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Photosynthetic response to green crown pruning in young plantation-grown *Eucalyptus pilularis* and *E. cloeziana*

Philip J. Alcorn [a,b,*], Jürgen Bauhus [b,c], Dane S. Thomas [d], Ryde N. James [b], R. Geoff B. Smith [d], Adrienne B. Nicotra [a]

[a] School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia
[b] Fenner School of Environment and Society, The Australian National University, Canberra, ACT 0200, Australia
[c] Institute of Silviculture, Freiburg University, D-79085 Freiburg, Germany
[d] Forests NSW, PO Box J19, Coffs Harbour, NSW 2450, Australia

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ABSTRACT

The loss of foliage through pruning of live branches may reduce tree growth or it may be compensated by photosynthetic up-regulation of the remaining crown. Here, the changes in light-saturated photosynthesis following pruning to remove 50% of green crown length were examined in 4-year-old *Eucalyptus pilularis* Sm. and *Eucalyptus cloeziana* F. Muell. trees. The objectives of the study were to: (1) compare leaf-level physiological (light-saturated photosynthesis (A_{max}), stomatal conductance (g), transpiration (T), dark respiration (R_d), quantum yield (Φ), light compensation point (Γ), water-use efficiency (WUE), nitrogen-use efficiency (NUE)) traits in species with contrasting crown dynamics and structure, (2) examine the effect of crown position on these traits, and (3) examine the effect of pruning on A_{max}, g, T, WUE, NUE, leaf N and P concentrations and specific leaf area (SLA). Prior to pruning there were no differences in R_d, Γ and Φ between *E. pilularis* and *E. cloeziana* but differences in A_{max}, T, g, leaf N, leaf P, WUE, NUE and SLA. Whereas the rate of physiological processes (A_{max}, T, and g) and leaf N and P concentrations increased with crown height, R_d, Γ, Φ and SLA declined along this vertical gradient, except in the upper crown of *E. cloeziana* where A_{max}, T and g were not different to the lower crown. No up-regulation of photosynthesis or changes in leaf physiology occurred between 6 and 13 months after pruning in either species. The results provide an important basis for modelling pruning effects in process-based tree growth models.

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1. Introduction

Pruning is a silvicultural treatment that directly reduces leaf area available for photosynthesis. Intuitively, one expects that pruning therefore reduces, at least in the short-term, the growth of trees. However, there may be up-regulation of photosynthesis in the remaining foliage leading to compensatory growth. The phenomenon of enhanced photosynthesis in the remaining foliage following reductions in the foliage has been shown in a wide-range of species (Heichel and Turner, 1983; von Caemmerer and Farquhar, 1984; Ovaska et al., 1992; Layne and Flore, 1995; Pinkard et al., 1998). The increased photosynthetic activity may occur because leaves often function below their maximum photosynthetic capacity (Wareing et al., 1968; Lovett Doust, 1989; Harbinson, 1994), in particular in the lower part of tree crowns, which are subject to pruning of live branches. A number of mechanisms have been identified that would allow for enhanced photosynthesis of remaining foliage, including changes in intracellular or stomatal carbon dioxide transport or rate of carboxylation in the cell (Hartt et al., 1964; Thorne and Koller, 1974; Nafziger and Koller, 1976), increased assimilate demand following loss of foliage (Hartt et al., 1964; Wareing et al., 1968; Thorne and Koller, 1974; Nafziger and Koller, 1976), elevated hormone concentrations in the remaining foliage (Ovaska et al., 1993) and/or increased foliar nitrogen concentrations (Hoogesteger and Karlsson, 1992). The result of this up-regulation of photosynthesis is an enhanced capacity to lead to compensatory growth (Senock et al., 1991).

There are many factors that may affect the magnitude and duration of photosynthetic response following defoliation. It has generally been observed that increasing the intensity of defoliation increases photosynthetic up-regulation (Pinkard et al., 1998), until the rate of biochemical reactions reaches a maximum (von Caemmerer and Farquhar, 1984). The species (Heichel and Turner, 1983), frequency of leaf-area removal (Wallace et al., 1984), leaf

* Corresponding author at: Nicotra Laboratory, School of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia.
Tel.: +61 2 6125 2671; fax: +61 2 6125 5573.
E-mail address: philip.alcorn@anu.edu.au (P.J. Alcorn).

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age (Pinkard et al., 1998), plant size and age (Reich et al., 1993) and nitrogen availability (Ovaska et al., 1993) can all influence the magnitude and duration of the photosynthetic response. Since pruning in plantation-grown trees removes the lower branches, which are generally of lower productivity due to shading from neighbouring trees and upper crown sections (Pinkard et al., 1998), the photosynthetic responses may be less through the removal of more productive upper crown foliage. While the magnitude and duration of the photosynthetic response to foliage removal is well-studied in glass-house seedlings, there have been few studies conducted on trees (e.g. Pinkard et al., 1998). Even fewer studies have examined the photosynthetic responses to pruning defoliation in *Eucalyptus* trees (e.g. Pinkard et al., 1998; Ngugi et al., 2004).

This paper describes a study carried out under field conditions at the Southgate research site, north-eastern New South Wales, Australia, to examine the physiological difference in leaf traits between *Eucalyptus pilularis* Sm. and *Eucalyptus cloeziana* F. Muell and response to pruning. The two species possess quite contrasting crown dynamics in young plantations. In *E. pilularis* branches are shed much sooner than in *E. cloeziana* trees of the same age. Consequently, *E. cloeziana* retains a deeper live crown than *E. pilularis* (Smith et al., 2006; Alcorn et al., 2007). This difference in crown dynamics suggests that *E. cloeziana* is more shade-tolerant than *E. pilularis*, since its foliage may endure a greater level of self-shading within crowns. Consequently, foliage in the lower crown of *E. cloeziana* might be expected to display the characteristics of greater tolerance to low light including foliage with lower light-saturated photosynthesis rates, lower dark respiration rates and lower light intensities at which photosynthesis and respiration are equal (light compensation point) (e.g. Larcher, 1995; Florence, 1996). In addition, the foliage in the lower crown of *E. cloeziana* may use water and nutrients more effectively as comparable foliage in *E. pilularis*, so that lower branches can contribute to whole tree assimilation for longer. Two common measures of foliage resource efficiency include the water-use and the nitrogen-use efficiency of photosynthesis (e.g. Larcher, 1995). The efficiency also depends largely on the foliage construction costs, which can be expressed as the ratio of leaf surface area to dry weight (specific leaf area) (e.g. Evans and Poorter, 2001). Therefore, we analysed how these measures of efficiency of foliage differed between the different crown positions in the two species.

An earlier experiment at the Southgate research site found that pruning to remove 50% of the green crown length in young *E. pilularis* and *E. cloeziana* did not result in significant reductions in the increment of height or stem diameter at breast height in trees of both species over a 2-year period (Alcorn et al., 2008). Given that growth rates were not reduced at this site, it is predicted that an up-regulation of leaf photosynthesis occurred to compensate for the loss of leaf area. This experiment therefore tested the hypotheses that (a) there are large effects of crown position on physiological leaf traits in both species, (b) there are differences in the physiological leaf traits between species with contrasting crown dynamics and (c) there would be evidence of up-regulation of photosynthesis following pruning to remove 50% of the live crown in both species.

2. Methods

2.1. Study site and treatment design

The pruning trial was established at Southgate, an experimental plantation located near Nana Glen in north-eastern New South Wales (30°1′S, 153°8′E) (for full site description see Alcorn et al., 2008).

Trees of *E. pilularis* and *E. cloeziana* located in four plots established at 1250 trees ha^{-1} were selected for this experiment. Eight trees of each species were selected from the co-dominant or dominant crown classes (Smith et al., 1997), they were straight and single stemmed, free of visible health defects and surrounded by eight immediate neighbours in each direction. On 6 October 2004, four selected trees were pruned to remove the lower 50% of the length of the green crown. Green crown length was visually defined as the distance between the tree height and the stem insertion height of the lowest green branch contained within a geometrically regular crown envelope (Soares and Tomé, 2001). Branches were pruned perpendicular to the branch axes as close to the stem as possible using pruning shears. Unpruned trees served as controls.

