PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Anatomical and physiological regulation of post-fire carbon and water exchange in canopies of two resprouting *Eucalyptus* species

Tarryn L. Turnbull · Thomas N. Buckley · Alexandra M. Barlow · Mark A. Adams

Abstract The great majority of *Eucalyptus* spp. are facultative resprouters, and they dominate the eucalypt forests of Australia. Despite this numeric and geographic dominance, there is a general lack of knowledge of their capacity for carbon capture and water loss during canopy reinstation. After a crown-removing fire, we measured leaf-level determinants of carbon and water flux in resprouting canopies of *Eucalyptus dives* and *E. radiata* over the 3 years that followed. Leaf anatomy and physiology changed markedly during canopy reinstation, and leaves produced in the second year (2010) were distinct from those produced later. Leaves produced in 2010 were thicker (all measures of leaf anatomy), yet more porous (increased intercellular airspace), causing specific leaf area also to be greater. Indicators of heterotrophic activity, leaf respiration rate and light compensation point, were twofold greater in 2010, whereas all measures of photosynthetic capacity were greatest in leaves produced in 2011 and 2012. Whilst stomatal density, vein density and leaf hydraulic conductance all progressively decreased with time, neither leaf water status nor carbon isotope discrimination were affected. We conclude that canopy reinstation is primarily limited by pre-fire carbon stores, rather than by post-fire edaphic conditions (e.g., water availability), and thus argue that capacity for recovery is directly linked to pre-fire forest health.

Keywords Photosynthesis · Respiration · Heteroblasty · Epicormic · Vertical gradient

Introduction

Any plant that relies on resprouting as a means of regeneration must have the ability to provide water, carbon and energy during reinstation of a photosynthetically active canopy after disturbance. A recent very active period of research on resprouting (the Clarke et al. 2013; Tansley review cites over 500 peer-reviewed articles published on the topic since 2000) focused on the protection and early ontogeny of resprouting canopies. Far less attention has been given to the ecophysiology of a reinstating canopy. For example, there have been many studies of the energetics of resprouting based on quantifying pools of carbohydrates and nutrients required for initial bud burst (see review by Clarke et al. 2013). On the other hand, very few studies have considered the role of exchanges of carbon and water in the rebuilding of canopies, and none have examined leaf physiology within mature trees resprouting via epicormic buds.

Available data suggest that leaves on resprouts exhibit increased light-saturated photosynthesis and/or stomatal conductance (Oechel and Hastings 1983; DeSouza et al. 1986; Kauppi et al. 1990; Reich et al. 1990; Busch and Smith 1993; Castell et al. 1994; Fleck et al. 1996a, b; Kruger and Reich 1997; Clemente et al. 2005; Schutz

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et al. 2009), decreased photoprotection (Fleck et al. 1998), enhanced leaf water status (Wellington 1984; Crombie 1997; Utsumi et al. 2010) and leaf nutrient status (DeSouza et al. 1986; Kauppi et al. 1990; Reich et al. 1990; Fleck et al. 1996b; Kruger and Reich 1997). These findings have been explained as originating either from increased water and nutrient availability due to increased root:shoot ratio following defoliation (Kruger and Reich 1997), or, in the instance of fire, from increased nutrient availability (Oechel and Hastings 1983; Reich et al. 1990; Fleck et al. 1996b). The interaction between photosynthesis and respiration in regenerating canopies has received passing attention in the literature (for two species of *Erica*; Goorman et al. 2011). The development of photosynthetic capacity during canopy reinstation thus remains unknown, as does how canopy reinstation via resprouting affects tree- or ecosystem-level carbon exchange.

Facultative resprouters (mature specimens resprout after crown-scorching fire and seedling recruitment is stimulated by fire), dominate the prolific and iconic Australian taxon *Eucalyptus*: over 90 % of all eucalypts resprout from dormant apical (epicormic) or basal (lignotuber) buds (Gill 1997). Evidence for the presence of epicormic buds (Jacobs 1955) and the link between the prevalence of epicormic sprouting and bark depth (Gill and Ashton 1968) are well established, and it is clear that epicormic buds sprout when a crown is removed, provided bud tissues are sufficiently deep within the stem (i.e., in the vascular cambium) to protect them from fire (Bennett 1992; Burrows 2002). Furthermore, eucalypts that resprout from meristematic strips produce juvenile leaves, which are morphologically and physiologically distinct from adult leaves ordinarily produced on mature trees. This ontogenetic progression, termed heteroblasty, is widely regarded as an adaptation to environmental change during growth (see also the review by Zotz et al. 2011). In eucalypts, persistence of juvenile leaves varies among species and provenances (e.g., up to 3 years for epicormic resprouts; Gill 1978; 2–4 years for seedlings; Beadle et al. 1989). Generally, it is believed that juvenile eucalypt leaves are adapted to exploit abundant resources (light, nutrients and water) in order to maximize carbon gain at the cost of water-use efficiency (Velikova et al. 2008), whereas adult leaves are thought to be better adapted for resilience under resource-limited conditions (James and Bell 2000, 2001). These conclusions are drawn from studies of seedlings rather than of juvenile leaves from resprouting branches. The persistence of juvenile leaves and the physiology of leaf gas exchange of carbon and water on resprouting canopy branches during canopy reinstation is virtually undescribed.

The objective of the present study was to examine dynamics of carbon and water exchange and their physiological and anatomical determinants in leaves developing on epicormic branches in a canopy of uniform light and leaf age. We examined the vertical profile of leaf anatomy, morphology and physiology for two common resprouting eucalypt species (*E. radiata* Sieber and *E. dives* Schauer) in the period of 2–4 years after crown-removing fire (the extensive Black Saturday fires in February 2009). As the resprouting canopy was comprised initially of juvenile leaves with good access to resources (light, water, nutrients), we hypothesized that (1) the canopy should display a high initial capacity for carbon gain and water loss to permit rapid canopy reinstation, and (2) capacity for carbon gain and water loss would diminish with ontogenetic progression to a mature leaf form. We hypothesized further that leaf properties would initially be vertically uniform, but that vertical gradients would arise over time as canopy development led to gradients in light and water availability.

