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EFFECT OF DIFFERENT SHADE PERIODS ON *NEOBALANOCARPUS HEIMII* SEEDLINGS BIOMASS AND LEAF MORPHOLOGY

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Neobalanocarpus heimii, a well-known heavy hardwood timber species, is categorised as Vulnerable in Peninsular Malaysia due to the extreme demand for its timber and poor regeneration of the species. Moreover, limited research on shading and its effect on biomass allocation, biomass ratio and leaf morphology pose great challenge for *N. heimii* restoration. A study was carried out to elucidate the effects of four shade periods on biomass allocation, biomass ratio and leaf morphology of *N. heimii* seedlings, namely 0, 6, 9 and 12 months under shade. Mass of stem, leaf, root and total plant were significantly reduced when the seedlings were grown under full sunlight for 12 months. Under reduced shade period, leaf mass ratio decreased, while root mass ratio and root to shoot mass ratio increased. Morphological properties of the leaf were significantly affected by various shade periods, where leaf area and leaf area ratio values were reduced by removing shade at different periods. Results indicated that *N. heimii* seedlings could acclimatise to direct sunlight particularly after they had been placed under the shade for 6 to 9 months.

Keywords: Dipterocarpaceae, acclimation, biomass allocation, leaf area, specific leaf area, leaf area ratio

INTRODUCTION

The Malaysian tropical forest was estimated to be about 20.45 million ha in 2010 (62% of total land area) (FAO 2011), and mostly dominated by trees belonging to the Dipterocarpaceae family (Widiyatno et al. 2013). However, from 1990 to 2010, about 1.92 million ha or 8.6% of the total forest area in Malaysian have been deforested (Blaser et al. 2011). The remaining forests are facing serious threat due to unsustainable management and illegal logging (WWF-Malaysia 2015). As a result, some shade-tolerant Dipterocarpaceae species, especially those which produce heavy hardwood timbers, face serious risk of extinction. One such species is *Neobalanocarpus heimii*, which is listed as Vulnerable in Peninsular Malaysia as a consequence of overexploitation and poor regeneration (Chua 1998).

Intensity of light affects plant growth (Kozlowski & Pallardy 1997). Most dipterocarps require shading to obtain optimum growth in the early establishment stage (Widiyatno et al. 2013). However, after establishment stage, they require more light and they can acclimate to full sunlight (Shono et al. 2007). Thus, age and growth stage of plants play critical roles in modulating shade tolerance (Martin & Gower 1996, Kozlowski & Pallardy 1997, Sheil et al. 2006). For example, *Dryobalanops aromatica* and *Shorea parvifolia* prefer shading at seedling stage (Barker et al. 1997, Itoh et al. 1999). However, they need moderate to high light in sapling and pole stages to grow well (Adjers et al. 1995, Schwarzwaller et al. 1999). In Borneo, after the formation of the artificial glade (i.e. artificial gap made by cutting trees) in dipterocarp forest, mortality rate of the small seedlings (*Hopea nervosa*, *Parashorea malaanonan* and *Shorea johorensis*) was more than of the large seedlings (Brown & Whitmore 1992).

Adaptation of shade-tolerant species to full sunlight in open area relates to the ability of the plants to adjust their biomass between leaves, stems and roots, and to change the morphology of their leaves accordingly. The seedlings of *Nothofagus alessandrii* grown under low light level allocated more biomass to shoot (stem and leaves), and as a result, root to shoot ratio decreased. In contrast, the seedlings exposed to full sunlight allocated more biomass to root. Therefore, root to shoot biomass ratio increased. Increased root to shoot biomass ratio under full sunlight may give higher survival rate of plants (Santelices et al. 2015). Generally, leaf area, specific leaf area and leaf area ratio of shade-tolerant plants decrease in response to increasing light intensity (Turner 2004, Cuzzuol & Milanez 2012).

Researches on various shade periods and their effects on biomass allocation, biomass ratio and leaf morphology of shade-tolerant tree species have never been conducted. Therefore, the objectives of this study were to investigate (1) the adaptation of *N. heimii* seedlings to direct sunlight and (2) the response of plant biomass and leaf morphology to full sunlight after each shade period treatments.

