**Impact of pathogenic fungi, herbivores and predators have on secondary succession of a tropical rainforest vegetation**

Piotr Szefer1,2, Kenneth Molem3, Austin Sau3, and Vojtech Novotny1,2

1 Faculty of Science, University of South Bohemia, Branišovská 31, 370-05 České Budějovice, Czech Republic.

2 Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

3 New Guinea Binatang Research Center, Madang, Papua New Guinea

**Key Words**: secondary forest, tropics, Papua New Guinea, insect herbivory, pathogenic fungi, predator exclosures, successional dynamics, top down factors, food webs.

**Abstract**

1. Successional theories emphasize plant performance and competitive abilities as key drivers of early successional dynamics, in contrast to top-down biotic factors maintaining plant diversity in mature tropical forests.

2. Here we are testing, for the first time, the effects of food web interactions on the early successional dynamics in a lowland tropical forest in Papua New Guinea. On 36 vegetation plots (25 sq. meters) we initiated secondary succession and for one year experimentally manipulated insect herbivores, fungal pathogens and predators using broad spectrum insecticides, fungicides, predator exclosures and herbivore introductions.

3. Insects were responsible for increased diversity and total plant biomass and shifts in community composition. Nonetheless, increasing pressure of generalist herbivores didn’t further amplify that effect and in turn strong herbivory pressure caused significant decrease in plant biomass. Our results showed that fungal pathogens are able to increase abundance of woody plants measured as stem density. Presence of both insects and top predators increased predictability of community assembly processes.

*4. Synthesis*. In early successional communities, hebivorous insects control diversity, biomass and community composition. Simplified or disturbed aboveground insect communities and their predators can increase randomness of the community assembly processes, which potentially can slow down regeneration of tropical forests. Effects of pathogenic fungi, causing increase in stem density, may affect later stages of succession.

**Introduction**

Ecological succession is one of the few complex, community-level processes that are underpinned by ecological theory allowing us to some extent predict its trajectory, both in terms of species composition and species traits of secondary vs. primary forest species (Turner 2001). Deterministic, rule based succession is believed to be driven mainly by inter-specific competition and environmental filtering (van Breugel et al. 2012, Asefa et al. 2017, Craven et al. 2018). Forest regeneration patterns are thus hypothesized to be mainly shaped by plant traits and abiotic conditions (Yamamoto 2000, Schnitzer & Bongers 2002), while the effects of top-down biotic interactions are rarely considered. This situation is in sharp contrast with increasing attention paid to top-down control of plant composition in primary tropical forest. The Janzen-Connell hypothesis suggests that diversity of these forests could be maintained by the density-dependent mortality caused by plant pathogens and herbivores (Janzen 1970, Connell 1971). In tropical forests herbivorous insects (Gillet 1962), their predators (Richards & Coley 2007) and pathogenic fungi (Augspurger 1983, Clark & Clark 1989) are ubiquitous, abundant and have the ability to affect plant population dynamics and ecosystem processes (Crawley 1989). Recent manipulative experiments in Neotropical old-growth forest have shown that pathogenic fungi, acting at the seedling stage, are important density-dependent mortality agents. Herbivorous insects were able to affect community compositon, but they were killing seedlings independently of their density (Bagchi et al 2010, 2014). To our knowledge there are no studies extending manipulative experiments to key predators of herbivores despite the fact that the importance of trophic cascades in tropical forests is well recognized (Letourneau et al 1998, Milton and Kaspari 2007, Leles et al. 2017).

Early successional communities assemble under specific ecological conditions: they arise as a result of unpredictable disturbance and, for a short period of time, offer environment rich in sunlight often also mineral resources, and competition free space. This environmental filtering favours traits that maximize dispersal and growth rate at the expense of anti-herbivore defences (Coley et al. 1985, Denslow 1987, Herms & Mattson 1992). Therefore, pool of early successional species is relatively small and more closely related phylogenetically than expected by chance (Norden 2009, Whitfeld et al. 2012). This can have important implications for the structure of interactions with their natural enemies.

Herbivorous insects are more abundant on vegetation in secondary than in primary forest because of the higher abundance of more palatable and poorly defended young foliage (Lepš et al. 2001, Whitfeld et al. 2012). On the other hand pathogenic fungi, potential density-dependent mortality factors, have higher infection rates in shade tolerant species (García-Guzmán & Heil 2014). Mobile natural enemies, like bats and birds, tend to follow more abundant prey into the canopy gaps (Richards & Coley 2007). Importantly, the impact of pathogens, herbivores, or predators on plants cannot be inferred solely from the frequency of trophic interactions. For instance, high herbivory could be compensated by fast growing pioneer species, but not slow-growing primary forest species (Trumble et al. 1993, Strauss and Agrawal 1999). Manipulative experiments are therefore key approach to assess the importance of top-down biotic information on plants.

