Getting to the Root: The Effects of Iron and Light on *Avicennia germinans* in Drought

Janice Yohannan
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Abstract

Mangroves, salt-tolerant wetland trees in the tropics and subtropics, provide many services to humans and the environment. Yet, as they are rapidly declining, examining how mangroves respond to stressors like water scarcity is essential for conservation efforts. Previous research has shown that iron is an important nutrient for mangroves, and that the *Avicennia germinans* (black mangrove) grows better in areas with greater light. However, it has been suggested that some nutrients are ineffective with high shade or aridity. The purpose of this study was to see how light affects the efficiency of iron as a micronutrient in the *Avicennia germinans*, and find its optimal conditions of iron and light under the stress of water scarcity. Through a full factorial design, with shade cloths and iron fertilizers as treatments, stem height, leaf number, and leaf color were recorded over a period of 3 months for young *Avicennia germinans* seedlings. A stressor period simulating a drought was also included for approximately 3.5 weeks. After the 3 months, each plant was harvested for wet biomass measurements. While light did not seem to significantly impact growth, the plants that received no additional iron fertilizer were healthiest across all parameters measured. The addition of iron fertilizer inhibited growth during the stressor period, and high concentrations of iron were shown to be toxic. Future research should be conducted in the field to further examine mangrove light tolerance in drought, as well as nutrient tolerance in the face of different hydrologic stressors.

I. Introduction

While mangroves are foundational pillars in thousands of communities and ecosystems worldwide, conservation and restoration efforts are becoming more urgent for these wetland plants. Mangroves are trees found in saline and brackish water throughout the tropics and subtropics. They provide a nursery area for many fish, filter pollutants, stabilize shorelines, and mitigate effects of strong winds and storms. Mangrove wood is even economically valuable to many local communities (Ewel, K.C. et al., 1998). Mangroves are vital ecosystems in terms of mitigating climate change, as well. Since they are productive carbon sinks, if mangrove forests are lost, the amount of carbon released could not be recovered by 2050 - when climate change becomes irreversible (Goldstein et al., 2020).

Yet, mangroves have been rapidly declining mostly due to anthropogenic stressors. Aquaculture and coastal development have been large contributors to mangrove deforestation. In fact, 20-35% of mangroves have been lost in the past 50 years (Polidoro et al., 2010). It is also important to consider that with the changing climate, mangrove mortality is a growing concern, considering extreme hydrologic changes like drought or flooding. In fact, drought conditions can lead to a decreased seedling establishment rate (Hoppe-Speer et al., 2013). Considering the need for restoring mangroves in ecosystems, conservation and planting efforts are being encouraged. It is vital to monitor the environmental conditions in which mangrove species in specific regions thrive, so these planting efforts are more successful. Moreover, examining these environmental factors under increasingly prevalent stressors like drought is crucial to identifying more vulnerable populations.

Halophytes (salt-tolerant plants) like mangroves are typically known to be shade intolerant, although some species of mangroves grow faster under shaded conditions (Dangremond et al., 2015). In particular, the *Avicennia germinans,* also known as the black mangrove, has been shown to prefer greater light availability in their early stages of growth (Pickens et al., 2018). Moreover, mangroves are influenced by macro- and micronutrients. Specifically, it has been determined that iron, a micronutrient, aids in the growth of mangroves (Alongi 2010), as it is involved in the photosynthesis process, specifically in chlorophyll synthesis. Iron deficiency can result in chlorosis, where plants cannot produce enough chlorophyll, which can be identified through leaves appearing more yellow in color. Nutrient deficiency can also lead to higher root-shoot ratios, with more mass allocated to the roots than typically healthier plants, as well as a comparatively smaller leaf area (Kang and Van Iersel, 2004).

However, data has suggested that some nutrients are somewhat ineffective with high salinity, aridity, and shade depending on species or other environmental conditions (Krauss et al., 2008). Since mangrove photosynthetic productivity simultaneously depends on the preferred thresholds of iron and light, it is important to see how these two variables act together for optimal growth. What are the optimal light intensity and iron concentration combinations for the growth of black mangroves (*Avicennia germinans*) when facing drought? How does light affect the efficiency of iron as a nutrient for the black mangrove? It is hypothesized that the presence of moderate concentrations of iron and high light availability will result in the highest growth rate in black mangroves in low water levels. Moreover, high concentrations of iron may decrease survivorship as the excess iron may become toxic. This information may help determine where to transplant mangroves and the effects of adding fertilizers with proper nutrients to enhance growth. It may also be helpful to decipher which areas, especially in drying mangrove forests, may be more vulnerable due to unfavorable light and nutrient levels, or iron-reducing bacteria. Ultimately, these results will add to the growing body of knowledge on environmental drivers for mangrove growth and the interactive effects of light and nutrients, which will aid scientists in taking action for effective mangrove conservation.

