

Effects of Fertilizer on Growth and Biomass Allocation of Three Evergreen Tree Species from Seasonally Dry Tropical Forests

Dia Panitnard Shannon¹, Pimonrat Tiansawat^{1,*},
Sirinapa Dasoon¹, Stephen Elliott¹ and Wittaya Pheera²

¹Forest Restoration Research Unit and Environmental Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

(*Corresponding author's e-mail: pimonrat.t@cmu.ac.th)

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Abstract

Tree planting is widely accepted as a strategy to mitigate climate change, with a strong focus on use of native tree species. Various kinds of fertilizer have been recommended to produce optimal quality planting stocks for forest restoration. This study tested the hypothesis that additional fertilizer could improve seedling growth and alter biomass allocation in seedlings of evergreen tree species. Three species were studied: *Aphanamixis polystachya* (a slow-growing species), *Eriobotrya bengalensis* (a pioneer species) and *Podocarpus neriifolius* (a slow-growing species). These species are used to restore seasonally dry tropical forests in northern Thailand. We applied 4 different treatments of fertilizer addition (0, 150, 300 and 600 mg per seedling) and measured relative growth rate (RGR) and biomass allocation. The 3 species responded differently to the fertilizer addition in both growth and biomass allocation. Only the pioneer species, *E. bengalensis*, showed a significant response to fertilizer addition at the highest dose. The 600 mg treatment increased *E. bengalensis*' RGR by 60 % but decreased root mass fraction by 4 %, compared with the control. Pioneer species respond to fertilizer addition with accelerated growth rate rather than by increasing nutrient stores. On the other hand, slow-growing species have a low annual requirement; therefore, they are not highly responsive to nutrient addition. Further investigation into the effects of fertilizer on growth and biomass allocation in pioneer species is needed to enable the propagation of cost-effective and high-quality planting stocks for forest restoration.

Keywords: Biomass distribution, Additional nutrients, Tropical forests, Forest restoration, Climate change mitigation, Northern Thailand

Introduction

With the United Nations (UN) having declared 2021 - 2030 the Decade on Ecosystem Restoration [1], it is crucial to extend our knowledge on how to produce high quality seedlings of diverse species. Seedling development is one of the most important stages of plant life history [2]. Their morpho-functional attributes are related to their ability to withstand environmental stressors, which is critical to the initial success of forest restoration projects [3]. Nursery practices, such as sowing, pruning, media, and fertilization, are usually standardized in order to grow high-quality planting stocks [4].

The addition of fertilizer has been found to increase drought tolerance via increasing root growth potential [5], and thus the ability to capture soil water [6]. In the past few decades, fertilizer application in forest nurseries has drawn growing attention throughout the world, as a result of the increased need for timber and CO₂ offsets [7]. However, adding too much fertilizer during nursery production can reduce growth because of salt accumulation [8], and can cause seedlings to grow too large for their containers and subsequently perform poorly after transplanting [9]. Therefore, proper addition of fertilizer is critical during the production of planting stock [10], due to its effects on the quality and traits of seedlings both in nursery [3] and field conditions [11], especially in degraded areas with low soil fertility [11].

Seedling response to different nutrient levels can be highly species-specific [12], relatively little is known to what extent soil nutrients contribute to seedling development and growth at early stages. The main aim of this study was to determine to what extent nutrient levels affect early growth and biomass allocation in *Aphanamixis polystachya* (Wall.) R. Parker (**Figure 1**), *Eriobotrya bengalensis* (Roxb.) Hook. f. (**Figure 2**) and *Podocarpus neriifolius* D. Don (**Figure 3**) in nursery conditions.

Materials and methods

Studied species and seedling propagation

Among the 3 studied species, *E. bengalensis* (Rosaceae) has been suggested to be a pioneer species while both *A. polystachya* (Meliaceae) and *P. neriifolius* (Podocaraceae) are reported as climax species due to their habitats and low relative growth rates [13]. In northern Thailand, *E. bengalensis* seedlings are usually found in canopy gaps and are unlikely to regenerate in mature forests [32]. However, *A. polystachya* and *P. neriifolius* are usually found in less disturbed forests [24-26] and tend to have slow growth potential [13].