MATERIALS AND METHODS

Location and species of research

The experiment was conducted at Universiti Putra Malaysia, Serdang, Selangor, Malaysia, from May 2014 till May 2015 under two conditions, namely, in the shade house (which provided approximately 70% shade) and in the open area. Seedlings of *N. heimii* of the same age (3 years and 4 months) and height (66 ± 2.4 cm) were bought from the Lentang nursery, Pahang, where the seedlings were grown under 50% shade. The seedlings were then transferred into bigger polybags, measuring 12 cm (diameter) × 20 cm (height), where each polybag was filled with potting media composed of topsoil and biochar of oil palm fruit in 2:1 ratio.

Light measurement

The plastic net used in the current research allowed 30% full sunlight (i.e. 70% shade). To confirm this amount of light under the shade house, light quantity, photosynthetic active radiation (PAR) (μmol m^{-2} s^{-1}) was measured in the open area and under the shade house at the begning of the study using a mini weather station containing light sensor. The manufacturer specification of the light penetration of the plastic net was verified by calculating relative light intensity (RIL) using the formula by Tong (2006):

$$\text{RIL} = \frac{\text{Light quantity under the shade house}}{\text{Light quantity in the open area}} \times 100$$

$$= \frac{332.17}{1006.50} \times 100$$

= 33% of full sunlight

Experimental design of the study

Randomised complete block design was used for this experiment, with four shade-period treatments and three replications. The four shade period treatments were 12 months under full sunlight (zero shade period) (T0), 6 months under 70%-shade and next six months under full sunlight (T6), 9 months under 70%-shade and next three months under full sunlight (T9) and 12 months under 70%-shade (without exposure to full sunlight) (T12). The plastic nets were fixed so that it covered all sides of the shading house to shield the seedlings from direct sunlight. The net was removed manually according to each shade period. A total of 36 *N. heimii* seedlings (of uniform size) were randomly assigned to the three blocks inside the four shade periods; each shade period treatment received 9 seedlings. The distance between blocks was 50 cm, between experimental units in a block was 30 cm and between seedlings in an experimental unit, 20 cm.

Measurements of the parameters

After 12 months, three seedlings of *N. heimii* were randomly selected and harvested under each shade-period treatment to obtain stem dry mass (g), leaf dry mass (g), root dry mass (g), total plant dry mass (g) and leaf area (cm^2). Seedling roots were washed carefully from the potting soil by washing the soil. The seedlings were separated into their components (stem, leaf, and root) and oven dried at 80 °C for 72 hours until constant dry weight (Zainudin 1990). The plant parts were then weighed using digital balance. Leaf area was calculated for fresh leaves using digital leaf area meter. Other parameters determined were stem mass ratio (stem dry mass/total plant dry mass), leaf mass ratio (leaf dry mass/total plant dry mass), root mass ratio (root dry mass/total plant dry mass), root to shoot mass ratio (root dry mass/(stem dry mass + leaf dry mass)), specific leaf area (leaf area/leaf dry mass) and leaf area ratio (leaf area/total plant dry mass) (Ogilvy 2004).

Data analysis

Analysis of variance (ANOVA) at $p \leq 0.05$ was used to consider significant differences in allocation of biomass (stem dry mass, leaf dry mass, root dry mass and total plant dry mass), biomass ratio (stem mass ratio, leaf mass ratio, root mass ratio, and root to shoot mass ratio) and leaf morphology (leaf area, specific leaf area and leaf area ratio) for the seedlings growing in the various shade periods. The ANOVA was performed using SAS version 9.3 (2002). Differences between means of each parmeter were examined by Tukey's studentised range test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Biomass allocation

Table 1 showed that stem mass, leaf mass, root mass and total plant mass of *N. heimii* seedlings were significantly influenced by different shade periods. There was no significant difference between the stem, leaf and total plant mass of the seedlings under T6, T9 and T12 (Figures 1a, b and d). These three shade periods produced significantly higher stem, leaf and total plant mass compared with T0. Root mass for T9 was significantly better than other shade periods. Root mass for T6 and T12 were significantly higher than T0 (Figure 1c).

This result indicates that *N. heimii* seedlings, which is considered as very shade-tolerant tree species, may suffer chronic photoinhibition to full direct sunlight (i.e. T0) (Kenzo et al. 2011) Seedlings under T0 accumulated lower total biomass than other shade treatments. However, after establishment stage, seedlings could acclimate to direct sunlight as observed under T6 and T9. The results of our study showed that *N. heimii* behaved similarly to *D. aromatica* and *S. parvifolia* whereby they require shading in the early establishment stage (Adjers et al. 1995, Barker et al. 1997, Itoh et al. 1999, Schwarzwaller et al. 1999). However, *D. aromatica* and *S. parvifolia* need moderate to high sunlight after their establishment stage in order to grow sufficiently.

