

# Increased photosynthesis following partial defoliation of field-grown *Eucalyptus globulus* seedlings is not caused by increased leaf nitrogen

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Received July 12, 2006; accepted January 18, 2007; published online July 3, 2007

**Summary** Increased photosynthetic rates following partial defoliation may arise from changes in leaf biochemistry, water relations or nutrient status. Twelve-month-old field-grown *Eucalyptus globulus* Labill. seedlings were pruned from below to reduce the green crown depth by 50 (D50) or 70% (D70). Photosynthetic responses to light and CO<sub>2</sub> concentration were examined before and one, three and five weeks after partial defoliation. One week after defoliation, photosynthetic rates were greater in seedlings in the D50 (21 μmol m<sup>-2</sup> s<sup>-1</sup>) and D70 (23 μmol m<sup>-2</sup> s<sup>-1</sup>) treatments than in control seedlings (15 μmol m<sup>-2</sup> s<sup>-1</sup>); however, there was little difference in photosynthetic rates between partially defoliated seedlings and control seedlings after 5 weeks. An analysis of the sensitivity of photosynthesis to biochemical parameters revealed that the transient increase in photosynthetic rate in response to partial defoliation was largely a function of the maximum rate of carboxylation (85–87%) and the maximum rate of RuBP regeneration (55–60%) rather than stomatal conductance (12–13%). Nitrogen increased in leaves following partial defoliation (increases of 0.6 and 1.2 g m<sup>-2</sup> for D50 and D70, respectively), but was accumulated in a non-photosynthetic form (i.e., there was no increase in nitrogen concentration of Rubisco or chlorophyll). Increased photosynthetic rates immediately following partial defoliation were primarily a result of increased activity rather than amount of photosynthetic machinery. There was no evidence that phosphorus was responsible for the increase in photosynthetic rates after partial defoliation.

**Keywords:** carbohydrates, chlorophyll, foliar respiration, phosphorus pools, pruning, Rubisco, sensitivity analysis, stomatal conductance.

## Introduction

For the past two decades, the evergreen species *Eucalyptus globulus* Labill. has dominated newly established hardwood plantations in southern Australia (totalling 155,000 ha; BRS 2007), and *E. globulus* plantations are expanding at about 25,000 ha per annum (Keenan et al. 2004), primarily on ex-agricultural land. *Eucalyptus globulus* is also an economically

important plantation species in other countries, especially Spain, Portugal, Chile and Brazil (Brown 2000).

Defoliation of *E. globulus*, either by insect herbivores (Jordan et al. 2002) or by deliberate management (branch-pruning to produce wood clear of knots; Pinkard 2003) is common and of increasing significance to production and environmental values. Rapid reductions in photosynthetic area usually initiate a range of physiological responses in plants, and the size of the response is generally a function of the severity of defoliation (Reich et al. 1993). Increased rates of photosynthesis after partial defoliation have been measured in a range of plant types including: grasses (Wallace et al. 1984); the herb *Phaseolus vulgaris* L. (Alderfeder and Eagles 1976, von Caemmerer and Farquhar 1984); conifers *Pinus resinosa* Ait. (Reich et al. 1993), *Larix decidua* Mill. and *Pinus uncinata* Mill. (Handa et al. 2005); broad-leaved deciduous trees *Populus tremuloides* Michx. (Hart et al. 2000), *Acer rubrum* L. and *Quercus rubra* L. (Heichel and Turner 1983); and broad-leaved evergreens *Prosopis juliflora* (Swartz) DC (Elfadl and Luukkanen 2003), *Eucalyptus nitens* (Deane and Maiden) Maiden (Pinkard and Beadle 1998) and *E. globulus* (Pinkard 2003).

Corresponding increases in stomatal conductance ( $g_s$ ) could indicate that enhanced water availability (e.g., because of increased root:leaf area) explains increased photosynthetic rates after partial defoliation (Fay et al. 1993, Reich et al. 1993, Morrison and Reekie 1995). Source–sink changes, such as the potential for partial defoliation to reduce feedback inhibition of photosynthesis, have also been proposed as causes of changes in photosynthetic rates after partial defoliation (Gezelius et al. 1981, Tschaplinski and Blake 1994, 1995). Although remobilization of assimilates is not strictly associated with defoliation (Lavigne et al. 2001), retained “source” leaves generally have reduced starch and soluble sugar concentrations when whole leaves are removed from above (Zhou and Quebedeaux 2003) or below (Cerasoli et al. 2004).

After partial defoliation, photosynthetic carbon fixation may increase in response to an increased flow of nitrogen (N) to remaining leaves (Neales and Incoll 1968, Gezelius et al. 1981, Lavigne et al. 2001, Ozaki et al. 2004, Handa et al. 2005). One mechanistic explanation for the dependence of in-

creased photosynthesis on N is the large proportion of leaf N in chlorophyll and Rubisco (Evans 1989). However, many studies have shown increased photosynthesis following defoliation with either no change in leaf N concentration (Ovaska et al. 1993a, 1993b, Reich et al. 1993, Pinkard et al. 1998, Volin et al. 2002) or reduced leaf N concentration (Vanderklein and Reich 2000). A few studies have examined the N–photosynthesis relationship in detail (Close et al. 2004). For example, relationships among partial defoliation, N allocation among photosynthetic pools within foliage and photosynthesis were examined in the perennial crop *P. vulgaris* (von Caemmerer and Farquhar 1984) and the deciduous tree *Betula pendula* Roth. (Ovaska et al. 1993a). In both studies, increased photosynthesis was associated with increased Rubisco activity rather than with a change in measured or estimated amounts of Rubisco.

Phosphorus (P) is also required for photosynthesis, and its foliar concentration may increase in response to partial defoliation, which could explain the photosynthetic responses to defoliation. However, the relationship is not well understood as recent studies of tree species found either no relationship between P and photosynthesis (*Eucalyptus nitens*; Pinkard et al. 1998) or a reduction in P despite increased photosynthesis (*Quercus ilex* L.; Cherbuy et al. 2001).

The response of photosynthesis to changing CO<sub>2</sub> concentration ([CO<sub>2</sub>]) is a useful guide to the biochemical determinants of photosynthesis, yet this approach has seldom been adopted in studies of defoliation. The limited available data show that enhanced rates of both RuBP regeneration ( $J_{\max}$ ) and Rubisco carboxylation ( $V_{\max}$ ) are related to increased photosynthesis in herbs and broad-leaved species (von Caemmerer and Farquhar 1984, Layne and Flore 1992, Ovaska et al. 1993a) including *E. nitens* (Pinkard and Beadle 1998). We lack definitive evidence as to whether increased  $J_{\max}$  and  $V_{\max}$  in eucalypts arise from an increased activation state (e.g., von Caemmerer and Farquhar 1984, Layne and Flore 1992, Ovaska et al. 1993a) or greater amounts of Rubisco.

We aimed to elucidate the relationships between photosynthesis and N, P and leaf N allocation between Rubisco and chlorophyll in young leaves of the evergreen species *E. globulus*. Specifically, we tested the hypotheses that, in response to partial defoliation, photosynthesis would: (1) initially increase as a function of rapid increases in  $g_s$ ; (2) as a function of a slower increase in biochemical capacity (as measured by  $J_{\max}$  and  $V_{\max}$ ), be correlated with increased concentrations of leaf N and P; and (3) be correlated with increased concentrations of Rubisco and chlorophyll.

