.* 75% shorter than the mean residence time of R_{crown} (*c.* 40 h), suggesting that woody tissues were respiring the ^{13}C label for a longer time period relative to leaves.

The $\delta^{13}C$ value of root respiration (R_{root}) was substantially higher than background within 24 h after labeling, at *c.* +125‰ in the ambient treatment and +275‰ in the warmed treatment (Fig. 4b). The mean residence time of ^{13}C of R_{root} was longer than R_{leaf} or R_{crown}, but not as long as R_{soil}. The mean residence time of ^{13}C of R_{root} was equivalent across treatments at 64.2 ± 10.3 (SE) and 51.1 ± 8.5 h in the ambient and warmed treatments, respectively ($P = 0.4$). One month after labeling the isotopic composition of R_{root} also remained marginally higher (−26‰) than background levels in unlabeled chambers (−30‰).

The isotopic composition of microbial biomass also changed rapidly after labeling, reflecting rapid ^{13}C transport belowground and incorporation by microbes (Fig. 4c). Microbial biomass isotopic composition was variable and did not spike as clearly as for R_{leaf} and R_{root}, but the $\delta^{13}C$ value in microbial biomass increased to −5‰ or +10‰ *c.* 3 d after labeling. The mean residence time of ^{13}C content of microbial biomass was more uncertain, given the high measurement variation, but was estimated to be 142 ± 122 (SE) and 42 ± 42 h in the ambient and warmed treatments, respectively. This trend towards a faster turnover of microbial biomass in the warmed treatment was not statistically significant ($P = 0.5$). Overall, the change in microbial biomass isotopic composition suggests that a portion of the ^{13}C label was respired heterotrophically; microbial metabolism was responsible for some $^{13}C–CO_2$ that contributed to the measured isotopic change in R_{soil} (Fig. 2d).

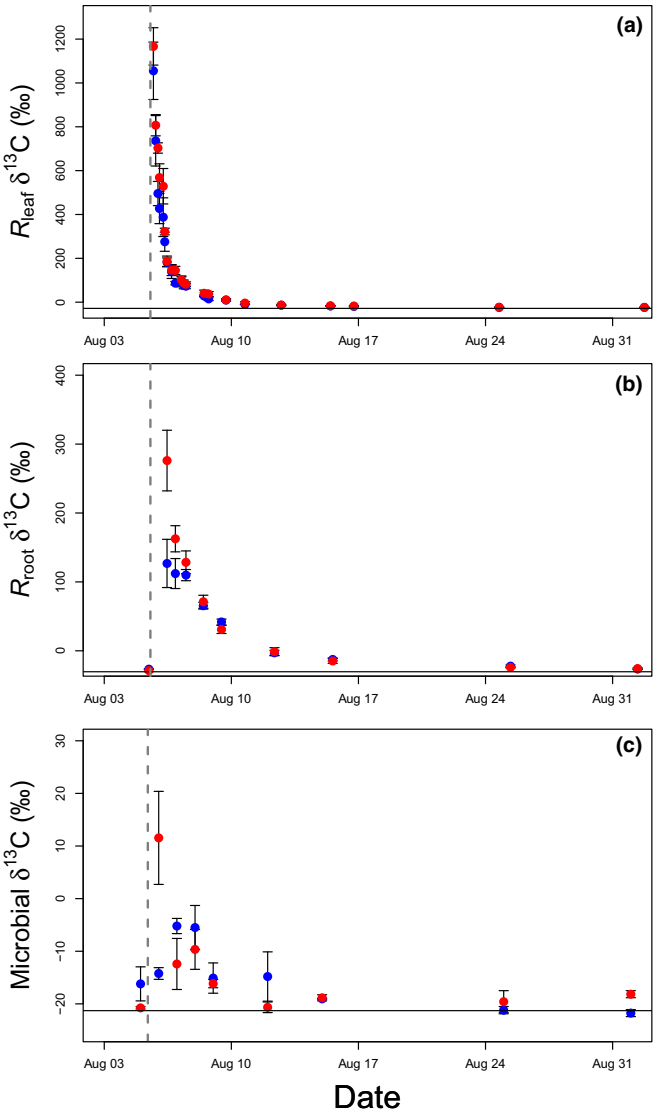

Fig. 4 The isotopic composition of respiration from leaves, roots (R_{leaf} and R_{root}) and carbon (C) content of microbes for a $^{13}C–CO_2$ pulse–chase experiment with *Eucalyptus parramattensis*. The isotopic composition of R_{leaf} was extremely enriched upon the first measurement, 4 h after labeling, but rapidly declined thereafter (a). The isotopic composition of root respiration was not enriched upon the first measurement, 5 h after labeling, but was highly enriched 24-h after labeling (b). The isotopic composition of soil microbial biomass was variable, but showed enrichment within 24 h of labeling (c). The vertical dashed lines denoted the labeling event, error bars reflect ± 1 SE, and the horizontal solid lines reflect the background isotopic composition measured on unlabeled reference chambers. Note the disparity in *y*-axis scales. Blue, ambient; red, warmed.

