Stable Isotopes Reveal the Contribution of Corticular Photosynthesis to Growth in Branches of Eucalyptus miniata$^{1[OA]}$

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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 aluminum foil for 4 years to block corticular photosynthesis and 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 aluminum foil for 4 years increased wood δ¹³C by 0.5‰ ($P = 0.002, n = 6$) and wood δ¹⁸O 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 δ³¹C 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.

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 CO$_2$, reducing the CO$_2$ 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 CO$_2$ from the atmosphere typically does not occur in the branches and stems of woody plants; therefore, the process has 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

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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*, 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 (Fig. 1A). A green, chlorophyllous layer of tissue is visible beneath the smooth bark surface in the upper stem and branches (Fig. 1B). We covered branch sections of mature *E. miniata* trees with aluminum foil for 4 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 (refixation), 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 I provides a summary of all symbols and abbreviations used in this paper. Equation 2 can then be divided through by \(R_o\), 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 \((R_X/R_o) - 1 = \Delta^{18}\text{O}_X\), 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:

\[
\frac{C_o}{W_o} = \frac{\Delta^{18}\text{O}_L - \Delta^{18}\text{O}_W}{\Delta^{18}\text{O}_C - \Delta^{18}\text{O}_W}
\]

To a very close approximation, the \(\Delta^{18}\text{O}\) of any component \(X\) can be calculated as:

\[
\Delta^{18}\text{O}_X \approx \delta^{18}\text{O}_X - \delta^{18}\text{O}_S
\]

where \(\delta^{18}\text{O}_X\) and \(\delta^{18}\text{O}_S\) are \(^{18}\text{O}\) values of component \(X\) and source water, respectively. The \(^{18}\text{O}\) enrichment of wood dry matter constructed from leaf photosynthate \((\Delta^{18}\text{O}_L)\) can be described as (Barbour and Farquhar, 2000; Cernusak et al., 2005):

\[
\Delta^{18}\text{O}_L = \Delta^{18}\text{O}_{LW}(1 - p_{wc}) + e_{wc} + e_{cp}
\]

where \(\Delta^{18}\text{O}_{LW}\) is the \(^{18}\text{O}\) enrichment of leaf water above source water, \(p_{wc}\) is the proportion of oxygen atoms exchanging with local water during the synthet-

![Figure 1](image-url)
sis of wood cellulose, $p_x$ is the proportion of unenriched source water at the site of wood synthesis, $e_{sw}$ is the equilibrium fractionation between organic oxygen and local water, and $e_{cp}$ 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., 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 7 should have a value of zero. Thus, for wood constructed from corticular photosynthesize, Equation 7 becomes:

$$\Delta^{18}O_C = e_{sw} + e_{cp} \quad (8)$$

The derivation presented above suggests that the difference in $^{18}O$ composition between wood dry matter constructed from leaf photosynthesize and that constructed from corticular photosynthesize 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

