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High nitrogen and elevated [CO$_2$] effects on the growth, defense and photosynthetic performance of two eucalypt species

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ABSTRACT

Atmospheric nitrogen deposition and [CO$_2$] are increasing and represent environmental problems. Planting fast-growing species is prospering to moderate these environmental impacts by fixing CO$_2$. Therefore, we examined the responses of growth, photosynthesis, and defense chemical in leaves of *Eucalyptus urophylla* (U) and the hybrid of *E. deglupta* x *E. camadulensis* (H) to different CO$_2$ and nitrogen levels. High nitrogen load significantly increased plant growth, leaf N, photosynthetic rate ($A_{growth}$), and photosynthetic water use efficiency (WUE). High CO$_2$ significantly increased $A_{growth}$, photosynthetic nitrogen use efficiency (PNUE) and WUE. Secondary metabolite (SM, i.e. total phenolics and condensed tannin) was specifically altered; as SM of U increased by high N load but not by elevated [CO$_2$], and vice versa for SM of H.

Keywords: Eucalypts, elevated [CO$_2$], nitrogen loading, defense chemical, resource allocation

Capsule: The two eucalypts differently allocate assimilates in response to high N; while elevated [CO$_2$] tended to increase condensed tannin and to decrease total phenolics.

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1. Introduction

Indonesia’s environment is in a severe state of degradation. Deforestation and industrialization that contribute to the degradation have taken place at an alarming rate. Deforestation in Indonesia is noted as the world’s third largest emitter of greenhouse gases (GHGs) (PEACE, 2007). Therefore, afforestation and reforestation have been initiated by government and environmentalists to reduce the GHGs emission and restore the environmental conditions. Moreover, rapid industrialization has contributed to pollutions and environment damages, including atmospheric nitrogen deposition (Gillett et al., 2000). In fact, the rapid increasing of nitrogen (N) deposition due to industrial development and the use of N fertilizer not only happens in Indonesia but is taking place in many parts of Asia (Galloway et al., 2004; Ogawa et al., 2006). Furthermore, the world atmospheric CO₂ concentration continuous to increase (e.g. Grubb, 2003; IPCC, 2007). Under these environmental conditions, we expect the high CO₂ fixation and storage capacity of fast growing species, such as eucalypts and acacias (e.g. Alves et al., 2002; Armstrong, 1998; Laclau et al., 2008; Novriyanti et al., 2012) to be able to moderate environment damages via afforestation and reforestation projects. Elevated [CO₂] usually stimulates plant growth (e.g. Ghannoum et al., 2010). However, the magnitude of plant response to elevated [CO₂] among species and conditions varies with soil nutrient status (Ainsworth and Long, 2005; Zhao et al., 2011). Since N is one of the main limiting resources for plant growth in nature, however, and most leaf N is allocated to photosynthetic organs (e.g. Feng et al., 2009; Schulze et al., 2005), the extent of plant development would be fostered by elevated [CO₂] in the presence of high supply of nutrient (i.e. nitrogen) is then questioned. The availability of N and C influences plant leaf chemistry, which in turn would define plant defense status (e.g. Bryant et al., 1983; Koike et al., 2003) as well as photosynthetic capacity. Increasing the availability of these resources may induce changes not only in plant growth traits but also in plant defense strategies (e.g. Gleadow et al., 1998). Several studies have reported that elevated [CO₂] and N deposition may change the physical and chemical defense traits of leaves (e.g. Gleadow et al., 1998; Koike et al., 2006). The certain behavior of plants with specified nutrient and resources availability can be explain by plant defense theory. The Growth Differentiation Balance hypothesis (GDB) predicts that trade-off exist between growth processes and differentiation processes (e.g. the production of SM) (Herms and Mattson, 1992). The trade-off has ecological consequences that affect the resources partitioning and allocation, thus not only soil nutrient condition (Bryant et al., 1983), but any environmental factor that retards growth more than photosynthesis can increase the availability of resource pool for allocation to SM. Therefore, the relationship between growth and SM production is nonlinear and has a peak in defense chemical production (Herms and Mattson, 1992).

Altered growth and photosynthesis under higher [CO₂] is well documented (e.g. Schulze et al., 2005). However, the growth and defense traits of eucalypts have been studied in relation to their growth condition. Low soil nutrient availability and elevated CO₂ increase C/N ratio that leads to higher level of defense chemicals, even though carbon...
allocation may differ between growth habits because of different investment in storage and structural components (Lawler et al., 1997).