Leaf and crown respiration rates throughout the warming experiment

Measurements of foliar respiration rates at the crown and leaf scales (R_{crown} and R_{leaf}) throughout the warming experiment were indicative of homeostatic acclimation of respiration in response to the +3°C warming treatment (Fig. 5). Nightly measurements of R_{crown} expressed per unit total crown leaf area were exponentially related to air temperature in a manner that was adequately described by Q_{10} functions (Fig. 5a). The long-term apparent Q_{10} values were equivalent across treatments at 1.52 (95% CI from 1.43 to 1.60) for the ambient treatment and 1.58 (95% CI from 1.49 to 1.69) for the warmed treatment. The basal rate parameter R_{15}, however, was significantly lower in the warmed treatment (0.64; 95% CI from 0.62 to 0.65 μmol CO_2 m^{-2} s^{-1}) relative to the ambient treatment (0.78; 95% CI from 0.76 to 0.81 μmol CO_2 m^{-2} s^{-1}). Therefore, the temperature−response curve was shifted downwards in

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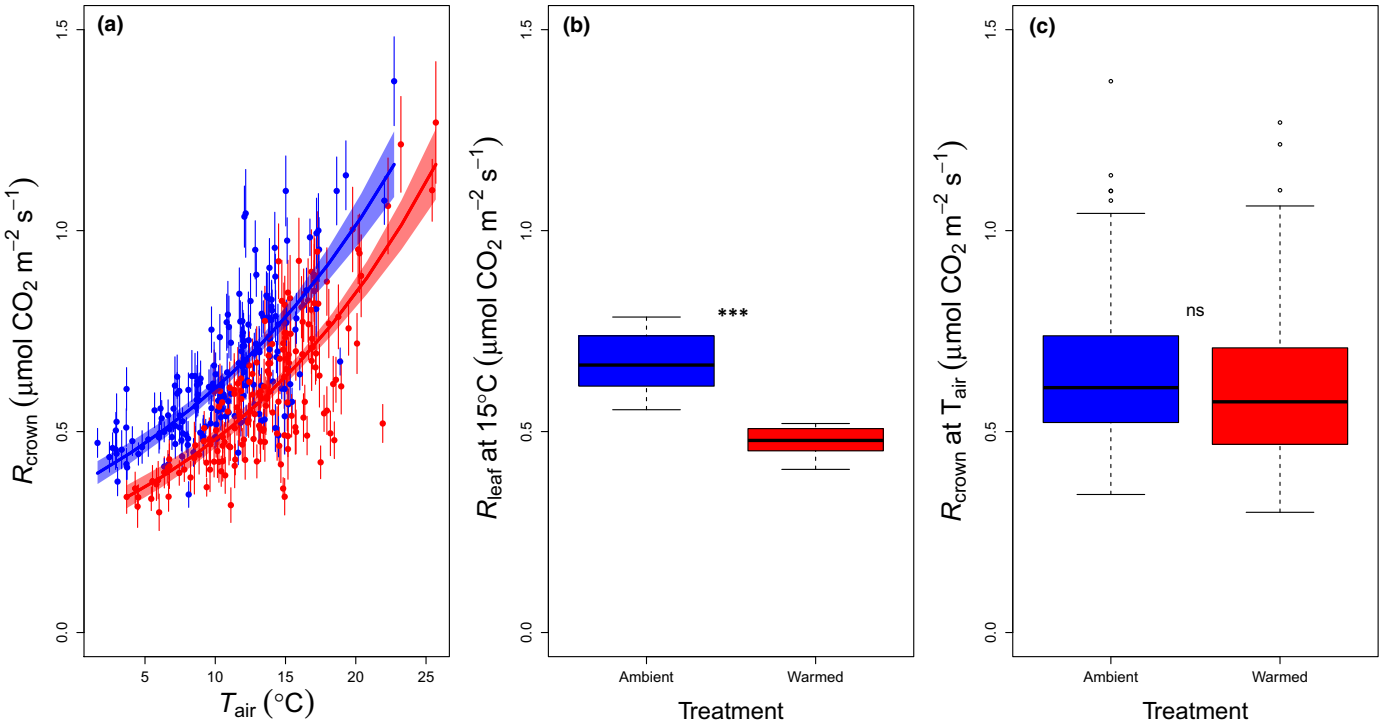

