Examiner’s commentary

This is a complex investigation with several independent variables making it challenging to maintain a focus. The research question is firmly placed in a clear biological context and there is strong evidence of engagement from the outset. Interesting, well researched and mostly relevant background is provided on the target species but there is insufficient information about what is meant by “degraded soil”. The research approach and method are modeled on work the candidate has read about, but no precise in-text reference is provided for this. Effective use is made of illustrative material including tables, graphs (with some weaknesses) and images. Overall data presentation is detailed, easy to follow and is done so in a way that relates directly to the research question. A justification is provided for the statistical approach, and this is performed and presented competently and with understanding. The findings resulting from the analysis of data are clearly explained and are assessed in the light of published work. The large standard deviations in some cases (50% of the mean and above) suggest that the study perhaps needed a more refined approach to data collection, focusing on fewer variables and generating levels of data that would allow more reliable conclusions to be reached.

To what extent do competition and the addition of nitrates and phosphates alter the relative growth rates of *Aquilaria microcarpa, Cinnamomum iners,* and *Melastoma malabathricum,* in degraded soil*?*

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Contents

Introduction

Research Question

The research question being investigated is **"To what extent do competition and the addition of nitrates and phosphates alter the relative growth rates of *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum,* in degraded soil?"**

Reasons for Topic Choice

There is an urgent need for reforestation in Southeast Asia. Singapore, for example, has less than 2% of its primary rainforest remaining due to the clearing of lowland dipterocarp forests for the purpose of agriculture (Sim et. al 1992).

At school, I partake in a "Rainforest Restoration Project" (RRP) which grows local rainforest species in a nursery, and shares the importance of trees with the community through species education and plantings. As a member of RRP, I am always interested in learning about local rainforest tree species and how to grow them effectively for the purposes of restoration.This investigation was a part of a research collaboration between RRP, National University of Singapore, and the National Parks Board (NParks), regarding practical reforestation in Southeast Asia. Assistance was kindly provided by Dr. Siew Chin Chua of the National University of Singapore.

RRP recently acquired *Aquilaria microcarpa* seedlings, and is growing them for future reforestation in degraded sites around the school campus. There is a dearth of reforestation research in Southeast Asia, and as a result, I chose to investigate the conditions which will best suit the survival of rainforest species such as *Aquilaria microcarpa*. I hope that my findings may aid the future reforestation efforts of RRP and others in the region.

Background Information

Aquilaria microcarpa

Aquilaria microcarpa is a primary rainforest species found in lowland dipterocarp forests (Fern, 2018). The species is found at late stages of secondary succession, thus requires a large supply of nutrients in the soil to thrive. The *Aquilaria* genus is regarded as being very valuable for when wood from the trees is infected with a fungus, a highly sought after substance known as Agarwood is produced. Many trees are intentionally infected with the fungus and cut down, and the species is considered to be in vulnerable condition by the IUCN (World Conservation Monitoring Center, 1998).

Aquilaria species are valuable candidates for reforestation in Singapore, as they are widely available and native to the region. However, whilst there have been some attempts in *Aquilaria* reforestation, it is proving to be a challenge. One of the reasons for this is that the species is often outcompeted by faster growing trees (Soehartono, 1999).

Soehartono's study found that *Aquilaria microcarpa* seedlings grew at a rate of around 15.7 cm per year, relatively slower than most other species of rainforest trees. Whilst it was found that the species grew well in a variety of light conditions (5 p. mol photons to 200 p. mol photons), the species' relative growth rates differed due to nutrient availability. All species of *Aquilaria* required the presence of the three essential macronutrients: nitrogen, phosphorus, and potassium.

Aquilaria microcarpa reforestation will most likely take place in the "Adinandra Belukar", a type of secondary forest prevalent in Singapore (National Parks Board Singapore, 2013). Adinandra Belukar forests are characterised by their poor nutrient conditions, with acidic soils containing low levels of both nitrogen (0.06%- 0.14%) and phosphorus (11-29 μg g-1) (Sim et. al 1992).

This research project investigated the effect of adding select nutrients to and varying the nature of competition on *Aquilaria microcarpa* seedling growth in Adinandra Belukar soil. Through measuring the relative growth rates of the species, the nutrient and species conditions most beneficial towards promoting *Aquilaria microcarpa* growth could be deduced.

Apart from *Aquilaria microcarpa*, the species investigated (*Cinnamomum iners* and *Melastoma malabathricum*) were chosen due to their presence in the Adinandra Belukar. The use of these species in the experiment mimics common ecological conditions in Singaporean rainforests, and the conditions which will be faced by *Aquilaria microcarpa* when reforestation plantings take place.

Cinnamomum iners

Cinnamomum iners is a common secondary forest species which thrives after pioneer growth (Bailey et al, 1976). The small trees grow well in a variety of conditions, ranging from poor nutrient soil with high levels of sunlight, to more shaded areas, and as a result, are quite versatile (National Parks Board, 2013).

Melastoma malabathricum

Melastoma malabathricum, a common rainforest shrub, is a pioneer species which survives well in high light intensities and soils with poor nutrients. The species is an "Aluminum accumulator", which enables it to thrive in degraded soils (Watanabe et al. 1998). Aluminum $^{3+}$ ions are generally toxic to plants in high quantities (Brunner & Sperisen, 2013), but these species are able to tolerate the conditions by storing large amounts of aluminum in their leaves. *Melastoma malabathricum* secretes organic acids into the rhizosphere that react with aluminum ions in the soil to form soluble compounds which it can then uptake (Ecological Society of America, 2017). This mechanism allows for the survival of *Melastoma* in acidic and nutrient deficient soils (Watanabe et al. 1998).

A primary mechanisms through which *Melastoma* utilises its aluminum accumulator abilities is by the secretion of Oxalate (an organic acid). Oxalate reacts with aluminum phosphate ions in the soil, making the ions soluble and therefore available for uptaking (Watanabe et al. 1998).

Melastoma malabathricum is dominant over other species in degraded soils, and due to competitive exclusion, its presence inhibits primary rainforest trees from returning to areas of secondary succession (Davies and Semui, 2006). Thus, it would be expected for the presence of *Melastoma* seedlings to affect and inhibit the relative growth rates of *Aquilaria* and *Cinnamomum* in all three nutrient conditions tested. However, prior research is unclear as to what extent this differs in non degraded soils. This information lead me to question: would altering the nutrient levels of an area give *Aquilaria microcarpa* seedlings a fighting chance at survival in Singapore's Adinandra Belukar forests? Or, is the reason behind reforestation difficulties due to competition with other, faster growing species such as *Melastoma malabathricum* and *Cinnamomum iners*?

Hypotheses

Based on the above research, two hypotheses were created:

1. The addition of nitrates results in higher relative growth rates of *Aquilaria microcarpa* and *Cinnamomum iners*, whereas the addition of phosphates results in higher relative growth rates of *Melastoma malabathricum*.
2. The aspect of competition increases growth rates of *Melastoma malabathricum*, and decreases growth rates of *Aquilaria microcarpa* and *Cinnamomum iners*.

Methodology

In order to test the hypotheses, an experiment was set up in the school rainforest nursery. The effect of nutrient addition on competitive interactions between seedlings of *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum* was assessed by comparing the relative growth rates of seedlings of each species in competitive environments in three trays with varied addition of nutrients. To assess the effect of competition on seedling growth, an additional tray was used to grow seedlings in a non competitive environment. The relative growth rates of each species in the non competitive tray was compared with the relative growth rates of the species in the tray with soil with no added nutrients and a competitive environment.

