Variations in relative stomatal and biochemical limitations to photosynthesis in a young blackbutt (Eucalyptus pilularis) plantation subjected to different weed control regimes

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Summary Foliar gas exchange and carbon (δ13C) and oxygen (δ18O) isotope ratios were measured in a young blackbutt (Eucalyptus pilularis Sm.) plantation subjected to four weed control treatments defined by the width of the weed-free strip maintained for the first 12 months after planting. Treatments were: 2-m-wide weed-free strip (50% of plot area, 2.0MWC), 1.5-m-wide weed-free strip (37.5% of plot area, 1.5MWC), 1-m-wide weed-free strip (25% of plot area, 1.0MWC) and no weed control (NWC). Our objectives were to determine (1) if decreasing the width of the weed control strip (decreasing herbicide use) affected growth and leaf photosynthesis of the plantation, and (2) the effects of the weed control regimes on variations in relative stomatal and biochemical limitations to photosynthesis. Trees in the 1.0MWC treatment had lower foliar light-saturated photosynthetic rate (A sat) than trees in the 2.0MWC treatment. An increase in metabolic limitation was responsible for the decrease in A sat in the 1.0MWC trees, which was also partly confirmed by the isotopic data. Compared with trees in the 1.0MWC, 1.5MWC and 2.0MWC treatments, A sat of NWC trees was significantly lower, a difference that was attributable mainly to stomatal limitation and to a lesser extent to biochemical limitation. The results support the conclusion that different weed control regimes cause differences in relative stomatal and biochemical limitations to photosynthesis. This report contributes to a growing body of literature on competition for soil resources between trees and weeds. Our results highlight the usefulness of the stable isotopic method in supporting analysis of the response of net photosynthesis to varying intercellular CO2 concentration for determining the relative stomatal and non-stomatal limitations to photosynthesis under experimental conditions in the field.

Keywords: A/Ci curve, δ13C, δ18O.

Introduction

Plant growth depends on a series of physical and biochemical processes that transfer CO2 from the surrounding air to organic molecules and, for this reason, much work has focused on the assessment of relative limitations to CO2 assimilation rates imposed by stomatal and non-stomatal processes in trees subjected to environmental stresses (Stewart et al. 1995, Wilson et al. 2000, Tissue et al. 2005). Changes in soil water and nutrient availability resulting from silviculture may influence both the physical and biochemical processes in tree crowns. Understanding the effects of silvicultural treatments on soil water and nutrient availability and, therefore, the physiology of tree crowns, is useful for the successful establishment of forest plantations in resource-limited environments. Leaf nitrogen (N) concentration is closely related to photosynthetic capacity in many eucalypt species (Leuning et al. 1991, Sheriff and Nambiar 1991, Macfarlane et al. 2004). Up to 75% of leaf N may be confined to the chloroplasts, and 30–50% of this N may be present in ribulose bisphosphate carboxylase (Rubisco) (Evans 1989). In addition to Rubisco, N is a necessary component of other photosynthetic enzymes involved in light harvesting and electron transfer (Evans and von Caemmerer 1996). Changes in soil water availability may affect tree water potential and therefore alter canopy-scale stomatal conductance, which is closely related to changes in plant water status (Herrick et al. 2004). In addition, trees subjected to water stress may suffer from foliar metabolic impairment (Flexas et al. 2004).

Weed control with herbicide treatment decreases the competition for water and nutrients and benefits the survival and productivity of forest plantations during establishment (Wilkinson and Neilsen 1990, Adams et al. 2003, Pitelli et al. 2003, Goncalves et al. 2004). The importance of competition for water or nutrients, particularly N, between weeds and trees and the impact of such competition on tree photosynthesis has been well documented (Sands and Nambiar 1984, Ellis et al. 1985, Woods et al. 1992). However, studies on how weed competition changes the relative stomatal and non-stomatal limitations to foliar photosynthesis are rare, especially under field conditions.
conditions, although an understanding of the processes involved in competition between weeds and trees is fundamental to defining appropriate weed control practice (Nambari and Sands 1993).