## Materials and methods

### Study site

The study was conducted in a 12-month-old *Eucalyptus globulus* plantation (1200 stems ha<sup>-1</sup>, mean tree height of 1.8 m) near Ballarat, south-west Victoria, Australia (37°3' S, 143°5' E, 470 m a.s.l.). Mean annual rainfall for the area is 600 mm (mean annual evaporation being 1200 mm), mean an-

nual maximum temperature is 15 °C, and mean annual minimum temperature is 6 °C. During the experiment, temperatures ranged from -0.3 to 35.3 °C (averaging 20 °C), and there was 100 mm of rainfall (Australian Bureau of Meteorology). Soils are silty clay loams derived from shallow Palaeozoic sediments (Robinson et al. 2003) that contained 6–12 mg kg<sup>-1</sup> N and 6–13 mg kg<sup>-1</sup> P before planting (D. Bristow, East Gippsland Plantation Company of Australia Pty., pers. comm.). Immediately following planting, trees were fertilized with N, P, potassium, sulfur, copper and zinc (18,9,15,1,1,1) at a rate of 19 kg ha<sup>-1</sup> of N (D. Bristow, pers. comm.).

### Partial defoliation

Five trees were randomly selected for each of three defoliation treatments: 70 (D70) and 50% (D50) of canopy height defoliated and undefoliated (control). Trees were partially defoliated in November 2003 by pruning whole branches from below.

### Sampling strategy

Photosynthetic measurements were conducted on recently detached leaves (recut under water, no effect on  $g_s$  was observed) immediately before partial defoliation (Week 0) and 1 (Week 1), 3 (Week 3) and 5 (Week 5) weeks after partial defoliation. The youngest fully expanded leaf was measured.

### Gas exchange measurements

Gas exchange was measured with a portable infrared gas analyzer (LI-6400 with 6 cm<sup>2</sup> chamber and LED light source, Li-Cor, Lincoln, NE). Leaves were exposed to a [CO<sub>2</sub>] of 350 μmol mol<sup>-1</sup>, leaf temperatures varied between 23 and 26 °C, and airflow through the chamber was 250 μmol s<sup>-1</sup>. Vapor pressure deficit (VPD) approximated ambient conditions, and, among all measurement dates, varied between 0.93 and 1.85 kPa. Treatments and replicates were measured randomly, and there was no difference in VPD among treatments.

Leaves were acclimated to a saturating photosynthetic photon flux (PPF; 2000 μmol m<sup>-2</sup> s<sup>-1</sup>) until photosynthetic rates stabilized. The PPF was then decreased, in 11 steps, to 0 μmol m<sup>-2</sup> s<sup>-1</sup>. At each step, three consecutive measurements were logged at 30-s intervals to obtain a mean value. The rate of photosynthesis at 2000 μmol m<sup>-2</sup> s<sup>-1</sup> was taken to be the maximum photosynthetic rate ( $A_{\max}$ ). Dark respiration rate ( $R_d$ ) was measured after leaves had been kept in darkness for at least 5 min, by which time gas exchange rates had stabilized. The CO<sub>2</sub> response curves were measured with a PPF of 2000 μmol m<sup>-2</sup> s<sup>-1</sup>, leaf temperatures of 23 to 26 °C and an airflow (through the chamber) of 250 μmol s<sup>-1</sup>. Leaves were first acclimated to a [CO<sub>2</sub>] of 350 μmol mol<sup>-1</sup> and a PPF of 2000 μmol m<sup>-2</sup> s<sup>-1</sup>, then the [CO<sub>2</sub>] was raised to 1800 μmol mol<sup>-1</sup> and decreased in 10 steps to 50 μmol mol<sup>-1</sup>. At each step, three consecutive measurements were logged at 30-s intervals to obtain a mean value.

Photosynthetic responses to [CO<sub>2</sub>] were fitted to the biochemical model developed by Farquhar et al. (1980), as modified by von Caemmerer and Farquhar (1981), Harley and Sharkey (1991) and Harley et al. (1992). Kinetic constants and

temperature dependencies of Harley et al. (1992) were used without including the limitation by triose phosphate utilization (TPU) because no limitation by TPU was observed at high  $[\text{CO}_2]$  (Wullschlegel 1993). We estimated  $V_{\text{cmax}}$  and  $J_{\text{max}}$  from photosynthetic rate–intercellular  $\text{CO}_2$  concentration ( $A-C_i$ ) curves using the minimization routine in Photosyn Assistant 1.1 to produce the best fit.

#### *Sensitivity analysis of the factors contributing to treatment effects on photosynthesis*

Sensitivity analyses as described by Warren and Adams (2004) were performed to rank the gas exchange parameters  $C_i$ ,  $g_s$ ,  $R_d$ ,  $V_{\text{cmax}}$  and  $J_{\text{max}}$  in relation to the responses of  $A_{\text{max}}$  at Weeks 1 and 3 after partial defoliation. This involved the mean value of a parameter for the treatment (D50 or D70) replacing that of the control in the model, after which  $A_{\text{max}}$  (at a PPF of  $350 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was recalculated with the Farquhar et al. (1980) model. Because  $g_s$  is not a direct parameter in the Farquhar et al. (1980) model, the sensitivity of  $A_{\text{max}}$  to  $g_s$  was calculated from  $C_i$ :

$$C_i = C_a - \frac{A}{g_{s\text{CO}_2}} \quad (1)$$

where  $C_a$  is ambient  $[\text{CO}_2]$  and  $g_{s\text{CO}_2}$  is stomatal conductance to  $\text{CO}_2$ , calculated from  $g_s$ :

$$g_{s\text{CO}_2} = \frac{g_s}{1.6} \quad (2)$$

Altering the biochemical capacity for photosynthesis through  $R_d$ ,  $V_{\text{cmax}}$  and  $J_{\text{max}}$  affects  $C_i$  unless  $g_s$  also responds. Although some species retain a constant  $C_i$  with altered biochemical capacity, others retain a constant  $g_s$  and thus vary  $C_i$  (Poorter and Evans 1998). *Eucalyptus globulus* has been shown to vary both  $C_i$  and  $g_s$  as the biochemical capacity for photosynthesis changes (Warren and Adams 2004), so both scenarios were considered. The case for constant  $C_i$  (and therefore varying  $g_s$ ) was calculated by direct substitution of new values into the model and recalculating  $A_{\text{max}}$ . For the case of constant  $g_s$  (and therefore varying  $C_i$ ), Equations 1 and 2 were substituted into the model, and equations were solved iteratively for  $A_{\text{max}}$ .

#### *Leaf harvest and specific leaf area*

Each leaf used for photosynthetic measurements was split along the midrib, and one half was frozen immediately to  $-20^\circ\text{C}$  for no longer than one day (during which no browning was observed) before storage at  $-80^\circ\text{C}$  pending chemical analyses. The area of the other half was measured before drying at  $70^\circ\text{C}$  for 72 h. Dry mass was measured for determination of specific leaf area (SLA), and the leaf was ground in a mixer mill (MM301, Retsch, Haan, Germany).