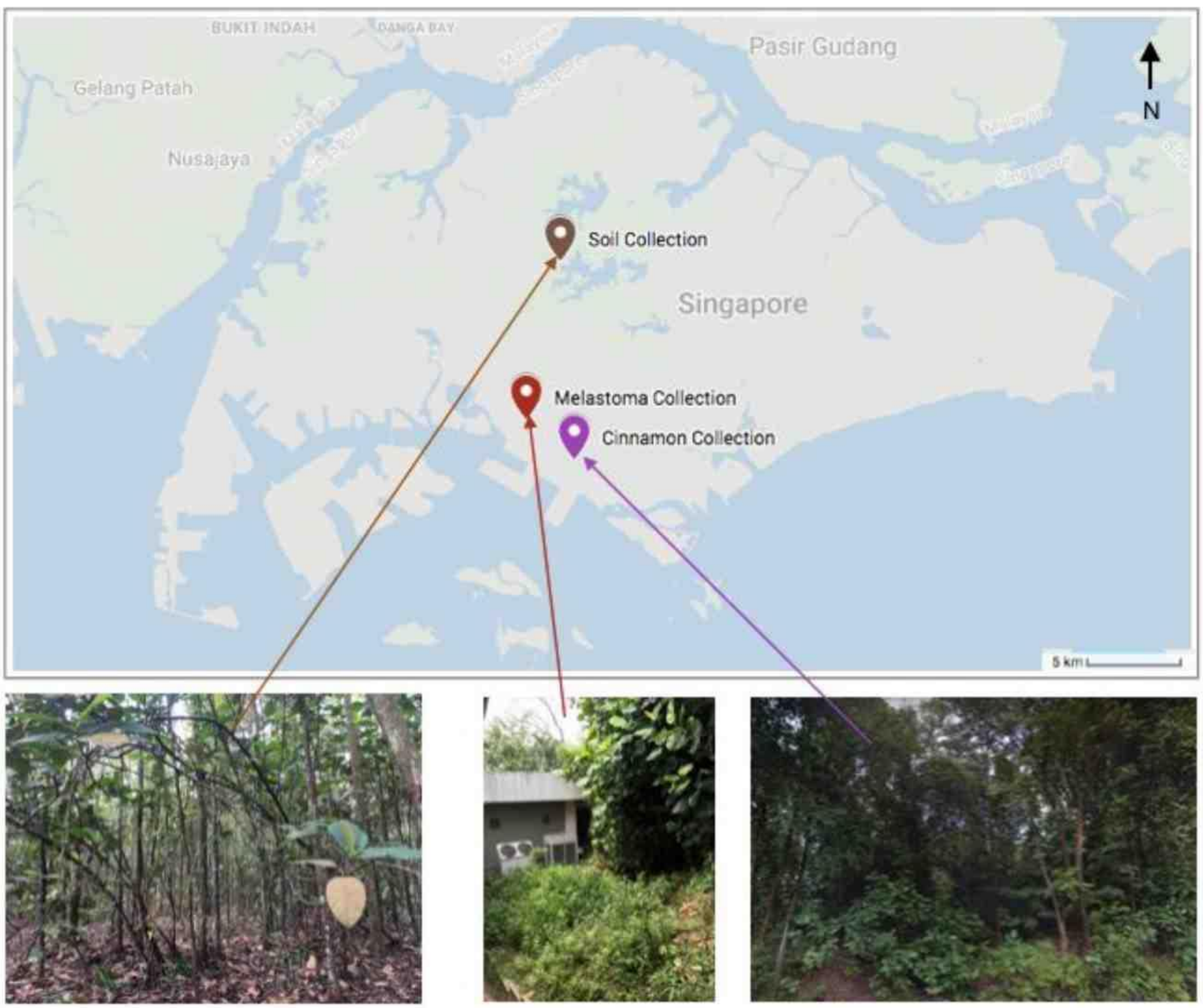
The species were kept in controlled low light conditions, to mitigate the impact of light intensity on growth rates. The low light levels did not favor the growth of any particular species. However, it should be noted that the nursery was subject to natural environmental conditions, such as climate. Thus, light intensity was monitored, using a lux meter, 5 times at 9 am on each day of data collection[1].

Prior to beginning the experiment, soil, *Melastoma malabathricum* seedlings, and *Cinnamomum iners* seedlings, were collected from a variety of locations around Singapore. To mimic the conditions in the Adinandra Belukar, approximately 30 grams of soil was collected, using chunkles and trowels, from a degraded site in Chestnut Nature Park. *Melastoma malabathricum*

[1]Monitored variable data shown in Appendix.

seedlings were collected from a site on National University of Singapore's campus, and *Cinnamomum iners* seedlings were collected using trowels from a mixed forest site in Kent Ridge Park (illustrated on Figure 1). Seedlings collected were between 2-10 cm to match the heights of the *Aquilaria microcarpa* already available in the nursery.

Figure 1: Annotated map to show the location of soil and specimens collected for the experiment (Google, 2018).



Experimental Design

The experimental design modified an experiment by Stuart J. Davies and Hardy Semui investigating competitive dominance by species such as *Melastoma malabathricum* in a secondary successional rainforest community in Borneo. Their experiment was considered suitable to this research project as both experiments investigated similar rainforest species, and, due to close geographic proximity, climatic conditions between studies were comparable.

Seedling trays were filled with a 5 cm layer of gravel to ensure that seedlings' exposure to the sun was not inhibited by the sides of the tray. A mesh sheet was laid across the gravel and a 3.5 cm layer of soil was poured over the sheet. Before pouring, the soil was sieved to remove roots and large particles which could affect infiltration rates and block the growth of seedlings.

Seedlings were transplanted on June 15th 2018. To reduce the impact of transplant stress on the results, data collection commenced one week after transplanting. Each seedling was planted 7 centimeters 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 and Semui, 2006)[2]. Seedlings on the edges of the trays were planted 3.5 cm from the edge of the tray, to keep distance constant. Seedlings were planted at a depth so that their roots were completely submerged by the soil, but the stems were not covered.