No significant differences were observed in stem mass, leaf mass and total plant mass between T6, T9 and T12 (Figures 1a, b and c). Significant biomass reduction in the seedlings under T0 indicated that biomass allocation of *N. heimii* seedlings responded and acclimated well to direct sunlight at least after they had been grown under shade for 3 years and 4 months in Pahang before this study followed by 6 months in this study. There were no previous study about the effect of shade periods or canopy opening at different times on biomass allocation. Several researches have been done on the effect of different rates of shade or canopy opening on biomass allocation of various shade tolerance species (e.g. Mohamad 1986, Zainudin 1990, Cornelissen et al. 1994, Minotta & Pinzauti 1996, Romell et al. 2008, Perrin & Mitchell 2013). Biomass allocation of shade-tolerant tree species may reduce or increase when they were exposed to direct sunlight. Optimum shoot and root biomass of *Shorea materialis* was achieved between 33–55% sunlight whereas both shoot and root mass were reduced under full sunlight condition (Mohamad 1986). After 12 months of planting, *N. heimii* under different light intensities (25, 35, 55, 70 and 100%), stem recorded the highest mass under 55% relative light intensity (Zainudin 1990). However, leaf mass and root mass were not significantly influenced by light treatments. Cornelissen et al. (1994) conducted a study by planting three shade-tolerant species under different light regimes (100, 55, 33 and 18% of full sunlight) and the results showed that the Highest plant biomass values of *Sloanea leptocarpa* and *Elaeocarpus japonicus* were observed at 33 and 18% relative light intensity respectively, whereas for *Castanopsis fargesii*, the greatest biomass was at 55 and 100% full sunlight. Stem mass, leaf mass, root mass, total plant mass and root to shoot ratio of *Fagus sylvatica* seedlings increased with increasing light intensity (Minotta & Pinzauti 1996). Plant biomass of four dipterocarp species (*Dipterocarpus applanatus*, *D. caudiferus*, *Shorea argentifolia* and *Shorea pauciflora*) increased with reduction in canopy and subcanopy densities (Romell et al. 2008). Increased sunlight influence stimulation of total dry weight and root to shoot ratio of *Taxus baccata* saplings (Perrin & Mitchell 2013).

The varied responses of species to the light treatments may be due to variation of age or plasticity between the species to direct sunlight. Shade-tolerant species has less ability to adapt to full sunlight compared with intermediate shade and shade-intolerant species. Even though *N. heimii* is considered as a very shade-tolerant tree

Table 1 ANOVA of stem mass, leaf mass, root mass and total plant mass of *Neobalanocarpus heimii* seedling under different shade periods

Source of variation	DF	p value			
		Stem mass	Leaf mass	Root mass	Total plant mass
Shade period	3	< 0.0001	0.001	0.001	< 0.0001