2.2. Gas exchange measurements

Light response curves were determined on a single occasion for three leaves from each of the four trees of each species randomly selected before pruning in late September 2004. Measurements were made on the youngest fully expanded leaf on the northern side (sun-exposed side) of the crown from the lower (25% of the green crown length), middle (60% of the green crown length) and upper (85% of the green crown length) positions. Leaf gas exchange was measured using a LI-6400 (LI-COR, Lincoln, Nebraska) portable gas exchange system with a 6400-02B LED leaf chamber. Leaves were initially dark adapted by wrapping the leaf and leaf cuvette in a black cloth to determine dark respiration (R_d) when the photosynthetic photon flux density (PPFD) level was 0 $\mu mol\ m^{-2}\ s^{-1}$. PPFD was progressively increased using the leaf chamber red and blue light diodes in 10 steps from 0 to a maximum of 3000 $\mu mol\ m^{-2}\ s^{-1}$, allowing for an acclimation period of 3–8 min following adjustment of light levels and recording measurements when the photosynthetic rates were stable. A flow rate of 200 $\mu mol\ s^{-1}$ was used and the reference CO_2 concentration was set at 370 $\mu mol\ CO_2\ mol^{-1}$ air. Leaf chamber humidity of 60–80% and leaf temperature of 26–28 °C was maintained during all measurements. Measurements were made over 4 days between 08:00 and 13:00 EST.

Apparent quantum yield (Φ) and R_d were estimated from measured data. Values of Φ, were calculated as the slope of photosynthesis (A) by irradiance between 50 and 100 $\mu mol\ m^{-2}\ s^{-1}$. Light compensation points (Γ) were estimated by extrapolating between measured data. Light saturation was defined as the photosynthetic photon flux density of 2000 $\mu mol\ m^{-2}\ s^{-1}$.

Measurements of light-saturated photosynthesis (A_{max}), transpiration (T) and stomatal conductance (g) were made prior to pruning (September 2004) and on three occasions following pruning in March, July and November 2005 on unpruned and 50% pruned treatments. Measurements of A_{max} were made at 1000 $\mu mol\ m^{-2}\ s^{-1}$, approximately 90% saturation (determined from light response curves) and the same leaf cuvette conditions as above. All measurements were made between 08:00 and 13:00 EST. Pre-pruning A_{max} measurements were derived from the light–response curves taken on leaves from four out of the eight trees selected for each species. Additional pre-pruning measurements of A_{max} were made on leaves from the four trees selected for the experiment from each species that were not measured for light–response curves. Measurements were made over 4 days, with two trees (unpruned and 50% pruned tree) of each species being randomly selected for measurement on the same day. Measurements were made on three fully expanded leaves selected from the northern side of the crown in the lower (25% of the green crown length), middle (60% of the green crown length) and upper (85% of the green crown length) crown positions at the time of pruning. An additional crown zone was added at the first post-pruning measurement to account for A_{max} on new foliage produced through the height growth after pruning

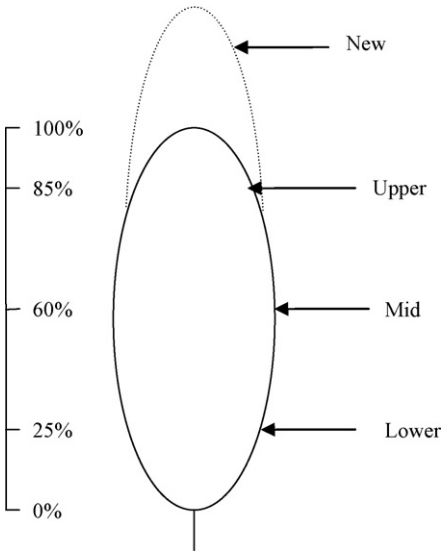

Fig. 1. Schematic diagram indicating the sampling positions within the crown for photosynthesis measurements. The percentages (%) refer to green crown length at the time of pruning.

(Fig. 1). Measurements were made in the middle of this additional crown zone. The order of measurement for the three or four canopy positions was randomly selected for each tree prior to measuring.

Transpiration was calculated using the equations derived by von Caemmerer and Farquhar (1981). Relationships between relative humidity and conductance and between vapour pressure deficit and conductance were examined using regression analysis. No significant differences were found for either species during all measurement periods.

Measurements of area-based A_{max} ($A_{max\ area}$) (μmol CO_2 m^{-2} s^{-1}) were used to derive mass-based estimates of A_{max} ($A_{max\ mass}$) (μmol CO_2 g^{-1} s^{-1}) using specific leaf-area (SLA; m^2 kg^{-1}) values. Instantaneous water-use efficiency (A_{max}/g) (WUE μmol CO_2 - mol^{-1} H_2O) was also determined for each leaf measured for A_{max} before and after pruning. Post-pruning $A_{max\ area}$ was calculated as a percentage of pre-pruning levels for each crown zone ($\Delta A_{max\ area}$).

2.3. Leaf nutrient analysis

Leaves measured for photosynthesis were collected for determination of leaf area, dry mass and nutrient content (nitrogen and phosphorus) on each sampling occasion. Fresh leaves were measured for leaf area using a Li-3000A Leaf Area Meter (Li-Cor Inc, Lincoln, Nebraska, USA). Leaves were stored at 5 °C (max. of 7 days) before being dried at 45 °C for 5 days, weighed and stored until analyses could be performed. SLA was determined as leaf area divided by leaf dry mass. Dry leaf material was finely ground and digested in an acid solution (potassium sulfate dissolved in concentrated sulfuric acid) at 200 °C for 20 min; hydrogen peroxide was then added and the temperature increased to 380 °C (Heffernan, 1985). Total N and P concentrations in digests were determined using a Technicon Auto-Analyser II (Technicon Instruments Corporation, Tarrytown, NY, USA) using an ascorbic acid/ammonium molybdate method for P and nitro-prusside method for N.

N and P concentrations were used to calculate instantaneous nitrogen-use efficiency (A_{max}/N) (NUE μmol CO_2 s^{-1} g^{-1} N) and instantaneous phosphorus-use efficiency (A_{max}/P) (PUE μmol CO_2 s^{-1} g^{-1} P) for each A_{max} measurement made before and after pruning.

2.4. Measurements of leaf water potential

Leaf water potential (Ψ) was measured using a Scholander-type pressure chamber, Plant Water Status Console Series 3000 (Soil Moisture Equipment Corp, Santa Barbara, USA). For each measurement a branchlet containing 8–10 leaves was excised from the canopy and placed into plastic bags within an insulated ice-cooled container, where it remained until measurement for up to 20 min, until three individual leaves were cut from the branchlet and measured following the methods of Turner (1988) within 1 min of excision.

Pre-dawn (03:30–05:30 EST) leaf water potential (Ψ_{pd}) and mid-day (11:00–13:00 EST) leaf water potential (Ψ_{md}) measurements were made over 2 consecutive days following the completion of the gas exchange measurements in the same crown positions. Two trees of each species and treatment were randomly selected for measurement on the same day.

2.5. Measurement of soil moisture, rainfall and air temperature

Soil moisture was measured using a Campbell Pacific Nuclear neutron probe meter CPN 503 hydroprobe (Campbell Pacific Nuclear Corp, Martinez, CA). Aluminium access tubes of 50 mm internal diameter were augured into the soil to a depth of 2.4 m. Placement of the six tubes was chosen to cover the site as completely as possible with three tubes allocated to plots of each species. Tubes were placed between the unpruned and pruned trees in each of the plots. Soil moisture measurements were made at depths of 0.2, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.1 m at approximate monthly intervals from August 2004 to November 2005.

In July 2005, two additional access tubes were installed 1 m either side of the measurement tubes at three of the plots for calibration purposes. At the completion of the measurement period, the three plots containing the additional tubes were sampled. The location of the additional access tubes were randomly selected, with two tubes located in *E. cloeziana* plots and a single in *E. pilularis*. Neutron probe readings were taken immediately before the start of excavation. Three soil cores from each neutron probe depth were removed down to 2.1 m. Volumetric soil water content (VSWC) estimates were obtained by weighing soil before and after oven-drying at a temperature of 105 °C and bulk density determined for each soil core. All probe counts were normalised to a water drum (WD) count taken at the time of measurement (McKenzie et al., 1990) and a surface soil (0.2–0.5 m) and sub-surface soil (0.8–2.1 m) calibration equation was obtained from mean VSWC taken at each depth at each of the nine access tubes excavated. Two equations were needed due to changes in texture and organic matter content with depth in the soil profile.