In this present study, *Eucalyptus urophylla* and hybrid *E. deglupta x E. camaldulensis* were examined. Their photosynthetic rate was expected to increase under elevated [CO2] and high N. Under N-rich environment, C/N ratio should decrease and apparent trade-off between high growth and low production of secondary metabolites (SM) may take place as predicted by GDB. In contrast, elevated [CO2] should increase C/N ratio that would lead to higher concentration of SM, but the growth is not necessarily to decrease since the enhanced resource use efficiency may cause the possibility of positive correlation between growth and SM production (Herms and Mattson, 1992).

In order to access these predictions, we investigated the response of chemical defense, leaf and photosynthetic traits of two eucalypts to high N load and elevated [CO2]. These results will provide a plausible understanding of the role of excess N accompanied by elevated [CO2] and exposed to the tropic/subtropic environments. The growth media were pumice soil and clay soil (1:1, v/v) in 7 liter pots that are commonly used in nursery practices. As basal dressing, we supplied 500-fold diluted liquid fertilizer (balanced nutrient; N: P: K = 6: 10: 5, Hyponex Corp. JAPAN, Osaka, Japan) at a rate of 1 kg N ha\(^{-1}\). The pots were watered periodically to sustain the soil moisture.

### 2. Materials and Methods

#### 2.1. Plant materials

We used seedlings of *Eucalyptus urophylla* (U) and cuttings of hybrid *E. deglupta x E. camaldulensis* (H). The latter was originally developed for materializing high growth performance to supply plant materials for pulpwood plantations, therefore cuttings were used due to mass production of seedlings with similar traits among them. The seed of U was obtained from Australia Tree Seed Centre of CSIRO, Kingston, Australia. *E. urophylla* is species native to Indonesia islands (e.g. ERDB, 2009), while based on the parent characteristics, H is expected to survive the tropic/subtropic environments.

At the initiation of the experiment that was lasted on January – May 2010, the average height and diameter of U were 40.5 cm and 4.1 mm, while of H were 57.0 cm and 4.9 mm. The seedlings and cuttings were 7 – 8 months old. The plants were grown in phytotron chambers of the Forest and Forestry Research Product Institute, Sapporo, Japan (43°0'N, 141°2'E, 180 m a.s.l.). The chambers were maintained at daily temperatures around 25/20°C under natural light, supplemented by sodium halide lamps for adjusting day-length of 14 hours.

The research design was factorial randomized 2 x 2, the factors were: N supply (N0 = 0 kg ha\(^{-1}\) and N1 = 50 kg ha\(^{-1}\) of (NH\(_4\))2SO\(_4\) + balance nutrient) and [CO2] (ambient (A) = 380 µmol mol\(^{-1}\) and elevated (E) = 760 µmol mol\(^{-1}\)). There were three replications for each plant species for the measurement.

#### 2.2. Measurement of gas exchange rates

The gas exchange rates were measured on mature leaf (counted third or fourth from the shoot top) by using an open gas exchange system (LI-6400, LI-Cor, 1992).
Lincoln, Nebraska, USA) in late April 2010. Measurement was carried out under a photosynthetic photon flux of 1500 µmol m⁻² s⁻¹. The leaf temperature and vapor pressure deficit (VPD) were maintained at 25°C ± 1°C and 1.2 kPa, respectively. We measured the net photosynthetic rate (Agrowth) (Farquhar et al., 1980; Long and Bernacchi, 2003), stomatal conductance (gs), and transpiration rate (E) at growth [CO₂] (i.e. 380 and 760 µmol mol⁻¹ for ambient and elevated treatments, respectively). The water use efficiency (WUE, mmol mol⁻¹) was calculated as Agrowth divided by E. All gas exchange parameters were expressed on the basis of the projected (one-sided) leaf area covered by the chamber area.

### 2.4. Leaf traits and chemical measurement

Subsequent to gas exchange measurements, leaves were excised from the plants for measurement of the leaf mass per area (LMA, g m⁻²) by dividing the leaf mass (oven-dried at 70°C for one week) with the leaf area. Leaf N and C content was determined by using combustion method with NC analyzer (NC-900, Sumica-Shimadzu, Kyoto, Japan). Photosynthetic N use efficiency (PNUE, µmol g⁻¹ s⁻¹) was calculated by dividing Agrowth by Narea. Total phenolics and condensed tannin were measured following the Folin-Ciocalteu method that modified by Jukunen-Tiitto (1985) and Matsuki et al. (2004). Lignin content in the leaf was measured followed Iiyama and Wallis (1990). Chlorophyll content was extracted with dimethyl sulfoxide (DMSO) and the absorbance of the extracts was measured by a spectrophotometer (Shinano et al., 1996).

