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High nitrogen and elevated [CO₂] effects on the growth, defense and photosynthetic performance of two eucalypt species

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ABSTRACT

Atmospheric nitrogen deposition and [CO₂] are increasing and represent environmental problems. Planting fast-growing species is prospering to moderate these environmental impacts by fixing CO₂. 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 CO₂ and nitrogen levels. High nitrogen load significantly increased plant growth, leaf N, photosynthetic rate (A_{growth}), and photosynthetic water use efficiency (WUE). High CO₂ 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 [CO₂], and vice versa for SM of H.

Keywords: Eucalypts, elevated [CO₂], nitrogen loading, defense chemical, resource allocation

Capsule: The two eucalypts differently allocate assimilates in response to high N; while elevated [CO₂] tended to increase condensed tannin and to decrease total phenolics.

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2 1. Introduction

3
4 Indonesia's environment is in a severe
5 state of degradation. Deforestation and
6 industrialization that contribute to the
7 degradation have taken place at an
8 alarming rate. Deforestation in Indonesia
9 is noted as the world's third largest
10 emitter of greenhouse gases (GHGs)
11 (PEACE, 2007). Therefore, afforestation
12 and reforestation have been initiated by
13 government and environmentalists to
14 reduce the GHGs emission and restore
15 the environmental conditions.

16 Moreover, rapid industrialization has
17 contributed to pollutions and environment
18 damages, including atmospheric nitrogen
19 deposition (Gillett et al., 2000). In fact,
20 the rapid increasing of nitrogen (N)
21 deposition due to industrial development
22 and the use of N fertilizer not only
23 happens in Indonesia but is taking place
24 in many parts of Asia (Galloway et al.,
25 2004; Ogawa et al., 2006). Furthermore,
26 the world atmospheric CO₂ concentration
27 ([CO₂]) continuous to increase (e.g.
28 Grubb, 2003; IPCC, 2007)

29 Under these environmental conditions,
30 we expect the high CO₂ fixation and
31 storage capacity of fast growing species,
32 such as eucalypts and acacias (e.g. Alves
33 et al., 2002; Armstrong, 1998; Laclau et
34 al., 2008; Novriyanti et al., 2012) to be
35 able to moderate environment damages
36 via afforestation and reforestation
37 projects.

38 Elevated [CO₂] usually stimulates
39 plant growth (e.g. Ghannoum et al., 2010).
40 Since N is one of the main limiting
41 resources for plant growth in nature,
42 however, and most leaf N is allocated to
43 photosynthetic organs (e.g. Feng et al.,
44 2009; Schulze et al., 2005), the extent of
45 plant development would be fostered by
46 elevated [CO₂] in the presence of high
47 supply of nutrient (i.e. nitrogen) is then
48 questioned.

49 The availability of N and C influences
50 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
magnitude of plant response to elevated
[CO₂] among species and conditions
varies with soil nutrient status (Ainsworth
and Long, 2005; Zhao et al., 2011).

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] give rise to changes in the defense chemical of eucalypts (i.e. total phenolics and condensed tannin). We hope to contribute our findings to ecosystem rehabilitation in Indonesia.

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/sub tropic 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 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.

2.2. Research design

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 (NH₄)₂SO₄ + 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.

2.3. 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,

Lincoln, Nebraska, USA) in late April 2010. Measurement was carried out under a photosynthetic photon flux of $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The leaf temperature and vapor pressure deficit (VPD) were maintained at $25^\circ\text{C} \pm 1^\circ\text{C}$ and 1.2 kPa, respectively. We measured the net photosynthetic rate (A_{growth}) (Farquhar et al., 1980; Long and Bernacchi, 2003), stomatal conductance (g_s), and transpiration rate (E) at growth $[\text{CO}_2]$ (i.e. 380 and $760 \mu\text{mol mol}^{-1}$ for ambient and elevated treatments, respectively). The water use efficiency (WUE, mmol mol^{-1}) was calculated as A_{growth} 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, $\mu\text{mol g}^{-1} \text{s}^{-1}$) was calculated by dividing A_{growth} by N_{area} . 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 spectrophotometer (Shinano et al., 1996).

