Stable isotopes reveal the contribution of corticular photosynthesis to growth in branches of *Eucalyptus miniata*¹

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Abstract

The deciduous bark habit is widespread in the woody plant genus *Eucalyptus*. Species with deciduous bark seasonally shed a layer of dead bark, thereby maintaining smooth-bark surfaces on branches and stems as they age and increase in diameter. This has a significant cost in terms of fire protection, because smooth-barked species have thinner bark than rough-barked species that accumulate successive layers of dead bark. Eucalypts are closely associated with fire, suggesting that the smooth-bark habit must also provide a significant benefit. We suggest that this benefit is corticular photosynthesis. To test this, we quantified the contribution of corticular photosynthesis to wood production in smooth-barked branches of *Eucalyptus miniata* growing in tropical savanna in northern Australia. We covered branch sections with aluminium foil for four years to block corticular photosynthesis, then compared the oxygen and carbon stable isotope composition of foil-covered and uncovered branch sections. We developed theory to calculate the proportion of wood constructed from corticular photosynthate and the mean proportional refixation rate during corticular photosynthesis from the observed isotopic differences. Coverage with aluminium foil for four years increased wood $\delta^{13}C$ by 0.5‰ ($P=0.002$, $n=6$) and wood $\delta^{18}O$ by 0.5‰ ($P=0.02$, $n=6$). Based on these data, we estimated that 11 ± 3% of wood in the uncovered branch sections was constructed from corticular photosynthate, with a mean $\delta^{13}C$ of -34.8‰, and that the mean proportional refixation rate during corticular photosynthesis was 0.71 ± 0.15. This demonstrates that corticular photosynthesis makes a significant contribution to the carbon economy of smooth-barked eucalypts.
**Introduction**

*Eucalyptus* is a large genus of woody flowering plants, containing more than 700 species. Most of these species only occur naturally in Australia, with a few species also found in Papua New Guinea, Indonesia, East Timor, and the Philippines. Eucalypts dominate the forests and woodlands of Australia. They also occur in arid shrublands, although typically not as canopy-dominant components. Eucalypts range in life form from shrubs to the tallest angiosperm trees in the world (Williams and Brooker, 1997).

A distinguishing characteristic of many eucalypt species is a deciduous bark, whereby an outer layer of dead bark tissue is seasonally shed to expose a smooth bark surface (Chattaway, 1953). These smooth-barked, decorticating species differ from the rough-barked species in which the dead, outer bark persists and accumulates on the tree. The decorticating process acts to maintain smooth-bark surfaces as the stems and branches increase in diameter with increasing age. Some species are decorticating in the upper branches and stem, but have persistent, rough bark on the lower stem. About half the eucalypt species are wholly smooth-barked over both the main stem and branches, and about three fourths have smooth bark over the canopy branches, including branches larger than about 8 cm diameter (Slee et al., 2006).

Woody plants that have smooth bark typically have a layer of green, chlorophyllous tissue just beneath the bark surface (Sprugel and Benecke, 1991; Pfanz et al., 2002). This photosynthetic tissue refixes respired CO₂, reducing the CO₂ efflux from the woody tissue in the presence of sunlight, thereby recycling part of the respired carbon that would have otherwise been lost from the plant to the atmosphere (Strain and Johnson, 1963; Benecke, 1985; Cernusak and Marshall, 2000; Pfanz and Aschan, 2000; Wittmann et al., 2006; McGuire et al., 2009). Net uptake of CO₂ from the atmosphere typically does not occur in the branches and stems of woody plants, and the process has therefore been termed refixation, or corticular photosynthesis, because most of the photosynthetic tissue is located in the bark cortex (Sprugel and Benecke, 1991; Nilsen, 1995).

Although many eucalypts maintain smooth-bark surfaces by seasonally shedding a layer of dead bark, little research has been conducted into corticular photosynthesis in these trees (Tausz et al., 2005; Cernusak et al., 2006; Cerasoli et al., 2009; Eyles et al., 2009). Of particular interest from an ecological and evolutionary perspective is the extent to which corticular photosynthesis contributes toward the carbon economy of smooth-barked eucalypts. In this study, we estimated the contribution of corticular photosynthesis to wood production in branches of *Eucalyptus miniata* Cunn. ex Schauer, a commonly occurring eucalypt in the mesic savannas of northern Australia (Brooker and Kleinig, 2004). *E. miniata*
maintains smooth bark on its upper stem and branches, while the lower stem accumulates a thick layer of dead, fibrous bark in mature trees (Figure 1A). A green, chlorophyllous layer of tissue is visible beneath the smooth bark surface in the upper stem and branches (Figure 1B). We covered branch sections of mature E. miniata trees with aluminium foil for four years to exclude sunlight and thereby block corticular photosynthesis. We then compared the stable oxygen and carbon isotope composition of the wood formed beneath the foil with that of wood formed in adjacent, uncovered branch sections. We used these isotopic differences to estimate 1) the contribution of corticular photosynthesis to wood production, and 2) the proportional refixation rate during corticular photosynthesis.

**Theory**

If wood is constructed from photosynthate contributed by both leaf photosynthesis and corticular photosynthesis (re-fixation), a mass balance for the oxygen in the wood can be written as

$$W_o = L_o + C_o,$$

where $W_o$ is the oxygen content of the total wood dry matter, $L_o$ is the oxygen content of the wood dry matter constructed from leaf photosynthate, and $C_o$ is the oxygen content of the wood dry matter constructed from corticular photosynthate. A similar mass balance can be written for $^{18}\text{O}$,

$$W_o R_w = L_o R_L + C_o R_C,$$

where $R_w$ is the $^{18}\text{O}/^{16}\text{O}$ ratio of total wood dry matter, $R_L$ is the $^{18}\text{O}/^{16}\text{O}$ ratio of wood dry matter constructed from leaf photosynthate, and $R_C$ is the $^{18}\text{O}/^{16}\text{O}$ ratio of wood dry matter constructed from corticular photosynthate. Table 1 provides a summary of all symbols and abbreviations used in this paper. Equation (2) can then be divided through by $R_S$, the $^{18}\text{O}/^{16}\text{O}$ ratio of source water, i.e. water absorbed from the soil by the roots. Next, applying the relationship $(R_X/R_S)-1=\Delta^{18}\text{O}_X$, where $R_X$ is the $^{18}\text{O}/^{16}\text{O}$ ratio of component $X$, and $\Delta^{18}\text{O}_X$ is the $^{18}\text{O}$ enrichment above source water of component $X$, gives the following

$$W_o (\Delta^{18}\text{O}_w + 1) = L_o (\Delta^{18}\text{O}_l + 1) + C_o (\Delta^{18}\text{O}_c + 1).$$

