

Effects of nutrient addition to adinandra belukar soil on seedling performance of early and late successional species

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Abstract. Currently, much of Singapore's land area is covered by secondary forests which have developed since the abandonment of degraded agricultural lands beginning from the 1950s. One common secondary forest type is adinandra belukar, which is characterised by soil with low pH and low concentrations of nitrates and phosphates. Previous studies have noted the lack of late successional rainforest seedlings in Singapore's secondary forest fragments, citing dispersal barriers and environmental filtering as probable causes of unsuccessful regeneration. We aimed to test the impact of two environmental barriers—soil nutrients and competition—on early and late successional forest species, with a focus on understanding conditions that facilitate the regeneration success of late successional species. In a greenhouse experiment, we grew seedlings of a late successional species, *Aquilaria microcarpa*, in adinandra belukar soil alongside a common secondary forest species, *Cinnamomum iners*, and a pioneer species, *Melastoma malabathricum*. Nitrates and phosphates were each added to one tray of seedlings to test the impact of each nutrient on competitive outcomes. Each species was also grown in an environment surrounded only by seedlings of the same species to test the impact of intraspecific competition on seedling growth. Surprisingly, we found that *Aquilaria microcarpa* seedlings performed best in environments with interspecific competition and no added nutrients. The addition of phosphate strongly favoured *Melastoma malabathricum* growth, but the addition of nitrate did not favour any species. Our findings show that *Aquilaria microcarpa* seedlings can successfully regenerate in adinandra belukar soil. As such, seed dispersal is likely to be the main reason for the poor regeneration success of *Aquilaria microcarpa* in Singapore's secondary forest fragments.

Key words. competition, ecology, gaharu, phosphorus, regeneration, tropical

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INTRODUCTION

Southeast Asia contains almost 15% of the world's tropical forests (Estoque et al., 2019). However, the region is undergoing rapid deforestation, having lost 80 million hectares of forest between 2005 and 2015 (Estoque et al., 2019). In Singapore, less than 1% of original primary rainforest remains (Yee et al., 2016) due to the clearing of lowland dipterocarp forests for the purposes of agriculture and logging (Corlett, 1992). Widespread deforestation occurred after British colonisation in 1819. By 1900, 90% of the nation's primary forest had been cleared for plantations of crops such as gambier and rubber (Corlett, 1997). Poor agricultural practices exhausted the soil, leaving many patches of land with degraded soil. Secondary succession on such land resulted in adinandra belukar, a type of native-dominated early successional secondary forest (Yee et al., 2019). In Singapore, these forests grow on land cleared before the 1950s (Yee et al., 2016) and are characterised by poor nutrient conditions, with acidic soils (pH = 3.3–3.9) containing low levels of both total nitrogen (0.06–0.14%) and phosphorus (11–29 $\mu\text{g g}^{-1}$) (Sim et al., 1992). They are dominated by *Adinandra dumosa* Jack. trees, but also contain species such as *Cyrtophyllum fragrans* (Roxb) DC., *Dicranopteris linearis* (Burm.f.) Underw., and *Dillenia suffruticosa* (Griff. ex Hook.f. & Thomson) Martelli (Yee et al., 2019). Other secondary forest types in Singapore include trema belukar, a native-dominated forest which grows on soils less degraded than adinandra belukar (Yee et al., 2016), and exotic-dominated secondary forests, which grow on land abandoned after the 1960s (Yee et al., 2016; 2019).

In Singapore, the recovery of seedlings of primary (late successional) forest species in secondary (early successional) forest fragments has had varied success (Chua et al., 2016). Secondary forests often have lower plant biodiversity and lower seedling densities than primary forests (Goldsmith et al., 2011), which corresponds to a lack of understory vegetation (Goldsmith et al., 2011; Chua et al., 2013; Chua et al., 2016). Several barriers to seedling recruitment for late successional species have been posited. At a landscape scale, dispersal limitations may impact regeneration success. Goldsmith et al. (2011) and Chua et al. (2016) found seedling recruitment levels to be inversely related to the distance seedlings travelled from their sources. Such a pattern may reflect dispersal limitation, which could result from the loss of local animal seed dispersers due to deforestation and hunting, and the fragmentation of primary forests from deforestation

and urbanisation, which prevent the movement of remaining seed dispersers (Goldsmith et al., 2011). This is seen not only in Singapore, but in forests across the world (Wade et al., 2003). At the site level, environmental filters such as soil infertility present barriers to secondary forest seedling recruitment (Chua et al., 2016). Secondary forests, such as adinandra belukar, tend to be lower in nutrients and higher in aluminium than primary forests (Grubb et al., 1994). As such, most common secondary forest species have low foliar nitrogen and phosphorus, and thick leaves (Chua et al., 2013), which are traits associated with slow decomposition rates. Leaf litter build-up is thus a common feature of adinandra belukar, and may also prevent seedling establishment (Goldsmith et al., 2011; Chua et al., 2013) by physically preventing seeds from reaching the soil. Additionally, the low light levels under the leaf litter prevent sufficient light from reaching seeds to cue germination (Chua et al., 2016). Furthermore, competition between seedlings post-germination may limit seedling success. Davies & Semui (2005) found that in nursery environments with interspecific competition between pioneer species, seedlings of *Melastoma malabathricum* L. accounted for 70% of total biomass at the end of the experiment and stunted the growth of other pioneer species that were grown together. The addition of nitrates increased the competitive performance of *Melastoma malabathricum* seedlings and reduced the concentration of nitrogen in the leaves of other species by 20–25%. Although there are several probable regeneration barriers, it is unknown whether the dominant barrier to the regeneration of late successional species in Singapore could be dispersal, environmental filtering, or both.