#### *Nitrogen fractions*

About  $0.120 \pm 0.005$  g of ground leaf was analyzed for total N by Dumas combustion at  $900^\circ\text{C}$  (Leco CHN-2000, St. Joseph,

MI). Leaf N concentration was initially calculated on a dry mass basis and was converted to an area basis ( $N_a$ ;  $\text{g m}^{-2}$ ) based on SLA.

Chlorophyll was extracted from frozen leaf discs with dimethyl sulfoxide (DMSO) at  $65^\circ\text{C}$  for 30 minutes (Hiscox and Israelstam 1979, Richardson et al. 2002). Preliminary experiments established that a single extract yielded  $>98\%$  of total chlorophyll. The sample was made up to 2 ml with DMSO before absorbance was measured at 645 and 663 nm. Chlorophyll a and b concentrations ( $\text{mol m}^{-2}$  leaf area) were calculated with the equations of Wellburn (1994). Chlorophyll N ( $N_{\text{Chl}}$ ) was calculated empirically: chlorophyll a = 6.3% N and chlorophyll b = 6.2% N (Hall and Rao 1999). Thylakoid N concentration ( $N_{\text{Thy}}$ ;  $\text{mmol m}^{-2}$ ) was calculated according to Evans and Seeman (1989):

$$N_{\text{Thy}} = 0.79J_{\text{max}} + 0.0331\text{Chl}_a \quad (3)$$

where  $J_{\text{max}}$  ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) was calculated from  $A-C_i$  curves according to the equations of Farquhar and von Caemmerer (1982) and  $\text{Chl}_a$  is area-based chlorophyll concentration ( $\text{mol m}^{-2}$ ).

Rubisco was quantified by capillary electrophoresis as described by Warren et al. (2000a) and modified by Warren (2004). Frozen leaf discs were ground with polyvinylpyrrolidone to remove polyphenols, and proteins were extracted from the mixture by shaking (1.5 min at 30 Hz) in cooled extraction buffer (50 mM Tris-HCl pH 8, 1% (w/v) SDS, 15% glycerol, 0.1 M 2-mercaptoethanol). After centrifugation, the supernatant was retained and the pellet was re-extracted with cooled extraction buffer. The pooled supernatant was purified by precipitation with methanol:chloroform:water (4:1:3; v/v) according to Wessel and Flügge (1984). The pellet was then redissolved in extraction buffer and denatured by incubation at  $100^\circ\text{C}$  for 10 minutes. Rubisco was detected by a capillary electrophoresis system (Beckman-Coulter, Fullerton, CA) at 220 nm and quantified against a standard curve generated with bovine serum albumin (BSA) as the standard. Standard curves were linear, with regression coefficients of 0.99 between 0.1 to 1 mg BSA  $\text{ml}^{-1}$ . Rubisco concentration was initially calculated on a mass basis and converted to an area basis ( $\text{Rub}_a$ ;  $\text{g m}^{-2}$ ) based on SLA values. Rubisco N concentration ( $N_{\text{Rub}}$ ) was calculated empirically:  $N_{\text{Rub}} = 16.7\%$  Rubisco.

#### *Phosphorus fractions*

About 0.25 g of dried and ground leaf tissue was digested in concentrated acid (1:2 nitric:perchloric acid) at  $200^\circ\text{C}$ . Samples were analyzed with an inductively coupled plasma-atomic emission spectrometer (VistaPro ICP-AES, Varian) fitted with an SPS5 Autosampler (Varian). Phosphorus was detected at 178 nm, and quantification was performed with ICP Expert software (Varian). Total P concentrations were calculated and converted to an area basis ( $P_a$ ;  $\text{g m}^{-2}$ ) as for total N.

Inorganic P in foliage was extracted by incubating 50 mg of dried leaf powder with 1.2 ml of Type 1 water at  $95^\circ\text{C}$  for 1 h. Samples were cooled, centrifuged and diluted 1:10 for quanti-

fication by high performance liquid chromatography (Dionex DX2500 HPLC system fitted with an IonPac AS-11 HC column). Inorganic phosphate concentration was calculated and converted to an area basis ( $P_i$ ;  $\text{g m}^{-2}$ ).

#### Nonstructural carbohydrates

Leaf starch and soluble sugars were determined colorimetrically with anthrone according to the methods of Hansen and Møller (1975), Marshall (1986) and Oren et al. (1988). Interfering pigments were extracted from 0.002 g dried and ground leaf with 100% acetone. Soluble sugars were extracted with 80% ethanol. Starch was subsequently extracted by incubating in 1.1% HCl at 100 °C for 30 min. Immediately after reacting with anthrone, sugars were quantified at  $A_{630}$ . Starch and soluble sugars were calculated and subsequently converted to an area basis.

#### Data analyses

Repeated measures analysis of variance (ANOVA) determined whether parameters were affected by partial defoliation. In cases where partial defoliation was significant, Tukey's HSD test (at  $P < 0.05$ ) determined the significance of differences between means. Relationships of photosynthetic characteristics with N, P and soluble carbohydrate pools were examined by linear regressions fitted to the raw data.

## Results

Specific leaf area was unaffected by treatment or time; for simplicity, therefore, all data are presented on a leaf area basis.

#### Nitrogen, chlorophyll and Rubisco

Partial defoliation significantly increased  $N_a$  (control < D50 < D70; Figure 1). One week after defoliation,  $N_a$  in the D70 treatment ( $2.6 \text{ g m}^{-2}$ ) was almost twice as high as the control value ( $1.5 \text{ g m}^{-2}$ ), and this difference was maintained through to Week 5. In contrast, neither  $\text{Chl}_a$  (ranging from 0.39 to  $0.49 \text{ mmol m}^{-2}$ ) nor  $\text{Rub}_a$  (ranging from 2.7 to  $4 \text{ g m}^{-2}$ ) was affected by partial defoliation (Figure 1), and thus,  $N_{\text{Chl}}/N_a$  and  $N_{\text{Rub}}/N_a$  decreased with partial defoliation.

#### Phosphorus

Foliar P concentration was slightly but significantly affected by partial defoliation (control < D50 < D70; Figure 2); however, treatment differences may be partly a result of differences in pretreatment concentrations (pretreatment values ranged between 0.17 and  $0.22 \text{ g m}^{-2}$ ). Reductions in  $P_i$  with partial defoliation were insignificant (values ranged between 97 and 62%; Figure 2). However, when coupled with the slight increase in  $P_a$ , the decrease in  $P_i$  produced a 9% increase in the organic P concentration ( $P_{\text{org}}$ ) in seedlings in both treatments.

#### Nonstructural carbohydrates

Concentrations of nonstructural carbohydrates ( $\text{NSC}_a$ ) and their component insoluble ( $\text{NSC}_i$ ) and soluble ( $\text{NSC}_s$ ) sugars were unaffected by partial defoliation but varied over time

(Table 1). Foliar soluble sugar concentration was 1.7-times higher in Week 3 than in Week 1 in the D50 treatment and twice as high in Week 3 than in Week 1 in the D70 treatment.

