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# Photosynthetic nitrogen and water use efficiency of acacia and eucalypt seedlings as afforestation species

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## Abstract

The ecophysiological traits of acacia and eucalypt are important in assessing their suitability for afforestation. We measured the gas-exchange rate, the leaf dry mass per area (LMA) and the leaf nitrogen content of two acacia and four eucalypt species. Relative to the eucalypts, the acacias had lower leaf net photosynthetic rate ($P_N$), lower photosynthetic nitrogen-use efficiency (PNUE), higher water-use efficiency (WUE), higher LMA and higher leaf nitrogen per unit area ($N_{area}$). No clear differences were observed within or between genera in the maximum rate of carboxylation ($V_{cmax}$) or the maximum rate of electron transport ($J_{max}$), although these parameters tended to be higher in eucalypts. PNUE and LMA were negatively correlated. We conclude that acacias with higher LMA do not allocate nitrogen efficiently to photosynthetic system, explaining why their $P_N$ and PNUE were lower than in eucalypts.

*Additional keywords*: Afforestation; fast growing species; photosynthetic nitrogen-use efficiency; photosynthetic water-use efficiency.

*Abbreviations*: $C_i$ – leaf intercellular $CO_2$ concentration; $E$ – leaf transpiration rate; $g_s$ – stomatal conductance; $J_{max}$ – maximum rate of electron transport; LMA – leaf dry mass per area; $N_{area}$ – leaf nitrogen per area; $N_{mass}$ – leaf nitrogen per dry mass; $P_N$ – leaf net photosynthetic rate; $P_{\mathrm{Nmax}}$ – light-saturated net photosynthetic rate; PNUE – photosynthetic nitrogen-use efficiency; $V_{\mathrm{c,max}}$ – maximum rate of carboxylation; VPD – vapour pressure deficit; WUE – photosynthetic water-use efficiency.

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## Introduction

Nitrogen (N) and water are essential for the photosynthetic and plant growth and survival. The efficiency with which these resources are used in the assimilation process is expressed by means of the photosynthetic nitrogen use efficiency (PNUE) and the photosynthetic water use efficiency (WUE). The PNUE and WUE are capable of predicting how the net photosynthetic capacity is optimized per unit of water and N in leaves (*e.g.* Castellanos *et al.* 2005). The PNUE is an important parameter in determining the efficiency of N utilization for growth (Garnier *et al.* 1995, Poorter and Evans 1998, Larcher 2003) so that it is a useful indicator of plant performance in relation to edaphic condition (Funk and Vitousek 2007, Huang *et al.* 2008). PNUE is a key characteristic of leaves of differing species, and is related to leaf economics, leaf physiology, strategy (*e.g.* Wright *et al.* 2004a), and competition (Robinson *et al.* 2001). Species with a high PNUE tend to have high growth rates (*e.g.* Reich *et al.* 1995, Hikosaka 2004). Poorter and Evans (1998) stated that variation in PNUE is linked with a suite of traits that determined the growth potential of a species.

The photosynthetic water use efficiency (WUE) is related to the optimum water use of plants (Robinson *et al.* 2001, Larcher 2003). The WUE underpins plant responses to the competition for water under xeric conditions (*e.g.* Schulze *et al.* 2005). In forest ecosystems, WUE is a critical link between wood production and forest water management (*e.g.* Cernusak *et al.* 2007).

Regardless of strong correlation between leaves N and photosynthetic rate; the ratio of photosynthesis rate to leaf N (PNUE) is often found to be variable. LMA is the inverse of specific leaf area (SLA) and as there is a strong correlation between PNUE and specific leaf area (SLA) (Poorter and Evans 1998, Schulze 2005), thus LMA can be used to describe the variation on PNUE (Poorter and Evans 1998). LMA interconnects with a suite of traits that are important determinant of variation in plants growth rate (*e.g.* Poorter and Evans 1998). Then, at the measured-LMA, a variation in PNUE may capable in explaining the difference in photosynthetic rate between species that divergent inherently (Reich *et al.* 1995), such as in acacia and eucalypt.

Acacia and eucalypt are both fast growing plants, yet differ inherently *e.g.* in leaf morphology and chemistry. Eucalypts bear true-leaves, whereas some acacias are characterized by phyllodes. The anatomical structure of the phyllodes and true leaves are very different (*e.g.* Leroy *et al.* 2009). Differences in leaf traits between acacias and eucalypts should influence their physiological function, including their photosynthetic activity.

Leaf morphological traits, *e.g.* leaf mass per unit area (LMA) (*e.g.* Niinemets 1999a, Lee *et al.* 2000) and chemical composition (*i.e.* leaf nitrogen content) are reportedly to be positively correlated with the photosynthetic capacity per unit leaf area. This shall assist in seedling selection for afforestation, taking into account the photosynthetic efficiency of the seedlings. This in turn depends on structural and biochemical parameters, and influences the plant growth (Barja *et al.* 2001, Alves *et al.* 2002, Kruger and Volin 2006).