Very few greenhouse experiments investigating these barriers have been conducted using local primary (late successional) and secondary (early successional) rainforest species, prompting our study to investigate the impact of two probable regeneration barriers: competition and soil conditions, on the growth of late successional species in Singapore's adinandra belukar. We grew seedlings of *Aquilaria microcarpa* Baill., a late successional species, alongside the secondary forest species *Cinnamomum iners* Reinw. ex Blume, and *Melastoma malabathricum* in a greenhouse experiment using adinandra belukar soil. These species were chosen for their different life history characteristics and because of their availability to us. *Aquilaria microcarpa* is a slow growing primary rainforest species found in lowland dipterocarp forests throughout Peninsular Malaysia, Singapore, Borneo, and Sumatra (Soehartono & Newton, 2001). *Cinnamomum iners* is a common secondary forest species occurring throughout Peninsular Malaysia, Singapore, Borneo, Sumatra, Java, Bali, the Philippines, Laos, Thailand and Southern China (de Kok, 2021). It is highly tolerant of a range of light and nutrient conditions (Chua, pers. obs.), including in adinandra belukar (Sim et al., 1992; Chua et al., 2016). *Melastoma malabathricum* is an aluminium accumulator and fast-growing pioneer species which survives well in high light intensities and leached soils with poor nutrients (Watanabe et al., 1998).

Owing to its rarity, not much is known about *Aquilaria microcarpa*. For *Aquilaria malaccensis* Lam., a congener, Soehartono (2002) found that it thrived in soils with relatively high pH environments (pH (H₂O) = 5.11, pH (KCl) = 4.13) and high cation exchange capacities, in a 15-week nursery experiment. By contrast, *Melastoma malabathricum* and *Cinnamomum iners* are often found in adinandra belukar. Given each species' native environment and that *Melastoma malabathricum* is a specialist of degraded soils, as well as the findings of Davies & Semui (2005), we expected that, without considering the effects of interspecific competition, *Melastoma malabathricum* would have faster growth rates than either *Cinnamomum iners* or *Aquilaria microcarpa* in adinandra belukar soil.

Tropical rainforest seedlings are usually phosphorus limited (Vitousek, 1984), and a nursery experiment by Burslem (1994) showed that in primary rainforest soil, *Melastoma malabathricum* seedlings grown alone showed a 60–90-fold increase in growth rates with the addition of phosphate and showed little response to the addition of nitrate. Given these results, we expected all three species to perform better in response to the addition of phosphate compared to their performance under the addition of nitrate.

Soehartono (2002) found that *Aquilaria malaccensis* seedlings had a high mortality rate in the field, potentially due to below-ground and above-ground competition from nearby fast-growing species. These results suggest that seedlings of similar slow growing late successional species such as *Aquilaria microcarpa* may not be able to attain sufficient light and nutrients when planted next to *Melastoma malabathricum*. Given the results of Soehartono (2002) and Davies & Semui (2005), we expected that, with the addition of nutrients, *Melastoma malabathricum* would further inhibit the growth rates and mortality of seedlings of *Aquilaria microcarpa*, and to a lesser extent, *Cinnamomum iners*.

We measured the growth rates of each species in soils with no added nutrients, added nitrate, and added phosphate, and with intraspecific and interspecific competition. We tested the following three hypotheses:

Hypothesis 1: When *Aquilaria microcarpa*, *Melastoma malabathricum*, and *Cinnamomum iners* are grown in adinandra belukar soil with intraspecific competition, *Melastoma malabathricum* will grow faster than *Cinnamomum iners* and *Aquilaria microcarpa*.

Hypothesis 2: Phosphorus has a greater positive effect on species performance than nitrogen for all three rainforest species in environments with interspecific competition.

Hypothesis 3: Interspecific competition has a negative effect on the performance of *Aquilaria microcarpa*, and a positive effect on the performance of *Melastoma malabathricum*, while *Cinnamomum iners* is unaffected.

METHODS

Soil and Seedling Collection. Soil and seedlings were collected from various locations around Singapore, a small island located at a latitude of 1°N. Singapore has a tropical wet climate, with high temperatures and rainfall throughout the year. The long term mean annual temperature is 27.5°C, and mean annual rainfall is 2165.9 mm (Meteorological Service Singapore, 2020).

Aquilaria microcarpa seedlings were collected from a primary forest patch in Chestnut Nature Park on 16 January 2018 (Fig. 1). *Melastoma malabathricum* and *Cinnamomum iners* seedlings were collected from adinandra belukar on the National University of Singapore campus and at Kent Ridge Park, respectively, on 9 June 2018. Seedlings collected were between 2 and 10 cm in height.

