

Controls on declining carbon balance with leaf age among 10 woody species in Australian woodland: do leaves have zero daily net carbon balances when they die?

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Summary

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Received: 1 October 2008

Accepted: 11 February 2009

New Phytologist (2009) **183**: 153–166
doi: 10.1111/j.1469-8137.2009.02824.x

Key words: Australia, carbon balance, leaf age, light, nitrogen, photosynthesis, respiration, woody species.

- Here, we evaluated how increased shading and declining net photosynthetic capacity regulate the decline in net carbon balance with increasing leaf age for 10 Australian woodland species. We also asked whether leaves at the age of their mean life-span have carbon balances that are positive, zero or negative.
- The net carbon balances of 2307 leaves on 53 branches of the 10 species were estimated. We assessed three-dimensional architecture, canopy openness, photosynthetic light response functions and dark respiration rate across leaf age sequences on all branches. We used YPLANT to estimate light interception and to model carbon balance along the leaf age sequences.
- As leaf age increased to the mean life-span, increasing shading and declining photosynthetic capacity each separately reduced daytime carbon gain by approximately 39% on average across species. Together, they reduced daytime carbon gain by 64% on average across species.
- At the age of their mean life-span, almost all leaves had positive daytime carbon balances. These per leaf carbon surpluses were of a similar magnitude to the estimated whole-plant respiratory costs per leaf. Thus, the results suggest that a whole-plant economic framework, including respiratory costs, may be useful in assessing controls on leaf longevity.

Introduction

As leaves age, their net photosynthetic capacity (A_{\max}) typically declines (Mooney *et al.*, 1981; Field & Mooney, 1983; Hom & Oechel, 1983; Koike, 1988; Reich *et al.*, 1991; Kitajima *et al.*, 1997; Oleksyn *et al.*, 1997; Mediavilla & Escudero, 2003). Changes in leaf net carbon exchange with ageing result from increased shading of the leaf's microenvironment (an unavoidable consequence of plant growth), internal resource redistribution by the plant and cumulative deterioration of tissue function, often associated with declining leaf nitrogen concentration (Mooney *et al.*, 1981; Field, 1983; Field & Mooney, 1983; Reich *et al.*, 1991). These processes are particularly interesting to consider for evergreen species and

fast-growing pioneers in which leaf senescence often occurs independent of seasonally deteriorating climate for photosynthesis (Kikuzawa, 1991; Ackerly, 1999), in contrast with deciduous leaves in autumn in northern temperate regions.

Although we know that shading and declining physiological capacity can both contribute to an overall decline in net carbon balance (hereafter 'carbon balance') with leaf age in evergreens (Field & Mooney, 1983; Ackerly & Bazzaz, 1995; Kitajima *et al.*, 1997, 2005; Kitajima *et al.*, 2002; Mediavilla & Escudero, 2003), the relative controls by physiological shifts with ageing vs resource availability shifts with leaf position are not well characterized (Field, 1983; Ackerly, 1999). In addition, whether leaves as old as the typical age of leaf death (i.e. the mean leaf life-span) have near-zero or substantially positive

net carbon balance is incompletely understood (for example, Schoettle & Smith, 1991; Kitajima *et al.*, 1997, 2005; Ackerly, 1999; Kitajima *et al.*, 2002; Mediavilla & Escudero, 2003). Thus, it is not clear whether leaves are held until they possess near-zero (leaf-level) net carbon balance or are shed whilst still having considerably positive net carbon balances. Moreover, if the latter is true, why should this pattern exist? Here, we address these issues using measurement-based carbon balance modelling of leaves from 10 woody species in semi-open woodland in eastern New South Wales, Australia.

In assessing age-related changes in net carbon balance, we first consider the relative contributions of shading and deterioration of net photosynthetic capacity in reducing the net carbon balance of leaves as they age. A number of studies have described decreasing light availability along age or position sequences along branches. For example, Kitajima *et al.* (2002) studied two fast-growing pioneer species with short leaf life-span in tropical forest. Leaves of each species began life with markedly different light environments (80% vs 30% of full light), but, over 90 d, each species lost approximately 1% per day of that initial value as a result of increased self-shading because of continued new shoot growth. By contrast, for late successional tropical tree species in deeply shaded environments, Kitajima *et al.* (2005) found that older leaves on a shoot were no more shaded than younger leaves on the same shoot. The late successional species are inherently slower growing and much more shaded in general than pioneers, and so the two studies by Kitajima and colleagues probably represent the end points on a continuum of shade gradients along leaf age and leaf position sequences. How strong are such gradients for relatively slow-growing plants in semi-open and open conditions is unclear, although Wright *et al.* (2006) reported significantly decreasing light with increasing leaf age in both semi-arid and moist open habitats in eastern Australia which were mid-way between the two extremes reported for tropical forests.

Declining light availability with increasing leaf age (typically as a result of self-shading) has been associated with declining net carbon exchange potential (Field & Mooney, 1983; Ackerly & Bazzaz, 1995; Mediavilla & Escudero, 2003), and alleviation of such shading by defoliation has increased photosynthetic carbon gain (Anten & Ackerly, 2001). However, the relative contribution of increased shading and decreasing photosynthetic capacity to declines in realized carbon balance has been minimally addressed. If plants use resources near-optimally, photosynthetic capacity would be expected to decline with increasing age to roughly match declining light environment (Field, 1983); the decline in achieved photosynthesis could then be directly explained by either altered physiology or deteriorating light environment.

Next, we consider whether plants keep leaves alive only as long as they are positive carbon sources, and shed them when they reach zero or negative carbon balance. Published reports on this issue, from both empirical evidence and studies, show

mixed results. Several field studies report that A_{\max} is substantially above zero at the typical age of leaf death (Kitajima *et al.*, 1997; Kitajima *et al.*, 2002; Mediavilla & Escudero, 2003). This was true in one glasshouse study only when both the light availability and nutrient supply were abundant (Ackerly & Bazzaz, 1995); by contrast, in another potted plant study, this was true when nutrient supply was low, but not when it was high (Oikawa *et al.*, 2006). Schoettle & Smith (1991) found that, despite marked differences in leaf life-span in *Pinus contorta* at different elevations, leaves of the average age of leaf death had a similar and very low available light level and approximately zero net carbon balance. Thus, there is empirical evidence both for and against the notion of zero net carbon balance at the typical age of leaf death. Analyses from a theoretical perspective regarding the optimum carbon gain at death have also provided a range of plausible outcomes depending on the assumptions of the model (for example, Ackerly, 1999).

In holistically examining the carbon balance context of individual leaves as they age, some argue that the whole-plant costs of maintaining a leaf also need to be considered (Kikuzawa & Ackerly, 1999). Givnish pioneered this idea in examining the constraints on total maximum plant height (Givnish, 1987), and also examined the role of maintenance costs in terms of contrasting competitive ability of deciduous vs evergreen species in a common habitat across a range of seasonality (Givnish, 2002). Moreover, Walters & Reich (2000) demonstrated that species' metabolic adaptations to shade resulted in a performance trade-off (with successful strategies identified across all light niches) only when whole-plant respiratory costs were incorporated into the analyses. Hence, as there are costs in roots and stems associated with maintaining the full population of leaves (and these are probably important *vis-à-vis* selection processes leading to varying economic strategies), we hypothesize that leaves are shed when their own (individual leaf) net carbon balance is greater than zero. This positive net carbon balance should be sufficiently large to offset (i.e. pay for) the respiratory costs of stem and root tissues associated with the maintenance of water flow and structural support for each leaf. Just how large an offset would be needed is uncertain, because it remains unclear how to allocate costs of plant infrastructure among multiple leaves, especially considering variation in leaf age. If the hypothesis is supported that leaves have a positive net carbon balance at death, we wish to determine how much extra respiration from stems and roots could be supported at the average age of leaf death for the leaf to have a zero carbon balance from the perspective of the whole plant.