Fig. 5 Respiration rates measured at the crown and leaf scale for *Eucalyptus parramattensis* were indicative of homeostatic acclimation of respiration to experimental warming of +3°C. Respiration of entire tree crowns measured at *in situ* nighttime temperatures on 205 nights using whole-tree chambers and expressed per total crown leaf area (R_{crown}) indicate a downwards shift in the temperature–response curve with long-term warming (a). Respiration was also measured at a constant air temperature of 15°C by placing leaves in cuvettes (R_{leaf}; b). The reduction of R_{leaf} with experimental warming when measured at a constant temperature ($P < 0.05$) was an indication of respiratory acclimation. Given respiratory temperature acclimation, average *in situ* rates of R_{crown} did not differ between ambient and warmed treatments ($P > 0.1$), despite the 3°C difference in temperature (c). Error bars (a) reflect ± 1 SE. ***Denotes statistical significance at $P < 0.01$, and 'ns' refers to differences that are not statistically significant. Box plots follow the standard convention; the solid line reflects the median, the hinges reflect the first and third quartiles, the whiskers reflect the first and third quartiles plus 1.5 times the interquartile range, and circles reflect extreme observations. Blue, ambient; red, warmed.

response to long-term warming. Physiological temperature acclimation was also apparent in the direct measurements of R_{leaf} in cuvettes at a controlled and constant temperature of 15°C, as R_{leaf} was significantly lower in the warmed group relative to ambient treatment at this common measurement temperature ($P < 0.01$; Fig. 5b). Given the respiratory acclimation to temperature, *in situ* rates of R_{crown} expressed per unit total crown leaf area were equivalent across treatments ($P > 0.5$; Fig. 5c), indicating that respiratory acclimation was completely homeostatic.

Discussion

We used the unique infrastructure of WTCs to deliver a whole-tree ^{13}C–CO_2 label to field-grown trees in ambient conditions as well as trees exposed to +3°C warming, and we tracked the label as it was respired by leaves, whole crowns, roots, and whole soil systems. Across treatments, we found no differences in the mean residence times or in the total amount of ^{13}C label that was respired. These results are consistent with the idea that homeostatic temperature acclimation of respiration prevented a reduction in tree CUE in response to experimental warming. The trees acclimated to the warming treatment, such that R_a consumed an equivalent fraction of GPP in both treatments and CUE was unaffected by warming.

Respiratory acclimation to warming temperatures is consistent with previous work across a wide range of plants (reviews by Atkin & Tjoelker, 2003; Smith & Dukes, 2013; Slot & Kitajima, 2015). Some have speculated that respiration and photosynthesis exhibited a coupled dynamic acclimation response, given their connected nature as processes that produce and consume carbohydrates, leading to a constant respiration to photosynthesis ratio across a range of temperatures (Dewar *et al.*, 1999). Respiration and photosynthesis may acclimate to temperature together such that their ratio (*R/A*) is constant. This is consistent with some measurements of an invariant *R/A* ratio across a wide range of temperatures, but with some additional variation at exceptionally high or low temperatures (Atkin *et al.*, 2007; Aspinwall *et al.*, 2016; Slot & Winter, 2017; Crous *et al.*, 2018; Dusenge *et al.*, 2019). Importantly, several studies indicated that the *R/A* ratio changed in response to a change in growth temperature, but that a homeostatic *R/A* ratio was restored following the development of new leaves (Ziska & Bunce, 1998; Loveys *et al.*, 2003; Campbell *et al.*, 2007). Temperature acclimation of respiration at the whole-plant scale, as measured here in trees grown under ambient and warmed (+3°C) conditions, is likely to be strongly affected

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by the continuous development of new foliage in these indeterminately growing evergreen trees. In summary, our results are consistent with complete homeostatic acclimation of respiration rates and acclimation in respiratory partitioning of photosynthate in response to a long-term warming experiment.