Analysis of A/Ci curves—the response of net photosynthesis (A) to varying intercellular CO₂ concentration (Ci)—has frequently been used to separate the stomatal and non-stomatal limitations to foliar photosynthesis of water- (Stewart et al. 1995, Wilson et al. 2000, Lawlor 2002, Flexas et al. 2004, Grassi and Magnani 2005) or nutrient-limited plants (Tissue et al. 2005). However, it is unclear whether this kind of analysis can be applied reliably to trees subject to weed competition because of two sources of uncertainty in the interpretation of the photosynthetic responses to Ci. The first involves the estimation of Ci; patchy stomatal closure (Buckley et al. 1997) and changes in cuticular conductance (gᵢ) to vapor pressure (Boyer et al. 1997) may result in the overestimation of Ci and invalidate the A/Ci curves. The second uncertainty involves the widely used Farquhar et al. (1980) model of photosynthesis that assumes that liquid-phase transfer conductance (gᵢ) is infinitely large, whereas more recent studies show that this assumption is incorrect and that gᵢ of eucalypt leaves is small enough to impose a significant limitation on photosynthesis (Harley et al. 1992a, Bernacchi et al. 2002, Ethier and Livingston 2004, Warren and Adams 2006). This suggests that the non-stomatal limitation to photosynthesis could be imposed by both a biochemical process and gᵢ.

Alternative approaches for determining relative stomatal and biochemical limitations to photosynthesis include analyses of leaf carbon (δ¹³C) and oxygen (δ¹⁸O) isotope compositions. The isotopic method provides a time-integrated measure of the gas exchange process (Farquhar et al. 1989, Xu et al. 2000, Prasolova et al. 2001). Furthermore, foliar δ¹⁸O provides an estimate of the assimilation-rate-weighted value of Ci, whereas gas exchange is a conductance-weighted value of Ci. Therefore, the overestimation of foliar Ci resulting from stomatal heterogeneity by instantaneous measurements of gas exchange can be avoided by analyzing foliar δ¹³C (Brugnoli and Lauteri 1991). Based on analyses of δ¹³C and δ¹⁸O in C₃ plants, Scheidegger et al. (2000) have developed a conceptual model of the relationship between stomatal conductance and biochemical capacity that may arise as a result of changing environmental conditions. Cernusak et al. (2005) suggested that the combination of δ¹³C and δ¹⁸O analyses allows interpretation of the response of leaf tissue δ¹³C to environmental effects on foliar Ci caused by variation in either biochemical capacity or stomatal conductance.

In subtropical Australia, it is a common practice to maintain a 2-m-wide weed-free strip along each planting row for the first 12 months during hardwood plantation establishment (DPI Forestry 2004). However, a goal of the plantation forestry industry is to reduce herbicide use to limit possible herbicide transport to surface and ground waters (DPI Forestry 2004). We established an experiment to examine the growth response of a young blackbutt (Eucalyptus pilularis Sm.) plantation to four weed control treatments (different quantities of herbicide use) and to determine the physiological mechanisms underpinning the response. Our objectives were to assess: (1) the growth response to four weed control regimes; (2) how foliar photosynthetic parameters are altered by weed control intensity; and (3) the relative limitations to photosynthesis imposed by stomatal and non-stomatal processes in response to the weed control treatments in the water- and nutrient-limiting environments of subtropical Australia.

Materials and methods

Experimental site and treatments

The experiment was located in Pechey, 140 km northwest of Brisbane, Australia. The site has freely draining, well-textured red Ferrosols (Soil Survey Staff 1999), with the predominant weather pattern giving cool dry winters and warm wet summers. Rainfall in this area is highly variable with a mean annual total of 851 mm. On average, winter temperatures range from 4 to 20 °C, and summer temperatures vary between 20 and 33 °C. Before plantation establishment in July 2005, the experimental site was covered with grass weeds dominated by kikuyu (Pennisetum clandestinum Chiov.). Trees were planted at 4 × 2.5 m spacing. Four treatments were arranged in a randomized block design, with four replicates. Each plot (24 × 67.2 m) had six rows of trees with 28 trees per row. Weeds were controlled with glyphosate (present as the isopropylamine salt) (1.5 kg ha⁻¹) in August and November 2005 and in August 2006 to keep the treatments free of weeds for at least 12 months. The four treatments were: (1) 2.0-m weed control (2.0MWC): a 2-m-wide strip centered on each tree row (50% of the plot area); (2) 1.5-m weed control (1.5MWC) a 1.5-m-wide strip centered on each tree row (37.5% of the plot area); (3) 1.0-m weed control (1.0MWC): a 1-m-wide strip centered on each tree row (25% of plot area); and (4) no weed control (NWC): no herbicide applied.