Seeds of all species were collected (July 2014 - June 2015) from remnant forests in Doi Suthep-Pui National Park, Chiang Mai, Thailand. Within 7 - 14 days, seeds were extracted and processed at Ban Mae Sa Mai nursery (18° 52.574' N 98° 50.880' E, at 950 m elevation) following the propagation protocols of the Forest Restoration Research Unit [14]. During this study, the average temperature was between 19.1 to 23.8 degree Celsius, and the average rainfall was 1,054 mm per year [15].

Germination occurred in modular trays, filled with forest soil, in a germination room. After seedlings had grown 3 - 4 true leaves, they were transplanted into polyethylene bags (6×23 cm²), which were placed in an open area, on plastic sheets, to prevent their roots from penetrating into the soil. The plants were watered on days when no rain fell.

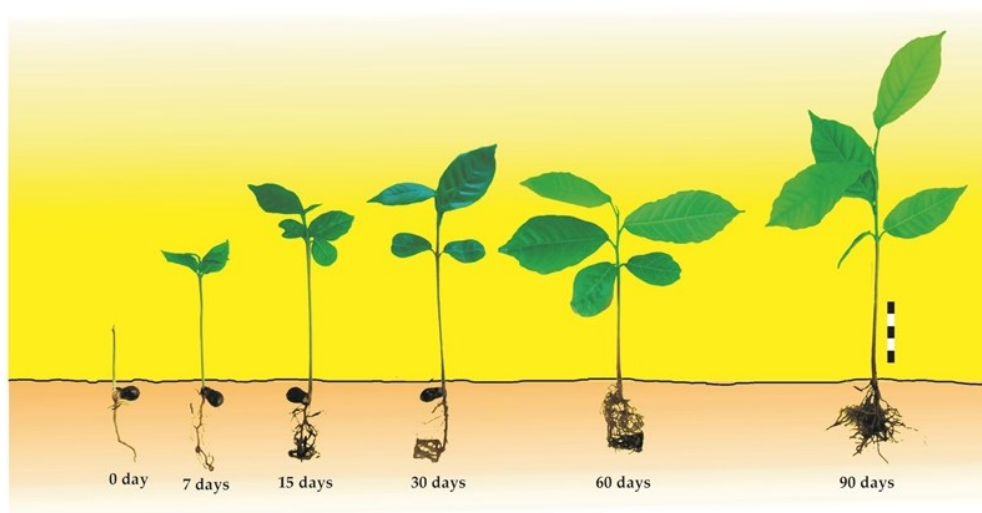


Figure 1 *Aphanamixis polystachya*.

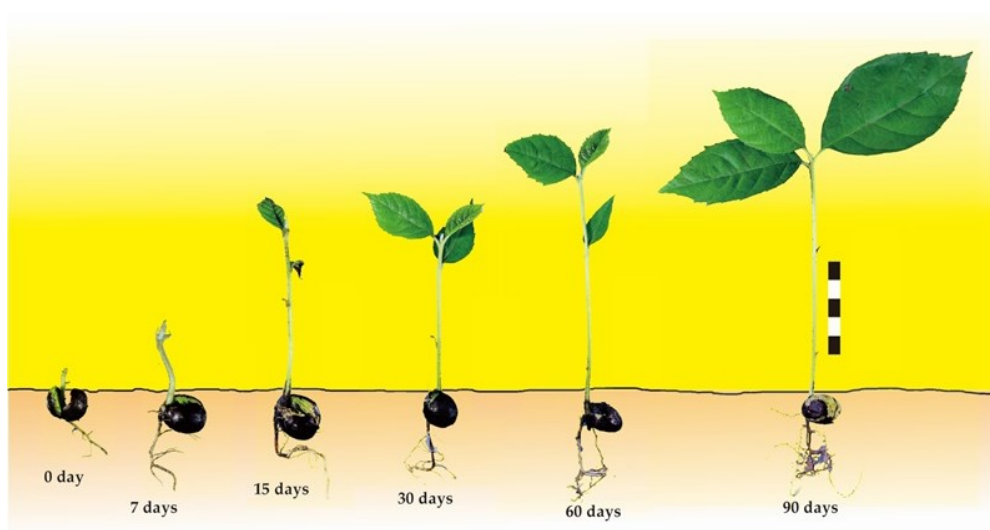


Figure 2 *Eriobotrya bengalensis*.