### Table 1. Symbols used in the text

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>$a$</td>
<td>$^{13}C^{18}O$ fractionation during CO$_2$ diffusion in air</td>
</tr>
<tr>
<td>$b$</td>
<td>$^{13}C^{18}O$ discrimination by photosynthetic enzymes in the bark</td>
</tr>
<tr>
<td>$C_C$</td>
<td>Carbon content of wood dry matter constructed from corticular photosynthesize</td>
</tr>
<tr>
<td>$C_L$</td>
<td>Oxygen content of wood dry matter constructed from corticular photosynthesize</td>
</tr>
<tr>
<td>$C_W$</td>
<td>CO$_2$ concentration in air outside the woody tissue</td>
</tr>
<tr>
<td>$C_p$</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>
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<td>$I_0$</td>
<td>Oxygen content of wood dry matter constructed from leaf photosynthesize</td>
</tr>
<tr>
<td>$P$</td>
<td>Cortical photosynthesize rate</td>
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<td>$p_x$</td>
<td>Proportion of oxygen exchanging with local water during cellulose synthesis</td>
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<td>$p_a$</td>
<td>Proportion of unenriched water in tissue where cellulose synthesis is occurring</td>
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<td>$R_C$</td>
<td>$^{18}O$ of wood dry matter constructed from corticular photosynthesize</td>
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<tr>
<td>$R_L$</td>
<td>$^{18}O$ of wood dry matter constructed from leaf photosynthesize</td>
</tr>
<tr>
<td>$R_S$</td>
<td>$^{18}O$ of source water (water absorbed by roots from the soil)</td>
</tr>
<tr>
<td>$R_W$</td>
<td>$^{18}O$ of total wood dry matter</td>
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<tr>
<td>$R_X$</td>
<td>$^{18}O$ of component X</td>
</tr>
<tr>
<td>$R_a$</td>
<td>$^{13}C$ of CO$_2$ in external air</td>
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<tr>
<td>$R_C$</td>
<td>$^{13}C$ of corticular photosynthesize</td>
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<tr>
<td>$R_D$</td>
<td>$^{13}C$ 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>$W_L$</td>
<td>Oxygen content of total wood dry matter</td>
</tr>
<tr>
<td>$\Delta^{18}C_{Cp}$</td>
<td>$^{13}C$ depletion of corticular photosynthesize relative to respired CO$_2$</td>
</tr>
<tr>
<td>$\Delta^{18}C_{C}$</td>
<td>$^{13}C$ depletion of CO$_2$ respired by woody tissues relative to CO$_2$ in external air</td>
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<tr>
<td>$\Delta^{18}O_{W}$</td>
<td>$^{18}O$ enrichment of leaf water</td>
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<td>$\Delta^{18}O_{X}$</td>
<td>$^{18}O$ enrichment of component X above source water</td>
</tr>
<tr>
<td>$\delta^{13}C$</td>
<td>$^{13}C$ of CO$_2$ in external air</td>
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<td>$\delta^{13}C_C$</td>
<td>$^{13}C$ of wood constructed from corticular photosynthesize</td>
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<tr>
<td>$\delta^{13}C_D$</td>
<td>$^{13}C$ of CO$_2$ respired by the woody tissue</td>
</tr>
<tr>
<td>$\delta^{13}C_L$</td>
<td>$^{13}C$ of wood constructed from leaf photosynthesize</td>
</tr>
<tr>
<td>$\delta^{13}C_W$</td>
<td>$^{13}C$ of total wood dry matter</td>
</tr>
<tr>
<td>$\delta^{18}O_X$</td>
<td>$^{18}O$ of source water (water absorbed by roots from the soil)</td>
</tr>
<tr>
<td>$\delta^{18}O_X$</td>
<td>$^{18}O$ of component X</td>
</tr>
<tr>
<td>$e_{sw}$</td>
<td>Difference between $\Delta^{18}O$ of wood dry matter and $\Delta^{18}O$ of cellulose</td>
</tr>
<tr>
<td>$e_{cp}$</td>
<td>$^{18}O$ fractionation between organic oxygen and local water</td>
</tr>
</tbody>
</table>
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 \( ^{13}C \)/\( ^{12}C \) ratio 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, 2009):

\[
\Delta^{13}C_C = (1 - \frac{P}{D}) \left( \frac{D}{D + g_c a} \right) \left( \frac{c_i}{c_i - c_a} - a - \Delta^{13}C_D \frac{c_a}{c_i - c_a} \right)
\]  

(9)