Materials and methods

Study site

Our study site was located in the Dingley Block of Stanley State Forest, near the town of Stanley (36°24′19″S, 146°45′19″E), NE Victoria, Australia. Mean annual rainfall between 1971 and 2000 was 1,014 mm, corresponding with mean average monthly temperature peaking at 20 °C in February and being least in July (6 °C, Australian Bureau of Meteorology). The climate is characterized as Oceanic in the Köppen climate classification system. The underlying geology comprised of Ordovician sediments containing sandstone, shale and mudstone horizons within fractured bedrock (Hough 1981).

Within all mixed-species eucalypt forest, the local proportions of each canopy species varies with aspect, soil depth and altitude. At our study site, the canopy was comprised mostly of *Eucalyptus radiata* Sieber (stocking of 224 ha^{-1}, basal area 23 m^2 ha^{-1}), *E. dives* Schauer (stocking of 288 ha^{-1}, basal area 11 m^2 ha^{-1}) and *E. mannifera* R. T. Baker, L. Johnson (stocking of 213 ha^{-1}, basal area 28 m^2 ha^{-1}). This forest was burnt in the extensive Black Saturday bushfires in February 2009 (see Pfautsch and Adams 2012); at our chosen site, the canopy was devoid of leaves and fine (tertiary and quaternary) branches immediately after the fire. In the months following, the overstorey trees began regenerating by sprouting from epicormic buds located along the entire tree bole. Of a group of trees readily accessible with an elevated work platform, we randomly selected six trees, three each of *E. radiata* and *E. dives*, for instrumentation and measurement. The selected *E. radiata* trees were 14–18 m tall with DBHs falling between 31–55 cm; *E. dives* trees ranged from 14 to 20 m tall and had a DBH range of 24–31 cm.

Atmospheric measurements

To assess vertical gradients in environmental variables, we measured air temperature (T_a, °C), relative humidity (RH, %) and photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) over 91 days from early autumn through early winter during the second year of the study (April–July 2011), at three heights (1, 11 and 18 m) within the canopy of the study trees using HOBO sensors (Onset, MA, USA). The light sensor at the lowest height became obscured by vegetation shortly after installation and we report PPFD only for the mid- and upper-canopy positions. We calculated vapour pressure deficit (VPD, kPa) from temperature and relative humidity as VPD = (1–0.01 RH) 0.6112·exp [17.62·T_a/(243.12 + T_a)] (World Meterological Organization data).

Distribution of leaf area during crown re-establishment

In April 2010, April 2011 and April 2012, we completed a full census on each study tree by recording the basal diameter class (<5, 6–10, 11–15, 16–20, 21–25 and >25 cm) and height (within 2-m lengths of bole) of each individual epicormic branch (data not shown). Species-specific allometric relationships (fourth-order homogeneous polynomial functions) between branch basal diameter and total leaf area (LI-3000; Li-Cor, NE, USA) were developed by sampling branch of each diameter class over the full tree height for each of the study trees. An earlier report (Paul et al. 2013) conclude that age-related change need only be incorporated into species-specific allometric relationships for eucalypts when comparing actively growing against senescent trees, so we pooled data over the three sampling campaigns (comprising 36–40 sprouts per tree in 2010, 10 per tree in 2011 and 15 per tree in 2012).

Leaf physiological measurements

In April 2010, April 2011 and April 2012, we assessed aspects of leaf physiology, all measurements being made in situ via an elevated work platform. On one leaf (the youngest full-expanded specimen, i.e., that closest to branch apex but with appearance and size comparable to that of older neighboring leaves) at three heights (low: 2–4 m; mid: 8–11 m; and top: 14–18 m) for each study tree, we measured carboxylation capacity (V_{m25}), electron transport capacity (J_{m25}) and dark-adapted (>12 h in darkness) leaf respiration (R_{d25}) using two recently calibrated infrared gas analysers (IRGA; Li-6400; Li-Cor), and corrected these values to 25 °C using temperature responses of Bernacchi et al. (2001, 2003). Over the course of 1 day (eight time-points per day in 2010 and 11 time-points in 2011 and 2012) during each campaign, on the same trees

and at the same measurement heights, we also measured stomatal conductance to water vapour (g_s) at both surfaces of each leaf, using two leaf porometers (SC-1; Decagon Devices, WA, USA). These leaves were then excised with clean razor blades and sealed in a Scholander-type pressure chamber (PMS model 1000; PMS Instrument, OR, USA) within 20 s of excision to measure leaf water potential (Ψ).

In 2012, we compared porometer and IRGA measurements of g_s on the same leaves, and found that porometer-g_s was reliably proportional to IRGA-g_s for a given measurement campaign (data not shown). We thus report vertical and diurnal trends in relative g_s within each campaign (g_{srel}, the ratio of g_s to its diurnal and vertical average within each campaign). Further description of absolute changes in stomatal conductance across the years of the study was based on measured carbon isotope discrimination (δ^{13}C), as described below. As a proxy for the balance of photosynthetic CO_2 fixation and CO_2 diffusion through stomata (Farquhar and Sharkey 1982; Farquhar et al. 1989), a qualitative estimate of trends in conductance is provided by concurrent δ^{13}C and photosynthetic capacity.

Leaf sampling for chemical, anatomical and morphological measurements

In each of April 2010, April 2011 and April 2012, we sampled young fully-expanded leaves growing on epicormic branches from each of our sample trees, at 2-m intervals up the bole of each study tree.