Acacias and eucalypts are pioneer species suitable for afforestation (*e.g.* Jøker 2000, Alves *et al*. 2002, Takahashi *et al.* 2002, Orwa *et al.* 2009). In particular, acacias through symbiotic nitrogen-fixing could improve the soil nitrogen (N) condition (Yang *et al.* 2009). Acacias (Armstrong 1998, Francis 2002, Forrester *et al.* 2006, Hai 2009) and eucalypts (Grattapaglia and Sederoff 1994) have been planted widely around the world because of their rapid growth traits and adaptability to diverse growth conditions.

Both acacia and eucalypts are known to have high photosynthetic rate, which is responsible for their high growth rate. Growth (*e.g.* Pinyopusarerk *et al*. 1996, Heriansyah *et al*. 2007, Hai 2009, Quang *et al*. 2010) and photosynthetic traits (*e.g.* Yu and Ong 2003, Grassi *et al*. 2002, Lewis *et al*. 2011) of species in both genera had been well described. Some studies had elucidated the relation of photosynthetic rate with leaf N and/or LMA of eucalypts and acacia (*e.g.* Prior *et al*. 2004, Harrison *et al*. 2009). However, little information is available to facilitate comparison of PNUE and WUE at the measured-LMA.

The two genera tend to grow in different natural environments (Tunstall 2005), and to maintain their growth and productivity they are believed to employ different strategies, as reflected in the PNUE and WUE. Species with high LMA almost invariably have low PNUE as a result of the higher $N_{\mathrm{mass}}$ (*e.g.* Poorter and Evans 1998). We therefore believe that acacias with nitrogen-fixing capacity would have greater leaf $N_{\mathrm{mass}}$ and $N_{\mathrm{area}}$ and higher LMA, and hence lower PNUE, than eucalypts. In this study we analyzed the variation of PNUE and photosynthetic WUE of two acacias and four eucalypts which have different LMA, so as to clarify the photosynthetic traits for assessing their suitability for afforestation.

## Materials and Methods

**Plant materials and growth conditions**: We studied two acacia and four eucalypt species: *Acacia auriculiformis, A. mangium, Eucalyptus camaldulensis, E. urophylla, E. grandis,* and *E. globulus.* These are among the most popular species used for man-made forests. The seeds were obtained from Australia Tree Seed Centre of CSIRO, Kingston, Australia. They were germinated in March 2008 and subsequently grown in a glasshouse equipped with vitreous silica (through which shorter wavelengths of light can pass) in the Agriculture School of Hokkaido University, Sapporo, Japan (43$^{\circ}$0’N, 141$^{\circ}$2’E). The seedlings were grown under identical conditions with a supply of nutrient, 100 ml of 500-fold diluted Hyponex (N: P: K = 6:10:5, Hyponex Corp. JAPAN, Osaka, Japan) which was applied to each plant once a week. The daily temperature and relative humidity (RH) in the glasshouse were maintained at 25±2$^{\circ}$C and 70%. The growing medium was a mixture of Kanuma pumice soil and clay soil (1:1) in the standard 7 liter pot commonly used in nursery practices. Pots were watered daily so as to sustain the soil moisture. The measurements comprised three replications for each species.

**Typical natural environment of the tested species:** *Acacia auriculiformis* and *A. mangium* are found naturally in Australia, Papua New Guinea, and Indonesia (*e.g.* Bino 1998). Although they grow better in areas with high rainfall, their natural habitat ranges from tropical dry to moist, and subtropical dry to wet, forest zone. They tolerate a wide range of soil types, from fertile to eroded soil, and prefer warm humid condition (*e.g.* Turnbull 1986).

Three of our eucalypts species are indigenous to Australia, and *E. urophylla* originates from Indonesia (Baltazar *et al*. 2009). All four species have been used for afforestation (Hengari 2007). Although their growth is enhanced in the warm region, they tolerate colder environment better than the acacias, thus can survive the harsh subtropics environment. *E. grandis* (Schonau 1984) and *E. urophylla* (Baltazar *et al*. 2009), however, are sensitive to frost, whereas *E. gobulus* can tolerate temperature as low as -8°C (Goudzwaard 2011). These eucalypts can grow on a wide range of soil types with varied fertility. They can be found in areas with mean-annual rainfall ranging from 250 mm to 2500 mm, but they prefer moist soil (Schonau 1984, Hengari 2007, Baltazar et al 2009, Goudzwaard 2011).

**Gas exchange rates** were measured on mature leaves (third and fourth leaves from the shoot top) using an open-type gas exchange system (*LI-6400*, *LI-Cor*, Lincoln, Nebraska, USA) in mid-August 2008. Measurements were carried out under a photosynthetic photon flux of 1500 μmol $m^{-2}$ $s^{-1}$, the light level corresponding to the light saturated photosynthetic rate for these plants in the growing environment. The leaf temperature was controlled at 25°C ± 1°C, and the leaf vapor pressure deficit (VPD) was maintained at 1.2 ± 0.2 kPa. The photosynthetic WUE [mmol $mol^{-1}$] was calculated as $P_N$ divided by $E$. The stomatal conductance in the measurements was regulated by maintaining the leaf temperature and vapour pressure deficit at values stated. It took 15 – 20 min for the stomata to equilibrate with the changed-condition after clipping the leaf to the chamber at every new measurement.