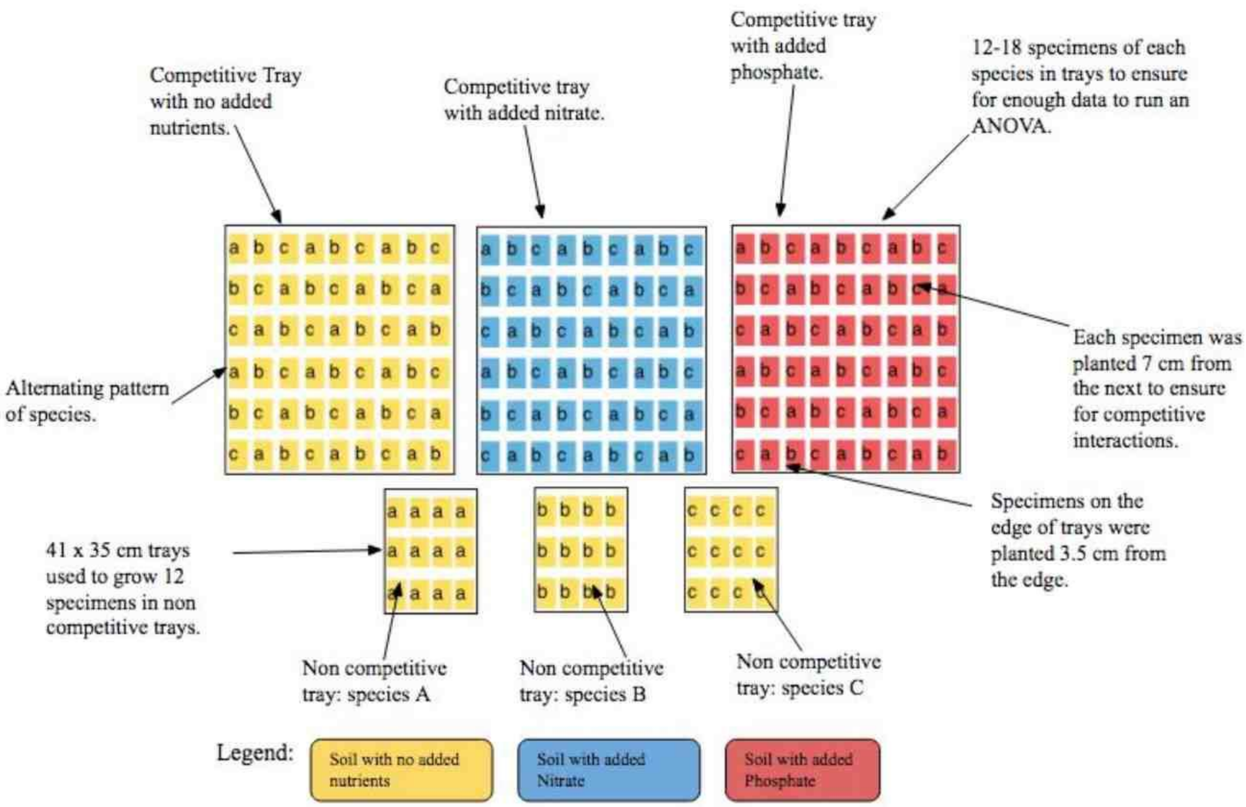
[2] The distance of 7 cm was decided upon based on Davies and Semui's experiment.

The experiment had two Independent Variables: the nutrients added to the trays of seedlings in competitive environments, and the competitive nature of the environment experienced by the *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum*.

The effect of nutrient addition on competitive interactions between seedlings of *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum* was assessed by comparing the relative growth rates of 12-18 seedlings of each species in competitive environments in three 58 cm by 39 cm trays. The seedlings were planted in a grid with a patterned formation such that a seedling of one species would be surrounded by at least 2 seedlings of each species. To assess the effect of competition on seedling growth, a 37 cm by 41 cm tray was used to grow 12[3] seedlings in a non competitive environment (as shown by Figure 2, where a, b, and c represent different species).

[3] More than 10 seedlings of each species were planted to ensure greater accuracy in results and for later use of the ANOVA test.

Figure 2: Diagram to show the arrangement of seedlings.



1 week after transplanting seedlings, nutrient solutions were applied to the three trays containing the seedlings in competitive environments. One tray contained soil with no added nutrients. 5 ml of 0.5 mol dm^{-3} ammonium nitrate solution was added to the soil surrounding each species in a second tray. 5 ml of 0.5 mol dm^{-3} sodium dihydrogen phosphate solution was added to the soil surrounding each species in a third tray.

The <u>dependent variable</u> investigated was the change in stem height of *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum* seedlings in centimeters. Seedling heights were measured twice over a 5 week period[4] commencing on June 22nd 2018 and finishing July 27th 2018. Height readings were taken at the beginning of week 1 and and of week 5 of the experiment. A 30 cm ruler was used to measure the height of each seedling from the soil to the highest apex on its stem. If the stem was bent, it was naturally straightened for the purposes of measuring its full height.

Results and Analysis[5]

Qualitative Observations

Figure 3: Annotated photos to show qualitative observations of seedlings on week 1 and week 5 of the experiment.

[4] A 5 week time period was decided upon due to the subsequent movement of the rainforest nursery due to renovations. The new location had significantly different environmental conditions, so for the sake of accuracy, timing for the experiment was limited to the weeks before the move.

[5] Raw Data in Appendix.

Processed Data[6]

For each paired data set, the percentage growth rates were calculated by use of the formula:

$$100\left(\frac{\text{Measurement 2 - Measurement 1}}{\text{Measurement 1}}\right)$$

Legend for Figure 4
A: Non Competitive
B: Competitive with controlled soil
C: Competitive with added ammonium nitrate
D: Competitive with added sodium dihydrogen phosphate

	Figure 4: A table to show the percentage growth rates of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a variety of conditions. (%)[7]											
	Aquilaria microcarpa				***Melastoma malabathricum***				***Cinnamomum iners***			
Specimen Number	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	22.4	75.0	20.0	3.7	57.7	48.9	46.7	165.9	23.6	3.9	14.7	18.4
2	27.8	3.1	25.0	19.6	15.4	22.5	18.0	113.0	26.7	14.1	6.3	31.6
3	18.2	50.0	10.0	30.3	17.8	9.5	14.7	92.3	18.6	4.0	26.9	19.7
4	25.8	30.0	57.5	24.4	65.9	10.0	22.0	158.5	1.3	55.6	21.5	12.9
5	13.3	35.9	21.6	27.6	57.4	22.9	12.3	59.5	8.0	20.3	19.4	11.6
6	16.9	42.0	33.3	14.5	88.4	0.0	47.9	76.9	27.3	21.1	45.6	20.3
7	37.8	0.0	2.6	9.1	25.7	7.5	14.6	106.7	4.0	14.7	6.0	11.7
8	61.8	42.5	37.0	20.7	60.0	8.7	20.7	105.7	1.7	13.6	12.7	45.5

[6] All measurements given to one decimal place.
[7] Discrepancies in number of specimens in conditions B, C, and D were created due to the death of seedlings.

9	50.0	130.0	20.6	20.3	48.3	22.1	17.2	45.8	3.1	32.8	29.6	12.5
10	16.9	30.0	10.2	15.4	14.9	15.5	23.3	146.3	10.0	5.0	6.1	5.6
11	27.9	9.2	14.8	7.9	47.7	15.0	14.3		7.7	69.8		40.8
12	12.7	46.7	18.6	9.8	61.9	3.3	10.6		20.0	28.9		
13		1.4				20.8				31.8		
14		36.1				29.2				26.8		
15		68.1				13.0				13.0		
16		60.0										
	Mean Percentage Height Change											
	27.6	41.3	22.6	16.9	46.8	16.6	21.9	107.1	12.7	23.7	18.9	21.0
	Standard Deviation											
	15.2	32.9	14.6	8.3	23.4	12.0	12.5	40.6	10.0	18.6	12.7	12.9

Figure 5: A Graph to show the average growth rates of *Aquilaria microcarpa seedlings* in different environments. Error bars show standard deviation.

