1. **Running head:** Biotic factors improve succession along tropical elevation gradient

**Title: The positive effects of pathogenic fungi, herbivores, and predators on pioneer successional vegetation in tropical forests vary with elevation.**

Piotr Szefer, Austin Sau, Kenneth Molem, Jonah Philip, Martin Volf, and Vojtech Novotny

**Abstract**:

In regenerating tropical forests, natural enemies significantly influence plant community assembly, their impact varying along ecological gradients. Our study, conducted at three sites (200m, 750m, and 1900m a.s.l.), explored the effects of insectivorous birds, bats, ants, herbivorous insects, and pathogenic fungi on richness, density, diversity, and biomass of pioneer tropical forest vegetation. Treatment effects were generally positive at low and high elevations but scarce at mid-elevation. Insects played a key role across all elevations, acting as filters for pioneer communities. Specific Leaf Area (SLA), Water Content (WtrC), and herbivory damage (HD) weakly predicted species' response. Local species richness and abundance emerged as an important predictors of biomass change of an individual species to predator exclusion and fungicide treatment. In conclusion, insects act as filters for pioneer communities, and biotic factors have a limited effect on further community development, beyond seedling establishment phase. Effects of biotic factors are more notable at higher and lower elevations, and high species richness at mid-altitudes seems to weaken their impact on pioneer succession.

**Key words**: tropical forest, pioneer succession, fungicide, insecticide herbivory, tri-trophic interactions, Papua New Guinea.

**Introduction**

Plant-natural enemy interactions were shown to have a profound impact on plant establishment, survival and competitive dynamics of tropical forest plants (Solé et al. 2019, Comita and Stump 2020, Williams et al. 2021). Multiple studies have demonstrated the importance of fungi and herbivorous insects as the key mortality factors, that increase diversity and richness of primary tropical forest seedling communities through density dependent and independent effects (Freckleton and Lewis 2006, Gripenberg et al. 2014, Bagchi et al. 2014, Krishnadas et al. 2018). Additionally, tri-trophic interactions, and specifically top-down control of insects by insectivorous birds, bats, and ants were shown to significantly affect competitive interaction in plant communities (Letourneau et al. 2004, Terborgh 2015).

These factors will likely be important also during pioneer stages of tropical forest succession. Plants, which are able to establish at initial stages of regeneration, will most likely determine the future trajectory of succession (Martínez-Garza et al. 2005, Mudrák et al. 2016). In contrast to primary tropical rain-forest, pioneer tropical plants thrive under starkly different ecological and environmental conditions and specialize in fast resource acquisition and growth at the cost of their resistance to natural enemies (Lai et al. 2021). This may result in changes in strength and direction of the effects of biotic factors during succession. Our previous study showed that insect effects are more important than those of fungi, and that insects and tri-trophic interactions affect pioneer succession by reducing randomness of the assembly process (Szefer et al. 2020). Therefore, including another tropic level while studying biotic interaction with plants, may improve predictability of successional processes. However, plant-natural enemy interactions are sensitive to changing ecological and landscape contexts (Kergunteuil et al. 2019). As accurately pointed out by (Rodríguez-Castañeda et al. 2010) ‘tropical forests are not flat’, and studying biotic interactions along altitude gradients may improve our knowledge on the rates and predictability of succession.

Tropical forest elevation gradient is characterized by reduction in temperature, increase in solar radiation and rainfall. This causes a shifts from resource acquisitive to conservative strategies with elevation (Silva et al. 2023). Tropical forest communities are also characterized by a clear elevational shift in functional trait characteristics. At higher elevations dominate slow growing, better defended, and resource conservative plants (Rasmann et al. 2014, Islam et al. 2024). By analogy, pioneers at the higher elevations may also have more resource conservative traits, and slow growing less palatable, longer living leaves compared to pioneers in the lowlands (Homeier et al. 2021). This in turn affects the plant-enemy interactions, including the Janzen-Connel effects (Kobe and Vriesendorp 2011, Jia et al. 2020). Insect activity is directly related to temperature and their general effect on the plant community should be diminished at higher elevations (Rasmann et al. 2014). In contrast, at high elevations pathogenic fungi activity is expected to increase, as with increased humidity and lower temperatures, plants should be more prone to necrotrophic fungi infections (Velásquez et al. 2018). Cascading effects of top vertebrate and invertebrate predators on plants are strong in primary tropical forests (Letourneau et al. 2004, Barber and Marquis 2011, Houska Tahadlova et al. 2023). However, arthropod predators (Longino et al. 2019), and bird predation risk is smaller at high elevations (Sam et al. 2015, 2019, Roslin et al. 2017). While evidence is abundant for the reduced pressure from vertebrate predators in primary forest, the top-down control may have a strong effect also on pioneer vegetation, and this effect can be weaker at high elevations.