Mean volumetric soil water contents (VSWC; g cm^{-3}) were normalised to a water drum neutron count using soil neutron count (CNT). The calibration equations obtained for the brown clay surface soil 0.2–0.5 m and mottled poorly structured clay sub-soil 0.8–2.1 m were:

$$\mathrm{VSWC}_{0.2-0.5\,\mathrm{m}} = 0.8489 \times \frac{\mathrm{CNT}}{\mathrm{WD}} - 0.1418 \quad (r^2 = 0.69, P < 0.001) \tag{1}$$

$$\mathrm{VSWC}_{0.8-2.1\,\mathrm{m}} = 0.5059 \times \frac{\mathrm{CNT}}{\mathrm{WD}} - 0.0404 \quad (r^2 = 0.70, P < 0.001) \tag{2}$$

One outlying volumetric soil moisture measurement was excluded from Eq. (2), which appeared to be a recording error associated with soil after drying. The above equations were applied to CNT/

Table 1
Summary of REML variance components analysis conducted to determine the effect of crown position and species on physiological and morphological leaf traits in *E. pilularis* and *E. cloeziana* trees prior to pruning

Leaf trait	No. trees	Crown position			Species			Crown position × species		
		d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value
R_d (μmol CO_2 m^{-2} s^{-1})	4	2	71.17	<0.001	1	0.01	0.943	2	1.12	0.570
Γ (μmol m^{-2} s^{-1})	4	2	50.86	<0.001	1	0.55	0.457	2	1.98	0.371
Φ (mol mol^{-1})	4	2	21.37	<0.001	1	0.70	0.403	2	2.14	0.343
Ψ_{pd} (MPa)	4	2	16.02	<0.001	1	0.00	0.974	2	5.40	0.067
$A_{max\ area}$ (μmol CO_2 m^{-2} s^{-1})	8	2	38.02	<0.001	1	1.38	0.239	2	18.63	<0.001
$A_{max\ mass}$ (μmol CO_2 g^{-1} s^{-1})	8	2	6.44	0.040	1	0.06	0.799	2	18.01	<0.001
T (mmol H_2O m^{-2} s^{-1})	8	2	8.14	0.017	1	0.10	0.751	2	10.77	0.005
g (mol H_2O m^{-2} s^{-1})	8	2	12.99	0.002	1	0.15	0.699	2	6.30	0.043
N (g m^{-2})	8	2	119.99	<0.001	1	0.70	0.403	2	11.03	0.004
P (g m^{-2})	8	2	87.39	<0.001	1	0.47	0.494	2	8.72	0.013
WUE (μmol CO_2 mol^{-1} H_2O)	8	2	3.38	0.185	1	0.02	0.899	2	6.69	0.035
NUE (μmol CO_2 s^{-1} g^{-1} N)	8	2	12.16	0.002	1	2.54	0.111	2	18.77	<0.001
PUE (μmol CO_2 s^{-1} g^{-1} P)	8	2	8.67	0.013	1	0.06	0.799	2	27.98	<0.001
SLA (m^2 kg^{-1})	8	2	108.55	<0.001	1	56.58	<0.001	2	40.57	<0.001

WD data collected over the course of the experiment to calculate VSWC as a percentage of total volume.

Measurements of rainfall were made using a 0.2 mm tipping-bucket rain gauge with a bucket located 200 m from the experimental site. A Starlog 6003B Portable Data Logger (Unidata Pty Ltd., Perth, Western Australia, Australia) was attached and programmed to record total rainfall at 15 min intervals. Maximum and minimum temperatures were derived from interpolated climate surfaces (Anon., 2007).

2.6. Data analysis

Residual maximum likelihood (REML) variance components analysis was conducted to examine the effect of crown position and species on fourteen leaf traits collected prior to pruning, including R_d, Γ, Φ, Ψ_{pd}, $A_{max\ area}$, $A_{max\ mass}$, T, g, N, P, WUE, NUE, PUE and SLA.

For each species, the effect of pruning treatment and crown position on leaf physiology was also examined using REML variance components analysis. Mean A_{max}, ΔA_{max}, T, g, N, P, WUE, NUE, PUE, SLA, Ψ_{pd} and Ψ_{md} were analysed separately 6, 10 and 13 months after pruning. All analyses were performed using Genstat (VSN International 2004, Hemel Hempstead, Herts, UK). Regression analysis was used to examine relationships between A_{max} and N.

3. Results

3.1. The effect of crown position on leaf-level photosynthesis

R_d, Γ, Φ) and Ψ_{pd} varied with crown position but not species prior to pruning (Table 1). With increasing height in the crown, R_d, Γ and Φ increased (Table 2).

$A_{max\ area}$, $A_{max\ mass}$, T, g, N, P, WUE, NUE, PUE and SLA varied with crown position and species. In both species $A_{max\ area}$, $A_{max\ mass}$, T, g, and N and P foliar concentrations increased with increasing height above the ground, except in the upper crown position of *E. cloeziana* where $A_{max\ area}$ and $A_{max\ mass}$, T and g were not significantly different from the lowest crown position. $A_{max\ area}$, $A_{max\ mass}$, T and g were significantly lower in the upper crown position of *E. cloeziana* than in the upper crown of *E. pilularis*. The ranges of leaf N and P concentrations measured in the crown were greater in *E. cloeziana* than *E. pilularis* (Table 2). N and P concentrations were lower in the lower crown of *E. cloeziana* than *E. pilularis*, while N concentrations in the mid-crown were higher in *E. cloeziana* than *E. pilularis*. WUE was significantly lower in the lower crown of *E. cloeziana* than *E. pilularis*, while NUE and PUE were significantly lower in the upper crown of *E. cloeziana* when compared to *E. pilularis*. SLA declined with increasing height in the crown of both species. In the middle and lower crown positions, SLA was significantly higher in *E. cloeziana* than in *E. pilularis* (Table 2).

Table 2
Predicted means from REML variance components analysis conducted to determine the effect of crown position (lower, middle, upper) and species on leaf physiology traits in *E. pilularis* and *E. cloeziana* trees prior to pruning

	E. pilularis			*E. cloeziana*			SED_{pos}	SED_{sp}
	Lower	Middle	Upper	Lower	Middle	Upper		
R_d (μmol CO_2 m^{-2} s^{-1})	−1.03 aA	−1.69 aA	−2.96 bA	−0.81 aA	−1.97 bA	−2.93 cA	**0.36**	0.34
Γ (μmol m^{-2} s^{-1})	22.56 aA	26.03 aA	38.66 bA	16.67 aA	27.33 bA	36.80 cA	**4.13**	3.62
Φ (mol mol^{-1})	0.020 aA	0.036 abA	0.045 bA	0.029 aA	0.048 aA	0.044 aA	**0.01**	0.01
Ψ_{pd} (MPa)	0.29 aA	0.19 bA	0.31 aA	0.26 aA	0.24 aA	0.28 aA	**0.05**	0.03
$A_{max\ area}$ (μmol CO_2 m^{-2} s^{-1})	6.07 aA	9.40 bA	12.45 cA	6.06 aA	10.76 bA	7.23 aA	**1.43**	1.09
$A_{max\ mass}$ (μmol g^{-1} s^{-1})	57.14 aA	77.32 aA	80.36 aA	80.20 aA	94.77 aA	53.86 bA	**11.68**	10.53
T (mmol H_2O m^{-2} s^{-1})	1.15 aA	1.77 abA	2.23 bA	1.56 aA	1.89 aA	1.42 aA	**0.38**	0.27
g (mol H_2O m^{-2} s^{-1})	0.07 aA	0.11 abA	0.15 bA	0.09 aA	0.13 aA	0.09 aA	**0.03**	0.02
N (g m^{-2})	1.18 aA	1.52 aA	2.22 bA	0.89 aA	2.02 bA	2.44 bA	**0.22**	0.17
P (g m^{-2})	0.10 aA	0.12 aA	0.13 aA	0.05 aA	0.15 bA	0.17 bA	**0.02**	0.02
WUE (μmol CO_2 mol^{-1} H_2O)	89.60 aA	86.47 aA	80.90 aA	68.39 aA	94.40 aA	96.55 aA	10.70	10.08
NUE (μmol CO_2 s^{-1} g^{-1} N)	5.31 aA	6.26 aA	5.86 aA	6.67 aA	5.30 aA	3.04 bA	**0.76**	0.68
PUE (μmol CO_2 s^{-1} g^{-1} P)	65.64 aA	84.20 aA	82.86 aA	114.61 aA	75.81 bA	52.10 bA	**15.69**	10.95
SLA (m^2 kg^{-1})	8.75 aA	7.91 abA	6.94 bA	14.53 aB	9.18 bB	7.90 cA	**0.60**	**0.60**

Means sharing the same lower case letters are not significantly different from other positions within a species and means sharing the same upper case letters are not significantly different from the same position on the alternate species ($P < 0.05$). Standard errors of difference between position (SED_{pos}) and species (SED_{sp}) are provided. Bold SED values indicate a significant effect (Chi $P < 0.05$).

