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# Growth performance of eucalypts and acacia seedling under elevated CO<sub>2</sub> load in the changing environment

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**Abstract.** Acacia and Eucalypt are important species in the global forest plantations. The resilience of those species under the changing environment would define their significance in the dynamic of forest plantation. This study was aimed to provide information on the growth performance of two acacias and two eucalypts seedlings under elevated CO<sub>2</sub> concentrations. The seedlings of *A. auriculiformis*, *A. mangium*, *E. camadulensis*, and *E. urophylla* were subjected to two levels of CO<sub>2</sub> and two levels of nutrient supply in the FACE system in Sapporo Experimental Forest, Japan. The eucalypts showed significantly higher growth performance than the acacias. The nutrient addition significantly increased the growth, yet the CO<sub>2</sub> and interaction between CO<sub>2</sub> and nutrients were not significantly different. LMA was not significantly affected by the elevated CO<sub>2</sub> and nutrient addition. Although nutrients significantly affected the C/N in the eucalypts, they showed no different effect on the acacias. As expected, N<sub>mass</sub> and N<sub>area</sub> were higher in the acacia than those in the eucalypts, although no significant responses were shown to elevated CO<sub>2</sub> and nutrient addition. The tested acacia and eucalypts showed relatively insensitivity to elevated CO<sub>2</sub>. Thus they might possess resilience capacity under the keep increasing level of the atmospheric CO<sub>2</sub> concentration.

## 1. Introduction

Human activities have greatly contributed to the change in atmospheric gases, including CO<sub>2</sub>, a glasshouse gas that allegedly plays a key role in global warming. Anthropogenic is responsible for the keep increasing atmospheric CO<sub>2</sub> concentration ([CO<sub>2</sub>]). The modern [CO<sub>2</sub>] is observed 45% higher than that at the pre-industrial era [1] and is predicted to have risen to reach 540 to 950 ppm (μmol mol<sup>-1</sup>) if the trend continues and no significant mitigation efforts are taken [2]. Unlike atmospheric reactive gases, e.g., ozone that is relatively short-lived, CO<sub>2</sub> hangs for a long time in the atmosphere, can be between 300-1000 years [3]. Therefore, the human population and ecosystems must endure the impacts of elevated [CO<sub>2</sub>] for a considerable long duration of time.

Aside from the association of CO<sub>2</sub> with atmospheric temperature and global warming, CO<sub>2</sub> is the main component in plant photosynthesis. Thus the change in the atmospheric concentration will



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directly affect the photosynthetic rate and, in turn, will affect plant growth and productivity. Hypothetically, elevated  $[\text{CO}_2]$  exposure will increase plant photosynthetic rate because  $\text{CO}_2$  is required for the process. However, the plant growth and productivity may not be enhanced due to the increase in other raise physiological stress, e.g., high temperature. Since the early time of realization on the increased  $[\text{CO}_2]$ , many studies have reported the impacts on plant growth and productivity, mainly for crops, because of the relation with human livelihood. Various studies on the elevated  $[\text{CO}_2]$  effects on forest tree growth were also reported. Plant responses to elevated  $[\text{CO}_2]$  are various, i.e., increased growth, alteration in biomass allocation, change in secondary metabolites, change in assimilates allocation, different water use efficiency (WUE), and photosynthetic nitrogen use efficiency (PNUE), or nutrients uptake rates [4, 5, 6].

The previous study reported that although the net photosynthetic rate of *E. urophylla* and hybrid of *E. deglupta* x *E. camadulensis* was increased in elevated  $[\text{CO}_2]$  conditions, the height and diameter were not significantly stimulated [7]. However, elevated  $[\text{CO}_2]$  was reported to enhance the growth of hybrid larch (*Larix gmelinii* var. *Japonica* x *L. kaempferi*) and induced more biomass allocation to shoot than root [8, 9] noticed several cases in which elevated  $[\text{CO}_2]$  is reported to alter the flowering time of crops and wild plants.

In this current study, the growth responses of acacia and eucalypt species to elevated  $[\text{CO}_2]$  and N load are reported, considering those species are the main forest plantation plants of the third world in recent decades. Acacia and eucalyptus are also known as fast-growing and adaptable species. Therefore those plants are widely planted in forest plantation timber estates in Asia and Africa, including Indonesia. The plantation timbers and the associated industries have a big economic contribution, nationally, regionally, and even globally. The capacity of these species to cope with the increase  $[\text{CO}_2]$  will define their fitness in the sustainability of forestry estates.