δ^{13}C and total N

δ^{13}C and total N were quantified from 3 mg of dried and finely ground leaves via a Flash HT (ThermoFisher Scientific, Bremen, Germany) modified to a dual reactor setup (reduction reactor at 680 °C on the left and oxidation reactor at 1,020 °C on the right), coupled to a Delta V Advantage isotope ratio mass spectrometer (IRMS) by a Conflo IV interface (ThermoFisher Scientific).

Light microscopy for leaf structure, leaf hydraulic conductance and vein density

Vertical distance from minor veins to the leaf epidermis (y-axis in Brodribb et al. 2007), cuticle thickness, leaf thickness, intercellular airspace and characteristics of palisade cells were measured on fixed and stained leaf sections. Leaf sections of 1 cm × 2 mm were fixed with 2.5 % glutaraldehyde solution in 0.1 M phosphate buffer in the field and retained at 4 °C for 4 days to enable penetration of fixative. Samples were dehydrated in a graded series of ethanol, cleared with xylene and infiltrated with paraffin using an automated processor (TissueTek VIP; Sakura, Tokyo).

Samples were embedded (paraffin) and cut into 4-μm sections using a microtome (RM2165; Leica Biosystems, NSW, Australia) and dried at 56 °C upon slides. Slides were de-paraffinized with xylene and rehydrated via an ethanol series, stained in 0.1 % toluidine blue for 1 min, rinsed with distilled water, dehydrated, cleared and mounted with Ultramount (Fronine, Sydney, NSW, Australia). Slides were mounted on the stage of a light microscope (Zeiss Axio Imager A1; NSW, Australia) to expose the leaf cross-section. Images were captured with a charge-coupled device (CCD; Retiga 1394; Q Imaging, BC, Canada) at ×20 magnification for measurement of vertical distance from minor veins to the leaf epidermis (equating to at least two measurements per slide, presented as an average value for each leaf) and ×40 magnification for measurement of palisade cell x and y dimensions (for four randomly chosen cells per slide, presented as an average value for each leaf). All images were analysed using Adobe Photoshop CS5 (San Jose, CA, USA).

We estimated leaf hydraulic conductance (K_{leaf}) in these leaves as described by Brodribb et al. (2007), based on a relationship reported by those authors between K_{leaf} and mesophyll hydraulic path length (D_m). We measured D_m by identifying the point within an areole that is most distant from the veins that border the areole, and computing the distance between that point and the nearest vein. These measurements were performed for 10 areoles from one leaf per height interval per species. Areoles were imaged in fresh leaves under transmitted light on a stereomicroscope fitted with a CCD camera (DMW-143-N2GG; Motic, Hong Kong) at 10× magnification. The same images were used to measure vein density on one 25-mm^2 section per leaf (using Adobe Photoshop CS5).

SEM of leaf surfaces for stomatal features

Leaves were dried to 60 °C without contact on a 5 × 5 mm section of the epidermal surface. This section was excised from the remaining leaf and mounted, using carbon tabs, on an aluminium stub. The section was then sputter-coated with gold and imaged via a Hitachi S4500 FEG scanning electron microscope (Hitachi, Tokyo, Japan) at ×1,100 for measurement of the cuticular ledge and size of stomatal complex (per England and Attiwill 2006), and at 150× for calculation of stomatal density and stomatal index. Images were analyzed using Adobe Photoshop CS5.

Statistical analysis

We used linear mixed models to quantify effects of species, year and height (where appropriate) as fixed factors, with tree as a random factor, on all anatomical and physiological parameters. Alternative models that included interaction terms and permutations of these fixed factors were fitted using maximum likelihood; the most parsimonious model was chosen as that with smallest corrected Akaike information criterion (AICc), and then refitted using restricted maximum likelihood (REML). Different levels of significant fixed effects were compared using Tukey's LSD post hoc tests. Where appropriate, results were presented using LS means and standard errors to correct for effect of confounding covariates. Additionally, to explore changes in leaf anatomy during post-fire crown development, we used principal components analysis (PCA) to partition variance among five anatomical parameters (palisade depth, palisade cell height and diameter, cuticle thickness and epidermal depth) into maximally orthogonal components. We used R 3.0.1 (R Core Team 2013), packages lme4 (Bates et al. 2013) and multcomp (Hothorn et al. 2008), for all statistical analyses.

Results

Trends in epicormic leaf biology over time

We observed sharp changes in total leaf area and the vertical distribution of leaves, as well as in the properties of individual leaves, over the 3 years of the study, from 2010 (second full growing season after the fire) through 2012. In general, changes were greatest between 2010 and 2011. Species differences were not significant for any of the results discussed below ($p > 0.05$), so we pooled results across both species. Leaf area per tree peaked at 75 m^2 during the third year after fire, having increased by 49 % after the second year (50 m^2), before declining by 20 % in the fourth year to 60 m^2. There was a marked shift in the vertical distribution of leaf area: most leaf area was below 8 m height in 2010, but by 2012 most leaf area was above 12 m (Fig. 1).