All the plots were fertilized with 275 kg ha⁻¹ MAP (N 10.0%, P 21.9%, S 2.3%) 4 weeks before planting. Blocks were geographically distinct, although there were no discernible gradients in site characteristics.

Growth and gas exchange measurements

Because of high variability and significant sampling error involved in measuring stem diameters of young trees, only height increments were measured as an indicator of growth. Tree heights were recorded in September 2005, March and December 2006 and June 2007.

Gas exchange measurements were conducted in December 2006 and June 2007 in fully expanded leaves located in the middle portion of the crown pointing north. Photosynthesis was measured with portable photosynthesis systems (Model LI-6400, Li-Cor) equipped with CO₂ control modules and light sources consisting of blue-red light-emitting diodes (Model 6400-02B). We determined A/Ci curves by measuring the response of A to varying Ci. External CO₂ partial pressures (Ci) were supplied in 11 steps, decreasing from 400 to 50 µmol mol⁻¹, and then increasing from 400 to 1200 µmol mol⁻¹, with irradiance (Q) maintained at a saturating value of 1500 µmol
Measurements were recorded automatically at each C	extsubscript{s} set point when photosynthesis had equilibrated, which was typically within 120 s. Photosynthetic light response curves (A/Q) were determined by measuring the response of A to varying Q at ambient CO	extsubscript{2} partial pressure. We reduced Q in nine steps from 2000 µmol s\(^{-1}\) to darkness. Measurements were recorded automatically at each value of Q when photosynthesis had equilibrated. The A/C	extsubscript{i} and A/Q curves were measured on the same foliage in the morning between 0900 and 1100 h over five consecutive days. The trees were measured in random order. Foliage temperature during determination of the A/C	extsubscript{i} and A/Q curves was maintained at 25 °C and humidity was near ambient. Leaf-to-air vapor pressure deficit was generally between 0.6 and 0.8 k Pa, reflecting ambient conditions. We measured four trees from each treatment giving a total of 16 A/C	extsubscript{i} and A/Q curves.

**Foliar N concentration, δ\textsuperscript{13}C, and δ\textsuperscript{18}O**

After the gas exchange measurements, leaves were collected for analyses of area, mass per area (LMA), N concentration, δ\textsuperscript{13}C and δ\textsuperscript{18}O. Leaf area was determined with a Li-Cor optical area meter (LI-3100). Leaves were oven dried at 60 °C for at least 76 h to determine dry mass. Dried leaf samples were ground, and analyzed for total N and C (% DW) by the Dumas ground, and analyzed for total N and C (% DW) by the Dumas ground, and analyzed for total N and C (% DW) by the Dumas ground, and analyzed for total N and C (% DW) by the Dumas ground, and analyzed for total N and C (% DW) by the Dumas ground, and analyzed for total N and C (% DW) by the Dumas ground. Foliar N, δ\textsuperscript{13}C and δ\textsuperscript{18}O were determined at Griffith University, Brisbane, Australia.

Photosynthetic parameters, stomatal and non-stomatal limitations to photosynthesis

Photosynthetic parameters \(V_{\text{max}}\) (maximum carboxylation velocity), \(J_{\text{max}}\) (maximum rate of electron transport) and \(R_{d}\) (mitochondrial respiration in the light per unit leaf area) were estimated from A/C\textsubscript{i} curves by nonlinear regression. Based on the general assumption that A is limited solely by the maximum rate of carboxylation at low C\textsubscript{i} (Farquhar et al. 1980), \(V_{\text{max}}\) and \(R_{d}\) were estimated from the lower region of the A/C\textsubscript{i} curves, where C\textsubscript{i} is < 150 µmol mol\(^{-1}\).