The unpredictability of successional processes in regenerating tropical forests is an ecological phenomenon of the highest importance for the restoration of tropical forests (Walker et al. 2007). Biomass accumulation patterns, directional changes in plant trait composition, and local environmental conditions can be more deterministic, than other aspects of plant diversity or ecosystem functioning (Zirbel et al. 2017). Recent advances in our understanding of the role of biotic factors in shaping species diversity and richness in tropical forests suggest, that fungi, insects and their predators play potentially a crucial role in resisting randomness of the community assembly processes.

The plant trait variation should be reduced at higher elevations due to increased environmental filtering (Homeier et al. 2021). This suggests, that succession at higher elevation may be more strongly affected by recruitment limitation and specific trait values, particularly growth rate and photosynthetic activity, due to plants’ conservative resource acquisition strategies. SLA can be used as a relatively good approximation of that measure of a plant fitness (Osone et al. 2008, Kleinschmidt et al. 2020) and water content (or leaf water mass) as a measure of a whole-leaf photosynthesis (Wang et al. 2022). Thus, predictability of a plant success by their functional traits could potentially be higher. Specifically, SLA and water content should be increasingly better at predicting changes in biomass of individual species with decreasing pressure from natural enemies and prevalence of more conservative traits related to increase in elevation.

Here we present results of replicated experimental exclusion of fungal pathogens, insects and spiders and their predators from pioneer (0-1.5 year) stages of regenerating gaps in tropical forest at three distinct elevations. We hypothesize that positive effect of the plant-natural enemy interactions increases in strength with elevation for fungi, and vertebrate predators, but decreases for insects that result in an increase in richness and diversity effects of fungi with elevation and reduced effect of insects, as well as stronger effects of predator exclusion. Stronger effects of fungi and predators will cause an increase in predictability of the community composition with elevation. Finally, we anticipate that plant traits related to growth, resource acquisition (leaf water content, SLA) will be increasingly better predictors of a species’ success in early stages of regenerating community with elevation, because of increasing trait integration and shift towards resource conservatism.

**Materials and methods**

*Experimental design*

Experiments were conducted in tropical rainforest areas surrounding Wanag village (200 m a.s.l., 145°5′32″E, 5°14′26″S), Numba village (750 m a.s.l.), and Ubi camp (1900 m a.s.l.). Throughout the text, we refer to these sites as low, mid-, and high altitudinal sites. The study took place in abandoned food gardens that had undergone approximately five years of regrowth. The advantages of this design are discussed in our previous paper (Szefer et al. 2020).

At each elevation, experimental blocks were established by clearing all above-ground vegetation in an area of approximately 400 m^2. Individual gardens were separated by a minimum distance of 200 m. Within each block, five experimental plots (5 x 5 m) were marked. All dead wood and rocks were removed from each plot to ensure at least 95% bare soil cover.

Plots were assigned to one of five experimental treatments:

1. Control (C): Sprayed weekly with an appropriate amount of water to simulate the application of a water solution of pesticides and fungicides on other plots.
2. Exclusion of pathogenic fungi (F): Contact fungicide was applied to above-ground plants at weekly intervals (12.5 g soluble powder per 5 L, commercial name: Mancozeb 80%).
3. Exclusion of insects (I): Two types of broad-spectrum insecticides, lambda-cyhalothrin (5 ml per 5 L, commercial name: Thunder 0.25%) and imidacloprid (1.5 ml per 5 L, commercial name: Mustang 24%), for both systemic and non-systemic protection, were applied weekly. Plots were surrounded by a 50-cm high plastic fence that continued 20 cm below the ground. Insect glue (tanglefoot) was repeatedly applied to the top of the fence to prevent insect re-colonization.
4. Exclusion of predatory ants, insectivorous birds, and bats (P): Wooden frames with a transparent fishing net (1.5–2.0 cm mesh size) were placed over plots to protect vegetation from foraging birds and bats. Poisonous tuna baits (permethrin 0.5%), enclosed in PVC tubes, were exposed at monthly intervals to reduce insectivorous ant abundance. The plot was surrounded by a plastic fence (as in treatment I) to prevent ants from re-colonizing the plot. Mesh size was chosen to allow wind-dispersed and larger seeds to fall through the netting. Additionally, every week, all litter-fall potentially containing seeds and insects was collected from the top of the exclosure and randomly placed inside the experimental plot.
5. Increased herbivory (H): We used two species of locally common, highly polyphagous, and flightless Oribius sp. weevils to increase herbivory pressure within the plot. Weevils from this genus were previously documented in the local secondary vegetation, and their wide polyphagy was established by feeding tests. Every two weeks, 125 weevils were collected in the surrounding forest, starved for 24 hours, and released into the plot to sustain their population size. Plots were additionally protected from predators with exclosures (see treatment P) and fenced off, with insect glue applied to the top of the fence to prevent weevils from dispersing to the surrounding plots.

