NUTRIENT EFFECTS ON LEAF BIOMASS ALLOCATION AND HERBIVORY ACROSS TROPICAL FOREST SUCCESSION

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Abstract. Biomass allocation is a strategy used by plants to overcome resource constraints. Plants can change their biomass allocation in fine roots and wood, but it is unclear if biomass allocation to leaves can be plastic. To determine if tropical trees can adjust leaf biomass allocation to address nutrient limitation, we analyzed nutrient effects on canopy leaf biomass—calculated by multiplying the leaf area index (LAI) and leaf mass per area (LMA)—over forest succession in Agua Salud, Panama. We used generalized linear mixed models (GLMM) to determine the effects of nutrient addition, forest age, and the forest landscape on LMA, LAI, leaf herbivory and leaf biomass. Our results showed that there are no significant effects of nutrient addition and forest age on LMA, LAI, leaf herbivory, and leaf biomass ($Pr(>F)$ and $p > .05$). These data suggest that plants do not adjust biomass allocation to leaves in response to nutrient additions and across forest age. Our findings are consistent with studies which have found that plants typically adjust allocation in fine roots and wood, but not to leaf biomass. This study contributes to our current understanding of plant nutrient strategies in tropical forests and supports carbon sink efforts such as reforestation and recovery.

INTRODUCTION

Tropical forests are a principal driver of Earth's climate system. A primary function of tropical forests is to offset anthropogenic CO_2 emissions. Although tropical forests only cover 7% of Earth's surface, they serve as a major terrestrial carbon sink, storing roughly ~70% of global carbon stocks (Pan et al. 2011; Wright 2019). Moreover, the tropical carbon sink is influenced by plant responses. Plant growth and productivity require nutrients, mainly nitrogen (N) and phosphorus (P) (Evans 1972). However, projections show that N and P availability will not be able to meet tropical nutrient demands in the future. Limitation by N and P could result in decreased carbon sequestration in forests (Poorter et al. 2011; Wieder et al. 2015). Therefore, analyzing plant functions in tropical forests and their responses under nutrient constraints and across forest succession will help reveal a better picture of the future tropical carbon sink. A major challenge is understanding how plants in tropical forests respond when there are nutrient constraints and particularly in how these responses shift the carbon sink.

Plants have developed various strategies to overcome nutrient limitation, but we do not know how these strategies are utilized across forest succession. Plant responses to nutrients can be dependent on forest age. Successional forests tend to be N-limited while intact old-growth forests tend to be P-limited or co-limited by N and P (Batterman et al. 2013; Nagy et al. 2017). Nutrient limitation is also stronger in successional forests than in old-growth forests (Wright 2019). Moreover, studies have indicated P to be the most significant driver of plant responses in tropical forests (Walker and Syers 1976; Vitousek 1984; Alvarez-Clare et al. 2013). Biomass allocation is one strategy that plants use to overcome nutrient constraints. Plants use this strategy to change the amount of biomass located in different organs like wood and fine roots which influences the uptake of carbon. However, it is still unclear if trees can adjust leaf biomass as a strategy to overcome nutrient limitation. In low nutrient conditions, plants may allocate less to leaf biomass and more to fine roots to get nutrients. Conversely, in high nutrient conditions, plants may allocate to leaf biomass to build more leaves, capturing more carbon and increasing photosynthesis (Poorter et al. 2011; Waring et al. 2019).

Whether or not biomass allocation to the leaves is plastic remains unclear. This is a significant question to address as nutrient limitation could affect leaf characteristics like leaf mass per area (LMA) and leaf area index (LAI) may affect photosynthesis and thus carbon sequestration. LMA is a measure of leaf thickness and tissue density and plays an important role in herbivory defenses (Wright et al. 2002, Poorter et al. 2009). LAI is a metric for the number of leaf layers in a canopy (Watson 1947). There is an initial increase in total photosynthesis with added leaf layers, but there are diminishing returns to a point where adding leaf layers allows little light and no additional photosynthesis. However, the addition of nutrients such as N may increase the rate of photosynthesis while sustaining carbon stocks in leaves (Poorter et al. 2009; Dybzinski et al. 2011).

Nutrient additions can also have an effect on leaf herbivory, which can also reduce leaf biomass. Leaves in tropical forests are typically protected from predators because of their low nutritional quality and dense tissue (Coley and Barone 1996). Herbivores generally prefer more nitrogen-rich leaves to meet their nutritional requirements. Nutrient additions—particularly N—increase leaf nutrient richness which could lead to increased leaf herbivory. Leaves may also adjust LMA in response to nutrient additions to increase toughness and defense against herbivores. Collectively, LMA, LAI, and leaf herbivory could have a profound effect on leaf biomass which reflects the ability of a forest to sequester carbon (McCarthy et al. 2009; Dybzinski et al. 2014).