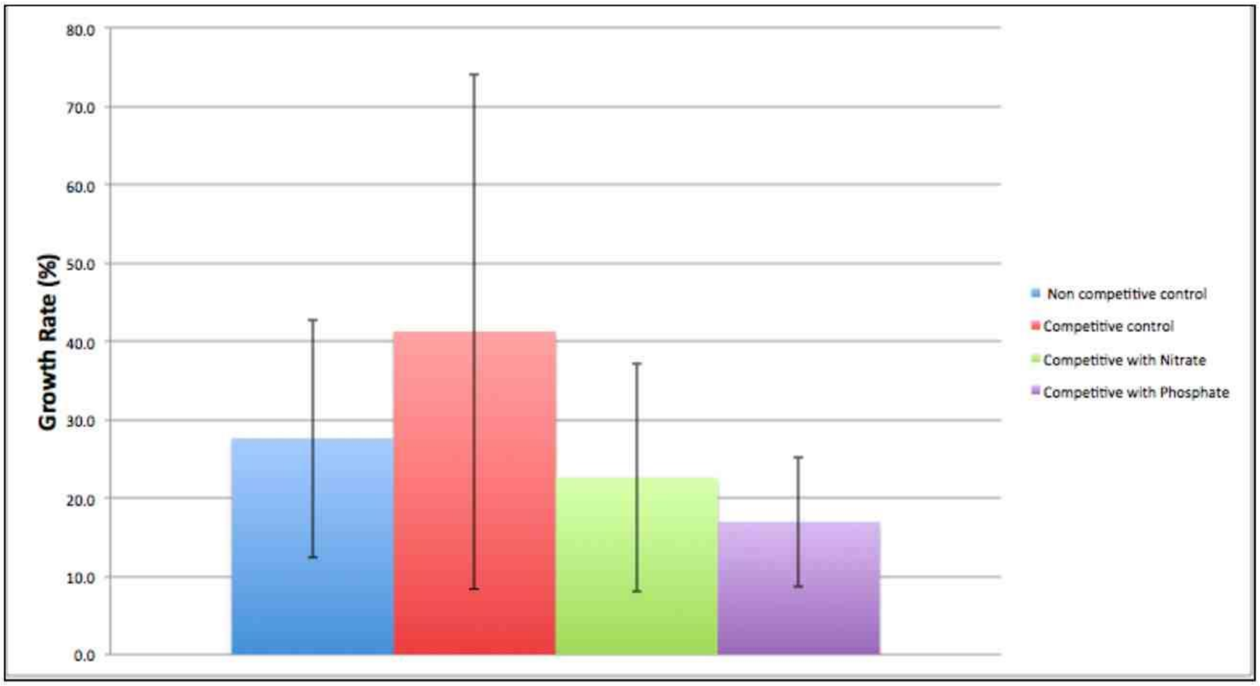


Figure 6: A Graph to show the average growth rates of *Melastoma malabathricum seedlings* in different environments. Error bars show standard deviation.

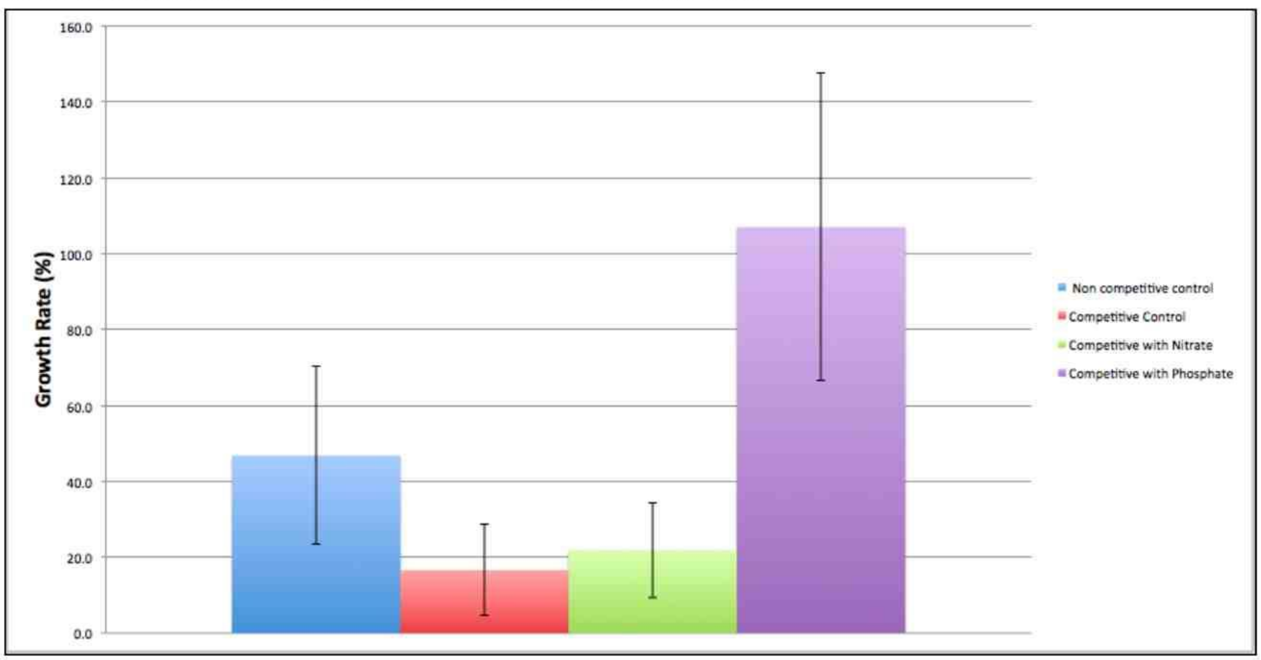
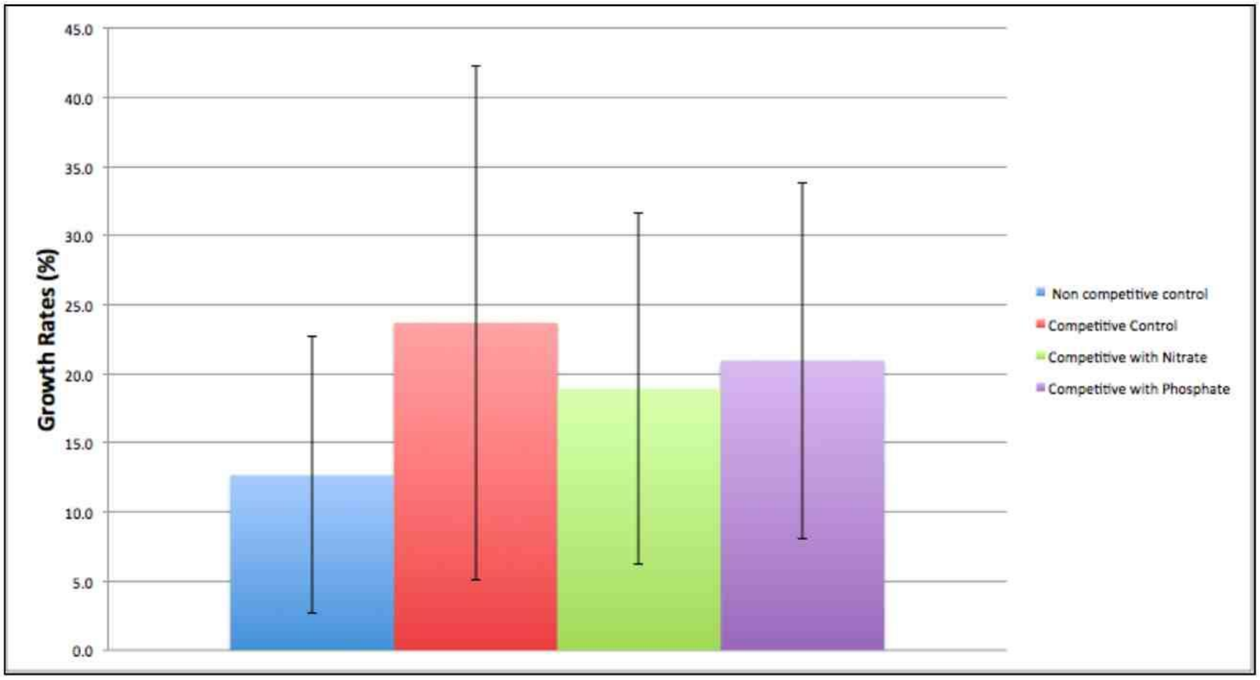


Figure 7: A Graph to show the average growth rates of *Cinnamomum iners seedlings* in different environments. Error bars show standard deviation.