Subtracting equation (1) from equation (3) gives

$$\Delta^{18}\text{O}_w W_o = \Delta^{18}\text{O}_l L_o + \Delta^{18}\text{O}_c C_o.$$

Substituting from equation (1), and solving equation (4) for $C_o/W_o$, the proportion of total wood dry matter constructed from corticular photosynthate, gives
To a very close approximation, the \( \Delta^{18}O \) of any component X can be calculated as
\[
\Delta^{18}O_X = \delta^{18}O_X - \delta^{18}O_S ,
\]
where \( \delta^{18}O_X \) and \( \delta^{18}O_S \) are \( \delta^{18}O \) values of component X and source water, respectively.

The \( ^{18}O \) enrichment of wood dry matter constructed from leaf photosynthate (\( \Delta^{18}O_L \)) can be described as (Barbour and Farquhar, 2000; Cernusak et al., 2005)
\[
\Delta^{18}O_L = \Delta^{18}O_{LW} \left(1 - p_{ex} p_x \right) + \varepsilon_{wc} + \varepsilon_{cp} ,
\]
where \( \Delta^{18}O_{LW} \) is the \( ^{18}O \) enrichment of leaf water above source water, \( p_{ex} \) is the proportion of oxygen atoms exchanging with local water during synthesis of wood cellulose, \( p_x \) is the proportion of unenriched source water at the site of wood synthesis, \( \varepsilon_{wc} \) is the equilibrium fractionation between organic oxygen and local water, and \( \varepsilon_{cp} \) is the \( \Delta^{18}O \) difference between wood cellulose and total wood dry matter. The \( \Delta^{18}O_{LW} \) can range between approximately 0 and 30\%, and varies primarily as a function of relative humidity (Craig and Gordon, 1965; Dongmann et al., 1974; Farquhar et al., 2007). The combined term \( p_{ex} p_x \) has been observed to be relatively constant in trees, having a value of about 0.4 (Roden et al., 2000; Cernusak et al., 2005; Cernusak et al., 2008). The \( \varepsilon_{wc} \) has a value of approximately 27\% (Sternberg and DeNiro, 1983; Sternberg et al., 1984; Yakir and DeNiro, 1990). Finally, the \( \varepsilon_{cp} \) has been observed to be relatively constant for wood dry matter in trees, having a value of approximately -5\% (Borella et al., 1999; Barbour et al., 2001; Cernusak et al., 2005).

It was previously observed that water in the bark of *Eucalyptus globulus* was not evaporatively enriched in \( ^{18}O \) compared to xylem water (Cernusak et al., 2005). This result likely applies to bark water generally, consistent with low evaporation rates from bark surfaces (Cernusak and Marshall, 2000; Cernusak et al., 2001; Wittmann and Pfanz, 2008). Xylem water has also been shown to have the same \( ^{18}O \) composition as water absorbed from soil by roots (Barbour, 2007). Therefore, in the case of corticular photosynthesis, the first term on the right side of equation (6) should have a value of zero. Thus, for wood constructed from corticular photosyntheate, equation (7) becomes
\[
\Delta^{18}O_C = \varepsilon_{wc} + \varepsilon_{cp} .
\]
The derivation presented above suggests that the difference in \( ^{18}O \) composition between wood dry matter constructed from leaf photosyntheate and that constructed from corticular photosyntheate should be determined by the magnitude of leaf water \( ^{18}O \) enrichment. Thus,
for a leaf water enrichment of 10‰, the predicted difference between $\Delta^{18}O_L$ and $\Delta^{18}O_C$ will be 6‰, and for a leaf water enrichment of 20‰, the predicted difference will be 12‰.

The carbon isotope signature of wood constructed from corticular photosynthate is also expected to differ from that of wood constructed from leaf photosynthate. During refixation in photosynthetic bark, photosynthetic enzymes discriminate against the heavier carbon isotope, $^{13}C$ (Cernusak et al., 2001). Because the source of CO$_2$ for refixation is primarily respired CO$_2$, refixed photosynthate is expected to have a $\delta^{13}C$ more negative than that of the respiratory CO$_2$. The $^{13}C$ depletion of refixed photosynthate relative to respired CO$_2$ can be described as (Cernusak et al., 2001; Cernusak et al., 2009)

$$\Delta^{13}C = \left(1 - \frac{P}{D}\right) \left(\frac{D}{D + gc_a}\right) \left(b\frac{c_i}{c_i - c_a} - a - \Delta^{13}C_D \frac{c_a}{c_i - c_a}\right). \quad (9)$$

The $\Delta^{13}C$ is defined as $(R'_D/R'_C)$-$1$, where $R'_D$ is the $^{13}C/^{12}C$ ratio of respired CO$_2$ in the woody tissue, and $R'_C$ is the $^{13}C/^{12}C$ ratio of refixed photosynthate. In equation (9), $P$ is the corticular photosynthesis rate (µmol CO$_2$ m$^{-2}$ s$^{-1}$), $D$ is the respiration rate (µmol CO$_2$ m$^{-2}$ s$^{-1}$), $g$ is the bark surface conductance to CO$_2$ (mol m$^{-2}$ s$^{-1}$), $c_a$ is the external CO$_2$ concentration (µmol mol$^{-1}$), $c_i$ is the CO$_2$ concentration inside the bark (µmol mol$^{-1}$), $b$ is the discrimination against $^{13}C$ by photosynthetic enzymes in the bark (~29‰ for Rubisco), and $a$ is the $^{13}C/^{12}C$ fractionation during diffusion of CO$_2$ in air (4.4‰). The $\Delta^{13}C_D$ is defined as $(R'_a/R'_D)$-$1$, where $R'_a$ is the $^{13}C/^{12}C$ ratio of CO$_2$ in air outside the branch or stem, and $R'_D$ is the $^{13}C/^{12}C$ ratio of respired CO$_2$.