\[
A = \left(1 - \frac{0.5O}{\tau C_{i}}\right) \frac{V_{\text{max}}C_{i}}{C_{i} + K_{c}(1 + O/K_{c})} - R_{d}
\]

where \(O\) is the partial pressure of oxygen in the intercellular air space, \(\tau\) is the Rubisco specific factor, \(K_{c}\) and \(K_{o}\) are the Michaelis–Menten constants for CO\textsubscript{2} and O\textsubscript{2}, respectively (\(K_{c} = 275 \mu\text{mol mol}^{-1}\) and \(K_{o} = 420 \mu\text{mol mol}^{-1}\), and \(= 2321\) (Harley et al. 1992b), and \(R_{d}\) represents CO\textsubscript{2} evolution from mitochondria in the light, rather than that from photorespiratory carbon oxidation (Farquhar et al. 1980).

At high C\textsubscript{i}, A is limited by the regeneration of RuBP via electron transport. Hence, \(J_{\text{max}}\) can be estimated from A/C\textsubscript{i} curves by nonlinear regression when C\textsubscript{i} is > 250 µmol mol\(^{-1}\).

\[
A = \left(1 - \frac{0.5O}{\tau C_{i}}\right) \frac{J_{C_{i}}}{4(C_{i} + O/\tau)} - R_{d}
\]

where \(J\) is potential rate of electron transport and dependent on photon flux and \(J_{\text{max}}\):

\[
J = \frac{\alpha I}{\sqrt{1 + (\alpha I/J_{\text{max}})^{2}}}
\]

where I is absorbed photon flux and \(\alpha\) (0.24) is the efficiency of light conversion.

We analyzed A/Q curves to determine the maximum rate of photosynthesis in saturating light and ambient CO\textsubscript{2} (\(A_{\text{sat}}\)), maximum stomatal conductance (\(g_{\text{st}}\)), light compensation point (\(Q_{\text{comp}}\)) and rate of CO\textsubscript{2} evolution in darkness (\(R_{d}\)). The parameters \(A_{\text{sat}}\) and \(R_{d}\) were calculated as (Prioul and Chartier 1977):

\[
A = \Phi Q + A_{\text{sat}} - \sqrt{(\Phi Q + A_{\text{sat}})^{2} - 4\Phi Q k A_{\text{sat}}} - R_{d}
\]

where A is net photosynthetic rate, \(\Phi\) is maximum quantum yield, Q is irradiance, \(A_{\text{sat}}\) is light-saturated photosynthesis, \(k\) is convexity, and \(R_{d}\) is rate of CO\textsubscript{2} evolution in darkness.

The relative stomatal limitation to photosynthesis (\(L_{s}\)), an estimate of the proportion of the reduction in photosynthesis attributable to CO\textsubscript{2} diffusion between the atmosphere and the site of carboxylation, was determined from A/C\textsubscript{i} curves as (Farquhar and Sharkey 1982):

\[
L_{s} = \left(1 - \frac{A}{A_{\text{sat}}}\right)100
\]

where A is net photosynthetic rate at the growth C\textsubscript{i} (360 µmol
mol\(^{-1}\)) and \(A_s\) is the photosynthetic rate when \(C_i\) (360 µmol mol\(^{-1}\)) equals the growth \(C_a\). Under these conditions, \(A_s\) is the rate of photosynthesis that would occur if resistance to CO\(_2\) diffusion from the bulk atmosphere to the site of carboxylation were zero. For this calculation, mesophyll conductance was assumed to be infinitely large. The relative non-stomatal limitation to photosynthesis was determined as 100 – \(L_s\). The \(C/C_s\) ratio was estimated from \(A/C_i\) curves measured at saturating irradiance and ambient CO\(_2\) partial pressure.

**Statistical analysis**

A two-way repeated measures analysis of variance (ANOVA) was used to test for the main effects and interactions of sampling time and treatment on all parameters. Linear regression analyses were used to determine the significance of the relationships between foliar N and \(A_{sat}\), \(V_{max}\) or \(J_{max}\). The Student-Newman-Keuls (SNK) tests were used to compare between-treatment means. Throughout the text, differences were considered significant if \(P < 0.05\) and marginally significant if \(P < 0.10\).