In the course of secondary succession specific leaf area (SLA) tends to decrease and leaf dry-matter content (LDMC) to increase (Buzzard et al. 2015, Boukili & Chazdon 2016). High community weighted mean (CWM) SLA values and low LDMC values often indicate low competitive pressure within the community (Kunstler et al. 2016). So far, these traits were shown to be connected to plant competition (Lasky et al. 2014), but their role in mediating plant community responses to biotic factor during succession has not been examined to date.

Even low variability in environmental conditions often leads to alternative, divergent successional pathways (Mesquita et al. 2001, Suding et al. 2004, Williamson et al. 2012). The unpredictability is assigned to random, neutral dynamics (Hubbel 2001) including colonization, extinction and ecological drift. Some of these random changes in the structure of early successional plant communities may persist for decades (Saldarriaga et al. 1988). Changed trajectories often lead to significant differences in community structure (Norden et al. 2015), species composition (Guariguata & Ostertag 2001, Barlow et al. 2007) and species turnover rates (Mesquita et al. 2015). Therefore, making predictions on the outcome of successional processes is a challenging task. Whether biotic factors can increase predictability of succession hasn’t been evaluated yet for secondary tropical rainforests.

In this paper we experimentally test the general hypothesis that above-ground biotic factors: fungal pathogens, insect herbivores and predators can have significant impact on the initial secondary succession of tropical rainforest vegetation. More specifically, we hypothesise that herbivores control productivity, richness and composition of the early successional plant community by mitigating inter-specific competition between plants (Chesson 2000). Contrarily predators could, by limiting herbivores abundance, cause simplification of the plant community composition through increased inter-specific competition between species. We also test if intensification of herbivory pressure without predators would further increase plant community richness and diversity. We hypothesize, that the impact of pathogenic fungi, at least for the initial stages of secondary succession, may be weaker than in primary forest because of the unfavourable micro-climatic conditions, but we consider this treatment as important in the view of their importance in primary forests. Finally we hypothesize that the biotic factors, by responding to the initial plant composition, determined mostly by dispersal, can in predictable manner shift succession trajectories by increasing determinism in community assembly process.

**Materials and methods**

*Study sites*

The experiment was conducted in tropical rainforest surrounding Wanang vilage (145°5′32″E, 5°14′26″S) located in the Wanang Conservation Area in the Ramu River basin, Madang Province, Papua New Guinea. The climate in the region is humid, with a mild dry season from July to September. Annual rainfall averages at around 3500 mm and mean monthly temperature is 26°C (McAlpine et al. 1983). The study was conducted in abandoned food gardens created during slash-and-burn subsistence agriculture within the primary forest matrix. Abandoned gardens originate from forest patches cleared for cultivation. After clearence vegetation is burned, gardens planted with mixed crops and after a few harvests, typically after two years, gardens are abandoned and left to spontaneous forest regeneration. This setup creates an excellent opportunity to study secondary succession. Old abandoned gardens have open canopy and mostly undisturbed seed bank, required for a secondary growth to initiate. Lack of tillage, usage of pesticides or fertilizers during cultivation leaves soil properties and microbial biomass unchanged, thus making local conditions practically indistinguishable form soil in naturally created forest gaps (Kukla et al. 2019). Usually structure of those regenerating forests resembles that of the nearby natural forest tree gaps (pers. obs.).

*Experimental design*

Within the radius of three km from the village center we selected six experimental gardens (blocks) 3-5 years old, each covering an area of approximately 400 m2, separated by a minimal distance of 200 m. Within each block we cleared all aboveground vegetation and removed all the rocks and dead wood in order to keep bare soil exposure at minimum 95% for each plot. We also removed all tree stumps and tree roots from top 30 cm of soil to prevent re-sprouting. These initial preparations were shown not to have an effect on the seed bank and soil conditions (Chazdon 2014). Within each block six 5x5 m experimental plots were established, resulting in total 36 experimental plots (six plots within each of the six blocks). Individual plots were separated by a one meter wide strip and three meter cleared margins around the block was established to minimize edge effects. Control plot and five experimental treatments were randomly assigned to plots within each block according to the randomized complete block design. Following treatments were applied continuously for 12 months:

1. Control (C) was sprayed weekly with appropriate amount of water simulating application of pesticides. In order to account for possible effect of fencing used in I, P, H1 and H2 treatments (see below) a 50 cm high dummy plastic fence with a 10-20 cm gap left at the ground level was build to allow for easy access to crawling insects, lizards and rodents.