The first term on the right side of equation (9) describes the departure from unity of the proportional refixation rate, $P/D$. When $P/D$ is small, the $\Delta^{13}C_C$ is large, and when $P/D$ is large, the $\Delta^{13}C_C$ is small. The second term on the right side of equation (9) accounts for the diffusion of CO$_2$ from air outside the branch or stem into the bark. If there is no CO$_2$ in the air outside the woody tissue, the term goes to unity. It is reduced from unity as $gc_a$ increases, which describes the one-way diffusive flux of CO$_2$ from the external air into the bark (Cernusak et al., 2009). The third term on the right side of equation (9) describes $^{13}C/^{12}C$ fractionations associated with enzymatic discrimination, diffusional fractionation, and variation in the $^{13}C/^{12}C$ ratio of the respired CO$_2$. A full derivation for equation (9) is given in Part 3 of the Appendix of Cernusak et al. (2001).

We used equation (5) to estimate the proportion of wood constructed from corticular photosynthate in the uncovered branch sections. In equation (5), the $\Delta^{18}O_L$ was determined from the wood sampled from the foil-covered branch sections. Wood in these sections was
assumed to have formed in the absence of any refixation, and therefore to have been derived entirely from leaf photosynthate. The $\Delta^{18}O_W$ was determined from the uncovered branch sections, where wood was assumed to have been constructed from both leaf and corticular photosynthate. The $\Delta^{18}O_C$ was calculated from equation (8), assuming $\varepsilon_{wc}=27^\circ$ and $\varepsilon_{cp}=-5^\circ$. For calculations of $\Delta^{18}O_L$ and $\Delta^{18}O_W$, the oxygen isotope composition of source water, $\delta^{18}O_S$, was assumed to be -5%. This is the amount-weighted mean $\delta^{18}O$ of rainfall for Darwin between 1962 and 2002 (International Atomic Energy Agency: http://www-naweb.iaea.org), approximately 30 km from the study site. The $\Delta^{18}O_L$ and $\Delta^{18}O_W$ were then calculated according to equation (6).

We then used the estimate of $C_o/W_o$, the proportion of wood dry matter constructed from corticular photosynthate, calculated from equation (5), to estimate the $\delta^{13}C$ of wood constructed from corticular photosynthate, $\delta^{13}C_C$. Following a derivation analogous to that given for equation (5), but for $\delta^{13}C$, leads to the following

$$
\delta^{13}C_C = \delta^{13}C_L - (\delta^{13}C_L - \delta^{13}C_W) \frac{W_c}{C_c},
$$

(10)

where $\delta^{13}C_L$ is $\delta^{13}C$ of wood constructed from leaf photosynthate, $\delta^{13}C_W$ is $\delta^{13}C$ of wood constructed from both leaf and corticular photosynthate, $W_c$ is the total wood carbon content, and $C_c$ is the wood carbon content derived from corticular photosynthate. The $C_c/W_c$ was assumed equal to $C_o/W_o$ calculated from equation (5), $\delta^{13}C_L$ was determined from wood sampled from the foil-covered branch sections, and $\delta^{13}C_W$ was determined from wood sampled from the uncovered branch sections. The $\Delta^{13}C_C$ was then calculated as

$$
\Delta^{13}C_C = \frac{\delta^{13}C_D - \delta^{13}C_C}{1 + \delta^{13}C_C},
$$

(11)

where $\delta^{13}C_D$ is the $\delta^{13}C$ of respired CO2 in the woody tissue. We assumed that $\delta^{13}C_D$ had the same value as $\delta^{13}C_W$.

Having estimated $\Delta^{13}C_C$, we then solved equation (9) for $P$, the corticular photosynthesis rate, in order to estimate $P/D$, the proportional refixation rate. This required estimates for $D$, $g$, $c_a$, $b$, $a$, $\delta^{13}C_D$, and $\delta^{13}C_a$. We assumed that $D=3$ µmol CO2 m$^{-2}$ s$^{-1}$, based on previous measurements in E. miniata (Cernusak et al., 2006), $g=0.001$ mol m$^{-2}$ s$^{-1}$ (Cernusak and Marshall, 2000; Cernusak et al., 2001; Ubierna et al., 2009b), $c_a=380$ µmol mol$^{-1}$, $b=29^\circ$, $a=4.4^\circ$, $\delta^{13}C_D=\delta^{13}C_W$, and $\delta^{13}C_a=-8^\circ$. The $\Delta^{13}C_D$ for equation (9) was then calculated as
\[ \Delta^{13}C_B = \frac{\delta^{13}C_a - \delta^{13}C_D}{1 + \delta^{13}C_D}, \]  

(12)

and \( c_i \) was calculated as

\[ c_i = \frac{D - P}{g} + c_a. \]  

(13)

We conducted a sensitivity analysis to investigate the effect of variation in these assumed parameter values on the estimate of the proportional refixation rate, \( P/D \).

The above calculations assumed that wood in the foil-covered branch sections was constructed exclusively from leaf-derived photosynthate. If corticular photosynthate was translocated into the foil-covered branch sections from the sun-exposed sections, this would have biased the calculations, such that we would have underestimated the contribution of corticular photosynthesis to wood production in sun-exposed branches. Further experimentation is required to determine the fate of corticular photosynthate, and whether it is likely to be translocated from its source to other parts of the plant.