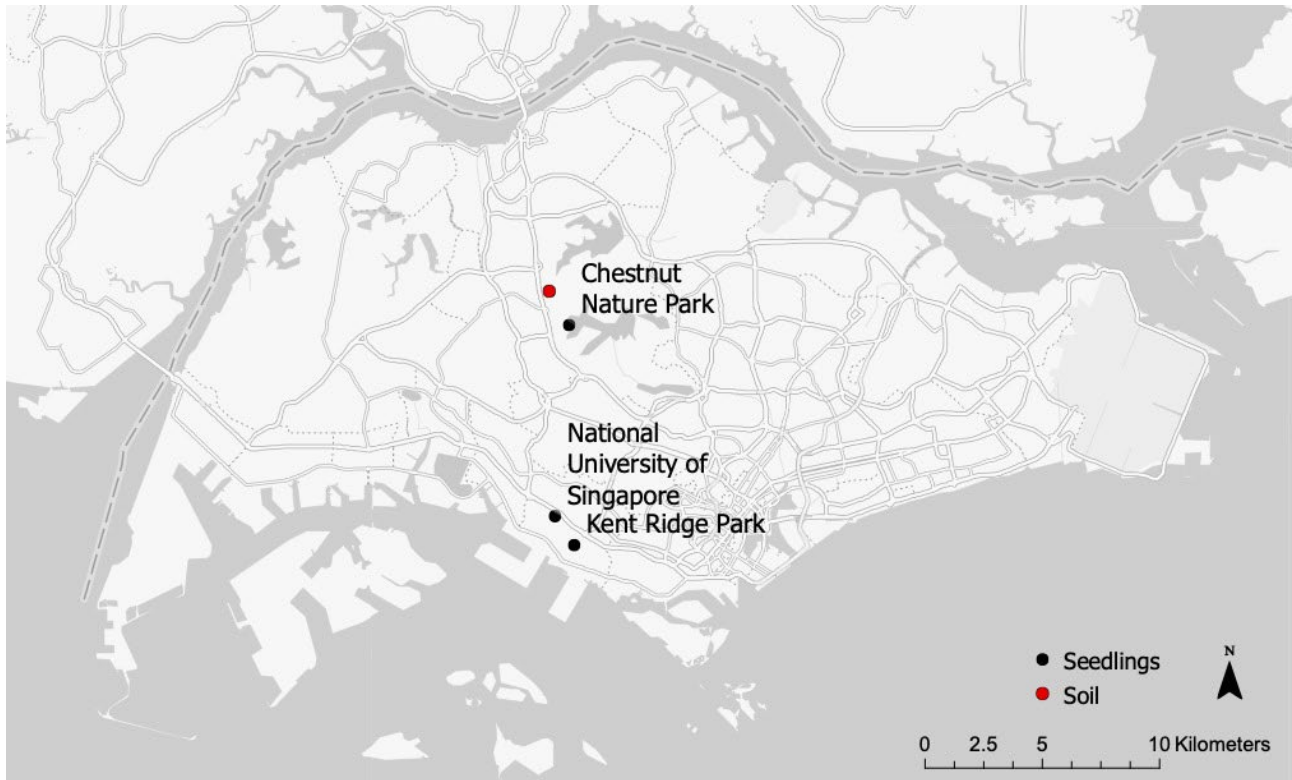


Fig 1. Map of soil and seedling collection sites on the main island of Singapore. Soil and *Aquilaria microcarpa* seedlings were collected from Chestnut Nature Park. *Melastoma malabathricum* and *Cinnamomum iners* seedlings were collected from the National University of Singapore campus and Kent Ridge Park.

Soil from a depth of 1–10 cm was collected from adinandra belukar near Chestnut Nature Park, at the western part of the Central Catchment Nature Reserve, on 9 June 2018 (site shown in Fig. 2). The site was the same as that studied by Chua et al. (2016) and contained secondary forest species including *Melastoma malabathricum* and *Cinnamomum iners* (Chua et al., 2016). In that study, the top 5 cm of organic soil beneath the leaf litter was analysed at the Smithsonian Tropical Research Institute, and was found to contain less than half of the average phosphate content in both primary and secondary forests in Singapore, and comparable nitrogen content to secondary forests in the Central Catchment Nature Reserve (0.304 % nitrogen, 2.08 mg/kg phosphate, and 576 mg/kg aluminium) (Chua et al., 2016). This soil was used to grow the *Aquilaria microcarpa*, *Cinnamomum iners* and *Melastoma malabathricum* seedlings.



Fig. 2. Image of soil collection site at Chestnut Nature Park (Photograph by: Naomi Schulberg).

Experimental Design. We set up a greenhouse experiment at the nursery of the United World College of South East Asia Dover campus. Seedling trays were filled with a 5 cm layer of gravel. A mesh sheet was laid across the gravel and a 3.5 cm layer of the collected and sieved soil from adinandra belukar was poured over the sheet.

Seedlings were transplanted into the trays on 15 June 2018. To account for transplant stress, data collection of plant growth commenced one week after transplantation. Each seedling was planted 7 cm apart from the next. This distance enabled the seedlings to be close enough to one another to have interaction, but not so close as to prohibit growth completely (Davies & Semui, 2005). Seedlings on the edges of the trays were planted 3.5 cm from the edge of the tray. Seedlings were planted at a depth so that their roots were completely covered by the soil.