To address these issues, we measured a variety of physiological and light environmental attributes for leaves varying in age and position for 10 woody species in eastern Australia. These measures included the decline in A_{\max} and associated light response characteristics with leaf age and the canopy architecture of light environment of branches of all 10 species. We used a

modified version of the YPLANT model (Pearcy & Yang, 1996) to estimate light interception and to model net carbon balance for leaves of each species across the entire leaf age sequence. These measures enabled us to address the above hypotheses generally and, specifically, to pursue the following objectives:

- To estimate the decline in leaf-level carbon balance with leaf age.
- To partition the total decline in leaf-level net carbon balance into component factors attributable to: the decline in the leaf light environment as a result of within-branch shading; the decline in the leaf light environment caused by within- and among-plant shading (e.g. beyond branch shading); age-related declines in leaf photosynthetic capacity across the full range of light levels.
- To assess whether leaves at the age of their average leaf life-span have projected zero net carbon balance or, rather, positive net carbon balance that can offset nonleaf respiratory costs.

Materials and Methods

Approach

To investigate age-related declines in light environment and leaf-level carbon balance and budgets, we employed a space-for-time substitution implemented along leaf sequences, with ages of individual leaves estimated by calibrating a leaf's position along the branch (i.e. the shoot) with known values (previously obtained from similar plants) for species leaf life-span. Three-dimensional digitizing of branch architecture and measurements of leaf physiological performance were then combined within the architectural model YPLANT (Pearcy & Yang, 1996) to estimate daily light interception and carbon assimilation rates across the life-span of a typical leaf in each species (Table 1). Overall, this study employed a complex set of measurements, which are described below.

Table 1 Overview of the different simulations run for each plant

	1	2	3	4	5	6	7
Simulation scenario	□	○	▲	■	●		
Effect included							
Leaf orientation	*	*	*	*	*	*	*
Within-branch shading	*		*		*	*	
Beyond-branch shading		*	*		*	*	
Physiological ageing				*	*	*	
Night-time respiration					*		

Outputs simulated for each plant include the incorporation of factorial combinations of within-branch self-shading, shading from adjacent plants (beyond-branch shading), the decreasing efficiency of leaf photosynthetic machinery with leaf age (physiological ageing) and night-time respiration. This design allows the magnitude of different influences on the leaf carbon budget to be assessed.

Study sites and species

The study site was Ku-ring-gai Chase National Park (33°41'38"S, 151°8'35"E), Sydney, NSW, Australia. This location was chosen because previous estimates of species' leaf life-span were available (Wright *et al.*, 2002). The vegetation is fire-prone temperate woodland with a high diversity of shrub species under a moderately open eucalypt canopy. The soils are derived from Hawkesbury Sandstone parent material, and soil fertility is low (94 mg kg⁻¹ total phosphorus; Wright *et al.*, 2002). Average annual rainfall is 1220 mm distributed throughout the year, and the mean daily temperatures are 22 and 13°C in summer and winter, respectively. Plant growth continues throughout the year, although some species may exhibit a flush of new growth in spring. Further site details are provided by Wright *et al.* (2002).

Ten woody plant species, chosen to span a range of leaf life-spans and with flat or simply folded leaves, a required assumption of YPLANT, were chosen for study. These included eight shrubs – *Acacia suaveolens* (Sm.) Willd., *Banksia marginata* Cav., *B. oblongifolia* Cav., *Eriostemon australasicus* Pers., *Grevillea buxifolia* (Sm.) R.Br., *Hakea dactyloides* (Gaertn.) Cav., *Lambertia formosa* Sm., *Persoonia levigata* (Cav.) Domin – and two trees – *Corymbia gummifera* (Gaertn.) K.D.Hill & L.A.S.Johnson, *Eucalyptus haemastoma* Sm. – which have a range of leaf sizes, phyllotaxy and branching patterns. Individual plants (1–4 m tall) were chosen randomly, with a total of five or six per species. We sampled the uppermost leading branch (i.e. shoot) on each plant. All branches were in semi-open conditions, with their mean light environment [percentage of total incoming photon flux density (PFD)] ranging from 28 to 50% among species, and averaging 36% for all 53 branches.

Sampling of architecture, leaf orientation and leaf light environment

On each individual plant, the first fully expanded leaf on the uppermost branch was identified. Following the leaf sequence down from this point, the position at which 50% of leaves had been lost (identifiable from leaf scars) was also located. These two locations have previously been used in studies estimating leaf life-span of the study species (Wright *et al.*, 2002), representing ages of zero and species' mean leaf life-span, respectively. The three-dimensional leaf arrangement of sequence (including any side branches) was recorded using a FASTRAK® 3D-digitizer (Polhemus, Colchester, VT, USA), in conjunction with the software package FLORADIG (CSIRO Entomology, Brisbane, Australia), as described by Falster & Westoby (2003). Hemispherical photographs were taken at several distances down the sequence, but with the branch itself removed from the field of view to estimate the shading from surrounding vegetation (see below for details).

Hemispherical canopy photographs

Hemispherical photographs used to describe canopy openness in the simulations were taken using a Nikon Coolpix 990 digital camera with a 183° fisheye attachment (Nikon Corporation, Tokyo, Japan). Photographs were taken on fully overcast days using standard methods for hemispherical photography (Rich, 1990; Pearcy & Yang, 1996). Photographs were analysed using the Gap Light Analyzer (GLA) software package (Frazer *et al.*, 1999) in conjunction with WinPhot 5 (Ter Steege, 1996), to provide diurnal sunfleck patterns for each plant on each simulation date.

Leaf photosynthetic capacity and nutrient measurements

Field photosynthesis data were collected on several dates in October and November 2001. Two datasets were collected: morning (generally between 09:00 and 11:30 h) maximum light-saturated photosynthetic rates (here called ' A_{\max} '), which represent the maximum field photosynthesis according to Reich *et al.* (1991, 1999) and many other studies, and light response curve ('LRC') data, which are measures of net photosynthetic rates across a large range of controlled light levels with other environmental variables held constant. To estimate dark respiration to A_{\max} ratios, we also included data collected in 1998 (Wright *et al.*, 2001) as available.

The decline in A_{\max} with leaf age was obtained using 509 measurements from 151 plants (three or four leaves per branch per plant) of 10 species. Measurements of net photosynthetic CO_2 uptake rates were made under saturating irradiances using portable photosynthesis systems equipped for automatic temperature, CO_2 concentration and water vapour

control (CIRAS-1, PP Systems, Hitchin, Hertfordshire, UK; and LI-6400 portable photosynthesis system, Li-Cor, Lincoln, NE, USA), and operated in an open configuration. At least two systems of each kind were used each day, with similar fractions of measurements for each species made with each kind of system to retain balance in the case of system bias. Leaves from three or four randomly selected branches (leaf age sequences) per species were finely ground and analysed for percentage nitrogen (Europa Scientific Integra isotope ratio mass spectrometer, University of California at Davis, Stable Isotope Facility, Davis, CA, USA) and percentage phosphorus (Inductively Coupled Plasma Emission Spectrometer model ARL 3560, at the University of Minnesota Research Analytical Laboratory, St. Paul, MN, USA).

Curve-fitting for leaf age and light responses In all species, A_{\max} decreased significantly (data not shown) with leaf position using analysis of covariance (with individuals and leaf position in the model). As we were unable to partition variance around the mean for each leaf position to error vs true variation, for each species we averaged all observations by position and then fitted the equations to these means. We did not make *a priori* assumptions about the shape of these relationships, other than that, from prior knowledge, rates should be similar or lower with increasing age and position. As the shape of the decline function could vary among species, we examined equations for linear or curvilinear decline (with no more than two terms), whilst meeting our assumption (noted above) about the shape of these functions. For each species, we chose to use simple linear fitting, unless the relation was visibly nonlinear and the best curvilinear function improved the overall fit by $\geq 2\%$. The curves adopted were all significant, with R^2 ranging from 0.95 to 0.99 (Table 2).

Table 2 Gas exchange parameters for each species used in conjunction with Eqn 1 to characterize species-specific light response curves and their dependence on leaf age (logarithm is natural). A slightly different set of Rd/A_{\max} values based on a larger data set measured under conditions more representative of night-time dark respiration were used to calculate night respiration. [Correction added on 24 April 2009, after first online publication: the legend for Table 2 was replaced with the legend now shown.]