Most measured features of leaf anatomy and morphology also changed greatly between 2010 and 2011: cuticle thickness, epidermal depth, total palisade depth, palisade cell height and palisade cell diameter (Fig. 2), as well as stomatal density (ρ_s) and vein density (ρ_v) (Fig. 3) and specific leaf area (SLA; Fig. 4) all declined significantly from 2010 to 2011, whereas stomatal index (SI) increased slightly (Fig. 3a). Subsequent changes from 2011 to 2012 were smaller or insignificant for many of these parameters. PCA on the anatomical parameters in Fig. 2 clearly segregated leaves from 2010 into a distinct group (Fig. 2 inset; Table 1), confirming a fundamental change in leaf anatomy between years 1 and 2 post-fire. Whilst second-year leaves were thicker in this study (total leaf thickness is the sum of palisade and epidermal depth and cuticle thickness, each of which were greater in year 1; Fig. 2), second-year
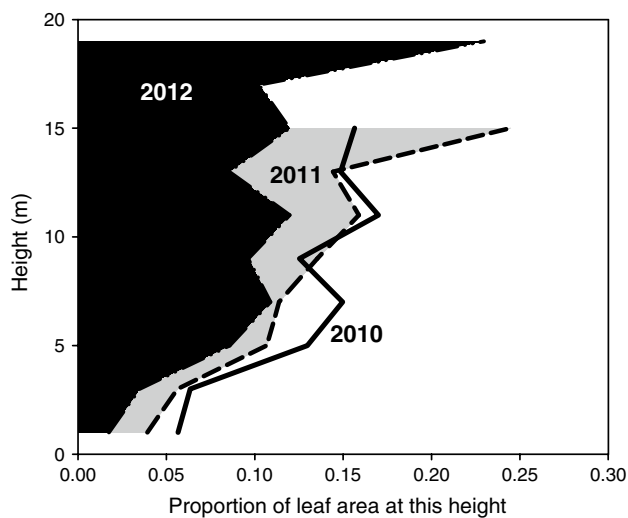
Fig. 1 Changes in the vertical distribution of leaf area on epicormic branches over a period of 2–4 years after crown-replacing fire in *Eucalyptus dives* and *E. radiata*: 2010 (*solid line*, *no shading*), 2011 (*dashed line*, *light gray diagonal hatches*), 2012 (*dash-dot line*, *dark gray cross-hatches*). Sample means of proportions of leaf area in 2-m increments centered at the value shown on the *vertical axis*

leaves also had nearly double the airspace fraction (25.6 %) than third- and fourth-year leaves (14.1 %) (not shown; $p < 0.001$).

These anatomical changes were complemented by substantial adjustments in leaf gas exchange. Two measures of heterotrophic activity or 'sink strength'—leaf respiration rate (R_d) and light compensation point (LCP)—both declined by over 50 % (Fig. 5a), whereas several measures of photosynthetic function increased, including carboxylation and electron transport capacities (V_{m25} and J_{m25}) and the maximum quantum yield of electron transport (ϕ) (Fig. 5b). There was a steady decline in leaf nitrogen concentration per unit area (N; Fig. 4) across all 3 years of the study. We found no evidence that the decline in stomatal density influenced stomatal limitations to leaf water status or photosynthesis, as both carbon isotope discrimination ($\delta^{13}C$, Fig. 4) and leaf water potential (ψ_l, not shown) were similar across years. Similarly, leaf hydraulic conductance (K_{leaf}, Fig. 3b) varied little across years, declining only marginally from 2011 to 2012 ($p = 0.047$).

Vertical trends in epicormic leaf biology

We observed vertical gradients in some parameters but not in others. Cuticle thickness increased with height (Fig. 6a) and palisade cell diameter (Fig. 6b) decreased with height, but all other anatomical parameters were unaffected by vertical position (not shown). Most gas exchange-related parameters increased marginally but insignificantly with height (Fig. 7). Carbon isotope discrimination declined
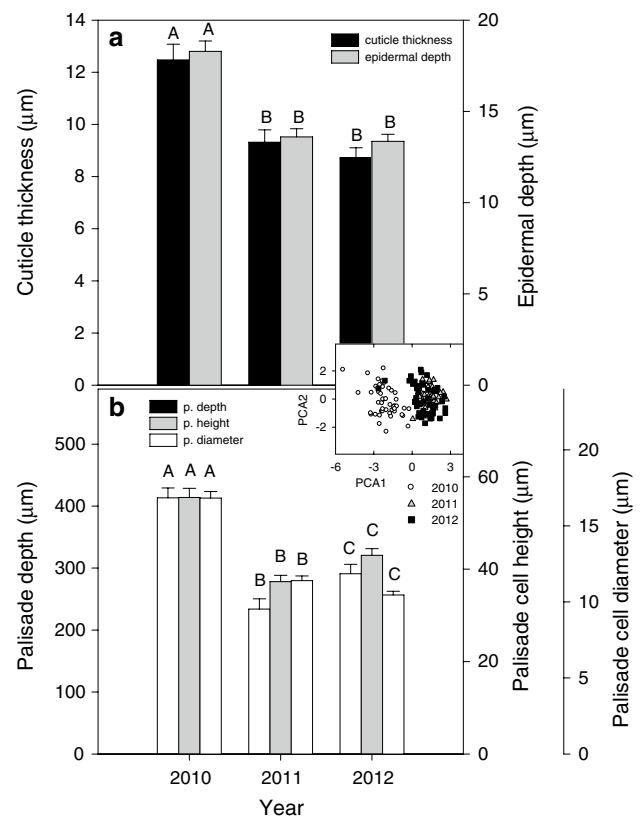

Fig. 2 Changes in leaf anatomical traits over a period of 2–4 years after fire in epicormic-resprouting *Eucalyptus dives* and *E. radiata*. LS mean ± SE. *Bars with the same letter* are not significantly different ($p \geq 0.05$). **a** Aspects of epidermal anatomy; **b** aspects of palisade mesophyll anatomy; *inset* first two components (PCA1 and PCA2) from principal components analysis (PCA) using the five parameters shown in (**a**) and (**b**), demonstrating that leaves in the second year (*open circles*) were anatomically distinct from those formed in the third and fourth years (*gray triangles* and *solid circles*, respectively). Full PCA results are given in Table 1

greatly with height (i.e., $\delta^{13}C$ became less negative; Fig. 6c), despite the vertical profiles of both stomatal conductance and photosynthetic capacity being similar to one another (Fig. 7a, d). There was no significant interaction of height and year for any parameter, indicating that vertical profiles were similar across the 3 years.