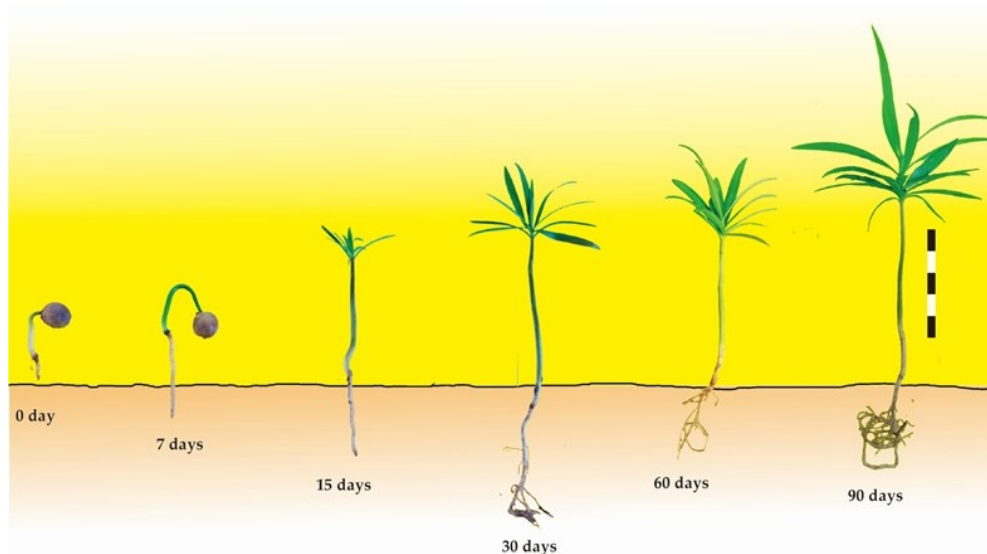


Figure 3 *Podocarpus neriifolius*.

Potting media and fertilizer

Potting media consisted of forest soil, peanut shells, and coconut husk (in the ratio 2:1:1 respectively) [14]. Before application of fertilizer treatments, concentrations of major nutrients (N, P, and K) in the potting media were compared to those in forest soil by the Kjeldahl method [16], molybdenum blue method [17] and flame photometry [18], respectively, in the Soil Science Laboratory, Faculty of Agriculture, Chiang Mai University. Three soil samples were randomly collected from forest soil and the potting media. Mean nutrient values in forest soil and potting media are shown in **Table 1**.

Table 1 Major nutrients (N, P, and K) in forest soil and potting media before application of fertilizer treatments.

| Nutrient | Forest soil | | | Potting media | | |
|------------------------|-------------|-------|-------|---------------|--------|--------|
| | n | Mean | SD | n | Mean | SD |
| Total N (g/100g) | 3 | 0.12 | 0.03 | 3 | 0.15 | 0.02 |
| Available P (mg/kg) | 3 | 1.46 | 0.43 | 3 | 8.13 | 3.35 |
| Exchangeable K (mg/kg) | 3 | 90.63 | 18.98 | 3 | 850.30 | 268.54 |

Experimental design

Forty-five days after potting, 3 fertilizer treatments were applied: 150, 300 and 600 mg of Osmocote fertilizer per pot. Slow-release fertilizer (Osmocote brand) was selected because it was recommended for producing forest native seedlings in northern Thailand [14]. One granule of Osmocote fertilizer contains 13 % total nitrogen, 13 % P₂O₅ and 13 % K₂O. The fertilizer treatments were applied twice: 45 and 90 days after potting. Control pots, with no fertilizer applied were also maintained. The experiment was a randomized complete block design with 3 species × 4 treatments (including control) × 3 replicates. Each replicate consisted of 16 potted seedlings, resulting in a total of 192 seedlings of each species being included in the experiment.

Measurement of seedling growth rate (RGR)

At the beginning of the experiment, 6 additional seedlings of each species were randomly harvested and dried to constant weight. The dry mass of each seedling was determined. For each species, the mean of the natural logarithm of the dry mass was used as the initial biomass for the calculation of RGR. RGR was calculated using a formula Eq. (1) modified from Hoffmann and Poorter [19].

$$RGR = \frac{\ln M_{final} - \ln M_{initial}}{(t_{final} - t_{initial})} \quad (1)$$

Here, $\ln M_{final}$ is the natural logarithm of the dry mass of a seedling at the end of the experiment. $\ln M_{initial}$ is the mean of the natural logarithm of the dry mass of the seedlings at the beginning. $(t_{final} - t_{initial})$ is the number of days the experiment was run (135 days).