Currently, knowledge of how LAI and LMA contribute to leaf biomass allocation and how in turn leaf herbivory affects leaf biomass in response to nutrient dynamics are limited. The possibility that nutrient constraints limit carbon sequestration in tropical forests poses a global ecological threat (Wieder et al. 2015). A mechanistic understanding of plant responses to nutrient additions will, therefore, be useful in maximizing future tropical carbon sink efforts such as reforestation and recovery. This study determines if tropical trees can adjust their leaf biomass as a strategy for overcoming nutrient constraints over forest succession, and whether nutrients affect herbivory rates on leaves. We analyzed hemispheric canopy photos and leaf scans to understand the effects of nutrient constraints and forest age on LAI, LMA, leaf herbivory and leaf biomass. Results from this study will also help answer the broader question of whether or not nutrients limit the carbon sink in tropical forests. We hypothesize that 1) Tropical trees adjust leaf biomass to overcome nutrient constraints over forest succession and that these 'adjustments' are less pronounced in old-growth forests than in successional forests 2) LAI increases with forest age and LMA is constant across forest age 3) LAI and LMA respond strongly to P across forest age and 4) leaf herbivory is highest in young forests and N plots.

METHODS

Data Collection

Data was collected from a 5-year fertilization experiment conducted with collaborators at the Smithsonian Tropical Research Institute. The study was designed to understand tropical forest responses to nutrient limitations and nutrient strategies across forest age. Seventy-six 0.1 hectare forest plots in Agua Salud are fertilized with N, P, or NP four times a year or left un-fertilized (control) (*Fig. 12*). To estimate LMA, five to ten leaves from each of the ten largest trees in every plot was collected, scanned to measure leaf area, dried at 65°C, and weighed. To estimate LAI, hemispheric canopy photos were taken of each plot in August and September 2019. Photos were taken during early morning or on cloudy days. Five photos were taken of each forest plot ≥10 years and ten photos were taken of each pasture plot.

Leaf Mass per Area (LMA) Assessment

Leaf area was calculated by analyzing leaf scans in a Java-based image processing software, ImageJ (https://imagej.nih.gov/ij/). Leaf scans were uploaded to the software and a macro was installed to identify the total leaf area, leaf area lost to herbivory, and the number of leaves in each photo. For leaves with herbivory, missing edges were drawn in using the pencil tool before calculating for area. The total leaf area was then divided from the dry mass of the leaves in each leaf scan to calculate LMA.

$$Leaf\ Mass\ per\ Area\ (g/cm^2) = \frac{leaf\ dry\ mass}{total\ leaf\ area}$$

Leaf Herbivory Assessment

Leaf herbivory was calculated by dividing the herbivory area by the total leaf area then converting to a percentage:

$$Leaf\ Herbivory\ (\%) = \frac{herbivory\ area}{total\ leaf\ area} * 100$$

Leaf Area Index (LAI) Assessment

Leaf area index was calculated by analyzing hemispheric canopy photos in Hemisfer (https://www.schleppi.ch/patrick/hemisfer/). Photos were analyzed using an adapted version of the standard Hemisfer LAI Analysis protocol (Dahlsjö et al. 2017). The following changes were made: RGB values were selected using ColorPix and then converted to percentages for input in Hemisfer, the "Lens" option was set to Nikon FC-8, and Center X, Center Y, and Radius was set to 1190, 840, and 800, respectively.

Leaf Biomass Assessment

Leaf mass per area data was converted into units of kg/m^2. Leaf mass per area and leaf area index data were collated by transect. Leaf biomass was then calculated as follows:

$$Leaf\ Biomass\ (kg/m^2) = Leaf\ Mass\ per\ Area \times Leaf\ Area\ Index$$

Statistics and Data Analysis

The effects of nutrients, forest age, and their interaction on LMA, LAI, leaf herbivory, and leaf biomass were analyzed in R using generalized linear mixed models (GLMM) with the plot nested within the site added as a random effect. Models were built using three variables (nitrogen, phosphorus, and forest age), two model types (aov and lmer), and two data levels (TreeID and Transect). Q-Q and residual plots were graphed to determine residual distribution and model fit. Data were transformed logarithmically to determine if there was a non-symmetric distribution or skew. Herbivory violated the assumptions of ANOVA, so we used both a logistic model and Kruskal-Wallis test to determine the effect of treatment and forest age on herbivory. We also used a GLMM with a gamma distribution with the fixed and random effects specified earlier to test the effects on herbivory rates. The Pr(>F), p, R^2m, and R^2c values from each of their respective models were recorded.

RESULTS

Leaf Mass per Area

Mean LMA ranged from 0.0080-0.016 g/m^2 with the smallest range across treatment in forest age 10 plots (*Fig. 1A-B*). NP plots trended slightly towards increasing across forest age but was not statistically significant. Control plots across all successional ages except forest age 10 were higher than nutrient treated plots. Mean LMA was highest in the forest age 30 control plots (mean LMA = 0.016 g/cm^2, *Fig. 1B*). LMA decreased in the forest age 30 plots in response to N, P, and NP by a factor of three. There were no significant effects of treatment and forest age on LMA (Pr(>F) > .05, *Table 1*).

Leaf Herbivory

Mean leaf herbivory rates ranged from 0.72-1.8% across all the plots, with some of the highest herbivory rates found in the P addition plots in the pasture (forest age 0) (*Fig. 2B*). Similar to LMA, the range of leaf herbivory was smallest for plots in forest age 10. Leaf herbivory increased in forest age 30 plots in response to P and NP by a factor of 2. There were no significant effects of treatment and forest age on leaf herbivory ($Pr(>F) > .05$, *Table 1*). A lmer logistic model showed similar p-values ($p > .05$, *Table 1*) to the Kruskal-Wallis test which supports no significance between treatment and forest age on leaf herbivory.