#### Gas exchange

Immediately after partial defoliation,  $A_{\text{max}}$  was significantly increased, but this effect decreased over time (Figure 3A). Differences were greatest one week after partial defoliation with  $A_{\text{max}}$  of seedlings in the D50 and D70 treatments 1.5- and 1.7-times higher, respectively, than the control value. By Week 3, treatment differences were small and insignificant. Photosynthetic N-use efficiency tended to decrease following partial defoliation, whereas water-use efficiency was unaffected (Table 2).

Dark respiration was significantly increased by partial defoliation. In Week 1,  $R_d$  was 1.7-times higher in seedlings in the D70 treatment compared with control seedlings, and twice as high as control values by Week 3 (Table 2). Values of  $R_d$  in seedlings in the D50 treatment differed from control values only in Week 3. By Week 5, seedlings in both treatments had

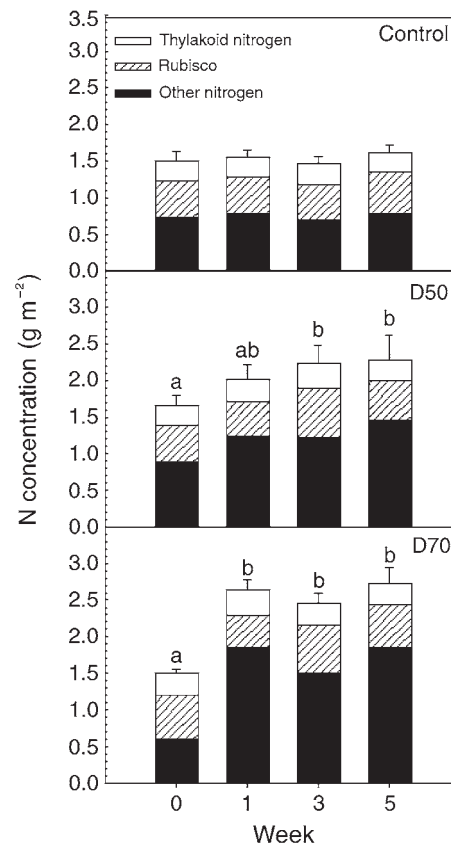


Figure 1. Effects of 50 and 70% canopy removal (D50 and D70, respectively) on nitrogen (N) allocation in leaves of field-grown 12-month-old *Eucalyptus globulus*. Data are the mean N concentrations per unit area allocated to Rubisco, thylakoid membranes and other forms in seedlings in the control, D50 and D70 treatments. Error bars are one SE for total leaf N concentration. Letters denote significant differences in leaf N concentration with time since partial defoliation.

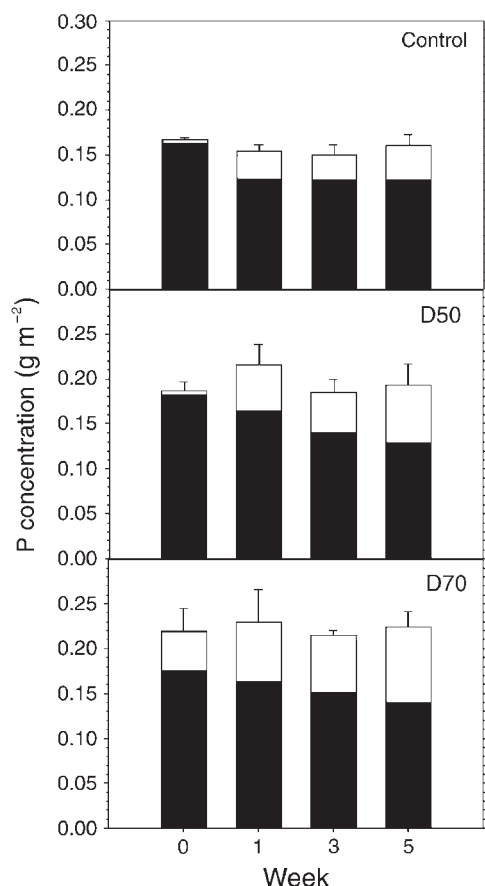


Figure 2. Effects of 50 and 70% canopy removal (D50 and D70, respectively) on phosphorus (P) partitioning in leaves of field-grown 12-month-old *Eucalyptus globulus*. Data are the mean P concentrations per unit area allocated to inorganic (filled bars) and organic (open bars) forms in seedlings in the control, D50 and D70 treatments. Error bars are 1 SE for total leaf P concentration.

lower  $R_d$  values than did control seedlings.

Stomatal conductance was marginally but not significantly higher in seedlings in the D50 and D70 treatments than in control seedlings (Figure 3B). Partial defoliation had no effect on  $C_i/C_a$  (Table 2).

Partial defoliation significantly increased  $J_{max}$  and  $V_{cmax}$  (Figures 3C and 3D). At Week 1,  $J_{max}$  and  $V_{cmax}$  were 30% higher in seedlings in the D50 treatment and twice as high in seedlings in the D70 treatment compared with control seedlings. The differences between treatments and controls decreased with time, and, by Week 3, the differences were less than 8%.

The light compensation point (LCP), light saturation point ( $L_{sat}$ ) and apparent quantum efficiency ( $\Phi$ ) were all significantly affected by partial defoliation (Table 2). The LCP was twice as high in seedlings in the D70 treatment as in control seedlings at both Weeks 1 and 3, whereas LCP of seedlings in the D50 treatment was within 4% of control values at Weeks 1 and 3. At Week 5, the LCP of the control seedlings was 30% higher than that of seedlings in both treatments. Treatment differences in  $L_{sat}$  were most clear at Week 1, when seedlings in the D50 and D70 treatments had 40 and 90% higher  $L_{sat}$ , respectively, than control seedlings. Partial defoliation affected  $\Phi$ , but there were no consistent trends among treatments over time.

Although  $A_{max}$  was positively correlated with  $N_a$  (Figure 4A) and  $Chl_a$  (Figure 4B), it was unrelated to  $Rub_a$  (Figure 4C). There was a negative relationship between  $N_{Rub}/N_a$  and  $A_{max}$  (Figure 4D). We found a positive relationship between  $A_{max}$  and  $P_a$  (Figure 5A), whereas  $P_i$  was unrelated to  $A_{max}$  (Figure 5B). There was no relationship between  $A_{max}$  and  $NSC_a$  or its components  $NSC_i$  and  $NSC_s$  (data not shown). Figures 6A–E show that  $A_{max}$  was positively correlated with  $g_s$  but not with  $C_i/C_a$ ,  $J_{max}$ ,  $V_{cmax}$  or in vivo specific activity of

Table 1. Effects of 50 and 70% canopy removal on nonstructural carbohydrates in leaves of 12-month-old field-grown *Eucalyptus globulus*. Data are mean values for nonstructural carbohydrate content per unit area ( $NSC_a$ ), soluble sugar content per unit area ( $NSC_s$ ), insoluble sugar content per unit area ( $NSC_i$ ) and the soluble:insoluble sugar ratio. One SE is shown in parentheses,  $n = 5$  for the control and D50, and  $n = 4$  for D70. Repeated measures ANOVA was used to test for significant effects of treatment, time and their interaction. The effect of time on  $NSC_a$  was significant at  $P < 0.05$ . All other effects were insignificant.