**Results**

The isotopic composition of the outer 3 mm of wood and of the bark in foil-covered and uncovered branch sections is shown in Table 2. Covering the branch sections with aluminium foil for four years resulted in relatively small, but consistent, shifts in both \( \delta^{18}O \) and \( \delta^{13}C \) of wood compared to that of the adjacent, uncovered branch sections. Wood \( \delta^{18}O \) was 0.5‰ higher in foil-covered compared to uncovered branch sections (\( P=0.02, n=6 \)), with differences for individual branches ranging from 1.1 to 0.1‰. Wood \( \delta^{13}C \) was also 0.5‰ higher in foil-covered compared to uncovered branch sections (\( P=0.002, n=6 \)), with differences for individual branches ranging from 0.7 to 0.3‰. The trend for bark \( \delta^{13}C \) was similar, with bark \( \delta^{13}C \) of foil-covered branch sections being 0.5‰ higher compared to that of uncovered sections (\( P=0.001, n=6 \)). Bark \( \delta^{18}O \) was 0.3‰ higher in foil-covered compared to uncovered branch sections, but the difference was not statistically significant (\( P=0.13, n=6 \)).

The nitrogen concentration of bark in foil-covered branch sections tended to be lower than that in uncovered sections (\( P=0.06, n=6 \)), with a mean difference of 0.3 mg g\(^{-1}\) (Table 2). The nitrogen concentration of wood in foil-covered branch sections was lower than that in uncovered sections by 0.2 mg g\(^{-1}\) (\( P=0.008, n=6 \)). Carbon concentrations of both bark and
wood were similar between foil-covered and uncovered branch sections ($P=0.27$ and $P=0.68$, respectively, $n=6$).

Applying equation (5), as described in the methods section, resulted in an estimate for $C_{o}/W_{o}$, the proportion of wood constructed from corticular photosynthate, of $0.11 \pm 0.03$ (mean ± 1 SE, $n=6$). Thus, the change in $\delta^{18}O$ of wood between foil-covered and uncovered branch sections indicated that corticular photosynthesis accounted for 11% wood dry matter production. This estimate is sensitive to the assumed value for $\delta^{18}O_S$, the $\delta^{18}O$ of source water. If $\delta^{18}O_S$ were assumed to be -4‰ instead of -5‰, the mean estimate for $C_{o}/W_{o}$ would be 0.14, and if $\delta^{18}O_S$ were assumed to -6‰ instead of -5‰, the mean estimate for $C_{o}/W_{o}$ would be 0.09.

Applying estimates of $C_{o}/W_{o}$ derived from wood $\delta^{18}O$ and the difference in wood $\delta^{13}C$ between foil-covered and uncovered branch sections, in conjunction with equation (9), resulted in a mean estimate for $P/D$, the proportional refixation rate, of $0.71 \pm 0.15$ (mean ± 1 SE, $n=6$). This $P/D$ corresponded to a discrimination during corticular photosynthesis, $\Delta^{13}C_c$, of $7.2 \pm 3.2‰$ (mean ± 1 SE, $n=6$). Assuming mean $\delta^{13}C_D$ of -27.9‰, this equates to a mean $\delta^{13}C_C$ of -34.8‰. Application of equation (9) in this context requires a number of assumed parameter values. A sensitivity analysis of the effect of changing the assumed parameter values is shown in Table 3. For a given $\delta^{13}C$ difference between foil-covered and uncovered branch sections ($\delta^{13}C_L-\delta^{13}C_W$), the estimate of $P/D$ is relatively sensitive to changes in $C_{o}/W_{o}$, $b$, and $\delta^{13}C_D$, and relatively insensitive to changes in $D$, $g$, $c_o$, and $a$. The estimate of $P/D$ is also sensitive to changes in $\delta^{13}C_L-\delta^{13}C_W$ (Table 3), but this was an observed parameter in our analysis, rather than an assumed parameter.

Discussion

Excluding sunlight from *E. miniata* branch sections by covering them with aluminium foil for four years resulted in increases in both $\delta^{18}O$ and $\delta^{13}C$ of underlying wood compared to that of adjacent, uncovered branch sections. The isotopic enrichments, although relatively small, were consistent among branches and statistically significant. The average increase in $\delta^{13}C$ of wood was 0.5‰. This can be compared with a $\delta^{13}C$ increase of 0.8‰ in wood of *Pinus monticola* branches following coverage with aluminium foil for one growing season (Cernusak et al., 2001). Additionally, three woody plant species native to California, USA showed increases in $\delta^{13}C$ of phloem sugars in branches and stems of 1 to 2‰ following light exclusion by aluminium foil in defoliated plants (Saveyn et al., 2010). We also observed an
increase in the \(\delta^{18}O\) of branch wood of *E. miniata* of 0.5‰ in response to long-term light exclusion. To our knowledge, this is the first time that the effect of corticular photosynthesis on woody-tissue \(\delta^{18}O\) has been quantified. Thus, our experiment clearly demonstrated the capacity of corticular photosynthesis to influence both the carbon and oxygen stable isotope composition of branch wood in *E. miniata*.

Based on the increase in wood dry matter \(\delta^{18}O\) of branch sections covered with aluminium foil and equation (5), we estimated that 11 ± 3% of wood in uncovered branch sections was constructed from corticular photosynthate (\(C_o/W_o=0.11 \pm 0.03\)). This estimate is sensitive to the assumed value of \(\delta^{18}O_S\), the \(\delta^{18}O\) of water absorbed by roots from the soil. We assumed a value for \(\delta^{18}O_S\) of -5‰, based on measurements of the \(\delta^{18}O\) of rainfall in Darwin between 1962 and 2002 (International Atomic Energy Agency: http://www-naweb.iaea.org). This assumed \(\delta^{18}O_S\) is also similar to measurements of xylem water \(\delta^{18}O\) recorded for mature canopy trees in the vicinity of our study site (Kelley, 2002). Changes to the estimate of \(C_o/W_o\) in uncovered branch sections would be relatively small if the assumed value for \(\delta^{18}O_S\) were shifted up or down by 1‰, as described above. Therefore, the mean estimate of \(C_o/W_o\) in branches of *E. miniata* of 0.11 is reasonably well constrained.