2. Exclusion of pathogenic fungi (F). To reduce infections by wide range of fungal pathogens, a contact fungicide mancozeb (12.5 g soluble powder per 5l, commercial name: Mancozeb) was applied on above ground plants according to manufacturer’s instructions at weekly intervals. A dummy fence was also used as in C.

3. Herbivorous insect exclusion (I). To achieve both systemic and non-systemic protection we applied two types of broad spectrum insecticides: lambda cychalothrin (5 ml per 5l, commercial name: Thunder) and imidacloprid (1.5ml per 5l**,** commercial name: Mustang). Because of intensive vegetation growth insecticide applications were repeated every week according to the manufacturer’s instructions. The plot was surrounded by a 50 cm high plastic fence which continued 20 cm below the ground. Insect glue (tanglefoot) was repeatedly applied on the top of the fence to prevented insect colonization.

4. Exclusion of predatory ants, insectivorous birds, and bats (P). Transparent fishing net (1.5-2.0 cm mesh size) was secured over the plot to protect vegetation from foraging birds and bats. To reduce key invertebrate predators – ants – we used poisonous tuna baits (Permethrin 0.5%) exposed inside the plot in monthly intervals. The plot was surrounded by a plastic fence as in the treatment I to prevent ants from re-colonizing the plot.

5. Increased herbivory – moderate (H1). We used locally common, highly polyphagous and flightless *Oribius* sp. weevils to increase herbivory pressure within the plot. Weevils from this genus were previously documented from the local secondary vegetation and their wide polyphagy was established by feeding tests [Supplementary Information - Vojta] . Every two weeks, 25 weevils were collected in 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 with insect glue applied on the top of the fence to prevent weevils from dispersing to the remaining plots.

6. Increased herbivory – high (H2). The same treatment was applied as above but with 125 *Oribius* sp. weevils per plot introduced every two weeks.

*Sampling*

After 12 months of undisturbed growth complete above-ground vegetation from all 36 plots was destructively sampled. At each plot all plant species were sorted and identified to species and weighed with 1g accuracy. During sampling all the stems with diameter at the breast height (DBH) ≥1cm were counted and their DBH value measured. Leaf area was calculated by sampling leaves from a pool of all leaves for each species at a given plot until 50x50 cm area (“leaf frame”) we filled. Frame was then photographed and processed using ImageJ software (Schneider et al. 2012). Herbivory, defined as a proportion of leaf area loss due to insect feeding, was obtained by comparing leaf blade area measured to the total estimated leaf area. Leaf fram samples were then oven dried, weighted and leaf dry matter content (LDMC) was recorded. Measurements of specific leaf area (SLA) were calculated as a ratio of leaf area to its dry mass. Further in the text, unless stated otherwise, community-weighted means (CWM) for LDMC and SLA values will be used. CWM’s are community level average trait values, where each species’ contribution is weighted by their biomass.

*Data analysis*

All analyses were performed in R (R Core Team 2018). To evaluate the effects of treatments on various community characteristics we developed linear and generalized linear mixed-models using the *lme4* package (Bates et al. 2015) where we considered block (garden) as a random factor. We modelled diversity, logarithm of the cumulative biomass on the plot and CWM for traits using *lmer* function. Herbivory and LDMC values were logit-transformed, and SLA values log transformed prior to the analyses to obtain Gaussian error distributions. For the number of species within the plot we used Poisson distribution and function *glmer*. Due to over-dispersion in the data we assumed negative binomial distribution distribution for the stem abundance and estimated parameters of the model using *glmer.nb* function. For the above analyses we obtained p-values for our models using *lmerTest* package (Kuznetsova et al. 2017).

To test for changes in community composition we used RDA ordination conditioned on block as implemented in *vegan* package (Oksanen et al. 2016). Significance of differences were assessed by permutation tests (999 randomizations) while preserving the random structure of blocks determined by the experimental design. Because of the differences in local plant communities biomass within each plot individual plant biomass was standardized by total biomass of the community. To test for dominance shifts in our communities we selected few species to compare their relative abundance in control plot and treatment plots. For meaningful comparisons we choose species which were present in at least three blocks for both control and treatment plots regardless of the block and which had average proportion in the community higher than 0.1 [run the code and select species again]. We compared average proportions for given species in a control and treatment combination using *betareg* function in the betareg package (Cribari-Neto and Zeileis 2010). To see if there were differences in trait values between species responsible for the community shifts we build a linear mixed-effect model wit trait values evaluated at the control plots as a response and garden as a random effect. For multiple comparisons we used *lsmeans* and *cdt* functions from the emmeans (Lenth 2019) package with Tukey’s multiplicity adjustment.