The plants were kept in low-to-moderate light conditions (average intensity = 657.5 lux, where full sunlight is 20,000+ lux). This light intensity was chosen as we intended to mitigate the impact of light intensity on growth rates. Previous studies found light intensity in secondary tropical forests to be relatively low, with an average transmittance of 1.57 %–33 % (Montgomery & Chazdon, 2001), which is in line with light conditions experienced by *Aquilaria microcarpa* and *Cinnamomum iners*. However, *Melastoma malabathricum* is often found along the edges of secondary forest areas, and canopy gaps, and it quickly gets shaded out once the canopy closes. Thus, relative to *Aquilaria microcarpa* and *Cinnamomum iners*, *Melastoma malabathricum* seedlings were probably grown in sub-optimal conditions. Light intensity was monitored using a lux metre at 9 am on each day of data collection.

We grew 16 seedlings of each species (*Aquilaria microcarpa*, *Melastoma malabathricum*, and *Cinnamomum iners*) together in three 58 × 39 cm trays (hereafter referred to as interspecific or “InterC, InterN, and InterP” trays, corresponding to the trays with no added nutrients, added nitrate, and added phosphate, respectively) and 12 seedlings of each species individually in a 37 × 41 cm tray (hereafter referred to as intraspecific-competitive or “IntraC” trays) (Fig. 3).

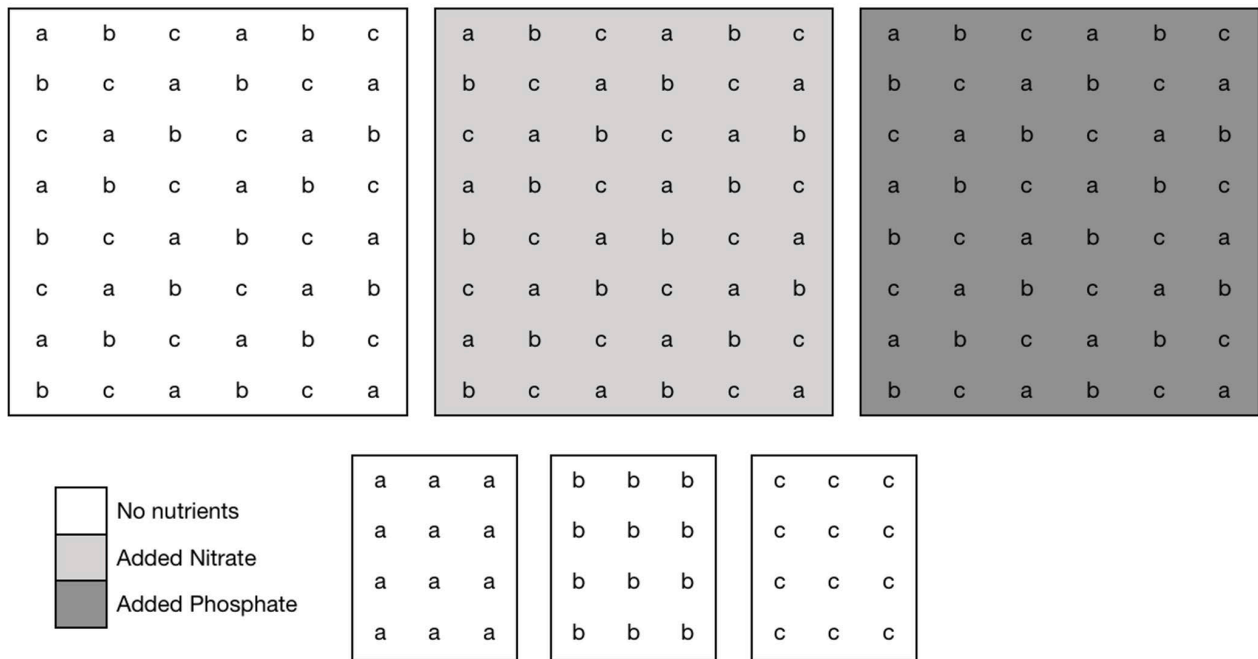


Fig. 3. Diagram showing the arrangement of seedlings. Labels a, b, and c represent *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum*, respectively.

One week after transplantation, 5 ml of 0.5 mol dm⁻³ ammonium nitrate (NH₄NO₃) solution and 5 ml of 0.5 mol dm⁻³ sodium dihydrogen phosphate (NaH₂PO₄) solution were each applied to the soil surrounding each seedling in to one of the three trays containing seedlings in interspecific competition environments, while 5 ml of water, with no added nutrients, was added to each seedling in the control tray with interspecific competition. The seedling trays were placed under sprinklers, which sprayed water for 15 minutes, three times per day.

Seedling heights were measured at the beginning and end of a nine-week period. A ruler was used to measure the height of each seedling from the soil to the highest apex on its stem. Nutrient solutions were added on weeks 1 and 5 of the experiment. The relative growth rate for each seedling was calculated to effectively compare the growth rates of seedlings with different initial heights. Relative growth rates were calculated using the formula:

$$\text{Relative Growth Rate (in days)} = (\ln (\text{final height}) - \ln (\text{initial height})) / \text{time in days.}$$

Data Analysis. To test each hypothesis, a two-way Analysis of Variance (ANOVA) was used to compare the growth rates of each species in each condition. To test for significant differences, the Tukey’s HSD test was used as a post hoc test. The software R, version 4.03 (R Core Team, 2023), was used to conduct this analysis.