Table 3
Summary of REML variance components analysis conducted to determine the effect of crown position and pruning treatment on leaf physiology traits in *E. pilularis* trees 6, 10 and 13 months (mos) post-pruning (pp)

Leaf trait	No. trees	Crown position			Treatment			Crown position × treatment		
		d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value
$A_{max\ area}$ (μmol CO_2 m^{-2} s^{-1})										
6 mos pp	8	2	107.88	**<0.001**	1	0.07	0.787	2	0.61	0.737
10 mos pp	8	1	12.54	**<0.001**	1	0.11	0.737	1	1.18	0.278
13 mos pp	8	1	18.20	**<0.001**	1	0.01	0.912	1	0.21	0.650
$\Delta A_{max\ area}$ (%)										
From 0 to 6 mos pp	8	1	3.5	0.061	1	1.47	0.225	1	1.87	0.172
From 0 to 10 mos pp	8	1	0.02	0.875	1	0.03	0.860	1	0.38	0.536
From 0 to 13 mos pp	8	1	0.13	0.717	1	0.15	0.702	1	0.01	0.907
$A_{max\ mass}$ (μmol CO_2 g^{-1} s^{-1})										
6 mos pp	8	2	69.3	**<0.001**	1	0.41	0.521	2	0.11	0.944
10 mos pp	8	1	0.13	0.720	1	2.57	0.109	1	1.40	0.237
13 mos pp	8	1	6.39	**0.011**	1	0.03	0.873	1	0.0	0.972
T (mmol H_2O m^{-2} s^{-1})										
6 mos pp	8	2	30.34	**<0.001**	1	0.08	0.781	2	0.61	0.736
10 mos pp	8	1	2.45	0.117	1	0.05	0.821	1	0.32	0.572
13 mos pp	8	1	25.56	**<0.001**	1	0.83	0.362	1	4.02	**0.045**
g (mol H_2O m^{-2} s^{-1})										
6 mos pp	8	2	45.89	**<0.001**	1	0.01	0.937	2	0.06	0.972
10 mos pp	8	1	4.19	**0.041**	1	0.55	0.458	1	0.09	0.763
13 mos pp	8	1	26.71	**<0.001**	1	0.11	0.742	1	2.60	0.107
N (g m^{-2})										
6 mos pp	8	2	41.44	**<0.001**	1	8.39	**0.004**	2	1.79	0.408
10 mos pp	8	1	14.79	**<0.001**	1	1.24	0.266	1	0.15	0.698
13 mos pp	8	1	20.56	**<0.001**	1	0.36	0.551	1	0.42	0.518
P (g m^{-2})										
6 mos pp	8	2	12.48	**0.002**	1	1.79	0.181	2	1.38	0.501
10 mos pp	8	1	0.04	0.849	1	0.25	0.614	1	0.01	0.927
13 mos pp	8	1	2.05	0.152	1	0.00	0.954	1	0.73	0.393
WUE (μmol CO_2 mol^{-1} H_2O)										
6 mos pp	8	2	7.62	**0.022**	1	0.46	0.499	2	0.48	0.787
10 mos pp	8	1	3.00	0.083	1	0.97	0.324	1	7.40	**0.007**
13 mos pp	8	1	27.48	**<0.001**	1	0.24	0.622	1	2.49	0.115
NUE (μmol CO_2 s^{-1} g^{-1} N)										
6 mos pp	8	2	12.27	**0.002**	1	1.24	0.266	2	0.55	0.759
10 mos pp	8	1	2.24	0.135	1	1.13	0.287	1	1.27	0.260
13 mos pp	8	1	21.41	**<0.001**	1	0.04	0.837	1	5.03	**0.025**
PUE (μmol CO_2 s^{-1} g^{-1} P)										
6 mos pp	8	2	108.64	**<0.001**	1	0.35	0.555	2	0.03	0.984
10 mos pp	8	1	11.11	**<0.001**	1	0.05	0.828	1	1.66	0.197
13 mos pp	8	1	6.06	**0.014**	1	0.01	0.931	1	1.73	0.188
SLA (m^2 kg^{-1})										
6 mos pp	8	2	2.16	0.339	1	3.86	**0.049**	2	1.00	0.608
10 mos pp	8	1	15.49	**<0.001**	1	3.18	0.075	1	0.05	0.831
13 mos pp	8	1	0.10	0.757	1	0.09	0.769	1	0.08	0.778
Ψ_{pd} (MPa)										
6 mos pp	8	2	40.82	**<0.001**	1	0.13	0.720	2	0.59	0.746
10 mos pp	8	1	0.02	0.900	1	0.56	0.455	1	2.72	0.099
13 mos pp	8	1	64.44	**<0.001**	1	0.05	0.819	1	0.01	0.942
Ψ_{md} (MPa)										
6 mos pp	8	2	7.48	**0.024**	1	0.02	0.896	2	0.64	0.725
10 mos pp	8	1	0.10	0.747	1	1.16	0.280	1	0.00	0.995
13 mos pp	8	1	31.88	**<0.001**	1	0.00	0.949	1	1.83	0.177

Note: crown position d.f. change with time since pruning due to the loss the leaves from the mid-crown position. Figures shown in bold are significant at the 0.05 significance level.

3.2. The effect of pruning on leaf-level photosynthesis

Leaf physiology and morphology traits showed strong differences between crown position but there was almost no effect of pruning treatment on leaf physiology and morphology 6, 10 or 13 months after pruning in either *E. pilularis* (Table 3) or *E. cloeziana* (Table 4). $A_{max\ area}$ was significantly different between crown position 6, 10 and 13 months after pruning in both species. Expressing $A_{max\ mass}$ as opposed to leaf-area basis did not change the results for both species.

In the unpruned trees of both species $A_{max\ area}$ varied over the 13-month experimental period (Fig. 2). In *E. pilularis*, $A_{max\ area}$ in the upper and new developed crown positions was relatively constant over the experimental period. Measurements in the lower

Table 4
Summary of REML variance components analysis conducted to determine the effect of crown position and pruning treatment on leaf physiology traits in *E. cloeziana* trees 6, 10 and 13 months (mos) post-pruning (pp)