To obtain the response curve of net assimilation rate ($P_N$) to intercellular $CO_2$ concentration ($C_i$), *i.e.*, the $P_N$ /$C_i$ curve, we established 12 values for the external $CO_2$ concentration in the chamber and determined the corresponding values of $P_N$ (from 60 to 1,500 μmol $mol^{-1}$). The $P_N$ /$C_i$ curve was used to estimate the light-saturated net photosynthetic rate ($P_{Nmax}$), stomatal conductance ($g_s$) and leaf transpiration rate ($E$) at 380 μmol $mol^{-1}$($CO_2$), and the maximum rate of carboxylation ($V_{cmax}$) and maximum rate of electron transport ($J_{max}$) (Farquhar *et al.* 1980, Long and Bernacchi 2003). The Rubisco Michaelis constants for $CO_2$ ($K_c$) and $O_2$ ($K_o$) and the $CO_2$ compensation point in the absence of dark respiration ($\Gamma^*$) for the analysis of the $P_N / C_\mathrm{i}$ curve were obtained from Bernacchi *et al.* (2001). All gas-exchange parameters were expressed on the basis of the projected (one-sided) leaf area covered.

**Leaf Nitrogen Measurement**: The leaves were excised from the plants in order to measure the leaf mass per area [LMA, g (DM) $\mathrm{m}^{-2}$], and to determine the N concentration per unit dry-mass [$N_\mathrm{mass}$, g $\mathrm{g}^{-1}$ or % (DM)] and per unit area [$N_\mathrm{area}$, g (DM) $\mathrm{m}^{-2}$]. After the leaf area had been measured, the leaves were oven dried at 60°C for one week to determine their dry mass. The leaf N content was determined using a combustion method with a NC analyzer (NC-900, Sumica-Shimadzu, Kyoto, Japan). The PNUE [μmol $\mathrm{mol}^{-1}$ $\mathrm{s}^{-1}$] was calculated by dividing $P_\mathrm{N}$ by the area-based leaf N content and molecular mass of nitrogen.

**The estimation of N allocation to photosynthetic system**: The nitrogen allocation to photosynthetic apparatus can be divided into three components: (*1*) Rubisco (N1), (*2*) bioenergetics or electron carriers except for photosystems, coupling factor and Calvin cycle enzymes except for Rubisco (N2), (*3*) light-harvesting complex and photosystems (N3). In this study we calculated only N1 and N2 to represent N allocation to the photosynthetic capacity (Westbeek *et al.* 1999). N1 was estimated from the following equation (Niinemets *et al.* 1999b; Tissue and Lewis 2010):

$$\mathrm{N1} = V_\mathrm{cmax} / (6.25\ V_\mathrm{cr}\ \mathrm{LMA}\ N_\mathrm{m}),$$

where $V_\mathrm{cr}$ denotes the specific activity of Rubisco (the maximum rate of RuBP carboxylation per unit Rubisco protein) which is equal to 20.5 μmol($\mathrm{CO_2}$) g(Rubisco)$^{-1}$ $\mathrm{s}^{-1}$ at 25°C for purified Rubisco enzyme from *Spinacia oleracea* (Jordan and Ogren 1984), and $N_\mathrm{m}$ is N per unit leaf mass, the factor of 6.25 [g Rubisco $\mathrm{g}^{-1}$(N) in Rubisco] would convert N content to protein content. The value of N1 from this calculation is underestimated because this method determines only the amount of activated Rubisco (Warren and Adams 2004).

The N2 was estimated according to the following equation (Kitaoka and Koike 2004; Takashima *et al.* 2004):

$$\mathrm{N2} = J_{\max} / (156 \times 9.53\ \mathrm{LMA}\ N_{\mathrm{m}}),$$

whereas N in bioenergetics is assumed to be proportional to $J_{\max}$, the coefficient 156 mmol $\mathrm{mol}^{-1}\ \mathrm{s}^{-1}$ is the ratio of $J_{\max}$ to the cytochrome *f* content (Niinemets and Tenhunen 1997), and the coefficient 9.53 mol $\mathrm{mmol}^{-1}$ represents N in bioenergetics per unit cytochrome *f* (Hikosaka and Terashima 1995).

**Statistical Analysis**: For each parameter measured, one-way analysis of variance (*ANOVA*) was used to test differences between the species. The net photosynthetic rate was compared between eucalypts and acacia using the two-tailed *t*-test. The test was performed using *SPSS 16.0.2* (*SPSS Inc*., USA).

## Results

**LMA, leaf N, PNUE, and Photosynthetic Response**: The LMA value was similar for the two acacia, and higher than that of the eucalypts (Fig 1). Among the eucalypts, the LMA was greater in *E. camaldulensis* than in the other species. For $N_{\mathrm{area}}$ the tendency was similar, with larger values for acacias than eucalypts. In the acacias, $N_{\mathrm{area}}$ was greater for *A. auriculiformis* than *A. mangium,* while *E. camaldulensis* had the largest $N_{\mathrm{area}}$ of the eucalypts. The $N_{\mathrm{mass}}$ did not differ significantly either within or between the genera. For PNUE the trend was opposite to that for LMA and $N_{\mathrm{area}}$. PNUE was lower for acacias than eucalypts, and of the eucalypts *E. camaldulensis* had the lowest PNUE (Fig. 1).