## 2. Materials and methods

The experiment was conducted in the Free Air  $\text{CO}_2$  Enrichment (FACE) system in Sapporo Experimental Forest, Hokkaido University, Japan ( $43^\circ 06' \text{ N}$ ,  $141^\circ 20' \text{ E}$ ). The location is in a transition zone between boreal and cold temperate forest [8], which is not the typical growing site of the tested species. However, the experiment was done during the growing season in that area, from June to September 2010.

### 2.1. Plant materials and experimental design

Seedlings of two acacias, i.e., *Acacia mangium* and *A. auriculiformis*, and two eucalypts, i.e., *Eucalyptus camadulensis* and *E. urophylla*, were assigned in this study. The seedlings originated from seeds obtained from the Australia Tree Seed Center of CSIRO, Kingston, Australia. The 6-7 months old seedlings were grown in pumice and clay soil (1:1, v/v) in 7 L pots commonly used in nursery practice. The pots were watered periodically to sustain the potted-soil moisture.

The seedlings were supplied with two levels of N ( $\text{N0} = 0 \text{ kg ha}^{-1}$  and  $\text{N1} = 50 \text{ kg ha}^{-1}$  of  $(\text{NH}_4)_2\text{SO}_4$  + balanced nutrients (N:P:K = 6:10:5, Hyponex Corp., Osaka, Japan) and two levels of  $[\text{CO}_2]$  (ambient (A) =  $370\text{--}380 \mu\text{mol mol}^{-1}$  and elevated (E) =  $500 \mu\text{mol mol}^{-1}$ ). The  $\text{CO}_2$  gas was supplied in the FACE system with three site replications for each ambient and elevated  $[\text{CO}_2]$ . In the elevated  $[\text{CO}_2]$  site,  $\text{CO}_2$  gas was fumigated during daytime when the photosynthetic photon flux exceeded  $70 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (the light compensation point of photosynthesis) in accordance with the fumigation regime of other FACE experiments with woody plants [8, 10].

### 2.2. Measurement of parameters

**2.2.1. Growth traits.** The height and stem diameter of the seedlings was measured once a month for four months. At the end of September 2010, all the plants were harvested to determine the dry mass of

leaves, stems, and roots. The dry weight of the plant organs was obtained by drying the leaves, stem, and root in an oven at 70 °C for a week [8].

**2.2.2. Leaf traits and chemicals.** The leaf mass area (LMA) was measured by taking the fifth leaves from the top shoot immediately scanned to get the leaf area (mm). The leaf was then oven-dried to get the dry weight (mg). The oven-dried was carried out at 70 °C for a week. The LMA was a result of dividing the leaf mass by the leaf area ( $\text{g m}^{-2}$ ) [5, 7].

The leaf N and C content were determined using a combustion method with the NC analyzer (NC-900, Sumica-Shimadzu, Kyoto, Japan). The C/N was obtained by dividing the leaf C content with the N content. The leave N per unit dry mass ( $N_{\text{mass}}$ ,  $\text{g g}^{-1}$  or %) was calculated by dividing the leaf N concentration with the dry weight. The leave N per unit area ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ) was calculated by dividing the leaf N concentration with leaf area.

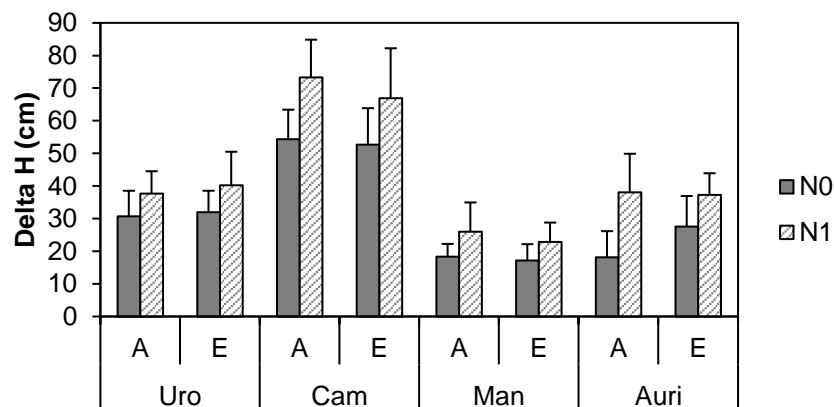
### 2.3. Statistical analysis

The one-way analysis of variance (ANOVA) was used to determine the difference between treatments for each parameter. The T-test was used to determine the different responses of the two genera, the  $[\text{CO}_2]$  treatment and N load. The statistical analysis was performed by using SPSS 16.0.2 (SPSS Inc., USA).