To test Hypothesis 1, the relative growth rates of each species were compared in trays with intraspecific competition, which did not have nutrient addition. To test Hypothesis 2, relative growth rates and mortality were compared in phosphate and nitrate treatments with interspecific competition. To test Hypothesis 3, relative growth rates and mortality of *Melastoma malabathricum* were compared against *Aquilaria microcarpa* and *Cinnamomum iners* seedlings in each of the three trays with interspecific competition.

RESULTS

We found no statistically significant differences between the growth rates of all species in the interspecific competition environment with nitrate, but statistically significant differences between species growth rates in all other treatments. (Fig. 4). The addition of phosphate heavily favoured the growth of *Melastoma malabathricum*. Additionally, without the addition of nutrients, *Aquilaria microcarpa* seedlings had significantly higher growth rates in environments with interspecific competition than intraspecific competition (Fig. 4, Table 1).

Mortality occurred across all species, and in all environmental growth conditions (Fig. 4). *Aquilaria microcarpa* mortality occurred in the interspecific competition environment with added nitrate. *Cinnamomum iners* seedlings had mortality in all environments except the tray with intraspecific competition. *Melastoma malabathricum* seedlings had mortality in the interspecific control and interspecific nitrogen-enriched environments.

The two-way ANOVA test (Table 2) showed significant differences ($p < 0.05$) between the growth rates of *Aquilaria microcarpa*, *Melastoma malabathricum*, and *Cinnamomum iners* seedlings based on species, treatment, and the interaction between species and treatment.

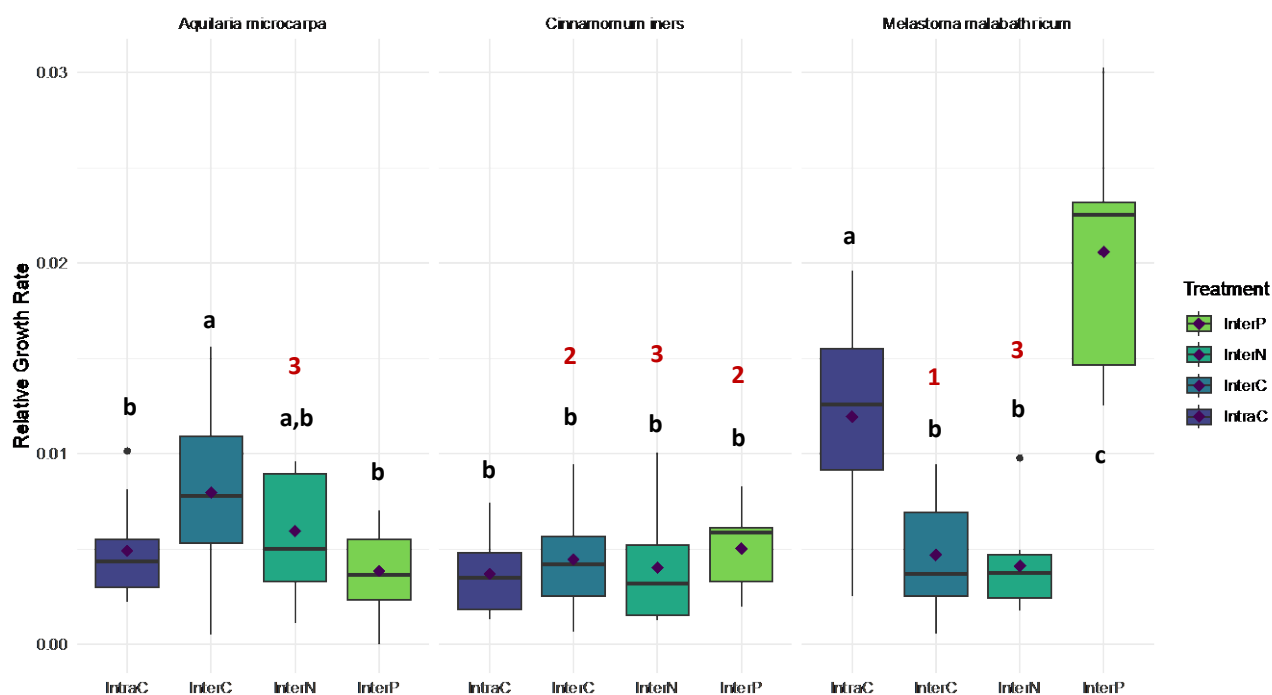


Fig. 4. Graph showing the relative growth rates of *Aquilaria microcarpa*, *Melastoma malabathricum* and *Cinnamomum iners* in all conditions. “InterC, InterN and InterP” refer to the trays with no added nutrients, added nitrate, and added phosphate, respectively. Error bars show standard errors. Number of seedlings per species = 12 for IntraC; 16 for all other treatments. The number of seedling deaths in each treatment is shown in red numbers above growth bars. Letters indicate significant differences ($p < 0.05$) in relative growth rates between and within species as given by the Tukey HSD post hoc tests.