II. Methodology

Greenspace Set-Up

The experiment was conducted at Stony Point Center (Stony Point, NY) with the two variables, light and iron. Forty *Avicennia germinans* seedlings were bought from a Louisiana wetland vendor and transported to New York. They were planted in 0.6 gallon pots. The pots, composed of 50:50 sand and potting soil, were placed in larger containers filled with water. With the drainage holes in the bottom, the soil was able to be inundated with saltwater. The salinity of the water was altered to 27 ppt using Instant Ocean Sea Salt and was monitored every few days.

Light Treatment

Half of the 36 plants used for the experiment were open to direct sunlight (S1), while half of them had shade cover (S2), imitating a canopy (**Figure 1b**). The plastic on the hoop house was removed so that natural sunlight could be blocked by the canopies. With the plastic, the light would have been likely distributed around the greenhouse. The three canopies were made of big pieces of mattress covers, and the covers were held up by cut pieces of bamboo acting as stakes. Rope was attached to the ends of each bamboo stake and the mattress cover was clothespinned over six plants in a row (**Figure 1b**).

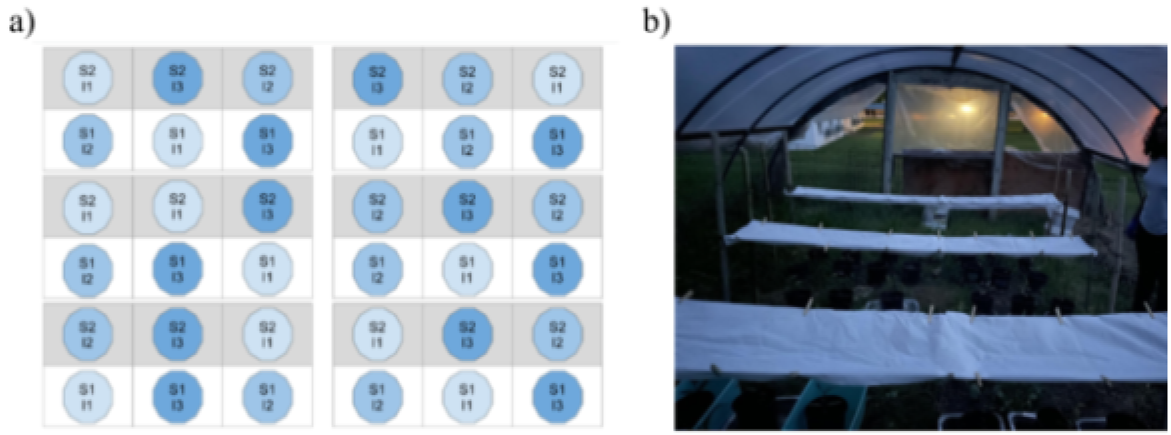


Figure 1a. Schematic design of set-up, with each group randomized to limit external factors affecting growth. S1 represents no shade, while S2 is for the pots that were shaded. I1 shows no iron treatment, I2 low iron treatment, and I3 high iron treatment.

Figure 1b. Image of the constructed shade cover, prior to all of the pots being in larger containers.

Iron Treatment

Three iron levels were tested. One level received no additional iron (I1), one received the amount recommended by the fertilizer (I2), and the third level had the plants receive double the amount recommended (I3). The iron was in the form of Fe-EDTA and added to water for plant uptake. There were two plants in each group of six that received the same iron treatment, resulting in a total of 12 plants for each iron level. However, determining which plants got which iron treatment was randomized to increase independence of each individual (**Figure 1a**).

Drought Stressor Period

On day 19, a stressor of lower water levels was applied to every plant. This was to mimic drought conditions. Rather than ensuring the plants were inundated with brackish water every 3-4 days, water levels were not monitored for about 7 days at a time. The stressor period ended on day 42.

1	2	3	4	5	6	7	8	9	10	11	12	13
dark brown	brown	bronze	bronzing	dull yellow	yellow	dull green	chartreuse	green and yellow	light green	bright green	true green	deep green

Figure 2. Leaf color chart for quantitative data analysis

Data Collection and Analysis

Stem height was measured biweekly with a ruler, as well as the number of leaves in each plant. Along with those measurements, color was noted as well as any signs of fungus or herbivory. Color was quantified using a handmade rating system (**Figure 2**), with higher numbers indicating a darker green, and lower numbers indicating a lighter or browner color. After 12 weeks, the plants were rinsed of soil, dried, and placed in individual paper bags. Leaves that had fallen off were also collected as dead biomass, to compare live-to-dead biomass ratio. Using a scale, biomass of each individual was measured. The roots and stem of each plant was also cut and were weighed separately to calculate above- and belowground biomass. A two-way ANOVA test was used in Excel to examine differences in biomass, net change in stem height, and net change in leaves.