Measurement of seedling biomass allocation

Six months after the start of the experiment, 2 seedlings per treatment (per replicate) of each species were harvested, and each individual seedling was divided into roots, stems (including non-green petioles) and leaves (including green petioles). The seedling parts were dried to a constant weight, and then root mass (RM), stem mass (SM) and leaf mass (LM) were measured. Total biomass was calculated as the sum RM+SM+LM. Root mass fraction (RMF), stem mass fraction (SMF) and leaf mass fraction (LMF) were calculated as the ratio of roots, stems, and leaves, respectively, to total seedling biomass.

Data analyses

Effects of fertilizer addition on RGR

All analyses were performed using the R Programming language 3.5.2 [20]. To determine the effect of fertilizer on the RGR, we used the *nlme* package [21] to perform a linear mixed effect analysis (*lme* function). The fixed effect was the fertilizer addition treatment and species, and blocking was a random effect. We started with a full model, in which species and treatment and the interaction between species and treatment were included in the model. A model in a reduced form without the interaction term was also fitted and compared with the full model. The model was fitted using Maximum Likelihood Estimation (MLE). Visual inspection of residual plots and quantile-quantile plots was used to diagnose the modeling assumption of homoscedasticity and normality of residuals, respectively. The Akaike's Information Criteria (AIC) of each model was calculated and the model with lowest AIC was selected. The likelihood-ratio test was also used to compare whether the 2 models were significantly different.

For each species, the differences of the RGR among the fertilizer addition treatments were further investigated using mixed effect models. The fixed effect was the fertilizer addition treatment, and blocking was a random effect. We also made pairwise comparisons of the means of the RGR under fertilizer treatments using the *lsm* function in package *lsmeans* [22] together with the *glht* function of package *multcomp* [23].

Effects of fertilizer addition on biomass allocation to leaf, stem, and root

The biomass allocation was analyzed separately by parts (LMF, SMF, and RMF). We asked 2 main questions: 1) Did biomass allocation differ among species and 2) Did fertilizer addition treatment affect biomass allocation.

The differences of biomass allocation among species were tested using a mixed-effects model. The LMF, SMF and RMF in the control treatment of each species were used as a response variable. Species was a fixed-effect factor and blocking was a random effect factor.

The effect of fertilizer addition was examined with a mixed-effects model analysis of covariance. The LMF, SMF and RMF were response variables. The treatment was a fixed effect factor and blocking was a random effect factor. The initial root collar diameter of the seedlings was used as a continuous covariate in the model. We used the same model checking and selection approach as used in the RGR analysis described above. For the best-fit models that exhibited a significant treatment effect, we made pairwise comparisons of the means of biomass allocation using the *lsm* function in package *lsmeans* [22] together with the *glht* function of package *multcomp* [23].

Results and discussion

Effects of fertilizer addition on seedling growth rate (RGR)

Among species, the RGR of seedlings in the control treatment varied from 0.003 to 0.009 mg g⁻¹ d⁻¹. There was no significant interaction between species and treatment ($F_{(6, 58)} = 1.8, P = 0.11$). The final adequate model included significant species effect ($F_{(2, 64)} = 79.9, P < 0.001$) and treatment effect ($F_{(3, 64)} = 6.2, P = 0.001$).

E. bengalensis and *P. neriifolius* did not differ significantly in RGR, while *A. polystachya* had significantly lower RGR (**Figure 4**). For *A. polystachya* and *P. neriifolius*, the fertilizer treatments did not have significant effects on the RGR (**Figure 4** and **Tables 2** and **3**). For *E. bengalensis*, the highest amount of fertilizer (600 mg) increased the RGR, but the RGR of seedlings given a smaller amount of fertilizer (150, 300 mg) was not significantly different than those of the control treatment (**Table 2**).