Analysis of Variance Test[8]

A Two way Analysis of Variance (ANOVA) test was run on data to examine the statistical variation between the differences in growth rates amongst the species with the addition of nutrients, and aspect of competition. An ANOVA was chosen as I wished to compare more than two sets of data, investigating two independent variables. The software *Statplus* was used to perform the statistical analysis on the data. Data was sorted into categories based on the factors investigated: species, nutrient/competitive condition (isolated control, competition control, competition nitrate, and competition phosphate), and percentage growth.

[8] All test data given to two decimal places.

Figure 8: A table to show results of ANOVA							
Source of Variation	**SS**	**d.f.**	**MS**	**F**	**P Value**	**F crit**	**Omega Sqr.**
Factor #1 (Species)	14,837.73	2	7,418.87	18.16	1.02×10^{-7}	3.06	0.11
Factor #2 (Condition)	10,904.94	3	3,634.98	8.90	0.00	2.67	0.073
Factor #1 + #2 (Species x Condition)	51,569.86	6	8,594.98	21.04	2.00×10^{-17}	2.17	0.37
Within Groups	55,559.92	136	408.53				
Total	132,872.45	147	903.89				
Omega squared for combined effect	0.55						

Fisher's Least Significant Difference Test

As the P Values were all below 0.05, there was 95% certainty of a statistically significant difference between the sets of data. Fisher's Least Significant Difference Test was used as a follow up to the ANOVA, so as to break down which categories of data within the sets had statistically significant differences. Fisher's Least Significant Difference test was chosen over other follow up tests, such as the T test, as it takes into account multiple variables.

Figure 9: A table to show Fisher's LSD results for Overall Growth Differences				
Group vs. Group (Contrast)	**Difference**	**Test Statistic**	**p-value**	**Significant**
Competition Control vs Competition Nitrate	6.24	1.37	0.17	No
Competition Control vs Competition Phosphate	-18.10	3.93	0.00	Yes
Competition Control vs Isolated Control	-1.72	0.38	0.70	No
Competition Nitrate vs Competition Phosphate	-24.35	4.93	2.20×10^{-6}	Yes

Figure 10: A table to show Fisher's LSD results for variations in *Aquilaria microcarpa* growth based on conditions				
Group vs. Group (Contrast)	**Difference**	**Test Statistic**	**p-value**	**Significant**
Competition Control vs Competition Nitrate	18.65	2.42	0.02	Yes
Competition Control vs Competition Phosphate	24.31	3.15	0.00	Yes
Competition Control vs Isolated Control	13.15	1.66	0.01	No
Competition Nitrate vs Competition Phosphate	5.66	0.69	0.49	No

Figure 11: A table to show Fisher's LSD results for variations in *Cinnamomum iners* growth based on conditions				
Group vs. Group (Contrast)	**Difference**	**Test Statistic**	**p-value**	**Significant**
Competition Control vs Competition Nitrate	4.81	0.58	0.56	No
Competition Control vs Competition Phosphate	2.73	0.34	0.73	No
Competition Control vs Isolated Control	11.03	1.40	0.16	No
Competition Nitrate vs Competition Phosphate	-2.08	0.24	0.81	No

Figure 12: A table to show Fisher's LSD results for variations in *Melastoma malabathricum* growth based on conditions				
Group vs. Group (Contrast)	**Difference**	**Test Statistic**	**p-value**	**Significant**
Competition Control vs Competition Nitrate	-5.27	0.67	0.50	No
Competition Control vs Competition Phosphate	-90.47	10.96	0.00	Yes
Competition Control vs Isolated Control	-30.17	3.85	0.00	Yes
Competition Nitrate vs Competition Phosphate	-85.20	9.85	0.00	Yes

Discussion and Conclusion

Through analysis of the relative growth rates of *Aquilaria microcarpa*, *Cinnamomum iners,* and *Melastoma malabathricum*, the research question "**To what extent do competition and the addition of nitrates and phosphates alter the relative growth rates of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum*, in degraded soil?**" can be answered.

Through use of the mean growth rates and ANOVA test, it is evident that, overall, the addition of phosphate significantly impacted the growth rates of species. The p values comparing the addition of phosphate and the addition of nitrate (2.21×10^{-6}) and comparing the addition of phosphate to the control soil (0.00013) were both below 0.05, showing a statistically significant difference in data sets. The addition of nitrate ($p=0.17$) and the competitive nature of the environment ($p=0.70$) did not have a significant impact on growth rates. Both p values were above 0.05, therefore there was not a statistically significant difference in growth rates. However, there were variations in results between the different species.

Aquilaria microcarpa

The growth rates of *Aquilaria microcarpa* seedlings were significantly affected by both the addition of nitrates and the addition of phosphates, shown by the respective p values of 0.01692 and 0.0199. Instead of aiding in growth, the addition of nutrients in competitive environments seemed to inhibit growth rates of *Aquilaria microcarpa*. The mean height change of *Aquilaria* seedlings with controlled soil was 41.3%, and the mean height changes were 22.6% in the tray with added nitrate and 16.9% in the tray with added phosphate.

The effect of the addition of nitrates against the addition of phosphate was not statistically significant ($p = 0.493$), and neither was the competitive nature of the environment ($p = 0.100$). Therefore, it can be concluded that for *Aquilaria microcarpa* seedlings, both hypotheses were disputed: rather than the addition of nitrate enhancing growth rates, the addition of either nutrient affected inhibited growth rates to a large extent, and the aspect of competition was not a large factor in determining growth rates.

As a primary rainforest species, *Aquilaria microcarpa* is only able to thrive in late stages of succession (Fern, 2018), so it was hypothesised that the species would thrive in soils with higher nutrient levels. However, nutrient levels in primary rainforest soils in Singapore are generally quite low -- approximately 0.94 - 1.31 mg g^{-1} nitrate and 0.05 - 0.06 mg g^{-1} phosphate-- due to high rates of loss through precipitation and leeching (Grubb, 1994). Thus, it is possible that by adding 0.1 M nutrient solutions to the soil, the nutrient levels deviated significantly from the ideal conditions for *Aquilaria microcarpa* growth*,* stunting seedling growth.

Although the ANOVA showed no statistically significant difference between growth rates with and without competition, the a difference in growth rates between *Aquilaria microcarpa* seedlings in competitive and non competitive environments did exist. The differences could be explained by *Aquilaria microcarpa* lack of aluminum accumulation abilities. In the trays with added nutrients, particularly phosphorus, *Melastoma* had high growth rates due to its aluminum accumulating abilities. As a result of *Melastoma malabathricum*'s high growth rates, less light and nutrients may have been available to the *Aquilaria* seedlings, which could have resulted in their diminished growth.

Cinnamomum iners

The growth rates of *Cinnamomum iners* were not affected significantly by changing the nutrient conditions, nor the change in competition. As no statistically significant differences existed, neither hypotheses regarding the effect of adding nutrients or competition on growth rates was supported. However, the mean percentage height change was the lowest in the non competitive environment (12.7%), and the greatest in the competitive environment with controlled soil (23.7%), suggesting that the *Cinnamomum* seedlings flourished slightly more in competitive environments.