III. Results

a) b)

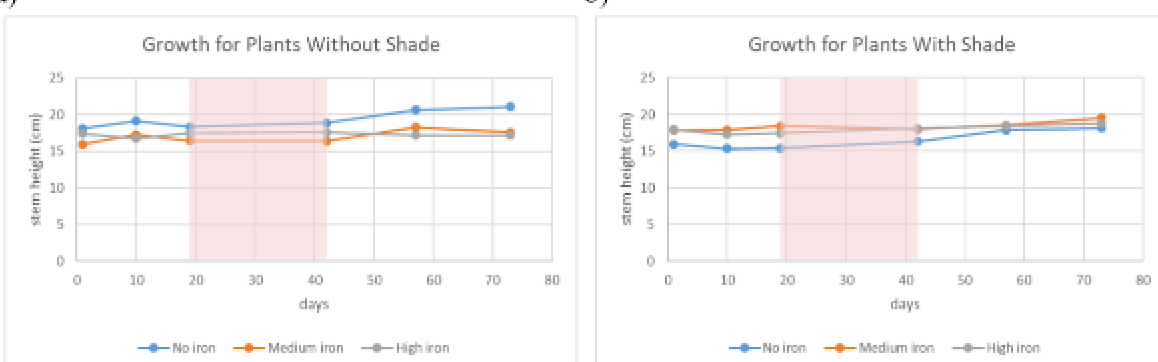


Figure 3. Graph showing stem height growth across duration of experiment. Highlighted portion represents the stressor period of water scarcity.

Stem Height

During the drought period, the mangroves in every group experienced little growth. However, following the drought period, the plants that grew the most were the ones that did not receive any iron treatment, followed by the ones that received some iron (**Figure 3**). The plants that received the high concentration of iron experienced minimal to no growth after the stressor period.

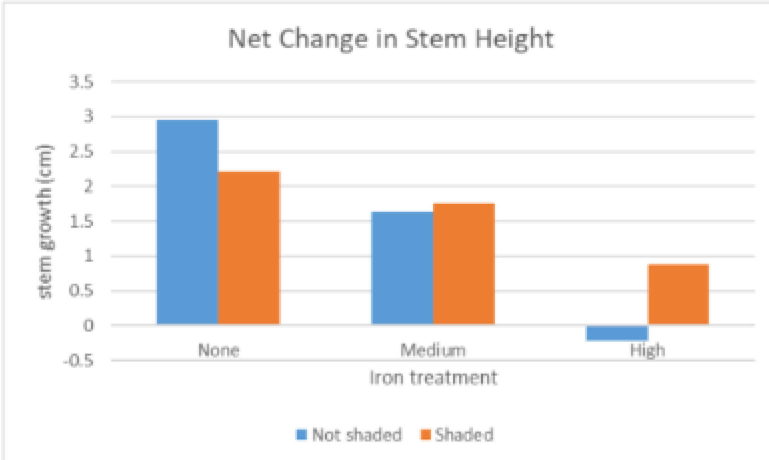
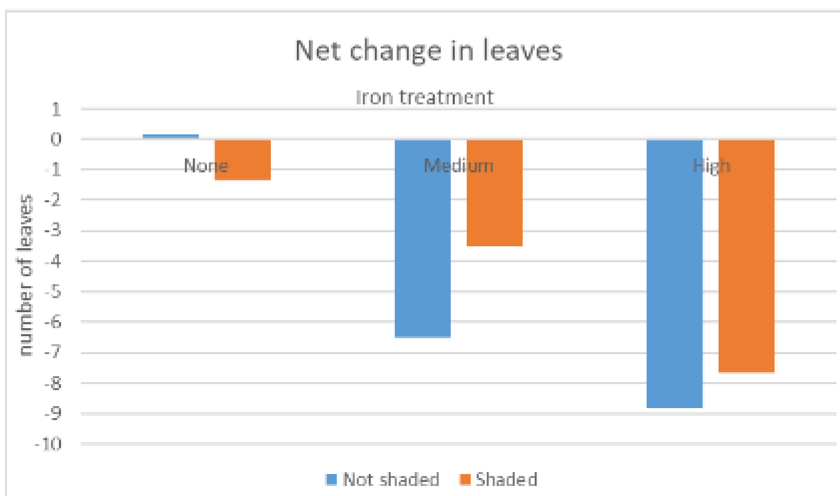


Figure 4. Graph of the net change in stem height from week 1 to week 11. P-value between iron treatments = 0.059653; p-value between light levels = 0.829777; p-value for interaction between light and iron = 0.60653.