The estimate of 11% for the contribution of corticular photosynthate to wood production in *E. miniata* branches is also consistent with a simple scaling of observed instantaneous refixation rates. At an irradiance of 1000 \(\mu\text{mol photons m}^{-2}\text{s}^{-1}\), a proportional refixation rate during corticular photosynthesis (\(P/D\)) of 0.55 was previously observed in excised branches of *E. miniata* (Cernusak et al., 2006). On a 24-hour basis, this refixation rate would scale to 0.275, if we assume that the estimated \(P/D\) took place for 10 hrs day\(^{-1}\) (Cernusak et al., 2006). If we then assume that branch carbon use efficiency is 0.6 (Gifford, 1994; Gifford, 2003), such that branch respiration accounts for 40% of total branch carbon allocation, the scaled refixation rate as a proportion of total branch carbon allocation would be 0.11. This scaled refixation rate would be expected to be the same as \(C_o/W_o\) if refixed photosynthate were not preferentially used for any one metabolic process over another. Thus, the \(\delta^{18}O\)-based estimate of \(C_o/W_o\) is in very good agreement with an estimate based on a simple scaling of observed instantaneous refixation rates in *E. miniata* branches.

We employed the estimate of \(C_o/W_o\) based on the \(\delta^{18}O\) measurements, along with the difference in wood \(\delta^{13}C\) between foil-covered and uncovered branch sections, to estimate the \(\delta^{13}C\) of wood constructed from corticular photosynthate, as described in equation (10). Our mean estimate for this parameter was -34.8‰. We then used this value to parameterize
equation (9) and estimate the mean $P/D$ during corticular photosynthesis in the uncovered branch sections. The resulting estimate was $0.71 \pm 0.15$. This application of equation (9) required assumed values for several parameters. However, a sensitivity analysis showed that changing many of these parameters had little effect on the estimate of $P/D$ (Table 3), and we therefore suggest that 0.71 is a realistic value. This value is higher than the instantaneous $P/D$ of 0.55 previously observed under unidirectional irradiance of 1000 µmol photons m$^{-2}$ s$^{-1}$ (Cernusak et al., 2006). This is to be expected, as the instantaneous rate of 0.55 was based on gas exchange measurements for whole branch sections. Thus, it represents an unweighted average of both the illuminated and shaded sides of the branch. The isotopic estimate based on wood $\delta^{13}C$, on the other hand, is a corticular photosynthesis-weighted average. The $P/D$ on the illuminated side of the branch in this case will be more highly represented than that on the shaded side of the branch, because the corticular photosynthesis rate will be higher on the illuminated side than the shaded side. Thus the $\delta^{13}C$-based estimate of $P/D$ would always be expected to be higher than that based on gas exchange measurements, unless the gas exchange measurements were made under isotropic illumination, such that all sides of the branch were evenly illuminated.

Most of the CO$_2$ fixed during corticular photosynthesis is likely derived from within woody tissues themselves. The $\delta^{13}C$ of this CO$_2$ source could therefore potentially be affected by processes such as variation in the $\delta^{13}C$ of CO$_2$ produced by respiration within woody tissues (Damesin et al., 2005; Maunoury et al., 2007; Kodama et al., 2008), or uptake by roots of CO$_2$ dissolved in soil water (Levy et al., 1999; Moore et al., 2008; Teskey et al., 2008; Ubiera et al., 2009a). The $\delta^{13}C$ of internally supplied CO$_2$ enters Equation 9 as $\delta^{13}C_D$, which is used to calculate $\Delta^{13}C_C$ and $\Delta^{13}C_D$. In our analysis, we assumed that internally supplied CO$_2$ had the same $\delta^{13}C$ as xylem wood, such that $\delta^{13}C_D$ was set equal to $\delta^{13}C_W$. There is clearly some uncertainty in assigning this value to $\delta^{13}C_D$. A shift of 3‰ in the assumed value of $\delta^{13}C_D$ caused a moderate shift in predicted $P/D$ in the sensitivity analysis (Table 3). Thus, a more refined understanding of the $\delta^{13}C$ dynamics of the internal CO$_2$ pool in woody tissues can contribute toward more robust $\delta^{13}C$-based estimates of $P/D$.

We observed small reductions in the nitrogen concentration of branch sections covered with aluminium foil compared to adjacent, uncovered sections (Table 2). A visual inspection of the foil-covered sections at harvest showed that there was no green tissue beneath the bark surface, in contrast to the uncovered sections. Coverage of stem sections with aluminium foil in other woody plant species caused significant reductions in stem
chlorophyll concentrations (Bossard and Rejmanek, 1992; Saveyn et al., 2010). We suggest that coverage of the E. miniata branch sections with aluminium foil for four years would have led to the disassembly of the photosynthetic machinery in the underlying bark and wood that would otherwise have been associated with corticular photosynthesis. The small reductions in nitrogen concentration of 0.3 mg g\(^{-1}\) for bark and 0.2 mg g\(^{-1}\) for wood as a result of foil coverage suggest that the amount of nitrogen required for corticular photosynthesis is small, being only 10 to 15\% of the nitrogen normally contained in the bark and outer 3 mm of wood. This suggests high nitrogen use efficiency for corticular photosynthesis. A high nitrogen use efficiency is consistent with the high CO\(_2\) concentrations found in woody tissues (Cernusak and Marshall, 2000; Teskey et al., 2008; Ubierna et al., 2009a), which would minimize photorespiration and maximize the efficiency of photosynthetic enzymes.