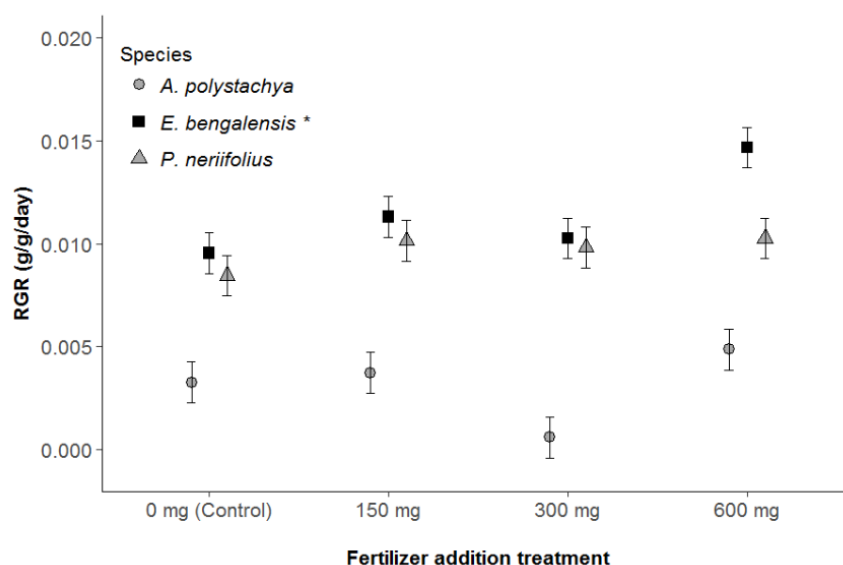


Figure 4 Comparisons of relative growth rate of 3 native tree species among different fertilizer treatments. The asterisk (*) after *E. bengalensis* indicates the significant effect of fertilizer treatment on the RGR.

Despite having different RGRs, both *A. polystachya* and *P. neriifolius* seedlings showed no response to the addition of fertilizer. Both species have been suggested as late successional or climax species [24-26], and their responses to changed nutrient availability is likely a function of the species' life strategy [27]. Slow-growing species have a low annual requirement; therefore, they are not highly responsive to nutrient addition [28]. One possibility is that the availability of nutrient resources in the surrounding environment may be more relevant to the plant response than the added quantity [29,30]. Species with slow growth rate are not highly responsive to nutrient addition, as when grown in fertile soils, they accumulate nutrient reserves but do not show a large increase in growth rate [31].

Table 2 Multiple comparisons of the mean of RGR among treatments of each species.

| Species | Treatment comparison | Estimate of differences | Standard error | t value | p-value |
|-----------------------|--------------------------|-------------------------|----------------|--------------|-------------------|
| <i>A. polystachya</i> | Control vs 150 mg | -0.0005 | 0.0012 | -0.38 | 0.98 |
| | Control vs 300 mg | 0.0027 | 0.0012 | 2.24 | 0.15 |
| | Control vs 600 mg | 0.0016 | 0.0012 | -1.32 | 0.56 |
| | 150 mg vs 300 mg | 0.0031 | 0.0012 | 2.62 | 0.07 |
| | 150 mg vs 600 mg | 0.0011 | 0.0012 | -0.93 | 0.79 |
| | 300 mg vs 600 mg | -0.0043 | 0.0012 | -3.56 | 0.01 |
| <i>E. bengalensis</i> | Control vs 150 mg | -0.0018 | 0.0011 | -1.62 | 0.39 |
| | Control vs 300 mg | -0.0007 | 0.0011 | -0.65 | 0.91 |
| | Control vs 600 mg | -0.0051 | 0.0011 | -4.72 | < 0.001 |
| | 150 mg vs 300 mg | 0.001 | 0.0011 | 0.97 | 0.77 |
| | 150 mg vs 600 mg | -0.0033 | 0.0011 | -3.1 | 0.03 |
| | 300 mg vs 600 mg | -0.0044 | 0.0011 | -4.06 | < 0.004 |
| <i>P. neriifolius</i> | Control vs 150 mg | -0.0017 | 0.0013 | -1.32 | 0.56 |
| | Control vs 300 mg | -0.0014 | 0.0013 | -1.07 | 0.71 |
| | Control vs 600 mg | -0.0018 | 0.0013 | -1.42 | 0.5 |
| | 150 mg vs 300 mg | 0.0003 | 0.0013 | -0.25 | 0.99 |
| | 150 mg vs 600 mg | -0.0001 | 0.0013 | -0.1 | 1 |
| | 300 mg vs 600 mg | -0.0004 | 0.0013 | -0.35 | 0.98 |