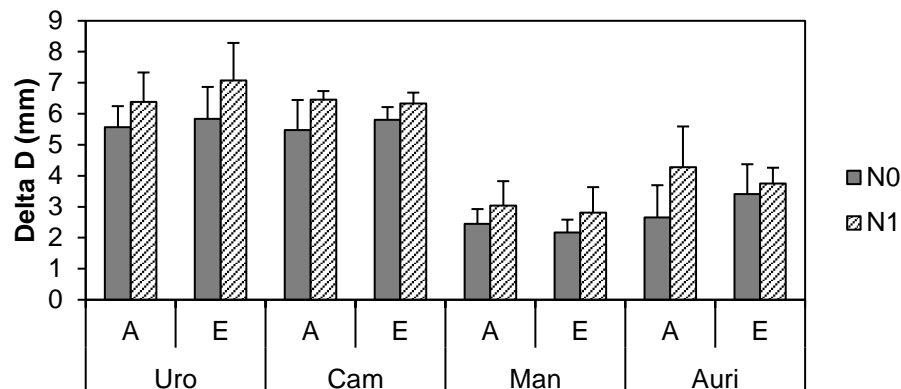
## 3. Results

### 3.1. Growth traits

In general, the genus eucalypts showed better growth performance than the acacias (figures 1, 2, and 3). The growth of the tested species was affected by nutrient addition, but the effect of  $[\text{CO}_2]$  was less pronounced.

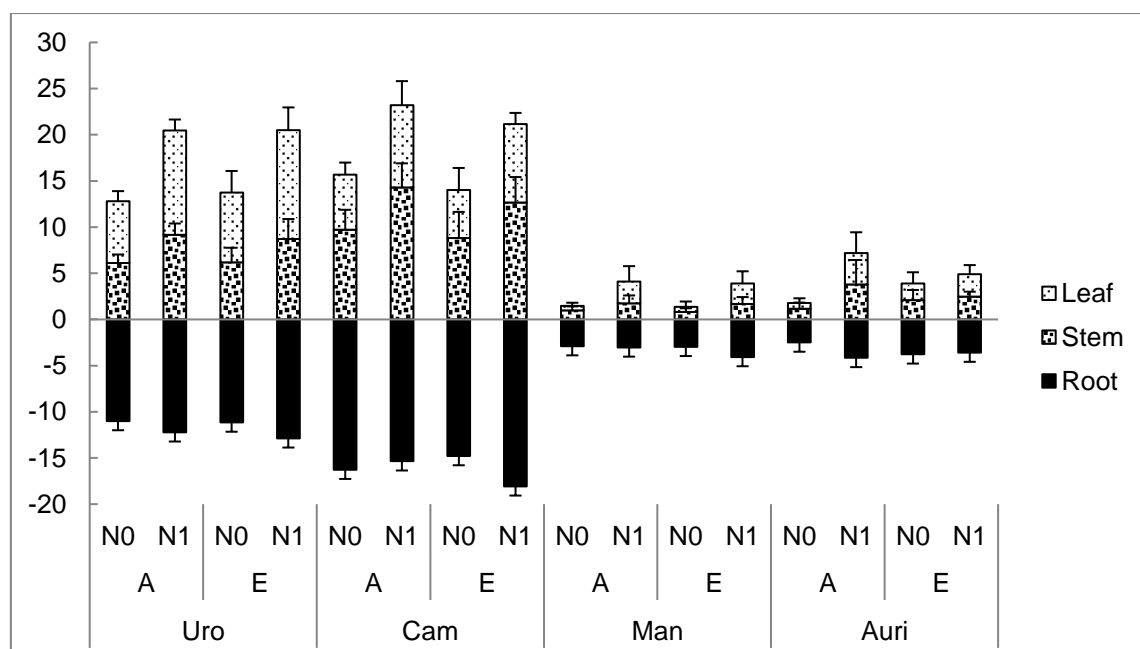


**Figure 1.** The height increment of the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient  $[\text{CO}_2]$ , E = elevated  $[\text{CO}_2]$ , N0 = no nutrient, N1 = N addition, data are average values  $\pm$  SD,  $P < 0.05$ .