Eucalypts rose to prominence in Australia in close association with increasing aridity and increasing occurrence of fire during the Pleistocene (Barlow, 1981; Hill, 1994). They are generally well adapted to frequent fire. Adaptations include woody capsules that release seeds after fire, dormant buds which can promote rapid recovery of the canopy following scorching, lignotubers, and thick insulating bark (Barlow, 1981; Williams and Brooker, 1997). The last of these is particularly interesting in the context of corticular photosynthesis. For a given diameter of branch or stem wood, smooth-barked eucalypts with decorticating bark have thinner bark than rough-barked species that accumulate successive layers of dead bark (Gill and Ashton, 1968; Vines, 1968; Cernusak et al., 2006). To a first approximation, the temperature rise at the stem or branch cambium for a given heat input depends only on the bark thickness (Vines, 1968). It follows that trees with thicker bark should be better protected from thermal damage to the cambium during fire events. Why then would the decorticating bark habit be so widespread among eucalypts? We suggest that corticular photosynthesis provides an explanation. Smooth-barked species, whereby smooth bark is maintained by seasonally shedding an outer layer of bark, can maintain their capacity to refix respired CO\(_2\) as woody tissues increase in size with increasing age. In rough-barked species, on the other hand, the accumulation of successive layers of dead bark significantly reduces the amount of sunlight that can penetrate to living cells that could contain chloroplasts.

These considerations suggest that corticular photosynthesis should provide a significant benefit to smooth-barked tissues, because the maintenance of smooth bark carries a significant cost in terms of reduced protection from fire. We have demonstrated that corticular photosynthesis contributed 11 ± 3\% of the carbon incorporated into wood in branches of E. miniata. We have also shown that the nitrogen allocation required to support
Corticular photosynthesis is apparently small, being only about 10 to 15% of the nitrogen present in the bark and outer wood. However, the most significant benefit of corticular photosynthesis likely derives from its water use efficiency. Because evaporation rates from smooth bark surfaces are very low, corticular photosynthesis proceeds with a minimum of water loss. It was estimated in branches of Pinus monticola that the water use efficiency of corticular photosynthesis was 50 times greater than the water use efficiency of leaf photosynthesis (Cernusak and Marshall, 2000). This fundamental difference in water use efficiency between leaves and bark results from the fact that leaves must expose moist tissues to the atmosphere in order to take up CO₂, whereas bark primarily uses internally produced CO₂. This advantage in terms of water use efficiency likely contributes toward the drought tolerance of smooth-barked eucalypts. Drought tolerance is presumably one of the key features that lead to the evolutionary success of eucalypts with the onset of increasing aridification in Australia during the Pleistocene (Barlow, 1981; Hill, 1994; Bowman, 2000).

Eucalypts typically have open canopies. Most species have isobilateral leaves that hang in a more or less vertical direction (Williams and Brooker, 1997). Thus, light penetration in eucalypt canopies is relatively high, and light interception by woody tissues is probably higher than in other woody plant taxa that tend to have higher leaf area indices. Thus, the characteristically open nature of most eucalypt canopies would maximize the contribution of corticular photosynthesis to the carbon economy of smooth-barked branches and stems (Tausz et al., 2005).

Some eucalypt species retain dead bark on the lower stem, but have decorticating bark on the upper stem and branches. E. miniata is an excellent example of such a species (Figure 1). This strategy would appear to provide the benefits of both fire protection by thick dead bark on the lower stem and maintenance of capacity for corticular photosynthesis on the upper stem and branches. In the mesic savannas where E. miniata occurs, the frequent fires are typically surface fires that consume the grassy fuel layer, but do not burn in the crowns of the over-story trees (Williams et al., 1999). Thus, the strategy of retaining dead bark on the lower stem and seasonally shedding bark from the upper stem and branches to maintain corticular photosynthesis may be particularly advantageous for a savanna tree such as E. miniata.

Conclusion

The deciduous bark habit is exceptionally widespread in the genus Eucalyptus. Species with decorticating bark have thinner bark than species that accumulate successive
layers of rough, dead bark. Eucalypts are generally well adapted to coexisting with fire, but
the prevalence of decorticating bark among eucalypts is counterintuitive in this context,
because thin bark allows the cambium temperature to increase more during fire events than
thick bark. Maintenance of smooth-bark surfaces by seasonally shedding a layer of dead bark
therefore carries a cost in terms of reduced protection from fire, which suggests that it must
also provide a benefit, given the close association between eucalypts and fire. We suggest
that this benefit is the maintenance of capacity for corticular photosynthesis as woody tissues
increase in diameter with increasing age. Corticular photosynthesis provides an effective
mechanism for recycling respired CO$_2$ that would otherwise be lost from woody tissues to the
atmosphere. We have demonstrated that corticular photosynthesis contributed 11 ± 3% of
wood production in branches of mature *E. miniata* trees, based on isotopic shifts in branch
wood following long-term light exclusion. Thus, corticular photosynthesis can make a
significant contribution to the carbon economy of eucalypts that maintain smooth bark on
their branches and stems by seasonally shedding a layer of dead bark. Corticular
photosynthesis is particularly advantageous in terms of its water use efficiency, and likely
contributes to the drought tolerance of smooth-barked eucalypts.

**Materials and Methods**

Our study site was located approximately 30 km south east of Darwin, Northern
Territory, Australia in a tropical savanna in the Howard River catchment (12°29.7′ S,
131°09.0′ E). The site has been recently described in detail (Hutley et al., 2000; O'Grady et
al., 2000; Cernusak et al., 2006). In order to explore the effect of refixation on the isotopic
composition of *E. miniata* branches, we covered branch sections with aluminium foil. The
foil was expected to block all sunlight from reaching the bark beneath it. Therefore, the
wood formed beneath the foil was expected to form in the absence of any photosynthetic
refixation. The branch sections covered with foil were approximately 30 cm long. Branch
diameters at the conclusion of the experiment ranged from 3.2 to 4.6 cm. The aluminium foil
was secured to the bark with adhesive tape at the ends of the foil-covered sections. The foil
was applied to branches at heights above the ground ranging from 6 to 10 m. The branches
were accessed with a 16-m elevated work platform (cherry picker). Foil was applied to the
branches in October 2004. The branches were harvested four years later, in September 2008.
Foil was initially applied to 12 branches. When we returned four years later, we were able to
re-locate six of the foil-covered branches spread across four mature *E. miniata* individuals.
We observed no evidence of fungal infection or insect attack on the foil-covered branch sections.