Species name	A_{\max} as a function of leaf position z ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	A_{\max} at position 1 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	$\text{R}_{\text{dark}}/A_{\max}$	Γ_Q ($\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$)
<i>Acacia suaveolens</i>	$[A_{\max}]^2 = (67.543 - 0.948e^z)$	8.06	-0.229	46.5
<i>Banksia marginata</i>	$A_{\max} = 14.291 - 1.825z$	12.47	-0.067	19.3
<i>B. oblongifolia</i>	$A_{\max} = 17.004 - 4.790 \log(z)$	17.00	-0.115	46.4
<i>Corymbia gummifera</i>	$A_{\max} = 13.424 - 1.148z$	12.28	-0.136	40.0
<i>Eriostemon australasicus</i>	$\text{sqrt}[A_{\max}] = 2.506 - 0.021e^z$	6.00	-0.115	17.0
<i>Eucalyptus haemastoma</i>	$A_{\max} = 17.649 - 2.631z + 0.239z^2$	15.26	-0.108	35.9
<i>Grevillea buxifolia</i>	$A_{\max} = 9.440 - 1.857z$	7.58	-0.151	23.0
<i>Hakea dactyloides</i>	$A_{\max} = 8.869 + 0.287z - 0.310z^2$	8.85	-0.198	42.1
<i>Lambertia formosa</i>	$A_{\max} = 14.917 - 2.023z$	12.89	-0.067	18.8
<i>Persoonia levis</i>	$A_{\max} = 9.789 - 1.313z$	8.48	-0.116	21.4

Leaf age is measured as the position along a leaf sequence, where position 0.5 corresponds to age 0 and position 3.5 to a leaf at the expected leaf life-span. A_{\max} is the light-saturated rate of net photosynthesis at ambient CO_2 , R_{dark} is the leaf dark respiration rate (measured at 25°C) and Γ_Q is the quantum flux at the photosynthetic compensation point. [Correction added on 24th April 2009, after first online publication: the ratio values provided in the column entitled ' $\text{R}_{\text{dark}}/A_{\max}$ ' were corrected to display as the negative ratio values now shown. Also, in the column entitled ' A_{\max} at position 1 ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)', the values were corrected to those now shown.]

Photosynthetic parameters were modelled using an empirical rectangular hyperbola model (Hanson *et al.*, 1987):

$$A = A_{\max} \left[1 - \left(1 - \frac{R_{\text{dark}}}{A_{\max}} \right)^{1 - \frac{Q}{\Gamma_Q}} \right] \quad \text{Eqn 1}$$

[A , net photosynthesis ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at a given level of Q ; Q , quantum flux density of photosynthetically active radiation (PAR) incident on the leaf ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$); A_{\max} , light-saturated rate of net photosynthesis at ambient CO_2 ; R_{dark} , leaf dark respiration rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; negative sign for CO_2 efflux from leaf); Γ_Q , quantum flux at the photosynthetic light compensation point ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)]. From LRCs (described below) and additional sampling of A_{\max} and respiration for each species (see above), the parameters A_{\max} , R_{dark} and Γ_Q , including their dependence on leaf age, were estimated for all species. This model was chosen because it enabled a tractable sampling regime to be performed that allowed us to evaluate LRC traits among a considerable number of species which vary in leaf life-span, leaf orientation and overall light capture. A number of alternative models would have required more parameters, and hence would have been poorly constrained from a model-fitting standpoint given the feasible sampling effort.

For 104 individual leaves (three to four per species for three or four leaf position classes), light response measurements were made across a range of light conditions, in which both A_{\max} and R_{dark} were determined directly: A_{\max} was taken as the rate of CO_2 assimilation measured at the highest quantum flux density, and R_{dark} was the rate of CO_2 evolution in the dark. The apparent quantum efficiency ϕ was also solved using least-squares regression techniques for data below 200 $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ (Singsaas *et al.*, 2001). From this and the estimate of respiration, the light compensation point (Γ_Q) was derived as $\Gamma_Q = R_{\text{dark}}/\phi$. The mean Γ_Q for each species was used in conjunction with the modelled A_{\max} for different leaf age classes (Table 2) and the species-specific R_{dark}/A_{\max} to generate all the parameters for modelling the light response of photosynthesis using the Hanson *et al.* (1987) model.

Architectural model and carbon balance simulation approach

A primary goal of this study was to partition the effects of different factors influencing light capture and potential carbon balance across the life-span of a leaf. These include changes in leaf orientation, shading within the branch, shading from surrounding vegetation and physiological capacity of the leaf with age. YPLANT was used to estimate light interception and potential carbon balance for different sample periods throughout a single day, and integrated across entire days using output from the architectural measurements. YPLANT output includes an estimate of the PFD ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) on the

shaded and unshaded portions of each leaf at each time step throughout the day. Shading can arise from other leaves within the same branch, or from adjacent branches or plants. The latter was incorporated by including a hemispherical photograph recording the proportion of sky blocked by surrounding foliage in the daily simulations. Typically, a single canopy photograph, taken at the top of the plant, is used in YPLANT simulations (Pearcy & Yang, 1996). However, as the shading characteristics of the surrounding vegetation can also vary with leaf age (as the vegetation grows up around the leaf), we simulated this effect by taking four photographs at a series of points down each leaf sequence. For each plant, identical simulations were then run under five canopy conditions: one assuming an open canopy and one each for the four canopy profiles. Using combinations of output from YPLANT simulations (Table 1), we were able to partition out four effects on daily light interception and carbon assimilation for each leaf: leaf orientation; canopy position; within-branch self-shading; and shading from adjacent branches and neighbouring vegetation (i.e. beyond-branch shading).

For each plant, we conducted several simulations under a range of scenarios incorporating different combinations of these factors (Table 1), allowing us to estimate their effects both independently and in combination. For example, scenario 1 includes the effects of leaf orientation only on light capture and carbon balance. This could be influential, hypothetically; for example, if leaves become more flatly or more vertically inclined further along the sequence (i.e. in older leaves), one could expect a change in light capture and carbon balance with leaf age, relative to leaves that retained the same orientation over time. In addition to leaf orientation, scenario 2 includes the effects of within-branch shading. Moving down a leaf sequence from top to bottom, leaf area accumulates above a given position, leading to lower levels of light capture for older leaves. Scenario 4 adds the effects of changes in beyond-branch shading. Typically, branches rise as they extend from the main trunk, with older leaves located deeper in the vegetation. By incorporating hemispherical photographs taken at different distances along the branch, we can capture the effect of changing shading from surrounding vegetation in simulations. Figure 1 shows example data on light capture for one individual from each of three different species corresponding to these three scenarios. Each point shows the mean daily light capture (see below) for leaves spread along the sequence (i.e. of different ages) under one scenario, and the lines represent the least-squares linear relationships fitted to the data. As the three factors in Fig. 1 are incorporated in an aggregate integrated fashion, the fitted lines become increasingly steep as more age-related effects are incorporated.

Carbon balance simulations for each plant were run on 4 days equally spaced between the longest (21 December) and shortest (21 June) days of the year. This approach would be most appropriate if annual photosynthetic climate averages out well