The \( \Delta^{13}C_C \) is defined as \( (R'_{dp}/R') - 1 \), where \( R'_{dp} \) is the \( ^{13}C/^{12}C \) ratio of respired CO\(_2\) in the woody tissue and \( R' \) is the \( ^{13}C/^{12}C \) ratio of refixed photosynthate. In Equation 9, \( P \) is the corticular photosynthesis rate (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \)), \( D \) is the respiration rate (\( \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \)), \( g \) is the bark surface conductance to CO\(_2\) (\( \text{mol m}^{-2} \text{s}^{-1} \)), \( c_a \) is the external CO\(_2\) concentration (\( \mu \text{mol} \text{ mol}^{-1} \)), \( c_i \) is the CO\(_2\) concentration inside the bark (\( \mu \text{mol} \text{ mol}^{-1} \)), \( b \) is the discrimination against \( ^{13}C \) by photosynthetic enzymes in the bark (approximately 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 \( (R'_{s}/R') - 1 \), where \( R'_{s} \) is the \( ^{13}C/^{12}C \) ratio of CO\(_2\) in air outside the branch or stem and \( R'_{dp} \) 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 \( g_c \) 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 \( \Delta^{18}C_C \) was calculated from Equation 8, assuming \( \varepsilon_{wc} = 27‰ \) and \( \varepsilon_{wp} = -5‰ \). For calculations of \( \Delta^{18}O_L \) and \( \Delta^{18}O_W \), the oxygen isotope composition of source water, \( \delta^{18}O_W \), 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 \( C_W/W \), the proportion of wood dry matter constructed from corticular photosynthate, calculated from Equation 5, to estimate the \( ^{13}C \) of wood constructed from corticular photosynthate, \( ^{13}C_C \). Following a derivation analogous to that given for Equation 5, but for \( ^{13}C \), leads to the following:

\[
\delta^{13}C_C = \delta^{13}C_L - \left( \delta^{13}C_L - \delta^{13}C_W \right) \frac{W_C}{C_W}
\]  

(10)

where \( \delta^{13}C_L \) is the \( ^{13}C \) of wood constructed from leaf photosynthate, \( \delta^{13}C_W \) is the \( ^{13}C \) of wood constructed from both leaf and corticular photosynthate, \( W_C \) is the total wood carbon content, and \( C_W \) is the wood carbon content derived from corticular photosynthate. The \( C_W/W \) was assumed equal to \( C_L/W \) 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:

\[
\Delta^{13}C_C = \frac{\delta^{13}C_D - \delta^{13}C_C}{1 + \delta^{13}C_D}
\]  

(11)

where \( \delta^{13}C_D \) is the \( ^{13}C \) of respired CO\(_2\) 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 \( \delta^{13}C_C \). We assumed that \( D = 3 \mu \text{mol CO}_2 \text{ m}^{-2} \text{s}^{-1} \), based on previous measurements in E. miniata (Cernusak et al., 2006), \( g = 0.001 \mu \text{mol m}^{-2} \text{s}^{-1} \) (Cernusak and Marshall, 2000; Cernusak et al., 2001; Ubierna et al., 2009b), \( c_a = 380 \mu \text{mol mol}^{-1} \), \( b = 29‰ \), \( a = 4.4‰ \), \( \delta^{13}C_D = \delta^{13}C_W \), and \( \delta^{13}C_a = -8‰ \). The \( \Delta^{13}C_D \) for Equation 9 was then calculated as:

\[
\Delta^{13}C_D = \frac{\delta^{13}C_a - \delta^{13}C_D}{1 + \delta^{13}C_D}
\]  

(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 photosynthetic. 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 photosynthate 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 II. Covering the branch sections with aluminum foil for 4 years resulted in relatively small, but consistent, shifts in both δ18O and δ13C of wood compared with the adjacent, uncovered branch sections. Wood δ18O was 0.5‰ higher in foil-covered compared with uncovered branch sections (P = 0.02, n = 6), with differences for individual branches ranging from 1.1‰ to 0.1‰. Wood δ13C was also 0.5‰ higher in foil-covered compared with uncovered branch sections (P = 0.002, n = 6), with differences for individual branches ranging from 0.7‰ to 0.3‰. The trend for bark δ13C was similar, with bark δ13C of foil-covered branch sections being 0.5‰ higher compared with that of uncovered sections (P = 0.001, n = 6). Bark δ18O was 0.3‰ higher in foil-covered compared with 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 II). 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 “Materials and Methods,” resulted in an estimate for C_Wa/W_o, the proportion of wood constructed from corticular photosynthate, of 0.11 ± 0.03 (mean ± se, n = 6). Thus, the change in δ18O of wood between foil-covered and uncovered branch sections indicated that corticular photosynthesis accounted for 11% of wood dry matter production. This estimate is sensitive to the assumed value for δ²⁸O_o, the δ18O of source water. If δ²⁸O_o were assumed to be −4‰ instead of −5‰, the mean estimate for C_Wa/W_o would be 0.14, and if δ²⁸O_o were assumed to −6‰ instead of −5‰, the mean estimate for C_Wa/W_o would be 0.09.