To determine if the relative importance of random and deterministic processes during community assembly was affected by our treatments we used null model approach proposed by Chase et al. (2011). It is based on the comparisons of dissimilarity matrices for randomly assembled plots with empirical ones. For a given pair of plots simple transformation (see below) of the number of randomizations with dissimilarity smaller or equal than its observed value defines the Raup-Crick (RC) index. This index has a useful interpretation and it can be used to measure importance of deterministic processes during community assembly. Values close to negative one indicate that the community converge deterministically resulting in higher than random chance of observing more similar plant composition. In contrast, RC values approaching one suggest that during assembly process different communities diverge deterministically, resulting in higher chance of observing more dissimilar plant community composition. Instead of using presence absence data we adapted procedure modified by Stegen et al. (2013) and later by Alberti et al. (2017) to species’ biomass. We performed randomizations separately for each of the five control and treatment combinations (i.e. C vs. F, C vs. I, C vs. P, C vs. H1, C vs. H2) each comprising 12 plots (six control plots and six treatment plots). This way we ensured that experimental treatment plots (30 plots vs. 6 control plots) were not over-represented in the procedure. We set several constraints to the random community assembly process for a single plot. (1) Number of species and total biomass for a given plot was restricted to its empirical values. (2) For each species its sampling probability was proportional to their occurrence frequencies among all 12 plots for a given control and treatment combination. To randomly assemble a community instead of individuals we were sampling units of biomass equal to 0.1 kg. This value was based on the approximated weight of the smallest woody plant individual among all experimental plots. (3) Probability of increasing biomass of a given species in the community by one unit was proportional to the relative biomass of that species evaluated across all 12 plots for a given control and treatment combination. We randomly assembled each pair of plots 999 times. During each iteration we quantified compositional turnover using Bray-Curtis (BC) dissimilarity, calculated with *vegdist* function from the *vegan* package. These randomized BC values were then compared to the empirically observed dissimilarity values and proportion of iterations where BC index was smaller or equal to its empirical value was recorded. To obtain the the RC index value we subtracted 0.5 from that proportion and multiplied this difference by two. This transformation constrain any proportional value to vary between negative one and one (Chase et al. 2011). Following procedure developed by Alberti et al. (2017), we tested treatment effects on community assembly processes using linear mixed models. RC values for pairwise comparisons of plots within the treatment (five comparisons for each treatment, i.e. plots 1 vs. 2, 1 vs. 3, 1 vs. 4, 1 vs. 5, 1 vs. 6) were the response value. Models were built using *lmer* function with a fixed factor of treatment and block as a random factor. We used likelihood-ratios to test the significant differences between the control and treatment plots.

**Results**

After one year of re-growth plant community richness across all experimental plots amounted to 96 species (Table S1), with 40 woody species. Average biomass per plot was 82.156 kg (95% CI: 46.815, 144.175). Most abundant species included *Trema orientalis*, *Melanolepis multiglandulosa* and *Pipturus argenteus*. Highest biomass, nearly 129 kg, was reached by *Melochia* sp. 1. However, this species was present only at one block. From non-woody plants *Mikania micrantha* vein had highest biomass (… kg) among the control plots (Figure S1). We observed high variability in community composition between blocks. Bray-Curtis dissimilarity of control plots averaged at 0.8 with minimum and maximum 0.42 and 0.99, respectively (similarly for woody species alone: mean 0.81, minimum 0.39 and maximum 0.99). One insecticide treated plot was overgrown by a *Calopogonium mucunoides* Desv. vine making up to 72% of the total biomass of the plot which prevented trees from colonizing that plot. Therefore, we treated this plot as an outlier and removed it from the analyses.

High herbivory increase (H2) was the only treatment significantly decreasing plant biomass (Fig. 1) both in the whole community and looking only at woody plants (t-value = -3.594, P = 0.001 and t-value = -3.576, P = 0.001 respectively). This treatment also significantly reduced the number of stems per plot (t-value = -6.707, P < 0.001). Woody plants density was also lower at the fungicide (F) treated plots (z-value = -2.507, P = 0.012). Insect removal (I) caused a decrease in diversity of the whole plant community measured by Shannon’s H’ index (t-value = -2.291 p = 0.031). Similar pattern was shown for Simpson’s diversity. Additionally, insecticide caused increase in LDMC (t-value = 2.228, p = 0.035) and marginally significant decrease in SLA (t-value = -1.991, p = 0.058). Similar pattern was observed in woody plant communities only for LDMC (t-value = 2.071, p = 0.049). No treatment had significantly affected evenness or species richness of experimental communities.