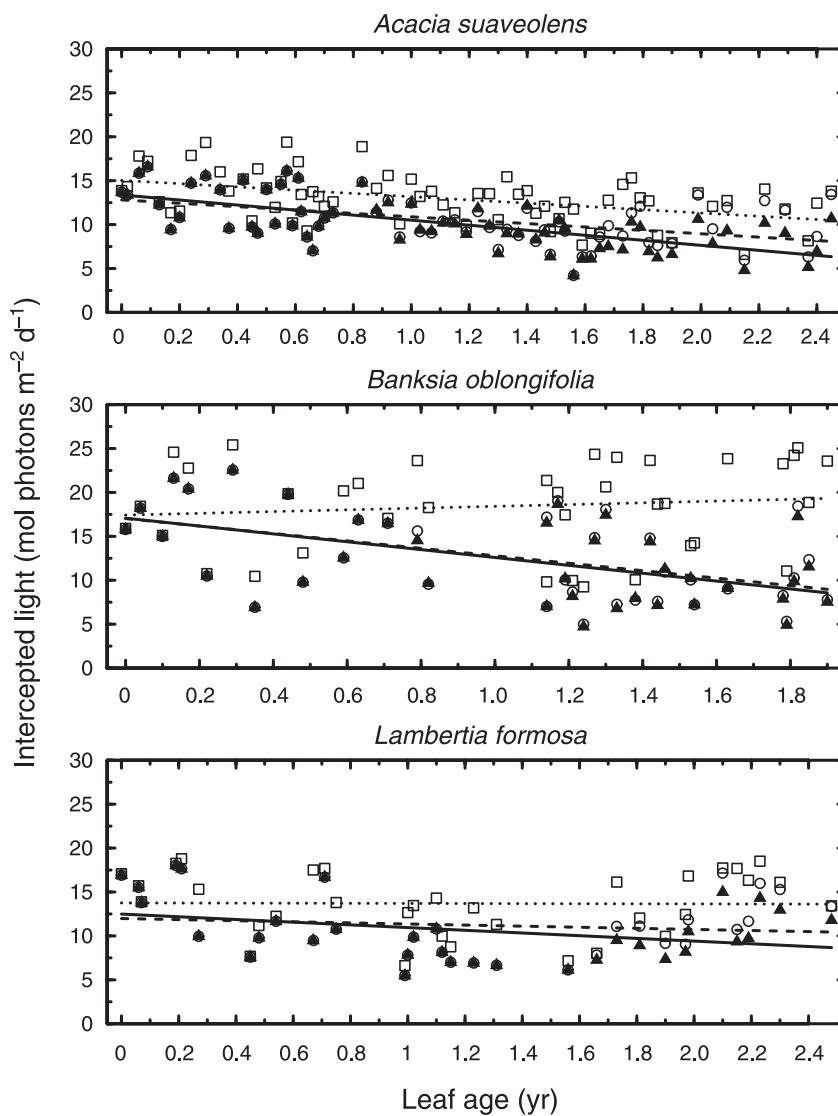


Fig. 1 Average daily light interception ($\text{mol m}^{-2} \text{d}^{-1}$) vs leaf age in one individual from each of three different species. Points represent different leaves spread along the leaf sequences of each individual. For each leaf, data for three different scenarios (see Table 1) incorporating effects of leaf orientation (scenario 1, squares), leaf orientation plus within-branch shading (scenario 2, circles) and both of these plus beyond-branch shading (scenario 4, triangles) are shown, together with fitted lines (dotted, broken and full lines, respectively).

across these four dates, and if leaves are produced randomly throughout the year, as seasonal leaf production could lead to seasonal cohorts that spend different lengths of time at different leaf positions along a branch. However, as neither climate nor leaf production is strongly seasonal in this system, and we have no specific information relevant to this study, this simplified approach seems parsimonious. Moreover, it is unlikely that our conclusions would be markedly different if such information were known and incorporated into the simulations. The four simulation dates (28 November, 13 October, 28 August, 13 July) span the full range of solar trajectories experienced at the site, corresponding also to complementary dates spaced in the alternate half of the year. Simulation output was averaged across the four dates to provide annual daily averages in light capture and photosynthetic yield for each leaf ($\text{mol m}^{-2} \text{d}^{-1}$). Light interception and gas exchange rates for all of the leaves on each branch were simulated under several different scenarios (see Table 1). For each scenario, annual

daily averages for light capture ($\text{mol photons m}^{-2} \text{d}^{-1}$) and carbon balance ($\text{mol CO}_2 \text{ m}^{-2} \text{d}^{-1}$) were calculated as the mean of simulations run on the four different dates selected to span the range of solar trajectories experienced throughout the year. The simulations are subject to several limitations. They considered only the steady-state effects of light availability and changes in photosynthetic capacity on photosynthetic performance, and thereby overlooked some potentially important features, such as climate variability, differences between diffuse and direct light-driven photosynthesis (Brodersen *et al.*, 2008), leaf absorbance, stress-induced stomatal closure and photosynthetic dynamics associated with the required induction of photosynthetic proteins and stomatal aperture (Naumburg *et al.*, 2001). There is, as yet, no comprehensive physiological model that handles all of these factors well.

Scenario 7 (Table 1) included night-time respiration costs that were estimated as follows. For each species and leaf, we

estimated an instantaneous dark respiration rate (R_{dark}) at a standard temperature (T_1 , 25°C). In our dataset, $R_{\text{dark}}/A_{\text{max}}$ did not vary with leaf age in a significant manner, and so we treated this ratio as a constant in modelling. As A_{max} decreases with age, this gives decreasing R_{dark} with age (called R_1 in our calculations). We took this approach because the age-specific R_{dark} rates include considerable uncertainty as a result of difficulties in measuring respiration precisely and the limited sample size. If the observed invariance in $R_{\text{dark}}/A_{\text{max}}$ is real, our approach smoothes out some of the error associated with estimates of R_{dark} for individual leaf ages (positions). The respiration rate (R_1) was then converted (following Tjoelker *et al.*, 2001) to a rate R_2 observed at mean night-time temperature (T_2):

$$R_2(\mu\text{mol m}^{-2} \text{s}^{-1}) = R_1 \left[3.09 - 0.0435 \left(\frac{T_2 + T_1}{2} \right) \right]^{\frac{T_2 - T_1}{10}}$$

Eqn 2

Night-time respiration was calculated on each of eight dates [the four simulation dates plus corresponding dates in the opposite half-year (to better characterize both intra-annual variation and mean temperatures)], and multiplied by night length to give daily night-time respiration:

$$R_N(\text{mol m}^{-2} \text{d}^{-1}) = \frac{R_2(\mu\text{mol m}^{-2} \text{s}^{-1}) * (24.0 - \text{day length(h)}) * 3600(\text{s h}^{-1})}{10^6(\mu\text{mol mol}^{-1})}$$

Eqn 3

This value was then averaged across the eight dates. Night-time temperatures were estimated using climate data from New *et al.* (2002). Monthly maxima and minima were calculated from daily mean and diurnal temperature range (dtr) ($t_{\text{min}} = t_{\text{mean}} - 0.5 \text{ dtr}$). Mean night-time temperature was estimated as $t_{\text{night}} = t_{\text{mean}} - 0.25 \text{ dtr}$.

Analysis

Data were analysed by fitting linear lines to light and carbon balance data for all scenarios (Table 1) in each plant. The fitted lines were used to estimate the average light and carbon balance of a typical leaf at the beginning and end of its life-span in each scenario. Because leaf orientation introduces considerable scatter about the mean trend, this is preferable to using data from the youngest/oldest leaf in each sequence, which may have an unusually favourable or unfavourable orientation. Comparing the predicted light/carbon capture at the beginning and end of life-span in scenario 1, we calculated the proportional change across the life-span arising from leaf orientation. Comparing predictions at the end of life-span

for scenarios 2, 3 and 5 with that for the end of life-span in scenario 1, we calculated the proportional change arising from within-branch shading, beyond-branch shading and leaf physiology, independent of effects of leaf orientation. Comparing the predicted light/carbon capture at the end of life-span for scenarios 4 and 6 with that from age zero in scenario 1, we calculated the cumulative total decline from shading and shading plus changes in physiology (declining A_{max} and changing light response functions).

Results and Discussion

Leaf age, shading and light interception

Changes in mean daily-integrated light interception with leaf age were a composite effect of changes in within-branch shading, beyond-branch shading and leaf orientation (e.g. Fig. 1). On average, light availability declined with increasing leaf age in all species (Table 3, last column), although with substantial variation within and among species. Light capture declined with age by 34% averaged across all 10 species (similar to results in Wright *et al.*, 2006), ranging from a high of 68% in *Grevillea buxifolia* to a low of 17% in *Corymbia gummifera*. Species' differences in light decline with leaf age were unrelated to any measured leaf trait (e.g. leaf life-span, percentage nitrogen or percentage phosphorus, or A_{max} of young mature leaves). The decline in light environment with leaf age was primarily a result of increased within-branch shading, which was observed in all 53 plants and averaged 31% across the 10 species (Table 3). Leaf orientation change with age resulted in both greater and lesser light interception among individuals of most species, with impacts averaged per species varying by as much as $\pm 20\%$. However, across all 10 species, the average effect of changes in leaf orientation on light interception as the leaf aged was near-zero (+1.6%) for this set of species. Similarly, changes in light environment caused by beyond-branch shading were also both positive and negative among individuals of most species and, on average, amounted to a decline of only -3% with age across these 10 species.