### 2.5. Statistical analysis

Two way analysis of variance (ANOVA) was used to evaluate the difference among treated seedling by using SPSS 16.0.2 (SPSS Inc., USA).

### 3. Results

#### 3.1. Net photosynthetic rate, growth, and defense chemical

For both eucalypts, Agrowth was increased by N load and elevated [CO₂]; interaction of both factors was also detected. High N load, but not elevated [CO₂], stimulated the diameter and height of both species (Fig. 1). The hybrid H contained a significantly higher concentration of condensed tannin (p < 0.05) and marginally lower total phenolics content (p < 0.1) by elevated [CO₂] but those were not changed by high N load. Total phenolics of H showed significant interaction between N load and elevated [CO₂]. The concentration of phenolics in H was reduced by elevated CO₂ under N0 condition, however, no difference was found between ambient and elevated CO₂ under N1 condition (Table 1). High N load significantly decreased the total phenolics and although not significant, it reduced the tannin content of U by 50.1%. However, the effect of elevated [CO₂] on the concentration of total phenolics and condensed tannins of U was insignificant (Table 1).

#### 3.2. Leaf and photosynthetic traits

For both species, elevated [CO₂] did not significantly affect the chlorophyll content, LMA, Nmass, Narea, and lignin content (Table 1), but significantly increased PNUE and WUE (Table 2). For U, high N load significantly increased chlorophyll content, Nmass, Narea, lignin content, and WUE, but decreased the LMA. For H, high N load increased the chlorophyll content, Nmass, Narea, and
WUE, but its effect was insignificant on LMA, lignin, and PNUE. Phosphorous (P) content in the H foliage was significantly increased by N addition but no effects of elevated [CO\textsubscript{2}] were found in either H or U foliage (Table 1).

Under high N condition, lignin content significantly increased in U and tended to increase by 36.6% in H. Elevated [CO\textsubscript{2}] also slightly increased foliar lignin content of the eucalypts, by 39.3% and 31.2% for U and H, respectively (Table 1).

### 3.3. Interaction effect

In general, lack evidences were found in this study for interaction effect of N load and elevated [CO\textsubscript{2}]. For U, interaction between N and [CO\textsubscript{2}] strengthened the increased A\textsubscript{growth}, and N\textsubscript{area}. Meanwhile for H, the interaction strengthened A\textsubscript{growth}, phenolics content but weaken E.

### 4. Discussion

#### 4.1. Net photosynthetic rate, growth and defense chemical

Growth increments of the two eucalypts were increased under high N load, but were not profoundly influenced by elevated [CO\textsubscript{2}], despite A\textsubscript{growth} were increased on both conditions. In many cases (e.g. Ghannoum et al., 2010), high [CO\textsubscript{2}] usually increases the photosynthetic rate and thereby the growth rate. However, in the present study and some other experiments (e.g. Arp et al., 1998), there were no positive effects of elevated [CO\textsubscript{2}] on growth of both eucalypts. Some environmental limitations may retard growth than photosynthesis via balance among growth and plant defense with development (Herms and Mattson, 1992). Thus, supposedly, assimilates were not allocated more to growth of those species, since assimilates might be allocated to other priorities than plant growth, e.g. defense system (e.g. Bryant et al., 1983; Hamilton et al., 2001; Herms and Mattson, 1992) or food reserve (e.g. Chapin et al., 1990; Herms and Mattson, 1992).

The lignin content and LMA of the plants were presumably credited for the insignificant effect of elevated [CO\textsubscript{2}] on the growth increments. The LMA of both eucalypts tended to decrease (17.2% in U and 8.4% in H) which indicated that leaf mass per unit area was decreased under high [CO\textsubscript{2}] (Table 1). Meanwhile the lignin content tended to increase (38.8% in U and 31.1% in H) under elevated [CO\textsubscript{2}]. Thus, the most likely reason for the insignificant growth under elevated [CO\textsubscript{2}] was that the plants which had the stimulated-A\textsubscript{growth} could not beneficially deploy the newly fixed carbohydrates into new growth, perhaps allocated to e.g. non-structural carbohydrates or lignin synthesis.