Discussion

Canopy processes in resprouting eucalypts

We found rapid and striking changes in leaf-level determinants of canopy carbon and water flux over the period of 2–4 years after a stand-replacing fire in two species (*E. dives* and *E. radiata*) that dominate large areas of the fire-prone, mixed-species eucalypt forests of southeastern Australia.
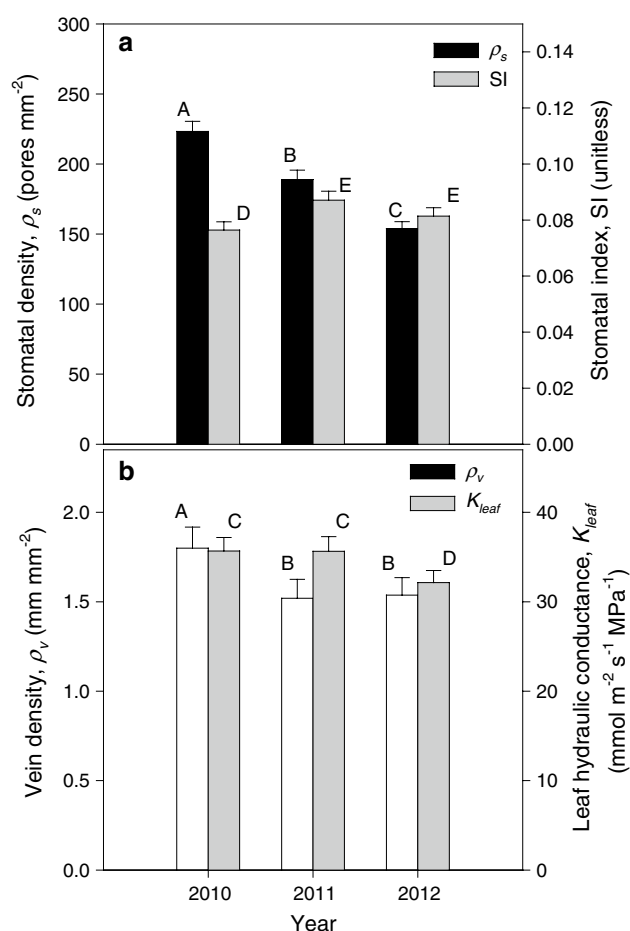
Fig. 3 Trends in leaf anatomy traits related to regulation of gas exchange in the period of 2–4 years after fire: **a** stomatal index (*SI*) and stomatal density (ρ_s), and **b** vein density (ρ_v) and leaf hydraulic conductance (K_{leaf}). LS mean ± SE. *Bars with the same letter* are not significantly different ($p \geq 0.05$)

However, the nature of these changes was largely opposite to our expectations. We hypothesized that the dense, rapid flush of juvenile leaves produced on epicormic branches immediately after fire would have characteristics similar to juvenile foliage such as that found on eucalypt seedlings regenerating after fire (Buckley et al. 2012), and in other genera known to resprout after fire (e.g., *Adenostoma* and *Quercus*, Oechel and Hastings 1983; *Rhus*, DeSouza et al. 1986; *Quercus*, Reich et al. 1990). We further hypothesized that the physical characteristics would be reflected in physiological changes common to foliage formed following en masse defoliation (Reich et al. 1993)—namely, we expected these leaves to have much greater photosynthetic capacity and stomatal conductance than leaves produced 3 years after the fires.

We found the opposite result: although leaf area peaked early in canopy development (during the third year), leaves produced in the second year had lesser photosynthetic
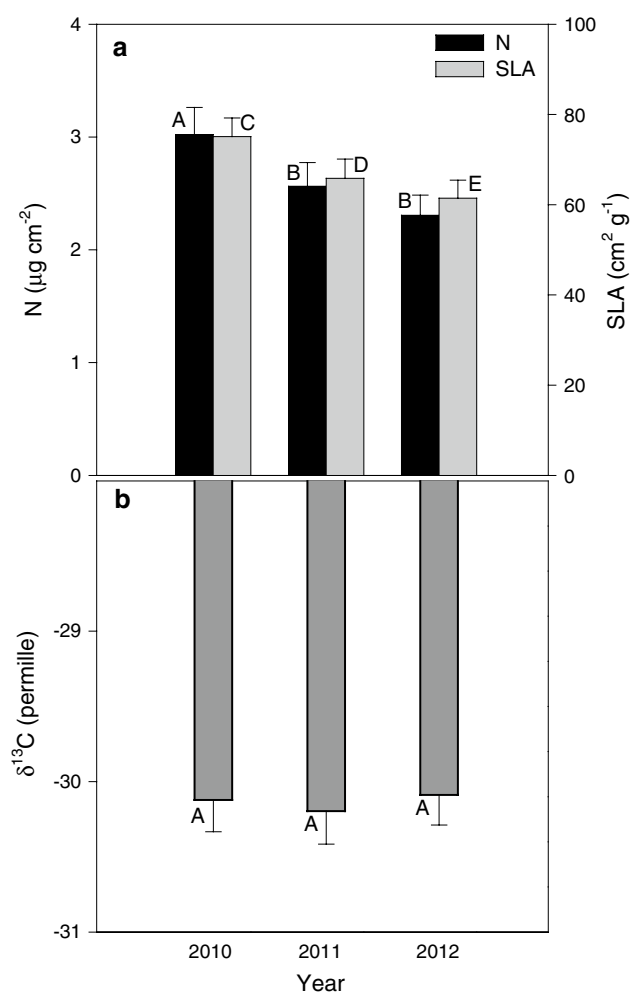