After the branches were harvested, a wood disk was taken from the centre of the foil-covered section. Disks were also taken from the same branches, but approximately 30 cm away from each end of the foil-covered section to provide samples that had been exposed to sunlight over the four year period. The disks had a width of approximately 1 cm. The bark was removed from each disk, and the outer-most circumference of wood (sapwood) was removed to a depth of approximately 3 mm. The wood and bark were oven-dried at 70°C for several days, then ground to a fine powder for isotopic and elemental analyses.

The $\delta^{13}$C and total nitrogen and carbon concentrations of the bark and wood were determined on subsamples of approximately 3 mg. Analyses were carried out in an elemental analyser (ECS 4010, Costech Analytical Technologies, Valencia, CA, USA) linked via a continuous-flow interface to a stable isotope ratio mass spectrometer (Delta XP, Finnigan MAT, Bremen, Germany). The $\delta^{18}$O of the wood and bark dry matter was determined on subsamples of approximately 1 mg, which was pyrolysed in a high-temperature furnace (Thermoquest TC/EA, Finnigan MAT) linked via continuous-flow interface to an isotope ratio mass spectrometer. Isotopic and elemental analyses were carried out in the Stable Isotope Core Laboratory, Washington State University, Pullman, WA, USA. The precision of isotopic analyses, based on the standard deviation of repeated measurements of working standards during the sample runs, was 0.2‰ for $\delta^{18}$O and 0.1‰ for $\delta^{13}$C. The $\delta^{18}$O and $\delta^{13}$C values have been expressed relative to the Vienna Standard Mean Ocean Water (VSMOW) and PeeDee Belemnite (PDB) international standards, respectively.

The stable isotope and elemental composition of wood and bark dry matter was compared between foil-covered and uncovered branch sections using paired $t$-tests. Results were considered statistically significant at $P<0.05$. Data for wood disks taken 30 cm from either end of the foil-covered section were averaged for the uncovered values.

**Literature Cited**


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Gill AM, Ashton DH (1968) The role of bark type in relative tolerance to fire of three central Victorian eucalypts. Aust J Bot 16: 491-498


Ubierna N, Kumar AS, Cernusak LA, Pangle RE, Gag PJ, Marshall JD (2009a) Storage and transpiration have negligible effects on δ13C of stem CO2 efflux in large conifer trees. Tree Physiol 29: 1563-1574


Figure Legends

Figure 1. (A) An individual of *Eucalyptus miniata* growing in tropical savanna near Darwin, Northern Territory, Australia. Note the stocking of thick, fibrous bark at the base of the tree that abruptly gives way to smooth, white bark part way up the main stem. Panel (B) gives a closer view of the transition from rough to smooth bark on the main stem. The outermost surface of the smooth bark has been scraped away from a square section, revealing the green, chlorophyllous layer of photosynthetic tissue just beneath the smooth bark surface.
Table 1. Symbols used in the text.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>$^{13}$C/$^{12}$C fractionation during CO$_2$ diffusion in air</td>
</tr>
<tr>
<td>$b$</td>
<td>$^{13}$C/$^{12}$C discrimination by photosynthetic enzymes in the bark</td>
</tr>
<tr>
<td>$C_c$</td>
<td>Carbon content of wood dry matter constructed from corticular photosynthate</td>
</tr>
<tr>
<td>$C_o$</td>
<td>Oxygen content of wood dry matter constructed from corticular photosynthate</td>
</tr>
<tr>
<td>$c_a$</td>
<td>CO$_2$ concentration in air outside the woody tissue</td>
</tr>
<tr>
<td>$c_i$</td>
<td>CO$_2$ concentration inside the bark</td>
</tr>
<tr>
<td>$D$</td>
<td>Woody-tissue respiration rate</td>
</tr>
<tr>
<td>$g$</td>
<td>Bark surface conductance to CO$_2$</td>
</tr>
<tr>
<td>$L_o$</td>
<td>Oxygen content of wood dry matter constructed from leaf photosynthate</td>
</tr>
<tr>
<td>$P$</td>
<td>Corticular photosynthesis rate</td>
</tr>
<tr>
<td>$p_{ex}$</td>
<td>Proportion of oxygen exchanging with local water during cellulose synthesis</td>
</tr>
<tr>
<td>$p_x$</td>
<td>Proportion of unenriched water in tissue where cellulose synthesis is occurring</td>
</tr>
<tr>
<td>$R_C$</td>
<td>$^{18}$$O$/^{16}O of wood dry matter constructed from corticular photosynthate</td>
</tr>
<tr>
<td>$R_L$</td>
<td>$^{18}$$O$/^{16}O of wood dry matter constructed from leaf photosynthate</td>
</tr>
<tr>
<td>$R_S$</td>
<td>$^{18}$$O$/^{16}O of source water (water absorbed by roots from the soil)</td>
</tr>
<tr>
<td>$R_W$</td>
<td>$^{18}$$O$/^{16}O of total wood dry matter</td>
</tr>
<tr>
<td>$R_X$</td>
<td>$^{18}$$O$/^{16}O of component X</td>
</tr>
<tr>
<td>$R'_a$</td>
<td>$^{13}$C/$^{12}$C of CO$_2$ in external air</td>
</tr>
<tr>
<td>$R'_C$</td>
<td>$^{13}$C/$^{12}$C of corticular photosynthate</td>
</tr>
<tr>
<td>$R'_D$</td>
<td>$^{13}$C/$^{12}$C of CO$_2$ respired by the woody tissue</td>
</tr>
<tr>
<td>$W_c$</td>
<td>Carbon content of total wood dry matter</td>
</tr>
<tr>
<td>$W_o$</td>
<td>Oxygen content of total wood dry matter</td>
</tr>
<tr>
<td>$\Delta_{13}C_C$</td>
<td>$^{13}$C depletion of corticular photosynthate relative to respired CO$_2$</td>
</tr>
<tr>
<td>$\Delta_{13}C_D$</td>
<td>$^{13}$C depletion of CO$_2$ respired by woody tissues relative to CO$_2$ in external air</td>
</tr>
<tr>
<td>$\Delta^{18}O_C$</td>
<td>$^{18}$O enrichment of wood dry matter constructed from corticular photosynthate</td>
</tr>
<tr>
<td>$\Delta^{18}O_L$</td>
<td>$^{18}$O enrichment of wood dry matter constructed from leaf photosynthate</td>
</tr>
<tr>
<td>$\Delta^{18}O_{LW}$</td>
<td>$^{18}$O enrichment of leaf water</td>
</tr>
<tr>
<td>$\Delta^{18}O_W$</td>
<td>$^{18}$O enrichment of total wood dry matter</td>
</tr>
<tr>
<td>$\Delta^{18}O_X$</td>
<td>$^{18}$O enrichment of component X above source water</td>
</tr>
<tr>
<td>$\delta_{13}C_a$</td>
<td>$\delta^{13}$C of CO$_2$ in external air</td>
</tr>
<tr>
<td>$\delta_{13}C_C$</td>
<td>$\delta^{13}$C of wood constructed from corticular photosynthate</td>
</tr>
<tr>
<td>$\delta_{13}C_D$</td>
<td>$\delta^{13}$C of CO$_2$ respired by the woody tissue</td>
</tr>
<tr>
<td>$\delta_{13}C_L$</td>
<td>$\delta^{13}$C of wood constructed from leaf photosynthate</td>
</tr>
<tr>
<td>$\delta_{13}C_W$</td>
<td>$\delta^{13}$C of total wood dry matter</td>
</tr>
<tr>
<td>$\delta^{18}O_S$</td>
<td>$\delta^{18}$O of source water (water absorbed by roots from the soil)</td>
</tr>
<tr>
<td>$\delta^{15}O_X$</td>
<td>$\delta^{15}$O of component X</td>
</tr>
<tr>
<td>$\epsilon_{cp}$</td>
<td>Difference between $\Delta^{18}O$ of wood dry matter and $\Delta^{18}O$ of cellulose</td>
</tr>
<tr>
<td>$\epsilon_{wc}$</td>
<td>$^{17}$O/$^{18}$O fractionation between organic oxygen and local water</td>
</tr>
</tbody>
</table>
Table 2. Oxygen and carbon stable isotope composition of branch sections covered with aluminium foil compared to adjacent sections on the same branch not covered with foil. Values are given for wood and bark separately. Concentrations of nitrogen and carbon for the same samples are also shown. Values are the mean of six branches, with one standard error given in parentheses.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$\delta^{18}$O (‰)</th>
<th>$\delta^{13}$C (‰)</th>
<th>[N] (mg g$^{-1}$)</th>
<th>[C] (mg g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood: foil-covered</td>
<td>21.8 (0.1)</td>
<td>-27.4 (0.2)</td>
<td>1.7 (0.2)</td>
<td>465 (4)</td>
</tr>
<tr>
<td>Wood: no foil</td>
<td>21.3 (0.2)</td>
<td>-27.9 (0.2)</td>
<td>1.9 (0.2)</td>
<td>463 (4)</td>
</tr>
<tr>
<td>Bark: foil-covered</td>
<td>21.1 (0.3)</td>
<td>-28.0 (0.2)</td>
<td>1.8 (0.1)</td>
<td>449 (6)</td>
</tr>
<tr>
<td>Bark: no foil</td>
<td>20.7 (0.2)</td>
<td>-28.5 (0.2)</td>
<td>2.1 (0.2)</td>
<td>442 (6)</td>
</tr>
</tbody>
</table>
Table 3. A sensitivity analysis of the effect of changing assumed parameter values on predicted estimates of \( P/D \), the proportional refixation rate during corticular photosynthesis.

Calculations were performed according to equation (9) of the main text. Parameters were varied one at a time, and all other parameters were fixed at the middle value when not under examination. The effect of a halving or a doubling of the middle value for each parameter is shown, except for \( \delta^{13}C_D \), which was varied by \( \pm 3\%e \). Symbols are as defined in Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range of values</th>
<th>Predicted ( P/D )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \delta^{13}C_L - \delta^{13}C_W ) (‰)</td>
<td>0.25, 0.5, 1</td>
<td>0.94, 0.83, 0.62</td>
</tr>
<tr>
<td>( C/W_c ) (mol mol(^{-1}))</td>
<td>0.05, 0.1, 0.2</td>
<td>0.59, 0.83, 0.95</td>
</tr>
<tr>
<td>( D ) (µmol m(^{-2}) s(^{-1}))</td>
<td>1.5, 3, 6</td>
<td>0.86, 0.83, 0.82</td>
</tr>
<tr>
<td>( g ) (mmol m(^{-2}) s(^{-1}))</td>
<td>0.5, 1, 2</td>
<td>0.82, 0.83, 0.86</td>
</tr>
<tr>
<td>( c_a ) (µmol mol(^{-1}))</td>
<td>190, 380, 760</td>
<td>0.82, 0.83, 0.86</td>
</tr>
<tr>
<td>( b ) (‰e)</td>
<td>15, 29, 58</td>
<td>0.45, 0.83, 0.99</td>
</tr>
<tr>
<td>( a ) (‰e)</td>
<td>2.2, 4.4, 8.8</td>
<td>0.85, 0.83, 0.80</td>
</tr>
<tr>
<td>( \delta^{13}C_D ) (‰e)</td>
<td>-25, -28, -31</td>
<td>0.70, 0.83, 0.95</td>
</tr>
</tbody>
</table>
Figure 1. (A) An individual of *Eucalyptus miniata* growing in tropical savanna near Darwin, Northern Territory, Australia. Note the stocking of thick, fibrous bark at the base of the tree that abruptly gives way to smooth, white bark part way up the main stem. Panel (B) gives a closer view of the transition from rough to smooth bark on the main stem. The outermost surface of the smooth bark has been scraped away from a square section, revealing the green, chlorophyllous layer of photosynthetic tissue just beneath the smooth bark surface.