**Figure 2.** The stem diameter increment of the tested species Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient [CO<sub>2</sub>], E = elevated [CO<sub>2</sub>], N0 = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .

The height and diameter increment of the eucalypts were significantly higher than those of the acacias. The ANOVA analysis showed that nutrient addition significantly increased the height of the tested species ( $P = 0.00$ ). However, the effect of elevated [CO<sub>2</sub>] and the interaction of those factors were less pronounced ( $P = 0.464$  and  $P = 0.357$ , respectively). The highest height increment was shown by *E. camaldulensis*, followed by *E. urophylla*, *A. auriculiformis*, and last by *A. mangium* (figure 1). Meanwhile, the stem diameter increment showed a positive response to nutrient addition ( $P = 0.00$ ) but not to elevated [CO<sub>2</sub>] ( $P = 0.531$ ). The diameter increment between the eucalypts was not significantly different, but they were significantly higher than *A. auriculiformis* and *A. mangium*.



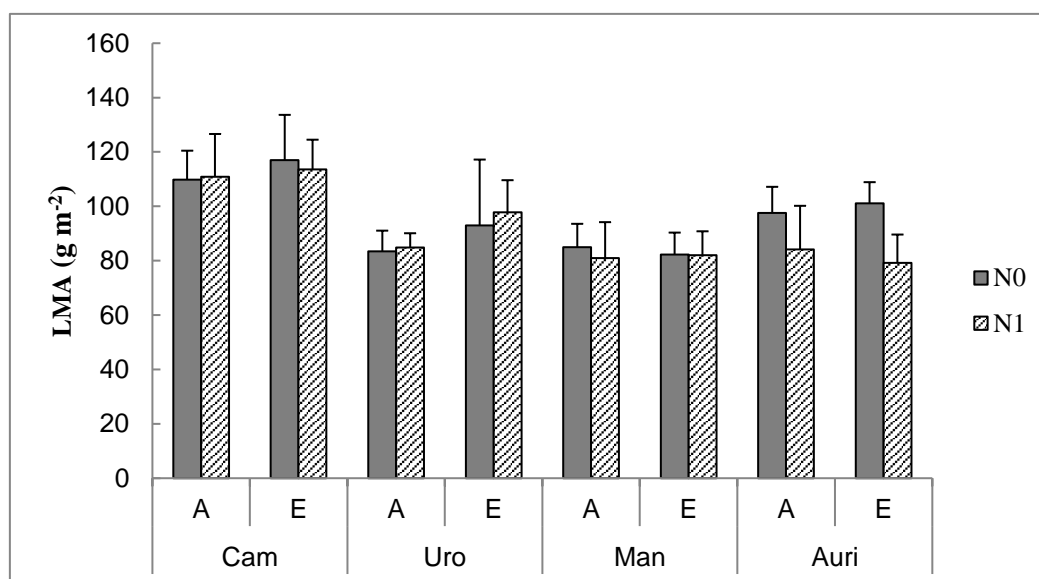
**Figure 3.** The biomass allocation in the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient [CO<sub>2</sub>], E = elevated [CO<sub>2</sub>], N0 = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .

The T-test showed similar results on biomass. Elevated [CO<sub>2</sub>] The biomass was not significantly affected the total biomass and the partial biomass of leaves, stem, and root. However, nutrient addition

significantly affected the total biomass and biomass of leaves and stem, except for root biomass that was not different. The P-value for total, leaves, stem, and root biomass were 0.000, 0.002, 0.326, and 0.008, respectively. The interaction effect of elevated  $[\text{CO}_2]$  and nutrient addition also showed no significant effect on the biomass of the tested species. The Pots hoc test showed that *E. camaldulensis* had the highest total biomass, followed by *E. urophylla*, *A. auriculiformis*, and *A. mangium*.