Unlike the other 2 species, for the pioneer species *E. bengalensis* [32], the RGR increased with the highest level of fertilizer addition. Positive-growth responses to nutrient addition have also been observed in *Bauhinia* species in pots [33] and in wild plants *in situ* [34]. Plants with rapid growth strategy or

pioneer species grow as rapidly as possible given their photosynthetic carbon and nutrient supply, so they respond to nutrient addition with greatly accelerated growth rate rather than by increasing nutrient stores. Thus, they maintain a competitive advantage under favorable conditions [28].

Biomass allocation and the effects of fertilizer addition

To compare the biomass allocation among the 3 species, the mean of LMF, SMF, and RMF of the control treatment were analyzed. The 3 species differed in their biomass allocation. *A. polystachya* allocated 36.4 ± 3.0 % of biomass to leaves, which was less than that of *E. bengalensis* (53.4 ± 1.7 %) and *P. neriifolius* (53 ± 1.6 %) ($F_{(2, 13)} = 32.4$, $P < 0.001$) (Figure 5 Control treatment bars). For stems, the SMF was 22.2 ± 1.3 , 24.7 ± 1.0 and 28.0 ± 2.2 % for *P. neriifolius*, *A. polystachya* and *E. bengalensis*, respectively. There were significant differences in the biomass allocation to stems among species ($F_{(2, 13)} = 5.3$, $P = 0.02$) (Figure 5 Control treatment bars). The SMF of *E. bengalensis* was significantly larger than that of *P. neriifolius*, but was not different than that of *A. polystachya*. For the allocation to root, *A. polystachya* allocated 38.8 ± 4.0 % to root, which was more than the other 2 species that allocated 24.2 ± 2.3 % (*P. neriifolius*) and 18.6 ± 0.5 % (*E. bengalensis*) of biomass to root ($F_{(2, 13)} = 35.0$, $P < 0.001$) (Figure 5 Control treatment bars).

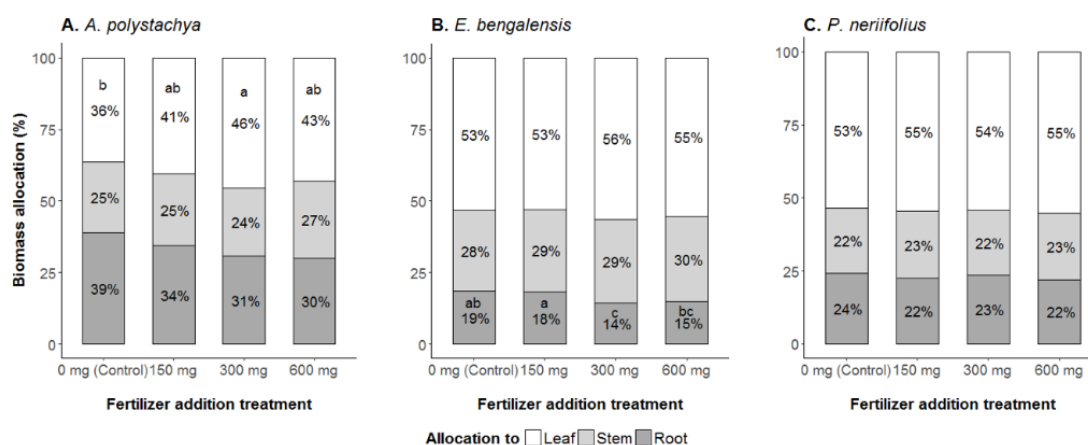


Figure 5 Biomass allocation of 3 native tree species in different fertilizer treatments; A) *A. polystachya* B) *E. bengalensis* and C) *P. neriifolius*.

The effect of fertilizer addition to biomass allocation was analyzed separately by species and by parts (LMF, SMF, and RMF). For *A. polystachya*, fertilizer addition did not affect the allocation to leaf ($F_{(3, 18)} = 3.1$, $P = 0.052$). In *A. polystachya*, the LMF in the 300-mg-fertilizing treatment was higher than the control, but in comparison to other treatments the difference was not significant. In *A. polystachya*, fertilizer addition did not affect the SMF ($F_{(3, 18)} = 0.7$, $P = 0.57$) or the RMF ($F_{(3, 18)} = 2.3$, $P = 0.11$) (Figure 5A).