Significant occurs when p value is ≤ 0.05, DF = degree of freedom

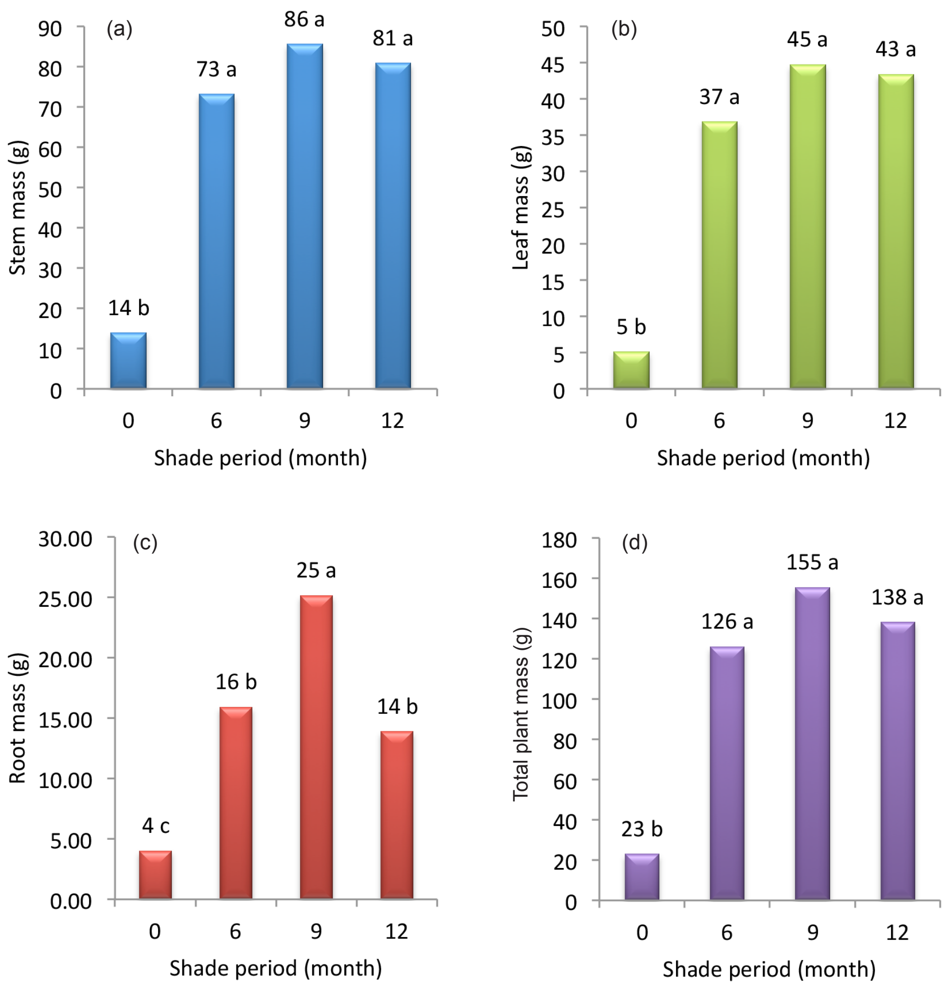

Figure 1 The means of (a) stem mass, (b) leaf mass, (c) root mass and (d) total plant mass of *Neobalanocarpus heimii* seedlings under different shade periods; means with the same letter are not significantly different by Tukey's studentised range test at p ≤ 0.05

species, it can grow well under full sunlight, especially after it has been grown under the shade for 9 months.

Biomass ratio

ANOVA of plant biomass ratio showed that stem mass ratio of the species was not significantly affected by different shade periods (Table 2). Shade period treatments had high significant effect on the leaf, root and root to shoot mass ratios (Table 2, Figures 2a–c) although leaf mass ratio showed no differences between treatments T6, T9 and T12 (Figure 2a). Root mass ratio was significantly higher in T0 than T6 and T12 (Figure 2b). Root to shoot mass ratio under T12 was significantly lower than the rest of the shade periods. The highest value of root to shoot mass ratio was achieved in seedlings exposed to full sunlight (T0) (Figure 2c).

Table 2 ANOVA of stem mass ratio, leaf mass ratio, root mass ratio and root to shoot mass ratio of *Neobalanocarpus heimii* seedling under different shade periods

Source of variation	DF	p value			
		Stem mass ratio	Leaf mass ratio	Root mass ratio	Root to shoot mass ratio
Shade period	3	0.073	0.006	0.005	0.009