**Results**

**Growth**

Weed control significantly affected growth, as determined by height increments in the establishing blackbutt plantation. Tree height increments increased with increasing intensity of weed control. For example, between December 2006 and June 2007, mean tree height increased by 81, 74 and 67 cm in the 2.0MWC, 1.5MWC and 1.0MWC treatments, respectively, whereas height of the NWC trees increased by only 6 cm in the same period (Figure 1).

**Photosynthetic parameters**

Foliar \(A_{sat}\) was significantly affected by treatments and sampling month. In both sampling months, foliar \(A_{sat}\) was the lowest in the NWC trees and increased with increasing weed control intensity. There were no significant differences in foliar \(g_{max}\) between trees in the 2.0 MWC, 1.5MWC and 1.0MWC treatments in either December 2006 or June 2007. The NWC trees had lower \(g_{max}\) than trees in the other treatments, but the differences were only marginally significant. In both sampling months, foliar \(Q_{comp}\) was significantly lower in the NWC trees than in trees in the other treatments. The NWC trees had less negative \(R_n\) values than trees in the other treatments (Table 1).

In both sampling months, the effects of treatments on \(V_{max}\) and \(J_{max}\) were pronounced. Among treatments, \(V_{max}\) was highest in 2.0MWC trees and significantly higher than in the 1.5MWC, 1.0MWC and NWC trees. In June 2007, \(J_{max}\) in trees in the 2.0MWC treatment was significantly higher than in the other treatments. There were no significant differences in \(V_{max}\) between trees in the 1.5MWC, 1.0MWC and NWC treatments in either sampling month. In December 2006, \(J_{max}\) was lower in NWC trees than in 1.5MWC and 1.0MWC trees, but the differences were only marginally significant (Table 2).

The \(L_n\) was significantly higher in NWC trees than in 2.0MWC, 1.5MWC and 1.0MWC trees. The \(C/C_s\) ratio was significant higher in NWC trees than in 1.5MWC and

![Figure 1. Effects of different weed control treatments on mean tree height growth September 2005 and June 2007. Within the same measuring month, means followed by the same letters do not differ significantly between treatments by the SNK test (\(P > 0.05\)). No significant differences in mean tree height among treatments were found in September 2005.](https://academic.oup.com/treephys/article-abstract/28/7/997/1674719)

**Table 1. Effects of different weed control treatments on photosynthetic parameters derived from \(A/Q\) curves.** Light-saturated photosynthetic rate (\(A_{sat}\)), maximum stomatal conductance (\(g_{max}\)), photosynthetic light compensation point (\(Q_{comp}\)) and night respiration (\(R_n\)). Significance of main effects of treatment and sampling month are shown as \(P < 0.05\), \(P < 0.10\) or not significant (ns). There were no significant interactions (\(P > 0.10\)) between treatment and sampling month on the selected photosynthetic parameters. Within a column, means followed by different uppercase letters indicate that means differ significantly (\(P < 0.05\)) between treatment (\(n = 4\)). Within a column, means followed by different lowercase letters indicate that differences between treatments are marginally significant at \(P < 0.10\).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(A_{sat}) (µmol m(^{-2}) s(^{-1}))</th>
<th>(g_{max}) (µmol m(^{-2}) s(^{-1}))</th>
<th>(Q_{comp}) (µmol m(^{-2}) s(^{-1}))</th>
<th>(R_n) (µmol m(^{-2}) s(^{-1}))</th>
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</thead>
<tbody>
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<td>Dec/06</td>
<td>Jun/07</td>
</tr>
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<td>15.5Aa</td>
<td>229Aa</td>
<td>215Aa</td>
</tr>
<tr>
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<td>15.3Aab</td>
<td>223Aa</td>
<td>210Aa</td>
</tr>
<tr>
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<td>14.5Aab</td>
<td>221Aa</td>
<td>213Aa</td>
</tr>
<tr>
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<td>Treatment &lt; 0.10</td>
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1.0MWC trees. Values of $R_d$ were significantly less negative in NWC trees than in trees in the other treatments (Table 2).

