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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 CO_2 concentration ($[CO_2]$) continuous to increase (e.g. Grubb, 2003; IPCC, 2007)

Under these environmental conditions, we expect the high CO_2 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 $[CO_2]$ usually stimulates plant growth (e.g. Ghannoum et al., 2010). 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 $[CO_2]$ 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 $[CO_2]$ 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 $[CO_2]$ is well documented (e.g. Schulze et al., 2005). However, the magnitude of plant response to elevated $[CO_2]$ 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 CO_2 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 [CO_2] 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 [CO_2] 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 [CO_2]. These results will provide a plausible understanding of the role of excess N accompanied by elevated [CO_2] 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 $(NH_4)_2SO_4$ + balance nutrient) and [CO_2] (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 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 (A_{growth}) (Farquhar et al., 1980; Long and Bernacchi, 2003), stomatal conductance (g_s), and transpiration rate (E) at growth [CO_2] (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 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, μmol g^{-1} 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 (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, A_{growth} was increased by N load and elevated [CO_2]; interaction of both factors was also detected. High N load, but not elevated [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 [CO_2] but those were not changed by high N load. Total phenolics of H showed significant interaction between N load and elevated [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 [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 [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

Rosado, 2011; Gonçalves et al., 2004; Xu et al., 2005). In fact, nutrient stress may enable the reduction of growth stimulation under elevated [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 [CO_2] are often greater than aboveground responses, therefore, allocations of assimilates to belowground (Jackson et al., 2009) is possible explanation

The higher A_{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_{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 of U to decline as growth received priority for resource allocation (Herms and Mattson, 1992). Further, the LMA of U decreased under high N supply (Table 1), 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 [CO_2] did not increase C/N ratio of the eucalypts. Despite of that, the [CO_2] treatment significantly decreased total phenolics and increased total tannin in H (Table 1). In regard to the increased tannin content, supposedly, elevated [CO_2] might actually increase the N available for tannin production because the efficiency of photosynthesis was increased. In other words, elevated [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 [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 [CO_2] (Table 1), however, a trade-off was only apparent between the hampered-growth and condensed tannins but not total phenolics.

The high [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 [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 [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 [CO_2] decreased the SM of H when N was limited. However, high N diminished the effect of elevated [CO_2] (Table 1). Supposedly, when N is not a limiting factor in the elevated [CO_2] environment, the plant could enhanced the A_{growth}, 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 [CO_2]. This fact further strengthens our consideration that both eucalypts respond differently to elevated [CO_2] and high N with regard to the resource allocation to secondary metabolites. Productions of secondary metabolite are deeply related to evolutionally processes, and therefore, the responses to environmental change 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 [CO_2] 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 eucalyptus species, despite the higher values of A_{growth} under elevated [CO_2]. We found that lignin tend to increase with N load and elevated [CO_2] (Table 1). Lignin is another end-products of the available-resources in plants (e.g. Herms and Mattson, 1992). Contrary to our result, the decreased C/N ratio by N load usually leads to the lower lignin content (e.g. Henry et al., 2005). Growth and lignin content usually show negative correlation (Novaes et al., 2010), yet both were increased in this study. GDB hypothesis (Herms and Mattson, 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 [CO_2] and N load (Blaschke et al., 2002; Cotrufo, 1994).

Some studies found increased chlorophyll content with elevated [CO_2], with or without N supply (e.g. Li and Gupta, 1993) or affected by only elevated [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 [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 [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 [CO_2] plant may have lower N tissue content. Although tended to slightly increased, there was no significant effect of elevated [CO_2] on N_{mass} and N_{area} 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 [CO_2]. 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 [CO_2]. 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 $(NH_4)_2SO_4$ 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 [CO_2] 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 [CO_2] may increase the PNUE independent of N content by increase in A_{growth} (Davey et al., 1999).

N load and elevated [CO_2] also significantly increased the WUE of both species. This finding consent with other observations (e.g. Cao et al., 2007) that elevated [CO_2] enhances the overall rate of A_{growth} and greatly increased the WUE, in regard to the decreasing of E and g_s in all N treatment. However, decreased E and g_s are observed only in the H under elevated [CO_2]. Under N load, the values of E and g_s of U in fact increased (Table 2).