Only insecticide treatment (I) altered species composition significantly (permutation test Fig 3, F = 3.135 p = 0.019) resulting in shifts in the dominance structure in comparison with the control (Fig. ...). The first ordination axis was related with decreasing herbivory and by itself explained 19.55 % of variation in community composition. Similar effects were present when we considered only woody plant communities (permutation test, F = 2.6154, p = 0.05, not shown in the figure). Except for the marginally significance of high herbivory plots (H2) community composition in other treatments didn’t show consistent and predictable patterns. Only few species were present in both control and one of the treatment plot allowing for meaningful comparisons of their relative abundance changes (Fig. 4). Shifts in dominance structure at the insecticide treated plots were mainly caused by a consistent increase in relative abundance of one of the woody plant dominants: *Pipturus argenteus (betareg results).* This pattern coincided with decreased dominance of vein *Mikania micrantha* (betareg results) and *Melanolepis multiglandulosa* (betareg results). *There were no differences in* suffered greater leaf area loss due to herbivory. However, these three species differed significantly in their SLA and LDMC values (Fig. SX Tukey) with *P. argenteus* having higher SLA and lower LDMC value than *M.micrantha* and *M. multiglandulosa* .

The RC index values among the control plots showed highly variable, but non-random communtiy composition (Fig. X). Insecticide treatment (χ2 = 3.892, P = 0.048) and predator exclusion (χ2 = 4.923, P = 0.026) significantly decreased RC values thus increasing randomness of community assembly (mean RC index values closer to zero) for woody plants.

**Discussion**

Our study showed that invertebrate herbivores, their predators (birds, bats and ants) and fungal pathogens, influence early successional rainforest vegetation in lowland Papua New Guinea. Our hypothesis that herbivorous insects control biomass, species richness and diversity of plant community, was partially supported by our results. Our experimental application of insecticide had, similarly to the results from the primary forest (Bagchi et al. 2014), strong effect on the plant community composition and Kempel et al. (2015) argued that these changes can be driven by preferences of invertebrate herbivores. Looking at the damage caused by herbivores for the species driving compositional shifts in our experimental communities we found some support for this explanation. Also previous studies showed that herbivorous insects usually prefer *P. argenteus* (Basset 1996) over *M. multiglandulosa* (Novotny et al. 2004). Insecticide treatment was also responsible for decreased diversity (but not richness and evenness) of our experimental plant communities, which is in partial agreement with majority of large herbivore exclusion experiments from temperate grasslands (Stein et al. 2010, Mortensen et al. 2018, Jia et al. 2018). The mechanism responsible for these patterns has been proposed by Agrawal et al. (2012). Authors showed that when herbivores were removed plant competition intensified and led to higher dominance of a few competitively superior plant species. Communities driven by strong inter-specific competition usually have low SLA and high LDMC (Kunstler et al. 2016). In our experiment smilar trait patterns suggest that diversity at insecticide treated plots could be affected by intensified inter-specific competition. Somewhat unexpectedly, herbivore removal showed no effect on stem density or biomass. Herbivore removal experiments in the tropics usually result in higher biomass and plant abundance (Jia et al. 2018). However, successional theory predicts no effect of herbivores on the biomass of pioneer stages if plants tend to maximize their growth in competition for light and, in accordance with resource availability hypothesis (Coley et al. 1985), they can compensate for biomass loss due to herbivores (Maron et al. 2014).

Artificially increased herbivory predictably led to lower biomass and lower stem density, although, only for the high level of herbivore additions. Interestingly, this large structural change did not indicated a response in species richness, diversity and evenness. This lack of impact may confirm conclusions based on simple simulations performed by Freckleton and Lewis (2006) which showed that density dependence caused by generalist natural enemies acting through total community density (as opposed to individual species density) would not affects richness, diversity and evenness. We show here that this might be the case at least for insect herbivores in the early successional communities in the tropics.

As discussed in review by Mooney et al. (2010) effects of vertebrate predators on plant communities are stronger, whenever predaceous arthropods are abundant and there is a strong intra-guild predation. However, decrease in plant biomass was only marginally significant and not detected in the woody plant community. The lack of effects on plant biomass caused by focal predators partially reducing herbivores is not surprising considering that even the complete removal of insect herbivores in the insecticide treatment did not have such effect. Another possible explanation is that the high abilities of pioneer trees to compensate for herbivory damage, as well as higher abundance of species least attractive to herbivores (e.g. *M. multiglandulosa*), may cause biomasses of experimental communities not to differ greatly from those in the control plots. Moreover, Schmitz et al. (2000) argued that top-down effects of predators are more likely to be present in communities where plants have anti-herbivore defences. In our experiment, despite some level of variation in herbivore defences, in general these are not expected to be strong (Kardol et al 2006, Lasky et al. 2014).