These findings support previous research on characteristics of *Cinnamomum* species. As *Cinnamomum iners* is very versatile, it would be expected for it to thrive in a wide variety of conditions. Thus, altering of environmental characteristics did not seem to have significantly affected the growth of seedlings.

Melastoma malabathricum

The growth rates of *Melastoma malabathricum* seedlings were affected significantly by both the addition of phosphate ($p=0$) and the effect of competition ($p = 0.00017$). The growth rates were not affected significantly by the addition of nitrate, as the p value comparing the competitive tray with controlled soil to the tray with added nitrate was 0.502.

From examining the mean height changes in *Melastoma* seedlings, it is clear that the addition of phosphate had a comparatively large impact on growth rates. The mean change in height with phosphate addition was 107.1%, whereas the addition of nitrate caused a 21.9% mean change in height. The qualitative observations show concurrent findings, as in the tray with added phosphate, *Melastoma* seedlings grew visibly more than those of *Aquilaria microcarpa* and *Cinnamomum iners*. These findings support the first hypothesis: that the addition of phosphate results in the competitive dominance of *Melastoma malabathricum*. *Melastoma* is able to uptake large amounts of phosphate through secreting oxalate into the rhizosphere (Watanabe et al. 1998), which may have accounted for its significantly higher growth rates with a greater supply of phosphate.

This agrees with prior studies conducted on the competitive dominance of *Melastoma*: Davies and Semui's study found that *Melastoma* accounted for 73-79% of total biomass in controlled experiments with added nutrients.

The aspect of competition significantly decreased growth rates of *Melastoma malabathricum* seedlings, as the mean growth rate in the non competitive tray was 46.8%, whereas in the competitive tray with controlled soil, it was 16.6%. This finding did not support the second hypothesis: that competition would cause the dominance of *Melastoma*, and contradicted previous literature suggesting it would be expected for the *Melastoma* seedlings to thrive in competitive environments due to their ability to withstand high levels of aluminum toxicity. The significant decrease in growth rates with competition could be resulting from initial differences in seedling heights. *Melastoma malabathricum* seedlings were, on average, 5.28 cm when planted. By contrast, *Cinnamomum iners* seedlings were 6.68 cm. These height differences may cause the other seedlings to block light from reaching *Melastoma* seedlings, resulting in less *Melastoma* growth. However, when the *Melastoma* seedlings were surrounded by others of a similar height, they were given more equal access to light, resulting in higher growth rates. Additionally, the subsequent development of *Cinnamomum iners'* roots may have been greater due to the initial height difference, and since the layer of soil was 3.5cm, lateral root growth would have occurred, leading to more competition.

Evaluation and Further Investigation

The research project did not take into account the preexisting conditions that the seedlings were subject to. For example, *Melastoma* seedlings were found in an area of degraded soil, *Cinnamomum* seedlings were extracted from a mixed forest site with soil of a higher quality than that of the *Melastoma* site (as evidenced by common indicator species) and *Aquilaria* seedlings had been grown in the nursery for almost five months prior to beginning the experiment. These initial differences in nutrient availability may have affected the health of the plants and thus their relative growth rates.

Although only seedlings of certain heights were collected, the range of heights collected was quite large. Initial seedling heights ranged from 2cm to 10cm, and there were large differences between the average heights of the species. This may have impacted the conclusions drawn, as *Cinnamomum iners* seedlings were significantly taller than those of *Melastoma malabathricum* and *Aquilaria microcarpa*. Having seedlings of differing heights may have caused the shading of some seedlings by others, resulting in unequal access to light. Greater access to light may lead to more leaf development, and a positive feedback loop resulting in higher growth rates for the taller seedlings. To reduce inequalities in light access, seedlings collected could have been more similar in height.

The apparatus used in the experiment was generally highly accurate: volumetric flasks were used to prepare the nutrient solutions, mitigating uncertainties due to random error. However, the uncertainty of the ruler used to measure seedling heights was relatively large (±0.1 cm). Additionally, the gaps in the fan head used to sieve large soil particles were wide, resulting in large variations in the soil particle size. Thus, some areas of the soil may have had higher porosities than others, affecting the seedlings' nutrient uptake.

Although the experiment aimed to provide the same conditions for all seedlings, the seedlings were subject to natural environmental conditions. Thus, the growth rates of all seedlings differed from one another, shown from the large standard deviations of the data (ranging from 8.3 to 40.6). Additionally, because the seedling heights were quite short, even a small difference in height would amount to a relatively large difference in growth rates, thus yielding larger standard deviations.

The experiment gave a good indication of the species' dominance in the short term, but due to the timescale of the research project the long term relative growth rates were not able to be assessed. This temporal limitation may have affected conclusions about the competitive outcomes of nutrient addition to strands. As *Melastoma malabathricum* is a shrub, its presence in an area will fade as it gets shaded out by larger trees, such as *Aquilaria microcarpa* (Chua, Siew Chin, personal communication, 2018), therefore, in the long term the effect of nutrient addition may differ[9].

To further the study of how nutrient uptake is affected by competition amongst the three species, it would be interesting to study the effect of each individual species on the others. Two additional trays of the species in non competitive environments could be set up, and nitrate and phosphate solutions could be added. From this data, a greater understanding of the effect of each nutrient on the individual species can be gathered. This information would aid in better understanding the reasons behind the differing growth rates of the seedlings.

The data raised a number of additional potential research questions. For example, the layer of soil placed in the trays was 3.5 cm deep, which may have forced lateral growth of the roots and could have resulted in greater competitive interaction. To test whether soil depth has an effect on

[9] The experiment continued throughout subsequent months to assess the long term effects of nutrient addition/competition on species growth rates.

the relative growth rates of the species, seedlings in trays with varying depths of soil could be measured.

The purpose of this research project was to try and better understand the conditions that benefit the reforestation of native rainforest species, such as *Aquilaria microcarpa*. Although not all of the results were conclusive, the findings from the experiment may certainly be helpful in determining the most suitable conditions in ex situ nursery settings to cultivate rainforest species. The results of the experiment show that *Aquilaria microcarpa* seedlings grew significantly more without nutrient addition than with the addition of nitrates and phosphates. This suggests that rather than adding large amounts of fertilizer to nursery seedling trays, a better way to promote *Aquilaria microcarpa* growth would be cultivate seedlings in the natural soil conditions, even if degraded.

Reference List

Bailey et al. (1976). The Cinnamon & Camphor Genus. FAO, 2012.

Brunner, I., & Sperisen, C. (2013). Aluminum exclusion and aluminum tolerance in woody plants. Frontiers in Plant Science, 4, 172.

Corlett, R. (1991). PLANT SUCCESSION ON DEGRADED LAND IN SINGAPORE. Journal of Tropical Forest Science, 4(2), 151-161.

Davies, S., Semui, H. Competitive Dominance in a Secondary successional rainforest community in Borneo. Journal of Tropical Ecology (2006) 53–64.

Ecological Society of America, Ecological Monographs, 87(4), 2017, pp. 685–701

Fern, Ken (2018). Tropical Plants Database, tropical.theferns.info.

Google. (2018). [Map of Singapore]. Retrieved from http://maps.google.com

Grubb, P., Turner, I., & D. F. R. P. Burslem. (1994). Mineral Nutrient Status of Coastal Hill Dipterocarp Forest and Adinandra Belukar in Singapore: Analysis of Soil, Leaves and Litter. Journal of Tropical Ecology, 10(4), 559-577.