Table 1. Mean relative growth rates for each species in all conditions.

Species	Treatment	Relative Growth Rate (d^{-1})	Standard Error (d^{-1})
<i>Aquilaria microcarpa</i>	IntraC	0.0049	0.00071
	InterC	0.0080	0.0012
	InterN	0.0060	0.0010
	InterP	0.0038	0.00065
<i>Cinnamomum iners</i>	IntraC	0.0037	0.00067
	InterC	0.0044	0.00069
	InterN	0.0040	0.00099
	InterP	0.0050	0.00073
<i>Melastoma malabathricum</i>	IntraC	0.012	0.0015
	InterC	0.0047	0.00075
	InterN	0.0041	0.00081
	InterP	0.021	0.0019

Table 2. Results of two-way ANOVA to compare growth rates of each species in each condition.

	df	Sum sq	Mean sq	F value	P value
Species	2	0.00075	0.00038	29.74	< 0.05
Treatment	3	0.00040	0.00016	10.78	< 0.05
Species × Treatment	6	0.0016	0.00027	21.70	< 0.05
Residuals	124	0.0016	0.000013		

Hypothesis 1. Tukey's HSD test (Fig. 4) showed that there were significant differences in the relative growth rates of *Melastoma malabathricum* compared to *Aquilaria microcarpa* and *Cinnamomum iners* in intraspecific competition environments, with *Melastoma malabathricum* having the highest growth rates.

Hypothesis 2. Tukey's HSD tests (Fig. 4) showed that there were no significant differences in relative growth rates between the interspecific competition environment with degraded soil and the environment with added nitrate for any species, though all species had relatively high mortality rates in the environment with added nitrate. *Aquilaria microcarpa* seedlings had significantly higher growth rates when grown with interspecific competition in the degraded soil of adinandra belukar without nutrient addition than in the soil with added phosphate (Fig. 4), and *Melastoma malabathricum* seedlings had significantly higher growth rates with added phosphate than in interspecific environments with no added nutrients (Fig. 4). *Cinnamomum iners* growth rates were similar in all nutrient environments, but it was the only species with mortality in the soil with added phosphate.

Hypothesis 3. Tukey's HSD tests (Fig. 4) showed that in the interspecific control environment, the growth rates of *Aquilaria microcarpa* were significantly higher than those of the other two species. In environments with added nitrate, the species all performed similarly. Only in environments with added phosphate did *Melastoma malabathricum* seedlings grow significantly faster than *Aquilaria microcarpa* and *Cinnamomum iners* seedlings. Additionally, comparing the growth rates in intraspecific competition and interspecific control environments, there was no significant difference for *Cinnamomum iners* seedlings. *Cinnamomum iners* had the highest mortality rates in all interspecific competitive environments, while *Aquilaria microcarpa* and *Melastoma malabathricum* had minimal mortality, an exception being in the tray with added nitrates.

DISCUSSION

We grew *Aquilaria microcarpa* seedlings alongside *Cinnamomum iners* and *Melastoma malabathricum* seedlings in an 8-week nursery experiment. Seedlings were grown in degraded adinandra belukar soil with 0.304% nitrogen and 2.08 mg/kg phosphorus (further site information given in Chua et al., 2016, supplementary data). We varied nutrient conditions and competition to investigate the factors limiting the regeneration of late successional species in secondary forest soils. Under intraspecific competition conditions, *Melastoma malabathricum* seedlings had significantly higher growth rates than the other two species. While the addition of nitrate corresponded with the highest mortality across all treatments for all species, the addition of phosphate heavily favoured the growth of *Melastoma malabathricum*. Additionally, interspecific competition with *Melastoma malabathricum* did not significantly affect the growth rates of *Aquilaria microcarpa* or *Cinnamomum iners* seedlings. *Cinnamomum iners* seedlings had the highest mortality (seven seedling deaths across all conditions), and *Aquilaria microcarpa* had the lowest mortality (three seedling deaths across all conditions). Overall, *Aquilaria microcarpa* grew surprisingly well in degraded adinandra belukar soil, even with interspecific competition. Our results suggest that nutrient levels in adinandra belukar soil and interspecific competition are not limiting factors in *Aquilaria microcarpa* growth, but growth may be limited in environments with added nutrients. Our discussions on key findings for each hypothesis are detailed below.

Hypothesis 1: *Melastoma malabathricum* grew significantly faster than *Aquilaria microcarpa* and *Cinnamomum iners* in adinandra belukar soil in environments with intraspecific competition. These results agreed with our expectations. The faster growth of *Melastoma malabathricum* aligns with its characteristics as an early successional pioneer species and a specialist of degraded soils, despite experimental light conditions being lower than in the native habitat of *Melastoma malabathricum*. However, *Cinnamomum iners*, while commonly found in adinandra belukar, also grows in various environments including other types of secondary forests and primary forests, and is not a specialist of degraded soils like *Melastoma malabathricum*. Moreover, as neither *Cinnamomum iners* nor *Aquilaria microcarpa* are pioneer species, they are less likely to have life history traits suited to early successional environments, such as fast growth rates.