All other plots were surrounded by a 50-cm high dummy plastic fence, with a 10–20 cm gap left between the ground and the lower edge of the fence to allow easy access for crawling insects, lizards, and rodents.

*Statistical analyses*

We utilized log-response ratios (LRR), representing the natural logarithm of the ratio between a plot with a specific factor and that without the factor for a given vegetation characteristic, to assess the treatment effects across sites. A zero-intercept linear model was employed to compare LRRs at individual sites. Bertellet's and Levene's homogeneity tests were conducted for LRR comparisons of all descriptors between sites. In the case of the fungi treatment on woody plant density, tests revealed a lack of homogeneity in LRR values between sites. This discrepancy was addressed in the statistical model by incorporating the *varIdent(form = ~1|site)* option as weights in the *gls* function. The significance of pairwise differences between sites was assessed using post-hoc Tukey tests.

At each elevation, a partial redundancy analysis (pRDA) was performed to evaluate the effect of biotic factors on plant community composition. Separate analyses on species were conducted for each altitude. The location dependency of individual plots/species was assessed by projecting coordinates in the ordination space (determined by the first two PCA axes) sonto the effect vector and correlating it with the trait values.

Bray-Curtis dissimilarities between control plots and each of the four treatments (fungicide, herbivore, insecticide, and exclusion of key predators) were employed to test the prediction that some treatments might exert a stronger impact on community composition at higher elevations. BC dissimilarities were further decomposed into balanced and gradient components, representing species turnover and changes in dominance structure, respectively (Baselga 2017). Differences between control values for BC and its components were tested using beta-regression, with BC values equal to 0 and 1 converted to 0.001 and 0.999.

We adopted the null model approach proposed by (Chase et al. 2011) and modified by (Alberti et al. 2017) to assess the extent to which differences in variability were attributed to stochastic and deterministic processes in community assembly. This approach aimed to evaluate the probability of observing a random pair of plots less dissimilar than in the experiment. A high probability implies that the compared empirical communities are strongly convergent in composition, while a low probability indicates a small chance of having a more dissimilar community by chance, suggesting greater divergence than expected. We calculated Bray-Curtis dissimilarity (BC) between all unique combination of plots within treatment and between blocks:, where *a* denotes a treatment, and *k* denotes number of blocks at a given location, and. We used the *vegdist* function from the *vegan* package (Oksanen et al. 2019). We then randomized the two plots and calculated randomized dissimilarity . The measure was the number of iterations iteration where smaller than.. The random sampling of plant communities from each plot was conducted through the following steps:

1. Calculate species sampling probabilities: All species within one of the three studied sites at different altitudes were combined. The probability of a species being sampled into the randomized community was determined proportionally to its occurrence frequencies across all plots from all experimental blocks within the site. This involved utilizing all species from a specific site, encompassing all gardens and treatments, to obtain a comprehensive sample of potential communities.
2. Calculate biomass sampling probabilities: The harvested biomass of species, aggregated across all plots within a given site, was used as the sampling probabilities for the biomass increase in the randomized plot.
3. Randomization process for each plot: Before randomization, the empirical richness and biomass of each plot were calculated. Species identities were sampled using the species sampling probability distribution (from step 1) up to an empirical limit. Biomass increase occurred in iterations. During each iteration, the biomass of each species in the randomized community was increased by one unit (accuracy = 0.001g), with the probability defined by the biomass sampling probability distribution (from step 2), until the empirical biomass limit for a given plot was reached. This iterative process ensured the generation of randomized communities with varied species composition and biomass levels, reflecting the characteristics observed in the original plots.

The obtained probability values were scaled to the Raup–Crick (RC) index by subtracting 0.5 and multiplying the obtained difference by two, to measure the importance of deterministic processes during community assembly. RC values near -1 indicate deterministic convergence, resulting in a higher chance of observing more similar plant community composition. In contrast, RC values approaching 1 suggest deterministic divergence during assembly, resulting in a higher chance of observing more dissimilar plant community composition. We tested significance of RC values between control site and each treatment using linear random-effect models with garden as a random effect. Models were build and tested using the *lme* function in the *nlme* package ().

Phylogeny [Martin…].

The Ornstein-Uhlenbeck (OU) model of trait evolution was selected based on the likelihood criterion using the *fitContinuous* function in the *geiger* library (ref). The estimated alpha parameter for this model was later used in the PGLS models to provide correlation structure generated with *corMartins* function in the *ape* library (ref).

We computed community weighted means (CWM) for specific leaf area (SLA) measured in m²/kg dry leaf mass and water content (percentage of weight lost after oven drying for 24 hours). Subsequently, we compared the average CWM values between treatment and control plots to assess the effects of treatments on the trait composition of the plant community. Additionally, we examined the success of individual species and how it was influenced by treatment and altitude.

Species success was quantified as the logarithm of the difference between biomass at the control and treatment plots, denoted as Δbiomass. This measure was modeled as a quadratic function of log(SLA) and water content (WtrC). The covariance structure between individual observations (plant species at a given pair of plots, control and treatment) was derived from the phylogeny, incorporating the *alpha* parameter as described earlier.

We conducted a backward model selection to identify traits with the strongest statistical correlation with species success (Δbiomass) and to determine the most appropriate relationship representation (linear or quadratic). Each plant species was associated with two trait values, one estimated at the control plot and the other at the treatment plot. Given the observed tendency of plant species to alter their specific leaf area (SLA), water content (WC), or herbivory damage in response to treatment, our models included SLA, WC, and herbivory plasticity as additional covariates.

The backward selection involved comparing multiple competitive statistical models explaining the relationship between species traits and their capacity to change biomass in response to treatment. These models encompassed both linear and quadratic relationships, as well as site interactions, while considering the phylogenetic relationships between species. We used the chi-square test of log-likelihoods of competing model to test for significant differences between their performance. In cases where two models were not significantly different, the model with the lower Akaike Information Criterion (AIC) was favored.

The procedure unfolded as follows:

1. Comparison of models with and without traits: We compared the null model (without traits) to a model including traits, allowing for a nonlinear (quadratic) trait vs. Δbio relationship.
2. Testing removal of a single trait: If the model with traits outperformed, we tested whether the removal of a single trait could improve the quadratic model.
3. Reduction of a two-trait model: In cases where a two-trait model was chosen, we endeavored to further reduce it to a one-trait model.
4. Testing necessity of quadratic terms: The final model underwent testing to determine the necessity of quadratic terms, and these terms were systematically removed in all combinations for two/three-trait models.
5. Testing removal of interaction terms: Finally, we examined whether interaction terms could be removed without compromising model performance.

This systematic approach enabled the identification of the most relevant traits and their relationships with species success, accounting for both linear and quadratic components, and considering potential interactions within the context of the species' phylogeny.

**Results**

We identified 157 woody plant species, totaling 8,594 kg of fresh biomass (Supplementary Information: Appendix I, Tab. S1). Our data revealed that diversity was highest at mid-elevation (Supplementary Information: Appendix I, Fig. S1). Predicted richness peaked around 1000 m a.s.l. Elevations were similar in their baseline productivity (total biomass), as evaluated at the control plot (Fig. S1). Plant abundance, richness, and diversity at mid-elevation were significantly higher than at low elevation. Only the woody plant density of P showed a signal of non-homogeneity in variances (Supplementary Information: Appendix I, Tab. S1).

Fungi had a positive effect on woody plant richness and density at both low and high elevations (Fig. 1). Insects reduced biomass only at mid-elevation. At high elevation, insects increased the diversity and richness of experimental plant communities, while at low elevations, only richness was increased. Predator removal resulted in an increase in diversity, richness, and density only at high elevation. The addition of herbivores reduced woody plant biomass and density at low elevation, as well as richness at both low and high elevations.

Insect exclusion had a significant impact on community composition across all elevations, and fungicide treatment only at the highest elevation (Fig 2, Supplementary Information: Appendix I, Tab. S4). At low and high altitudes, the changes were primarily driven by an increase in *Pipturus argenteum* in the insecticide-treated plots. At mid-altitude, *Trema orientale* showed a strong positive response to the insecticide treatment.

At low elevation, control plots exhibited higher within-treatment Bray-Curtis dissimilarity in community composition compared to the mid-altitude. Only the addition of herbivores resulted in a consistent decrease in dissimilarity with increasing elevation (Supplementary Information: Appendix I, Fig S2). At the low elevation, species composition changed primarily through species turnover (high balanced component, low gradient component of dissimilarity). However, at the other two altitudes, changes through shifts in the abundance structure dominated in communities. All treatments, except fungi, had influenced quantities of the beta-diversity partitions. At the low elevation, insects reduced dissimilarity mainly by reducing the gradient component (Fig. 3). At the mid elevation reduction of the balanced component by insect, predator presence and increased levels of herbivory were leveled out by increase in the gradient components, except for the herbivore addition treatment. This resulted in no changes to general dissimilarity. At the highest elevation increase in balanced component caused by insects, and presence of predators were both reflected in the increase in general dissimilarity. At this elevation, treatment did not caused decrease in the gradient component.

At the highest studied elevation, all treatments caused a shift from deterministically divergent (predictably unpredictable) towards random community assembly (Fig. 4). Similar but weaker shift were observed at the low altitude in cases of insecticide and fungicide, and the opposite patterns were found at the mid-elevation. There communities showed rather random assembly and only addition of herbivores significantly increased divergence of the community composition.

At mid and high elevation, plants with higher water content tend to increase in abundance along the effect vectors of fungi and insecticide treatments. At the mid elevation, plants with lower SLA tend increase along the same vectors (Supplementary Information: Appendix I,Tab. S5).

CWM for water content and SLA was linearly, negatively related to species richness i.e., species CWMSLA and CWMWC were lower at high species richness sites, and higher at low species rich sites (Supplementary Information: Appendix I, Fig S3). This relationship was the same for all sites, and treatments.

Three studied traits varied in their importance in predicting the change in biomass (Δ biomass) in response to a treatment (Fig. 5, Tab. 1). SLA was significant, nonlinear only in the predator exclusion treatment. Species with higher water content tend to increase biomass in response to the fungicide treatment, similarly to the results of RDA. Water content, although not significant as a predictor, increased performance of all the models as a co-variate in all models. Majority of variation in the biomass change was explained by abundance, site identity, intra-specific shifts in SLA values.

**Discussion**

Our results indicate that biotic factors influence pioneer vegetation, but the direction and magnitude of their effects are varying depending on local plant community characteristics and elevation. With the exception of a strong negative effect of insects at the mid-elevation, biomass remained resilient to experimental treatments, suggesting a strong bottom-up control of community biomass. This was not coupled with any positive effect of predators, which could suggest a lack of top-down cascades affecting plant biomass.

Contrary to our expectations, fungi and insects exhibited similar patterns in their effects on plant community diversity, density, and richness at all three elevations. Both high and low elevations showed an increase in richness and density, possibly due to negative density dependence, which opened up niche space for additional plant species. However, it is puzzling that these effects were absent at the mid-elevation. It is possible, though, that at the mid elevation, fungi and insects can compensate for each other's effects, resulting in a nullification of individual treatment effects (ref). If there is a strong compensation by either fungi or other factors, and we remove insects from a plot, then the treatment effect would not be detected for both of these treatments. Moreover, there will be no correlation between magnitudes of their effects. To be certain about the presence of these effects additional treatment combining insecticide and fungicide treatment would have to be evaluated. Certainly, we cannot exclude also other factors, like rodents (ref) and the importance of the soil natural-enemy communities, which were not studied here. This calls for more thoughtful experimental designs. Huge within-treatment variation in the Jaccard index of plant community similarity compared to low and high elevations may suggests, that local diversity may play a significant role in mediating the factors’ effect sizes and directions.

Surprisingly, elevated abundances of herbivores had no effect on plant biomass in Numba when there was a clear effect of insects at this elevation. This lack of consistency because the ability to reduce plant biomass is attributed mainly to highly specialized herbivores. That would, however result in some effects of insects on richness and diversity. These were not observed. Numba is the most species rich from our sites. These species have lowest SLA from all sites (). These species may have limited compensation abilities. In the absence of herbivores, plants with higher SLA tend to dominate the community (ref), which may cause the increase in biomass.

Significant effects of top predators on richness and diversity of top-predators occurred more often at the highest elevation. Their effects are positive, as predicted by classic, mechanistic model of the top-down control (ref). However, in these situation we also observe positive effects of insects on these sites, instead of negative effects. Moreover, a lack of effect does not necessarily mean that the cascading effects are not present. However, negative correlation of the predator and insect effects is expected at minimum. In our case, significant correlations of herbivorous insects and their predators effects on vegetation biomass, diversity, richness, and density were either not significant nor positive, especially at the high elevation (). Positive correlation means, that the exclosure treatment (no top-predators, expected higher insect abundances) had the same effect on vegetation, as the insecticide treatment (reduced insect abundance). It is possible, that in the exclosure spiders are released from the predatory pressure and exert stronger pressure on herbivores (intermediate predation that modifies the effect). Due to limitations of surveying the plots for insects to see exactly what had happened. Exclusion intensifies spider predation either by creating conditions for web building spiders, or by reducing predatory spider mortality, which intensifies predation on herbivores (Schmitz 2010). At least in Wanang no cosistent increase in spider abundance was observed, and we concluded a weak top-down control there (basically no effect of the exclosure). (Szefer et al. 2022). This might be different for N and Y (). However, intermediate predators can increase their predatory pressure also by behavioral changes. However, change in alternative predator community may as well be also behavioural (Schmitz).

Only insects affected the plant species composition at all elevations, and fungi also at the highest elevation. The analysis of beta diversity components showed, that all insect-related treatments (P, P+H, I) affect species turnover and dominance structure in a very similar way within elevation, but differently among elevations. At low altitudes, only insects reduced changes in dominance structure of plant communities. At the mid altitude insects acted as a filter for species – reduced species turnover and increased dominance shifts, and at the high altitude only increased species turnover. These effects are qualitatively different at each elevation and may be related to the humped-shaped patterns in regional species richness. In a rich regional pioneer species pool, the competitive landscape for plants may become more complex. In that case it can be more difficult for species-specific natural enemies to accumulate (ref), because the community composition is unpredictable, as may be suggested by high variation in Jaccard similarity at the mid altitude (). Assembly analysis confirms higher randomness in plant community composition at the mid altitude. Therefore, when species pool of pioneers is smaller, which means a more predictable composition, insects may open up space for new species and increase richness. Moreover, insects reduce dissimilarity for highly variable communities.

The CWM of SLA and WtrC was negatively correlated with species richness, which means that dominant species in more rich communities had lower values of these traits. This pattern has already been observed for the old growth forest and was attributed to shifts from resource acquisitive to conservative strategies with accumulating species (Zhang et al. 2019). This explanation would only be true if in more species rich successional communities trait were shifts were occurring faster than in species poor ones. If that is not the case, than resource acquisition (higher SLA and WtrC) would be a better strategy, or it would result from a weaker inter-specific competition at species poor sites. Our results, however, show that this relationship is present at all experimental and control plant communities. It is also not a result of trait value shifts by individual species. This might be caused by an increased competition between plants for some limited resources.

If traits were better at predicting species success at higher elevations, as predicted in case of weak top-down control, we should see significant correlation between SLA and Log(TotBio) at the control plot and our model fit measure (R2) should increase with the elevation. This trend is not present at all (). Instead, plants with lower SLA tend to have higher biomass irrespective of the elevation ().

In the more complex analysis, when we controlled for baseline (evaluated at the control plot) abundance, richness, site identity, trait plasticity (LRR SLA), and phylogeny we found rather limited effect of studied traits. Differential effects of elevation found only for the SLA and water content, at predator an P+H sites. It seems that the effects of insects are not trait mediated. SLA is a moderately good predictor of insect herbivory (Whitfeld et al. 2012, Zhang et al. 2023). We also showed nonlinear trends in the relationship. In Wanang, plants with average SLA will respond positively, or at least non-negatively. When we add herbivores to the exclosure, their additional effect will affect water content, but not SLA. It will promote high water content plants in the exclosure with herbivores. Similar effect was also found for fungi. This is different in the mid, and high altitudes. There, for the predator removal treatment the relationship between biomass change and SLA has an opposite effect, and at the high altitude biomass change decreases linearly with SLA.

At least for richness, density and diversity measures, patterns seem to be correlated with the local plant species richness at each sites: low species richness plots have stronger positive effects and high species richness site (Numba, mid elevation) has weak to no effects. However, species richness was a poor predictor of a LRR at the level of individual gardens (). In case of strong bottom-up control, response of plant community to a treatment should not be correlated with its biomass or richness at the control plot. Whenever changes in community are driven by the initial community we should be able to explain much of the LRR effect based on the initial community composition (control plots). The direction and strength of the effects seems to be richness-related. This is another evidence for bottom-up control.

In summary, the effects of insects and other biotic factors are weakest at the mid-elevation. The effects of herbivores and pathogenic fungi seem to affect trait community composition, but are absent at the mid altitude. High diversity of plants at the mid altitude reduces the effects of fungi and herbivores and top predators. Only there insects had negative effect on biomass, but increase in generalist abundance did not. The effect were most prevalent at the high elevation. There we got mostly positive effects of fungi, insect herbivores (both addition and removal) as well as tropic cascade of top-predators. Cascades seem to increase in strength at the highest elevation (and affect richness and diversity). Birds bats and ants somehow can affect arthropod predators, that releases insects from the pressure of their arthropod predators and this may have positive effect on plants. There is also limited trait-mediation of the effects. Models suggest, that the initial and local conditions may modulate the strength of biotic factors.

**Literature**

Alberti, J., E. S. Bakker, R. van Klink, H. Olff, and C. Smit. 2017. Herbivore exclusion promotes a more stochastic plant community assembly in a natural grassland. Ecology 98:961–970.

Bagchi, R., R. E. Gallery, S. Gripenberg, S. J. Gurr, L. Narayan, C. E. Addis, R. P. Freckleton, and O. T. Lewis. 2014. Pathogens and insect herbivores drive rainforest plant diversity and composition. Nature 506:85–88.

Barber, N. A., and R. J. Marquis. 2011. Light environment and the impacts of foliage quality on herbivorous insect attack and bird predation. Oecologia 166:401–409.