Fig. 4 Trends in leaf ecophysiological parameters over the course of the study: negative of carbon isotope discrimination, $-\delta^{13}C$; specific leaf area (*SLA*); leaf nitrogen concentration per unit area (*N*). LS mean ± SE. *Bars with the same letter* are not significantly different ($p \geq 0.05$)

Table 1 Results of principal components analysis (*PCA*) of leaf anatomical traits (cuticle thickness, epidermal depth, palisade depth, palisade height and palisade diameter)

Variable	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5
Cuticle thickness	−0.35	0.77	−0.24	−0.36	0.30
Epidermal depth	−0.44	0.27	0.74	0.43	−0.10
Palisade depth	−0.51	−0.04	−0.44	0.10	−0.73
Palisade diameter	−0.45	−0.46	0.31	−0.70	0.05
Palisade height	−0.48	−0.35	−0.32	0.43	0.60
% of variance	62.9	17.7	10.2	5.7	3.5
Cumulative % of variance	62.9	80.6	90.8	96.5	100

capacity and greater heterotrophic activity than leaves produced in the fourth year after fire. Additionally, carbon isotope discrimination was similar across years, indicating
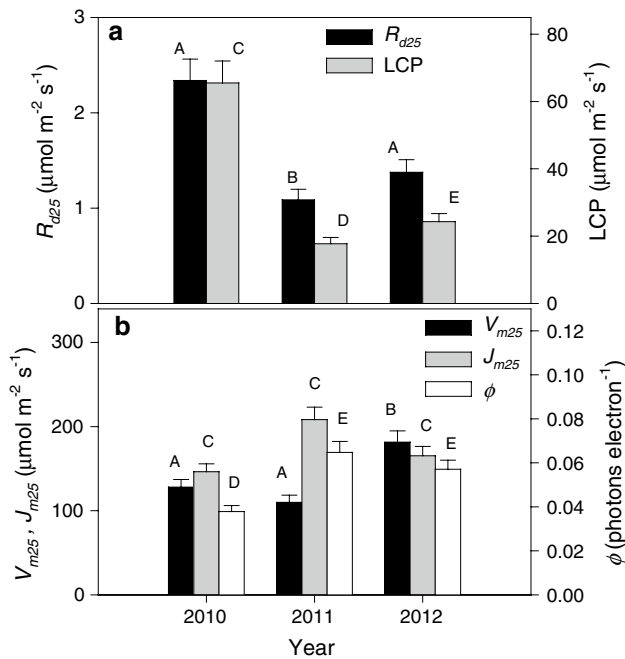
Fig. 5 Physiological parameters of leaf CO_2 exchange across the 3 years of the study: *A* rate of non-photorespiratory CO_2 release in the light (R_{d25}) and light compensation point (LCP); *B* carboxylation capacity (V_{m25}), electron transport capacity (J_{m25}) and initial slope of potential electron transport rate versus incident irradiance (ϕ). LS mean $\pm$ SE. *Bars with the same letter* are not significantly different ($p \geq 0.05$)
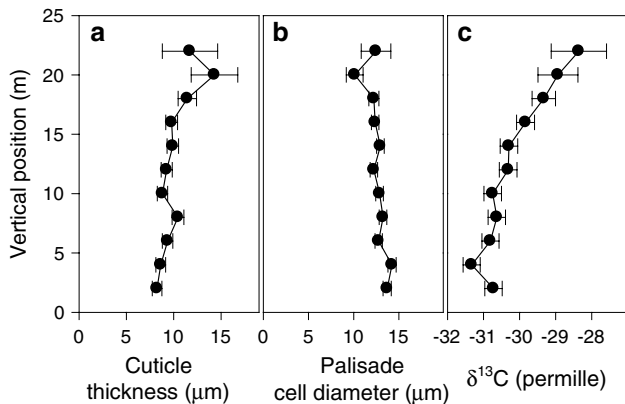

Fig. 6 Vertical profiles of leaf parameters that were measured at one-metre intervals along the bole (LS mean $\pm$ SE). *A* cuticle thickness (effect of height significant at $p = 0.0024$); *B* palisade cell diameter ($p = 0.0019$); *C* carbon isotope discrimination, $\delta^{13}C$ ($p < 0.0001$)

that stomatal conductance was approximately proportional to photosynthetic capacity across the study (Farquhar and Sharkey 1982; Farquhar et al. 1989), and was thus slower in the initial flush of leaves. In a concurrent companion study at the same site, leaves produced in the fourth year of canopy reinstation had similar photosynthetic capacity, stomatal conductance and leaf water status as leaves in nearby

mature canopies that escaped fire damage (Gharun et al. 2013). We also found that leaves of both ontogenetic forms displayed adaptations for resilience such as increased SLA and increased leaf thickness (James and Bell 2000; James and Bell 2001), and, unlike previous studies in relatively open eucalypt canopies (Turnbull et al. 2007), that SLA was unrelated to canopy position.

That we observed no vertical profiles in most anatomical and physiological parameters, despite large increases in leaf area in the period of 2–4 years after fire, may in part be attributable to the small gradient in irradiance (PPFD; Fig. 8a, 18.3 % greater in the upper canopy than in the middle canopy) (O'Grady et al. 2008) and negligible difference in evaporative demand (VPD) and air temperature (Fig. 8b, c) across canopy positions.

Our results contrast with most previous studies of resprouting plants, which have recorded faster rates of stomatal conductance and enhanced leaf water status during canopy reinstation (Oechel and Hastings 1983; DeSouza et al. 1986; Kauppi et al. 1990; Reich et al. 1990; Busch and Smith 1993; Castell et al. 1994; Kruger and Reich 1997; Clemente et al. 2005; Schutz et al. 2009; Wellington 1984; Crombie 1997; Utsumi et al. 2010). The differences shown here between second-year and third- or fourth-year leaves also contrasts with recognized patterns in ontogenetic change from juvenile to adult leaf form in eucalypts (Velikova et al. 2008). Ontogenetic shifts from juvenile to adult leaves in eucalypts are not necessarily coupled to a decrease in the capacity for carbon and water exchange, as previously thought.