A significant negative correlation between PNUE and LMA was observed for both genera, implying that the higher the LMA of the species, the lower the PNUE (Fig. 2*A*). The lower PNUE of acacias is presumably due to their higher N leaf content per unit area.

The $P_N$ was significantly lower for acacias than for eucalypts (Table 1). Of the two acacias, $P_N$ of *A. auriculiformis* was slightly higher than for *A. mangium*. Among the eucalypts, the highest value was for *E. urophylla* and lowest for *E. grandis*. Values of $P_N$ did not differ statistically within genera.

Although $P_N$ tended to increase with increasing leaf N in both genera, the correlation was not significant. However, the $P_N$ of eucalypts were higher than that of the acacias at similar value of the leaf $N_{area}$ (Fig. 2B).

$V_{cmax}$ and $J_{max}$ were not statistically different, either within or between genera (Table 1); however, values were lower for acacias than for eucalypts. Although not all differences were statistically significant, *A. mangium* had the lowest values of $P_N$, $V_{cmax}$, and $J_{max}$, while *E. camaldulensis* had the highest. The $V_{cmax}$ and $P_N$ were significantly positively correlated. There was a positive correlation, though not statistically significant, between the $P_N$ and $J_{max}$, (Fig. 3).

In this study, the measurement of N1 and N2 represents N allocation to photosynthetic system. The N allocated to photosynthetic system was significantly greater in the eucalypts than the acacias (Fig. 4). Both N1 and N2 were the greatest for *E. globulus*, followed in order of *E. grandis, E. urophylla*, *E. camaldulensis, A. mangium*, and *A. auriculiformis* (Table 1).

We found that $P_N$, $g_s$, and *E* were lower in acacias than in eucalypts (Table 1). Among the tested species, the lowest values of $g_s$ and *E* were for *A. mangium,* and the highest was for *E. globulus*.

**WUE**: On the genus basis, an independent two-tailed *t*-test indicated that the WUE of acacias was significantly higher than that of eucalypts. Conversely, there was little variation of WUE among the observed species (Table 1). Although there was no strong correlation, WUE increased as $N_{\mathrm{area}}$ (Fig. 5) and LMA (Fig. 6) increased.

## Discussion

**Photosynthetic traits in relation with stomatal gas diffusion, nitrogen, and LMA**: As the studied-species are all capable of growing in the subtropical environment (Thin *et al*. 1998, Bino 1998, Arnold and Luo 2002, Bauhus 2004, Yang *et al*. 2009), they were considered to be comparable as potential-species for afforestation and reforestation projects.

The lower $P_{\mathrm{N}}$ was apparently due to the significantly lower $g_{\mathrm{s}}$ for acacias than for eucalypts (Table 1 and Fig. 1). It is well known that $g_{\mathrm{s}}$ is positively correlated with the rate of photosynthesis (*e.g.* Larcher 2003, Schulze *et al.* 2005). There was almost 50% reduction in stomatal conductance of acacias than that of eucalypts (Table 1). Higher $N_{\mathrm{area}}$ may be responsible for the lower $g_{\mathrm{s}}$ of acacias relative to eucalypts, leading to lower $CO_2$ uptake through the stomata and hence a lower $P_{\mathrm{N}}$ (*e.g.* Lambers *et al*. 2008). Stomatal closure presumably played a large role in limiting the photosynthetic capacity of acacias relative to eucalypts, since there were no differences in $V_{\mathrm{cmax}}$ and $J_{\mathrm{max}}$. Although $V_{\mathrm{cmax}}$ and $J_{\mathrm{max}}$ were positively correlated with $P_{\mathrm{N}}$ (Fig. 3), they did not differ between acacias and eucalypts, nor within genus (Table 1). This implies that the photosynthetic capacity in chloroplast was not different among the tested species and was not the cause of the lower $P_{\mathrm{N}}$ of acacias than that of eucalypts.

The relation between $P_{\mathrm{N}}$ and $N_{\mathrm{area}}$ was even corroborated when species were compared within the same genus. *A. mangium* of the acacias and *E. camaldulensis* of the eucalypts showed the highest $P_{\mathrm{N}}$ among their respective genus due to the highest $N_{\mathrm{area}}$. As acacias acquire N via symbiosis with N-fixing microbes from the environment, they have greater N content in foliage. However, a comparison of the two genera gave an unexpected result; different from eucalypts, the high $N_{\text{area}}$ of acacias did not give rise to a high $P_{\text{N}}$. Despite their higher leaf N, acacias allocated a smaller portion of the leaf N to the photosynthetic system (defined by N1 and N2) than the eucalypts (Fig. 4). This may explain the lower $P_{\text{N}}$ of the acacias than the eucalypts.

Leaf structure and N concentration affect photosynthetic capacity. A higher LMA usually implies greater investment in leaf dry-mass per unit leaf area, thicker leaf blade and denser tissue (Wright *et al.* 2004a, Reich *et al.* 1995). The increase in LMA roughly coincides with the decrease in photosynthetic capacity. High LMA corresponds to longer paths for diffusion from stomata to chloroplast, and greater internal shading of deeper chloroplasts, limiting the necessary $CO_2$ supply and light irradiance for photosynthesis (Wright *et al.* 2004a, Terashima *et al.* 2001). Moreover, N is probably assigned less to photosynthetic machinery in acacias, because of competition with N allocation to non-photosynthetic leaf components in high LMA species. These facts probably explain why acacias with higher LMA have lower $P_{\text{N}}$ than eucalypts (Fig. 1).