Declines in A_{max} with leaf age

All 10 species showed significant declines in A_{max} with increasing leaf age (Fig. 2), although the response surfaces ranged from linear to nonlinear and the magnitude of the declines varied among species. Decreases in A_{max} with leaf age were accompanied by declines in leaf percentage nitrogen and percentage phosphorus (data not shown), as seen in many previous studies (e.g. Field & Mooney, 1983; Reich *et al.*, 1991). At the leaf age equivalent to the mean leaf life-span, A_{max} ranged from as little as 39% of that in young leaves (*Grevillea buxifolia*) to as much as 85% in *Banksia oblongifolia* (Fig. 2). Like the age-related declines in light availability, the

Table 3 Proportional changes in daily light capture with increasing leaf age as a result of leaf orientation, within-branch shading, beyond-branch shading and all sources of shading and leaf orientation combined

Species	n	LL (yr)	Incident light, age 0 (mol m ⁻² d ⁻¹)	Proportional change in daily light capture with increasing leaf age			
				Leaf orientation gradient	Within-branch shading	Beyond-branch shading	All shading + leaf orientation
<i>Acacia suaveolens</i>	6	2.45	12.64	0.03 (0.37, -0.30)	-0.25 (-0.07, -0.37)	-0.05 (0.22, -0.18)	-0.26 (0.20, -0.58)
<i>Banksia marginata</i>	5	3.03	10.93	-0.04 (0.14, -0.25)	-0.35 (-0.23, -0.48)	0.07 (0.73, -0.48)	-0.32 (0.13, -0.70)
<i>B. oblongifolia</i>	5	1.9	14.81	0.23 (0.47, -0.05)	-0.48 (-0.31, -0.65)	-0.16 (-0.01, -0.32)	-0.51 (-0.16, -0.71)
<i>Corymbia gummifera</i>	5	1.22	13.27	0.13 (0.83, -0.12)	-0.22 (-0.02, -0.38)	-0.03 (0.21, -0.16)	-0.17 (0.20, -0.37)
<i>Eriostemon australasicus</i>	5	1.01	13.15	0.09 (0.49, -0.33)	-0.19 (-0.04, -0.26)	-0.07 (0.11, -0.22)	-0.18 (0.40, -0.40)
<i>Eucalyptus haemastoma</i>	5	1.55	14.76	-0.14 (0.31, -0.37)	-0.16 (-0.04, -0.32)	-0.02 (0.22, -0.14)	-0.28 (0.06, -0.55)
<i>Grevillea buxifolia</i>	5	1.25	17.61	-0.17 (-0.04, -0.29)	-0.57 (-0.47, -0.56)	-0.12 (0.08, -0.35)	-0.68 (-0.53, -0.78)
<i>Hakea dactyloides</i>	5	3.45	12.71	-0.14 (0.01, -0.43)	-0.30 (-0.13, -0.43)	0.11 (0.66, -0.15)	-0.36 (-0.16, -0.60)
<i>Lambertia formosa</i>	7	2.48	10.00	0.15 (0.45, -0.03)	-0.30 (-0.23, -0.40)	-0.08 (0.00, -0.17)	-0.26 (-0.02, -0.44)
<i>Persoonia levis</i>	5	3.79	9.90	0.02 (0.29, -0.11)	-0.25 (-0.05, -0.34)	-0.11 (0.09, -0.35)	-0.33 (-0.14, -0.41)

Values [mean (min, max)] are the proportional change in light capture as a result of different factors, calculated as $(X_{\text{old}} - X_{\text{young}})/X_{\text{young}}$. LL, leaf life-span from Wright & Westoby (2002).

differences in the magnitude of the declines in A_{max} among species were unrelated to any leaf trait measured in our study. Declines in A_{max} from the youngest to oldest age class averaged roughly 70% across the 10 species. Thus, leaves at the typical age of leaf death retained a sizeable proportion of their maximum photosynthetic capacity, suggesting the potential for continued positive net carbon balance.

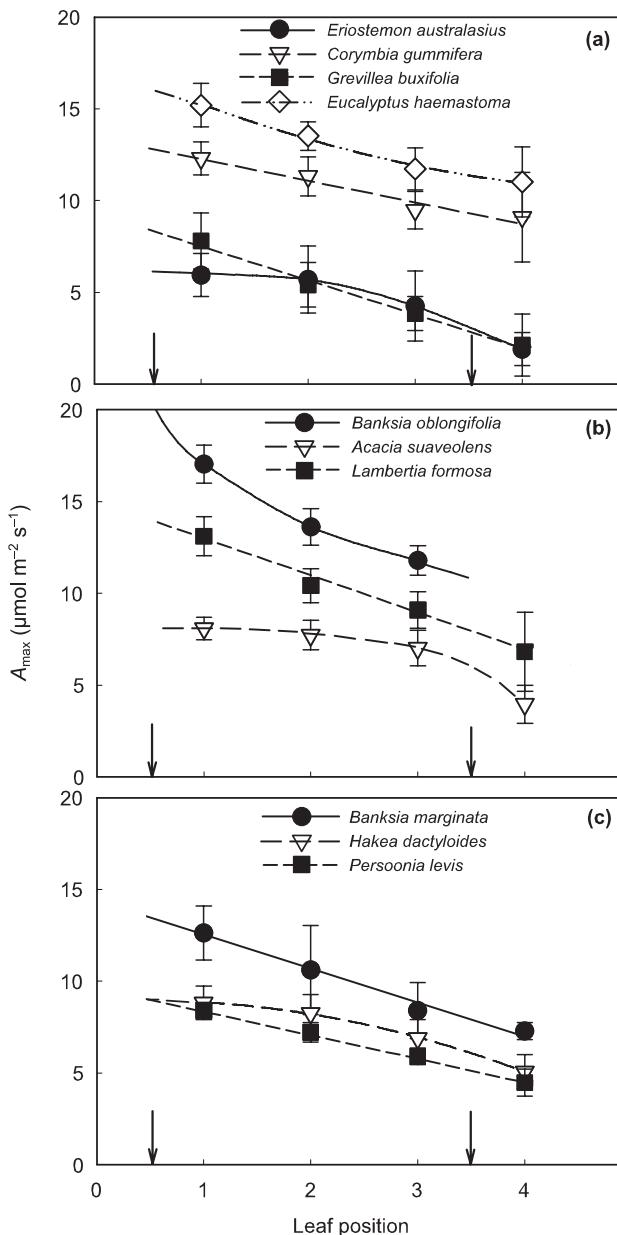


Fig. 2 Light-saturated maximum net photosynthetic capacity (A_{max} , mean \pm standard error) of leaves at four positions (i.e. corresponding to four leaf ages) for each of 10 Australian woodland species. Leaf positions represent locations evenly scaled with reference to the average mean leaf life-span; thus positions represent different chronological ages, but in all cases the arrows at positions 0.5 and 3.5 represent leaves at age 0 and the approximate age of the mean life-span, respectively.

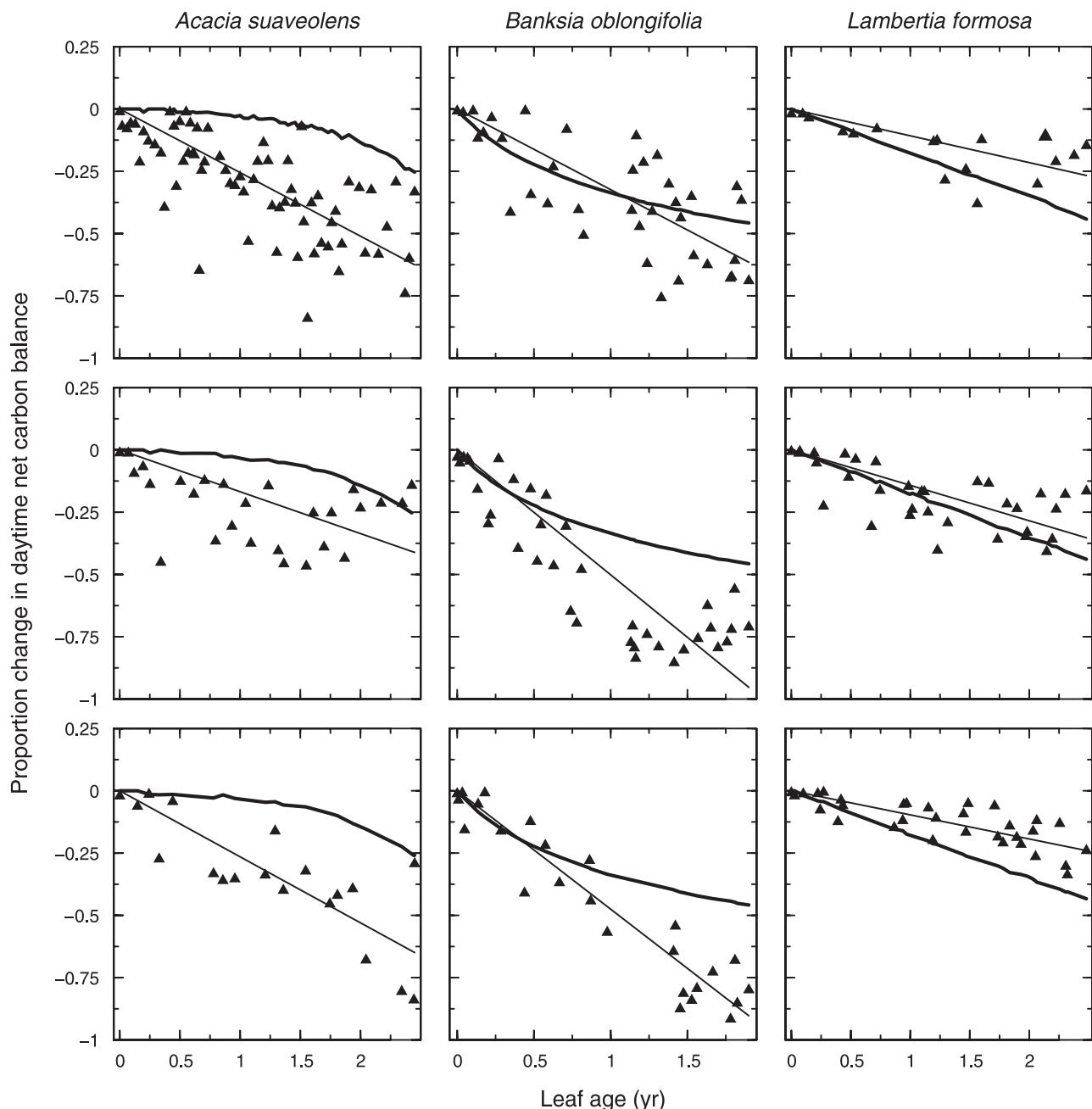


Fig. 3 Proportional change in net daily carbon balance vs leaf age in three different species. Data are for scenario 4 (triangles, thin full line), incorporating combined effects of leaf orientation, within-branch and beyond-branch shading, and scenario 5 (thick full line), incorporating the effects of leaf orientation with leaf physiology only. Because scenario 5 is driven almost entirely by the effect of leaf physiology, there is no scatter in the proportional change data. Consequently, symbols are not shown. The curvature in the lines for scenario 5 reflects the curvature of functions describing age-related declines in A_{\max} (Table 2).

Effects of leaf age-related shading and declining photosynthetic capacity on net carbon balance

Estimated mean daily net carbon balance declined with leaf age in all species because of increased shading, declining photosynthetic capacity (A_{\max}) and associated light response functions, and their combined effects (Figs 3, 4, Table 4).

On average across the 10 species, daily net carbon balance declined across the leaf life-span by 64% as a result of the combined effects of all factors, with declining photosynthetic function and increased shading being almost entirely responsible for this, given the modest impact of leaf orientation (Table 4, Fig. 4). In each species, the overall decline in net carbon balance was greater than that which would have occurred

Table 4 Proportional changes in net daily carbon balance with increasing leaf age as a result of leaf orientation, beyond-branch shading, leaf physiology, all sources of shading plus leaf orientation and all factors

Species	n	LL (yr)	Carbon balance age 0 (mol m ⁻² d ⁻¹)	Proportional change in daily carbon balance with increasing leaf age				
				Leaf orientation gradient	Within-branch shading	Beyond-branch shading	Leaf physiology	All shading + leaf orientation
<i>Acacia suaveolens</i>	6	2.45	0.07	-0.01 (0.15, -0.20)	-0.34 (-0.13, -0.49)	-0.06 (0.52, -0.27)	-0.18 (-0.16, -0.19)	-0.40 (0.03, -0.68)
<i>Banksia marginata</i>	5	3.03	0.11	-0.05 (0.01, -0.18)	-0.34 (-0.24, -0.46)	0.09 (0.86, -0.59)	-0.42 (-0.41, -0.42)	-0.31 (0.25, -0.76)
<i>B. oblongifolia</i>	5	1.9	0.19	0.09 (0.24, -0.03)	-0.52 (-0.34, -0.69)	-0.25 (-0.01, -0.46)	-0.50 (-0.49, -0.51)	-0.61 (-0.24, -0.92)
<i>Corymbia gummifera</i>	5	1.22	0.12	0.04 (0.29, -0.12)	-0.24 (-0.04, -0.43)	-0.04 (0.30, -0.25)	-0.27 (-0.26, -0.28)	-0.89 (-0.71, -1.02)
<i>Eriostemon australasius</i>	5	1.01	0.08	0.04 (0.14, -0.04)	-0.16 (-0.07, -0.27)	-0.08 (0.11, -0.27)	-0.34 (-0.32, -0.36)	-0.47 (-0.21, -0.64)
<i>Eucalyptus haemastoma</i>	5	1.55	0.19	-0.08 (0.15, -0.24)	-0.19 (-0.06, -0.38)	-0.04 (0.20, -0.17)	-0.33 (-0.31, -0.36)	-0.28 (-0.04, -0.47)
<i>Grevillea buxifolia</i>	5	1.25	0.11	-0.24 (-0.12, -0.32)	-0.52 (-0.36, -0.67)	-0.10 (0.12, -0.24)	-0.73 (-0.68, -0.79)	-0.67 (-0.55, -0.83)
<i>Hakea dactyloides</i>	5	3.45	0.08	-0.13 (0.03, -0.34)	-0.47 (-0.19, -0.81)	0.24 (1.31, -0.21)	-0.28 (-0.26, -0.31)	-0.43 (-0.07, -0.73)
<i>Lambertia formosa</i>	7	2.48	0.11	0.03 (0.14, -0.03)	-0.25 (-0.18, -0.38)	-0.09 (0.00, -0.18)	-0.44 (-0.43, -0.45)	-0.30 (-0.16, -0.42)
<i>Personia levis</i>	5	3.79	0.08	0.00 (0.07, -0.06)	-0.23 (-0.08, -0.35)	-0.14 (0.11, -0.41)	-0.43 (-0.43, -0.44)	-0.35 (-0.18, -0.48)

Values [mean (min, max)] are the proportional change in carbon balance as a result of different factors, calculated as $(X_{\text{old}} - X_{\text{young}})/X_{\text{young}}$. LL, leaf life-span from Wright & Westoby (2002).

as a result of either increased shading or deterioration in physiological performance taken separately, although it was always less than the sum of the two effects. On average, across the 10 species, mean daily carbon income would decline by 39% over the leaf life-time because of decreased light interception, even if photosynthetic performance hypothetically remained at its peak throughout the leaf lifetime. Similarly, on average across the 10 species, mean carbon income would decline by 39% over the leaf life-time because of declining photosynthetic performance, even if light interception remained constant.

Species varied in the magnitude of the decline in net carbon balance with age, and in the causes of the decline (Table 4). When both local architecture and physiological deterioration with leaf position/ageing were considered together, declines were as great as 99% (*Grevillea buxifolia*) and as low as 47% (*Eriostemon australasius*). Among the 10 species, declines in carbon income as a result of physiological decline and of the changes in light environment were correlated ($P < 0.05$, $r = 0.62$), with the slope not far from the 1 : 1 line (data not shown). These results are consistent with the hypothesis (Field, 1983) that the reallocation of nitrogen in ageing leaves (to enhance, if not optimize, whole-plant photosynthetic nitrogen use) should generally occur to match shifts in light environment.

An important question is whether the observed differences among species in the decline with age in net carbon balance are associated with aspects of their ecophysiology? The variation in the magnitude of decline in net carbon balance among species was, however, unrelated to any measured leaf trait (e.g. A_{max} , leaf life-span, percentage nitrogen, percentage phosphorus, leaf mass per area). Nor was there any discernible pattern regarding the known ecology of the species that could help us explain the species' differences. Thus, it is impossible to deduce whether differences among species are real (but unexplained) or largely reflect random experimental error.

Net carbon balance at the leaf age equal to the average leaf life-span

For all species, leaves at ages equal to their average leaf life-span retained positive predicted daytime net carbon balance, although just slightly for *Grevillea* (Table 4, Figs 4, 5). The carbon balance of an ageing leaf was also evaluated including leaf respiratory costs from a 24-h perspective. At a leaf age equal to the average leaf life-span, leaves of seven of the species had positive predicted 24-h leaf net carbon balance, and the other three species had near-zero balances (24-h_{leaf}, Fig. 5). Ackerly (1999) also made carbon balance calculations that included leaf respiration costs on a whole-day (24-h) basis. In doing so, he examined two different optimality models involving self-shading, net carbon balance and leaf dynamics. One model, based largely on light declines (caused by leaf position), provided plausible predictions that also matched

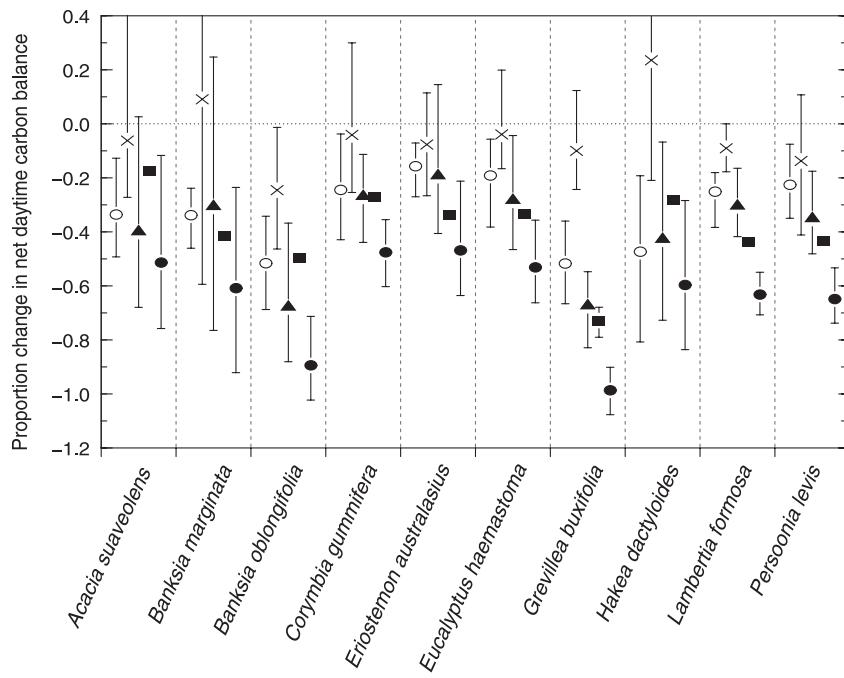


Fig. 4 Proportional change in net daytime carbon balance across the entire leaf life-span for each species. Points represent the mean effect size for different factors: within-branch shading (open circles), beyond-branch shading (crosses), leaf orientation, within-branch shading and beyond-branch shading combined (triangles), leaf physiology (squares) and total decline (filled circles). Bars show the range observed across individuals for each factor.

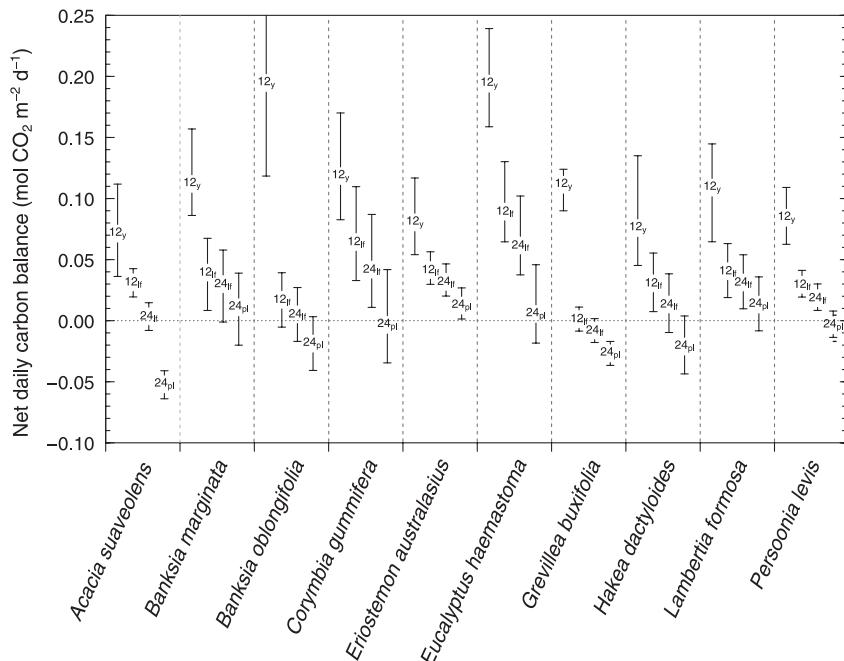


Fig. 5 Estimated net daily carbon balance for leaves of each species at the end of the life-span (other than for young leaves on a daytime basis). Symbols show the daytime net carbon balance of the leaf alone when young (12_y) and when at the mean age of the leaf life-span (12_{lf} , with lf short for 'leaf'), the 24-h net carbon balance of the leaf alone (24_{lf}) and the 24-h net carbon balance of the leaf examined from the whole-plant perspective (24_{pl}). The 24-h net carbon balance of the leaf alone includes night-time leaf respiration. The 24-h net carbon balance of the leaf from the whole-plant perspective includes both night-time leaf respiration and 24-h respiration of roots and stems estimated from a simple scenario (see text for details). Bars show the range observed across individuals.

field measurements of saplings of three species. This model and the field data suggested that 24-h daily net carbon assimilation per unit leaf should be near-zero at the mean age of leaf death (as with three of our 10 species). By contrast, however, the other model, based largely on age declines in net photosynthesis, suggested leaves should be shed when they have a positive carbon balance (as with seven of our 10 species), but this model predicted infinite values for some properties, and did not match well with empirical field data

(Ackerly, 1999). Several theoretical studies on optimal leaf life-span also considered construction costs, leaf production rates and carbon export vs leaf production functions (Kikuzawa, 1991; Ackerly, 1999; Kikuzawa & Ackerly 1999). Such issues are potentially influential, but are beyond the scope of this paper.

On average, across all leaves for our 10 study species, the predicted positive 24-h leaf net carbon balance was approximately $0.025 \text{ mol m}^{-2} \text{ d}^{-1}$. This surplus is roughly the

equivalent of the net carbon gained by photosynthesis for 1 h at a rate of approximately $7 \mu\text{mol m}^{-2} \text{s}^{-1}$. Such a surplus might imply that leaves are typically shed for reasons unrelated to their net carbon-generating benefits to the plant. However, a plant incurs costs elsewhere in the plant to sustain a given leaf, including respiratory costs in root and stem tissues that supply the leaf with resources, transport carbon away from leaves and physically support the leaf (Givnish, 1987).

Given the positive leaf-level carbon balances, how much root and stem respiration can a leaf at the age equal to the average leaf life-span hypothetically support? We addressed this question using output from the simulations by calculating the amount of beyond-the-leaf respiration that would be required to reduce the net carbon balance to zero. On average, leaves at the mean age of leaf death had sufficient net carbon balance surplus on a 24-h basis to support total respiratory costs roughly three times the night-time respiration of the leaf itself (data not shown).