Parameter	Treatment (% crown removal)	Week 0	Week 1	Week 3	Week 5
$NSC_a$ (g m <sup>-2</sup> )	0	14.8 (1.0)	17.4 (2.3)	15.6 (0.7)	10.7 (2.3)
	50	15.9 (3.8)	16.2 (2.5)	22.6 (3.2)	11.7 (2.6)
	70	12.5 (0.8)	13.2 (0.6)	20.4 (3.5)	5.8 (1.6)
$NSC_s$ (g m <sup>-2</sup> )	0	5.84 (0.85)	6.68 (1.06)	5.73 (0.68)	4.98 (0.61)
	50	5.62 (1.07)	6.70 (1.10)	6.33 (0.90)	4.18 (1.45)
	70	4.60 (0.64)	7.20 (0.47)	7.50 (0.94)	2.43 (0.72)
$NSC_i$ (g m <sup>-2</sup> )	0	9.0 (0.8)	10.7 (1.7)	9.9 (0.4)	5.7 (2.2)
	50	10.3 (2.7)	9.6 (1.7)	16.2 (2.5)	7.5 (2.7)
	70	7.9 (0.9)	5.9 (0.1)	12.9 (3.0)	3.4 (1.4)
$NSC_s/NSC_i$ (g g <sup>-1</sup> )	0	0.68 (0.12)	0.65 (0.11)	0.58 (0.08)	1.21 (0.37)
	50	0.59 (0.07)	0.74 (0.09)	0.40 (0.05)	3.25 (2.70)
	70	0.63 (0.17)	1.21 (0.05)	0.75 (0.24)	1.25 (0.46)

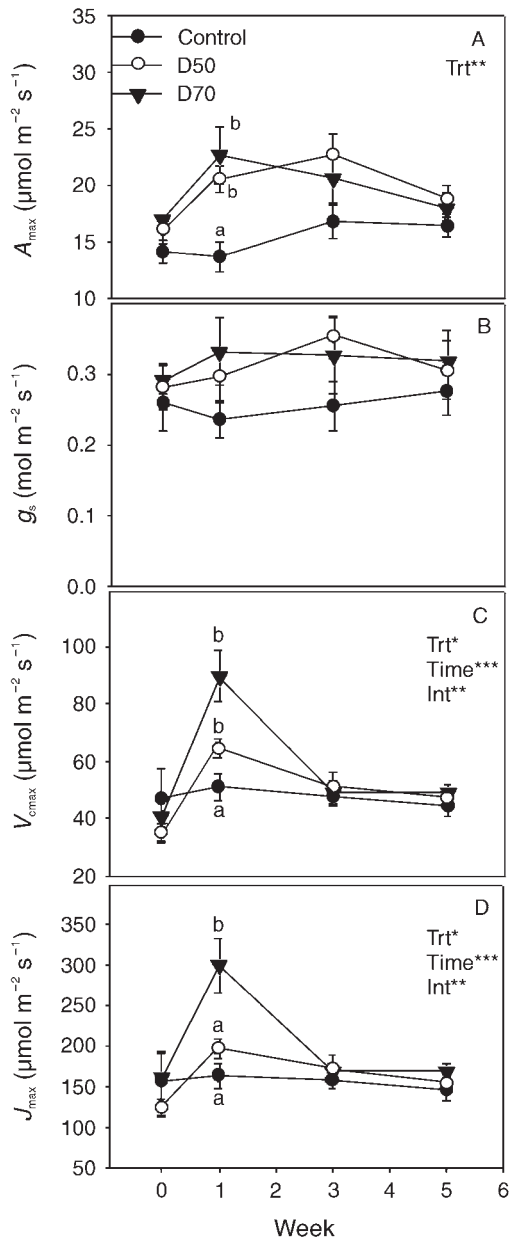


Figure 3. Effects of 50 and 70% canopy removal (D50 and D70, respectively) on photosynthetic parameters in leaves of field-grown 12-month-old *Eucalyptus globulus*. Data are the mean values for (A) maximum rate of photosynthesis ( $A_{\max}$ ), (B) stomatal conductance ( $g_s$ ), (C) maximum rate of carboxylation ( $V_{\text{cmax}}$ ) and (D) maximum rate of RuBP regeneration ( $J_{\max}$ ). Error bars are one SE,  $n = 5$  for the control and D50, and  $n = 4$  for D70. Asterisks indicate significance of treatment (Trt), time or their interaction (Int): \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ ; and \*\*\*,  $P \leq 0.001$ . Repeated measures ANOVA was used to test for significant effects of treatment, time and their interaction. Different letters denote a significant treatment ( $\alpha = 0.05$ ) effect within a measurement period.

Rubisco ( $V_{\text{cmax}}/\text{Rub}_a$ ).

#### Sensitivity analysis

Sensitivity analyses revealed that  $A_{\max}$  was most sensitive to

$V_{\text{cmax}}$  (Table 3), especially in Week 1. When  $V_{\text{cmax}}$  values in seedlings in the D50 and D70 treatments were substituted for the control value,  $A_{\max}$  increased by 30 and 85–87%, respectively. Compared with  $V_{\text{cmax}}$ ,  $A_{\max}$  was less sensitive to  $J_{\max}$  (increase of 55–60% with substitution of the D70 treatment value for the control value),  $g_s$  (increase of 12–13% with substitution of the D70 treatment value for the control value) and  $R_d$  (decrease of 10% with substitution of D70 treatment value for the control value).

#### Discussion

Photosynthetic rates increased significantly within one week of removing 50 or 70% of the canopy. The speed of response was comparable with that recorded for conifers, e.g., *Pinus radiata* D. Don (Whitehead et al. 1996) and *Pseudotsuga menziesii* (Mirb.) Franco (Warren et al. 2003), but was twice as quick as reported for *Eucalyptus nitens* (Pinkard et al. 1998). As expected (after Reich et al. 1993), the increase in photosynthesis was short-lived compared with previous studies of older plants, with rates returning to control values by the fifth week after defoliation.

Compared with previous studies (Reich et al. 1993, Pinkard and Beadle 1998, Pinkard et al. 1998), the changes in  $A_{\max}$  were small given the proportion of canopy removed. This finding is at odds with other studies of young plants (Reich et al. 1993) and may result from the heteroblastic nature of eucalypt leaves. Typically, growth following defoliation produces leaves of high SLA (Oesterheld and McNaughton 1988). In our study, however, trees were already displaying juvenile leaves and did not alter their SLA during regrowth after partial defoliation. Unlike previous studies, we found that the increase in  $A_{\max}$  was not directly proportional to the loss of leaf area. Instead, both treatments resulted in an increase in  $A_{\max}$  of 30% within one week. Although this finding suggests a limit to the size of the photosynthetic response, regardless of severity of defoliation, it was likely also influenced by environmental conditions and therefore we cannot assume that it is typical. Partial defoliation significantly increased  $R_d$ , but  $A_{\max}$  was sensitive to  $R_d$  in seedlings in the D50 treatment (Table 3), allowing for the possibility that photosynthesis and respiration are balanced such that carbon gain varied little between treatments.