Leaf Area Index

Mean LAI had a range of 4.2-7.0 m^2/m^2 across all plots with the highest LAI values in the P treated, forest age 30 plots (*Fig. 3*). There were no significant effects of treatment and forest age on LAI ($Pr(>F) > .05$, *Table 1*). LAI trended towards increasing with forest age in P plots but was not statistically significant.

Leaf Biomass

Mean leaf biomass ranged from 0.40-0.94 kg/m^2 with the highest values in the forest age 30 control and P plots. As reflected in LMA and LAI results, leaf biomass increased with forest age in P treated plots but was not statistically significant (*Fig. 4*). Mean leaf biomass was higher in the control than in treated plots across forest age with the exception of P and NP in forest age 10. Mean leaf biomass increased with forest age. There were no significant effects of treatment and forest age on leaf biomass ($Pr(>F) > .05$, *Table 1*).

DISCUSSION

This study observes nutrient effects on LMA, LAI, leaf herbivory, and leaf biomass across forest age to determine if biomass allocation to leaves is plastic. Generalized linear mixed models of data from leaf scans and hemispheric canopy photos show that nutrient additions and forest age did not have strong, significant effects on LMA, LAI, leaf herbivory, and leaf biomass. Our findings are consistent with studies that detected allocation between wood and fine roots but not leaves (Dybzinkski et al. 2011; Dybzinski et al. 2014). Leaf litterfall—a proxy for leaf biomass—studies have also suggested that nutrient additions have a significant effect on the growth and abundance of wood and fine roots but not leaves, consistent with our models showing N, P, and treatment having $Pr(>F)$ and p-values $> .05$ (*Table 1*) (Alvarez-Clare et al. 2013; Waring et al. 2019; Sayer et al. 2020). The similar ranges for LMA, LAI, and leaf biomass across all plots also suggest that these variables are constant across forest age and treatment. Across forest age, biomass increases strongly with higher relative allocation to leaves in young forests than in old-growth forests (Brown and Lugo 1990; Batterman et al. 2013; Poorter et al. 2016). Leaf biomass was similar across forest age, suggesting fast recovery. Although we did not observe an absolute change in leaf biomass, there may be a relative change in leaf biomass if wood and fine root biomass changed across treatment and forest age. Future studies will have to consider leaf biomass relative to wood and fine root biomass.

There was evidence in support of the hypothesis that LMA is constant across forest age. This complements data suggesting that allocation to leaf biomass is constant (Dybzinski et al. 2014). There was no evidence in support of the hypothesis that LMA and LAI respond strongly to N. Nitrogen did not have a significant effect on LMA or LAI ($Pr(>F) = 0.77$ and 0.70, respectively, *Table 1*). One reason that we did not see strong effects may be because plants have adapted to low nutrient conditions which may explain why there were no strong responses to nutrient additions. Another reason may be because while plants are not adjusting absolute leaf biomass, they may be adjusting carbon to nutrient ratios, which would indicate plasticity in nutrient use efficiency. Thus, observing nutrient concentrations in leaves would provide key insights on nutrient effects on leaf biomass. Lastly, while plants may not be adjusting total absolute leaf biomass, another key step is to examine changes in relative leaf biomass to other organs.

Surprisingly, there was no evidence showing a significant effect of forest age or N on leaf herbivory as initially hypothesized (p = 0.91 and 0.41, respectively, *Table 1*). Our study observed leaf herbivory on the transect level. One factor to consider in future studies is if leaf herbivory changes across tree species. Young leaves have not developed defenses that can protect them from predators which makes them more vulnerable to damage (Aide et al. 1993; Coley and Barone 1996). There may be other plant defenses that play a role in determining leaf herbivory across forest age, for instance, calcium is often used as oxalate crystals to deter herbivory (Korth et al. 2006).

The rise in climate and anthropogenic emissions poses a threat on the tropical carbon sink. There is a possibility that nutrient constraints could limit the tropical carbon sink even further, warranting the research of plant nutrient strategies in tropical forests. Leaf biomass allocation is one plant nutrient strategy that may change in response to nutrient addition and forest age. For instance, in high nutrient conditions, plants may allocate to leaf biomass to increase carbon capture and photosynthesis. Thus, understanding plant nutrient strategies in tropical forests allows us to maximize carbon sink efforts like reforestation and recovery and lets us know if we can rely on the tropical carbon sink to offset human carbon emissions now and into the future.

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APPENDIX

TABLE 1. Pr(>F) or p-values for LMA, leaf herbivory, LAI and leaf biomass in response to five years of fertilization in secondary forests of Agua Salud, Panama using the following parameters: (LMA): lmer model, TreeID data level. (Leaf Herbivory): Kruskal-Wallis test, Transect data level. (LAI): lmer model, Transect data level. (Leaf Biomass): lmer model, Transect data level. All models were tested with factors nitrogen, phosphorus, and forest age with the addition of a treatment factor for leaf herbivory. * = *Log-transformed.*