### 3.2. Leaf mass area (LMA)

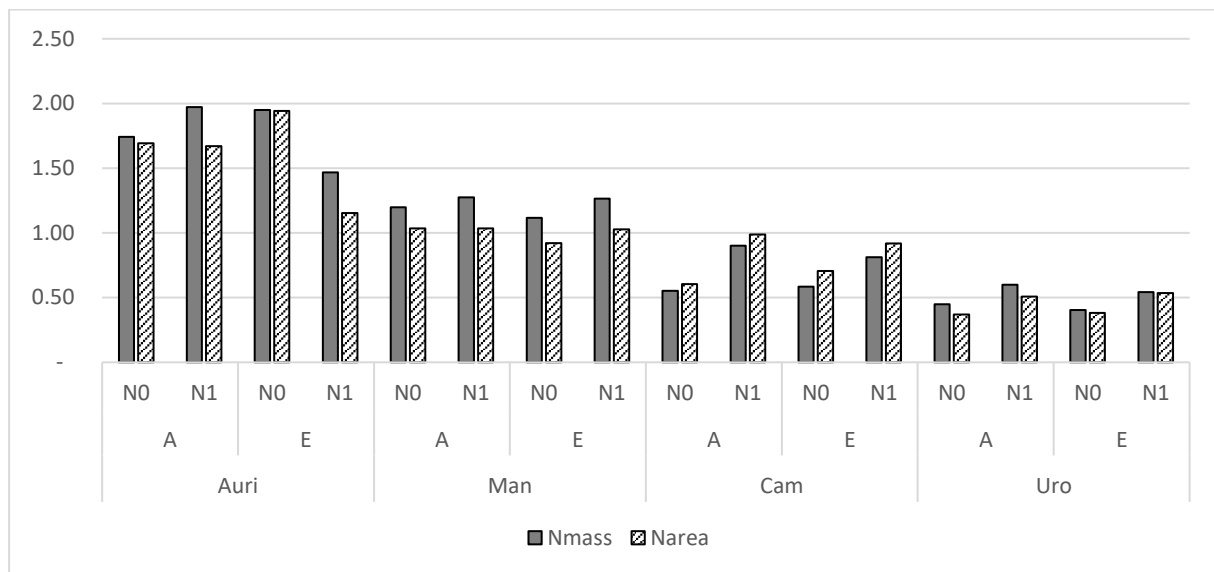
The LMA of the tested species responded similarly to exposure to elevated  $[\text{CO}_2]$ , nutrient addition, and interaction of both factors in which nutrient addition showed a significant effect ( $P = 0.033$ ) while the effect of elevated  $[\text{CO}_2]$  and the interactions were not significant ( $P = 0.72$  and  $0.733$ , respectively. (figure 4).



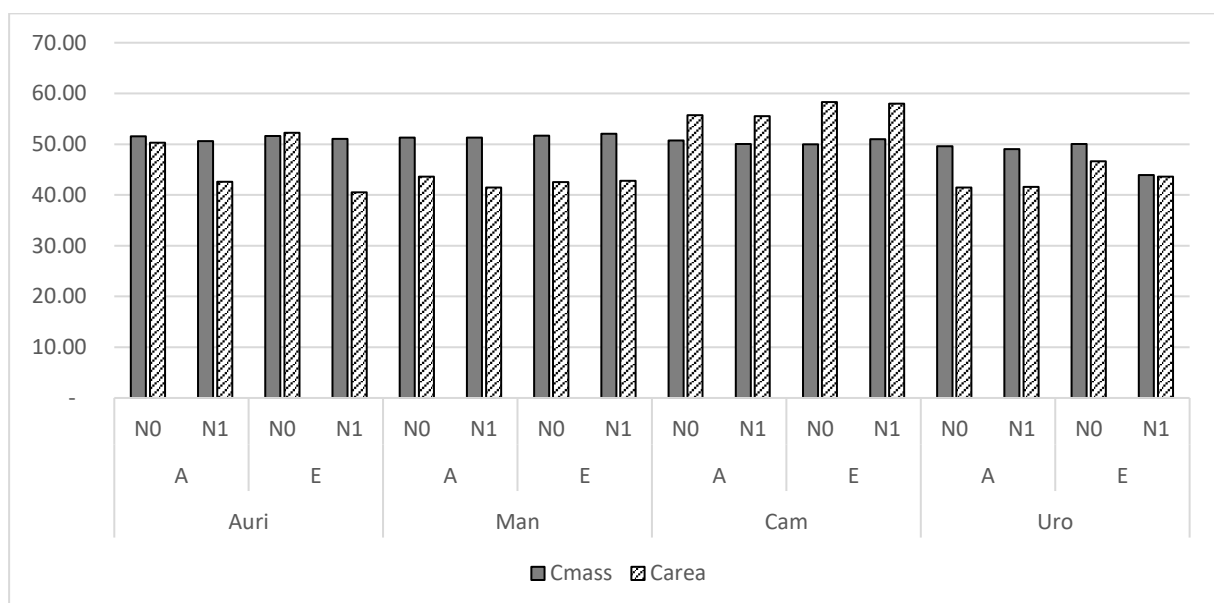
**Figure 4.** The leaf mass area (LMA) of the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient  $[\text{CO}_2]$ , E = elevated  $[\text{CO}_2]$ , N0 = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .

### 3.3. Leaf N and C content

The leaf N and C content of the tested species, based on both area and dry weight, were not affected by elevated  $[\text{CO}_2]$ , nutrient addition, and the interaction of both factors (figure 5 and 6) (all  $P > 0.05$ ).



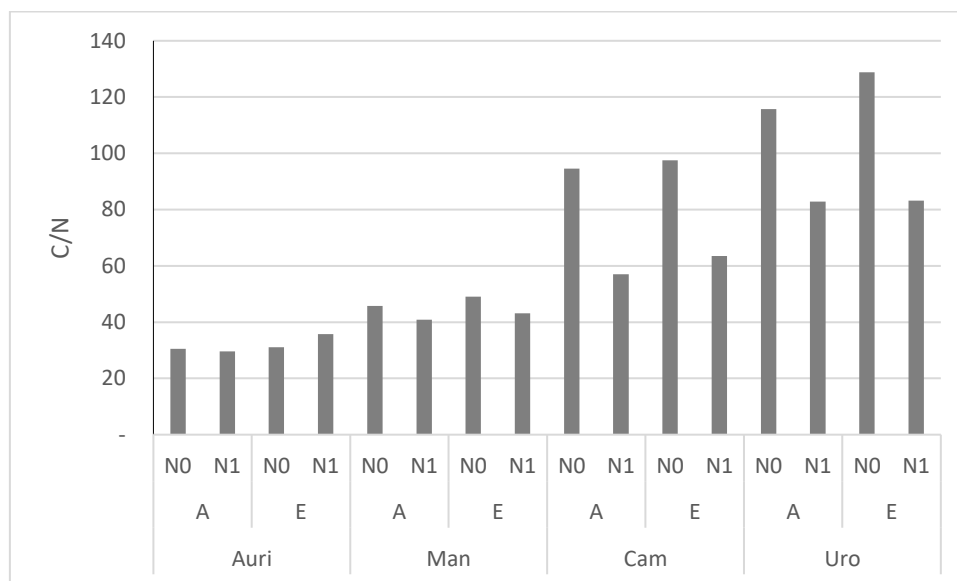
**Figure 5.** The leaf N content of the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient [CO<sub>2</sub>], E = elevated [CO<sub>2</sub>], N0 = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .



**Figure 6.** The leaf C content of the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient [CO<sub>2</sub>], E = elevated [CO<sub>2</sub>], N0 = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .

### 3.4. C/N

Similar responses were shown by the C/N of the tested species to the treatments (figure 7). The tested species responded positively ( $P = 0.000$ ) to nutrient addition but not to elevated [CO<sub>2</sub>] ( $P = 0.118$ ). The interaction of elevated [CO<sub>2</sub>] and nutrient addition was also showed no significant impact ( $P = 0.829$ ).



**Figure 7.** The leaf C/N of the tested species. Remarks: Uro = *E. urophylla*, Cam = *E. camaldulensis*, Man = *A. mangium*, Auri = *A. auriculiformis*, A = Ambient [CO<sub>2</sub>], E = elevated [CO<sub>2</sub>], NO = no nutrient, N1 = N addition; data are average values  $\pm$  SD,  $P < 0.05$ .