P is a macronutrient that most frequently limits plant growth next to N (e.g Schachtman et al., 1998, Bueneman et al. 2011). The foliar P content of the eucalypts varied 4-fold and was less than 0.20% of dry weight (Table 1). It was lower than P-requirement for the optimal growth of common plants, 0.30 - 0.50% of dry weight (Marschner, 1995).

Although eucalypts generally could survive in lower P soil (e.g. Beadle, 1962; Dell et al., 1983; Mulligan, 1988); however, P deficiency likely hampered the growth of the eucalypts under elevated [CO\textsubscript{2}] despite of higher rate of A\textsubscript{growth}. This may be attributed to the fact that shoot growth usually more severely impaired than photosynthetic rate in plants under P deficiency (e.g. Dell et al., 1987; Plénet et al., 2000). Several studies have reported that growth reduction of eucalypts seedlings is caused by P-starvation (e.g. Godoy and da Silva...
Rosado, 2011; Gonçalves et al., 2004; Xu et al., 2005). In fact, nutrient stress may enable the reduction of growth stimulation under elevated \([\text{CO}_2]\) (Conroy, 1992; Lynch and St.Clair, 2004; Poorter and Pérez-Soba, 2001; Tobita et al., 2010). P-deficiency also stimulates the root growth despite the shoot growth declines (e.g. Hawkesford et al., 2012). Further, since in general root responses to elevated \([\text{CO}_2]\) are often greater than aboveground responses, therefore, allocations of assimilates to belowground is possible explanation (Jackson et al., 2009) is possible.

The higher \(A_{\text{growth}}\) of the eucalypts under high \(N\) load sustained high growth rate despite of P-deficiency because plant under excessive \(N\) condition may enhance the efficiency of P-resorption (Conroy et al., 1992; Lü and Han, 2010). Under high \(N\) supply, both eucalypts exhibited enhanced-\(A_{\text{growth}}\) and growth increments (height and diameter), but their chemical defense responded differently. In the U both total phenol and total tannin tended to decline. The increased nutrient uptake would decreased the C/N ratio in U, lead the SM to decline as growth received priority for resource allocation (Herms and Mattson, 1992). Further, the LMA of U decreased under high \(N\) supply (Table 31), thus the concentration of total phenolics and condensed tannin were not diluted by biomass of the leaves, suggested that SM synthesis was reduced more than biomass accumulation (e.g. Koricheva, 1999; Lavola et al., 1998).

In contrast to U, the defense compound of H did not respond to high \(N\); although C/N ratio also significantly decreased and growth was increased as it received priority for the available resource (Fig. 1, Table 1). Therefore, the resource allocation might be species specific trait. While H showed a trade-off between high growth rate and lower SM concentration, H maintained high growth rate but did not decrease SM concentration. It may imply that H is probably more resistant to herbivory than U.

Contrary to our prediction, elevated \([\text{CO}_2]\) did not increase \(C/N\) ratio of the eucalypts. Despite of that, the \([\text{CO}_2]\) treatment significantly decreased total phenolics and increased total tannin in H (Table 1). In regard to the increased tannin content, supposedly, elevated \([\text{CO}_2]\) might actually increase the \(N\) available for tannin production because the efficiency of photosynthesis was increased. In other words, elevated \([\text{CO}_2]\) may allow the plant to reallocate \(N\) from photosynthesis to secondary metabolites, specifically tannin (Hamilton et al., 2001; Jones and Hartley, 1999).

Another possible explanation for the increased condensed tannins is a shifting resource allocation from retarded-growth under elevated \([\text{CO}_2]\). Environmental constrain can mitigate the cost of defense as when the growth is retarded more than the photosynthesis then it will increase the pool of resource availability for SM production with little or no trade-off with growth (Herms and Mattson, 1992). P deficiency likely limited the growth under elevated \([\text{CO}_2]\) (Table 1), however, a trade-off was only apparent between the hampered-growth and condensed tannins but not total phenolics.

The high \([\text{CO}_2]\) tended to decrease total phenolics in H. The decreased total phenolics may be partly attributed to the tendency of increased leaf \(N\) content and of decreased \(C/N\) ratio (by 3.81% in H) (Table 1). Elevated \([\text{CO}_2]\) usually decreases leaf \(N\) content because it inhibits the assimilation of nitrate into organic nitrogen compound in leaves (e.g. Bloom et al., 2010). Consequently, total phenolics seems to be decreased under elevated \([\text{CO}_2]\) as \(C/N\) ratio tended to decreased (Herms and Mattson, 1992).
In the case of H, the increased total phenolics content was influenced significantly by high N load. Elevated [CO₂] decreased the SM of H when N was limited. However, high N diminished the effect of elevated [CO₂] (Table 1). Supposedly, when N is not a limiting factor in the elevated [CO₂] environment, the plant could enhance the A₉growth, thus there were enough assimilates to be allocated to phenolics synthesis.

Unlike H, total phenolics and condensed tannins of U did not respond significantly to the elevated [CO₂]. This fact further strengthens our consideration that both eucalypts respond differently to elevated [CO₂] and high N with regard to the resource allocation to secondary metabolites. Productions of secondary metabolite are deeply related to evolutionally processes, and therefore, may be varied widely even among the same genus.

4.2. Characteristics of leaf and photosynthetic responses

A higher supply of C and N in elevated [CO₂] and N load could promote a higher photosynthetic rate, and hence a higher growth rate (e.g. Poorter and Pérez-Soba, 2001). In this study, however, no significant increase was found in the growth of either eucalypts respond differently to elevated [CO₂] and high N with regard to the resource allocation to secondary metabolites. Productions of secondary metabolite are deeply related to evolutionally processes, and therefore, may be varied widely even among the same genus.

Higher N content in leaves (Nmass and Narea) was found in the high N environment (Table 1). Although N is usually diluted in plant at high [CO₂] (Coleman et al. 1993), the increased growth rate of both species by high N load (Fig. 1) did not offset N tissue content by a dilution effect. Both species could therefore maintain their high N status despite the greater biomass. The N tissue content usually reduces at high [CO₂], because the high growth rate would dilutes the N tissue concentration and necessitates further N uptake in plant (Johnson, 2006), thus under high N and high [CO₂] plant may have lower N tissue content. Although tended to slightly increased, there was no significant effect of elevated [CO₂] on Nmass and Narea in either species, or in their growth.
increments (height and diameter). The seedlings could presumably maintain a high N content due to the balance nutrients we supplied together with N. PNUE of both species increased significantly with elevated [CO₂]. In general, high PNUE would be attained by species which have lower leaf N content. However, both species sustained high PNUE despite the foliar N content was insignificant under elevated [CO₂]. This higher PNUE was probably due to the lower LMA of the eucalypts in this environment (e.g. Harrison et al., 2009) as shown in Table 2. We assumed that the balance-nutrient supplied together with (NH₄)₂SO₄ also allowed those seedlings to maintain high PNUE. The increased N mass and N area under high N load to some extent decreased the PNUE of both species (5.5% and 10.8% for U and H, respectively), although the effects were insignificant (Table 2). It consistent with many studies, high N load usually increases leaf N content and thereby decreases PNUE (e.g. Poorter and Evans, 1998). However, elevated [CO₂] were likely sustained the increased-PNUE of both eucalypts (by 27.0% in U and 22.4% in H) despite high N was supplied (Table 1). Presumably, elevated [CO₂] may increase the PNUE independent of N content by increase in A growth (Davey et al., 1999).

5. Conclusion

Resources allocation in the two eucalypts, especially to defensive chemicals, was affected differently by N load and elevated [CO₂], suggesting that induced defense vary widely in eucalyptus species. The different response of the two eucalypts to the environmental change might imply their competitiveness in the growing environment. While at the same time it could maintain rapid growth, unaltered defensive chemicals of H may favorable to overcome the frequent herbivory attack in tropical/subtropical environment. Moreover, another environmental constraints could hamper the fertilizer effect of elevated [CO₂] on growth of the eucalypts. The retarded-growth is then could further define the concentration of defense chemicals as resources allocation is shifted to defense system. Despite the photosynthetic rate of the two eucalypts performed well under
elevated N and [CO₂]. The strategy of the eucalypts in allocating the available resource to growth and defense under altered-environment should define their prosperity as afforestation plants. Considering the advantageous response to high N load and its interaction with elevated [CO₂], H is therefore the more promising material for afforestation and reforestation to repair degraded areas in Indonesia.