Applying estimates of C_Wa/W_o derived from wood δ18O and the difference in wood δ13C 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 ± 0.15 (mean ± se, n = 6). This P/D corresponded to a discrimination during corticular photosynthesis, Δ13C_D of 7.2 ± 3.2‰ (mean ± se, n = 6). Assuming mean δ13CL-D of −27.9‰, this equates to a mean Δ13C_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 III. For a given δ18O difference between foil-covered and uncovered branch sections (δ13CL-δ13CW), the estimate of P/D is relatively sensitive to changes in C/W_a, b, and δ13CL-D and relatively insensitive to changes in D, g, c, and a. The estimate of P/D is also sensitive to changes in δ13CL-δ13CW (Table III), 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 aluminum foil for 4 years resulted in increases in both δ18O and δ13C of underlying wood compared with that of adjacent, uncovered branch sections. The isotopic enrichments, although relatively small, were consistent among branches and statistically significant. The average increase in δ18O of wood was 0.5‰. This can be compared with a δ13C increase of 0.8‰ in wood of Pinus monticola branches following coverage with aluminum foil for one growing season (Cernusak et al., 2001). Additionally, three woody plant species native to California showed increases in δ13C of phloem sugars in branches and stems of 1‰ to 2‰ following light exclusion by aluminum foil in defoliated plants (Saveyn et al., 2010). We also observed an increase in the δ18O 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 δ18O 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 δ18O of branch sections covered with aluminum foil and Equation 5, we estimated that 11% ± 3% of wood in uncovered branch sections was constructed from corticular photosynthate (C_Wa/W_o = 0.11 ± 0.03). This estimate is sensitive to the assumed value of δ²⁸O_o, the δ18O of water absorbed by roots from the soil. We assumed a value for δ²⁸O_o of −5‰ based on measurements of the δ18O of rainfall in Darwin between 1962 and 2002 (International Atomic Energy Agency; http://www-naweb.iaea.org). This assumed δ²⁸O_o is also similar to measurements of xylem water δ18O recorded for mature canopy trees in the vicinity of our study site (Kelley, 2002). Changes to the estimate of C_Wa/W_o in uncovered branch sections would be relatively small if the assumed value for δ²⁸O_o 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 corticale photosynthate to wood production in \( E. miniata \) branches is also consistent with a simple scaling of observed instantaneous refixation rates. At an irradiance of 1,000 \( \mu \)mol photons \( m^{-2} s^{-1} \), a proportional refixation rate during corticale photosynthesis (\( P/D \)) of 0.55 was previously observed in excised branches of \( E. miniata \) (Cernusak et al., 2006). On a 24-h basis, this refixation rate would scale to 0.275 if we assume that the estimated \( P/D \) took place for 10 h \( d^{-1} \) (Cernusak et al., 2006). If we then assume that branch carbon use efficiency is 0.6 (Gifford, 1994, 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 corticale 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 corticale photosynthesis in the uncovered branch sections. The resulting estimate was 0.71 ± 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 III); therefore, we 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 1,000 \( \mu \)mol 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 corticale 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 corticale photosynthesis rate will be higher on the illuminated side than on 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 corticale photosynthesis is likely derived from within woody tissues themselves. The \( \delta^{13}C \) of this CO\(_2\) source, therefore, could 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; Ubierna 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