#### 4. Discussion

Species in the genus *Acacia* and *Eucalyptus* are well known and usually fast-growing species with high productivity, thus preferable for timber estate plantation. The wood of those species is relatively easy to work in pulping, engineering, etc. The species are also relatively easy to adapt to the new, even harsh and marginal environments [11], sometimes even considered as invasive, especially the acacias. The capacity of those species to adapt to the changing environment, e.g., elevated concentration of atmospheric CO<sub>2</sub> gas, will further define their importance in the global forest plantation dynamic. Plants would not only have to physiologically adapt to the elevated [CO<sub>2</sub>] but also have to adapt to the change of the associated parameters, i.e., the rising air temperature, drought, available soil N and P, etc.

The eucalypts showed a better growth performance than the acacias. Both eucalypts performed higher stem height and diameter increments than the acacias. The total biomass and biomass per organ (leaves, stem, and root) were also significantly higher in the eucalypts than those in the acacias. These results are probably due to the typical environment of the tested species. All of the tested species were originated from Australia. The acacias natural habitats ranges from dry to moist tropical area and dry to wet subtropical area. Those acacias adapt well to a wide range of soil types, however, prefer high rainfall and humid condition to grow better [5, 12]. Although the two eucalypts can grow in various soil types and varied fertility, they tend to grow better in warm, moist, and high rainfall conditions. However, the eucalypts can tolerate colder environments better than the acacias thus can survive the harsh subtropical environments [5, 12, 13]. The toleration of the two eucalypts to the colder condition of the growing sites presumably had made them had better growth than the tested acacias.

The growth of the tested species responded similarly to the treatments, i.e., elevated [CO<sub>2</sub>] dan N load. The N load or nutrient addition significantly increased the growth traits of all tested species. However, the growth of all tested species seemed rather insensitive to the elevated [CO<sub>2</sub>]. A similar response to elevated [CO<sub>2</sub>] was observed in the previous study, whereas *E. urophylla* and a hybrid of *E. deglupta* x *E. camaldulensis* showed no difference in growth performance under elevated [CO<sub>2</sub>] of 760  $\mu\text{mol m}^{-1}$  [5].



Similarly, the LMA was significantly affected by nutrient addition but rather insensitive to the elevated  $[\text{CO}_2]$ . Nutrient addition tended to increase the LMA of the eucalypts while it tended to decrease the LMA of the acacias. When a comparison is made between the two genera, then the LMA of the eucalypts was not different than the acacias. These findings are different from the results of the previous study. In contrast, the LMA of *A. mangium* and *A. auriculiformis* was significantly higher than *E. globulus*, *E. grandis*, and *E. urophylla* [5]. These different results were probably because the species were subjected to elevated  $[\text{CO}_2]$  and N load in this present study. A higher LMA implied the bigger investment of dry mass per unit leaf area, thicker blade, and denser tissue [5, 14]. The exposure to elevated CO and/or to N load presumably increased the LMA of the eucalypts.

Leaf N content was higher in the acacias than in the eucalypts. However, this fact was expected because the acacias are known as legume species with N-fixing capacity, therefore, have higher N content than that in the eucalypts. Although not significantly different, the nutrient addition tended to increase  $N_{\text{mass}}$  and  $N_{\text{area}}$  of the eucalypts. A no different result was observed in the acacias though that was perhaps due to the N-fixing capacity of the acacia, which is not the traits of the eucalypts.

The N addition significantly reduced the C/N. The N addition supplied additional N to the leaves while there was no differential effect of elevated  $[\text{CO}_2]$ , thus the lower C/N. The Leaf C/N is higher in the eucalypts than that in the acacias, which is understandable considering the higher leaf N content.

## 5. Conclusion

The eucalypts showed significantly higher growth performance than the acacias. The growth performance and leaf traits of the two acacias and two eucalypts seedlings were not significantly affected by elevated  $[\text{CO}_2]$  but responded positively to nutrient addition. The combined effect of elevated  $[\text{CO}_2]$  and nutrient addition was also less pronounced on the tested acacia and eucalypt. The tested acacia and eucalypts showed relatively insensitivity to elevated  $\text{CO}_2$ . Thus they might possess resilience capacity under the keep increasing level of the atmospheric  $\text{CO}_2$  concentration, one of the greenhouse gases that responsible for global warming.

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