Acknowledgements

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Figure 1. Net photosynthetic rate ($A_{\text{growth}}$), $\Delta$ diameter and height of *E. urophylla* (U) and hybrid *E. deglupta* × *E. camaldulensis* (H). $A = $ ambient [CO$_2$] 380 µmol m$^{-1}$, $E = $ elevated [CO$_2$] 760 µmol m$^{-1}$, $N_0 = $ no N addition, $N_1 = $ N supply at rate of 50 kg ha$^{-1}$, $N = $ nitrogen treatment, $CO_2 = $ CO$_2$ treatment, $NxCO_2 = $ interaction of nitrogen and CO$_2$ treatment. P values are presented in the table next to each graph. Data are average values ± SD (n = 3).
<table>
<thead>
<tr>
<th></th>
<th>E. urophylla</th>
<th></th>
<th>E. deglupta x E. camaldulensis</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>E</td>
<td>N CO2</td>
<td>N x CO2</td>
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<tr>
<td><strong>Total phenolics (mg g⁻¹)</strong></td>
<td>131.75±26.50</td>
<td>109.55±12.33</td>
<td>134.83±18.78</td>
<td>76.85±9.46</td>
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<tr>
<td><strong>Condensed tannins (mg g⁻¹)</strong></td>
<td>5.63±3.64</td>
<td>4.23±1.97</td>
<td>9.34±7.49</td>
<td>3.23±2.32</td>
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<tr>
<td><strong>Lignin (%)</strong></td>
<td>4.53±1.09</td>
<td>6.24±3.57</td>
<td>4.97±1.42</td>
<td>9.98±2.11</td>
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<tr>
<td><strong>LMA (g m⁻²)</strong></td>
<td>111.85±5.53</td>
<td>76.27±24.55</td>
<td>93.90±20.05</td>
<td>66.58±24.38</td>
</tr>
<tr>
<td><strong>Chlorophyll(µg mg⁻¹)</strong></td>
<td>1.92±0.67</td>
<td>5.91±1.19</td>
<td>1.51±0.35</td>
<td>5.23±0.18</td>
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<tr>
<td><strong>N_mass (%)</strong></td>
<td>0.58±0.08</td>
<td>3.04±0.36</td>
<td>0.76±0.16</td>
<td>3.38±0.55</td>
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<tr>
<td><strong>N_area(g m⁻²)</strong></td>
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<td>2.94±0.34</td>
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<td><strong>C/N</strong></td>
<td>57.88±39.18</td>
<td>33.54±31.48</td>
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<td><strong>P (%)</strong></td>
<td>0.05±0.02</td>
<td>0.13±0.07</td>
<td>0.11±0.04</td>
<td>0.15±0.12</td>
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**E. urophylla**

Total phenolics (mg g⁻¹) 131.75±26.50 109.55±12.33 134.83±18.78 76.85±9.46 0.005 0.192 0.124
Condensed tannins (mg g⁻¹) 5.63±3.64 4.23±1.97 9.34±7.49 3.23±2.32 0.180 0.610 0.384
Lignin (%) 4.53±1.09 6.24±3.57 4.97±1.42 9.98±2.11 0.033 0.145 0.245
LMA (g m⁻²) 111.85±5.53 76.27±24.55 93.90±20.05 66.58±24.38 0.027 0.270 0.732
Chlorophyll(µg mg⁻¹) 1.92±0.67 5.91±1.19 1.51±0.35 5.23±0.18 0.000 0.339 0.804
N_mass (%) 0.58±0.08 3.04±0.36 0.76±0.16 3.38±0.55 0.000 0.225 0.688
N_area(g m⁻²) 0.53±0.24 2.94±0.34 0.67±0.26 2.36±0.39 0.000 0.255 0.084
C/N 57.88±39.18 33.54±31.48 68.82±19.76 14.95±2.39 0.037 0.812 0.372
P (%) 0.05±0.02 0.13±0.07 0.11±0.04 0.15±0.12 0.167 0.361 0.636