To interpret the implication of a leaf as old as the mean leaf life-span supporting a respiratory flux three times larger than the total nightly leaf respiration cost, it may be useful to provide some perspective on the relative respiratory costs per unit leaf at both leaf and whole-plant scales. To do so, we used some of the few whole-plant data available for both leaf and whole-plant respiration rates. Using data from Reich *et al.* (2006), we estimated the ratio of whole-plant respiratory cost per gram of leaf to the night-time leaf respiratory cost per gram of leaf, based on a re-analysis of 248 plants from 12 species. For these 248 plants, nonleaf respiratory flux rates (i.e. instantaneous respiration rates of roots and stems combined) per plant averaged just slightly more than the total instantaneous leaf dark respiratory flux rate per plant (data not shown). However, when extrapolated over a 24-h period (and assuming a 12-h dark period relevant to the Australian simulations), the total respiratory flux per plant averaged slightly more than three times (3.2) the night-time aggregate respiration flux of all foliage, as root and stem respiration fluxes occur for 24 h per day and leaf respiration during the day is already incorporated into estimates of net photosynthesis. This summary of data for 248 plants thus provides an estimate of the total 24-h respiratory costs per leaf (approximately 3.2 times the nightly leaf respiration) that roughly matches the amount of respiration that the average leaf at the mean age of its life-span can pay for from its 24-h carbon surplus (approximately three times the nightly leaf respiration), as suggested by our simulations.

As both the respiration estimates (based on data in Reich *et al.*, 2006) and our YPLANT simulations include a variety of uncertain simplifications and assumptions, their values and the comparison between them should be taken merely as a rough correspondence rather than a reliable quantitative estimate. Nonetheless, the comparison suggests that, at a first approximation, the average leaf at the mean age of leaf death produces just enough surplus carbon to pay for its share (i.e.

its proportion) of total plant respiration. This suggests that the notion of old leaves being shed when they reach a zero carbon balance can be supported, but only when respiratory carbon costs per leaf are accounted for at the whole-plant scale. To visualize what supporting root and stem respiratory costs would do to the estimated 24-h carbon balances, we also display the 24-h net carbon balance of the leaf examined from the whole-plant perspective ($24\text{-h}_{\text{plant}}$) for each of the 10 species (Fig. 5), under a scenario in which stem and root respiration costs were double those of each leaf.

The results of our analysis therefore suggest that, on average, for these 10 species, ageing leaves are shed when they can no longer pay for both their own ongoing costs and for the respiratory load in other parts of the plant that is required to support their activities. If leaves were maintained until each leaf, unto itself, had a zero net carbon balance, the plant would run a carbon deficit to maintain that leaf. This interpretation assumes that whole-plant respiratory costs are adjusted to reflect the size of the canopy. Although it is probably true that, at the moment a single leaf is senesced, such support costs do not instantaneously change, in our view it is equally likely that a plant continuously adjusts the balance of plant organs that play different roles in resource acquisition, physical structure, metabolic processing, and the like (Brouwer, 1962a,b). Thus, from an integrated perspective, the assumption that total respiratory costs can be considered as shared among all leaves is probably appropriate. Plainly, measurements for a more complete whole-plant carbon balance would be preferable, but such data are absent from most (perhaps almost all) studies. The approximate analysis undertaken here represents a step in refining carbon balance for native species to incorporate whole-plant infrastructure, as first suggested by Givnish (1987). An obvious caveat is that our study includes simplifications made in estimating LRCs, standardizing for temperature, assuming well-watered conditions and ignoring light quality, among others. Therefore, we recognize considerable uncertainty in our results, but point out that they are based on a dataset with at least as much, if not more, empirical foundation than most other attempts to address questions of this kind. Hence, we present our results as a first approximation and to provide a stepping-stone for future work that is yet more rigorous in its empirical basis.

One additional caveat to these analyses and interpretations is that the subpopulation of leaves that are alive at the mean age of leaf death may not be representative of the original cohort for each species (Reich *et al.*, 2004). A subset of the initial population may tend to suffer high mortality because of innate or acquired weakness, and the survivors might differ in their photosynthetic attributes. Leaves alive to be sampled for photosynthetic performance beyond the mean age of leaf death must, by definition, live longer than the mean leaf age, and perhaps might sustain a positive net carbon balance to an older age than leaves that died at an earlier age. There is no technical way to overcome this source of bias, which is present

in studies of ageing populations of all living (and even nonliving) entities (Vaupel *et al.*, 1998). One can only bear it in mind for interpretation. In our study, this phenomenon would not influence the interpretation of the implications of net carbon balance at the age of the mean leaf life-span (i.e. whether a plant should keep an old leaf or not), but it could influence the interpretation of the proportional declines in net carbon balance as a function of ageing (as ostensibly the subset of leaves that are alive have higher rates than those that are dead – which should be zero – but which are not counted).

In several respects, the issue of how long to hold onto individual ageing leaves parallels the question of how large a canopy to make and how fast to turn over its various strata. The consideration of the optimal leaf area index (e.g. Anten *et al.*, 1995; Hikosaka, 2003) is in part the problem of when to produce and drop leaves, in terms of levels of foliage (i.e. leaf area index) rather than leaf number on a stem. Boonman *et al.* (2006) reported that shade-induced leaf senescence of wild tobacco leaves low in a canopy leads to greater whole-plant carbon gain under competitive conditions, because of the advantages of reallocating nitrogen from senesced foliage to new leaf area at the canopy top. These results parallel the conclusions of Ackerly (1999) based on a model employing leaf population dynamics rather than whole-canopy optimization. However, Hikosaka (2003) concluded from his simulations and results of Anten *et al.* (1995) that old leaves might be dropped at quite different irradiance levels depending on the magnitude of the nitrogen supply, implying that there might not be a predictable association of light level and leaf senescence. The approach based on the canopy as a system (Anten *et al.*, 1995; Hikosaka, 2003; Boonman *et al.*, 2006), however, differs from the approach taken herein, in that optimization of canopy carbon gain is used to constrain multiple leaf traits as well as whether, and when, to drop old leaves. In the current study, we do not consider the entire canopy – instead we evaluate individual leaves, as their light supply is influenced by the rest of the canopy and as their photosynthetic capacity is diminished with age. It is interesting, therefore, to note that the results of these different studies are relatively compatible. In Australian woodland, species are characterized by low nutrient supply and low nutrient concentrations, and the most shaded leaves that are retained are appreciably less shaded than the most shaded leaves in more fertile forests with higher leaf nutrient concentrations and leaf area indices. It is impossible to conclude, however, whether the shedding of old leaves with positive 24-h leaf-level carbon balances (as generally noted in this study) is the result of needing to offset other respiratory costs (in roots and stems) or of the benefit of optimizing canopy carbon gain in a realistic competitive context (Ackerly, 1999; Boonman *et al.*, 2006). Of course, both could occur simultaneously. Additional studies that combine a consideration of canopy optimization (as in Hikosaka, 2003) with a more rigorous treatment of leaf-level environment and physiology than that performed in this study,

and with the direct assessment of total plant carbon balance considerations, could go a long way towards unravelling some of these issues.

Conclusions

The results support our hypothesis that leaf-level carbon balance should still be above zero at the leaf age of the typical leaf life-span, because leaves must support not only their own carbon costs, but also those of other plant parts that are required to sustain the canopy (including the costs of constructing and maintaining root systems, vascular systems and support structure). The results also suggest alignment across species between a decline in physiological carbon balance potential and a decline in light environment with increasing leaf age. Finally, the proportional age-related declines in light environment, leaf physiology and total carbon balance seem to be a common feature across the 10 species, but without any discernible pattern to the differences observed between species.

Acknowledgements

Warm thanks go to members of the Comparative Ecology Group who assisted with data collection. Nico Houter and Hans ter Steege kindly provided support for the use of WinPhot. This work was supported by US National Science Foundation International Program funding to Reich and Australian Research Council funding to Westoby.

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