2.5. Statistical analysis

Two way analysis of variance

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, A_{growth} was increased by N load and elevated $[\text{CO}_2]$; interaction of both factors was also detected. High N load, but not elevated $[\text{CO}_2]$, 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 $[\text{CO}_2]$ but those were not changed by high N load. Total phenolics of H showed significant interaction between N load and elevated $[\text{CO}_2]$. The concentration of phenolics in H was reduced by elevated CO_2 under N0 condition, however, no difference was found between ambient and elevated CO_2 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 $[\text{CO}_2]$ 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 $[\text{CO}_2]$ did not significantly affect the chlorophyll content, LMA, N_{mass} , N_{area} , and lignin content (Table 1), but significantly increased PNUE and WUE (Table 2). For U, high N load significantly increased chlorophyll content, N_{mass} , N_{area} , lignin content, and WUE, but decreased the LMA. For H, high N load increased the chlorophyll content, N_{mass} , N_{area} , 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$. For U, interaction between N and $[CO_2]$ strengthened the increased A_{growth} , and N_{area} . Meanwhile for H, the interaction strengthened A_{growth} , phenolics content and g_s 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 $[CO_2]$, despite A_{growth} were increased on both conditions. In many cases (e.g. Ghannoum et al., 2010), high $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ (Table 1). Meanwhile the lignin content tended to increase (38.8% in U and 31.1% in H) under elevated $[CO_2]$. Thus, the most likely reason for the insignificant growth under elevated $[CO_2]$ was that the plants which had the stimulated- A_{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 $[CO_2]$ despite of higher rate of A_{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

1 Rosado, 2011; Gonçalves et al., 2004; Xu 49
 2 et al., 2005). In fact, nutrient stress may 50
 3 enable the reduction of growth 51
 4 stimulation under elevated $[\text{CO}_2]$ (Conroy, 52
 5 1992; Lynch and St.Clair, 2004; Poorter 53
 6 and Pérez-Soba, 2001; Tobita et al., 2010). 54
 7 P-deficiency also stimulates the root 55
 8 growth despite the shoot growth declines 56
 9 (e.g. Hawkesford et al., 2012). Further, 57
 10 since in general root responses to 58
 11 elevated $[\text{CO}_2]$ are often greater than 59
 12 aboveground responses, therefore, 60
 13 allocations of assimilates to belowground 61
 14 (Jackson et al., 2009) is possible 62
 15 explanation 63

16 The higher A_{growth} of the eucalypts 64
 17 under high N load sustained high growth 65
 18 rate despite of P-deficiency because plant 66
 19 under excessive N condition may 67
 20 enhance the efficiency of P-resorption 68
 21 (Conroy et al., 1992; Lü and Han, 2010). 69

22 Under high N supply, both eucalypts 70
 23 exhibited enhanced- A_{growth} and growth 71
 24 increments (height and diameter), but 72
 25 their chemical defense responded 73
 26 differently. In the U both total phenol and 74
 27 total tannin tended to decline. The 75
 28 increased nutrient uptake would 76
 29 decreased the C/N ratio in U, lead the SM 77
 30 of U to decline as growth received 78
 31 priority for resource allocation (Herms 79
 32 and Mattson, 1992). Further, the LMA of 80
 33 U decreased under high N supply (Table 81
 34 1), thus the concentration of total 82
 35 phenolics and condensed tannin were not 83
 36 diluted by biomass of the leaves, 84
 37 suggested that SM synthesis was reduced 85
 38 more than biomass accumulation (e.g. 86
 39 Koricheva, 1999; Lavola et al., 1998). 87

40 In contrast to U, the defense 88
 41 compound of H did not respond to high N, 89
 42 although C/N ratio also significantly 90
 43 decreased and growth was increased as it 91
 44 received priority for the available- 92
 45 resource (Fig. 1, Table 1). Therefore, the 93
 46 resource allocation might be species 94
 47 specific trait. While H showed a trade-off 95
 48 between high growth rate and lower SM 96

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).

1 In the case of H, the increased total 49
 2 phenolics content was influenced 50
 3 significantly by high N load. Elevated 51
 4 $[\text{CO}_2]$ decreased the SM of H when N 52
 5 was limited. However, high N diminished 53
 6 the effect of elevated $[\text{CO}_2]$ (Table 1). 54
 7 Supposedly, when N is not a limiting 55
 8 factor in the elevated $[\text{CO}_2]$ environment, 56
 9 the plant could enhanced the A_{growth} , thus 57
 10 there were enough assimilates to be 58
 11 allocated to phenolics synthesis. 59

12 Unlike H, total phenolics and 60
 13 condensed tannins of U did not respond 61
 14 significantly to the elevated $[\text{CO}_2]$. This 62
 15 fact further strengthens our consideration 63
 16 that both eucalypts respond differently to 64
 17 elevated $[\text{CO}_2]$ and high N with regard to 65
 18 the resource allocation to secondary 66
 19 metabolites. Productions of secondary 67
 20 metabolite are deeply related to 68
 21 evolutionally processes, and therefore, 69
 22 the responses to environmental change 70
 23 may be varied widely even among the 71
 24 same genus. 72

25 73 26 4.2. Characteristics of leaf and 74 27 photosynthetic responses 75 28 76