The mangroves that had no iron added experienced the most growth over the course of the entire experiment, and the ones that received a high level of iron experienced the least amount of growth (**Figure 4**). While growth was ultimately higher for the plants without iron fertilizer and high light intensity, whereas the plants with iron fertilizer performed better in shaded conditions, the p-values between light levels was 0.829777, indicating that there was no statistically significant difference between the growth of the plants with and without the shade treatment. The p-value for the interaction between iron and light was 0.60653, also above the significance level (0.05). The p-value between iron treatments was 0.059, only slightly above significance level.

Figure 5. Graph showing net change in leaves from Week 1 to Week 11. P-value between iron treatments = 0.001034; p-value between light levels = 0.559125; p-value for interaction between light and iron = 0.479272.



Leaf count

In the case of both light treatments, the plants with the highest iron concentration experienced the most drastic reduction of leaves in comparison to the other iron treatments. (**Figure 5**). According to **Figure 5**, when given iron fertilizer, the seedlings with shade dropped less leaves than the seedlings without. However, shade did not appear to actually affect the number of leaves gained or lossed, as the p-value (=0.559125) was above significance level.

Leaf color

a) b)

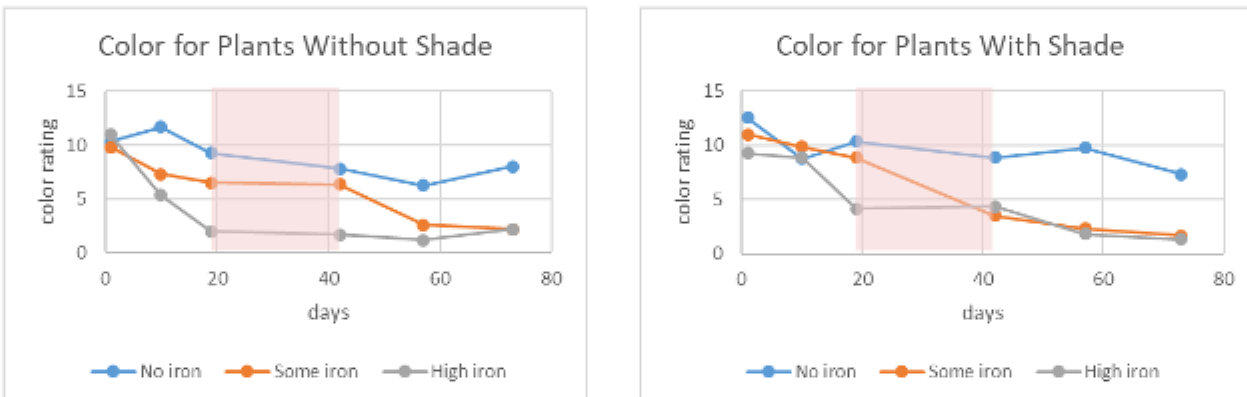


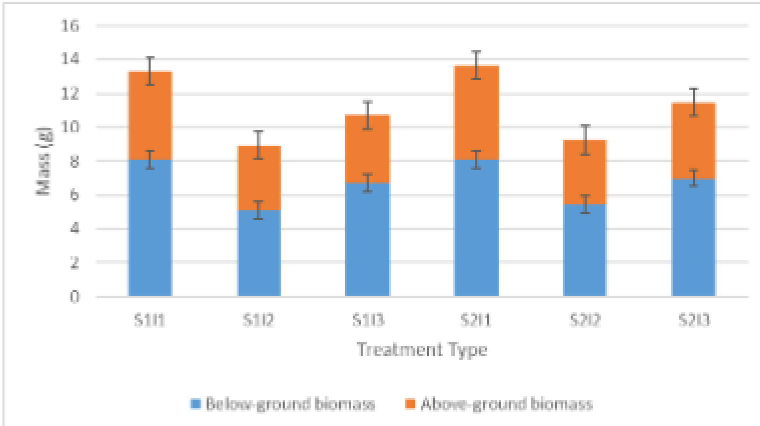
Figure 6. Line graph of color throughout duration of experiment.