No effect of pathogenic fungi removal on community composition supports our prediction of their generally less significant impact early in the succession. Fungal pathogens can drive species turnover through accumulation of host specific pathogens in time (Kardol et al. 2006). For example Mangan et al. (2010) showed that old-growth forest dynamics are driven by plant-soil feedbacks mediated by pathogenic fungi. Importance of fungal pathogens could, therefore, be expected to increase later in the succession and to correspond with growing density of mature leaves and changes in environmental conditions facilitating fungal infections (Marquis et al. 2001). Effect of pathogenic fungi on community richness might not be noticeable also in the situation where dominant pathogens are generalists (Freckleton & Lewis 2006), which is a safe assumption for early successional plant communities as host specialists usually need more time to establish their viable populations within a community (Koziol & Bever 2016). Moreover, fungicide application decreased the number of stems per plot, exactly as generalist natural enemies did in predator exclusion (P) and increased herbivory (H2) treatments.

RC values at the control plots revealed that species composition of the communities showed higher than random variability between sites (divergent but deterministic community composition). This result confirms predictions that in highly productive plant communities growing in nutrient rich environment would show deterministic but divergent assembly processes due to priority effects (Chase and Liebold 2002, Chalcraft et al 2004, Chase 2010). The RC values for communities without herbivores and with predators excluded were closer to zero, similarly as in herbivore exclosures from grassland communities (Alberti et al. 2017). More random community assembly with suppressed herbivores might be caused by random colonization of the plots by plant species from the forest matrix. In case of predators exclusion Chase et al. (2009) argued that abundant consumers, in our case herbivorous insects, can increase stochasticity during community assembly process by decreasing number of individuals in the community, and boost their chance of local extinction. We found partial support for this hypothesis in our data, where we observed lower number of stems in plots without predators. Fungicide treatment did not increase randomness of the community assembly processes despite their negative effect on stem density. Mordecai (2011) presented two hypotheses for pathogenic fungi diversity promoting mechanisms, which would produce similar patterns to the ones observed in our data: (1) reduction of fitness differences between pioneer trees and (2) changing the competitive structure between species. Second explanation is less probable as it should also result in significant and consistent changes in plant community structure. Therefore, we conclude that pathogenic fungi promote richness through differential effects on dominant plant species, where abundant species experience negative feedback, and rare species are positively affected or are neutral to fungal infections. Additionally, fungal infection can change herbivore preferences and performance on infected plants (Fernandez-Conradi et al. 2018), which introduces another level of complexity to fungi-plant-herbivore interactions.

Despite our considerable effort to define and execute treatments few pitfalls regarding our methods were identified. During evaluation of the effectiveness of ant eradication treatment we didn’t find significant decrease in ant abundance on the ground. However, harvested vegetation generally had low abundance of small sized, non-predatory canopy ants. Fungicide used in our experiment can potentially accumulate in the soil, which at high concentration can suppress ammonification and nitrification processes (Walia et al. 2014). However, we expected that these undesirable effect would be minimized due to short half-life times of mancozeb (Xu 2000) and easy biodegradability in tropical soil conditions (Racke et al. 1997). In addition to the predicted increase of arachnids abundances under their natural enemies suppression, netting used for the exclosure constructions could potentially boost the abundance of web-building spiders. Number of blocks used for the experiments was limited by the number of suitable locations available and our abilities to maintain and sample them all at the same time. Therefore, some effects may have not been detected and increased sample size could result in higher statistical power of performed tests.

Our results point out on an important role of local scale interaction webs of pathogenic fungi, predators and herbivorous insects during initial successional processes which shape future plant community composition. We identify potential factors which, if taken into account, can be used to decrease high unpredictability of successional trajectories found in empirical data. From our study we conclude that without a rich network of consumers successful regeneration of plant communities may be inhibited and strength of contingent effects during plant communities assembly processes can increase due to intensified inter-specific competition. We show that in the tropics herbivorous insects have the ability to change the competitive structure within regenerating communities, resulting in secondary effects on the community composition. Moreover, different species responses to biotic factors can be related to their traits. Importantly we showed, that pathogenic fungi, previously identified as important factors in shaping diversity of primary forests have limited influence on the plant assembly process. However it should change later in the succession. To get deeper insights into the mechanisms at place additional information on seed rain, mortality of newly established seedlings in the route of succession would have to be collected. Natural step forward would be to study effects of these biotic factors in combinations to identify possible complementary effects on plant composition and succession. Detailed demographical data for individual species combined with information on their competitive abilities, preferably tested in the greenhouse experiments, would allow for better understanding of the exact mechanisms of biotic control of successional communities. In the future disentangling complex relationships of individual plant species functional traits with biotic factors could allow for a better understanding of how can we facilitate more effective regeneration of tropical forests.

**Acknowledgments**

Tereza Holicova for processing leaf frames data. Jan Leps, Juan Alberti, Leonardo Re Jorge, Nick Pardikes and Moria Robinson for useful discussion, Binatang Rerearch Center staff in Papua New Guinea for support during field work. This article was funded by grants: GACR 17-23862S and the ERC grant 669609 to VN.

**Authors’ contribution**

VN and PS conceived the ideas and designed methodology; KM, AS and PS managed experiments and collected the data; KM identified plants; PS analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**References**

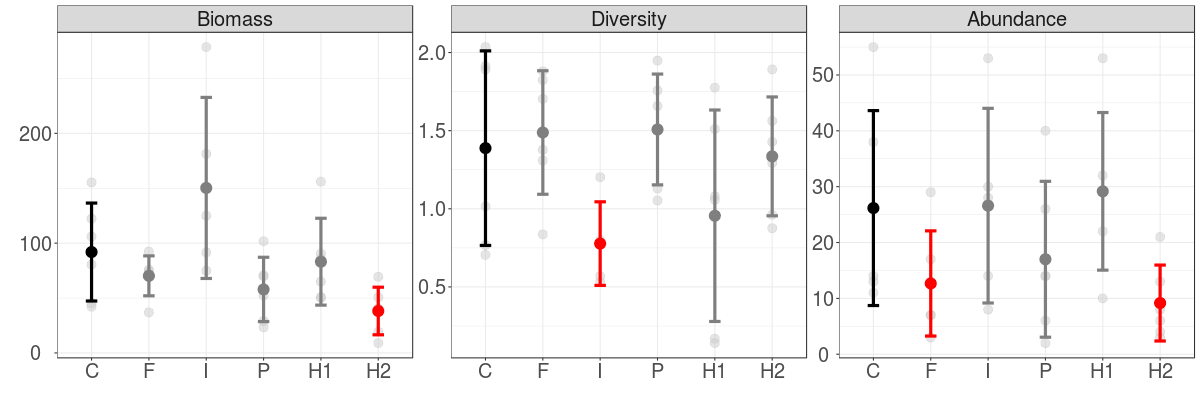


Figure 1. Community descriptors based on all species present in the experimental plot from Wanang. Red colour indicates that the mean for a treatment is significantly different from the control at the alpha = 0.05 level. Biomass is measured in kilograms. To evaluate diversity of vegetation plots we used Shannon’s index. Abundance represents number of stems above one cm DBH per plot. C – control, F – fungicide, I – insecticide, H1 – moderate increase in herbivory pressure, H2 – high increase in herbivory pressure.

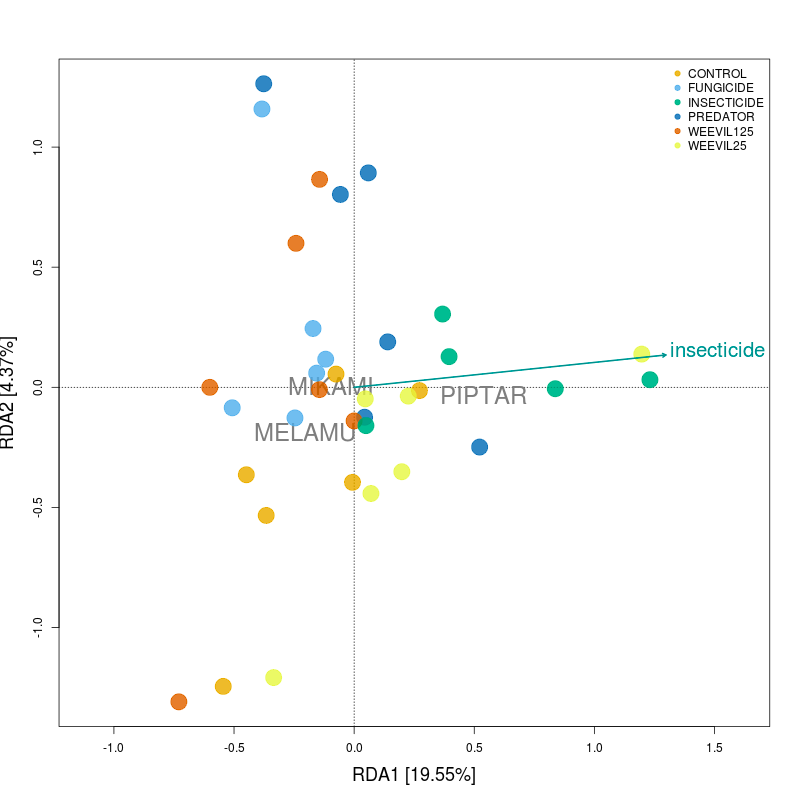


Figure 3. Redundancy analysis of the plant biomass data, conditioned on their blocks. Only significant factor is plotted. Each axis is described by their percentage of explained variance. Species

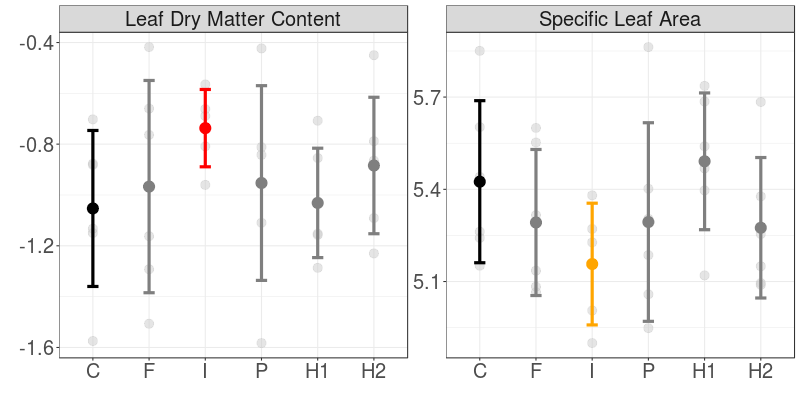


Figure 2. Community weighted means of measured leaf traits for the whole community and for the woody plant species. Grey point represents experimental plot overgrown by *Callopogonium mucunoides*, and was excluded from the analyses. Values of LDMC and herbivory are logit transformed, and SLA log transformed.

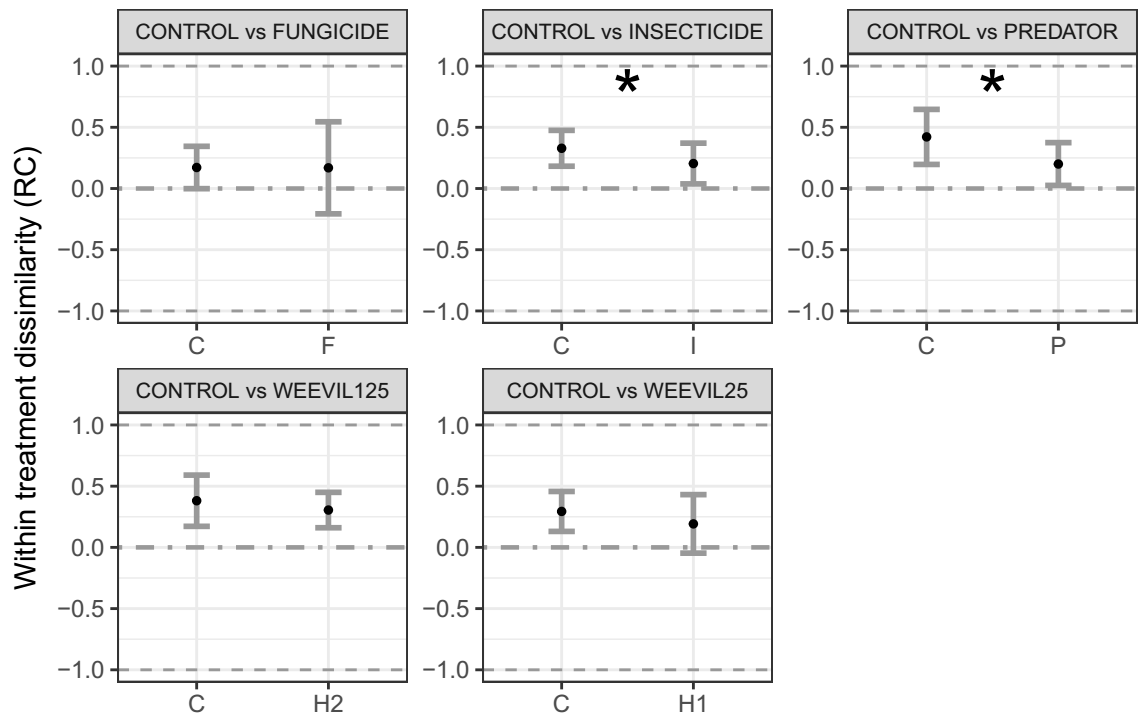


Fig 4. [plot only significant ones?]Within treatment dissimilarity (mean ± SE) according to a modified Raup-Crick dissimilarity metric (RC) for the woody plant community (no significant differences were found for the whole community). This metric ranges from -1 to 1, indicating whether a pair of plots are more similar (approaching 1), , or less similar (approaching -1) than expected by chance. Dashed horizontal line denotes a stochastic community assembly. Asterisks denote significant differences between treatments (P<0.05). The whole plant community including also herbaceous vegetation and grasses are shown in the Supplementary Information.