factor(variable)	LMA*	Leaf Herbivory	LAI*	Leaf Biomass*
factor(Nitrogen)	0.7740	0.9051	0.6995	0.2303
factor(Phosphorous)	0.9290	0.8954	0.8240	0.8303
factor(Forest.Age)	0.6399	0.4092	0.3494	0.3436
factor(Nitrogen):factor(Forest.Age)	0.4967	NA	0.6819	0.6327
factor(Phosphorous):factor(Forest.Age)	0.3160	NA	0.3553	0.8990
factor(Treatment)	NA	0.6633	NA	NA
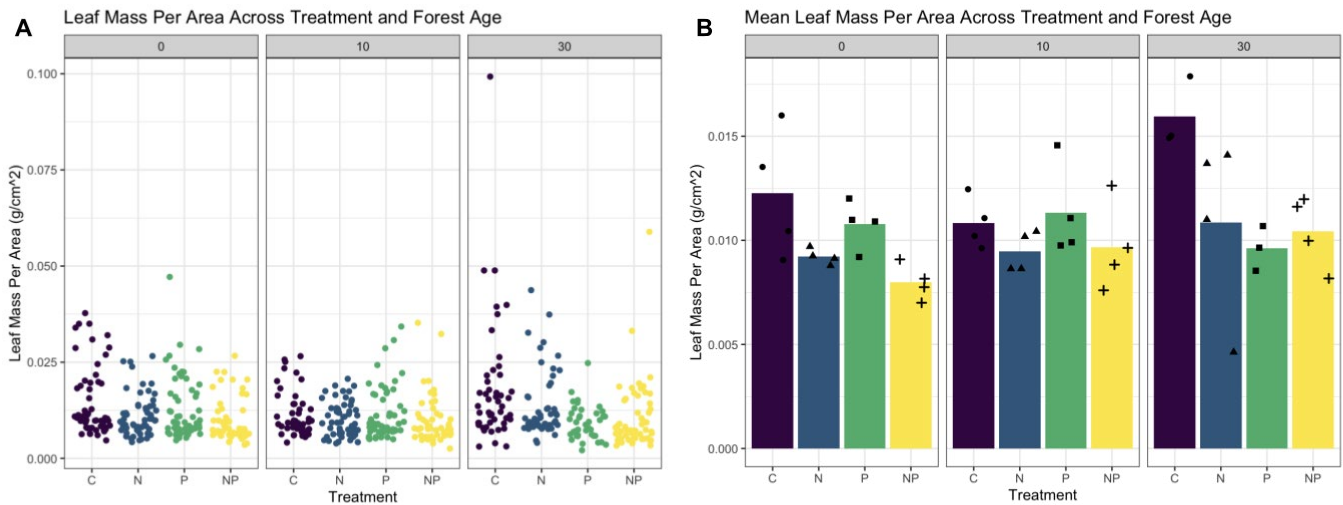
FIGURE 1. Leaf mass per area (g/cm^2) across treatment and forest age in secondary tropical forests in Agua Salud, Panama. (A) Tree level LMA across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years). (B) Transect level LMA across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years). Individual points are the LMA for each transect (n=4 in a given treatment and forest age with the exception of n=3 for C and P in forest age 30).
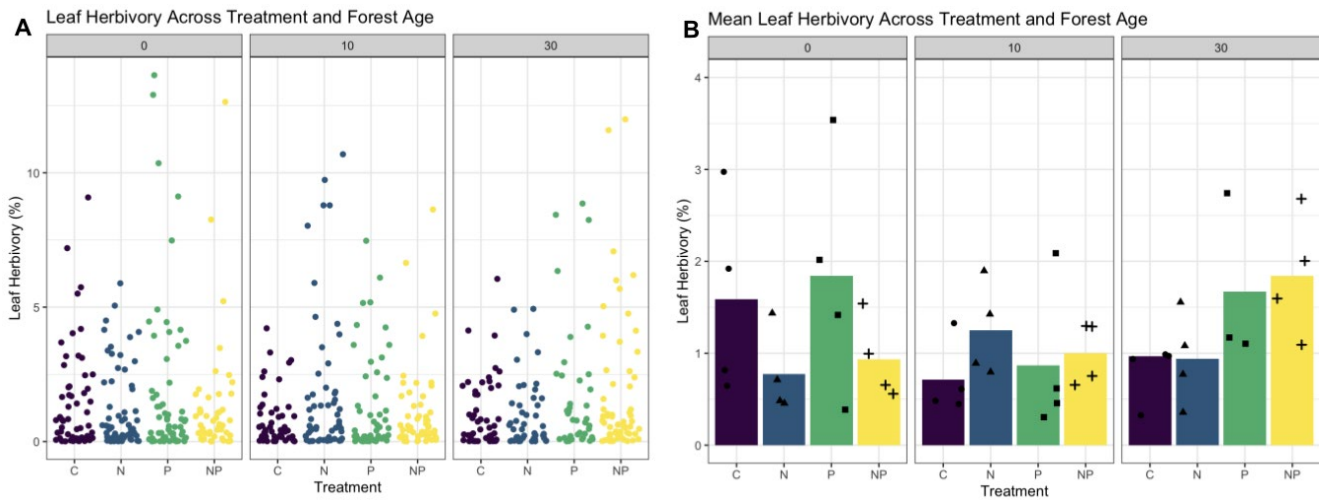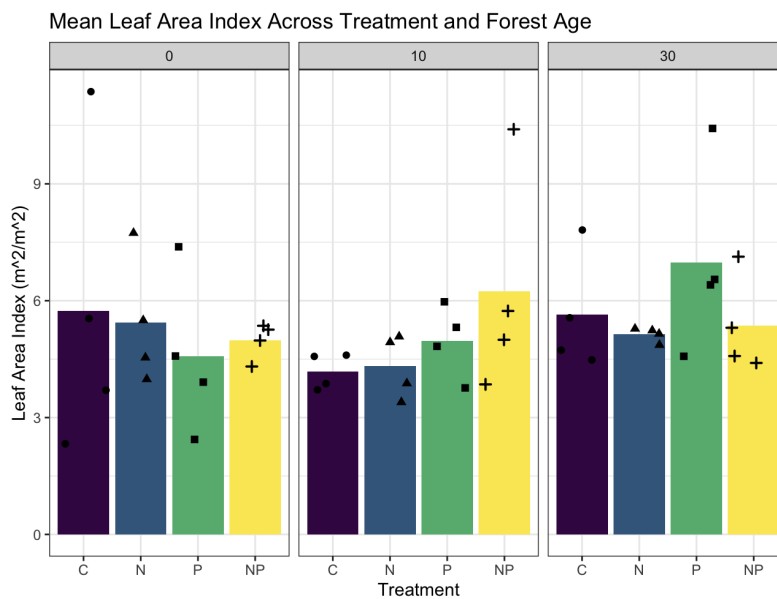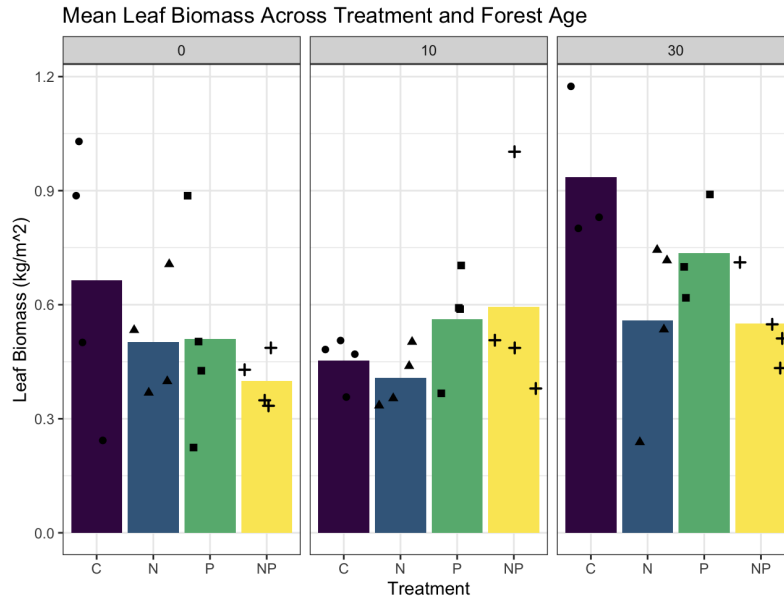
FIGURE 2. Leaf herbivory (%) across treatment and forest age in secondary tropical forests in Agua Salud, Panama. (A) Tree level leaf herbivory across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years). (B) Transect level leaf herbivory across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years). Individual points are the leaf herbivory for each transect (n=4 in a given treatment and forest age with the exception of n=3 for C and P in forest age 30).