Immediately after the treatments were applied, leaf color noticeably changed for plants with high iron concentration, while color remained relatively constant for the other treatment groups. However, once the stressor period began, the plants with iron fertilizer (regardless of concentration) became more bronze and decreased on the color scale (**Figure 6**).

Biomass

Across all 6 groups, the plants with the highest overall mass were typically the plants that received no additional iron, but neither iron (p-value = 0.629169) or light (p-value = 0.806475) appeared to have an effect on biomass. The proportion of aboveground and belowground biomass were approximately equal across every group, as well (**Figure** 7). It is important to note that while the ratio was the same, leaf count was different across groups, possibly indicating that some leaves may have been thicker or heavier for plants with higher iron concentration.

Figure 7. Biomass across groups. S1 - not shaded; S2 - shaded. I1 - no additional iron; I2 - medium iron concentration; I3 - high iron concentration. P-value between iron treatments = 0.629169; p-value between light levels = 0.806475; p-value for interaction between iron and light = 0.966135.



IV. Discussion

Generally, in every parameter measured, the seedlings with no additional iron grew and maintained a healthy leaf color to a greater extent than the other treatment groups. Only the differences in iron concentrations ultimately appeared to be significant, but further research should be completed with more replicates to more closely examine how shade affects black mangrove growth. This is encouraged as the data from this study is in conflict with a vast portion of the literature on light and the *Avicennia germinans*. Light intensity levels with and without the shade cover should also be measured. In terms of final biomass, the proportion of aboveground and belowground biomass appeared relatively the same across all treatment groups, but considering that leaf count was greater for certain treatment groups, additional analysis should be done analyzing leaf thickness and weight, an important indicator in determining carbon sequestration rates.

One reason for the reduction in leaves in most plants was likely the reduced water intake. After day 19, the stressor of lower water levels caused a drastic change in the growth and health of most seedlings. This is in line with previous research (Hoppe-Speer et al., 2013) that has shown water scarcity decreasing mangrove seedling establishment. The first plants to lose their greenish color and become bronze were the plants with the highest iron treatment (I3), regardless of shade. However, after day 19, while there was no significance between light levels in any of the measurements, the seedlings that appeared to retain their color were slightly more likely to be shaded. It is possible that in this experiment, shade provided cooler temperatures for the mangroves, leading to a slower evaporation rate. More water evaporating also could have led to salinization; leftover salt from the saline water may have stayed in the pot as the water had evaporated, causing a high, toxic salinity to shock the plants. Future research examining light levels and water availability in plants should record the temperature of the microclimate under a canopy, as well as the temperature without any shade. Soil salinity should also be measured to evaluate salinization.

Furthermore, some of the plants with the I2 treatment only started significantly shedding leaves after Week 3. Further analysis must be run to support this, but it is possible the addition of iron may have become more damaging to the plant after the lack of water. If not toxic, the plants did not grow significantly compared to the ones without any iron treatment, which may suggest that conditions like aridity decreased the efficiency of the nutrient. Future research should involve finding a specific threshold for iron tolerance, and investigate further whether limited water will decrease iron tolerance. In terms of stem height growth, while mostly all of the seedlings grew an insignificant amount during the stressor period, the plants that received no iron or some iron grew afterwards, while the plants with the high concentration of iron did not experience any growth. This may allude to a recovery period for the plants, as long as they are not exposed to toxic levels of nutrients.

V. Conclusions

The purpose of this study was to see how light affects the efficiency of iron as a micronutrient, and find the optimal conditions of iron and light for the *Avicennia germinans* under the stress of water scarcity. While there was no data to suggest an interaction between light and iron, trace amounts of iron appeared to optimize seedling growth. Furthermore, while there was not enough data to show the effect of shade on growth in the face of drought conditions, it is possible that shade decreased the stress of plants from high water scarcity, and future research should further investigate this observation.

Drought poses a large challenge for the growth of young *Avicennia germinans*. As climate change exacerbates the increase in frequency and intensity of drought, studies observing mangrove response to water scarcity should be conducted in the field itself. Conservation efforts should target areas that might be vulnerable to drought, especially the places that are overexposed to light. Moreover, hydrologic changes should be carefully monitored along coastlines and swamps, and ways to mitigate the impacts of drought in these ecosystems should be greatly considered. Replanting efforts should also take into account areas in the intertidal zone that will be properly inundated with water so as to not stress newly planted mangrove seedlings.

Mangroves are vital to communities, ecosystems, and the environment. While these plants are champions in sequestering carbon and thus mitigating climate change, droughts, intensified by climate change, pose a threat to their survival. More attention for wetland ecosystems like mangrove forests should be given in order to effectively maintain the important functions they serve to the earth.

VI. References

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