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**Table II.** Oxygen and carbon stable isotope composition of branch sections covered with aluminum foil compared with adjacent sections on the same branch not covered with foil

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( \delta^{18}O )</th>
<th>( \delta^{13}C )</th>
<th>[Nitrogen]</th>
<th>[Carbon]</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>

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**Table III.** A sensitivity analysis of the effect of changing assumed parameter values on predicted estimates of \( P/D \), the proportional refixation rate during corticale photosynthesis

<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 ) (mol mol(^{-1}))</td>
<td>0.05, 0.1, 0.2</td>
<td>0.59, 0.83, 0.95</td>
</tr>
<tr>
<td>( D ) (( \mu )mol m(^{-2}) s(^{-1}))</td>
<td>1.5, 3, 6</td>
<td>0.86, 0.83, 0.82</td>
</tr>
<tr>
<td>( g ) (( \mu )mol m(^{-2}) s(^{-1}))</td>
<td>0.5, 1, 2</td>
<td>0.82, 0.83, 0.86</td>
</tr>
<tr>
<td>( c_i ) (( \mu )mol mol(^{-1}))</td>
<td>190, 380, 760</td>
<td>0.82, 0.83, 0.86</td>
</tr>
<tr>
<td>( b ) (%)</td>
<td>15, 29, 58</td>
<td>0.45, 0.83, 0.99</td>
</tr>
<tr>
<td>( a ) (%)</td>
<td>2.2, 4.4, 8.8</td>
<td>0.85, 0.83, 0.80</td>
</tr>
<tr>
<td>( \delta^{13}C_D ) (‰)</td>
<td>-25, -28, -31</td>
<td>0.70, 0.83, 0.95</td>
</tr>
</tbody>
</table>
predicted $P/D$ in the sensitivity analysis (Table III). 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 concentrations of branch sections covered with aluminum foil compared with adjacent, uncovered sections (Table II). 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 aluminum 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 aluminum foil for 4 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 photos respiration 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 that 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, wherein 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 *P. 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 CO$_2$, whereas bark primarily uses internally produced CO$_2$. This advantage in terms of water use efficiency likely contributes to the drought tolerance of smooth-barked eucalypts. Drought tolerance is presumably one of the key features that led 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 characteristic 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 (Fig. 1). This strategy would appear to provide the benefits of both fire protection by thick dead bark on the lower stem and maintenance of the 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 overstory 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*. 

Corticular Photosynthesis in a Smooth-Barked Eucalypt
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 a capacity for corticular photosynthesis as woody tissues increase in diameter with increasing age. Corticular photosynthesis provides an effective mechanism for recycling respired CO₂ 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 southeast 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 recently been 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 aluminum 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 aluminum 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 branches at heights above the ground ranging from 6 to 10 m. The branches were harvested 4 years later, in September 2008. Foil was initially applied to 12 branches. When we returned 4 years later, we were able to relocate 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 disc was taken from the center of the foil-covered section. Discs had a width of approximately 1 cm. The disc was ground to a fine powder for isotopic and elemental analyses. The disc was accessed with a 16-m elevated work platform (cherry picker). Foil was applied to branches at heights above the ground ranging from 6 to 10 m. The branches were harvested 4 years later, in September 2008. Foil was initially applied to 12 branches. When we returned 4 years later, we were able to relocate 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.

The δ13C 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 analyzer (ECs 4010; Costech Analytical Technologies) linked via a continuous-flow interface to a stable isotope mass spectrometer (Delta XP; Finnigan MAT). The δ13C of the wood and bark dry matter was determined on subsamples of approximately 1 mg, which was pyrolyzed 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 at Washington State University in Pullman, Washington. The precision of isotopic analyses, based on the sd of repeated measurements of working standards during the sample runs, was 0.2‰ for δ13C and 0.1‰ for δ18O. The δ13C and δ18O values have been expressed relative to the Vienna Standard Mean Ocean Water and PeeDee Belemnite 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 discs taken 3 cm from either end of the foil-covered section were averaged for the uncovered values.

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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