FIGURE 3. Mean LAI across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years) in secondary tropical forests in Agua Salud, Panama. Individual points are the LAI for each transect (n=4 in a given treatment and forest age).

FIGURE 4. Mean leaf biomass across treatment (control, nitrogen, phosphorus, nitrogen plus phosphorus) and forest age (0, 10, 30 years) in secondary tropical forests in Agua Salud, Panama. Individual points are the mean leaf biomass for each transect (n=4 in a given treatment and forest age with the exception of n=3 for C and P in forest age 30).
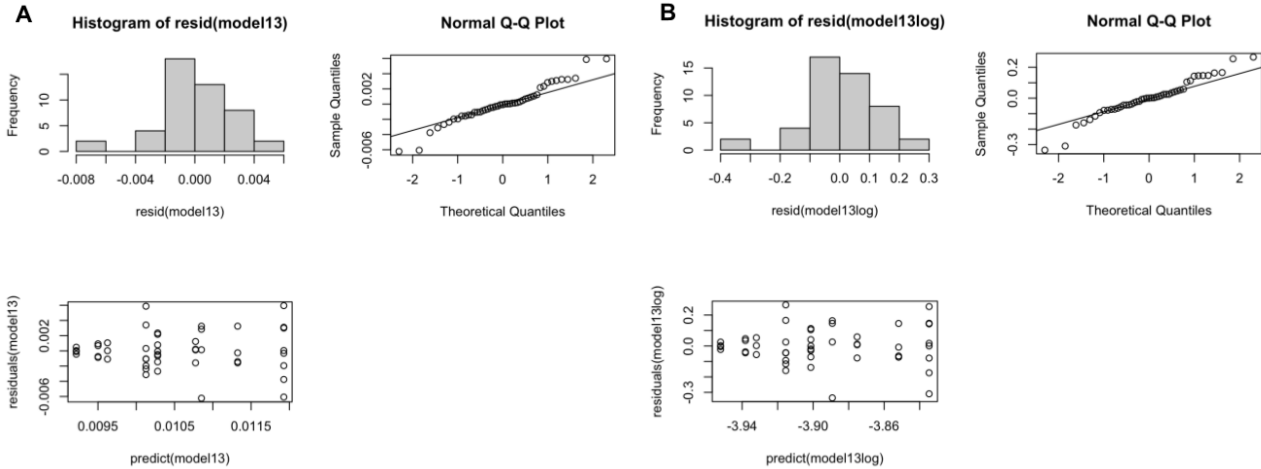