#### Biochemical rate-limiting parameters

The increase in  $A_{\max}$  following partial defoliation can be largely attributed to increases in  $V_{\text{cmax}}$  and  $J_{\max}$  (Table 3, see also Sharkey 1985). Resistance imposed on photosynthesis by biochemical processes was reinstated within three weeks of partial defoliation, which is more rapid than observed in other studies (Ovaska et al. 1992, Pinkard and Beadle 1998). In our study, Rubisco concentrations were unaffected by partial defoliation and thus the cause of increased  $V_{\text{cmax}}$  was increased specific activity (i.e.,  $V_{\text{cmax}}/\text{Rub}_a$ ) and not increased activity (von Caemmerer and Farquhar 1984, Ovaska et al. 1993a) or amount of Rubisco (Wareing et al. 1968). Increased  $A_{\max}$  was accompanied by increased  $R_d$ , and both slowed toward the end

Table 2. Effects of 50 and 70% canopy removal on photosynthetic characteristics of leaves from 12-month-old field-grown *Eucalyptus globulus*. Data are mean values for dark respiration rate ( $R_d$ ), ratio of intercellular CO<sub>2</sub> concentration to ambient CO<sub>2</sub> concentration ( $C_i/C_a$ ), the ratio of maximum rate of RuBP regeneration to nitrogen content ( $J_{max}/N_a$ ), in vivo specific activity of Rubisco ( $V_{cmax}/Rub_a$ ), light compensation point (LCP), light saturation point ( $L_{sat}$ ), apparent quantum efficiency ( $\Phi$ ), instantaneous photosynthetic nitrogen-use efficiency (PNUE) and instantaneous water-use efficiency (WUE). One SE is shown in parentheses,  $n = 5$  for the control and D50, and  $n = 4$  for D70. Repeated measures ANOVA was used to test for significant effects of treatment (Trt), time and their interaction (Int).

Parameter	Treatment (% crown removal)	Week 0	Week 1	Week 3	Week 5	P		
						Trt	Time	Int
$R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0	2.41 (0.35)	2.53 (0.43)	1.90 (0.22)	2.41 (0.16)	< 0.01	< 0.01	< 0.01
	50	1.81 (0.24)	2.49 (0.19)	2.25 (0.28)	1.89 (0.35)			
	70	2.69 (0.46)	4.28 (0.19)	3.80 (0.43)	1.58 (0.15)			
$C_i/C_a$ (mol mol <sup>-1</sup> )	0	0.818 (0.029)	0.770 (0.038)	0.762 (0.031)	0.816 (0.030)	ns	ns	ns
	50	0.806 (0.028)	0.754 (0.016)	0.830 (0.014)	0.802 (0.033)			
	70	0.833 (0.023)	0.768 (0.022)	0.848 (0.030)	0.810 (0.023)			
$J_{max}/N_a$ (mmol g <sup>-1</sup> s <sup>-1</sup> )	0	109 (22)	106 (6)	108 (3)	91 (6)	ns	< 0.001	< 0.05
	50	77 (9)	103 (11)	79 (9)	72 (9)			
	70	103 (26)	113 (8)	69 (4)	64 (7)			
$V_{cmax}/Rub_a$ (mmol mol <sup>-1</sup> s <sup>-1</sup> )	0	8.66 (1.83)	9.70 (0.67)	9.40 (0.86)	7.32 (0.39)	ns	< 0.001	< 0.05
	50	7.18 (1.29)	13.72 (1.68)	7.06 (0.41)	8.52 (0.83)			
	70	6.25 (0.28)	20.22 (3.96)	7.23 (0.45)	7.95 (0.82)			
LCP ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0	36.5 (6.3)	38.7 (6.8)	29.8 (3.6)	41.1 (2.9)	< 0.01	< 0.01	ns
	50	25.8 (3.5)	37.3 (2.7)	30.4 (3.5)	28.9 (5.5)			
	70	34.1 (5.7)	73.0 (5.3)	54.5 (7.5)	26.8 (3.0)			
$L_{sat}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	0	247 (24)	245 (17)	295 (33)	319 (13)	< 0.01	ns	< 0.01
	50	250 (14)	345 (18)	340 (29)	316 (6)			
	70	246 (8)	455 (29)	347 (39)	328 (8)			
$\Phi$ (mmol mol)	0	0.068 (0.004)	0.067 (0.005)	0.065 (0.004)	0.059 (0.003)	< 0.01	ns	ns
	50	0.072 (0.002)	0.067 (0.001)	0.074 (0.003)	0.065 (0.003)			
	70	0.080 (0.003)	0.059 (0.002)	0.071 (0.003)	0.059 (0.003)			
PNUE (mmol mol <sup>-1</sup> s <sup>-1</sup> )	0	136 (15)	124 (8)	162 (17)	145 (16)	ns	< 0.05	< 0.05
	50	141 (18)	150 (17)	145 (10)	122 (13)			
	70	158 (8)	121 (12)	117 (12)	95 (12)			
WUE (mmol mol <sup>-1</sup> )	0	57 (4)	60 (7)	70 (9)	62 (6)	ns	ns	ns
	50	59 (6)	72 (5)	65 (3)	66 (9)			
	70	58 (3)	69 (4)	67 (7)	60 (9)			

of the measurement period. The dependence of both  $A_{max}$  and  $R_d$  on leaf N has recently been highlighted (Turnbull et al. 2005, Machado and Reich 2006, Reich et al. 2006) and is an area for future research.

#### Stomatal conductance

Our results support the paradigm that  $g_s$  is coordinated with the biochemical capacity for photosynthesis (Figure 6A), but there was no evidence that  $g_s$  explained the responses to defoliation (Table 3). Although it is possible that treatment effects were masked by rehydration of the leaves before the gas exchange measurements, the large within-treatment variation in  $g_s$  obscured any increases in  $g_s$  in response to partial defoliation. In contrast, many other studies have shown that partial defoliation increases  $g_s$  (Wallace et al. 1984, Hart et al. 2000, Elfadl and Luukkanen 2003, Handa et al. 2005), although this response is not universal (Syvertsen 1994).

Increased photosynthesis was not a function of a change in

$C_i/C_a$  (Figure 6B, Table 2), as has been found in some (Cerasoli et al. 2004) but not all (Lavigne et al. 2001, Volin et al. 2002, Zhou and Quebedeaux 2003) previous studies. The insensitivity of  $A_{max}$  to  $C_i$  may be explained by the small range in  $C_i$  (40  $\mu\text{mol mol}^{-1}$ ) and the modest stomatal limitations, given that  $C_i$  was about 50  $\mu\text{mol mol}^{-1}$  greater than found in other studies of field-grown eucalypts (Pinkard et al. 1998, Warren et al. 2000b).