Baselga, A. 2017. Partitioning abundance-based multiple-site dissimilarity into components: balanced variation in abundance and abundance gradients. Methods in Ecology and Evolution 8:799–808.

Chase, J. M., N. J. B. Kraft, K. G. Smith, M. Vellend, and B. D. Inouye. 2011. Using null models to disentangle variation in community dissimilarity from variation in α-diversity. Ecosphere 2:art24.

Comita, L. S., and S. M. Stump. 2020. Natural Enemies and the Maintenance of Tropical Tree Diversity: Recent Insights and Implications for the Future of Biodiversity in a Changing World1. Annals of the Missouri Botanical Garden 105:377–392.

Freckleton, R. P., and O. T. Lewis. 2006. Pathogens, density dependence and the coexistence of tropical trees. Proceedings. Biological Sciences 273:2909–2916.

Gripenberg, S., R. Bagchi, R. E. Gallery, R. P. Freckleton, L. Narayan, and O. T. Lewis. 2014. Testing for enemy-mediated density-dependence in the mortality of seedlings: field experiments with five Neotropical tree species. Oikos 123:185–193.

Homeier, J., T. Seeler, K. Pierick, and C. Leuschner. 2021. Leaf trait variation in species-rich tropical Andean forests. Scientific Reports 11:9993.

Houska Tahadlova, M., O. Mottl, L. R. Jorge, B. Koane, V. Novotny, and K. Sam. 2023. Trophic cascades in tropical rainforests: Effects of vertebrate predator exclusion on arthropods and plants in Papua New Guinea. Biotropica 55:70–80.

Islam, T., M. Hamid, I. A. Nawchoo, and A. A. Khuroo. 2024. Leaf functional traits vary among growth forms and vegetation zones in the Himalaya. Science of The Total Environment 906:167274.

Jia, S., X. Wang, Z. Yuan, F. Lin, J. Ye, G. Lin, Z. Hao, and R. Bagchi. 2020. Tree species traits affect which natural enemies drive the Janzen-Connell effect in a temperate forest. Nature Communications 11:286.

Kergunteuil, A., G. Röder, and S. Rasmann. 2019. Environmental gradients and the evolution of tri-trophic interactions. Ecology Letters 22:292–301.

Kleinschmidt, S., W. Wanek, F. Kreinecker, D. Hackl, D. Jenking, A. Weissenhofer, and P. Hietz. 2020. Successional habitat filtering of rainforest trees is explained by potential growth more than by functional traits. Functional Ecology 34:1438–1447.

Kobe, R. K., and C. F. Vriesendorp. 2011. Conspecific density dependence in seedlings varies with species shade tolerance in a wet tropical forest. Ecology Letters 14:503–510.

Krishnadas, M., R. Bagchi, S. Sridhara, and L. S. Comita. 2018. Weaker plant-enemy interactions decrease tree seedling diversity with edge-effects in a fragmented tropical forest. Nature Communications 9.

Lai, H. R., D. Craven, J. S. Hall, F. K. C. Hui, and M. van Breugel. 2021. Successional syndromes of saplings in tropical secondary forests emerge from environment-dependent trait–demography relationships. Ecology Letters 24:1776–1787.

Letourneau, D. K., L. A. Dyer, and G. V. C. 2004. Indirect Effects of a Top Predator on a Rain Forest Understory Plant Community. Ecology 85:2144–2152.

Longino, J. T., M. G. Branstetter, and P. S. Ward. 2019. Ant diversity patterns across tropical elevation gradients: effects of sampling method and subcommunity. Ecosphere 10:e02798.

Martínez-Garza, C., V. Peña, M. Ricker, A. Campos, and H. F. Howe. 2005. Restoring tropical biodiversity: Leaf traits predict growth and survival of late-successional trees in early-successional environments. Forest Ecology and Management 217:365–379.

Mudrák, O., J. Doležal, and J. Frouz. 2016. Initial species composition predicts the progress in the spontaneous succession on post-mining sites. Ecological Engineering 95:665–670.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2019, May 12. vegan: Community Ecology Package.

Osone, Y., A. Ishida, and M. Tateno. 2008. Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with nitrogen absorption rate of roots. The New Phytologist 179:417–427.

Rasmann, S., L. Pellissier, E. Defossez, H. Jactel, and G. Kunstler. 2014. Climate-driven change in plant–insect interactions along elevation gradients. Functional Ecology 28:46–54.

Rodríguez-Castañeda, G., L. A. Dyer, G. Brehm, H. Connahs, R. E. Forkner, and T. R. Walla. 2010. Tropical forests are not flat: how mountains affect herbivore diversity. Ecology Letters 13:1348–1357.

Roslin, T., B. Hardwick, V. Novotny, W. K. Petry, N. R. Andrew, A. Asmus, I. C. Barrio, Y. Basset, A. L. Boesing, T. C. Bonebrake, E. K. Cameron, W. Dáttilo, D. A. Donoso, P. Drozd, C. L. Gray, D. S. Hik, S. J. Hill, T. Hopkins, S. Huang, B. Koane, B. Laird-Hopkins, L. Laukkanen, O. T. Lewis, S. Milne, I. Mwesige, A. Nakamura, C. S. Nell, E. Nichols, A. Prokurat, K. Sam, N. M. Schmidt, A. Slade, V. Slade, A. Suchanková, T. Teder, S. van Nouhuys, V. Vandvik, A. Weissflog, V. Zhukovich, and E. M. Slade. 2017. Higher predation risk for insect prey at low latitudes and elevations. Science 356:742–744.

Sam, K., B. Koane, D. C. Bardos, S. Jeppy, and V. Novotny. 2019. Species richness of birds along a complete rain forest elevational gradient in the tropics: Habitat complexity and food resources matter. Journal of Biogeography 46:279–290.

Sam, K., B. Koane, and V. Novotny. 2015. Herbivore damage increases avian and ant predation of caterpillars on trees along a complete elevational forest gradient in Papua New Guinea. Ecography 38:293–300.

Schmitz, O. J. 2010. Resolving Ecosystem Complexity (MPB-47). Princeton University Press.

Silva, N., A. J. P. Coelho, and J. A. A. Meira-Neto. 2023. Functional traits patterns along an altitudinal gradient in a large tropical forest region. Flora 308:152403.

Solé, R., S. Gripenberg, O. T. Lewis, L. Markesteijn, H. Barrios, T. Ratz, R. Ctvrtecka, P. T. Butterill, S. T. Segar, M. A. Metz, C. Dahl, M. Rivera, K. Viquez, W. Ferguson, M. Guevara, and Y. Basset. 2019. The role of herbivorous insects and pathogens in the regeneration dynamics of Guazuma ulmifolia in Panama. Nature Conservation 32:81–101.

Szefer, P., K. Molem, A. Sau, and V. Novotny. 2020. Impact of pathogenic fungi, herbivores and predators on secondary succession of tropical rainforest vegetation. Journal of Ecology 108:1978–1988.

Szefer, P., K. Molem, A. Sau, and V. Novotny. 2022. Weak effects of birds, bats, and ants on their arthropod prey on pioneering tropical forest gap vegetation. Ecology n/a:e3690.

Terborgh, J. W. 2015. Toward a trophic theory of species diversity. Proceedings of the National Academy of Sciences of the United States of America 112:11415–11422.

Velásquez, A. C., C. D. M. Castroverde, and S. Y. He. 2018. Plant and pathogen warfare under changing climate conditions. Current biology : CB 28:R619–R634.

Walker, L. R. R., J. Walker, and R. del Moral. 2007. Forging a New Alliance Between Succession and Restoration. Pages 1–18 *in* L. R. Walker, J. Walker, and R. J. Hobbs, editors. Linking Restoration and Ecological Succession. Springer, New York, NY.

Wang, Z., H. Huang, H. Wang, J. Peñuelas, J. Sardans, Ü. Niinemets, K. J. Niklas, Y. Li, J. Xie, and I. J. Wright. 2022. Leaf water content contributes to global leaf trait relationships. Nature Communications 13:5525.

Whitfeld, T. J. S., V. Novotny, S. E. Miller, J. Hrcek, P. Klimes, and G. D. Weiblen. 2012. Predicting tropical insect herbivore abundance from host plant traits and phylogeny. Ecology 93:S211–S222.

Williams, P. J., R. C. Ong, J. F. Brodie, and M. S. Luskin. 2021. Fungi and insects compensate for lost vertebrate seed predation in an experimentally defaunated tropical forest. Nature Communications 12:1650.

Zhang, S., G.-R. Xu, Y.-X. Zhang, W.-F. Zhang, and M. Cao. 2023. Canopy height, rather than neighborhood effects, shapes leaf herbivory in a tropical rainforest. Ecology 104:e4028.

Zhang, X., C. van Doan, C. C. M. Arce, L. Hu, S. Gruenig, C. Parisod, B. E. Hibbard, M. Hervé, C. A. M. Robert, R. A. R. Machado, and M. Erb. 2019. Plant defense resistance in natural enemies of a specialist insect herbivore. bioRxiv:710681.

Zirbel, C. R., T. Bassett, E. Grman, and L. A. Brudvig. 2017. Plant functional traits and environmental conditions shape community assembly and ecosystem functioning during restoration. Journal of Applied Ecology 54:1070–1079.