Leaf trait	No. trees	Crown position			Treatment			Crown position × treatment		
		d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value	d.f.	Wald statistic	Chi *P*-value
$A_{max\ area}$ (μmol CO_2 m^{-2} s^{-1})										
6 mos pp	8	2	32.66	**<0.001**	1	2.45	0.118	2	0.18	0.915
10 mos pp	8	2	46.46	**<0.001**	1	0.72	0.398	2	0.22	0.898
13 mos pp	8	2	38.38	**<0.001**	1	0.41	0.522	2	1.44	0.486
$\Delta A_{max\ area}$ (%)										
From 0 to 6 mos pp	8	1	32.44	**<0.001**	1	0.21	0.649	1	0.23	0.634
From 0 to 10 mos pp	8	2	35.01	**<0.001**	1	0.11	0.738	2	0.36	0.837
From 0 to 13 mos pp	8	2	53.14	**<0.001**	1	0.14	0.703	2	0.44	0.881
$A_{max\ mass}$ (μmol CO_2 g^{-1} s^{-1})										
6 mos pp	8	2	15.11	**<0.001**	1	3.09	0.079	2	0.94	0.625
10 mos pp	8	2	16.94	**<0.001**	1	0.09	0.768	2	0.20	0.907
13 mos pp	8	2	6.72	**0.001**	1	0	0.974	2	0.46	0.630
T (mmol H_2O m^{-2} s^{-1})										
6 mos pp	8	2	16.41	**<0.001**	1	0.64	0.425	2	0.99	0.611
10 mos pp	8	2	0.44	0.804	1	0.02	0.888	2	0.54	0.763
13 mos pp	8	2	7.16	**0.028**	1	0	0.985	2	0.08	0.962
g (mol H_2O m^{-2} s^{-1})										
6 mos pp	8	2	18.03	**<0.001**	1	0.53	0.467	2	1.12	0.572
10 mos pp	8	2	5.19	0.075	1	0.20	0.656	2	0.12	0.941
13 mos pp	8	2	5.17	0.075	1	0.09	0.764	2	0.20	0.904
N (g m^{-2})										
6 mos pp	8	2	21.55	**<0.001**	1	0.35	0.552	2	0.45	0.797
10 mos pp	8	2	56.57	**<0.001**	1	2.70	0.100	2	2.77	0.250
13 mos pp	8	2	18.52	**<0.001**	1	1.73	0.188	2	3.70	0.157
P (g m^{-2})										
6 mos pp	8	2	18.91	**<0.001**	1	0.04	0.850	2	0.34	0.845
10 mos pp	8	2	5.99	**0.050**	1	0.03	0.856	2	0.82	0.663
13 mos pp	8	2	10.30	**0.006**	1	0.50	0.478	2	0.45	0.800
WUE (μmol CO_2 mol^{-1} H_2O)										
6 mos pp	8	2	16.63	**<0.001**	1	0.50	0.480	2	2.47	0.291
10 mos pp	8	2	44.47	**<0.001**	1	1.23	0.267	2	0.61	0.736
13 mos pp	8	2	7.44	**0.024**	1	0.36	0.547	2	0.24	0.886
NUE (μmol CO_2 s^{-1} g^{-1} N)										
6 mos pp	8	2	20.90	**<0.001**	1	0.78	0.377	2	0.74	0.690
10 mos pp	8	2	10.18	**0.006**	1	1.47	0.225	2	1.32	0.517
13 mos pp	8	2	3.94	0.139	1	0.88	0.349	2	3.19	0.203
PUE (μmol CO_2 s^{-1} g^{-1} P)										
6 mos pp	8	2	33.93	**<0.001**	1	0.76	0.383	2	0.98	0.613
10 mos pp	8	2	42.88	**<0.001**	1	0.16	0.685	2	0.17	0.919
13 mos pp	8	2	7.77	**0.021**	1	1.90	0.168	2	2.38	0.305
SLA (m^2 kg^{-1})										
6 mos pp	8	2	1.62	0.455	1	0.60	0.439	2	0.93	0.630
10 mos pp	8	2	22.44	**<0.001**	1	0.01	0.904	2	0.57	0.750
13 mos pp	8	2	42.85	**<0.001**	1	0.22	0.643	2	1.50	0.473
Ψ_{pd} (MPa)										
6 mos pp	8	2	16.49	**<0.001**	1	0.36	0.549	2	2.29	0.319
10 mos pp	8	2	0.82	0.665	1	0.06	0.812	2	10.53	**0.005**
13 mos pp	8	2	50.89	**<0.001**	1	0.02	0.890	2	1.14	0.567
Ψ_{md} (MPa)										
6 mos pp	8	2	0.65	0.722	1	1.22	0.269	2	2.32	0.314
10 mos pp	8	2	1.19	0.550	1	0.00	0.988	2	0.25	0.882
13 mos pp	8	2	3.07	0.215	1	0.13	0.713	2	0.42	0.811

Figures shown in bold are significant at the < 0.05 signficance level.

and mid-canopy positions of *E. pilularis* could not be continued after the 6-month post-pruning period as leaves within the lower and mid-crown positions were shed from stems between 6 and 10 months after the first measurement. A slight increase in $A_{max\ area}$ was observed in the lower crown of *E. pilularis* 6 months after the first measurement, while decreases were observed in the mid-crown. Very few leaves remained on the lower and mid-crown branches during this measurement. A_{max} in *E. cloeziana* trees were relatively constant within the mid-crown over the experiment, however large changes were observed in the upper and new developed crown. No leaves were present on the northern side of the lower crown 10 months after first measurement.

To further examine the change in A_{max} over the 13-month measurement period, post-pruning measurements of $A_{max\ area}$ were expressed as a percentage of pre-pruning levels ($\Delta A_{max\ area}$). Data for the newly developed crown zone (>100%) was expressed

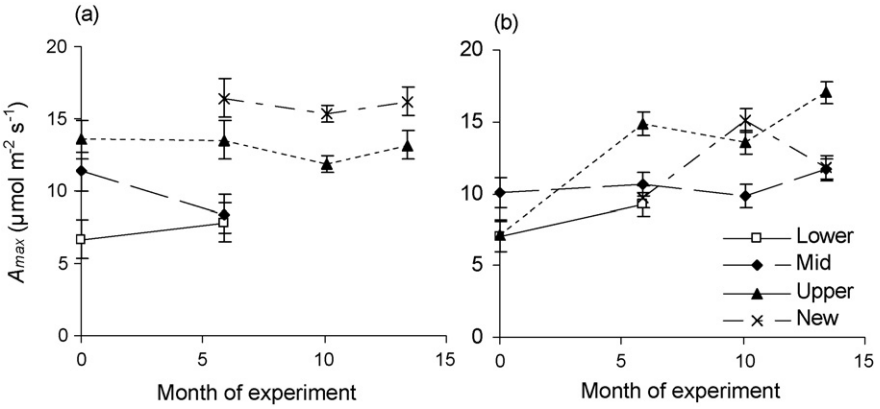

Fig. 2. Light-saturated photosynthesis (A_{max}) (μmol CO_2 m^2 s^{-1}) for leaves of unpruned (a) *E. pilularis* and (b) *E. cloeziana* trees from the lower (25% green crown length), mid (60% green crown length), upper (85% green crown length) and newly developed (>100% green crown length) crown positions. Error bars indicate standard errors of means.

as a percentage of measurements taken 6 months after pruning. Measurements from the lower crown position were excluded from the analysis in both species due to limited data from post-pruning measurements as a result of leaf shedding from the lower zone. Similarly, the further loss of leaves from the mid-crown in *E. pilularis* trees necessitated the exclusion of the mid-crown position from the analyses of measurements after 10 and 13 months. The $\Delta A_{max\ area}$ at 6, 10 and 13 months after pruning in *E. pilularis* trees were not significantly different between crown position or pruning treatment (Fig. 3), while in *E. cloeziana* trees, $\Delta A_{max\ area}$ was significantly different between crown position 6, 10 and 13 months after pruning (Fig. 3 and Table 4).

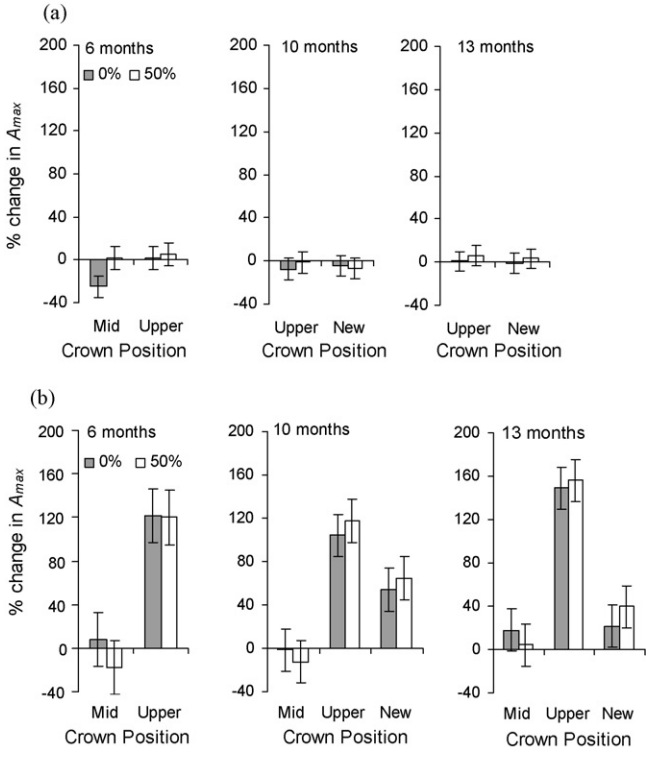

Fig. 3. Percentage change in pre-pruning light-saturated photosynthesis (A_{max}) 6, 10 and 13 months after pruning in leaves from unpruned (0%) and pruned (50%) trees of (a) *E. pilularis* and (b) *E. cloeziana* in the mid (60% green crown length), upper (85% green crown length) and newly developed (>100% green crown length) crown positions. Error bars represent the standard error of mean. There were no significant differences between pruning treatment but there were significant differences between crown position at 0.05 significance level.

Six months after pruning, N and SLA were significantly different between pruning treatments in *E. pilularis* (Table 3). Leaf N concentrations were 1.92 and 1.65 g m^{-2} in the 0 and 50% treatments, respectively, and SLA was 6.76 and 7.57 m^2 kg^{-1} in the 0 and 50% treatments, respectively. All other leaf traits were not significantly different between pruning treatments in either species.

T and NUE varied with crown position and pruning treatment 13 months after pruning and WUE varied with crown position and pruning treatment 10 months after pruning in *E. pilularis* (Table 3). Similarly Ψ_{pd} varied with crown position and treatment 10 months after pruning in *E. cloeziana*. The absence of significant treatment effects when significant interactions were present suggests treatment effects were weak.

A positive relationship between foliar N and $A_{max\ area}$ was evident in both species throughout the measurement period (Fig. 4). Foliar N data from the leaves collected from the upper crown of six (circled data points Fig. 4c) of the eight *E. cloeziana* trees prior to pruning were removed from the analysis as they displayed lower rates of $A_{max\ area}$ for a given N than leaves from all other crown positions. These leaves are likely to have been younger than the other sample leaves from the lower crown.

3.3. Soil moisture and weather

Soil moisture availability at all depths was lowest prior to pruning, but remained relatively similar at all depths for the following three post-pruning gas exchange measurements (Fig. 5a). The relatively constant availability of water to trees over the experimental period is reflected in the relatively constant Ψ measurements (Fig. 6).

A large amount (225 mm) of rain fell just after the pruning treatment in October 2004 (Fig. 5c). This was reflected in mean soil water content at all depths, with a sharp increase in soil moisture in October 2004 (Fig. 5).

3.4. Leaf water potential

Ψ_{pd} was relatively constant across the measurement period (Fig. 6). Diurnally, Ψ declined from pre-dawn to mid-day. The minimum mean Ψ_{md} reached across all crown positions and was −1.97 and −1.94 for *E. pilularis* and *E. cloeziana* leaves, respectively. Ψ_{pd} values were significantly lower in leaves from the upper crown 6 and 13 months after pruning in both species. Similar trends were observed in Ψ_{md} measurements, with lower values recorded for leaves from the upper crown than the middle or lower crown, however, only significant differences were present in *E. pilularis* 6

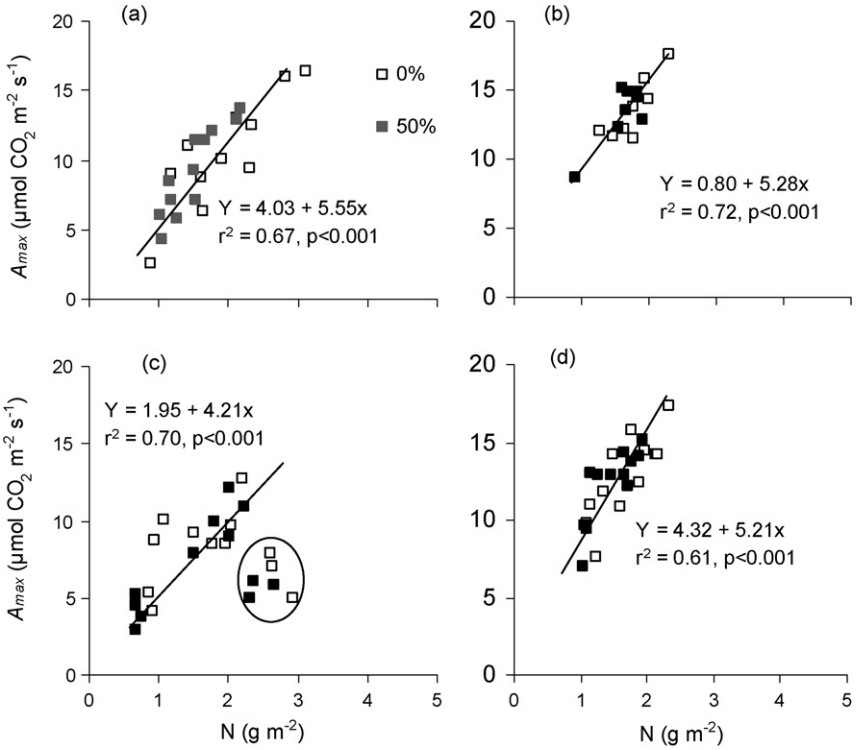

Fig. 4. Strong linear relationships between mean light-saturated photosynthesis rate (A_{max}, μmol CO_2 m^2 s^{-1}) and foliar nitrogen concentration (N g m^{-2}) for leaves from pruned (50% crown length removal) and unpruned (0% crown length removal) *E. pilularis* and *E. cloeziana* trees 0 (a and c) and 10 months (b and d) after pruning. Pruning treatment was not significant ($P < 0.05$). Circled values in graph (c) are the upper crown measurements excluded from the regression analysis.

and 13 months after pruning. The lack of difference in Ψ_{pd} (Fig. 6a and b) Ψ_{md} and between crown positions at 10 months after pruning in both species is a likely reflection of mild temperatures in July (Fig. 5b). In contrast, lower Ψ measurements recorded in March (6 months) and November (13 months) suggest that the newly developed crown was more water stressed than the upper crown, which is likely a reflection of the hotter weather during these measurements.

4. Discussion

The results of this study confirm that there are strong positional effects in physiological and morphological leaf traits in both species. We found little difference in physiological leaf properties between the two study species. There was no evidence of up-regulation of photosynthesis 6 months after pruning that removed 50% of the live crown.

4.1. The effect of crown position on leaf-level photosynthesis

Leaf position within the crown had a strong influence on physiological leaf traits in both species. Declining R_d and Γ with increasing depth in the canopy are typical responses to a light gradient. It has been suggested that the prolonged survival of heavily shaded leaves and branches in the lower crown may relate to lower R_d (Sprugel et al., 1991). Furthermore, lower Γ in the lower crown means a lower level of light is required to support a net gain of carbon uptake (Ehleringer and Björkman, 1977). Lower R_d and Γ may contribute to the survival of leaves at the base of crowns in both species.

Higher Φ means greater ability to fix carbon under low light conditions. The lower Φ in the lower crown position compared to the upper and mid-crown positions was a little surprising as quantum yield often decreases in leaves grown at high-light compared to low light (Björkman, 1981; Turnbull, 1991; Sims and Pearcy, 1992). Turnbull (1991) reported decreased quantum yield in five out of six rain forest trees when grown at the lowest light level at which they survived. The longer duration of shading in the lower leaves of both species due to branch shading from surrounding trees within the closed-canopy stand may have contributed to the lower quantum yield when compared to other parts of the crown, or these species may not develop true shade leaves (Leverenz and Jarvis, 1980) and do not possess the structures (e.g. thin epidermis and thick mesophyll) and mechanisms to facilitate a high quantum yield (Chazdon and Kaufmann, 1993).

$A_{max\ area}$ rates were similar in the lower and mid-crown positions of both species, however much lower rates were measured in the upper crown of *E. cloeziana* than *E. pilularis* prior to pruning. This may be a leaf age effect because leaves measured in the upper crown of *E. cloeziana* appeared to be much younger (a recent leaf flush had occurred just prior to sampling) than sample leaves used during subsequent measurements and younger than those sampled in *E. pilularis*. This observation is supported by the similarity in $A_{max\ area}$ measured in the upper crown of unpruned *E. cloeziana* and *E. pilularis* 6, 10 and 13 months after the initial measurements (Fig. 2). Overall the similarity in leaf physiological traits between *E. pilularis* and *E. cloeziana* suggests that there is little physiological difference in shade tolerance between the two species.

4.2. Photosynthetic response to pruning

Contrary to predictions, we did not observe an increase in light-saturated photosynthesis in leaves of *E. pilularis* and *E. cloeziana* trees 6–13 months after the removal of 50% of the green crown

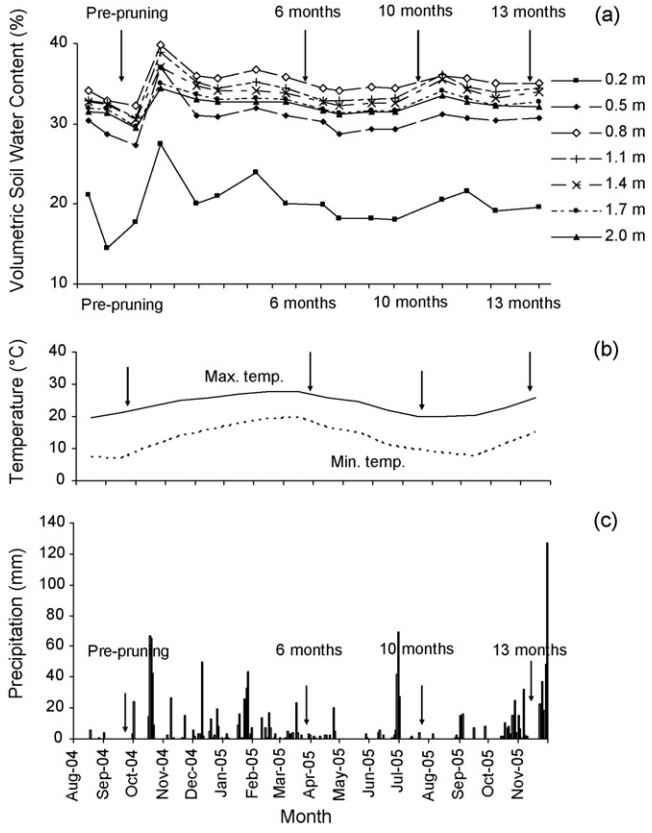

Fig. 5. Changes in mean soil water content (%) with depth below the ground (a), daily maximum and minimum temperature (°C), (b) precipitation (mm) and (c) at the Southgate Experimental Site from 1 August 2004 to 30 November 2005. Arrows indicate the 4 periods when gas exchange measurements were made. Temperature data was derived from modelled climate surfaces (NRM 2007).

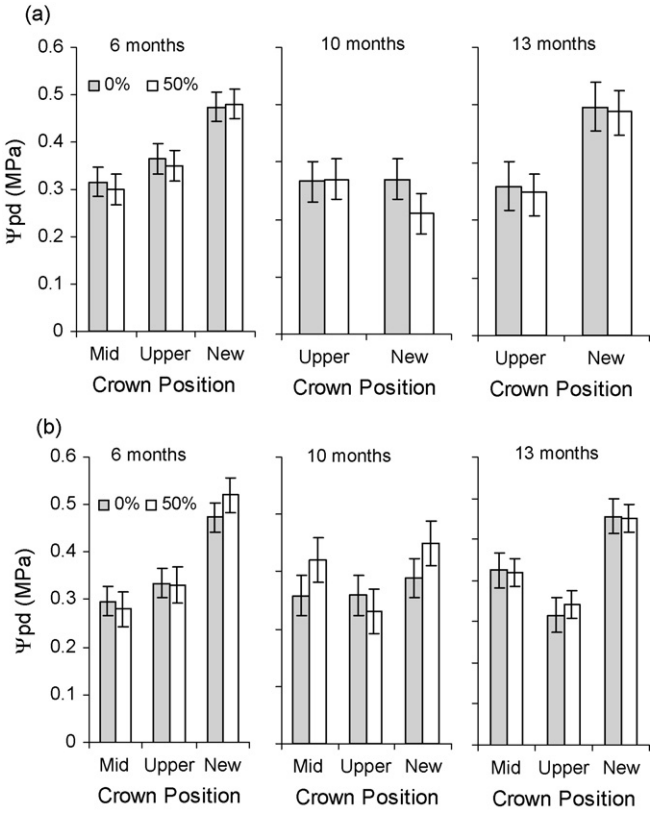

Fig. 6. Pre-dawn leaf water potential (MPa) measured 6, 10 and 13 months after pruning in leaves from unpruned (0%) and pruned (50%) trees of (a) *E. pilularis* and (b) *E. cloeziana* in the mid (60% green crown length), upper (85% green crown length) and newly developed (>100% green crown length) crown positions. Error bars represent the standard error of mean. There were no significant differences between pruning treatments but there were significant differences between crown position 6 and 13 months after pruning at 0.05 significance level.

length. Unfortunately, equipment failure prevented data being collected from the time of pruning up to 6 months after pruning. However, we can conclude that no change in A_{max} was apparent in the fully expanded apical foliage of pruned trees of both species 6 months after pruning. These results contrast with a study in pruned *E. nitens*, which found long-term (>16 months) increases in A_{max} throughout the crown in all foliage types (Pinkard et al., 1998). It is not possible to know whether a change in A_{max} occurred within the first 6 months after pruning, however, given the favourable soil moisture and temperature conditions in the field following pruning (Fig. 6a and b) there is a possibility that the duration of the response may have been very rapid (<6 months). For example, under favourable controlled temperature and moisture conditions, the duration of up-regulation reported following partial defoliation of seedlings of *Pinus resinosa* was 20 weeks (Heichel and Turner, 1983) and for *E. pilularis* and *E. cloeziana* seedlings it was found to be 7 weeks (Ivakou et al., unpublished data). Thus, if up-regulation did occur in these two sub-tropical eucalypt species during the first 6 months following pruning, the effect has been lost by 6 months and the duration was considerably shorter than findings from other artificial defoliation studies on young trees in field conditions (e.g. Reich et al., 1993; Pinkard et al., 1998; Medhurst et al., 2006).

The shedding of leaves from the lower crown of the controls of both species may have contributed to the inability to detect photosynthetic up-regulation. Reductions in leaf area may have improved the light environment and potentially photosynthetic capacity (Trumble et al., 1993). While higher rates of photosynthesis were measured in the upper crown of *E. cloeziana* and new crown of *E. pilularis* 6 months after pruning than prior to pruning, these effects cannot be separated from the possible seasonal or climatic effects on photosynthetic capacity measurements over time.

A limited number of defoliation studies have reported no change or a decrease in photosynthetic capacity in response to artificial defoliation (e.g. Troeng and Langström, 1991). Other studies have reported the photosynthetic activity remaining unchanged initially (e.g. Hall and Ferree, 1976; Hall and Brady, 1977; Karban and Courtney, 1987) and then increase following leaf-area recovery to levels above those plants that have not been defoliated (e.g. Meidner, 1970; Detling et al., 1979). Such variable or changing responses over time may also complicate the measurement of possible photosynthetic changes (Trumble et al., 1993). Given whole tree leaf area was restored to pre-pruning levels within 9 months after 50% crown removal in both species (Alcorn, unpublished data), it is unlikely that up-regulation could have occurred after 13 months following pruning.

Measurements indicate that the maximum rates of $A_{max\ area}$ across all foliage types for both species were approximately 17 μmol CO_2 m^{-2} s^{-1} under PPFD of 1100 μmol m^{-2} s^{-1}. There is a dearth of published photosynthetic data for comparison of *E. pilularis* and *E. cloeziana*. Maximum $A_{max\ area}$ in the upper crown of *E. pilularis* were within the range of 11.7–23.1 μmol m^{-2} s^{-1} reported for *E. grandis* growing under varying levels of P (Kirschbaum and Tompkins, 1990). The maximum $A_{max\ area}$ in the upper crown of *E. cloeziana* were slightly higher than the ca. 14 μmol CO_2 m^{-2} s^{-1} measured in the morning during summer in

the upper foliage of *E. cloeziana* growing at a sub-tropical site (Ngugi et al., 2004). $A_{max\ area}$ in the upper crown of *E. cloeziana* prior to pruning in this study (8 μmol CO_2 m^{-2} s^{-1}) were similar to those recorded in the same crown position in the morning in winter by Ngugi et al. (2004) (ca. 5–7 μmol CO_2 m^{-2} s^{-1}). Maximum values of $A_{max\ area}$ were lower than those reported for temperate plantation eucalypt trees such as *E. nitens* (22–25 μmol CO_2 m^{-2} s^{-1}) (Battaglia et al., 1996; Hunt et al., 2006) and *E. globulus* (14–20 μmol CO_2 m^{-2} s^{-1}) (Battaglia et al., 1996; Henskens et al., 2001).