The acacias have significantly higher LMA than the eucalypts, due presumably to the inherent difference in their typical leaf traits of xeromorphic adaptation. Whereas eucalypts bear true leaves, the foliage of the acacias is a modification of the leaf-petiole functioning like leaf blades, known as phyllodes (Niinemets 1999a, Leroy *et al*. 2009); these are thicker and stiffer than the true leaves of the eucalypts due to their denser mass per unit area. The higher LMA may account for a greater investment of resources in structural components of acacia foliages. Anatomically, phyllodes have thick cell wall and a two-layered palisade parenchyma at the abaxial and adaxial surfaces (Duarte and wolf 2005, Leroy *et al*. 2009), while the thickest sclerophyllous-true leaves of *E. camaldulensis* consist of a single layer of palisade parenchyma at the abaxial side (Souza *et al*. 1999).

There was a significant positive correlation between $N_{\text{area}}$ and the LMA (data are not shown). Yet, the LMA difference between the two genera may not be due directly to the difference in leaf $N_{\text{area}}$, but more to the differing anatomical structure of the foliage, because, despite of the higher LMA, the $N_{\text{mass}}$ at the acacias foliage did not differ with that at the eucalypts (Fig. 1).

**Photosynthetic nitrogen and water use efficiency**: Lambers *et al.* (2008) stated that the PNUE at the growth irradiance would be greatest in leaves with lowest N concentration. For high PNUE, a species should allocate a reasonable amount of total leaf N to the photosynthetic system, and should deploy most N efficiently in photosynthesis. With higher $N_{\text{area}}$, acacias were found to have lower PNUE than the eucalypts. In this case, presumably a smaller proportion of leaf N allocated to photosynthetic capacity (N1 and N2) accounted for the lower PNUE of the acacias than eucalypts (Fig 4). Further, although it was not confirmed by the $N_{\text{mass}}$, the highest $N_{\text{area}}$ of *E. camaldulensis* caused this species to have the lowest PNUE among the eucalypts (Fig. 1).

PNUE is strongly correlated with LMA (Poorter and Evans 1998, Hikosaka 2004, Harrison *et al.* 2009). Acacias with higher LMA had lower PNUE than the eucalypts (Fig. 2*B*). This result confirms that plants with high LMA almost invariably have low PNUE (*e.g.* Poorter and Evans 1998, Ponds and Westbeek 2004). These high LMA species were less N-efficient due to, either they overinvest in photosynthetic N (Poorter and Evans 1998,Westbeek *et al*. 1999), or they invest N in other functions (*e.g.* the structural N of cell wall protein) more than in photosynthesis protein and machinery (Ellsworth *et al.* 2004, Hikosaka 2004, Takashima *et al.* 2004). Lower N allocation to photosynthetic system *i.e.* N1 and N2 (Fig. 4) would be partly responsible for the negative correlation between LMA and PNUE.

Low PNUE is usually observed in stress-tolerant species (*e.g.* Hikosaka and Hirose 2000) and in late successional species (Ellsworth and Reich 1996). According to Yang *et al.* (2009), *Acacia mangium* and *A. auriculiformis* have high tolerance to bare soil. Their low PNUE may therefore be related to their high stress-tolerance traits.

There was little variation of photosynthetic WUE among the observed species. However, WUE of the acacias was significantly higher than that of the eucalypts. Eucalypts with low $N_{\mathrm{area}}$ allocated a larger portion of N to photosynthetic system (Fig. 4) which would determine the $CO_2$ drawdown inside the leaf, so that $g_s$ increases to facilitate the higher $CO_2$ uptake for photosynthetic capacity (Schulze *et al*. 1994, Farquhar *et al*. 2002). As $g_s$ for the eucalypts increased, transpiration through stomata also increased, so that these plants had lower WUE than the acacias (Table 1).

There was a positive correlation between WUE and LMA (Fig. 6). Since we maintained the leaf temperature and VPD stable, the variation in $E$ was not affected by leaf temperature and VPD but was influenced more by diffusion resistance. A high LMA with thicker and more compact tissue could enhance water diffusion resistance (Givnish 1998), thus lower $g_s$ of acacias should be attributed to their high LMA, causing greater leaf resistance to the transpirational water diffusion (*i.e.* lower $E$).

There are strong correlations of LMA with both PNUE and WUE. Species with high LMA tend to have high WUE but lower PNUE (*e.g.* Poorter and Evans 1998, Mediavilla 2001, Lambers *et al.* 2008). The high LMA of phyllodes of acacias cause these plants to maintain higher WUE but consequently decline the PNUE (Fig. 2B and 6).