**E. deglupta x E. camaldulensis**

Total phenolics (mg g⁻¹) 108.39±20.63 73.55±13.81 53.15±11.29 98.92±6.01 0.506 0.097 0.001
Condensed tannins (mg g⁻¹) 5.99±1.79 3.36±1.33 8.77±4.00 8.51±1.91 0.391 0.040 0.476
Lignin (%) 7.07±3.69 8.53±3.87 8.17±3.40 12.28±1.01 0.171 0.227 0.495
LMA (g m⁻²) 92.19±3.41 80.80±20.94 83.00±12.21 76.65±15.06 0.316 0.445 0.769
Chlorophyll(µg mg⁻¹) 1.75±0.62 4.60±0.15 2.16±0.32 4.12±0.58 0.001 0.437 0.673
N_mass (%) 0.92±0.09 2.23±0.27 0.88±0.68 2.68±0.38 0.000 0.176 0.115
N_area(g m⁻²) 0.85±0.55 1.77±0.37 0.81±0.65 1.83±0.29 0.000 0.954 0.723
C/N 51.27±5.53 21.55±2.19 51.90±4.28 18.14±2.13 0.000 0.547 0.388
P (%) 0.06±0.02 0.20±0.08 0.07±0.03 0.19±0.06 0.010 0.918 0.712

**LMA** = leaf mass per area, **N_mass** = leaf N content per unit dry mass, **N_area** = N leaf content per unit area, **C/N ratio** = carbon to nitrogen ratio, **P** = phosphorous, **N** is nitrogen treatment (N0 = 0 kg/ha, N1 = 50 kg/ha of (NH₄)₂SO₄ + balance nutrient), CO₂ is CO₂ treatment (A = ambient: 380 µmol m⁻¹, E = elevated: 760 µmol m⁻¹). Data are mean values ± SD (n = 3).
Table 2.
Photosynthetic traits of *E. urophylla* and hybrid *E. deglupta x E. camaldulensis*.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>E</th>
<th>P-value</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0</td>
<td>N1</td>
<td>N0</td>
<td>N1</td>
<td>N</td>
<td>CO₂</td>
</tr>
<tr>
<td><em>E. urophylla</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNUE (µmol mol⁻¹ s⁻¹)</td>
<td>103.86±94.53</td>
<td>98.12±34.94</td>
<td>142.01±29.53</td>
<td>194.62±40.81</td>
<td>0.492</td>
<td>0.071</td>
</tr>
<tr>
<td>WUE (mmol mol⁻¹)</td>
<td>4.04±0.82</td>
<td>6.79±0.99</td>
<td>5.10±2.57</td>
<td>9.84±0.74</td>
<td>0.002</td>
<td>0.044</td>
</tr>
<tr>
<td>gₛ (mol m⁻² s⁻¹)</td>
<td>0.04±0.01</td>
<td>0.25±0.15</td>
<td>0.12±0.08</td>
<td>0.25±0.02</td>
<td>0.012</td>
<td>0.480</td>
</tr>
<tr>
<td>E (mol m⁻² s⁻¹)</td>
<td>0.70±0.23</td>
<td>3.06±1.12</td>
<td>1.64±1.08</td>
<td>3.26±0.31</td>
<td>0.003</td>
<td>0.253</td>
</tr>
<tr>
<td><em>E. deglupta x E. camaldulensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNUE (µmol mol⁻¹ s⁻¹)</td>
<td>157.71±44.57</td>
<td>140.75±62.11</td>
<td>198.83±47.28</td>
<td>256.23±42.59</td>
<td>0.501</td>
<td>0.026</td>
</tr>
<tr>
<td>WUE (mmol mol⁻¹)</td>
<td>2.35±0.50</td>
<td>4.75±0.93</td>
<td>6.30±2.68</td>
<td>9.10±1.22</td>
<td>0.021</td>
<td>0.002</td>
</tr>
<tr>
<td>gₛ (mol m⁻² s⁻¹)</td>
<td>0.37±0.17</td>
<td>0.26±0.07</td>
<td>0.143±0.08</td>
<td>0.37±0.15</td>
<td>0.451</td>
<td>0.451</td>
</tr>
<tr>
<td>E (mol m⁻² s⁻¹)</td>
<td>4.12±1.06</td>
<td>3.54±0.45</td>
<td>2.00±0.71</td>
<td>3.68±0.68</td>
<td>0.249</td>
<td>0.055</td>
</tr>
</tbody>
</table>

PNUE = photosynthetic nitrogen use efficiency, WUE = photosynthetic water use efficiency, gₛ = stomatal conductance, E = leaf transpiration rate, N is nitrogen treatment (N₀ = 0 kg/ha, N₁ = 50 kg/ha of (NH₄)₂SO₄ + balance nutrient), CO₂ is CO₂ treatment (A = ambient: 380 µmol m⁻² s⁻¹, E = elevated: 760 µmol m⁻² s⁻¹). Data are mean values ± SD (n = 3).