J.W. Dalling et al. (2016). Nutrient Availability in Tropical Rain Forests: The Paradigm of Phosphorus Limitation. *Tropical Tree Physiology*, 261-273.

National Parks Board Singapore, Melastoma malabathricum L. 2013.

Sim, J., Tan, H., & Turner, I. (1992). Adinandra Belukar: An Anthropogenic Heath Forest in Singapore. Vegetatio, 102(2), 125-137.

Soehartono. (1999). *Conservation and sustainable use of tropical trees in the genus Aquilaria I. Status and distribution in Indonesia*.

Tee, S. P., Young, W. K., & Ng, J. S. (2009). Trees of our garden city: A guide to the common trees of Singapore. Singapore: National Parks.

Watanabe, T., M. Osaki, T. Yoshihara and T. Tadano. 1998a. Distribution and chemical speciation of aluminum in the Al accumulator plant, Melastoma malabathricum L. Plant Soil 201:165–173.

World Conservation Monitoring Centre. 1998. Aquilaria microcarpa. The IUCN Red List of Threatened Species 1998:

Appendix

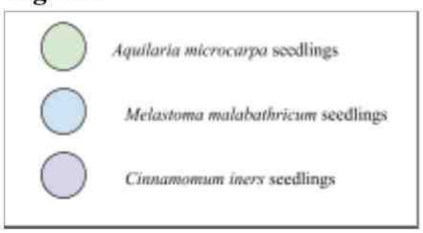
Apparatus List

Equipment	Quantity	Size	Uncertainty
Trays	5 (3 large, 2 small)	58x39 cm 37x41 cm	N/A
Gravel	3 bags	7x3 cm pieces	N/A
Mesh sheets	5 (3 large, 1 small, 1 extra small)	64x45 cm 43x47 cm 24x30 cm	N/A
Wooden board	1	37 cm	N/A
Melastoma malabathricum seedlings	52	2-10 cm	N/A
Aquilaria microcarpa seedlings	62	2-10 cm	N/A
Cinnamomum iners seedlings	58	2-10 cm	N/A
Trowel	2	N/A	N/A
Chunkel	2	N/A	N/A
Plastic bag	3	10x20 cm	N/A
Soil	3 bags (approx. 30 kg)	N/A	N/A
Fan head	1	55x55 cm	N/A
Ammonium Nitrate (s)	40 g	N/A	N/A
Sodium Dihydrogen Phosphate (s)	78 g	N/A	N/A

Weighing Scale	1	N/A	± 0.01 g
Volumetric Flask	2	500 ml	±0.25 ml
Dropping Pipette	2	5 ml	± 0.3 ml
Lux meter	1	N/A	± 1 lux
Ruler	1	30 cm	± 0.5 mm

Raw Data

Legend:



Collection 1: 22/06/18

Figure A.1: A table to show the raw data collected in week 1 of the experiment measuring heights of *Aquilaria microcarpa* seedlings in non competitive environment. (cm) (±0.1 cm)			
4.9	6.6	4.5	7.1
5.4	6.0	3.4	4.3
5.5	6.5	3.0	5.5

Figure A.2: A table to show the raw data collected in week 1 of the experiment measuring heights of *Melastoma malabathricum* seedlings in non competitive environment. (cm) (±0.1 cm)			
2.6	4.1	3.5	4.7
2.6	4.7	3.5	4.4
7.3	6.9	5.8	4.2

Figure A.3: A table to show the raw data collected in week 1 of the experiment measuring heights of *Cinnamomum iners* seedlings in non competitive environment. (cm) (±0.1 cm)			
5.5	7.5	7.5	7.0
6.0	5.0	6.0	6.5
5.9	5.5	6.5	6.5

Figure A.4: A table to show the raw data collected in week 1 of the experiment measuring heights of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a competitive environment with controlled soil. (cm) (±0.1 cm)							
4.0	5.0	3.5	6.9	6.1	6.0	6.9	8.0
7.7	6.3	3.9	6.8	6.8	7.6	6.6	6.5
4.5	4.2	5.9	4.0	3.0	6.3	4.8	4.7
6.5	4.5	3.0	4.0	8.0	6.0	3.6	9.2
9.2	4.0	5.0	6.6	5.8	3.0	5.6	5.4
4.0	5.0	9.0	8.0	5.0	9.0	7.0	3.0

Figure A.5: A table to show the raw data collected in week 1 of the experiment measuring heights of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a competitive environment with added ammonium nitrate. (cm) (±0.1 cm)					
3.0	3.0	6.7	8.2	6.8	dead
3.0	5.2	6.5	7.8	7.9	7.0
7.5	6.8	3.7	5.7	6.4	5.4
5.0	4.0	8.7	2.9	4.9	6.6
6.0	6.5	4.8	4.6	8.1	8.5
8.0	5.0	4.5	6.7	7.3	4.3

Figure A.6: A table to show the raw data collected in week 1 of the experiment measuring heights of *Aquilaria microcarpa, Cinnamomum iners, and Melastoma malabathricum* seedlings in a competitive environment with added disodium phosphate. (cm) (±0.1 cm)					
4.4	3.3	6.9	3.9	5.9	7.1
5.4	7.1	4.1	4.4	4.4	5.9
7.6	dead	2.9	6	5.3	6.3
5.4	4.5	6.4	3	5.2	4.9
4.6	6.2	4.2	2.9	5.6	6.7
7.6	3.9	5.5	5.9	5.1	5.1

Collection 2: 27/07/18

Figure A.7: A table to show the raw data collected in week 5 of the experiment measuring heights of *Aquilaria microcarpa* seedlings in non competitive environment. (cm) (±0.1 cm)			
6.0	8.3	6.2	8.3
6.9	6.8	5.5	5.5
6.5	7.6	4.5	6.2

Figure A.8: A table to show the raw data collected in week 5 of the experiment measuring heights of *Melastoma malabathricum* seedlings in non competitive environment. (cm) (±0.1 cm)			
4.1	6.8	4.4	5.4
3.0	7.4	5.6	6.5
8.6	13.0	8.6	6.8

Figure A.9: A table to show the raw data collected in week 5 of the experiment measuring heights of *Cinnamomum iners* seedlings in non competitive environment. (cm) (±0.1 cm)			
6.8	7.6	7.8	7.7
7.6	5.4	6.1	7.0
7.0	7.0	6.7	7.8

Figure A.10: A table to show the raw data collected in week 5 of the experiment measuring heights of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a competitive environment with controlled soil. (cm) (±0.1 cm)							
7.0	5.2	4.3	6.9	8.1	6.9	7.0	dead
8.0	6.9	5.3	7.8	8.3	8.3	8.7	8.4
6.7	6.3	7.1	4.3	6.9	10.7	5.8	7.9
6.7	7.0	3.0	5.7	8.4	6.2	4.9	10.4
10.5	4.4	7.1	7.5	6.7	4.4	7.1	6.1
4.9	6.5	10.9	8.7	6.5	11.6	dead	4.8