FIGURE 5. Histogram, Q-Q, and residual plot for (A) LMA using ANOVA (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age and (B) log-transformed LMA using ANOVA (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age).
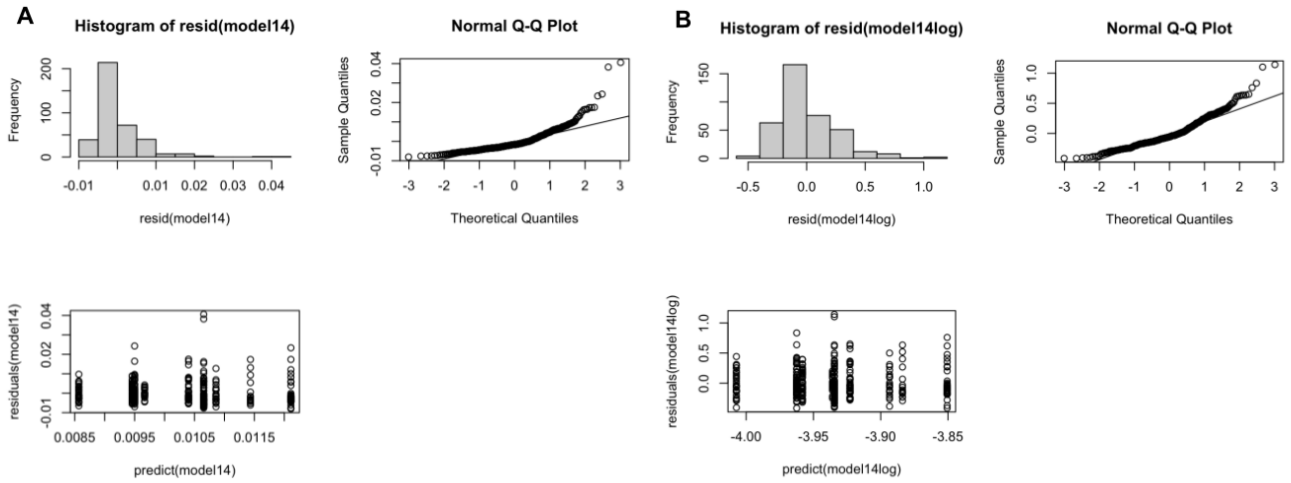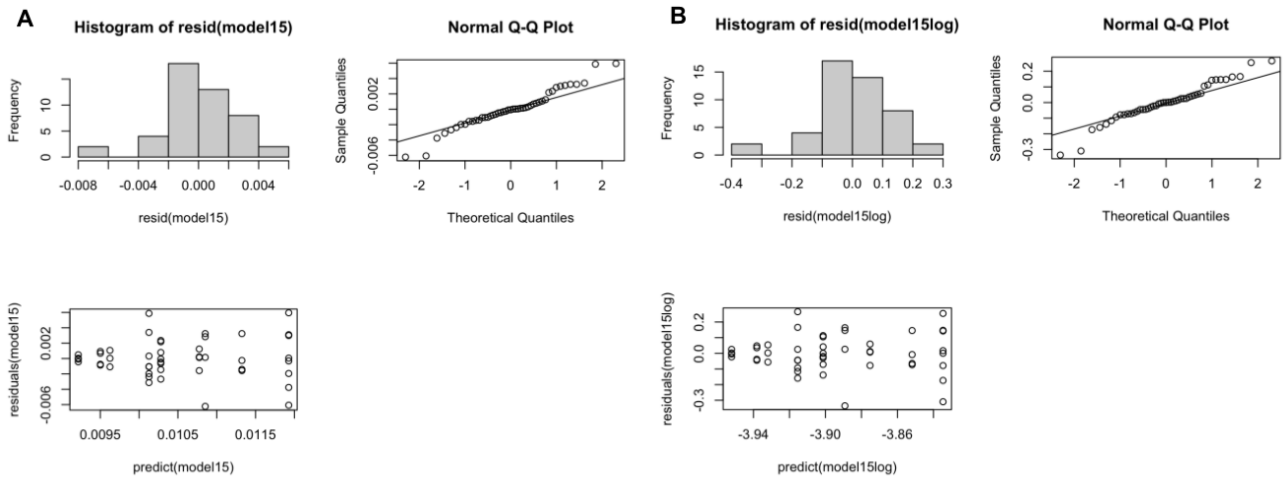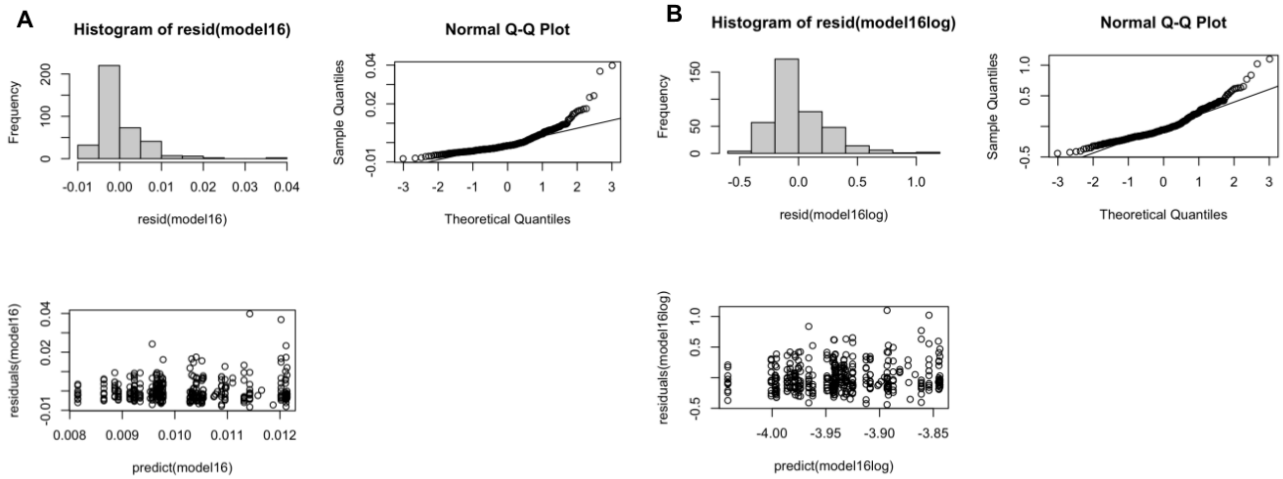
FIGURE 6. Histogram, Q-Q, and residual plot for (A) LMA using ANOVA (Data Level: TreeID, Variables: nitrogen, phosphorus, and forest age) and (B) log-transformed LMA using ANOVA (Data Level: TreeID, Variables: nitrogen, phosphorus, and forest age).