#### Allocation of nitrogen and phosphorus

Increased photosynthesis following partial defoliation was related to increased amounts of N in the remaining leaves, as has been found in some (Gezelius et al. 1981, Lavigne et al. 2001) but not all (Ovaska et al. 1993a, Reich et al. 1993, Pinkard et al. 1998, Volin et al. 2002) previous studies. A mechanistic basis for correlation of increased photosynthesis with increased N is obscure, however, because increased N did not translate

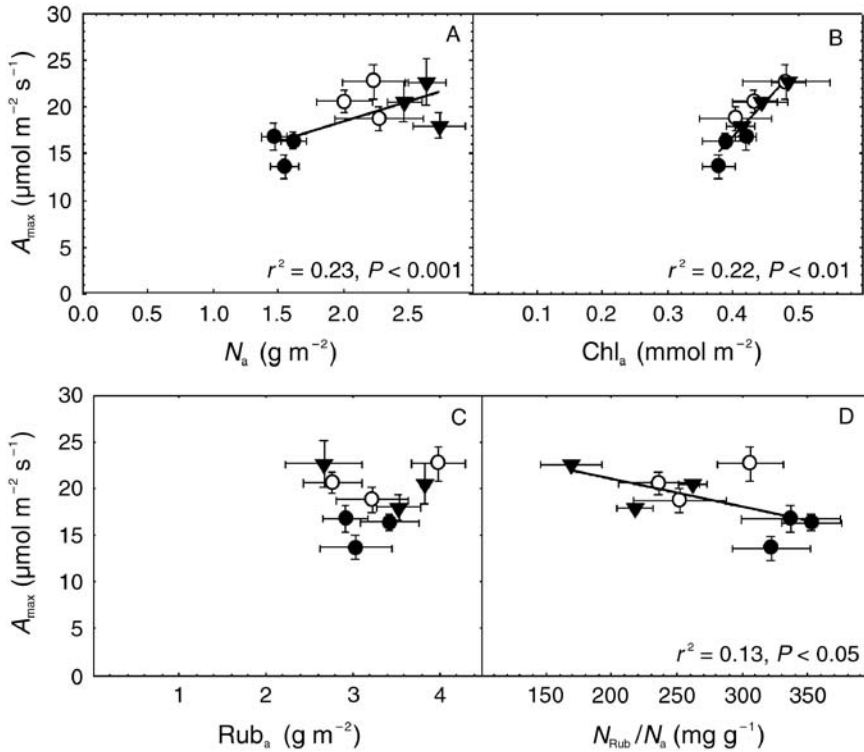


Figure 4. Relationships of maximum rate of photosynthesis ( $A_{\max}$ ) with nitrogen, Rubisco and chlorophyll following 50 or 70% canopy removal. Data are means  $\pm$  1 SE for (A) nitrogen per unit area ( $N_a$ ), (B) chlorophyll per unit area ( $\text{Chl}_a$ ), (C) Rubisco per unit area ( $\text{Rub}_a$ ) and (D) Rubisco nitrogen per unit leaf nitrogen ( $N_{\text{Rub}}/N_a$ ). Regressions are based on raw data, but mean data are presented for clarity. Symbols: ● = control; ○ = 50% canopy removal; and ▼ = 70% canopy removal.

into more Rubisco or chlorophyll. Photosynthesis was limited by Rubisco concentration in our control treatment (Table 3) but increased photosynthesis following defoliation was the result of increased  $V_{\text{cmax}}/\text{Rub}_a$  rather than increased amounts of Rubisco (Gezelius et al. 1981, Ovaska et al. 1993a, Lavigne et al. 2001).

*Eucalyptus globulus* has weak relationships between  $A_{\max}$  and  $N_a$  across leaf age-classes and vertical and horizontal planes (Close et al. 2004), perhaps because storage of N is in excess of requirements (Warren et al. 2000b, Close et al. 2004). This seemingly challenges the theory that N is optimally distributed to maximize whole-canopy photosynthesis (e.g., Field 1983). Even so, it would be erroneous to assert that partial defoliation of *E. globulus* results in an optimal distribution of N for photosynthesis, because N did not accumulate in Rubisco or chlorophyll.

Leaves remaining on partially defoliated trees were larger sinks for N than leaves on control trees. This N likely accumulated as a soluble protein (e.g., Ovaska et al. 1993a) other than Rubisco. It is well established that leaves remaining on plants subjected to pruning or defoliation tend to exhibit extended longevity (Nowak and Caldwell 1984, Crafts-Brandner 1991, Martín del Molino et al. 1995), and it seems reasonable to suggest that N loaded into leaves might first be retained in a form requiring little maintenance. Further analysis of storage proteins (and other forms of stored N) in foliage would provide insight into leaf N economics.

Unlike N, we found P was not loaded into leaves after partial defoliation (Figure 2), which is consistent with previous studies of eucalypts (Pinkard et al. 1998) and other genera (Cherbuy et al. 2001). Apart from woody tubers, which *E. globulus* does not form, there is little evidence of stores of P

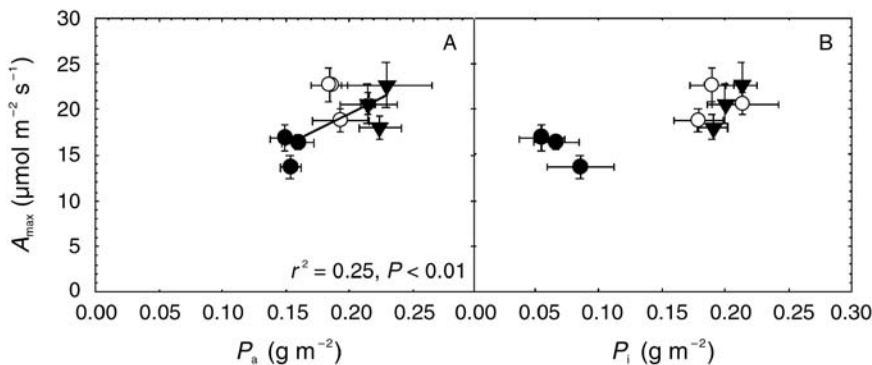


Figure 5. Relationships of maximum rate of photosynthesis ( $A_{\max}$ ) with leaf phosphorus fractions following 50 or 70% canopy removal. Data are means  $\pm$  1 SE for (A) phosphorus per unit area ( $P_a$ ) and (B) inorganic phosphate per unit area ( $P_i$ ). Regressions are based on raw data, but mean data are presented for clarity. Symbols: ● = control; ○ = 50% canopy removal; and ▼ = 70% canopy removal.