Aquilaria microcarpa and *Cinnamomum iners* did not exhibit significantly different growth rates in the study. In the context of facilitating the reforestation of primary rainforest species such as *Aquilaria microcarpa*, these results suggest that the growth rate of *Aquilaria microcarpa* in secondary forest soil may be similar to that of some secondary forest species.

Hypothesis 2: With added phosphate in the environment with interspecific competition, *Aquilaria microcarpa* seedlings had depressed growth compared to their growth in the interspecific control environment, and *Melastoma malabathricum* seedlings were competitively superior. These results agree with previous understanding of *Melastoma malabathricum* characteristics for two reasons. Firstly, *Melastoma malabathricum* can uptake large amounts of phosphate through secreting oxalate into the soil (Watanabe et al., 1998), which may have accounted for its higher growth rates with a greater supply of phosphate. Secondly, as a pioneer species, *Melastoma malabathricum* is likely to grow fast and respond quickly to nutrient addition. As such, the addition of phosphate is likely to affect the growth of *Melastoma malabathricum* more than that of *Aquilaria microcarpa* seedlings.

Our results regarding the performance of *Aquilaria microcarpa* in conditions with nutrient addition indicate that it is not aided by the addition of either nitrate or phosphate. *Aquilaria microcarpa* seedlings grew fastest in the interspecific competition environment with no added nutrients. Our results agree with previous studies by Turner et al. (2018) and Shannon et al. (2023). Turner et al. (2018) found that despite low nutrient availability, certain primary rainforest species grow well on unfertilised soil, and growth rates did not increase significantly with nutrient fertilisation. Shannon et al. (2023) conducted a nursery fertilisation experiment on three tropical rainforest species in Southeast Asia, one pioneer species and two slow-growing climax species, finding that the pioneer species responded strongly and positively to fertilisation, whereas the slow-growing species did not respond strongly to fertilisation because of their lower annual nutrient requirements. Our results suggest that *Aquilaria microcarpa* behaves similarly to the slow-growing species studied by Shannon et al. (2023) and is not aided by nutrient fertilisation.

Additionally, owing to the high growth rates of *Melastoma malabathricum* seedlings under added phosphate, less nutrients, light and water, may have been available to the *Aquilaria microcarpa* and *Cinnamomum iners* seedlings. This may not have affected the growth of *Cinnamomum iners* seedlings, relative to growth in the control environment with interspecific competition, since the species is tolerant of a wide range of environmental conditions. On the other hand, increased competition from the rapid growth of the *Melastoma malabathricum* seedlings under phosphate addition could have depressed the growth of *Aquilaria microcarpa* seedlings as compared to their growth in the interspecific competition control.

While phosphate addition significantly favoured *Melastoma malabathricum* growth over *Aquilaria microcarpa* growth, nitrate addition may have negatively impacted the performance of all three species. Nitrate addition appears to correspond with seedling mortality, as all species had a 25% mortality rate under added nitrate. However, the growth rates among surviving seedlings remained similar to growth rates in other nutrient environments. In a factorial nutrient fertilisation experiment, Santiago et al. (2012) found that nitrogen addition to lowland tropical forest seedlings led to the decrease of soil pH by 0.5 units. Such a decrease could make the soil conditions less suitable for the chosen species in our experiment, or could lead to other nutrients becoming limiting (Santiago et al., 2012), thereby affecting their survival. Given the low sample size in our experiment and the maximum mortality of three seedlings per tray, it is difficult to establish the significance of these mortality results, and the effects of nitrate on seedling mortality should be examined in further experiments.

Hypothesis 3: *Melastoma malabathricum* is competitively superior to both *Aquilaria microcarpa* and *Cinnamomum iners* under phosphorus addition, but in environments with interspecific competition but no added nutrients, *Aquilaria microcarpa* seedlings outperformed those of *Melastoma malabathricum*. The growth rates of *Cinnamomum iners* did not seem to be affected by any competitive interactions, but *Cinnamomum iners* seedlings had higher mortality rates than the other two species. Our results regarding the competitive interactions between *Aquilaria microcarpa* and *Melastoma malabathricum* were contrary to expectations. As a specialist of degraded soil, and a pioneer species which is commonly found in adinandra belukar, we expected *Melastoma malabathricum* seedlings to grow faster than seedlings of a late successional species like *Aquilaria microcarpa* under all conditions. As an aluminium accumulator, *Melastoma malabathricum* possesses characteristics that allow it to thrive in acidic soils (Watanabe, 2022), such as adinandra belukar soil. Additionally, due to the competitive dominance of *Melastoma malabathricum* in previous nursery experiments (Davies & Semui, 2005), we expected *Aquilaria microcarpa* growth rates to be negatively impacted by the presence of *Melastoma malabathricum*. However, *Aquilaria microcarpa* seedlings grew faster than expected, and *Melastoma malabathricum* seedlings grew slower than expected, under interspecific competition environments with no added nutrients. Our results regarding the growth rates of *Cinnamomum iners* aligned with our expectations. Because *Cinnamomum iners* is native to secondary forest environments, it commonly grows in environments with *Melastoma malabathricum* and can survive without being completely outcompeted. However, *Cinnamomum iners* had the highest mortality rates of all species in all interspecific competitive environments, compared to no mortality in the intraspecific competition growth environment, which may be a result of interspecific competition.