FIGURE 7. Histogram, Q-Q, and residual plot for (A) LMA using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age) and (B) log-transformed LMA using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age).

FIGURE 8. Histogram, Q-Q, and residual plot for (A) LMA using lmer (Data Level: TreeID, Variables: nitrogen, phosphorus, and forest age (B) log-transformed LMA using lmer (Data Level: TreeID, Variables: nitrogen, phosphorus, and forest age).
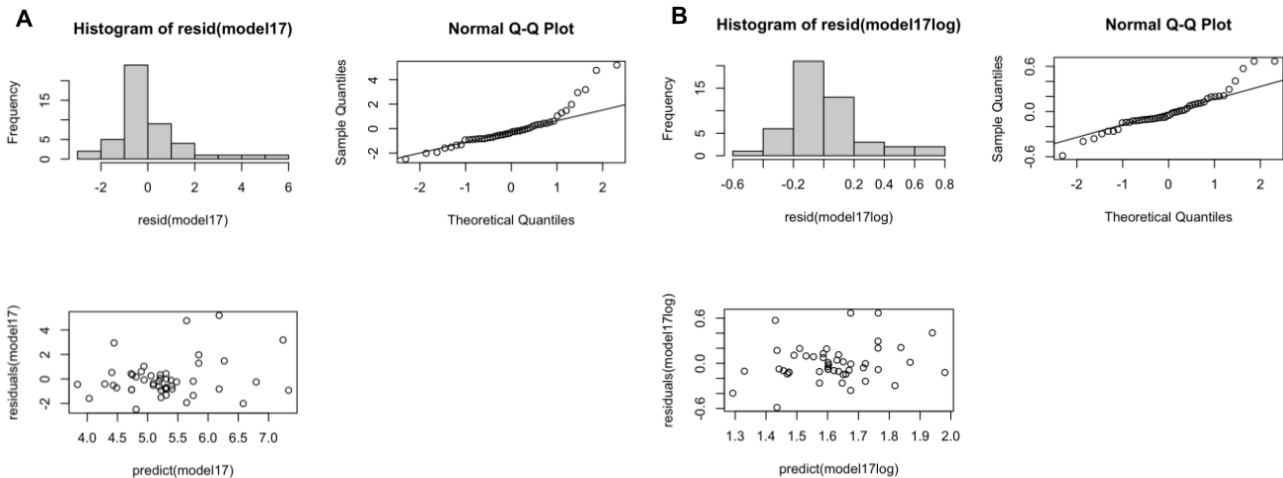