Our results indicated that a significant fraction of photosynthate is rapidly respired belowground. This is consistent with classic work demonstrating the rapid and close connection between photosynthesis and R_{soil} (Högberg *et al.*, 2001) and other isotopic studies showing short time scales between ^{13}C–CO_2 label uptake and belowground respiration (reviewed by Epron *et al.*, 2012). Our study extends this work by estimating that just 10% of ^{13}C label uptake was respired aboveground by leaves, branches, and stems, while nearly 40% of the ^{13}C label was respired belowground (Fig. 3). This partitioning term for crown respiration (10%, or 0.1) appears low compared with claims that foliar respiration is the largest autotrophic respiratory flux at the global scale at *c.* 50% of the total R_a flux (DeLucia *et al.*, 2007; Piao *et al.*, 2010; Atkin *et al.*, 2015). Our previous work in this facility using a different species and nonisotopic methods indicated that the fraction of daily GPP returned to the atmosphere via aboveground autotrophic respiration was highly variable from 0.18 to 0.8, with a time-integrated average of 28% (Drake *et al.*, 2016). Therefore it is possible that the isotopic estimate of 10% presented here may be a snapshot of a dynamic and variable partitioning term. A previous review of C partitioning in forests based on ecosystem-scale mass-balance calculations concluded that foliar respiration was 18% of GPP, and that allocation belowground was dynamic, with increasing partitioning belowground when soil resources were strongly limited (Litton *et al.*, 2007). Therefore, the low nutrient availability of the sandy and highly weathered soil at this site may have contributed to the high respiratory partitioning belowground and the low respiratory partitioning aboveground observed here (Fig. 3). We further emphasize that the rapid (within 3 d) labeling of soil microbial biomass indicates that the belowground respiratory partitioning value presented here (*c.* 40%; Fig. 3) was likely influenced by microbial metabolism of rhizodeposited C. Nevertheless, belowground respiration of recent photosynthesis was much larger than crown respiration in this study.

This study had several limitations. First, the low replication ($n = 3$) is relatively typical in isotope pulse–chase studies (Epron *et al.*, 2016), but had likely constrained our ability to detect effects of experimental warming for small effect sizes. For example, although not statistically significant, there were trends towards reduced mean residence times for ^{13}C in R_{crown}, R_{root}, R_{soil} and MB (but not R_{leaf}) with experimental warming (Table 1), which would be consistent with an increased velocity of metabolism. However, independent measures of R_{crown} with higher replication ($n = 6$) also revealed no evidence of increased respiration rate with warming (Fig. 5). Therefore, we recognize that limited replication constrained our ability to detect small treatment effects via the isotopic method, but the lack of any difference between ambient and warmed trees in ^{13}C partitioning or R_{crown} rates indicated that warming did not strongly affect CUE in these trees. Second, we recognized the uncertainty in the amount of ^{13}C–CO_2 label assimilated by these trees. Our three methods agreed that the warmed treatment took up more ^{13}C–CO_2 than the ambient treatment, but they did not agree on the absolute magnitude. We acknowledge this uncertainty and encourage future work to focus on quantifying the pulse, as well as the chase. Frequent measurements of chamber air during labelling using CRDS (instead of infrared gas analysis) would improve the precision of $^{13}CO_2$ uptake estimates. Third, we acknowledge that this labeling study took place during the relatively moderate environmental conditions of the Australian winter. Carbon allocation, and potential warming impacts, may still vary seasonally or in response to other environmental drivers. Finally, we did not harvest the trees at the end of the chase study to provide an independent estimate of the ^{13}C label retention.

We effectively delivered a whole-tree ^{13}C–CO_2 label to six large trees grown in field conditions and documented rapid transport and respiration of the label through the crown, root, and soil system. We documented homeostatic respiratory acclimation of foliar and whole-crown respiration rates; the trees adjusted to experimental warming such that leaf-level respiration rates were not increased. Experimental warming had no detectable effect on respiratory partitioning or mean residence times. These results suggested that these trees were fully capable of physiologically adjusting to warming temperatures in a manner that would strongly moderate a positive feedback between warming temperatures and the respiratory release of CO_2 to the atmosphere. This is somewhat at odds with the evidence for a positive feedback between warming and CO_2 release from land ecosystems at the Earth system scale; such studies have documented an atmospheric CO_2 concentration growth rate that is higher under warm years than under cool years (Frank *et al.*, 2010; Wang *et al.*, 2013). Our results suggested that such a feedback perhaps does not arise from the autotrophic respiration of trees directly, but such a feedback could still exist related to plant mortality and heterotrophic respiration of soil organic matter (Wieder *et al.*, 2013; Karhu *et al.*, 2014; Carey *et al.*, 2016).

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Author contributions

JED contributed to the design of the warming and ^{13}C–CO_2 experiments, collected and analyzed data, and led the writing. MEF contributed to the design of the ^{13}C–CO_2 experiment, collected and analyzed data, and contributed to the writing. MGT was the senior scientific lead on the warming experiment, helped with data interpretation, and contributed to writing. YC led the microbial biomass measurements and data interpretation, and contributed to the writing. CVMB built and maintained the whole-crown gas exchange system, contributed to the ^{13}C–CO_2 experiment, and assisted with writing. EP was the senior scientific lead on all aspects of the ^{13}C–CO_2 experiment, led the root respiration data collection, contributed to other data collection and interpretation, and contributed to writing.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Climate variables on the day of the $^{13}CO_2$ pulse.

Fig. S2 Climate variables during the month of the $^{13}CO_2$ pulse.

Fig. S3 Methodological comparison of carbon partitioning.

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See also the Commentary on this article by Ryan & Asao, **222**: 1167–1170.

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