Some studies state that enhanced [CO_2] alters plant photosynthetic traits (e.g. Ghannoum et al., 2010) and growth or defense traits (Mattson et al., 2005); other state that the effect of [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 [CO_2].

When N was not a limiting factor, the performance of A_{growth} under elevated [CO_2] increased. Given a high N supply, elevated [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 [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 [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 [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

elevated N and [CO_2]. 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 [CO_2], H is therefore the more promising material for afforestation and reforestation to repair degraded areas in Indonesia.

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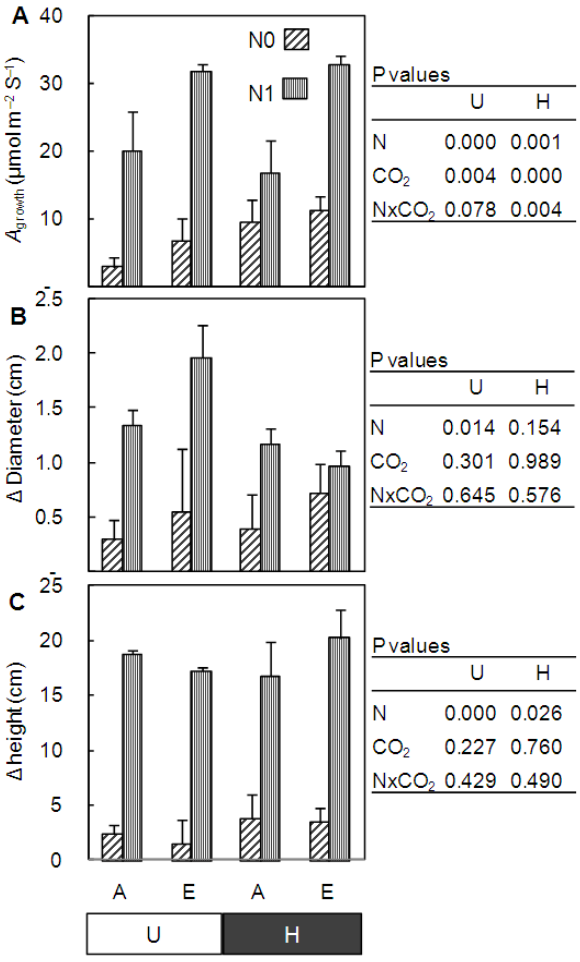

Figure 1. Net photosynthetic rate (A_{growth}), Δ diameter and height of *E. urophylla* (U) and hybrid *E. deglupta* x *E. camaldulensis* (H). A = ambient [CO_2] 380 µmol m^{-1}, E = elevated [CO_2] 760 µmol m^{-1}, N0 = no N addition, N1 = N supply at rate of 50 kg ha^{-1}, N = nitrogen treatment, CO_2 = 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 1.

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

	A		E		P-value		
	N0	**N1**	**N0**	**N1**	**N**	**CO_2**	**N x CO_2**
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. degluptaxE. 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±0.02	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 $(NH_4)_2SO_4$ + balance nutrient), CO_2 is CO_2 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. camaldulenis*.

	A		E		P-value		
	N0	**N1**	**N0**	**N1**	**N**	**CO_2**	**N x CO_2**
E. urophylla							
PNUE (μmol mol^{-1} 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 (mol m^{-2} 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 (mol m^{-2} s^{-1})	0.70±0.23	3.06±1.12	1.64±1.08	3.26±0.31	0.003	0.253	0.450
E. degluptaxE. camaldulensis							
PNUE (μmol mol^{-1} 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 (mol m^{-2} 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 (mol m^{-2} 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 $(NH_4)_2SO_4$ + balance nutrient), CO_2 is CO_2 treatment (A = ambient: 380 μmol m^{-2} s^{-1}, E = elevated: 760 μmol m^{-2} s^{-1}). Data are mean values ± SD (n = 3).