In *E. pilularis*, $A_{max\ area}$ increased with increasing height in the crown. This is consistent with the gradient of leaf age, as highest rates of photosynthesis are generally found in apical or mature foliage when compared to older foliage (Pereira et al., 1992; Pinkard et al., 1998; Medhurst and Beadle, 2005). In *E. cloeziana*, $A_{max\ area}$ was more variable between crown positions, however, this effect may also be attributable to leaf age. It was evident that seasonal flushes of leaves occurred at the tips of branches in the mid, upper and extra canopy positions of *E. cloeziana* between measurements. For this reason we did not obtain a consistent gradient in leaf age by measuring the external leaves only. A better approach would be to separate foliage into age classes along the branch and measure assimilation across age classes. This approach would not be possible and was not required in *E. pilularis* as leaf flushes were not evident on the lower and mid-crown branches. In contrast to *E. cloeziana*, there was a clear gradient in leaf age over the experimental period.

Leaf age is often closely related to leaf N. Studies have shown decreasing foliar N concentrations with age (Field and Mooney, 1983; Mooney et al., 1983; Hirose et al., 1989; Reich et al., 1994). Nitrogen is an essential component of chlorophyll, protein and amino acids required for photosynthesis (Grundon et al., 1997). Not surprisingly, $A_{max\ area}$ was positively correlated with leaf N in both species over the experimental period (Fig. 5). Positive correlations between N and $A_{max\ area}$ is consistent with findings in a number of *Eucalyptus* species, including *E. grandis* (Leuning et al., 1991), *E. globulus* (Sheriff and Nambiar, 1991; Pereira et al., 1992) and *E. nitens* (Medhurst and Beadle, 2005), as well as other plant species (Field and Mooney, 1983; Mooney et al., 1983; DeJong and Doyle, 1985; Reich et al., 1994). The lower rates of $A_{max\ area}$ observed in the upper crown of six *E. cloeziana* trees before pruning were also associated with lower intercellular CO_2 and stomatal conductance, and were comparable to field measurements of A_{max} from the upper crown leaves of *E. cloeziana* trees in Queensland, Australia (Ngugi et al., 2004). The higher levels of total leaf nitrogen in these young leaves, may have been associated with higher levels of N in storage compounds that do not play a direct role in photosynthesis (Dietz and Harris, 1997), however, this cannot be confirmed without more detailed examination of the leaf chemistry.

Foliar N and P concentrations in *E. pilularis* were towards the lower end of the range reported for plantation eucalypts (Judd et al., 1996). This may be because the majority of plantation eucalypts such as *E. nitens* and *E. globulus* belong to the *Symphyomyrtus* subgenus (Beadle and Turnbull, 1992), which are generally found on more fertile soils than the *Monocalyptus* subgenus (Noble, 1989), to which *E. pilularis* belongs. Concentrations of many foliar nutrients have been correlated with concentrations in the soil, including N and P (Lambert and Turner, 1983). Mean foliar N and P in the uppermost crown position of all treatments across all measurements were 1.38 and 0.08% oven dry weight for *E. pilularis*. These foliar N concentrations were in the lower end of the concentration range considered adequate for vigorous growth (1.3–1.6%) while P was in the upper range (0.06–0.12%) from the same crown position (Boardman et al., 1997). Mean foliar N and P concentrations in the lower crown of pruned and unpruned *E. pilularis* trees for all times were 1.01 and 0.09%. These N concentrations were towards the lower end of the range (0.9–1.3%) considered adequate for vigorous growth in mature foliage collected from the top of the crown and P was towards the middle of the range (0.05–1.2%) (Boardman et al., 1997). Mean foliar N and P in *E. cloeziana* in the uppermost crown position of all treatments across all measurements were 1.75 and 0.11%, respectively. This represented the beginning of the levels considered adequate in young *E. cloeziana* foliage, which were N > 1.7% and P > 0.1% (Olsen and Bell, 1990). Mean foliar N and P concentrations in mature *E. cloeziana* foliage from the lower crown position of all treatments and measurements was 1.28 and 0.10%, respectively. For N this was below the >1.6% considered adequate in mature foliage, while P was at the level required (>0.10) (Olsen and Bell, 1990). Since foliar N concentrations were not optimal for vigorous growth in either species, N may have constrained the photosynthetic capacity.

Foliar N limitation may have contributed to our inability to detect photosynthetic up-regulation after pruning in both species. Adequate N supply is critical to photosynthetic carbon gain (Pinkard et al., 2006), however, other studies have shown that even under N limiting conditions, defoliation can still result photosynthetic up-regulation (e.g. Lovett and Tobiessen, 1993; Ovaska et al., 1993). Under high N levels plants may be able to maintain up-regulated photosynthetic rates for a longer period of time (Lovett and Tobiessen, 1993). The low foliar N levels observed here therefore may have led to a short duration of any up-regulation response, but probably would not have inhibited it altogether.

E. pilularis showed differences in SLA and leaf N concentration 6 months after pruning. The higher SLA of leaves of pruned trees may be a response to maximise the area of leaves available for light capture for every unit of biomass invested (Evans and Poorter, 2001). The lower observed nitrogen in pruned treatments may have been the effect of a temporary reduction in canopy nitrogen following the removal and deposition of branches on the ground. Generally eucalypts withdraw nutrients from leaves before being shed (Florence, 1996), however, an instantaneous defoliation would not enable this process to occur. The cause of such changes in SLA and leaf N can only be speculative without further investigation.

Differences in SLA may influence comparisons between treatments if SLA was due to different amounts of photosynthetically active plant material (Kirschbaum and Tompkins, 1990). In both species, SLA was only different between crown position but not between treatments (except in *E. pilularis* prior to and 6 months after pruning). For this reason it was not surprising that expressing photosynthesis on a leaf-mass rather than leaf-area basis did not alter the effect of pruning treatment.

Ψ_{pd} measurements suggest that trees were not water stressed. White et al. (1996) showed that Ψ_{pd} in *E. nitens* leaves under non-stress conditions were −0.25 MPa while in *E. globulus* Ψ_{pd} of −0.6 MPa caused a decrease in g (Pereira et al., 1987). Since Ψ_{pd} in all crown positions did not exceed −0.6 MPa at any measurement period, it is unlikely that trees experienced water stress over the experimental period. This was despite drier soil conditions prior to pruning when compared to post-pruning (Fig. 6a). The drier soil conditions were the result of low rainfall in August and September 2004 (Fig. 6c) and may be reflected in the lower g measurements prior to pruning than post-pruning. Throughout the mornings, leaf water potentials became more negative in all crown positions. The lack of difference in pre-dawn or mid-day Ψ suggests that differences in plant water status were not responsible for the observed thinning of leaves in the lower and middle crown or photosynthesis results following pruning.

In this study, Ψ was not consistently different between vertical crown positions. Ψ has generally been found to decrease with increasing height in the crown in very tall trees (Koch et al., 2004), however, Ψ is also influenced by how much T has occurred (Myers, 1997), leaf age, branch type (e.g. lateral vs. main) and environmental conditions such as irradiance, vapour pressure deficit and wind speed (Hellkvist et al., 1974). Other studies have observed limited vertical differences in Ψ in other young plantation-grown eucalypts (e.g. Hunt et al., 2006).

There was no indication that pruning influenced g, T, WUE or NUE. Differences observed at other measurement times were not consistent to indicate pruning altered these parameters.

4.3. Application and conclusions

There was little prior information about the physiological characteristics of *E. pilularis* and *E. cloeziana*. A comparison of physiological characteristics showed limited difference between leaves of the species, suggesting no difference in shade tolerance. However, morphological differences in leaves may explain observed differences in crown structure (Smith et al., 2006). This study showed that there are strong positional effects on leaf physiology in both *E. pilularis* and *E. cloeziana* and therefore future experiments should account for large vertical gradients in leaf physiology.

While we did not find an up-regulation of photosynthesis 6 months after pruning, detailed and useful photosynthesis, transpiration and conductance data have been presented for both *E. pilularis* and *E. cloeziana*. These new data provide an important basis required for modelling pruning effects in process-based tree growth models.

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