Leaves produced on epicormic branches in the second year of growth after fire thus appear to serve a different purpose than leaves produced just 1 or 2 years later. For example, the early peak in canopy cover coincided with increased respiration rate and nitrogen concentration per unit area, but preceded full development of photosynthetic capacity. Increased respiration arguably reflects the cost of mobilizing carbon stores used to rebuild the canopy (Chapin et al. 1990), and delays replenishment of depleted starch reserves (Goorman et al. 2011). Leaves on epicormic branches in eucalypts (Marsh and Adams 1995) and other resprouting tissues in general (DeSouza et al. 1986; Kauppi et al. 1990; Reich et al. 1990) are known to be rich in nitrogen. Despite the known general relationship between leaf nitrogen and rates of photosynthesis, including leaves of some resprouting plants post-fire (DeSouza et al. 1986; Reich et al. 1990), we found no increase in rates of photosynthesis. Leaves produced in the second year after fire for these two *Eucalyptus* spp. seemingly provide functions other than the immediate replenishment of depleted carbon stores. Those functions likely include absorption of radiation and acquisition of nutrients that might otherwise support competitors in the understory. Tracking the
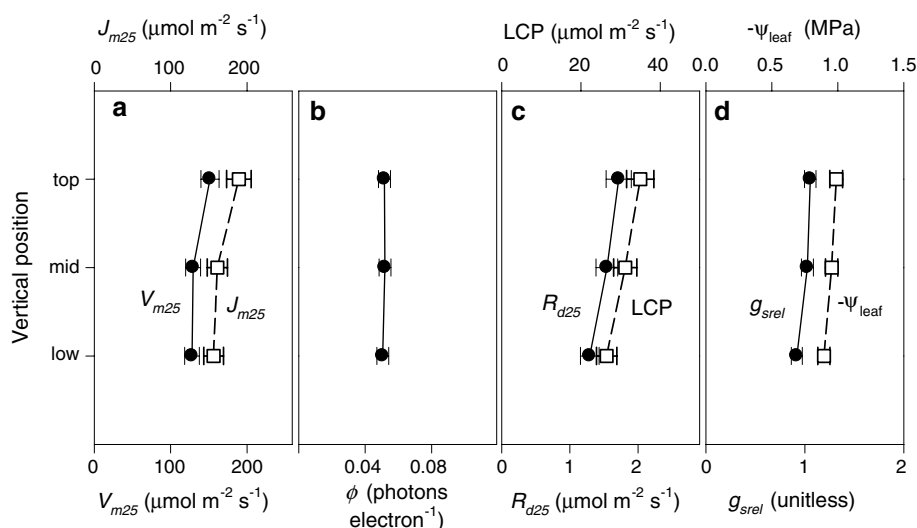
Fig. 7 Vertical profiles of physiological parameters related to leaf gas exchange: **a** carboxylation capacity (V_{m25}, *closed symbols*) and electron transport capacity (J_{m25}, *open symbols*); **b** initial slope of the response of potential electron transport rate to light (ϕ); **c** rate of non-photorespiratory CO_2 release in the light (R_{d25}, *closed symbols*) and light compensation point (*LCP*, *open symbols*); **d** relative stomatal conductance (g_{srel}, *closed symbols*) and minus leaf water potential ($-\psi_{leaf}$, *open symbols*). LS mean ± SE. None of the vertical trends shown here were statistically significant ($p > 0.05$)

origin and fate of nitrogen and the utilization and replenishment of carbon stores by isotopic methods could test these hypotheses.

Implications for forest carbon and water exchange, and forest management

This study has several implications for the impact of fire on ecosystem function in mixed-species eucalypt forests, which are dominated by facultative resprouting species. Whilst seedling recruitment can follow fire for such species, the results of our companion study (Gharun et al. 2013) show this to be minimal in this particular forest type (i.e. seedlings regenerating after this fire account for only 6.5 % of stand basal area). Our results suggest that the flush of new leaves in resprouters immediately after fire is unlikely to significantly increase forest water use, nor therefore to contribute to major changes in catchment water yield. This contrasts strongly with effect of fire on water exchange in ash-type forests, where rapid growth of a dense stand of seedlings leads to large and sustained increase in forest water use in the decades after stand-replacing fire (Langford 1976; Kuczera 1987; Buckley et al. 2012; Brookhouse et al. 2013).

While the present study encompassed the period of 2–4 years after fire, it seems unlikely that water use in mixed-species eucalypt forest would increase in later years. In contrast to the strong increase in water use in ash-type forests after fire, which is driven by an increase in sapwood area index (SAI, the total conducting sapwood area in the

stand per unit ground area), with smaller contributions from increases in leaf area index (LAI; Buckley et al. 2012), such increases in SAI is improbable in mixed-species eucalypt forest. Unlike young ash saplings, surviving mature trees in mixed-species eucalypt forest are subject to considerable constraints on continued stem growth, as encountered by all mature trees (Ryan et al. 2006). The overall effect of fire on water use by mixed-species eucalypt forest is likely to be small (and possibly even positive for water yield). This distinction between ash-type and mixed-species forests has large implications for catchment management and planning, and further studies of stand-level water balance are needed.