One explanation for our results regarding the competitive interactions between *Aquilaria microcarpa* and *Melastoma malabathricum* is that the mid-light conditions in the nursery favoured *Aquilaria microcarpa* growth over *Melastoma malabathricum* growth. The nursery experiment of Davies & Semui (2005) found *Melastoma malabathricum* to be the best competitor when grown in degraded soil from a mixed dipterocarp forest, among other pioneer species (*Dillenia suffruticosa*, *Duabanga moluccana*, *Ficus grossularioides*, *Nauclea maingayi*, *Trema cannabina* and *Vitex pubescens*). These species are all light-demanding and commonly regenerate in abandoned open areas at different stages of succession (Davies & Semui, 2005). Their experiment was conducted in full sunlight, whereas our experiment was conducted in partial sunlight. Compared with pioneer and early successional species, seedlings of *Aquilaria* species can survive better in low-light conditions (Soehartono, 2002), and *Cinnamomum iners* seedlings tolerate a broad range of environmental conditions. Soehartono (2002) found that although growth rates were reduced in low light environments, seedlings of another *Aquilaria* species (*Aquilaria malaccensis*) were able to survive in low light conditions, whereas *Melastoma malabathricum* is a relatively light-demanding species (Watanabe et al., 1998) which is often found to be relatively shade-intolerant (Chua, pers. obs.). As *Melastoma malabathricum* seedlings may require higher light intensities to thrive than

were provided in the nursery, our choice of species may have favoured late successional species in competitive outcomes in environments with no added nutrients. Our results thus suggest that once a canopy is established and light levels on the forest floor are relatively low, *Aquilaria microcarpa* and other primary forest species could successfully regenerate, even in degraded soil. Since many adinandra belukar sites with poor seedling regeneration have established canopies, including the site where we collected soil (Fig. 2), such conditions appear to be suitable for seedling establishment. Dispersal is thus likely to be the more important factor limiting recruitment in such sites.

Implications for Forest Restoration. Our findings imply that late successional species such as *Aquilaria microcarpa* regenerate more successfully when no nutrients are added to adinandra belukar soil. The addition of phosphate may favour the regeneration of pioneer species, such as *Melastoma malabathricum*. Secondary forest species such as *Cinnamomum iners* appear to be generalists and regenerate equally successfully regardless of fertiliser addition. These findings contrast the prevailing view of tropical rainforest species being phosphorus-limited (Vitousek, 1984; Vitousek & Sanford, 1986; Alvarez et al., 2013), and agree with the findings of Turner et al. (2018): certain primary tropical rainforest species grow rapidly on infertile soils despite low phosphorus availability.

Soil nutrients were not measured during the experiment, so it is difficult to estimate how much nutrient leaching occurred, and how much of the nutrients were utilised by the seedlings. However, mortality rates were relatively low and consistent across treatments, and the seedling growth rates were higher during the first four weeks than during the second four weeks, indicating that the seedlings responded well to the amount of nutrients added, and were likely not over-fertilised. Future trials could test the responses of the seedlings to different nutrient concentrations, as responses to nutrient addition may be different under lower or higher concentrations of nutrients. Despite studying just one treatment level of nutrient addition, our findings can inform the reforestation of late successional species in secondary forest soils.

Dispersal limitation and degraded soil are commonly cited as potential barriers to the regeneration of primary rainforest species (Goldsmith et al., 2011; Chua et al., 2016). Our experiments show that *Aquilaria microcarpa* seedlings can grow successfully in adinandra belukar soils, implying that the lack of successful regeneration is unlikely to be because of unsuitable soil conditions. As such, seed dispersal is likely to be the main reason for poor regeneration success. This is supported by the poor dispersal of primary forest species across a path from primary to secondary vegetation in the Bukit Timah Nature Reserve (Chua et al., 2013). Soehartono & Newton (2001) examined the reproductive ecology of several *Aquilaria* species, including *Aquilaria microcarpa*, and found that more than 65% of seedlings occurred within 5 metres of adult trees, showing further support for the impact of dispersal limitation on *Aquilaria microcarpa* regeneration. Seeds of some *Aquilaria* species, such as *Aquilaria sinensis* (Chen et al., 2016) and *Aquilaria malaccensis* (Soehartono, 2001; Manohara, 2013), are dispersed by hornets and wasps, respectively, but the role of larger animals which can travel longer distances (such as birds and animals) in their seed dispersal requires further research (Soehartono & Newton, 2001). If we assume similar insect-mediated dispersal across the genus, it is possible that dispersal presents a barrier to *Aquilaria microcarpa* regeneration, if these insects do not travel from primary to secondary forest fragments because of the distances between them.

Although the effects of interspecific competition are evident in our experiment, interspecific competition between seedlings may increase on timescales beyond the eight-week period of our experiment. Additionally, our conclusions regarding *Aquilaria microcarpa* do not represent the response of all late successional species to nutrient addition and interspecific competition, thus, study of additional late successional species is necessary to better inform our understanding of barriers to successful regeneration. Further long-term studies of additional species will improve our understanding of tropical rainforest regeneration.

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