### Foliar nitrogen, stable isotope compositions and leaf mass per area

Weed control and sampling month significantly affected foliar N concentration on both a mass ($N_{mass}$) and an area basis ($N_{area}$) (Table 3). In both sampling months, $N_{mass}$ was significantly higher in the 2.0MWC trees than in the 1.0MWC and NWC trees. There were no significant differences in $N_{mass}$ between trees in the 1.5 MWC, 1.0MWC and NWC treatments. However, $N_{area}$ was significantly lower in the NWC trees than in the 1.5MWC or 1.0MWC trees in December 2006. Compared with leaf samples in December 2006, $N_{mass}$ sampled in June 2007 declined by 0.21, 0.10 and 0.08% for trees in the 2.0MWC, 1.5MWC and 1.0MWC treatments, respectively. The correlations between foliar $N_{area}$ and $A_{sat}$, $V_{max}$ or $J_{max}$ suggest that 76, 53 and 43% of the variation in $A_{sat}$, $V_{max}$ and $J_{max}$ are attributable to foliar $N_{area}$ respectively (Figure 2).

Foliar $\delta^{13}C$ in NWC trees was significantly higher than in 2.0MWC, 1.5MWC and 1.0MWC trees, and averaged $-26.1\%e$ in December 2006 and $-25.9\%e$ in June 2007. However, there was no significant difference in foliar $\delta^{13}C$ among the 2.0MWC, 1.5MWC and 1.0MWC treatments in December 2006 or in June 2007. The weed control treatments significantly affected foliar $\delta^{18}O$, and the NWC trees had significantly higher foliar $\delta^{18}O$ than the 2.0MWC, 1.5MWC and 1.0MWC trees in both sampling months. Foliar $\delta^{18}O$ values in the 1.5MWC and 1.0MWC trees were higher than in the 2.0MWC trees, but the differences were only significant at $P < 0.10$. Foliar mass/area ratio (LMA, g m$^{-2}$) was significantly affected by the weed control treatments, but not by the sampling month (Table 3).

### Discussion

Growth and leaf-level physiology of the young blackbutt plantation were responsive to the weed control regimes. The observation that tree growth increased with weed control intensity is not unique; foresters throughout the world rely on the use of herbicides to improve the growth of eucalypts, especially in the early stage of forest plantations (George and Brennan 2002, Adams et al. 2003, Pitelli et al. 2003, Little and van Staden 2005). The water and N status of trees subjected to dif-

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**Table 2. Effects of different weed control treatments on photosynthetic parameters derived from A/C$_{i}$ curves. Maximum rate of Rubisco carboxylation ($V_{\text{max}}$), maximum electron transport rate ($J_{\text{max}}$), relative stomatal limitation to photosynthesis ($L_s$), the ratio of internal CO$_2$ partial pressure to atmospheric CO$_2$ partial pressure ($C_{i}/C_{a}$), and dark respiration ($R_d$). Significance of main effects of treatment and sampling month are shown as $P < 0.05$, $P < 0.10$ or not significant (ns). There are no significant interactions ($P > 0.10$) between treatment and sampling month. Within a column, means followed by different uppercase letters indicate that differences between treatments are marginally significant at $P < 0.10$.**

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<th>Parameters</th>
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<th>$V_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$) Jun/07</th>
<th>$J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$) Dec/06</th>
<th>$J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$) Jun/07</th>
<th>$L_s$ (%) Dec/06</th>
<th>$L_s$ (%) Jun/07</th>
<th>$100 - L_s$ (%) Dec/06</th>
<th>$100 - L_s$ (%) Jun/07</th>
<th>$C_{i}/C_{a}$ Dec/06</th>
<th>$C_{i}/C_{a}$ Jun/07</th>
<th>$R_d$ (µmol m$^{-2}$ s$^{-1}$) Dec/06</th>
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**Table 3. Effects of different weed control treatments on foliar N concentration, $\delta^{13}C$, $\delta^{18}O$ and leaf mass per area (LMA) as measured in December 2006 and June 2007. Significance of main effects of treatment and sampling month are shown as $P < 0.05$, $P < 0.10$ or not significant (ns). There are no significant interactions ($P > 0.10$) between treatment and sampling month. Within a column, means followed by different uppercase letters (A, B) indicate that means differ significantly ($P < 0.05$) between treatment ($n = 4$). Within a column, means followed by different lowercase letters (a, b) indicate that differences between treatments are marginally significant at $P < 0.10$.**