**Consideration of acacia and eucalypt for afforestation:** Acacias can be found in arid and semiarid environments, whereas eucalypts grow naturally in more favourable sites and therefore have a relatively higher growth rate. Eucalypts demand soil with moist and short water deficit conditions (*e.g.* Hengari 2007, Baltazar *et al*. 2009, Goudzwaard 2011). The ecophysiological characteristics determine how the photosynthetic rate of these genera develops. Acacias expend energy in fixing N and maintain a high LMA (Wright *et al.* 2004b, Tunstall 2005). They can adapt better to drought and aridity, and effectively prevent water loss (*i.e.* high WUE) due to the phyllode structure with high LMA (*e.g.* Hansen 1996, Eamus and Cole 1997, Sommerville *et al*. 2011), at the expense of lower PNUE and $P_N$. The eucalypts used N more efficiently than the acacias so as to maintain a higher rate of $P_N$, but they utilized water more generously in the photosynthetic process.

Despite of the advantages, acacias have high N in leaves, this tend to attract herbivore attack (Novriyanti *et al.* 2010), which should be anticipated in afforestation and reforestation projects. Further, there are deliberation to plant both acacia and eucalypts as complementary rather than as competitors (*e.g.* Forrester *et al*. 2006, Schiavo *et al*. 2009, Zahid *et al*. 2010) so as to recover degraded areas.

**Conclusion**: The variation in the LMA was due to the underlying difference in foliage anatomical structure and biomass density between acacias and eucalypts. This differing LMA entailed variation in PNUE and WUE within and among these two genera. Considering acacia and eucalypt as material for afforestation, they employ different strategies in utilizing N and water in their photosynthetic system. Under conditions of unlimited water and N, the eucalypts surpassed the acacias with higher $P_N$, as a result of higher PNUE, in the expense of photosynthetic WUE. However, acacias may benefit from their capability to acquire N from the air for their needs, which could improve the soil N status. The acacias also had higher WUE, which implies their moderate water usage in photosynthesis relative to eucalypts.

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Table 1. Leaf net photosynthetic rate ($P_N$), maximum rate of carboxylation ($V_{cmax}$), maximum rate of electron transport ($J_{max}$), stomatal conductance ($g_s$), transpiration rate ($E$), water use efficiency (WUE), and the photosynthetic capacity (N1 and N2) of the leaves of two acacias and four eucalypt seedlings. Data are mean values ± SD ($n = 3$); values followed by *the same letter* do not differ significantly; $P < 0.05$ (*ANOVA* and *Tukey* HSD test). Differences in the mean value of acacias and eucalypts were analyzed by the two-tailed *t*-test; ns – non significant differences, * – significant differences ($^{***}$– $P$<0.001; $^{**}$– 0.001<$P$< 0.01).

| | Individual species | | | | | | Average | | |
|---|---|---|---|---|---|---|---|---|---|
| | *A. auriculiformis* | *A. mangium* | *E. camaldulensis* | *E. globulus* | *E. grandis* | *E. urophylla* | Acacia | Eucalypt | *t*-test |
| $P_N$ [μmol m$^{-2}$ s$^{-1}$] | $15.3\pm3.3^{ab}$ | $13.0\pm3.6\ ^{a}$ | $19.37\pm2.1^{b}$ | $17.87\pm1.7^{ab}$ | $17.59\pm0.9^{ab}$ | $18.60\pm0.7^{ab}$ | 14.1±3.3 | 18.4±1.4 | $^{***}$ |
| $V_{cmax}$ [μmol m$^{-2}$ s$^{-1}$] | $82.5\pm16.7^{a}$ | $75.3\pm11.5^{a}$ | $89.2\pm8.1^{a}$ | $86.7\pm8.6^{a}$ | $83.8\pm4.6^{a}$ | $90.5\pm10.3^{a}$ | 78.9±13.4 | 87.5±7.1 | $^{\text{ns}}$ |
| $J_{max}$ [μmol m$^{-2}$ s$^{-1}$] | $133.7\pm14.3^{a}$ | $126.7\pm24.2^{a}$ | $150.2\pm19.6^{a}$ | $134.6\pm6.5^{a}$ | $130.4\pm16.0^{a}$ | $134.9\pm14.0^{a}$ | 130.2±18.2 | 137.53±14.2 | $^{\text{ns}}$ |
| $g_s$ [mol m$^{-2}$ s$^{-1}$] | $0.29\pm0.06^{ab}$ | $0.24\pm0.19^{a}$ | $0.50\pm0.09^{ab}$ | $0.56\pm0.14^{b}$ | $0.48\pm0.19^{ab}$ | $0.44\pm0.17^{ab}$ | 0. 26±0.13 | 0.49±0.14 | $^{***}$ |
| $E$ [mmol m$^{-2}$ s$^{-1}$] | $3.76\pm1.18^{ab}$ | $2.79\pm1.46^{a}$ | $5.20\pm1.39^{b}$ | $5.13\pm0.30^{b}$ | $5.30\pm1.45^{b}$ | $4.77\pm0.80^{ab}$ | 3.28±1.30 | 5.10±0.95 | $^{***}$ |
| WUE [mmol mol$^{-1}$] | $4.13\pm0.41^{ab}$ | $5.03\pm1.10^{b}$ | $3.85\pm0.78^{ab}$ | $3.51\pm0.52^{a}$ | $3.50\pm0.98^{a}$ | $3.96\pm0.55^{ab}$ | 4.59±0.89 | 3.71±0.66 | $^{**}$ |
| N1 [%] | $11.45\pm3.1^{a}$ | $17.07\pm4.36^{a}$ | $26.76\pm0.26^{a}$ | $75.53\pm4.61^{c}$ | $60.49\pm16.79^{bc}$ | $56.39\pm10.41^{b}$ | 14.26±4.57 | 54.04±19.54 | $^{***}$ |
| N2 [%] | $1.59\pm0.27^{a}$ | $2.50\pm0.82^{ab}$ | $3.89\pm0.44^{b}$ | $9.73\pm0.44^{d}$ | $7.89\pm0.98^{cd}$ | $7.45\pm2.48^{c}$ | 2.04±0.74 | 7.24±2.50 | $^{***}$ |

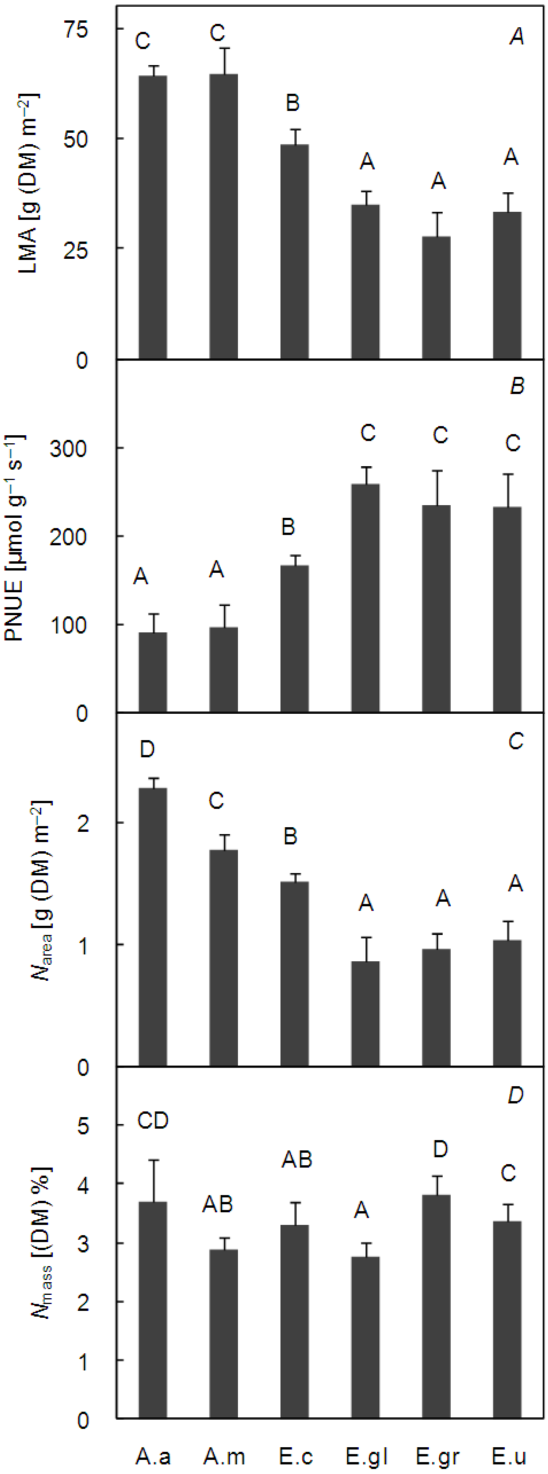

Figure 1. Leaf mass per area (LMA), photosynthetic nitrogen use efficency (PNUE), nitrogen content per leaf area ($N_{\mathrm{area}}$) and nitrogen content per leaf mass ($N_{\mathrm{mass}}$) of two acacias (A.a = *A. auriculiformis*; A.m = *A. mangium*) and four eucalypts (E.c = *E. camaldulensis*; E.gl = *E. globulus*; E.gr = *E. grandis*; E.u = *E. urophylla*). Data are average values ± SD (n = 3). Bars that have the same letter do not differ significantly according to the Tukey HSD test at $P < 0.05$.

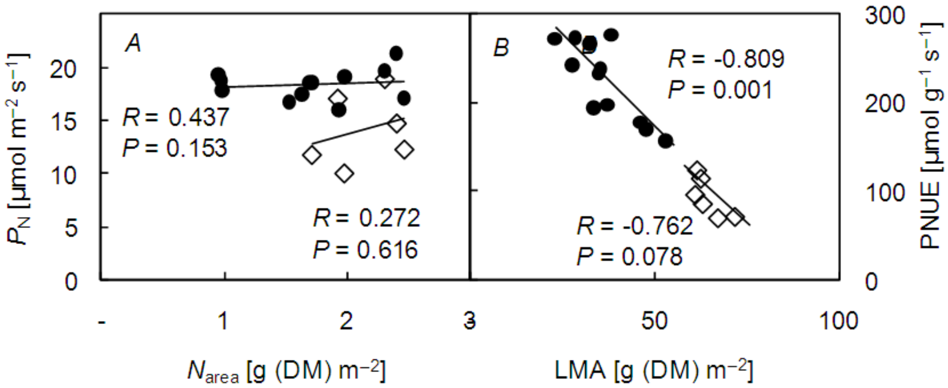

Figure 2. Relations between leaf nitrogen content per leaf area ($N_{area}$) and light saturated photosynthetic rate ($P_N$) (*A*), and the relation between leaf mass per area (LMA) and photosynthetic nitrogen use efficency (PNUE) (*B*).(□: acacias,●: eucalypts)

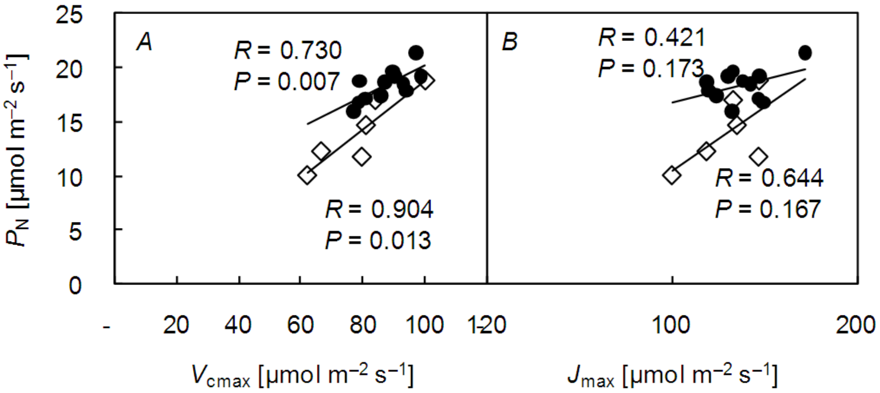

Figure 3. Variation of the maximum rate of carboxylation ($V_{cmax}$) (*A*) and maximum rate of electron transport ($J_{max}$) (*B*) with net photosynthetic rate ($P_N$) (□: acacias, ●: eucalypts)

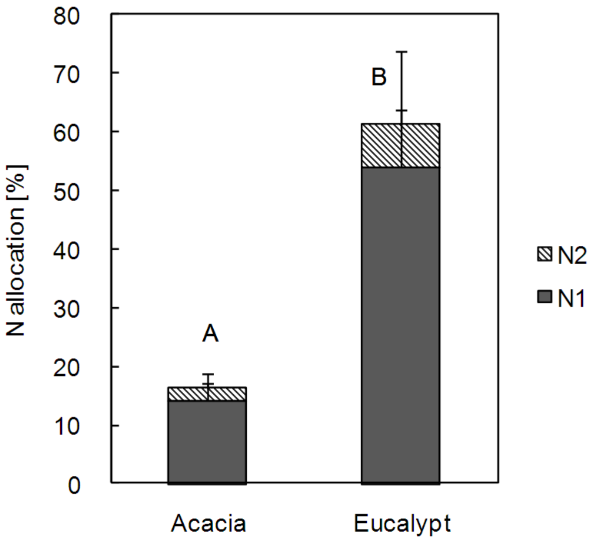

Figure 4. Nitrogen allocation in the leaves of tested species. N1, nitrogen allocated to Rubisco; N2, nitrogen allocated to electron carriers except for photosystems, coupling factor and Calvin cycle enzymes apart from Rubisco; Values with different letters attached differ significantly for the sum of N1 and N2, with $P< 0.05$.

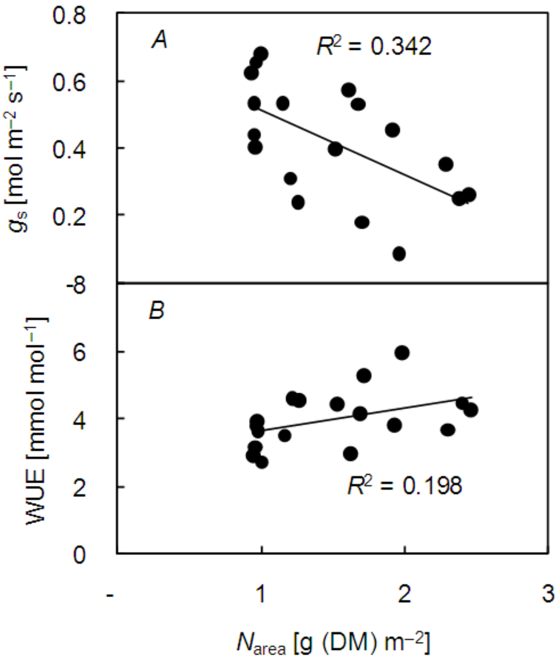

Figure 5. Variation of $N_{area}$ with stomatal conductance ($g_s$) (*A*) and photosynthetic water use efficiency (WUE) (*B*).

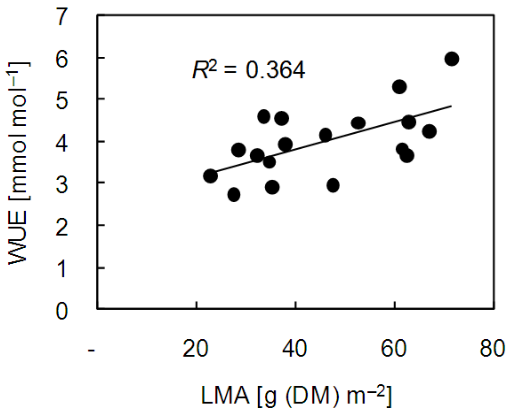

Figure 6. The relation between LMA and WUE.