Our results also indicate that the regenerating epicormic canopy in mixed-species eucalypt forest is a weak carbon sink after fire. Second-year leaves were less energetically self-sufficient than leaves produced in later years, implying that the initial flush of leaves in resprouting eucalypts is highly reliant on stored carbon. This in turn suggests strong interactions between pre-fire conditions and post-fire carbon and water exchanges in mixed-species eucalypt forest; the effects of climate on carbon storage in the years before a fire are likely to strongly affect canopy reinstatement after fire. In this way, fire may propagate and amplify climate impacts on carbon and water exchange, for at least the first few years after fire. For example, drought-limited carbon uptake for several years before fire might continue to suppress carbon uptake for a year or two after the fire, despite post-fire rainfall. Another implication is that carbon 'saved' from fire by survival of carbon stores in mature
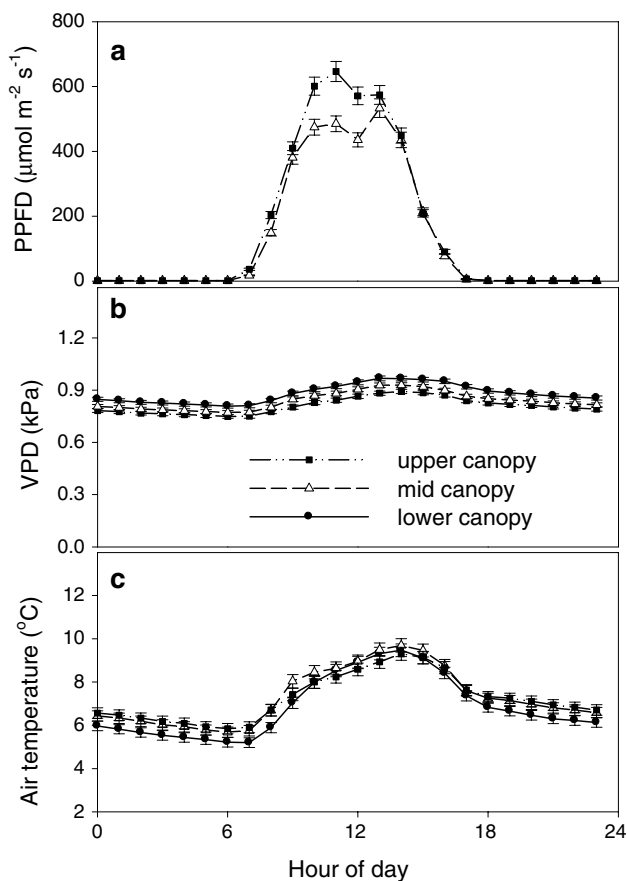
Fig. 8 Vertical profiles of environmental variables measured between April and July of 2011, at three canopy heights (*upper*, 18 m; *mid*, 11 m; *lower*, 1 m). **a** Photosynthetic photon flux density (PPFD); **b** air vapour pressure deficit (VPD); **c** air temperature. Data are omitted for PPFD at the lower canopy position because the PPFD sensor at that location became obscured by foliage shortly after installation, rendering the data not meaningful. Values shown are sample mean ± SE

mixed-species eucalypt forest trees is offset by reduced carbon gain in the regenerating canopy after fire. The size of this effect is unclear. We can estimate its magnitude by applying the changes we observed in leaf respiration rate and photosynthetic capacity during the period of 2–4 years after fire (−42 % for respiration and +43 and +12 % for carboxylation and electron transport capacities, respectively; Fig. 5) to published estimates of the fraction of gross primary productivity (GPP) that is lost by leaf respiration in eucalypts (12 %; Ryan et al. 2010). Assuming GPP is proportional to photosynthetic capacity across years, our results suggest the ratio of leaf respiration to GPP was 1.9–2.4 times greater in the second year than in the fourth year. This equates to a reduction of 11–17 % in GPP, or roughly 22–35 % in net carbon storage (assuming the latter is half of GPP; Waring et al. 1998). These calculations treat the fourth year after fire as a proxy for the pre-fire "baseline"

carbon flux; if the true baseline is even greater, which is likely, then the impact of fire on carbon storage would be even greater than indicated above.

Conclusions

Our original hypothesis—that leaves produced on epicormic branches initially after fire in eucalypts would have greater capacity for carbon gain and water loss than leaves produced in later years—was not supported by our results. This hypothesis was based on the premise that leaves produced on epicormic branches after fire should be well supplied with resources, due to elimination of shade cast by adjacent canopies, availability of endogenous carbon and nutrients stores, and access to the large water transport infrastructure of a mature tree. By contrast, our results suggest that the anatomical and ecophysiological properties of the early epicormic canopy may be controlled primarily by endogenous developmental "programming" (Bond 2000) or canopy conditions prior to the fire, rather than by favourable conditions during early regeneration.

Our results also suggest that fire is likely to affect carbon and water cycles very differently in mixed-species eucalypt forests than in ash-type eucalypt forests. Contrary to our hypothesis, we found that the initial flush of juvenile foliage after fire in resprouting eucalypts lacked the enhanced gas exchange observed in juvenile ash-type forests, and that subsequent increases in leaf gas exchange coincided with a reduction in total leaf area. These findings have three implications. First, it is unlikely that water use will increase in mixed-species eucalypt forest in the manner reported for ash-type forests during recovery from fire, because mixed-species eucalypt forest lack the heavily-transpiring foliage and capacity to rapidly increase sapwood area index that drive increased water use in ash-type forests after fire. Second, successful regeneration is contingent on forest health preceding the fire because epicormic canopy growth in mixed-species eucalypt forest relies heavily on pre-fire carbon stores. Conditions that deplete stores of carbon (e.g., ongoing drought, defoliation) will likely lengthen the period of recovery from fire in mixed-species eucalypt forest. Lastly, promoting resprouting eucalypts for their superior capacity as a carbon store in in the face of repeated burning (e.g., Crisp et al. 2011) might be premature in light of their heavy reliance on utilising stored carbon for recovery.

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