29 A higher supply of C and N in 77
 30 elevated $[\text{CO}_2]$ and N load could promote 78
 31 a higher photosynthetic rate, and hence a 79
 32 higher growth rate (e.g. Poorter and 80
 33 Pérez-Soba, 2001). In this study, however, 81
 34 no significant increase was found in the 82
 35 growth of either eucalyptus species, 83
 36 despite the higher values of A_{growth} under 84
 37 elevated $[\text{CO}_2]$. We found that lignin tend 85
 38 to increase with N load and elevated 86
 39 $[\text{CO}_2]$ (Table 1). Lignin is another end- 87
 40 products of the available-resources in 88
 41 plants (e.g. Herms and Mattson, 1992). 89
 42 Contrary to our result, the decreased C/N 90
 43 ratio by N load usually leads to the lower 91
 44 lignin content (e.g. Henry et al., 2005). 92
 45 Growth and lignin content usually show 93
 46 negative correlation (Novaes et al., 2010), 94
 47 yet both were increased in this study. 95
 48 GDB hypothesis (Herms and Mattson, 96

1992) explains that the enhanced- A_{growth}
 under condition of increased-resource
 availability may create the possibility for
 a positive correlation between the
 aforementioned variables (i.e. growth and
 lignin synthesis) despite competition for a
 common resource base. Similar tendency
 was reported that lignin content was
 increased by elevated $[\text{CO}_2]$ and N load
 (Blaschke et al., 2002; Cotrufo, 1994).

Some studies found increased
 chlorophyll content with elevated $[\text{CO}_2]$,
 with or without N supply (e.g. Li and
 Gupta, 1993) or affected by only elevated
 $[\text{CO}_2]$ or only N deposition (Zhao et al.,
 2011). However, this study found no
 difference in chlorophyll content with
 CO_2 treatment, but high chlorophyll
 content were detected with high N supply.
 Leaf chlorophyll concentration is
 sensitive to N supply, and it decreases
 markedly in condition of low N (Burns et
 al., 2002). We therefore expect the
 opposite effect of increasing chlorophyll
 under high N. High N supply
 significantly increased leaf N, and
 therefore increased the total chlorophyll
 content (Table 1).

Higher N content in leaves (N_{mass} and
 N_{area}) was found in the high N
 environment (Table 1). Although N is
 usually diluted in plant at high $[\text{CO}_2]$
 (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
 $[\text{CO}_2]$, 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 $[\text{CO}_2]$ plant may have lower N tissue
 content. Although tended to slightly
 increased, there was no significant effect
 of elevated $[\text{CO}_2]$ on N_{mass} and N_{area} in
 either species, or in their growth

1 increments (height and diameter). The 49
 2 seedlings could presumably maintain a 50
 3 high N content due to the balance 51
 4 nutrients we supplied together with N. 52
 5 PNUE of both species increased 53
 6 significantly with elevated $[\text{CO}_2]$. In 54
 7 general, high PNUE would be attained by 55
 8 species which have lower leaf N content. 56
 9 However, both species sustained high 57
 10 PNUE despite the foliar N content was 58
 11 insignificant under elevated $[\text{CO}_2]$. This 59
 12 higher PNUE was probably due to the 60
 13 lower LMA of the eucalypts in this 61
 14 environment (e.g. Harrison et al., 2009) 62
 15 as shown in Table 2. We assumed that the 63
 16 balance-nutrient supplied together with 64
 17 $(\text{NH}_4)_2\text{SO}_4$ also allowed those seedlings 65
 18 to maintain high PNUE. 66

19 The increased N_{mass} and N_{area} under 67
 20 high N load to some extent decreased the 68
 21 PNUE of both species (5.5% and 10.8% 69
 22 for U and H, respectively), although the 70
 23 effects were insignificant (Table 2). It 71
 24 consistent with many studies, high N load 72
 25 usually increases leaf N content and 73
 26 thereby decreases PNUE (e.g. Poorter 74
 27 and Evans, 1998). However, elevated 75
 28 $[\text{CO}_2]$ were likely sustained the 76
 29 increased-PNUE of both eucalypts (by 77
 30 27.0% in U and 22.4% in H) despite high 78
 31 N was supplied (Table 1). Presumably, 79
 32 elevated $[\text{CO}_2]$ may increase the PNUE 80
 33 independent of N content by increase in 81
 34 A_{growth} (Davey et al., 1999). 82

35 N load and elevated $[\text{CO}_2]$ also 83
 36 significantly increased the WUE of both 84
 37 species. This finding consent with other 85
 38 observations (e.g. Cao et al., 2007) that 86
 39 elevated $[\text{CO}_2]$ enhances the overall rate 87
 40 of A_{growth} and greatly increased the WUE, 88
 41 in regard to the decreasing of E and g_s in 89
 42 all N treatment. However, decreased E 90
 43 and g_s are observed only in the H under 91
 44 elevated $[\text{CO}_2]$. Under N load, the values 92
 45 of E and g_s of U in fact increased (Table 93
 46 2). 94

47 Some studies state that enhanced $[\text{CO}_2]$ 95
 48 alters plant photosynthetic traits (e.g. 96

Ghannoum et al., 2010) and growth or
 defense traits (Mattson et al., 2005);
 other state that the effect of $[\text{CO}_2]$ is
 coordinated with the presence of high
 nutrient supply (i.e. nitrogen) (e.g. Crous
 et al., 2008; Finzi et al., 2006). However,
 present study found only few interaction
 effects of N load and enhanced $[\text{CO}_2]$.