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<tr>
<th>Parameters</th>
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<th>$N_{mass}$ (%) Jun/07</th>
<th>$N_{area}$ (g m$^{-2}$) Dec/06</th>
<th>$N_{area}$ (g m$^{-2}$) Jun/07</th>
<th>Leaf $\delta^{13}C$ (%e) Dec/06</th>
<th>Leaf $\delta^{13}C$ (%e) Jun/07</th>
<th>Leaf $\delta^{18}O$ (%) Dec/06</th>
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<th>LMA (g m$^{-2}$) Dec/06</th>
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<td>1.32Bb</td>
<td>1.24B</td>
<td>1.98B</td>
<td>1.89Ab</td>
<td>-27.19A</td>
<td>-26.50A</td>
<td>22.45Ab</td>
<td>22.06Ab</td>
<td>149.7A</td>
<td>152.4A</td>
</tr>
<tr>
<td>NWC</td>
<td>1.24B</td>
<td>1.25B</td>
<td>1.66C</td>
<td>1.75B</td>
<td>-26.10B</td>
<td>-25.90B</td>
<td>25.78B</td>
<td>25.61B</td>
<td>133.8B</td>
<td>139.6B</td>
</tr>
</tbody>
</table>

ANOVA Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05 Treatment < 0.05
and 17.1 µmol m⁻² s⁻¹ in the two sampling months, which was 2.0MWC trees, the 1.0MWC trees had a lower age (Clearwater and Meinzer 2001, Whitehead and Beadle 2005). Compared with the 2.0MWC trees, implying that the biochemical limitation may be more important than stomatal limitation in controlling the apparent decline in A_sat in 1.0MWC trees (Farquhar and Sharkey 1982, Xu 1997).

We examined the long-term effects of the weed control treatments on photosynthetic biochemical capacity and stomatal conductance by estimating C_i from δ¹³C and transpiration from δ¹⁸O. The absence of significant differences in foliar δ¹³C between the 2.0MWC and 1.0MWC trees in either sampling months suggests little variation in C_i between these treatments (Farquhar et al. 1982, 1989). This balance arises if (1) increases in photosynthetic capacity are accompanied by proportional increases in stomatal conductance, or (2) decreases in stomatal conductance are accompanied by proportional decreases in photosynthetic capacity (Farquhar et al. 1982). In both sampling months, foliar δ¹⁸O values were higher (P < 0.10) in 1.0MWC trees than in 2.0MWC trees, suggesting the latter possibility. The results from the isotopic analysis partly agree with the results of our A/C_i analysis and suggest a lower foliar biochemical capacity in the 1.0MWC trees than in the 2.0MWC trees. We hypothesize that this may be attributed to lower foliar N concentration in the 1.0MWC trees (Table 3). The positive relationships between A_sat, V_cmax or J_max and foliar N concentration that we found (Figure 2) are a well-known consequence of the large N investment in proteins associated with the photosynthetic system (Evans 1989, Grassi et al. 2002).

Internal conductance was not taken into account to predict time-integrated C_i through δ¹³C in this study. However, studies have revealed that the value of δ¹³C is primarily determined by the CO₂ concentration within the chloroplast (C_i) rather than by C_i (Evans et al. 1986, Flanagan et al. 1994, Gillon and Griffiths 1997). Thus, the foliar δ¹³C values are affected by photosynthetic capacity, internal conductance to CO₂ diffusion from intercellular air spaces to the site of carbon fixation (internal transfer conductance, g_i), and stomatal conductance. Some studies (Vitousek et al. 1990, Macfarlane et al. 2004) have shown that LMA has a significant relationship with foliar g_i. The lack of significant differences in LMA (Table 3) between the 2.0MWC and 1.0MWC trees, therefore, suggests that the variation in foliar δ¹³C may mainly be associated with variations in photosynthetic capacity and stomatal conductance in this study. However, further investigations on foliar g_i are needed to substantiate the conclusion.