FIGURE 9. Histogram, Q-Q, and residual plot for (A) LAI using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age) and (B) log-transformed LAI using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age).
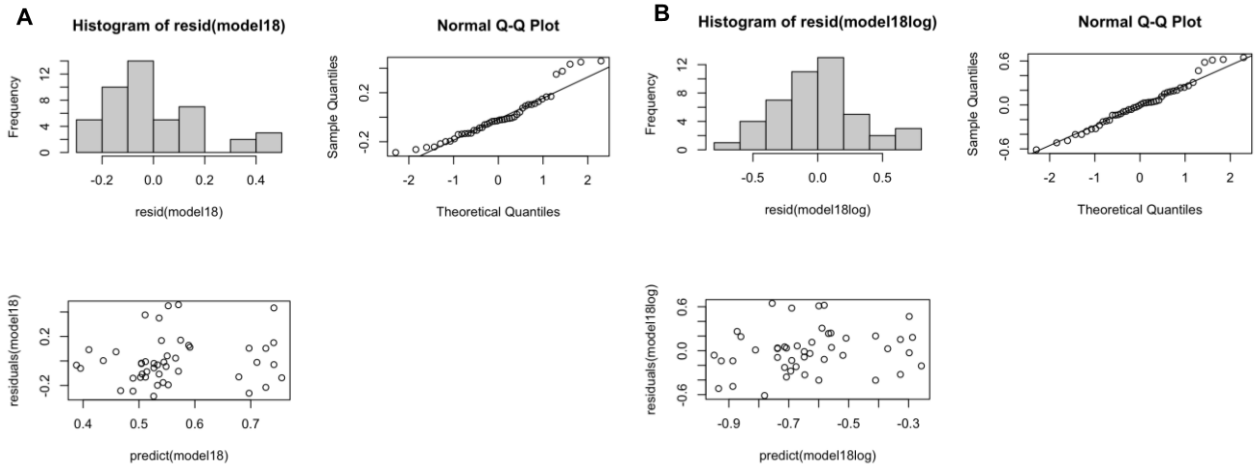
FIGURE 10. Histogram, Q-Q, and residual plot for (A) leaf biomass using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age) and (B) log-transformed leaf biomass using lmer (Data Level: Transect, Variables: nitrogen, phosphorus, and forest age).
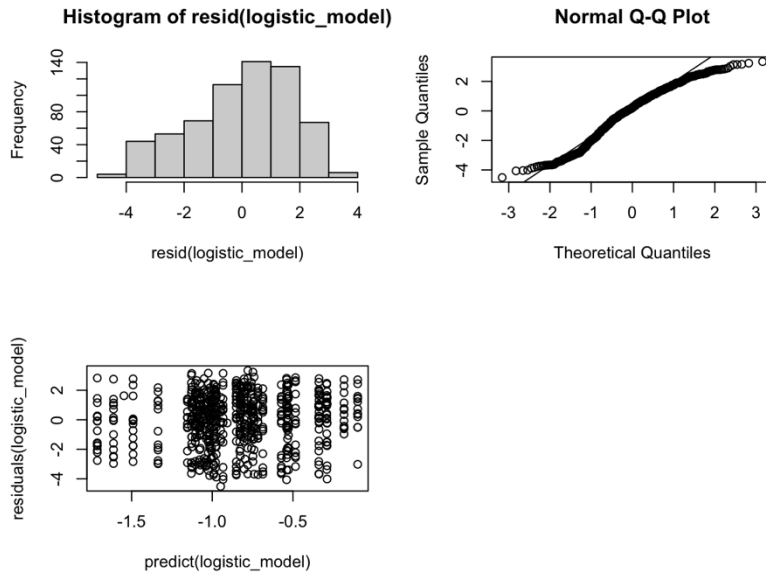

FIGURE 11. Histogram, Q-Q, and residual plot for LMA using logistic model (Data Level: TreeID, Variables: nitrogen, phosphorus, and forest age).
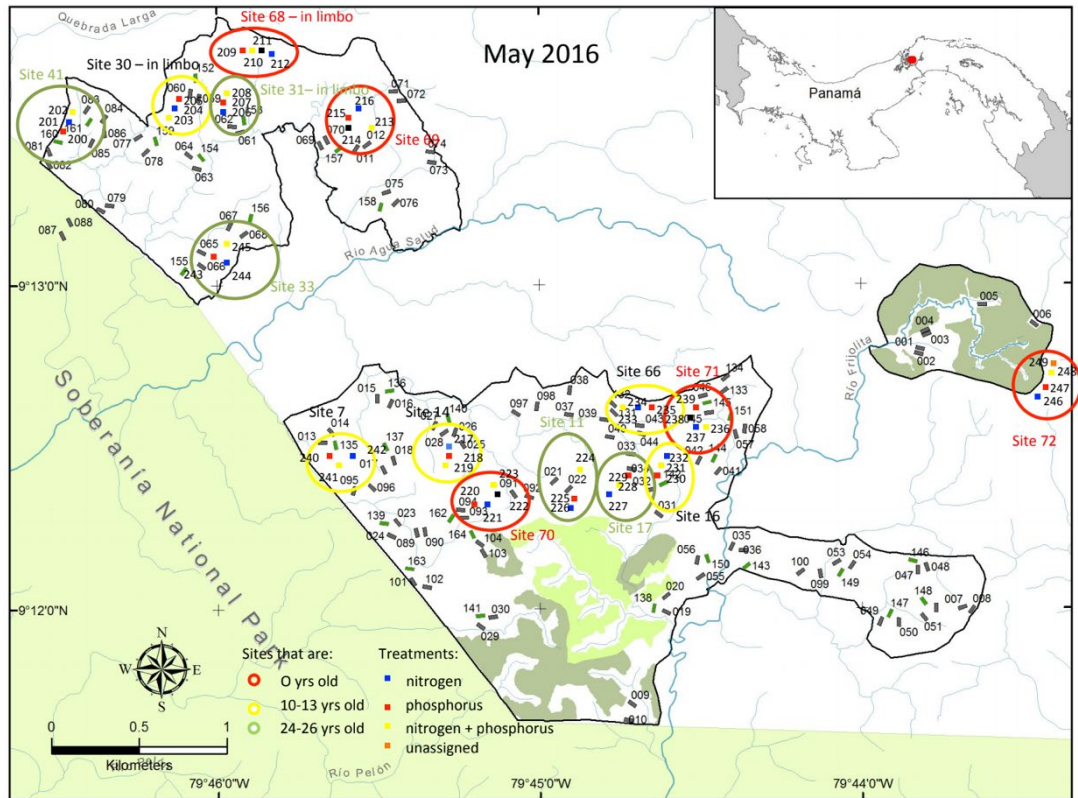
FIGURE 12. Map of Agua Salud fertilization experiments marked by site (n=76), transect (n=60), forest age (0, 10, and 30 years), and treatments (control, nitrogen, phosphorous, nitrogen plus phosphorous).