When N was not a limiting factor, the
 performance of A_{growth} under elevated
 $[\text{CO}_2]$ increased. Given a high N supply,
 elevated $[\text{CO}_2]$ increased A_{growth} of the U
 by 58.93% and of the H by 95.20%. For
 the H, high A_{growth} was associated with
 lower phenolics content when the N
 supply was high. The presence of high N
 would probably allow the H to sustain
 high A_{growth} , thus it ameliorated the effect
 of elevated $[\text{CO}_2]$ in which adequate
 assimilates could be allocated to SM
 synthesis (e.g. Bryant et al., 1983;
 Mattson et al., 2005; Simon et al., 2010).

5. Conclusion

Resources allocation in the two
 eucalypts, especially to defensive
 chemicals, was affected differently by N
 load and elevated $[\text{CO}_2]$, 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 $[\text{CO}_2]$ 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

1 elevated N and [CO₂]. The strategy of the
 2 eucalypts in allocating the available
 3 resource to growth and defense under
 4 altered-environment should define their
 5 prosperity as afforestation plants.
 6 Considering the advantageous response to
 7 high N load and its interaction with
 8 elevated [CO₂], H is therefore the more
 9 promising material for afforestation and
 10 reforestation to repair degraded areas in
 11 Indonesia.

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26
27 Figure 1. Net photosynthetic rate (A_{growth}), Δ diameter and height of *E. urophylla* (U)
28 and hybrid *E. deglupta* x *E. camaldulensis* (H). A = ambient [CO₂] 380 $\mu\text{mol m}^{-1}$, E =
29 elevated [CO₂] 760 $\mu\text{mol m}^{-1}$, N0 = no N addition, N1 = N supply at rate of 50 kg ha⁻¹,
30 N = nitrogen treatment, CO₂ = CO₂ treatment, NxCO₂ = interaction of nitrogen and CO₂
31 treatment. P values are presented in the table next to each graph. Data are average
32 values $\pm$ SD (n = 3).

Table 1.

Leaf traits and chemicals of *E. urophylla* and hybrid *E. deglupta* x *E. camaldulensis*.

	A		E		P-value		
	N0	N1	N0	N1	N	CO ₂	N x CO ₂
<i>E. urophylla</i>							
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
<i>E. deglupta</i>x<i>E. camaldulensis</i>							
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±0.02	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*.

	A		E		P-value		
	N0	N1	N0	N1	N	CO ₂	N x CO ₂
<i>E. urophylla</i>							
PNUE ($\mu\text{mol mol}^{-1} \text{s}^{-1}$)	103.86±94.53	98.12±34.94	142.01±29.53	194.62±40.81	0.492	0.071	0.392
WUE (mmol mol^{-1})	4.04±0.82	6.79±0.99	5.10±2.57	9.84±0.74	0.002	0.044	0.282
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.04±0.01	0.25±0.15	0.12±0.08	0.25±0.02	0.012	0.480	0.480
E ($\text{mol m}^{-2} \text{s}^{-1}$)	0.70±0.23	3.06±1.12	1.64±1.08	3.26±0.31	0.003	0.253	0.450
<i>E. deglupta</i> x <i>E. camaldulensis</i>							
PNUE ($\mu\text{mol mol}^{-1} \text{s}^{-1}$)	157.71±44.57	140.75±62.11	198.83±47.28	256.23±42.59	0.501	0.026	0.232
WUE (mmol mol^{-1})	2.35±0.50	4.75±0.93	6.30±2.68	9.10±1.22	0.021	0.002	0.829
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	0.37±0.17	0.26±0.07	0.143±0.08	0.37±0.15	0.451	0.451	0.055
E ($\text{mol m}^{-2} \text{s}^{-1}$)	4.12±1.06	3.54±0.45	2.00±0.71	3.68±0.68	0.249	0.055	0.033

PNUE = photosynthetic nitrogen use efficiency, WUE = photosynthetic water use efficiency, g_s = stomatal conductance, E = leaf transpiration rate, N is nitrogen treatment (N0 = 0 kg/ha, N1 = 50 kg/ha of $(\text{NH}_4)_2\text{SO}_4$ + balance nutrient), CO₂ is CO₂ treatment (A = ambient: 380 $\mu\text{mol m}^{-2} \text{s}^{-1}$, E = elevated: 760 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Data are mean values $\pm$ SD (n = 3).