Compared with the 1.0MWC, 1.5MWC and 2.0MWC trees, the A_sat in NWC trees was significantly lower (P < 0.05, Table 1), which may be attributable to both higher stomatal and non-stomatal limitations. However, we argue that greater relative stomatal limitation apparently dominated the significant decline of A_sat in NWC trees because we found that L_s was significantly higher in NWC trees than in 1.0MWC, 1.5MWC or 2.0MWC.
2.0MWC trees (Table 2) (Farquhar and Sharkey 1982, Xu 1997). In addition, lower C/Ci ratios in NWC trees in both sampling months compared with the 1.0MWC, 1.5MWC or 2.0MWC trees, indicate greater stomatal limitation in the NWC trees (Table 2) (Ball and Berry 1982, Tissue et al. 2005). We measured δ13C and δ18O of leaf samples to compare the variations in time-integrated stomatal conductance and biochemical capacity as affected by different weed control regimes. The significant increase in foliar δ13C in the NWC trees compared with the 1.0MWC, 1.5MWC or 2.0MWC trees, indicates a significant decrease in C. This can be caused either by increased photosynthetic capacity (at a constant g.), or by decreased stomatal conductance (at a constant photosynthetic capacity) (Scheidegger et al. 2000). The NWC treatment resulted in significantly higher foliar δ18O values compared with the 1.0MWC, 1.5MWC and 2.0MWC treatments (P < 0.05), indicating that the significant decreases in foliar Ci in the NWC trees were mainly attributable to a significant stomatal reaction, whereas biochemical capacity was relatively less affected (Barbour and Farquhar 2000, Scheidegger et al. 2000). Thus, the isotopic data agree with our A/Ci analysis.

The Qcomp may be seen as a balance between photosynthesis and respiration. The lower Qcomp in the NWC trees than in the 1.0MWC, 1.5MWC or 2.0MWC trees (Table 1) may be partly related to a low respiration rate (Tables 2 and 3) and, in turn, a low foliar N concentration (Prióul and Chartier 1977, Reich et al. 1998, Tjoelker et al. 1999). Generally, variation in LMA has been attributed to variation in irradiance in the forest canopy (Niinemets et al. 1998, Bond et al. 1999). Our NWC trees had less crown biomass and received higher irradiances per leaf than the 1.0MWC, 1.5MWC and 2.0MWC trees (data not shown). However, LMA in the NWC trees was significantly smaller than in trees in the weed control treatments, suggesting that other factors, for example competition for soil water, are more important determinants of LMA in this young blackbutt plantation (Niinemets et al. 1998, Koch et al. 2004).

Identification of the mechanism driving improved tree growth in response to weed control is complicated by simultaneous changes in water and nutrient availability (Robin and Ketchum 2002). We did not determine the leaf biomass responses of the young blackbutt plantation to the different weed control treatments. However, our analyses of foliar A/Ci curves and N concentration agree with Woods et al. (1992), who suggested that decreasing the width of the weed-control strip spanning the tree row (e.g., from 2.0 m to 1.0 m) might significantly decrease N uptake by the trees. Therefore, more N (e.g., through fertilization) is needed when less intensive weed control is applied during the first two years of plantation establishment. Compared with the 1.0MWC, 1.5MWC or 2.0MWC trees, the relative stomatal limitation to A_sat in the NWC trees significantly increased, perhaps indicating that the NWC trees experienced severe water stress as suggested by Adams et al. (2003) and Sand and Nambiar (1984). Estimating stomatal and non-stomatal limitations to photosynthesis under different weed control regimes, as shown in our study, provides a useful basis for optimizing the management of blackbutt plantation in both water- and nutrient-limiting environments, and the potential exists for their application in other tree species plantations under similar environmental conditions. Our data add to a growing body of literature on competition for soil resources between trees and weeds and highlight the usefulness of stable isotopic method in supporting the A/Ci analysis of stomatal limitation to photosynthesis in the field.

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Reference