Significant occurs when p value is ≤ 0.05, DF = degree of freedom

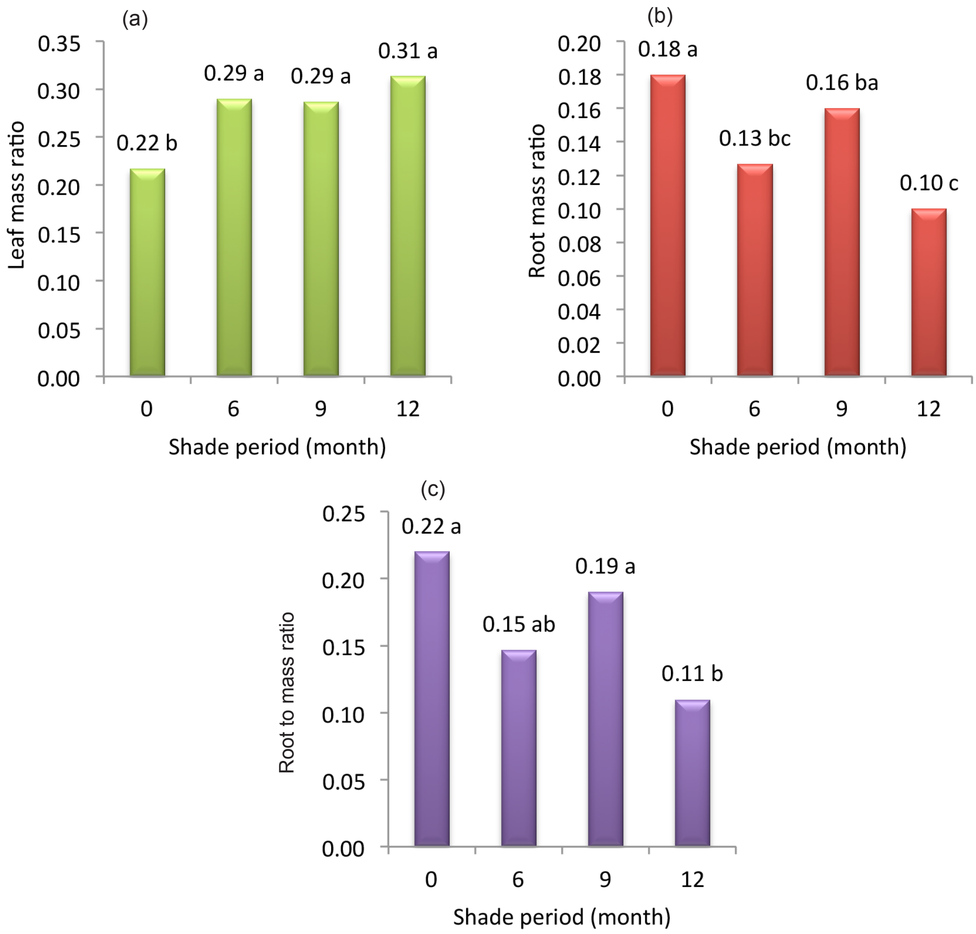

Figure 2 Mean of (a) leaf mass ratio, (b) root mass ratio and (c) root to shoot mass ratio of *Neobalanocarpus heimii* seedlings under different shade periods; means with the same letter are not significantly different by Tukey's studentised range test at p ≤ 0.05

These results indicated that *N. heimii* seedlings displayed typical responses to direct sunlight from the first shade period treatment (T0) such as reduction in leaf mass ratio and increase in root mass ratio and root to shoot mass ratio. Furthermore, modification of biomass ratio towards full sunlight suggests that this shade-tolerant tree species can adapt to high light intensity. Shade-tolerant and shade-intolerant species grown under low light conditions allocate relatively less biomass to roots compared with shoots (Perrin & Mitchell 2013). Plants are able to acclimate to their light environment by modification of biomass accumulated in roots, stems and leaves, So they show an increase in irradiance resulting in decreased biomass ratio assigned to leaves and increased biomass ratio invested to roots (Poorter & Nagel 2000, Evans & Poorter 2001). Seedlings of *Nothofagus alessandrii* grown under low light level allocated more biomass to shoot (stem and leaves), consequently, root to shoot ratio declined (Santelices et al. 2015). Seedlings exposed to full sunlight assigned more biomass to root and as a result, root to shoot biomass ratio increased. Increase in root to shoot biomass ratio under full sunlight conditions may give more chance of survival to the plant because at this situation, the plant has more ability to adsorb water from the soil and, at the same time, has more ability to reduce transpiration from the foliage. This way, plants are able to protect itself from water deficiency.

Leaf morphology

The analysis of variance for the effect of different shade periods on the leaf morphology of *N. heimii* demonstrated that leaf area and leaf area ratio were significantly impacted by different shade periods (Table 3); however, specific leaf area was not. Early net removal or reducing shade period caused significant reduction in leaf area of the seedlings, where the reduction in leaf area were found in T0 and T6 when compared to T12. The greatest leaf area was recorded for T12, while the lowest, T0 seedlings (Figure 3a). Leaf area ratio was significantly different only between T0 and T12 seedlings (Figure 3b).

Results of the leaf morphology showed that *N. heimii* seedlings responded accordingly to the increasing light from the first shade period treatment (T0). For example, reduction in leaf area and leaf area ratio were observed after the net was removed (T0, T6, T9) compared with T12 seedlings. Adjustment of leaf form and structures in response to high light suggested that the species can acclimate to direct sunlight. The findings of leaf morphological characteristics in this study were similar to those reported by Burton and Mueller-Dombois (1984) who found that both leaf area ratio and specific leaf area of *Metrosideros polymorpha* tree seedlings were negatively correlated with canopy opening. Leaf area of *S. materialis* decreased with the increasing light intensity (Mohamad 1986). Leaf area of *N. heimii* was greater under 55% relative light intensity than under full sunlight conditions (Zainudin 1990). Cornelissen et al. (1994) showed that leaf area and leaf area ratio of three shade-tolerant tree species (*Sloanea leptocarpa Elaeocarpus japonicus* and *C. fargesii*) were reduced by increasing light intensity. Minotta and Pinzauti (1996) found that *Fagus sylvatica* responded to high light intensity by decreasing mean leaf area, leaf area ratio and specific leaf area. Ishida et al. (1999) demonstrated that specific leaf area of *N. heimii* seedlings was greater in the gap than in the open site. Evans and Poorter (2001) showed that plants can adapt to various ranges of light through adjustment of their leaf features, e.g. reduction in irradiance led to decrease in leaf thickness and increase in specific leaf area and vice versa. Increasing light percentage was negatively correlated with specific leaf area, leaf length and leaf width of *Taxus baccata* saplings (Perrin & Mitchell 2013). Santelices et al. (2015) showed that the specific leaf area of *N. alessandrii* was recorded higher value under 80% shade compared with 0 and 18% shade.

CONCLUSIONS

The present study showed that the shade periods affected biomass allocation, biomass ratio and leaf morphological traits of *N. heimii* seedlings. Seedlings of *N. heimii* grew well under full sunlight as well as under shade after they had been grown under the shade for 6 to 9 months. In addition, the seedlings were grown under shade for about 4 years prior to the study. Variables of biomass fractions and leaf morphology indicated that this shade-tolerant species can acclimate to strong sunlight as well as under the appropriate shade. The seedlings displayed typical responses to increasing light from the first shade period treatment such as reduction in leaf mass ratio, leaf area, specific leaf area and leaf area ratio, and increase root mass ratio and root to shoot mass ratio. Although a shade-tolerant species, this study showed that *N. heimii* can be planted in the open after shading period of almost 4 years. In fact growth was still good after additional shade of 6 to 9 months.

Table 3 ANOVA of leaf area, specific leaf area and leaf area ratio of *Neobalanocarpus heimii* seedling under different shade periods

Source of variation	DF	p value		
		Leaf area	Specific leaf area	Leaf area ratio
Shade period	3	< 0.0001	0.624	0.032

Significant occurs when p value is ≤ 0.05, DF = degree of freedom

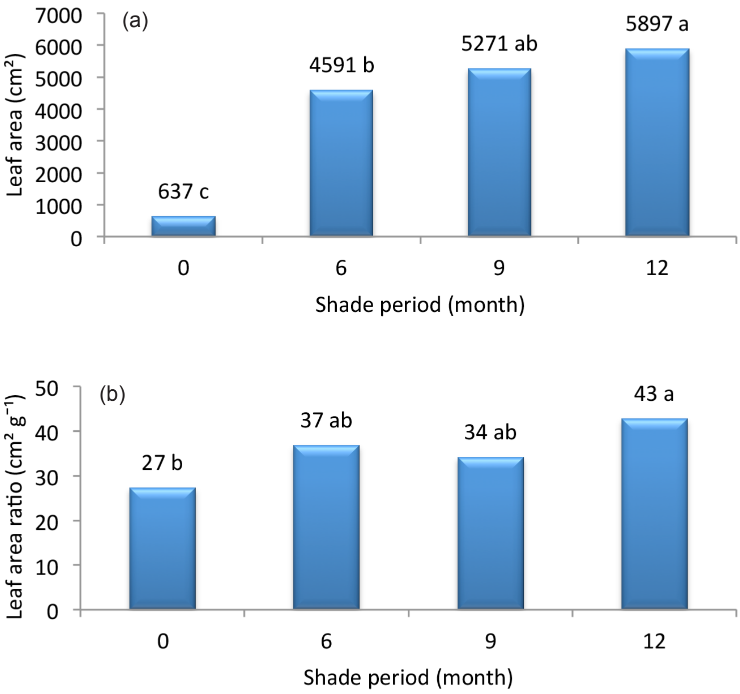

Figure 3 The mean of (a) leaf area and (b) leaf area ratio of *Neobalanocarpus heimii* seedling under different shade periods; means with the same letter are not significantly different by Tukey's studentised range test at $p \leq 0.05$

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