For *E. bengalensis*, fertilizer addition did not affect the allocation to leaf ($F_{(3, 18)} = 0.5$, $P = 0.70$) and stem ($F_{(3, 18)} = 0.2$, $P = 0.90$). On the other hand, the RMF of *E. bengalensis* was affected by initial size and fertilizer treatment. Larger seedlings were more likely to have higher RMF ($F_{(1, 17)} = 6.4$, $P = 0.02$). The RMF of the 600 mg fertilizer treatment was significantly lower than the control and 150 mg fertilizer treatments ($F_{(3, 17)} = 3.5$, $P = 0.04$) (Figure 5B). For the third species, *P. neriifolius*, fertilizer addition did not significantly affect LMF ($F_{(1, 18)} = 0.2$, $P = 0.86$), SMF ($F_{(1, 18)} = 0.2$, $P = 0.88$), or RMF ($F_{(1, 18)} = 0.6$, $P = 0.6$).

Biomass allocation varies depending on how seedlings accumulate carbohydrates to aboveground tissues vs. roots [35,36]. *A. polystachya* allocated more biomass to root compared to the other 2 species. This could be an adaptation to generally low nutrient availability in its natural habitat as a late-successional species. Enhanced allocation to roots suggests that carbon and other elements are mainly stored in belowground organs to improve seedlings survival under very low light intensity at the forest floor [37].

Under similar environmental conditions in the nursery, *E. bengalensis* and *P. neriifolius* allocated more than 50 % biomass to their leaves. Mensah *et al.* [38] reported in adult plants that per unit of wood

mass, more biomass is allocated to the foliage in the species with the larger leaf area. This might not be true during the seedling stage because both *E. bengalensis* and *P. neriifolius* have smaller leaf area compared to *A. polystachya* (126.65, 95.66 and 146.99 cm², respectively) [39]. At different development stage [40,41], tree species with different competitive abilities [42,43] can respond dissimilarly in their biomass allocation. However, low-nutrient species tend to have roots that live a long time compared to their leaves, causing biomass to accumulate preferentially belowground over time [44].

In both *A. polystachya* and *P. neriifolius*, the fertilizer did not affect biomass allocation during the young seedling stage. In this study, we focused only on nursery conditions, which provided limited space for the seedlings to grow their roots. We used potting media (forest soil, coconut husk and peanut shells) recommended for propagating native seedlings for restoration [14]. Even in the control treatment the potting media provided a certain amount of essential nutrients. Species with slow growth rate are not highly responsive to nutrient addition, as when grown in fertile soils they accumulate nutrient reserves but do not show much difference in biomass allocation [31]. This finding is in contrast with the result of meta-analyses that showed a compensatory change in carbon allocation to aboveground tissues when soil nutrient availability increased [40].

Biomass allocation is driven not only by environmental factors but also by a combination of many internal factors such as tree diameter, species identity, leaf area and wood density [38]. In this study, the RMF of *E. bengalensis* was affected by initial size and fertilizer addition. The RMF of *E. bengalensis* decreased with increasing nutrients (600 mg fertilizer treatment). This could be the result of decreasing root tissue density [45-47]. It is still a matter of debate to what extent plants benefit from and are affected by additional nutrient uptake and different types of nutrients (nitrogen, phosphorus, potassium) [47]. Therefore, further research is needed if we want to better understand how and why the allocation pattern varies based on different internal and external factors.

Conclusions

Proper addition of fertilizer to seedlings in the nursery is important for preparing high quality planting stocks for successful restoration programs. In this study, climax and pioneer species differed in their response to fertilizer addition. Additional soil nutrients did not boost the growth of either climax species in this study. However, the pioneer species was sensitive to additional soil nutrients. The highest dose of added fertilizer increased RGR but decreased RMF. Less biomass allocation to root could contribute to a lower chance of survival for pioneer species in degraded areas after transplanting. Fertilizer experiments in nurseries together with field monitoring of the same seedlings are needed to determine if increased nutrient availability in nurseries can promote seedling establishment and growth in degraded lands. This study emphasizes the differential responses of seedlings' growth and biomass allocation among species and its application in nursery management.

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