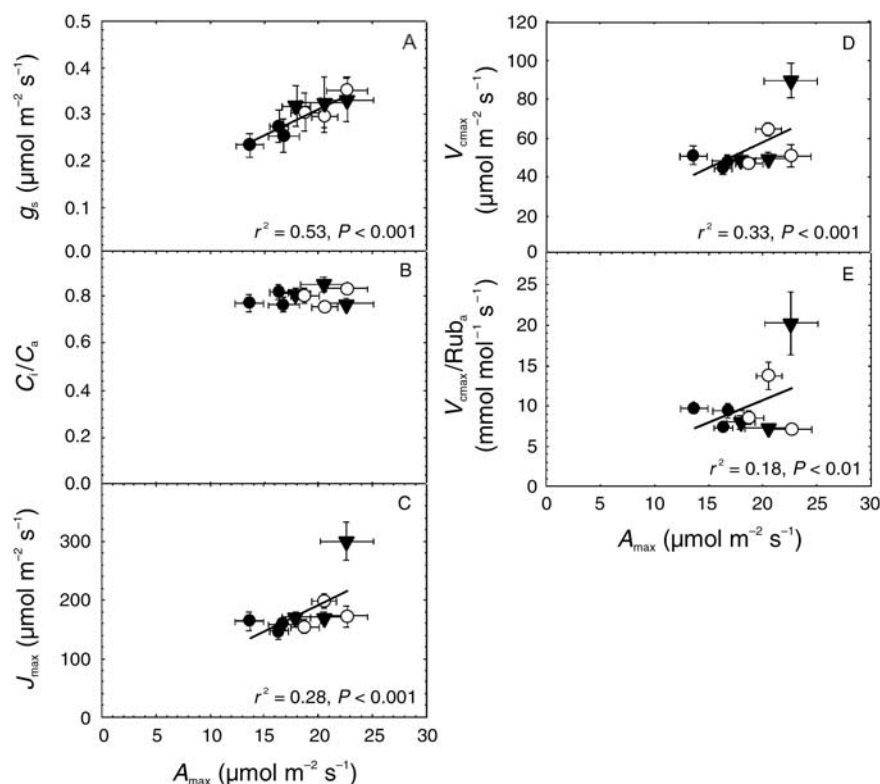


Figure 6. Relationships of maximum rate of photosynthesis ( $A_{\max}$ ) with photosynthetic parameters following 50 or 70% canopy removal. Data are means  $\pm 1$  SE for (A) stomatal conductance ( $g_s$ ), (B) ratio of intercellular  $\text{CO}_2$  concentration to ambient  $\text{CO}_2$  concentration ( $C_i/C_a$ ), (C) maximum rate of RuBP regeneration ( $J_{\max}$ ), (D) maximum rate of carboxylation ( $V_{\text{cmax}}$ ) and (E) the specific activity of Rubisco ( $V_{\text{cmax}}/\text{Rub}_a$ ). Regressions are based on raw data, but mean data are presented for clarity. Symbols: ● = control; ○ = 50% canopy removal; ▼ = 70% canopy removal.

in woody plants except in foliage. Even so,  $A_{\max}$  was never limited by  $P_a$  (Figure 5), and the proportion of organic P in remaining foliage increased after partial defoliation. Numerous organic forms of P are both stable and readily transformed in eucalypt leaves (Hawkins and Polglase 2000). We found no evidence that organic P was related to increased photosynthesis after partial defoliation, as it was unrelated to  $A_{\max}$  or to the amount of thylakoid protein (as estimated by chlorophyll concentration; data not shown). Instead, organic P paralleled the increase in non-photosynthetic N ( $r^2 = 0.28$ ,  $P = 0.001$ ; data not shown) and is likely involved in other metabolic processes.

#### Modifying carbohydrate reserves

The reduction in photosynthetic rate caused by the accumulation of carbohydrates such as sucrose, glucose, fructose and triose-phosphates is attributed to end-product inhibition (Neales and Incoll 1968, Stitt 1991). Several authors have argued that partial defoliation, either by pruning whole branches or reducing the areas of individual leaves, reduces end-product inhibition, resulting in increased photosynthesis, probably because of increased demand for carbohydrates (Neales and Incoll 1968, Azcon-Bieto 1983, McNaughton 1983, von Caemmerer and Farquhar 1984, Ovaska et al. 1993a, Layne

Table 3. Sensitivity analyses of the factors contributing to treatment differences in  $A_{\max}$ . Each value is the percent change in  $A_{\max}$  after substitution of a model parameter ( $C_i$ ,  $g_s$ ,  $R_d$ ,  $V_{\text{cmax}}$  or  $J_{\max}$ ) in the 50 or 70% canopy removal treatments (D50 and D70, respectively) for the corresponding control parameter. The mean value of a parameter for a partial defoliation treatment replaced that of the control and photosynthesis at  $350 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  was recalculated with the Farquhar et al. (1980) model. In considering the effects on  $A_{\max}$  of  $R_d$ ,  $V_{\text{cmax}}$  and  $J_{\max}$ , the cases of  $C_i/C_a$  and  $g_s$  being invariant were considered. Abbreviations:  $A_{\max}$ , maximum rate of photosynthesis;  $C_i$ , intercellular  $\text{CO}_2$  concentration;  $g_s$ , stomatal conductance;  $R_d$ , dark respiration rate;  $V_{\text{cmax}}$ , maximum carboxylation rate; and  $J_{\max}$ , maximum rate of RuBP regeneration.

Substituted parameter	Week 1				Week 3			
	D50		D70		D50		D70	
	$C_i$ fixed	$C_i$ varies	$C_i$ fixed	$C_i$ varies	$C_i$ fixed	$C_i$ varies	$C_i$ fixed	$C_i$ varies
$C_i$ ( $\mu\text{mol mol}^{-1}$ )	-2	-	0	-	+7	-	+8	-
$g_s$ ( $\text{mol m}^{-2} \text{ s}^{-1}$ )	-	+12	-	+13	-	+5	-	+6
$R_d$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	0	+9	-10	0	-2	+1	-11	-7
$V_{\text{cmax}}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	+30	+38	+87	+85	+6	+8	+3	+6
$J_{\max}$ ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	+18	+17	+60	+55	+7	+7	+6	+6

and Flore 1995). In contrast, we found that increased photosynthetic rates in *E. globulus* following partial defoliation were unrelated to carbohydrate concentrations (Table 1). Although this finding is consistent with those for other evergreen species (Gezelius et al. 1981, Lavigne et al. 2001) and contrasts with those for deciduous species (Zhou and Quebedeaux 2003, Handa et al. 2005), it is at odds with the majority of the literature and may result, in part, from altered source–sink dynamics following excision of leaves for photosynthetic measurements. Even though we observed no change in measures of bulked carbohydrates, rates of synthesis and efflux were not quantified and end-product inhibition may pertain more to a specific carbohydrate than to the total carbohydrate pool (Azcon-Bieto 1983). Therefore, we cannot dismiss the possibility of release from end-product inhibition following partial defoliation without more detailed knowledge of carbohydrate biochemistry.

In conclusion, increases in photosynthesis following partial defoliation in 12-month-old field-grown *E. globulus* were rapid and transient: one week after defoliation, photosynthesis increased by 30%, but it returned to control values after five weeks. Nitrogen was loaded into leaves in response to partial defoliation, but, despite the strong relationship between  $N_d$  and  $A_{max}$ , it was not recovered in either Rubisco or chlorophyll. Hence, increased biochemical capacity for photosynthesis in response to partial defoliation was not a function of the amount of Rubisco or chlorophyll, but was largely a function of the increased specific activity of Rubisco.

### Acknowledgments

TT was supported by an MRS/DSE scholarship. This work was supported by funding from the Australian Research Council. We gratefully acknowledge the East Victorian Plantation Company of Australia Pty. Ltd. for providing access to their Canadian Forest Estate and in particular Derek Bristow for providing soil nutrient data, and the assistance of Glenn Ogston from Treecorp during site selection. We also thank Najib Ahmady for analyzing total P.

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