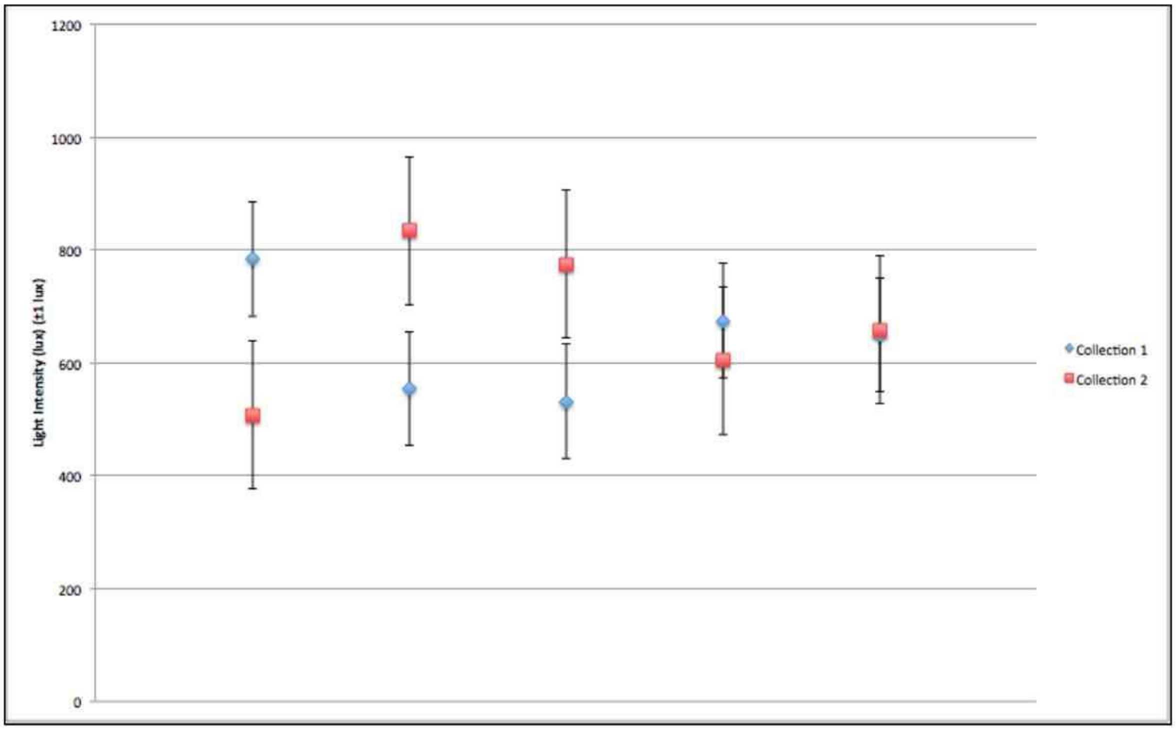
Figure A.11: A table to show the raw data collected in week 5 of the experiment measuring heights of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a competitive environment with added ammonium nitrate. (cm) (±0.1 cm)					
4.4	3.3	8.0	9.4	8.2	dead
3.6	6.6	7.3	8.0	8.9	8.0
8.6	7.8	4.5	8.3	7.5	6.2
5.9	6.3	dead	3.5	5.4	7.0
7.5	7.9	7.1	6.3	10.5	9.4
8.5	6.1	6.0	7.1	9.0	5.1

Figure A.12: A table to show the raw data collected in week 5 of the experiment measuring heights of *Aquilaria microcarpa*, *Cinnamomum iners*, and *Melastoma malabathricum* seedlings in a competitive environment with added disodium phosphate. (cm) (±0.1 cm)					
11.7	4.3	7.7	6.9	7.1	7.5
5.6	8.5	10.6	4.8	6.4	8.6
9.0	dead	3.7	6.7	10.9	6.8
11.5	5.6	7.7	6.2	6.0	6.9
5.5	7.0	6.7	3.5	6.3	16.5
10.0	7.5	6.3	dead	dead	5.6

Monitored Variable Data

Figure A.13: A table to show raw and processed quantitative data collected on light intensity (lux) (±1 lux)		
Raw measurements	**Collection 1**	**Collection 2**
	784	508
	555	834
	532	775
	675	604
	650	659
Mean	639	676
Standard Deviation	101	131

Figure A.14: A graph to show light intensity measurements taken at 9am on dates of data collection. Error bars show standard deviation.



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Extended essay - Reflections on planning and progress form

Candidate: *This form is to be completed by the candidate during the course and completion of their EE. This document records reflections on your planning and progress, and the nature of your discussions with your supervisor. You must undertake three formal reflection sessions with your supervisor: The first formal reflection session should focus on your initial ideas and how you plan to undertake your research; the interim reflection session is once a significant amount of your research has been completed, and the final session will be in the form of a viva voce once you have completed and handed in your EE. This document acts as a record in supporting the authenticity of your work. The three reflections combined must amount to no more than 500 words.*

The completion of this form is a mandatory requirement of the EE for first assessment May 2018. It must be submitted together with the completed EE for assessment under Criterion E.

Supervisor: *You must have three reflection sessions with each candidate, one early on in the process, an interim meeting and then the final viva voce. Other check-in sessions are permitted but do not need to be recorded on this sheet. After each reflection session candidates must record their reflections and as the supervisor you must sign and date this form.*

First reflection session

Candidate comments:

I plan to examine the impact of soil degradation on growth and nutrient uptake of different rainforest species. I chose a biolog y essay to allow for an in depth focus on natural systems and apply the findings towards conservation.

At school, I'm part of a practical Rainforest Restoration activity. My findings may aid the project in understanding the importa nce of choosing non degraded sites for restoration, and the extent to which this varies between species.

I hope to get permission from the National Parks Board for material collection from national parks. This may raise ethical con cerns, but the collected species will be raised rather than harvested for biomass.

I plan to collect primary data by growing rainforest species under varied soil conditions, comparing the results of varied soil q uality on two or three species (depending on seedling availability). I will supplement my findings with secondary research pa pers on South East Asian reforestation, however there is a dearth of research in reforestation in this region to finding appropr iate sources may prove challenging. Additionally, I may face challenges in refining my research question, and using seedling size as a controlled variable.

Date: May 9 2018

Supervisor initials:

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Interim reflection

Candidate comments:

As my research has progressed, I have altered my research question. The species investigated have changed due to limitations in accessing seedlings and the question now focuses on the competitive interactions between rainforest species, as understanding interactions between the species would provide greater insight on mechanisms for effective reforestation.

I have read numerous research papers on conditions needed for the growth of the different species investigated, as well as a particularly helpful paper on the results of the competitive interactions between Melastoma malabathricum and other rainforest species. Additionally, I have worked with a researcher from NUS, who has been invaluable in providing information about different rainforest species and Singapore's natural history.

I have planted the seedlings, and will start the experiment next week by applying the first dose of nutrients. Over the summer, I will be able to write the introduction and methodology of the essay, but will need to wait until after the experiment is complete (upon returning to school) to finish the draft.

Date: June 18 2018 Supervisor initials:

Final reflection - Viva voce

Candidate comments:

Through the process of writing my EE, I arrived at several conclusions, some of which differed from my expectations. Due to my research I expected certain species to thrive in competitive environments and others to fare relatively worse, however the results showed the opposite of what I anticipated.

I've made inferences as to what may have caused the discrepancies between my results and pre-existing literature, and, having met with a Biologist, have come to the conclusion that these inferences were likely correct.

In order for my research to add to "reforestation" knowledge, further analysis is needed. I plan to further analyse my data to compare the growth rates of each species in each environmental condition to assess each species' relative dominance in each condition. As the Singaporean government is planning reforestation of secondary forest, my findings could be helpful in aiding their efforts.

Date: November 14 2018 Supervisor initials: